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Individualising the exposure of -110°C whole body cryotherapy: The effects of sex and body composition.

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## Abstract

The purpose of this study was to investigate the effects of whole body cryotherapy (WBC) on a range of thermoregulatory measures. We also sought to examine the influence of sex and body composition. A convenience sample of 18 healthy participants (10 males and 8 females) (27±6 yrs) volunteered for this study. Temperature (core, tympanic, skin and mean body), heart rate, blood pressure, and thermal comfort and sensation were recorded pre- and post- (immediately and every 5 min until 35 min post) exposure to a single bout of WBC (30s at -60°C, 150s at 110°C). Anthropometric data (height, weight, body surface area, body mass index, fat mass and fat free mass) were also recorded. No significant differences in temperature (core, tympanic, skin and mean body), heart rate, blood pressure, or thermal comfort / sensation were observed between male and females at baseline. Immediately post WBC mean body (male: 31.9 ± 0.8°C; female: 31.0 ± 0.9°C; Δ mean body temperature: 0.9 ± 0.1°C; P ≤ 0.05, d = 0.64) and mean skin (male: 22.1 ± 2.2°C; female: 19.6 ± 2.8°C; Δ mean skin temperature: -2.5 ± 0.6°C; d = 0.99, P ≤ 0.05) temperature was significantly different between sexes. Sex differences were also observed in regional skin temperature (male thigh, 20.8 ± 1.1°C; female thigh, 16.7 ± 1.1°C, Δ mean thigh skin temperature: -4.1°C; d = 3.72; male calf, 20.5 ± 1.1°C; female calf, 18.2 ± 1°C, Δ mean calf skin temperature: -2.3 ± 0.1°C; d = 3.61; male arm, 21.7 ± 1°C; female arm, 19 ± 0.4°C, Δ mean arm skin temperature: -2.7 ± 0.3°C; d = 3.54; P ≤ 0.05). Mean arterial pressure was significantly different over time (P ≤ 0.001) and between sexes

(male 0 mins:  $94 \pm 10$ mmHg; female 0 mins:  $85 \pm 7$ mmHg; male 35 mins:  $88 \pm 7$ mmHg; female 35 mins:  $80 \pm 6$ mmHg;  $P \leq 0.05$ ). Combined data set indicated a strong negative relationship between skin temperature and body fat percentage 35 minutes' post WBC ( $r = -0.749$ ,  $P \leq 0.001$ ) and for core temperature and body mass index in males only ( $r = 0.726$ ,  $P \leq 0.05$ ) immediately after WBC. There were no significant differences between sexes in any other variables (heart rate, tympanic and perceptual variables). We observed sex differences in mean skin and mean body temperature following exposure to whole body cryotherapy. In an attempt to optimise treatment, these differences should be taken into account if whole body cryotherapy is prescribed.

Keywords: Thermoregulation; sexual dimorphism; sex differences; temperature; cold exposure.

## 1. Introduction

Whole body cryotherapy (WBC) involves a short exposure to cold air, which is growing in popularity with athletes and coaches (Hauswirth, Louis et al. 2011, Costello, Algar et al. 2012). The majority of protocols repeatedly expose individuals to extremely cold air ( $-110^{\circ}\text{C}$  to  $-140^{\circ}\text{C}$ ) in an environmentally controlled chamber for short periods of time (2-4 minutes) (Costello, Culligan et al. 2012). WBC has been claimed to treat depression (Rymaszewska, Tulczynski et al. 2003), rheumatic conditions (Hirvonen, Mikkelsson et al. 2006), ankylosing spondylitis (Banfi, Lombardi et al. 2010), and exercise induced muscle damage (Hauswirth, Louis et al. 2011) in both athletic and clinical populations (Hammond, Cuttell et al. 2014). A range of claims based on thermoregulatory responses have been stated about the benefits of WBC, however, the evidence supporting these claims is limited in both quality and statistical power (Costello, Culligan et al. 2012).

During WBC, the interaction between the cold environment and the body mainly occurs at the skin. The heat lost at the skin represents the balance of heat loss of metabolically active tissue to the environment (Cholewka, Stanek et al. 2012). Both core temperature and skin temperature have specific responses to a cold environment, and the magnitude in response is determined by the change in skin or core temperature (Kakitsuba, Mekjavic et al. 2007). Thermo afferent signals from the core to the periphery are integrated centrally to establish normothermia by means of skin vasoconstriction and increasing metabolic rate via shivering (Kakitsuba, Mekjavic et al. 2007). Tissue cooling and the transfer of heat from the body depends on several factors including relative mass, size of contact area, difference in starting temperatures and the relative heat capacity and rewarming of metabolically active tissue (Zhang, Huizenga et al. 2001). Skin temperature has been shown to reduce significantly after WBC, with concomitant decreases in core and muscle temperature (Costello, Culligan et al. 2012, Selfe, Alexander et al. 2014). Despite what is known about WBC, there is still a paucity of data regarding WBC treatment times, optimal temperatures, and how these relate to sex differences.

