Abstract:
This study compared the effect of passive rest (CON) and water immersion at 8.6±0.2°C (CWI9), 14.6±0.3°C (CWI15) and 35.0±0.4°C (thermoregulatory water immersion [TWI]) on post-exercise heart rate variability (HRV) indices. In a climate chamber (32.8±0.4°C, 32±5% relative humidity), nine men completed 25 min of cycling at the first ventilatory threshold and repeated 30-second bouts at 90% of peak power followed by a 5-minute recovery treatment in a randomised crossover manner. All water immersion re-established the HRV indices (natural logarithm of the square root of the mean sum squared differences between RR intervals [ln rMSSD], low-frequency [lnLF] and high-frequency power densities [lnHF] and Poincaré plot-derived measures [lnSD1 and lnSD2]) to the pre-exercise levels at 60 min post-immersion; however, only CWI9 accelerated parasympathetic reactivation during immersion. CWI9 increased lnLF and lnSD2 during immersion when compared with CON (p<.05). Although CWI9 had a large positive effect size (ES>0.80) on all HRV indices during immersion when compared with CON, between-conditions differences were observed only in lnLF and lnSD2 (p=.017-.023). CWI15 had a large positive ES on ln rMSSD and lnSD1 when compared with CON (both p=.064). Sympathovagal antagonism (i.e., SD ratio<0.15) did not occur during CWI9 and CWI15. Hence, both CWI treatments are effective means of enhancing post-exercise parasympathetic reactivation, but CWI9 is likely to be more effective at increasing post-exercise cardiac vagal tone.

Key words: autonomic cardiovascular control, cooling, recovery, hydrotherapy, vagal modulation

Introduction
Heart rate variability (HRV) allows non-invasive monitoring of cardiac autonomic activity by quantifying the beat-by-beat variations in heart rate (RR intervals), and is widely used to monitor the recovery of cardiovascular homeostasis following exercise (Buchheit, Peiffer, Abbiss, & Laursen, 2009; Flouris, et al., 2014; Stanley, Peake, Coombes, & Buchheit, 2014). Cold water immersion (CWI) is a popular post-exercise strategy amongst athletes to enhance recovery to preserve exercise performance across training periods (Ihsan, Watson, & Abbiss, 2016; Versey, Halson, & Dawson, 2013). Additionally, hydrostatic pressure and cold-induced peripheral vasoconstriction during CWI are believed to facilitate the recovery of cardiac parasympathetic activation, as inferred from the short-term estimate of HRV time-domain analysis (i.e., square root of the mean sum squared differences between RR intervals [rMSSD]) (Al Haddad, et al., 2010; Buchheit, et al., 2009; Stanley, Buchheit, & Peake, 2012). The immersion temperature may have an important influence on the degree to which CWI affects post-exercise cardiac vagal tone. Indeed, CWI at 14°C has been shown to be more effective in the recovery of cardiac vagal tone, compared with immersion temperature within the range of 28-35°C (Al Haddad, et al., 2010; Ottone, et al., 2014). There are, however, some disparate findings with regards to immersion temperature ≤14°C. For instance, a moderate-to-large effect size (ES=0.6-1.2) of post-exercise CWI at 14°C on rMSSD has been observed...
(Buchheit, et al., 2009; Stanley, et al., 2012), while others have documented minimal effect of post-exercise CWI at 2°C on the same HRV index (Flouris, et al., 2014). Moreover, rMSSD has been shown to remain lower than pre-exercise levels following post-exercise CWI at 10°C (Stanley, et al., 2014), while recovery to pre-exercise levels has been observed during CWI at 14-15°C (Ottone, et al., 2014; Stanley, et al., 2012). Yet, a direct comparison across these studies is difficult due to the differences in study designs (e.g., exercise protocols, ambient temperature and CWI strategies). Specifically, many of these studies completed the exercise task in temperate environments (20-23°C) (Al Haddad, et al., 2010; Bastos, et al., 2012; Ottone, et al., 2014; Stanley, et al., 2012), at low-intensity (Flouris, et al., 2014) or performed short bouts of exercise (Buchheit, et al., 2009). Given that exercise intensity and heat stress can aggravate vagal withdrawal (Brenner, Thomas, & Shephard, 1998), further investigation of the influence of CWI at different immersion temperatures following intense, prolonged exercise in the heat is warranted.

