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**Title: Physiological responses to partial-body cryotherapy performed during a concurrent strength and endurance session**

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## **Abstract**

This investigation examined the effect of partial-body cryostimulation (PBC) performed in the recovery time between a strength training and an interval running (IR). Nine rugby players [age  $23.7 \pm 3.6$ , BMI  $28.0 \pm 2.6$  kg/m<sup>2</sup>] were randomly exposed to two different conditions: i) PBC: 3-min at  $-160^{\circ}\text{C}$ ; ii) passive recovery at  $21^{\circ}\text{C}$ . We recorded the bioelectrical impedance analysis (BIA), temperature, and cardiac autonomic variables in three moments: at baseline, after strength training (R0) and after 90-min of recovery (R90). Additionally, the blood lactate concentration was measured 1-min before and 2.5-min after the IR. The heart rate, energy cost, minute ventilation, oxygen uptake and metabolic power were assessed during the IR. The homeostatic hydration status was affected by the execution of intense strength training sub-session. Then, after PBC the BIA vector was restored back, close to normohydration status. Autonomic variables changed over time in both conditions, although the mean differences and effect sizes were higher in the PBC condition. During IR, the heart rate was 3.5% lower after PBC, and the same result was observed for the oxygen uptake ( $\sim 4.9\%$ ) and ventilation ( $\sim 6.5\%$ ). The energy cost measured after cryotherapy was  $\sim 9.0\%$  lower than after passive recovery. Cryotherapy enhances recovery after a single strength training, while during the subsequent interval running it shows a reduction in cardiorespiratory and metabolic parameters. PBC may be used in those athletes who compete or train more than once in the same day to improve recovery between successive training sessions or competitions.

**Keywords:** baroreflex sensitivity; energy cost; heart rate variability; interval running.



## **Introduction**

A recent strategy used for post-exercise recovery in sports is cryotherapy, which involves local, partial-body (PBC), and whole-body exposures (WBC). The major differences in the two systems are the temperatures ( $-110^{\circ}\text{C}$  for WBC and  $-160^{\circ}\text{C}$  during PBC), the head exposure (only during WBC), and the source of cold stimulation (compressor for WBC and nitrogen gas during PBC) (Hauswirth et al., 2013). The cryotherapy has a vasoconstrictive effect during recovery after exercise, reducing the inflammation responses through a decrease of the cell metabolism, leading to improvements in blood and oxygen supply if performed between two consecutive training sessions. Moreover, stimulates the autonomic nervous parasympathetic activity favouring acute recovery (Hauswirth et al. 2013). Instead, performed before submaximal exercise reduces heart rate leading to higher stroke volumes.

In sports, athletes combine strength and endurance training within the same training cycle, a procedure defined 'concurrent training' (Leveritt et al. 1999). As pointed out by Leveritt et al. (1999), some studies have addressed the issue of assessing the inhibition in strength development after concurrent strength and endurance training with respect to strength training alone. This matter is still controversial (Fyfe and Loenneke 2018). A recent systematic review and meta-analysis (Sabag et al. 2018) has showed that high intensity interval training (HIIT) can be performed with resistance exercise without negatively impacting hypertrophy, and that any attenuation of lower body muscular strength might be improved by prescribing running instead of cycling, with adequate rest between HIIT and resistance sessions. Possible causes of the above-mentioned interferences could be the residual fatigue in the neuromuscular system and the overtraining produced by imbalances in the athlete recovery processes. Rugby players, one time a week,

perform two-a-day workouts, where the interval between training sessions represents their actual recovery time. Deficiency of proper recovery may result in the athlete being unable to train at the required intensity or complete the required load in extra time (Barnett 2006). Therefore, during these training sessions, a key issue is the recovery modalities that should be followed by the athletes. Until now, only two studies have investigated the effects of cryotherapy on recovery between two training sessions. Schaal et al. (2013) demonstrated that WBC yields a significant increase in vagal-related heart rate variability (HRV) indices and a decrease in heart rate (HR) during 70 min of recovery between two synchronized swimming performances. Ferreira-Junior et al. (2014) found a significant increase, after 40 min of strength recovery, on eccentric peak torque and total work when subjects were exposed to PBC with respect to passive recovery.

Partial-body cryotherapy has become a popular mode of cryotherapy (Hauswirth et al. 2013), but only few studies have investigated this method on recovery ( Ferreira-Junior et al. 2014; Hauswirth et al. 2013; Holmes and Willoughby 2016). Moreover, no study has examined the influence of cryotherapy during concurrent training in team sports. Therefore, the present study aimed to evaluate the effect of cryotherapy performed during the recovery time between a strength training and an interval running in a group of rugby players. We hypothesized that the physiological responses to this treatment will accelerate strength recovery, improving the subsequent interval running in athletes who train more than once on the same day. The rationale of our hypothesis is that, cryotherapy would induce lower submaximal heart rate due to lower thermal stress, as well as the increase of cardiac parasympathetic activity (Stanley et al. 2014), favouring acute recovery after strength training. In addition, cryotherapy causes a peripheral vasoconstriction that leads to improvements in blood and oxygen supply in the

working musculature (Krüger et al. 2015) improving the cardiorespiratory efficiency or less cardiovascular stress during the subsequent intermittent exercise. From a practical perspective, PBC might speed up recovery in those athletes who compete or train more than once in the same day.