Metabolic rate and heat production have been reported to be significantly reduced in overweight, compared to lean, participants during mild air cooling (15°C) and a rewarming period after cooling (Ooijen, Westerterp et al. 2006). Given that WBC uses vastly cooler temperatures (typically less than -100°C) and that higher fat mass/adipose tissue impacts upon cooling time, metabolic heat production may be amplified with leaner, compared to overweight, participants. Larger skin folds have been shown to require longer ice exposure than smaller skin folds to elicit similar reductions in deep tissue temperature (Otte, Merrick et al. 2002), and differences in the degree of skin temperature cooling experienced have been reported between high and low body mass index in individuals following WBC (Cholewka, Stanek et al. 2012). Consistent with the link between body mass index and cooling, there have been a number of attempts to examine the impact of WBC on skin temperature (Cholewka, Drzazga et al. 2006, Klimek, Lubkowska et al. 2011, Cholewka, Stanek et al. 2012, Hammond, Cuttell et al. 2014) along with core temperature (Westerlund, Oksa et al. 2003, Costello, Culligan et al. 2012), heart rate and blood pressure (Westerlund, Smolander et al. 2004, Westerlund, Uusitalo et al. 2006), but with little attention paid to sexual dimorphism, morphological and protocol differences. It is surprising that sexual dimorphism has received relatively little attention in WBC research, especially as WBC has been used in a wide variety of populations, with varying results, in both clinical and well trained individuals. Given that sexual dimorphism such as adiposity (Jutte, Hawkins et al. 2012) and the menstrual cycle (Coyne, Kesick et al. 2000) are likely to alter core and tissue cooling, understanding the effectiveness of WBC is of paramount importance to clinicians, athletes and coaches. In addition female participation is vastly underrepresented in sport and exercise science based research (Costello, Bieuzen et al. 2014) and even more so in thermoregulatory based areas such as WBC. The effects of this short duration WBC exposure (~3min) and sex differences in thermoregulatory and cardiovascular measures has not been studied in depth.

Therefore, this investigation sought to examine the role of sex differences on thermoregulatory and cardiovascular responses following WBC. It was hypothesised that greater reductions in tissue temperature would be observed in females. Additionally, in an attempt to establish a best practice for individualised WBC treatments a secondary aim was to explore predictive equations (Hammond, Cuttell et al. 2014) for the reduction in skin temperature following exposure.

## **2. Method**

### **2.1. Participants**

A convenience sample of 18 untrained participants (10 males and 8 females) volunteered for this study. A summary of anthropometric characteristics of the participants can be seen in Table 1. The study was approved by the Moulton College Human Research Ethics Committee and, in accordance with the Declaration of Helsinki, participants were informed of the requirements of the study prior to signing a consent form. Each participant also completed a medical consent form and declared that

they were free from medical conditions including Raynaud's phenomenon and other cold sensitivities, heart conditions, claustrophobia, and allergy to adhesive tape. Female participants were asked to inform investigators on the stage of their menstrual cycle in attempt to record and standardise the stages of menses.

## 2.2. Pre-experimental protocol

Participants were instructed to abstain from consuming caffeine, smoking, or taking part in exercise on the day of testing, and also not to consume food within 2 hours of WBC exposure. The participants were asked to remain hydrated. Upon arrival to the laboratory, height (SECA 213, UK) and weight (SECA Scales, UK) were recorded. Body composition was measured using bioelectrical impedance at a frequency of 50kHz (Biostat 1500, Isle of Man) and skin fold (Harpenden) thickness at four sites (tricep, calf, scapula and suprailliac crest). Body Mass Index, body surface area (Du Bois and Du Bois 1916) and body surface area to mass ratio were also calculated. Heart rate was measured (Polar heart rate FT2, Finland) in the supine position on a horizontally positioned massage bed (Metron elite 3-section exam couch, USA). Similarly, blood pressure was measured using an electronically calibrated blood pressure monitor (Omron M6, Matsuyama Mie, Japan) by the researcher while the participant was in a supine position.

Rectal temperature was recorded after the participants self-inserted a thermistor (Grant Instruments, Cambridge, UK), ~10cm beyond the anal sphincter. Tympanic temperature was assessed using a hand-held device (ThermoScan, Kaz Europe SA). Skin temperature was recorded using a FLIR Thermal Imaging Camera (E40BX FLIR systems, Danderyd, Sweden). The thermal images were taken according to the standard protocol for infrared imaging in medicine (Matos, Neves et al. 2015, Ring and Ammer 2015). The camera was positioned on a tripod, 3.8 metres from the participants, with an emissivity factor of 0.98 which is appropriate for skin thermographs. Thermally inert markers were attached to four sites on the body (chest, thigh, arm and lower leg) to create regions of interest (ROI) for analysis as previously described (Costello et al., 2012). Sites were determined by area box measurements (chest, thigh, calf, arm) using computerised software (Thermalcam Researcher software 2.8–2.9; designed by FLIR Systems, Danderyd, Sweden). The four-site equation of Ramanathan (1964) was used to determine mean skin temperature from the four temperature sites, and images were analysed using FLIR Quick Tools to establish mean skin temperature within each region of interest. In addition, predictive equations were used to measure the accuracy of change in skin temperature and sex differences (Hammond, Cuttell et al. 2014). Mean body temperature  $[0.64 \times T_{rec}] + [0.36 \times T_{sk}]$  (Burton 1935) and body heat content was also calculated ( $\Delta H_b = \Delta_{MBT} \cdot BM \cdot C_p$ ); where  $\Delta_{MBT}$  is the change in mean body temperature, BM is the specific body mass (Kg) and  $C_p$  is the specific heat of tissue (3.47 kJ/kg/K) (Burton 1935, Jay, Garipey et al. 2007). Thermal sensation and thermal comfort were rated with a 9-point scale. Thermal sensation ranged from 0 (considered as

unbearably cold) to 8 (unbearably hot), with 4 (neutral) and thermal comfort ranging from 4 (very comfortable) to -4 (very uncomfortable) with 0 (neutral) (Gagge, Stolwijk et al. 1967).