There is a distinct possibility that rapid reduction in heart rate during strong cardiac sympathetic activation (i.e., sympathovagal antagonism) may precipitate cardiac arrhythmias during CWI (Buchheit & Laursen, 2009; Tulppo, Mäkikallio, Seppänen, Airaksinen, & Huikuri, 1998). No apparent sympathovagal antagonism has been documented in studies using immersion temperature ≥14°C (Al Haddad, et al., 2010; Buchheit, et al., 2009). However, to date, we are unaware of any studies that have examined sympathovagal antagonism during CWI below 14°C. On the basis that excessive cooling following high-intensity exercise may accentuate sympathovagal antagonism (Buchheit & Laursen, 2009), further examination of the post-exercise HRV response during CWI below 14°C is warranted. Accordingly, this study aimed to compare the effects of passive rest (CON) and water immersion at 8.6±0.2°C (CWIw), 14.6±0.3°C (CWIw) and 35.0±0.4°C (thermoneutral water immersion [TWI]) on the HRV response following prolonged exhaustive exercise in the heat. We hypothesised that CWIw and CWIw would improve cardiac parasympathetic reactivation, but sympathovagal antagonism would be observed during CWIw.

Methods

Participants

All experimental procedures were approved by the Edith Cowan University Human Research Ethics Committee, and were undertaken in accordance with the ethical standards established by Declaration of Helsinki. Data reported herein were collected as part of a parallel study (Choo, et al., 2016). On separate occasions, nine untrained men (age 29±9 years; body height 172±5 cm; body mass 72.7±6.6 kg; body surface area 1.85±0.10 m²; body fat 19±4%; VO2max 40.4±3.6 mL·kg⁻¹·min⁻¹; mean±SD) completed an incremental exercise test followed by four experimental trials. Participants were non-smokers and free of any known cardiovascular diseases. Written consent was obtained after participants were informed of all experimental procedures and associated risks. Additionally, participants were instructed to record and replicate a 24-hour food log, refrain from exercise for 24 h, and avoid caffeine or alcohol for 12 h. They were also instructed to pre-hydrate with 500 mL of water 2 h before each trial.

Incremental cycling test

Participants performed the incremental exercise on an electronically braked Velotron cycle ergometer (Racermate, Seattle, WA, USA) in a laboratory (24.8±0.8°C and 33±7% RH), with the ergometer configuration adjusted for individual’s comfort and replicated for subsequent trials. After 5 min of self-paced warm-up, the incremental exercise was commenced at 70 W and increased by 35 W every minute until volitional exhaustion. Expired O2 and CO2 were analysed using a metabolic cart (TrueOne 2400, ParvoMedics, Utah, USA), calibrated according to the known gas mixtures (16% O2 and 4% CO2; Airgas Mid South, Tulsa OK, USA) and a 3-L flowmeter calibration syringe (Series 5530, Hans Rudolph Inc., Kansas City, USA). Peak power during the incremental test was determined based on the power achieved during the last completed stage (P) and time in seconds of the uncompleted stage (t) using the following equation: $P + (t/60 × 35)$ (Buchheit, Abbiss, Peiffer, & Laursen, 2012). First ventilatory threshold (VT1) and associated power output (PV1) were visually determined using the ventilatory equivalents method (Gaskill, et al., 2001).

Experimental protocols

All experimental procedures were undertaken in a climate chamber (32.8±0.4°C and 32±5% RH), and each participant reported to the laboratory at the same time of the day separated by 2-14 days. Euhydration was determined by urine specific gravity index (USG; Atago hand refractometer, model UNC-NE, Atago, Tokyo, Japan) ≤1.020 (Casa, et al., 2000). Upon entering the climate chamber, all participants had a mandatory 30-minute seated rest period on a massage table with their backs supported by a wedge-shaped cushion before measurement of resting RR intervals. Participants then commenced an exercise task modified from a previous study (Peiffer, Abbiss, Watson, Nosaka, & Laursen, 2010). Participants cycled for 25 min at PV1 followed two minutes later by repeated 30-second bouts of cycling at 90% of peak power inter-
spersed by 30-second cycling at 70 W. Participants performed the high-intensity intervals to exhaustion during their first trial and the number of intervals performed were replicated during subsequent trials. Each participant was allowed to drink non-chilled water *ad libitum* during the first trial, and volume and timing of consumption were replicated during subsequent trials.