## **Methods**

### ***Subjects***

An a priori power analysis was conducted to determine the sample size for the study (G\*Power 3.1.9.2, Germany). The following design specifications were considered:  $\alpha = 0.05$ ;  $(1-\beta) = 0.8$ ; effect size  $f = 0.5$ ; test family = F test and statistical test = ANOVA repeated measures, within-between interaction. The sample size estimated according to these specifications was 8 subjects. Thus, we selected 9 rugby league players who volunteered to participate in this study (Table 1). All subjects were instructed to continue normal daily activities and to refrain from training the day before testing. Additionally, all the exclusion criteria suggested by Podbielska et al. (2006) were respected. This study was approved by the Bioethic Committee of the University of Bologna, and all participants were informed of the benefits and risks of the investigation prior to signing an institutionally approved informed consent document to participate in the study.

### ***Testing procedure***

The athletes visited the laboratory 4 times. All tests were performed at the same time of the day (9:00 – 12:00 AM), in a quiet room with stable temperature (21°C; 52% of humidity). During the first visit, the athletes completed an incremental running test to determine their maximal oxygen uptake ( $\dot{V}O_2\text{max}$ ) and to familiarize with the interval running on treadmill. The second visit was used to



determine their maximal whole-body muscular strength (1-RM). The recording sessions were performed during the third and fourth visits when the athletes were tested in a randomized, counterbalanced, crossover design (Figure 1).

**Maximal oxygen consumption assessment ( $\dot{V}O_2\text{max}$ ).** The expired gas analysis (Quark CPET, Cosmed, Italy) was performed on motor-driven treadmill (Cosmed, Italy) at 8 km/h for 3-min as a warm-up, followed by 1 min at 10 km/h with 1% of incline, and an instantaneous increase of 0.5 km/h every min until exhaustion. The maximal exercise test lasted until attainment of oxygen uptake ( $\dot{V}O_2$ ) plateau or the attainment of at least one of the two additional criteria: (i) a plateau of heart rate despite an increased velocity, or (ii) exercise cessation due to substantial fatigue.  $\dot{V}O_2$  plateau was defined as an increase in  $\dot{V}O_2 \leq 50 \text{ ml min}^{-1}$  during the last 30 s despite increased velocity (Yoon et al. 2007). The highest  $\dot{V}O_2$  values reached during the exercise phase of the incremental test were considered as the  $\dot{V}O_2\text{max}$ .

**Strength assessment (1-RM).** Maximal whole-body muscular strength was assessed using the following five multiarticular exercises: bench press, squat, barbell row, deadlift, and overhead press. The participants first completed ten warm-up repetitions, and after a suitable rest period, one repetition maximum (1-RM) values were assessed according to the following equation:  $1\text{-RM} = \text{weight lifted} / [1.0278 - (0.0278 \times N^\circ \text{ repetitions})]$  (Brzycki 1993). It has been easy to establish with accuracy their 1-RM as they were athletes accustomed to strength exercises. Proper exercise execution was followed and enforced by one of the investigators who supervised and documented each testing session.

**Recording sessions.** During the third and fourth visits, separated by one-week, the athletes were tested in a randomized, counterbalanced, crossover design (Figure 1): 1) PBC; partial body cryotherapy: 3-min at  $-160^\circ\text{C}$  during 90-min of recovery; 2) CON; control: 90 min of passive recovery at  $21^\circ\text{C}$ . We measured bioelectrical-

impedance standards, temperature, and cardiac autonomic regulation parameters in three moments: at baseline, after strength training (start of recovery - R0), and after 90-min of recovery (R90). Additionally, the blood lactate concentration (La) was measured 1-min before and 2.5-min after the IR sub-session, while heart rate (HR), energy cost (EC), minute ventilation (VE), oxygen uptake ( $V'O_2$ ), and metabolic power (Pmet) were assessed during the IR sub-session (Figure 1). PBC was performed 15 minutes after strength training in a head-out cryochamber using gaseous nitrogen (Space Cabin Cryomed, Slovakia) at  $-160^{\circ}\text{C}$  for 3-min. Participants wore bathing suits, gloves, socks and shoes with thermic protection for their extremities. For the remaining recovery period, athletes seated at the laboratory with a temperature of  $21^{\circ}\text{C}$ . The CON condition consisted of passive recovery, during which athletes remained seated on a chair in the laboratory at  $21^{\circ}\text{C}$ . During the entire procedure (exercise and recovery), drinking or eating was forbidden.

Body temperature (head, right hand, right foot) was measured by an infrared thermometer (Tecnimed, Italy) in three moments: at baseline, after strength training (R0), and after 90-min of recovery (R90). The measurement areas were marked with a pen before each condition.

\*\*\*\*\*Figure 1 near here\*\*\*\*\*

### ***Exercise Programs***

***Strength protocol.*** Each sub-session started with a 5-min warm-up. The conditioning phase involved the same five resistance exercise stations used for the maximal whole-body muscular strength assessment. The program involved performing five sets of progressively heavier weights and lower reps, separated by 3 min of rest. It was an “*ascending pyramid*” program (increase weight, decrease reps), and consisted of performing a light set (50% of 1RM) for 10 reps, followed by 8 reps at 60%, 6

reps at 70%, 4 reps at 80%, and 2 reps at 90% of 1RM (Brown and National Strength & Conditioning Association (U.S.), 2007).