### **2.3. Baseline measurement**

Following instrumentation participants, wearing shorts (males) or shorts and a vest (females) rested for twenty minutes to acclimate to the environment (26°C and 33% RH) (Costello, Culligan et al. 2012). After this baseline measurement of skin temperature, core temperature and tympanic temperature, heart rate, blood pressure, thermal comfort and sensation were recorded. Participant's heart rate and blood pressure were again collected in the supine position on a horizontally adjusted massage bed.

### **2.4. WBC protocol**

Participants walked from the laboratory to the adjacent WBC facilities and donned gloves, socks, clogs, tubular bandages to cover elbows and knees, headband to cover the ears, and surgical mask over the mouth. Glasses, jewellery and piercings were removed before entering the chamber.

Following a safety briefing from the chamber (Cryogenic chamber; JUKA Poland) operators, participants were required, in pairs, to enter the antechamber for 30 seconds at -60°C, and transferred through an internal door to the main chamber for 2 minutes at -110°C. This protocol replicates previous reported times and temperatures (Dugué, Smolander et al. 2005, Smolander, Westerlund et al. 2006, Leppäluoto, Westerlund et al. 2008, Gong, Ma et al. 2011, Klimek, Lubkowska et al. 2011, Hammond, Cuttall et al. 2014). At the completion of the WBC exposure, participants transferred immediately to the adjacent laboratory (26°C and 33% RH) to capture the post WBC data.

### **2.5. Post WBC**

Participants' skin, core and tympanic temperature along with heart rate, blood pressure and perceptual data were recorded immediately post WBC and for every 5-minute period up to 35 minutes thereafter.

### **2.6. Statistical analysis**

All data are expressed as means and standard deviations. Normality was assessed using descriptive methods (skewness, outliers, and distribution plots) and inferential statistics (Shapiro–Wilk test). A two way repeated measures ANOVA (sex x time) was used to investigate changes in time with one between subject's variable, sex, with two levels (male and female) and one within subject variable, time, with 9 levels (-5, 0, 5, 10, 15, 20, 25, 30, 35 minutes) for skin temperature, core temperature, mean body temperature, tympanic temperature, change in body heat content, heart rate and blood pressure. If a significant interaction between factors was found Bonferroni comparison were undertaken in the post hoc analysis. When the assumption of sphericity was violated significance was

adjusted using the Greenhouse Geisser method. Similarly, where significant differences were found the effect sizes were calculated (Cohen's *d* test) (Cohen, 1988). All anthropometric variables (Body surface area, body surface area to mass ratio, fat free mass index, fat mass index, body mass index, body fat percentage, sum of skin folds) and change in skin temperature, core temperature, tympanic temperature and mean body temperature at 0 minutes and 35 minutes' post treatment were analysed using a Pearson's correlation. Where predictive equations were used (Hammond, Cuttall et al. 2014), the errors in measurements were calculated for actual change in skin temperature and predicted values. For non-parametric data, a Friedman two-way ANOVA was used to detect differences over time obtained from Likert scale measurement for thermal sensation and comfort. All statistical analyses were performed in SPSS (Statistical Package for the Social Sciences) version 22.0 (SPSS Inc, Chicago, IL). Significance was accepted at  $P \leq 0.05$ .

### 3. Results

#### 3.1. Core temperature

There was no significant interaction between sex and time for core temperature however a significant effect over time following WBC exposure was observed ( $F_{(1,16)} = 83.578$ ,  $P < 0.001$ ,  $d = 3.14$ ), but no difference between sexes was evident ( $F_{(1,16)} = 0.278$ ,  $P = 0.605$ ). Baseline core temperature was similar between sexes (male:  $37.3 \pm 0.1^\circ\text{C}$ ; female:  $37.3 \pm 0.2^\circ\text{C}$ ;  $P = 0.932$ ). Core temperature reduced to a minimum of  $36.7^\circ\text{C}$  and  $36.3^\circ\text{C}$  at 35 minutes after exposure respectively for males and females. However, there was significant inter individual differences. Post hoc analysis did not reveal any differences between sexes over the rewarming period however immediately post WBC there was a small increase in core temperature for both male and female participants (male:  $37.5 \pm 0.2^\circ\text{C}$ ; female:  $37.5 \pm 0.3^\circ\text{C}$ ;  $P = 0.741$ ; Figure 1). Pearson's correlation revealed the male data set indicated a strong negative relationship between core temperature and lean body mass ( $r = -0.718$ ,  $P = 0.019$ ), fat mass index ( $r = -0.695$ ,  $P = 0.026$ ), body surface area ( $r = -0.653$ ,  $P = 0.041$ ) and body mass index ( $r = 0.726$ ,  $P = 0.017$ ) at immediately after the WBC and a positive relationship for core temperature and body surface area to mass ratio ( $r = 0.636$ ,  $P = 0.048$ ). The female data set indicated a strong positive relationship between core temperature and body mass index ( $r = 0.878$ ,  $P = 0.004$ ), fat free mass index ( $r = 0.822$ ,  $P = 0.012$ ), body fat percentage ( $r = 0.810$ ,  $P = 0.015$ ). Combined data set indicated that core temperature at 35 minutes and sum of skin folds had a positive relationship ( $r = 0.537$ ,  $P = 0.022$ ).