In a crossover manner, participants completed 5 min of water immersion up to the midsternal level at CWI₉, CWI₁₅, TWI or CON (i.e., passive rest in an empty bath). The transition between exercise and the recovery treatments was 9 min 48 s±1 min 36 s during which RR intervals were recorded. A portable cooling unit (iCool Portacover, Gold Coast, Australia) was used to maintain the temperature during CWI, and CWI₁₅, and warm tap water was used to maintain the temperature for the TWI trials to elicit minimal body cooling effect. Water temperature was monitored by a data logger fixed with a thermistor (Squirrel 2020 data logger series, Grant Instruments, Shepreth Cambridge, UK). The temperature and immersion duration for CWI and CWI₁₅ were within the recommended range for the post-exercise recovery (Versey, et al., 2013) and allowed for comparison with relevant research (Al Haddad, et al., 2010; Bastos, et al., 2012; Buchheit, et al., 2009; Stanley, et al., 2012; Stanley, et al., 2014). Upon exit from water, participants towelled dry and moved .1 m to rest in the same seated position on the massage table during which RR-intervals were recorded at 30 min and 60 min post-immersion.

**Short-term HRV recordings**

RR interval data were recorded at 1000 Hz by a Polar S810i heart rate monitor (Polar Electro Oy, Kempele, Finland). Each 5-minute RR series were corrected for aberrant beats and errors using the default Polar ProTrainer 5 software correction algorithm (i.e., moderate filter power and minimum protection zone of 6 bpm). Validity and reliability of such data acquisition and correction algorithm have been established (Nunan, et al., 2009). The corrected RR series were further processed with Kubios HRV software v.2.2 (Biomedical The Signal Analysis Group, Department of Applied Physics, University of Kuopio, Finland). The signals were detrended using the smoothness prior approach and resampled at 4 Hz. Power spectrum analysis was performed with fast Fourier transform using Welch’s periodogram method. The low frequency (LF) band (0.04-0.15 Hz) and high frequency (HF) band (>0.15-0.40 Hz), expressed in ms², were calculated. Analysis was performed on the last three minutes of each 5-minute RR series recorded at baseline, the end of exercise, during immersion, and 30 min and 60 min post-immersion. Respiratory rate was not controlled to allow spontaneous recovery of HR since rMSSD and SD1 have been shown to be minimally influenced by respiratory rate (Penttilä, et al., 2001); however, possible influence of hyperventilation on RR intervals cannot be ruled out with confidence (Tulppo, et al., 2011). Instantaneous beat-to-beat (SD1) and continuous long-term (SD2) variability in the RR intervals, as well as SD ratio (SD1/SD2) were determined by plotting each RR interval as a function of the previous one in a Poincaré plot. Presence of sympathovagal antagonism was indicated by a torpedo-shaped (i.e., small SD1 with SD ratio < 0.15) or parabola-shaped Poincaré plot (Tulppo, et al., 1998). Time-domain (rMSSD), frequency domain (LF and HF) and Poincaré plot-derived HRV indices (SD1, SD2 and SD ratio) were retained for statistical analysis.

**Statistical analyses**

As Shapiro-Wilk tests indicated non-Gaussian distributions, all HRV indices were transformed by natural logarithm. A 2-way (condition × time) repeated measures analysis of variances (ANOVA) was performed to assess for differences in the HRV indices. A 1-way repeated measures ANOVA was performed to assess for differences in the whole body sweat loss and pre-exercise body mass. If the data violated the assumption of sphericity, Greenhouse-Geisser or Huynh-Feldt correction was applied to the degree of freedom based on the epsilon values. When a significant effect (i.e., p≤.05) was observed, pairwise comparisons were performed with the p-values adjusted with Bonferroni correction. If no significant effect was observed, but the p-value was less than 0.1, then an effect size (ES) was calculated (Cohen, 1988). A large ES (>0.80) and low statistical power indicated a likelihood of a type II error. Data are reported as mean ± SD, and statistical analysis was performed using SPSS v.21.0 (SPSS Inc., Chicago, IL, USA).