***IR protocol.*** Each sub-session was performed on the same motor-driven treadmill used during the  $\dot{V}O_2\text{max}$  test and monitored through the expired gas analysis and HR monitoring. It consisted of 1-min sprints at 95% of  $\dot{V}O_2\text{max}$  followed by 1-min of active rest at 45% of  $\dot{V}O_2\text{max}$  repeated for a total of 10-min of running (Denadai et al. 2006). Blood lactate samples were performed 1-min before and 2.5-min after interval running. Both the strength and IR sessions were supervised by three investigators.

### ***Body composition assessment***

All body composition measurements were performed in resting conditions. Body height was measured by a wall stadiometer to the nearest 0.1 cm and body mass was measured using a balance to the nearest 0.1 kg. Fat-free mass and fat mass were determined with the equation of Kotler et al.(1996).

Bioelectrical impedance vector analysis (BIVA), studied and validated for healthy population, as well as for specific patient subgroups and athletes (Campa and Toselli 2018; Lukaski 2013; Micheli et al. 2014) was performed with a phase-sensitive impedance plethysmograph (BIA-101, Akern-RJL Systems, Italy) at baseline; after strength training; and after 90 min of recovery. The electrodes were placed on the right hand and foot dorsum while the subjects lied in a supine position. The bioelectrical-impedance vector analysis calculates the body composition considering the impedance components like resistance (R) that depends on lean tissue mass and tissue hydration, and reactance (Xc), associated with cell size and integrity of the cell membranes (Lukaski, 2013). We standardized R and Xc by the subjects' height (H) to remove the effect of conductor length, thus expressing values

in Ohm/m. The combination of R and Xc return an impedance vector (arc tangent of Xc/R expressed in degrees), and its direction is defined as the phase angle (PA°). PA° is an indicator of cellular health and an index of fluid distribution between the intracellular and extracellular compartments. A main advantage of the use of PA° is that it can be applied even under unstable tissue hydration conditions (Selberg and Selberg 2002), because it indicates progressive changes in tissue hydration; from dehydration to hyperhydration with apparent edema (Lukaski 2013).

### ***Cardiac autonomic regulation assessment (HRV and BRS)***

Athletes were asked to stay in a supine position for 15 min, in a comfortable bed, with a respiratory frequency at 12-15 breaths/min. Only 5 min, from 10' to 15' were used for analysis (Camm et al. 1996). Participants underwent non-invasive continuous blood pressure monitoring using the servo-controlled infrared finger plethysmography (Portapres device; TNO/BMI) for analysis of HRV and baroreflex sensitivity (BRS) (Piras et al. 2017; Piras and Gatta 2017).

***HRV analysis.*** The Portapres recordings were used to extract time series of R-R intervals and systolic as well as diastolic pressures, to analyse HRV and BRS. Data were analysed with Kubios HRV software (v. 3.0, 2017, Finland), in which all time series were filtered to exclude artefacts. Time domain indices for HRV analysis were the standard deviation of the R-R interval (SDNN) and the square root of the mean squared differences of successive R-R intervals (RMSSD). The power spectrum density of HRV was estimated by an autoregressive method with model validation. Powers in the bands of low frequency (LF, 0.04–0.15 Hz) and high frequency (HF, 0.15–0.4 Hz) were calculated. It has been shown that the HF spectral component of HR variability (HFRR) is an index of the vagal tone, whereas both sympathetic and vagal activities contributed to the LF (LFRR) spectral component of HRV (Camm et

al. 1996). Both indices were expressed in absolute and normalized units. Such normalized units are obtained by dividing the power of each component by total variance from which the very-low-frequency component (below 0.04 Hz) had been subtracted and multiplying this value by 100 (Pagani et al. 1986).

**BRS analysis.** Baroreflex sensitivity was computed from RR intervals and systolic blood pressure sequence subtracted from the finger arterial pressure waveform. These data were then used to define the oscillations in both heart rate and systolic arterial pressure measures. Beatscope version 1.1a (TNO/BMI, The Netherlands) was used to evaluate spontaneous BRS, with a BRS add-on module that computes the time-domain cross correlation BRS.

### ***Energy cost assessment during IR***

In both running conditions, HR,  $\dot{V}O_2$ ,  $\dot{V}E$  and respiratory exchange ratio were collected breath by breath (Quark CPET, Cosmed, Italy). A blood sample was obtained from the index finger to determine  $La$  (Lapre; Lapost) (Lactate Scout, Germany) 1-min before and 2.5-min after running.

Figure 2 shows a typical tracing of a subject's oxygen uptake as a function of time during IR in both CON and PBC conditions.  $\dot{V}O_2$  increases during each bout of exercise at about  $95\%VO_{2max}$  and decreases during active recovery at about  $45\%VO_{2max}$ . Metabolic data collected during the working phase were averaged and used in the following analysis.

The energy cost (EC) of interval running was calculated based on values of  $\dot{V}O_{2net}$  and of blood lactate concentration (Zamparo et al. 2015):

1. The breath-by-breath net oxygen uptake ( $\dot{V}O_{2net}$ , ml/kg/min), was calculated by subtracting the resting  $\dot{V}O_2$  from  $\dot{V}O_2$  values. The energy

derived from aerobic energy sources ( $EO_2$ , ml/kg) was calculated by multiplying  $V'O_{2net}$  for the total exercise duration (min).