#### 3.2. Mean skin temperature

There was no significant interaction between sex and time for mean skin temperature however a significant effect over time was observed ( $F_{(1,16)} = 43899$ ,  $P < 0.001$ ) and between sexes ( $F_{(1,16)} = 17.1$ ,  $P = 0.001$ ; Figure 1). Baseline mean skin temperature were similar between sexes (male:  $33.5 \pm 0.5^\circ\text{C}$ ; female:  $33.1 \pm 0.5^\circ\text{C}$ ;  $P = 0.08$ ). Post hoc analysis revealed skin temperature was lower in females immediately after the WBC (male:  $22.1 \pm 2.2^\circ\text{C}$ ; female:  $19.6 \pm 2.8^\circ\text{C}$ ), 5 minutes (male:

28.8 ± 1.5°C; female: 27.2 ± 1.5°C) and 10 minutes (male: 30.4 ± 0.9°C; female: 28.9 ± 0.6°C) post WBC ( $P \leq 0.05$ ). Thereafter, until 35 minutes, there were no significant differences ( $P > 0.05$ ) between the males and the females. Pearson's correlation revealed the combined data set indicated a strong negative relationship between skin temperature and fat mass index at 35 minutes' post WBC ( $r = -0.651$ ,  $P = 0.003$ ) and body fat percentage ( $r = -0.749$ ,  $P < 0.001$ ). Similarly, a strong negative relationship was found for skin temperature at 35 minutes' post WBC for the sum of skin folds ( $r = -0.702$ ,  $P < 0.001$ ) and body fat mass ( $r = -0.662$ ,  $P = 0.003$ ).

### 3.3. Local skin temperature (thigh, calf, arm and chest)

Baseline local skin temperature for thigh, calf, arm and chest regions were similar (male thigh: 33.2 ± 0.8°C, female thigh: 32.4 ± 0.5°C; male calf: 32.9 ± 0.8°C, female calf: 32.3 ± 0.4°C; male arm: 32.8 ± 0.8°C, female arm: 32.5 ± 0.9°C; male chest: 34.5 ± 0.6°C, female chest: 34.8 ± 0.6°C;  $P \geq 0.05$ ).

Immediately after WBC exposure there were site specific differences observed between sexes in the thigh, calf and arm regions (male thigh: 20.8 ± 1.1°C; female thigh: 16.7 ± 1.1°C,  $d = 3.72$ ; male calf: 20.5 ± 1.1°C; female calf: 18.2 ± 1°C,  $d = 3.61$ ; male arm: 21.7 ± 1°C; female arm: 19 ± 0.4°C,  $d = 3.54$ ;  $P \leq 0.05$ ). There was a significant difference over time ( $F_{(1,16)} = 18029$ ,  $P \leq 0.001$ ) and between sexes ( $F_{(1,16)} = 21.8$ ,  $P \leq 0.001$ ; Figure 2) for thigh skin temperature. Similarly, there was a significant difference over time ( $F_{(1,16)} = 40787.4$ ,  $P \leq 0.001$ ) and between sexes ( $F_{(1,16)} = 19.8$ ,  $P \leq 0.001$ ) for arm skin temperature. A significant difference over time ( $F_{(1,16)} = 16102.6$ ,  $P \leq 0.001$ ) and between sexes ( $F_{(1,16)} = 20.1$ ,  $P \leq 0.001$ ) for calf skin temperature was also found. There was a difference over time for chest skin temperature ( $F_{(1,16)} = 29830.2$ ,  $P \leq 0.001$ ) but no difference between sexes ( $F_{(1,16)} = .553$ ,  $P = 0.468$ ) (Figure 2).

### 3.4. Mean body temperature

There was no significant interaction between sex and time for mean body temperature however there was a significant difference over time ( $F_{(1,16)} = 3.757$ ,  $P = 0.003$ ) and between sexes ( $F_{(1,16)} = 15.416$ ,  $P = 0.001$ ,  $d = 0.64$ ; Figure 2) post WBC. Baseline mean body temperature were similar between sexes (male: 35.9 ± 0.1°C; female: 35.8 ± 0.3°C;  $P \geq 0.05$ ). Post hoc analysis revealed the largest differences ( $P \leq 0.05$ ) between sexes for mean body temperature occurred at 0 minutes (male: 31.9 ± 0.8°C; female: 31.0 ± 0.9°C) at 5 minutes (male: 34.3 ± 0.5°C, female: 33.8 ± 0.4°C) and at 10 minutes (male: 34.9 ± 0.2°C, female: 34.4 ± 0.1°C). Pearson's correlation revealed a moderate negative relationship for mean body temperature and sum of skin folds ( $r = -0.537$ ,  $P = 0.022$ ) a moderate positive relationship for mean body temperature and lean body mass ( $r = 0.538$ ,  $P = 0.021$ ).

### 3.5. Tympanic temperature

Baseline tympanic temperature ( $T_y$ ) were similar between sexes (male: 36.9 ± 0.2; female: 37.1 ± 0.2°C;  $P \geq 0.05$ ). There was a significant difference over time ( $F_{(1,16)} = 171974.7$ ,  $P \leq 0.001$ ) but no

differences between sexes ( $F_{(1,16)} = .534, P = 0.477$ ) post WBC (Figure 2). There were no significant correlations between any body composition measures and tympanic temperature.