**Results**

**Exercise duration and hydration status**

Total exercise duration (33.4±3.0 min) and number of high-intensity intervals (8±3) were replicated for all experimental trials. Pre-exercise body mass and USG were not different between the conditions (Table 1).

<table>
<thead>
<tr>
<th>Table 1. Body mass and urine specific gravity (USG) before exercise</th>
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<td><strong>CON</strong></td>
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<td>Body mass (kg)</td>
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<td>USG (g mL⁻¹)</td>
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Effect on time domain HRV indices

A condition x time interaction was observed for ln rMSSD (p<.001). Figure 1a shows that ln rMSSD increased during immersion in CWI15 compared with TWI (p=.050, ES=0.80). At the same time point, the differences between CWI15 and CON (p=.064, ES=0.89), and between CWI9 and CON (p=.077, ES=0.95) or TWI (p=.083, ES=0.80) did not reach statistical significance. Ln rMSSD was not different from resting values during immersion for CWI9 (p=.432), and at 60 min post-immersion for CWI15 (p=.330) and TWI (p=.133); however, it remained lower than resting values for CON at 60 min post-immersion (p=.007).

Effect on frequency domain HRV indices

A condition x time interaction was observed for lnLF (p=.003) and lnHF (p=.002). Figure 1b shows that lnLF increased during immersion in CWI9 when compared with CON (p=.017, ES=1.24). LnLF was not different from resting values during immersion in CWI9 (p=.697), and at 30 min post-immersion for all other conditions (p≥.430). Post-hoc analysis showed that differences in lnHF between CWI9 and CON (p=.082, ES=0.96) or TWI (p=.062, ES=1.01) during immersion did not reach statistical significance (Figure 1c). At the same time point, the differences between CWI9 and TWI (p=.077, ES=0.72) did not reach statistical significance. LnHF was not different from resting values during immersion for CWI9 (p=.99) and at 60 min post-immersion for TWI (p=.200), while it remained lower than resting values at 60 min post-immersion for CON (p=.003). For CWI15, lnHF tended to be lower than resting values during immersion (p=.070, ES=1.08), but was not different from resting values at 30 min post-immersion (p=.211).

Effect on Poincaré plot-derived HRV indices

A condition x time interaction was observed for lnSD1 and lnSD2 (p<.001). During immersion, lnSD1 for CWI15 was higher compared with TWI (p=.050, ES=0.80), while the differences between CWI15 and CON (p=.064, ES=0.89), and between CWI9 and CON (p=.077, ES=0.95) or TWI (p=.084, ES=0.80) did not reach statistical significance (Figure 1d). LnSD1 was not different from resting values during immersion for CWI9 (p=.431), and at 60 min post-immersion for CWI9 (p=.329) and TWI (p=.132); however, it remained lower than resting values for CON at 60 min post-immersion (p=.007). During immersion, lnSD2 was higher for CWI9 when compared with CON (p=.023, ES=1.07, Figure 1e). LnSD2 was not different from resting values during immersion for CWI9 (p=.358), and at 30 min post-immersion for CWI9 (p=.218) and TWI (p=.152); however, it remained lower than resting values for CON at 60 min post-immersion (p=.101). During immersion, the SD ratios for all conditions were >0.15 (CON: 0.36±0.10, TWI: 0.38±0.16, CWI15: 0.42±0.14, CWI9: 0.35±0.08). A main time