2. The energy derived from anaerobic lactic energy sources ( $ELa$ , ml/kg) was calculated by multiplying  $La_{net}$  (mM), e.g. the difference between the largest value of  $La$  recorded at the end of the test and  $La$  recorded at rest, by an energy equivalent of 3.3 ml/kg/mM (Di Prampero and Ferretti 1999).

3. The net energy cost of interval running was then calculated as follows:

$$EC = [EO_2 + ELa] / d, \quad (1)$$

where  $d$  is the total distance covered.  $EC$  was expressed in J m/kg using an energy equivalent (J ml/ $O_2$ ) which considers the respiratory exchange ratio.

Moreover, the instantaneous  $P_{MET}$  expressed in W/kg, was obtained multiplying  $EC$  by the running speed (expressed in m/s).

\*\*\*\*\*Figure 2 near here\*\*\*\*\*

### ***Data Analysis***

Shapiro-Wilk tests were used to check the normal distribution of data. Measures with skewed distribution were log transformed (Ln) before analysis.

Time (baseline; R0; R90) x condition (PBC; CON) repeated measures ANOVAs were performed to analyse temperature, HRV, and BRS.

For all metabolic variables recorded during IR sub-sessions, the mean values of the PBC vs. CON condition were compared with Student's t test for paired data. Effect sizes were calculated as the mean difference standardised by the between-subject standard deviation and interpreted according to the following thresholds: trivial, <0.20; small, >0.20-0.60; moderate, >0.60-1.20; large, >1.20-2.00; very large, >2.00-4.00; extremely large, >4.00 (Hopkins et al. 2009). Statistical significance was set at  $p < 0.05$ . Post hoc tests were corrected with the Bonferroni procedure. Data

were analysed with SPSS v22.0 (SPSS, Chicago, IL, USA). Intra-class correlation coefficient was used to test the reliability of mean speed of both IR tests.

## **Results**

### ***Subjects***

The mean speed maintained by the athletes during both IR sub-sessions was  $2.76 \pm 0.17$  m/s, with high degree of reliability between measurements, in which the average measure ICC was 0.959 with a 95% confidence interval from 0.819 to 0.991.

\*\*\*\*\*Table 1 near here\*\*\*\*\*

### ***Body composition and temperature***

Repeated measure analysis indicated no significant differences between conditions at baseline and after strength training (Table 2, all  $P > 0.05$ ). After 90 min of recovery, a significant time x condition interaction effects was observed. Post-hoc tests showed that, after PBC, R/H ( $P < 0.001$ ,  $d = 0.78$ , moderate), Xc/H ( $P < 0.001$ ,  $d = 0.90$ , moderate), and PA ( $P = 0.013$ ,  $d = 0.52$ , small) increased, meanwhile, hand ( $P = 0.000$ ,  $d = -11.95$ , extremely large) and foot temperature ( $P < 0.001$ ,  $d = -14.19$ , extremely large) decreased. From baseline to R0 the vector decreased in both PBC and CON condition ( $P < 0.001$ ), meanwhile, a significant increase was observed between R0 and R90 only after PBC (Table 2).

\*\*\*\*\*Table 2 near here\*\*\*\*\*

### ***Cardiac autonomic regulation***

ANOVAs showed a significant time main effect for all autonomic variables investigated ( $P < 0.05$ ), meaning that variables changed over time in both CON and PBC condition. In addition, mean differences and effect sizes were higher on PBC in all cardiac autonomic regulation parameters (Table 3).

\*\*\*\*\*Table 3 near here\*\*\*\*\*

### ***Energy cost analysis***

The metabolic data obtained from the IR are reported in Table 4. Respiratory exchange ratio,  $\dot{V}_E$ , and  $\dot{V}'O_2$  were not significantly affected by cryotherapy ( $0.469 < P > 0.116$ ;  $0.63 < d > 0.15$ ), while all other metabolic parameters were lower during IR performed after PBC ( $0.006 < P > 0.000$ ;  $1.40 < d > 0.44$ ). Heart rate was 3.5% lower during IR performed after PBC than in the CON condition, and the same result was observed for  $\dot{V}'O_2$  (~4.9%) and  $\dot{V}'E$  (~6.5%). The EC measured after cryotherapy was ~9.0% lower than after passive recovery ( $P < 0.001$ ,  $d = -1.40$ , large). Figure 3 displays the EC of the athletes during IR in the two testing conditions, in which individual differences ranged from -3.98 to -17.27%. Although the variable of main interest in this study was EC, a comparison of  $P_{MET}$  mean values was also performed, and analysis showed that  $P_{MET}$  was higher in CON than in PBC (~6.7%).