### 3.6. Change in body heat content

There was no significant interaction between sex and time for change in body heat content however there was a significant difference over time ( $F_{(1,16)} = 490.5, P \leq 0.001, d = 5.12$ ) for change in body heat content but no difference between sexes ( $F_{(1,16)} = 0.56, P = 0.816$ ). Baseline change in body heat content was similar between sexes (male:  $-1085.7 \pm 223$  Kj; female:  $-1030.3 \pm 246$  Kj;  $P \geq 0.05$ ). Mean differences between sexes for change in body heat content post WBC were highly varied (5 minutes = 4.5Kj, 10 minutes = 19.07Kj, 15 minutes = 9.5Kj) which may help explain the lack of significance (Figure 3). Pearson's correlation revealed the female data set and change in body heat content had a strong negative relationship with sum of skin folds at 0 minutes ( $r = -0.739, P = 0.036$ ).

### 3.7. Heart rate and mean arterial pressure

Heart rate and mean arterial pressure were similar between sexes at baseline (male:  $60 \pm 13$  b $\cdot$ min $^{-1}$ ; female:  $61 \pm 13$  b $\cdot$ min $^{-1}$ ; male:  $95.1 \pm 12.5$  mmHg; female:  $81.8 \pm 4.8$ mmHg; all  $P \geq 0.05$ ; Table 2). There was no significant interaction between sex and time for heart rate and mean arterial pressure ( $P \geq 0.05$ ). Mean arterial pressure was significantly different over time ( $F_{(1,16)} = 2496.4, P \leq 0.001$ ) and between sexes (male 0 mins:  $94 \pm 10$ mmHg; female 0 mins:  $85 \pm 7$ mmHg; male 35 mins:  $88 \pm 7$ mmHg; female 35 mins:  $80 \pm 6$ mmHg;  $F_{(1,16)} = 5.338, P \leq 0.05$ ). There was a noticeable difference over time for heart rate ( $F_{(1,16)} = 659.5, P \leq 0.001$ ) but no difference between sexes for heart rate ( $F_{(1,16)} = 0.86, P = 0.773$ ) post WBC treatment (Table 2).

### 3.8. Perceptual variables

Thermal sensation and comfort were similar between sexes at baseline (thermal sensation male 2(1-2.25); thermal sensation female 1.5(0-2.75); thermal comfort male 3(2.75-4); thermal comfort female 3(2-4): Table 3). Males tended to find WBC 'cool' immediately post treatment (thermal sensation males 0 (-2-0.25)) whereas females found WBC 'slightly warm' (thermal sensation females 1.5(1-2)) at 0 minutes. There was a significant difference between thermal sensation and sexes immediately post WBC ( $X^2(2) = 77.97, P \leq 0.001$ ). There were no differences over time with thermal sensation and thermal comfort (Table 3).

### 3.9. Change in skin temperature prediction

Our data suggests that the predictive equation suggested by Hammond et al. (2014) is up to 90% accurate when adjusted. This adjustment is derived from the mean error of the fat free mass index (2-2.02°C) which helped to predict change in skin temperature in the male data set. The simple linear regression analysis for change in skin temperature for males should therefore be adjusted for

differences between high and low body fat percentages and relative fat free mass index (Figure 5 and 6).

#### 4. Discussion

The purpose of this study was to investigate the impact of sex differences on thermoregulatory and cardiovascular responses to cold exposure after WBC. Additionally, we investigated the accuracy of predictive equations to help explain the variability in skin temperature after WBC. Our data suggested that there are differences between male and female mean body temperature, simultaneously with regional skin temperature when using 3-min exposure of WBC. Specifically, females demonstrated lower mean skin and mean body temperatures post exposure.

The difference in mean body temperature may be explained by sex related site specific regional skin temperature (thigh, calf and arm: Figure 2) and also the relative body surface area and body surface area to mass ratio. As females had a larger body surface area to mass ratio the capability to lose heat would be increased (Hammond, Cuttall et al. 2014); this coupled with a tendency to have higher concentration of body fat percentage (see Table 1) females may have a greater insulative response (Cypess, Lehman et al. 2009, Pfannenber, Werner et al. 2010). Chudecka et al. (2014) reported a similar insulative response for females with the use of WBC. Chudecka et al. (2014) suggested that female adipose tissue transferred less heat to the periphery, whereas males had a greater muscle mass capable of generating more heat. Fat provides a greater insulation and protection from cold exposure (Young, Castellani et al. 1998), and potentially impacting upon metabolic heat produced both these factors would have likely altered mean body temperature and the magnitude of change in body heat content. The body heat content produced would certainly be relative to the average specific heat generated from different tissues attributable to sex variations (e.g. fat free mass and fat mass) (Jay et al., 2007; Stephens et al., 2014). The difference in relative anthropometric variables between sexes may be exemplified by change in body heat content from immediately post WBC up to 15 minutes (Figure 3) (mean differences between sexes; -4.5Kj, -19Kj; -9.5Kj). Males had an increased lean mass comparatively to females (Table 1), this may also indicate a propensity to produce metabolic heat quicker which could help explain the higher male skin temperatures post WBC.