Figure 1. Mean ± SD ln rMSSD (a), lnLF (b), lnHF (c), lnSD1 (d), lnSD2 (e) and lnSD ratio (f) at rest, at the end of exercise (Ex), during water immersion (WI), and 30 min (Post-WI15) and 60 min post-immersion (Post-WI60). †† significant difference between CWI9 and TWI; ††† significant difference between CWI9 and CON; † significant difference between CWI15 and TWI; †††† significant difference between CWI15 and CON with large ES (>0.80); †△ difference between CWI9 and TWI with large ES (>0.80); †# difference between CWI9 and TWI with large ES (>0.80); †* significant difference versus rest for CWI9; †* significant difference versus rest for CWI15; † † † significant difference versus rest for TWI; † † † † significant difference versus rest for CON.
effect (p<.001) but no condition (p=.168) or condition × time interaction (p=.154) was observed for lnSD ratio. Figure 1f shows that lnSD ratio increased during exercise (p=.009) and decreased (p≤0.001) at 30 min and 60 min post-immersion when compared with pre-exercise values. Poincaré scattergrams in a representative participant are illustrated in Figure 2a. Most participants demonstrated increased dispersion in the scattergrams for the CWI conditions (i.e., CWI9 and CWI15) when compared with TWI and CON; however the scattergrams remained narrow during CWI9 for two participants (Figures 2b and c).

Discussion and conclusions

This study examined the influence of 5-minute water immersion within the range of 9-35°C on cardiac parasympathetic reactivation after exercise in the heat. The results from the present study showed that: 1) all water immersion conditions accelerated the recovery of cardiac vagal tone (i.e., ln rMSSD, lnSD1, lnHF) to the pre-exercise levels compared with CON, but only CWI9 had a large positive effect on all HRV indices during immersion; 2) there were no differences (i.e., p>.05 and ES<0.80) between CWI1 and CWI15, except that CWI9 accelerated parasympathetic reactivation during immersion; and 3) somewhat contradictory to our hypothesis, sympatovagal antagonism was not observed during CWI9, but persistence of sympathetic activity was observed in two participants as indicated by the narrow Poincaré scattergrams.

Although all the water immersion conditions accelerated post-exercise cardiac vagal tone when compared with CON, only CWI9 had a large positive effect on all HRV indices during immersion. However, there were no differences between the CWI conditions, except that an accelerated parasympathetic reactivation was observed in CWI9. Previous studies have reported the ES for rMSSD to be within the range of 0.25-0.75 with CWI at 2°C or 10°C (Bastos, et al., 2012; Flouris, et al., 2014; Stanley, et al., 2014), while others have observed ES ranged from 0.48 to 1.83 with CWI at 14-15°C (Al Haddad, et al., 2010; Buchheit, et al., 2009; Ottone, et al., 2014; Stanley, et al., 2012). Moreover, rMSSD has been found to remain lower than the pre-exercise values following CWI at 10°C (Stanley, et al., 2014), while recovery to the pre-exercise levels has been observed during CWI at 14-15°C (Ottone, et al., 2014; Stanley, et al., 2012). However, comparison between the aforementioned studies is inappropriate due to the differences in the study designs (e.g., ambient temperature and exercise tasks). Moreover, relevant studies that compared different immersion temperatures were delimited to 14°C and above (Al Haddad, et al., 2010; Ottone, et al., 2014). It is also worth noting that In rMSSD, lnSD1 and lnHF remained lower than the pre-exercise levels at 60 min post-immersion in the CON trials, supporting the notion that exhaustive exercise performed in the heat can reduce cardiac vagal-related activity for prolonged duration (Brenner, et al., 1998). In contrast, all HRV indices have been shown to return to
The degree of cardiac parasympathetic reactivation has been associated with perceived recovery; however, the influence of increased cardiac parasympathetic activity on exercise performance is less clear (Al Haddad, Parouty, & Buchheit, 2012; Buchheit, et al., 2009; Stanley, et al., 2012; Stanley, Peake, & Buchheit, 2013). Stanley et al. (2014) found that increased cardiac parasympathetic activity following CWI at 10°C resulted in cardio-deceleration at the onset of exercise and decreased muscle oxygen uptake during the exercise bout. Conversely, others observed improved exercise performance following CWI at 14°C when compared with non-cooling conditions (Peiffer, et al., 2010; Yarger, et al., 2006). Accordingly, the present results require careful interpretation. For instance, CWI at 9°C may be effective as a post-exercise recovery treatment to enhance cardiac parasympathetic reactivation, but may not be advisable if preceding a bout of exercise.