\*\*\*\*\*Figure 3 and Table 4 near here\*\*\*\*\*

### **Discussion**

In the present study we investigated, in rugby players, the influence of a single session of cryotherapy performed during the 90-min of recovery between a strength training and a high-intensity interval running sub-sessions on body composition, temperature, and cardiac autonomic responses, and its effects on energetic cost during the subsequent interval running. The main findings of this study were as follows: (i) Bioelectric impedance analysis showed that strength training affected hydration parameters and PBC enhanced recovery compared to passive rest. (ii) PBC after strength training influenced the pattern of autonomic function recovery, in which indexes reflecting tonic cardiac vagal outflow such as SDNN, RMSSD, and HF power reflected moderate to large effect sizes. Furthermore, consistent changes were observed in BRS, which is a measure of reflex cardiac vagal



responsiveness (Piras et al. 2017; Piras et al. 2015; Piras and Gatta, 2017). (iii) Cardiorespiratory and metabolic parameters were reduced during a single bout of IR after PBC, indicated by lowered HR,  $\dot{V}O_2$ ,  $\dot{V}E$ , EC, and PMET with respect to CON.

### ***Bioelectrical impedance analysis***

The bioelectrical impedance analysis is commonly used to monitor changes in hydration and body composition induced by training (Piccoli et al. 1995). In our study, the mean of the impedance vector between baseline and R0 differed in the same way in both conditions, returning to normal when repeated 90-min later only after PBC. An intense physical training may affect cellular membrane stability, and passive recovery is unable to restore homeostasis (Korthuis 2011). Immediately after strength training, we found a decrease of the phase angle in both conditions, a situation characterised by an increase in body fluid volume, maybe due to muscle swelling. Then, only after PBC the  $PA^\circ$  was restored close to normohydration status. Because the rate of fluid movement across the microcirculation initially exceeds the drainage capacity of the lymphatic system, interstitial fluid volume can almost double within 15 min of the onset of intense rhythmic exercise (Korthuis 2011). Intense training could induce muscle damage and subsequent inflammation indicated by muscle soreness, swelling, and prolonged loss of muscle function. It has been already demonstrated that PBC stimulated physiological reactions of an organism which result in analgesic, anti-swelling, antalgic immune and circulatory system reactions and then could improve recovery after muscle injury from muscular trauma (Ferreira-Junior et al. 2015). A possible reason for these results may be related to a decrease in core, muscle, and skin temperatures after cryotherapy exposure (Bleakley et al. 2014). This physiological response may lead to increased vasoconstriction,

reducing blood vessel permeability and thus decreasing the cellular inflammatory process (Ferreira-Junior et al. 2015; Hausswirth et al. 2011).

In our study, temperature decreased after cryotherapy exposure. We are aware that the lack of muscle and core temperatures is a limitation because such measurements may have provided useful information on the body heat variations during each recovery procedure. However, skin temperature of hand and foot in the present study dropped respectively  $3.4\pm 0.8$  and  $4.2\pm 0.6$  °C immediately after PBC compared to the basal level.

### *Autonomic responses*

Regardless of the great sympathetic response necessary to support maximal strength production rates during exercise, we demonstrated that autonomic function recovered fully at the cardiac level within 90-min in both CON and PBC conditions. Furthermore, we found higher effect sizes after cold treatment in all cardiac autonomic regulation parameters, mainly on vagal-mediated HRV and BRS indices (Table 3). Schaal et al. (2013), examining the influence of WBC during recovery between two training sessions, showed that swimmers were able to repeat the same maximal workload with similar autonomic, metabolic, and subjective responses. Hausswirth et al. (2013) determined whether PBC was as effective as a WBC in stimulating a parasympathetic activation. Whatever the cryotherapy technique used, results showed that a single 3-min cryostimulation induced a strong autonomic response. Same results were found by Westerlund et al. (2006) on how an extreme cold air exposure influences cardiac autonomic regulation and its adaptation effects on healthy women.

Perhaps, the high level of training and endurance at high loads of our athletes resulted in an immediate recovery of the cardiac autonomic parameters. Therefore,

parasympathetic reactivation after maximal exercise was not a limiting factor to recovery but could have affected the subsequent high intensity exercise capacity, seen that a link was found between the magnitude of parasympathetic reactivation and the cardiorespiratory and metabolic parameters recorded during subsequent interval running after PBC compared to passive recovery.

### ***Oxygen uptake***

Cryotherapy had induced a reduction of the cardiorespiratory and metabolic parameters recorded during IR. Heart rate was about 3.5% lower after PBC than CON condition, and the same result was observed for  $\dot{V}O_2$  (~4.9%),  $\dot{V}E$  (~6.5%), EC (~9.0%) and PMET (~6.7%). These results are in agreement with those of Krüger et al. (2015) who found, after 3-min of WBC at -110°C, lowered values of heart rate,  $\dot{V}O_2$ , rate of perceived exertion, and a higher muscle oxygenation during running at submaximal intensity. The beneficial effects of cold treatment are commonly believed to be associated with rapid reduction in core temperature, which in turn may reduce fatigue associated with hyperthermia and suppressed muscle blood flow and metabolic activity (Holmes and Willoughby 2016). Schmidt and Bruck (1981) claimed that decreasing the body temperature at the onset of exercise, the critical environmental heat stress limits for exercising would decrease. This may result in performance enhancement of both endurance time and increased work rate (Ferretti et al. 1995). Metabolic load was reduced during running after PBC, indicated by lower  $\dot{V}O_2$ , energy cost, and metabolic power. We observed an average additional cost of 0.34 J/kg/m during running after passive recovery. Reduced submaximal  $\dot{V}O_2$  suggests that running after cold treatment seems somewhat less energy demanding than after passive recovery, with less effort needed to complete the intermittent exercise or a lower  $\dot{V}O_2$  of the passive muscles due to peripheral

vasoconstriction and therefore lower blood and oxygen directed to non-working musculature (Krüger et al. 2015). Ferretti et al. (1995) found that the minimum mechanical power necessary to elicit  $\dot{V}O_2\text{max}$  during cycling is about 21 W lower at 31°C than at 36°C. Stanley et al. (2014) found that  $\dot{V}O_2$  kinetics and muscle oxygen utilization were reduced after cold water immersion compared with passive recovery.