A strong negative relationship between skin temperature at 35 minutes and body fat percentage for both male and female participants was observed; from this observation, it may be prudent to suggest that an enhanced metabolic response is observed in individuals with a lower body fat % with the concomitant increase in skin temperature after exposure to WBC. This interpretation may be validated by the findings of Tikuisis, Bell and Jacobs (1991), who reported greater onset of a shivering and metabolic response in their male lean fat group compared to average body fat group after being cooled with 10°C air for 2 hours (Tikuisis, Bell et al. 1991). Our study used a very different protocol,

including different temperatures and duration of treatment, the likelihood therefore of leaner participants experiencing a greater shivering and metabolic response may have been enhanced. Similarly, our male data set showed a strong positive relationship between change in skin temperature and body fat percentage / fat free mass index at 35 minutes supporting the premise of the larger the body fat %, the greater the decrease in skin temperature. In comparison, the same time point in the female data did not have the same magnitude change in skin temperature but did show a strong positive relationship with fat free mass index, change in core temperature, body fat mass, body mass index, and body fat percentage at 35 minutes' post WBC. It is possible the reason for a lack of change in skin temperature may be due to larger body surface area and body surface area to mass ratio of female participants which may help further exemplify the change in core temperature. After cooling the ambient environment was quite warm (26°C, RH 33%) which may have worked opposite to a cold environment, as the body temperature would seek to establish a thermal equilibrium.

The change in skin temperature was observed to be different between body fat percentage and also sexes. We found a significant correlation with body fat % but also the sum of skin folds. This allowed us to test a new predictive model for change in skin temperature. Despite only having 18 participants for the study, a significant regression analysis was found to help predict these changes based on anthropometric variations and sex. Given the sample size, it is important to interpret the findings with caution. However, the predictive equations are encouraging and worthy of further investigation, particularly as they may help to optimize treatments and therefore influence recovery and performance. Of additional interest was the change in core temperature and tympanic. There was a concomitant decline in core temperature but an incline in tympanic (Figure 4). The difference may be explained by cooled ambient air entering the ear canal affecting tympanic temperature measurements. Therefore, the use of tympanic measures of core temperature may not be the most effective in studies of WBC.

Future studies should address the use of a new and improved predictive calculation of body heat content using calorimetry and thermometry to assess the exact differences in body heat content when using WBC. Despite a difference in mean body temperature between sexes there was no difference in change in body heat content even though an obvious thermal imbalance occurs with the use of WBC. A potential limitation in the prediction of change in body heat content using the thermometry approach may be due to the thermal influences of muscle tissue not being considered independent of core and skin temperature (Jay and Kenny, 2007). Certainly, the use of muscular temperature would have improved the estimation of change in body heat content, however the thermometry model also does not take into account the specific tissue heat capacity (e.g. fat free mass index and fat mass index). Therefore, future studies may want to take into account, if plausible, intramuscular temperatures when attempting to calculate body heat content using WBC for male and female

participants. This study has provided initial data on body heat content in males and females following WBC exposure. However, there remains a lack of knowledge on thermoregulatory responses to WBC, in particular with female participants. Additionally, we were unable to use menstrual cycle information provided by the female participants. It may be advisable to attempt to group female participants by stages of their menstrual cycle for future studies as the effect on thermoregulatory measures and WBC remains unanswered.

## 5. Conclusion

To our knowledge this is the first study to investigate the role of sex differences on change in body heat content, core, and mean body temperature following WBC. Our data suggests that sex related differences do have an influence on thermoregulatory responses after WBC. Female participants experienced a greater reduction in skin temperature than their male counterparts, which may provide tentative support for a greater insulative response to WBC. In addition, in this study it was demonstrated that tympanic measures of core temperature are ineffective when using WBC (Figure 4). Therefore, we recommend that tympanic measures of core temperature should not be utilised in studies using WBC. Finally, as guidelines for recovery strategies are often sought by athletes, coaches and sport scientists, there seems to be very little accounting for sex differences and the variation in anthropological, thermoregulatory and metabolic responses associated. Thus, practitioners need to be cognisant of sex related differences as they are pivotal when using whole body cryotherapy.

## Conflict of interest

The authors declare they have no conflict of interest on the content of this paper.

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### **Lists of Figures and Tables**

Figure 1. Thermoregulatory responses (core, tympanic, skin and mean body temperature) between sexes over time (mean  $\pm$  SD). \*indicates significantly different between sexes at respective comparative time points ( $P \leq 0.05$ ). Dashed line indicates where data was not comparable to terminal data.

Figure 2. Skin temperature ( $^{\circ}\text{C}$ ) and site specific regional differences between sexes (chest, thigh, calf, and arm, mean). \* indicates a significant difference between sexes ( $P \leq 0.05$ ).

Figure 3. Change in body heat content (Kj) over time and between sexes (mean  $\pm$  SD).

Figure 4. Change in core temperature and change in tympanic temperature ( $^{\circ}\text{C}$ ) over time (mean  $\pm$ SD).

Figure 5. Predictive versus actual change in skin temperature values ( $^{\circ}\text{C}$ ) for male body fat percentage at 0 minutes.

Figure 6. Predictive versus actual change in skin temperature values ( $^{\circ}\text{C}$ ) for male fat free mass index at 0 minutes.

Table 1. Anthropometric characteristics of study participants (mean  $\pm$  SD). \*indicates significant difference between sexes ( $P \leq 0.05$ ).