CWI may modulate the HRV response through a direct temperature effect on the sino-atrial node, as well as central blood volume expansion facilitated by hydrostatic effect and cold-induced peripheral vasoconstriction (Buchheit & Laursen, 2009; Mourot, et al., 2008). Additionally, activation of nociceptive cutaneous receptors by hand immersion at 7°C, but not 14°C, has been shown to increase muscle sympathetic nerve activity (MSNA) and arterial pressure (Kregel, Seals, & Callister, 1992), which in turn can accentuate cardiac vagal activity (Tulppo, et al., 2011; Yamazaki & Sone, 2000). Hence, while it is likely both CWI conditions facilitate central blood volume expansion, we suggest that CWI may have accentuated cardiac vagal activity through elevated MSNA. However, reduced baroreflex control of MSNA (Halliwill, Taylor, & Eckberg, 1996) and paradoxical increase in HR responsiveness to baroreflex control (Halliwill, Taylor, Hartwig, & Eckberg, 1996) have been observed following moderate exercise. As we were unable to assess MSNA in the present study, more detailed investigation is warranted to ascertain its contribution to cardiac parasympathetic activity during CWI at 9°C.

Although SD ratio is believed to be influenced by sympathetic activity, the decrease observed here-in could be ascribed to important decrease in lnSD1 (parasympathetic index) relative to lnSD2 which is a non-specific HRV index (Mourot, Bouhaddi, Perrey, Rouillon, & Regnard, 2004). Presence of sympathovagal antagonism during CWI was assessed by qualitative and quantitative Poincaré plot analysis. Sympathovagal antagonism is defined as a rapid reduction in HR during strong cardiac sympathetic activation (Buchheit & Laursen, 2009; Tulppo, et al., 1998). A narrow scattergram indicates sympathetic predominance while torpedo-shaped (i.e., small SD1 and SD ratio < 0.15) and parabola-shaped scattergrams are associated with cardiac instability (Mourot, Bouhaddi, Perrey, Rouillon, & Regnard, 2004; Tulppo, et al., 1998). Indeed, scattergrams at the end of the exercise were narrow and elongated for all participants (Figure 2), indicating an increase in cardiac sympathetic activity. Contradictory to our hypothesis, presence of sympathovagal antagonism was not supported by visual inspection of the Poincaré plots or lnSD ratio, as an increased dispersion was observed in CWI and CWI for most participants during immersion. However, two participants demonstrated persistence of sympathetic activity during CWI for all participants (Figure 2), indicating an increase in cardiac sympathetic activity. Contradictory to our hypothesis, presence of sympathovagal antagonism was not supported by visual inspection of the Poincaré plots or lnSD ratio, as an increased dispersion was observed in CWI for most participants during immersion. However, two participants demonstrated persistence of sympathetic activity during CWI, while it is possible that CWI resulted in elevated plasma norepinephrine concentration in some individuals, thus blunting the HR response to vagal stimulation via the α-adrenergic mechanism, the interaction between parasympathetic and sympathetic limbs in modulating the HR response is dependent on neural and hormonal stimulation (Miyamoto, et al., 2003, 2004). As we did not assess MSNA or plasma catecholamines in the present study, it is difficult to speculate on the exact mechanisms involved. Regardless, our findings demonstrated that CWI resulted in parasympathetic predominance, while CWI might cause heightened sympathetic activity in some individuals as indicated by the width of the Poincaré plots.

To conclude, the present study showed that all the water immersion conditions accelerated the recovery of cardiac vagal tone to the pre-exercise levels compared with CON, but only the CWI conditions (CWI and CWI) had large positive effects on vagal-related HRV indices during immersion. Although there were no differences between CWI and CWI, to justify the use of one temperature over the other, CWI accelerated parasympathetic reactivation during immersion. Hence, both CWI treatments are effective means of enhancing post-exercise parasympathetic reactivation, but CWI is likely to be more effective at increasing post-exercise cardiac vagal tone. However, based on the visual inspection of Poincaré plots, some individuals may demonstrate persistence of cardiac sympathetic activity during immersion for CWI, and will benefit from more rigorous investigation involving assessment of MSNA or plasma catecholamines.
References


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