Schaal et al. (2013) found an equal or greater  $VO_{2\text{peak}}$  on WBC in comparison to passive recovery, where swimmers were able to repeat the same maximal workload with similar autonomic, metabolic, and subjective responses. Conversely, Drust et al. (2000) found no significant differences for the oxygen consumption or heart rate under normal (20°C), heated (26°C) or pre-cooled (cold shower at 24°C) conditions during intermittent exercise in soccer players, although there was a tendency for heart rate to be lower during the pre-cooled condition. These data therefore suggest that cold treatment might improve oxygen uptake efficiency at muscle level with respect to passive recovery, possibly through different mechanisms. First, cooling results in reduced muscle perfusion and edema formation, all factors that reduce the transit distance between capillaries and muscle fibers facilitating oxygen delivery to the muscle cells (Ihsan et al. 2013). Second, decreased peripheral blood flow may increase central blood volume and enhance blood delivery to the working muscles (Lee and Haymes 1995), resulting in a greater contribution of the aerobic system to energy supply, combined with the low level of blood lactate that we have found at the end of the IR effort. Third, the increase in muscle blood flow redistribution and reduction in maximal heart rate, together with the left shift of the oxygen dissociation curve known to occur when temperature is decreased, can explain the observed lower  $\dot{V}O_2$  during running (Ferretti et al. 1995).

## **Conclusions**

In conclusion, cryotherapy enhances recovery after a single strength training sub-session, while during the subsequent interval running it shows a reduction in cardiorespiratory and metabolic parameters. These findings agree with the suggested mechanisms by which cooling between successive workouts has been recommended to enhance muscle recovery and as such it can be used in sports. Therefore, from a practical perspective, cryotherapy may be used in those athletes who compete or train more than once in the same day (i.e. soccer, rugby) to improve recovery between successive trainings or competitions.

**Conflict of Interests:** The author(s) declare(s) that there is no conflict of interests regarding the publication of this article.

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	<i>Mean</i>	<i>SD</i>
<i>Age</i>	23.67	3.61
<i>Weight (Kg)</i>	91.94	11.36
<i>Height (m)</i>	181.04	5.40
<i>BMI (kg/m<sup>2</sup>)</i>	27.99	2.58
<i>Training week (h)</i>	8.50	1.80
<i>Experience (yr)</i>	12.22	5.26
<i>VO<sub>2</sub>max (ml/kg/min)</i>	47.16	3.69
<i>VO<sub>2vel</sub> (95%) (km/h)</i>	13.11	0.78
<i>VO<sub>2vel</sub> (45%) (km/h)</i>	6.56	0.46
<i>Bench Press (kg)</i>	116.22	18.14
<i>Squat (kg)</i>	155.56	21.63
<i>Barbell Row (kg)</i>	108.33	15.39
<i>Deadlift (kg)</i>	142.11	26.04
<i>Overhead Press (kg)</i>	75.78	9.01

**Table 1.** Anthropometric, training (1-RM) and physiological parameters

**Table 2.** Mean

	Baseline		R0		R90		± SD of body composition parameters
	CON	PBC	CON	PBC	CON	PBC	
<b>FM (kg)</b>	15.35±5.39	16.42±5.14	13.84±5.25	13.42±4.68	15.84±4.53	15.43±4.58	
<b>FFM (Kg)</b>	75.50±7.84	75.32±7.15	77.29±8.54	78.32±7.90	76.92±10.38	75.10±8.65	
<b>R/H (Ohm/m)</b>	218.99±22.18	220.78±19.46	211.68±22.61	207.07±18.96	217.49±29.90	222.69±24.13*	
<b>Xc/H (Ohm/m)</b>	31.52±3.76	31.54±3.40	29.38±3.48	28.78±3.55	30.89±4.24	32.19±4.64*	
<b>PA (degrees)</b>	8.20±0.60	8.13±0.52	7.90±0.60	7.90±0.62	8.10±0.34	8.23±0.74*	
<b>Head temperature (°C)</b>	36.38±0.40	36.38±0.28	36.20±0.28	36.06±0.35	36.46±0.33	35.76±0.83	
<b>Hand temperature (°C)</b>	33.94±2.51	33.40±2.64	35.21±0.59	35.24±0.62	34.94±0.52	30.03±0.44*	
<b>Foot temperature (°C)</b>	34.45±1.87	34.17±1.67	35.03±0.35	34.73±0.47	34.96±0.41	30.10±0.51*	

*Abbreviations:* FM, fat mass; FFM, fat free mass; R/H, resistance divided by body height; Xc/H, reactance divided by body height; PA, phase angle.

\*Significant time x condition interaction effect ( $P<0.05$ ) after 90 min of recovery.