Table 1.	Males		Females		P Value
	Mean	SD	Mean	SD	
Height (cm)	182	0.7	163	0.8	$\beta$ 0.001
Age (years)	27.7	6.9	27.6	6.3	0.989
Mass (kg)	78.8	13	62.5	7.7	*0.005
Body Mass Index (kg/m <sup>2</sup> )	23.6	2.4	23.2	2.8	0.756
Fat Free Mass Index (kg/m <sup>2</sup> )	28.6	2.1	22.4	2	*0.001
Fat Mass Index (kg/m <sup>2</sup> )	3	1.2	6.1	2	*0.003
Fat Mass (kg)	10.3	5	16.3	4.2	*0.014
Lean Mass (kg)	68.4	8.6	46.1	7	$\beta$ 0.001
Body fat percentage	13.7	6.9	26.2	5.4	$\beta$ 0.001
Body Surface Area (m <sup>2</sup> )	1.9	0.1	1.6	0.1	*0.002
BSA to Mass Ratio (cm <sup>2</sup> /kg)	2.5	0.1	2.6	0.1	*0.043

\*indicates a significant difference between sexes  $P \leq 0.05$

$\beta$  indicates a significant difference between sexes  $P \leq 0.01$

Table 2. Heart rate and Mean Arterial Pressure over time (MAP; mmHg). \* indicates a significant difference ( $P \leq 0.05$ ).

Table 2.	Time (Minutes)									
	-5	0	5	10	15	20	25	30	35	
Male HR	59 $\pm$ 13	62 $\pm$ 8	59 $\pm$ 9	57 $\pm$ 9	58 $\pm$ 9	59 $\pm$ 9	58 $\pm$ 10	59 $\pm$ 9	58 $\pm$ 7	
Female HR	61 $\pm$ 13	62 $\pm$ 15	59 $\pm$ 10	61 $\pm$ 10	59 $\pm$ 10	59 $\pm$ 10	59 $\pm$ 8	60 $\pm$ 10	60 $\pm$ 11	
Male MAP	95 $\pm$	94 $\pm$	88 $\pm$ 7*	91 $\pm$	86 $\pm$ 8	89 $\pm$ 8	86 $\pm$ 9	87 $\pm$ 8	88 $\pm$ 7	
Female MAP	12*	10*		11*						
Male MAP	81 $\pm$ 4*	85 $\pm$ 7*	83 $\pm$ 6*	82 $\pm$ 5*	80 $\pm$ 6	81 $\pm$ 6	82 $\pm$ 5	80 $\pm$ 5	80 $\pm$ 6	

\*Indicates significant difference ( $P \leq 0.05$ )

Table 3. Perceptual data (thermal sensation and comfort) over rewarming period (Median (25 – 75th interquartile range)). \*indicates a significant difference ( $P \leq 0.05$ ).

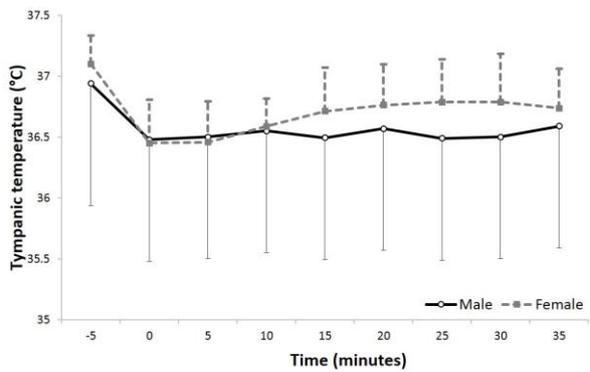
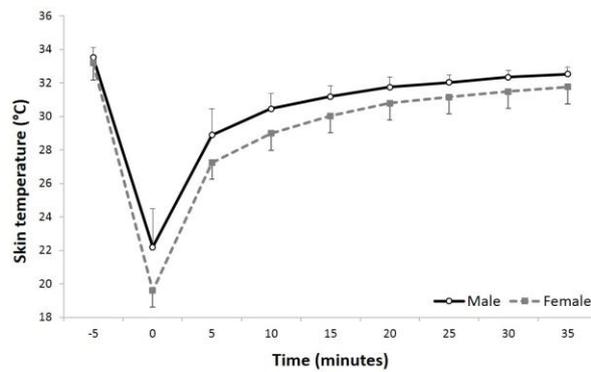
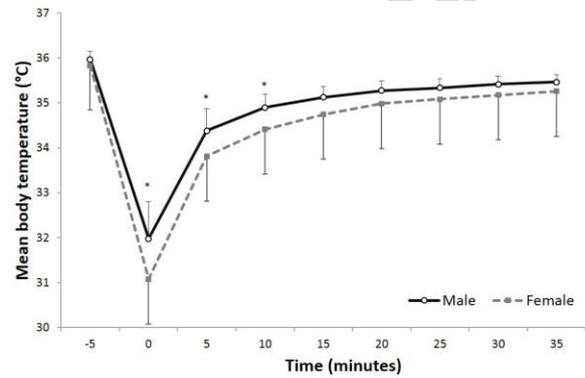
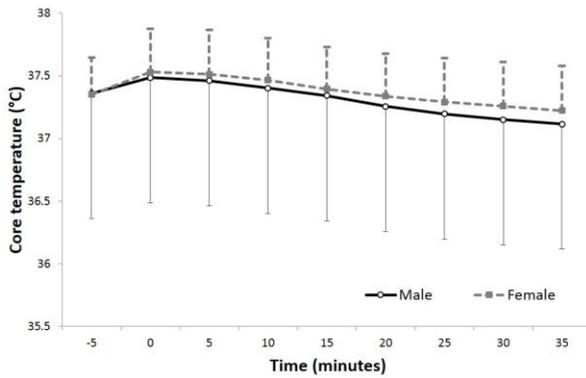
Table 3.	Time (minutes)									
	-5	0	5	10	15	20	25	30	35	
Male TS	2 (1–2.25)	0 (-0.25)*	1 (0.25–2)	1 (0–2)	2 (1–2)	2 (1–2)	2 (1–2)	2 (1.75–3)	2 (1–2.25)	
Female TS	1.5 (0–)	1.5 (1–)	1.5 (-3–)	0 (-1–)	0 (-)	0 (0–)	1 (1–)	1 (1–2)	2 (0.5–)	