**Table 3.** Mean differences (with Cohen's *d*) of autonomic nervous system parameters after recovery (R0 vs. R90) in both conditions

	<b>LF/HF</b>	<b>LF (nu)</b>	<b>HF (nu)</b>	<b>HR (bpm)</b>	<b>SDNN (ms)</b>	<b>RMSSD (ms)</b>	<b>BRS (ms/mmHg)</b>
<b>CON</b>	-1.08 (-0.75)	-11.77 (-0.72)	11.81 (0.72)	-8.75 (-1.15)	23.36 (0.95)	17.41 (1.01)	5.86 (0.78)
<b>PBC</b>	-1.19 (-0.95)	-12.11 (-0.73)	12.14 (0.73)	-9.20 (-1.21)	24.50 (1.00)	21.13 (1.34)	9.41 (0.96)

*Abbreviations:* LF, low frequency; HF, high frequency; HR, heart rate; RMSSD, root mean square of standard deviations; BRS, baroreflex sensitivity.

**Table 4.** Physiological data (mean  $\pm$  SD) collected during the IR sub-sessions

	CON	PBC	diff %	<i>P</i>	Cohen's <i>d</i>
<b>V'O<sub>2</sub> (ml/min/Kg)</b>	35.94 $\pm$ 3.81	34.23 $\pm$ 3.12	-4.9	0.006*	-0.49
<b>V'E (l/min)</b>	88.75 $\pm$ 12.44	83.41 $\pm$ 11.91	-6.5	0.005*	-0.44
<b>HR (bpm)</b>	142.62 $\pm$ 10.98	137.71 $\pm$ 9.76	-3.5	0.001*	-0.47
<b>RER</b>	0.95 $\pm$ 0.05	0.93 $\pm$ 0.05	-2.3	0.469	-0.38
<b>La<sub>pre</sub> (mmol/l)</b>	2.02 $\pm$ 0.43	2.30 $\pm$ 0.45	9.9	0.116	0.63
<b>La<sub>post</sub> (mmol/l)</b>	2.67 $\pm$ 0.65	2.89 $\pm$ 0.69	4.4	0.335	0.33
<b>E'O<sub>2</sub> (ml/kg(min))</b>	306.23 $\pm$ 42.58	311.94 $\pm$ 311.94	2.0	0.348	0.15
<b>EC (J/Kg/m)</b>	4.11 $\pm$ 0.29	3.77 $\pm$ 0.18	-9.0	0.000*	-1.40
<b>Pmet (W/Kg)</b>	11.28 $\pm$ 1.25	10.57 $\pm$ 1.02	-6.7	0.006*	-0.62

*Abbreviations:* V'O<sub>2</sub>, oxygen uptake; V'E, minute ventilation; HR, heart rate; RER, respiratory exchange ratio; La, blood lactate concentration (pre- and post-IR); E'O<sub>2</sub> net energy expenditure derived from aerobic energy sources; EC net energy cost; P<sub>met</sub>, metabolic power.

\* Significant differences between conditions ( $P < 0.05$ ).

### **Figure legends**

**Figure. 1** Graphical overview of the testing protocol with the timeline of events. Exercise bout 1 (strength training), exercise bout 2 (IR), start of recovery (R0), end of recovery (R90), heart rate variability (HRV), baroreflex sensitivity (BRS), temperature ( $T^{\circ}$ ), bioelectric impedance (BIA), partial-body cryotherapy (PBC), control condition (CON), energy cost (EC), minute ventilation ( $V'E$ ), heart rate (HR), oxygen consumption ( $V'O_2$ ), metabolic power ( $P_{MET}$ ).

**Figure. 2** Representative profile of an athlete's oxygen uptake as a function of time (sec) during IR in both CON (black) and PBC (grey) conditions.  $V'O_2$  increases during each bout of exercise at about  $95\%V'O_{2max}$  and decreases during active recovery at about  $45\%V'O_{2max}$ .

**Figure. 3** Individual differences (9 subjects) in energetic cost (EC) between control (CON) and partial-body cryotherapy (PBC) conditions.



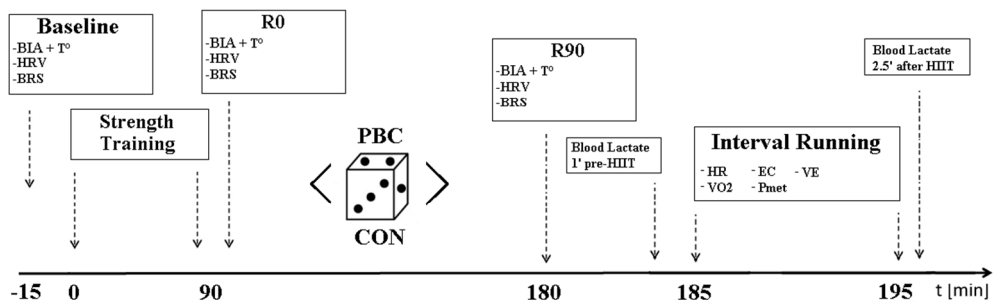


Figure. 1 Graphical overview of the testing protocol with the timeline of events. Exercise bout 1 (strength training), exercise bout 2 (IR), start of recovery (R0), end of recovery (R90), heart rate variability (HRV), baroreflex sensitivity (BRS), temperature (T°), bioelectric impedance (BIA), partial-body cryotherapy (PBC), control condition (CON), energy cost (EC), minute ventilation (V'E), heart rate (HR), oxygen consumption (V'O2), metabolic power (PMET).

101x31mm (300 x 300 DPI)

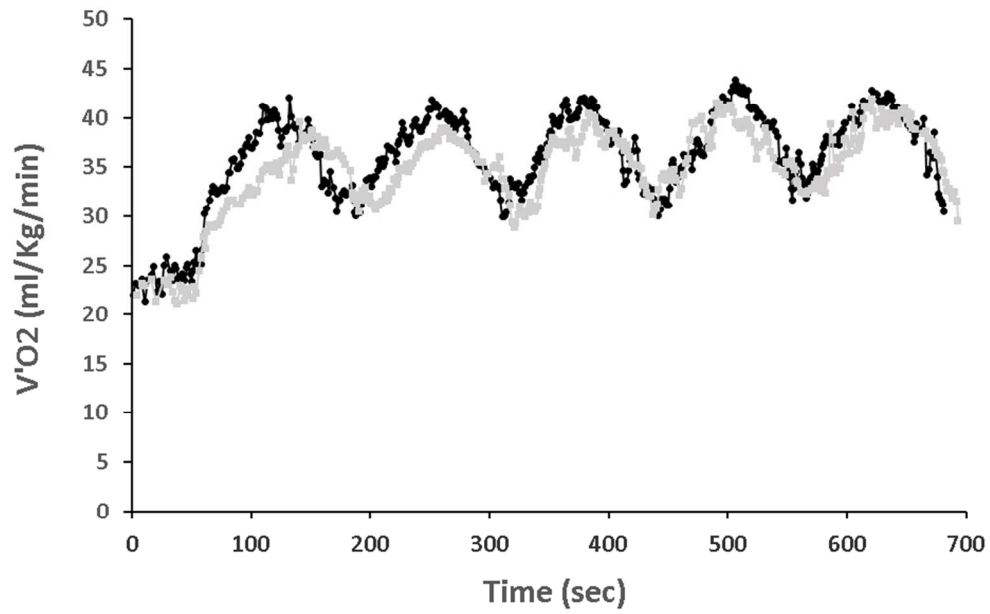


Figure. 2 Representative profile of an athlete's oxygen uptake as a function of time (sec) during IR in both CON (black) and PBC (grey) conditions.  $\dot{V}O_2$  increases during each bout of exercise at about 95% $\dot{V}O_{2max}$  and decreases during active recovery at about 45% $\dot{V}O_{2max}$ .

80x49mm (300 x 300 DPI)

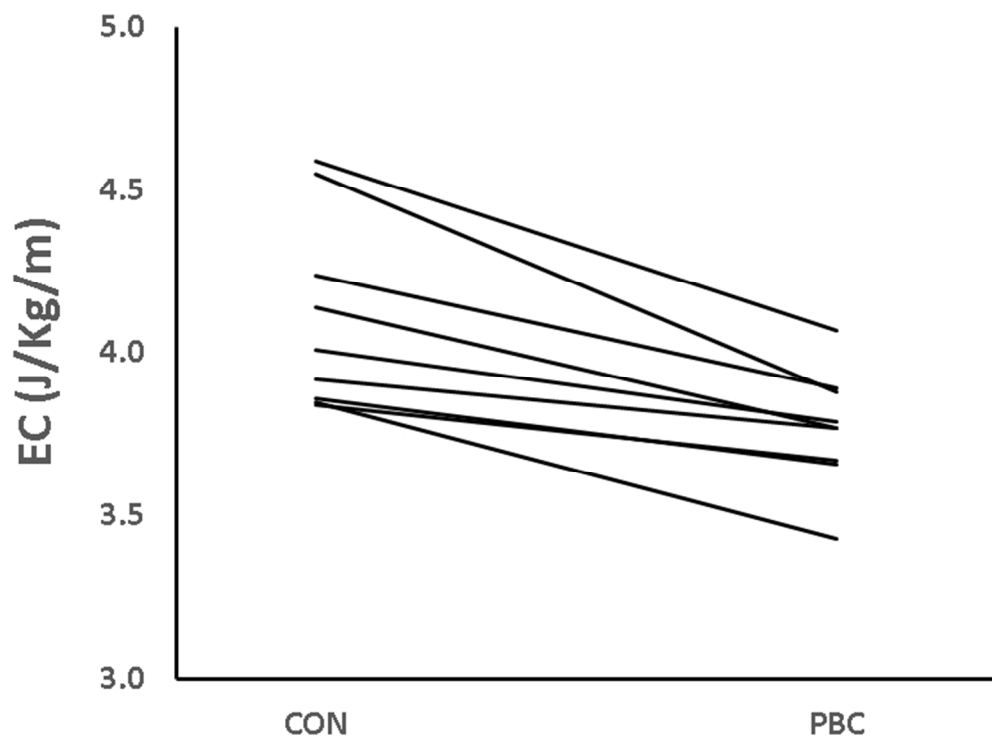


Figure. 3 Individual differences (9 subjects) in energetic cost (EC) between control (CON) and partial-body cryotherapy (PBC) conditions.

65x49mm (300 x 300 DPI)