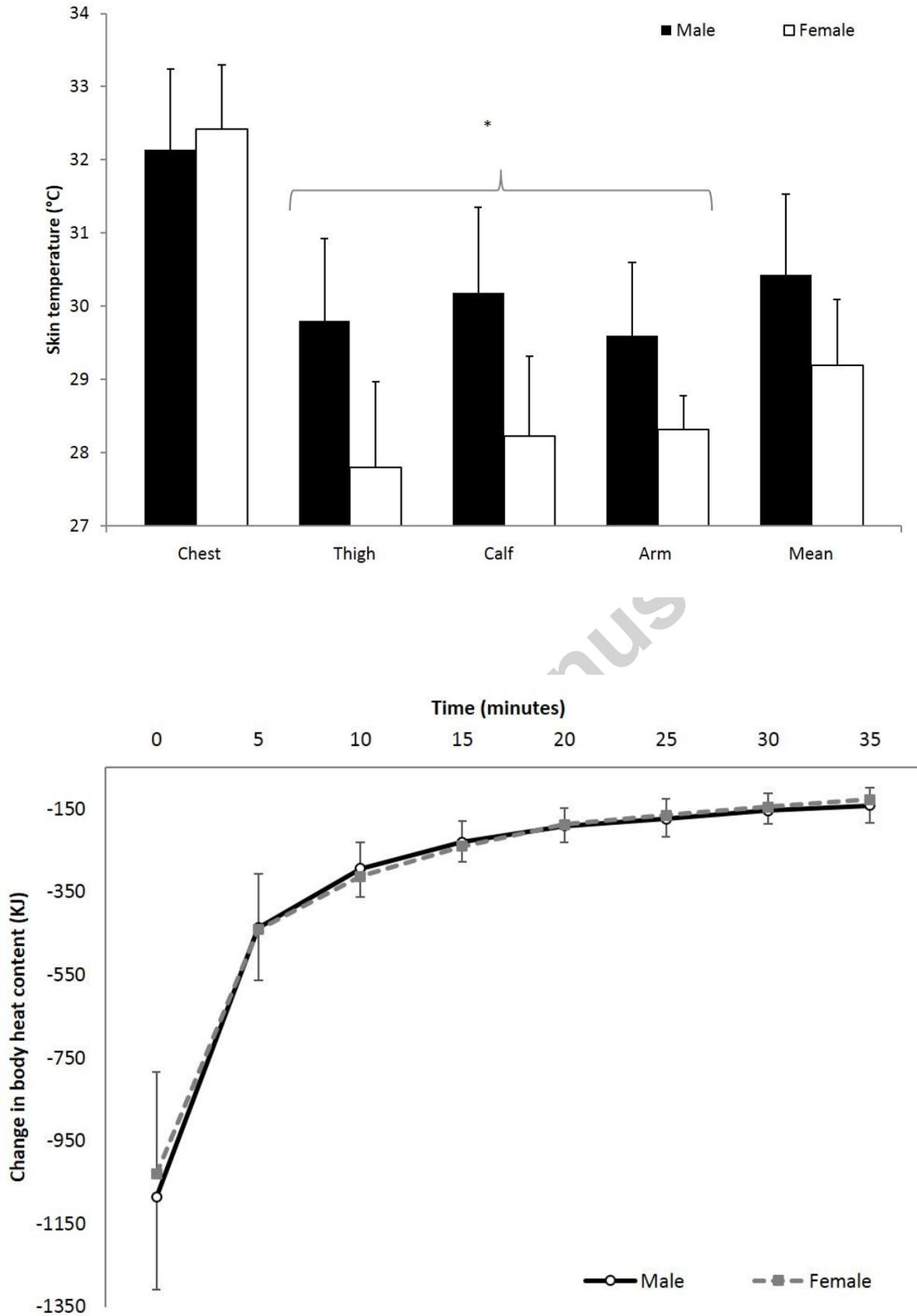
<b>TS</b>	2.75)	2)*	0)	0.75)	0.75-1)	2)	2)		2)
<b>Male TC</b>	3 (2.75-4)	1 (1-2.25)	-0.5 (-1.25-0)	1 (0-2)	1 (1-2.25)	2 (1-3)	2 (1-3)	3 (1.75-3)	3 (1.75-3)
<b>Female TC</b>	3 (2-4)	2.5 (1-3.25)	-1 (-2.25-0)	1 (0.5-1.25)	1 (1-2.25)	1.5 (1-3)	2 (1-3)	2 (2-3.25)	3 (1.75-3.25)

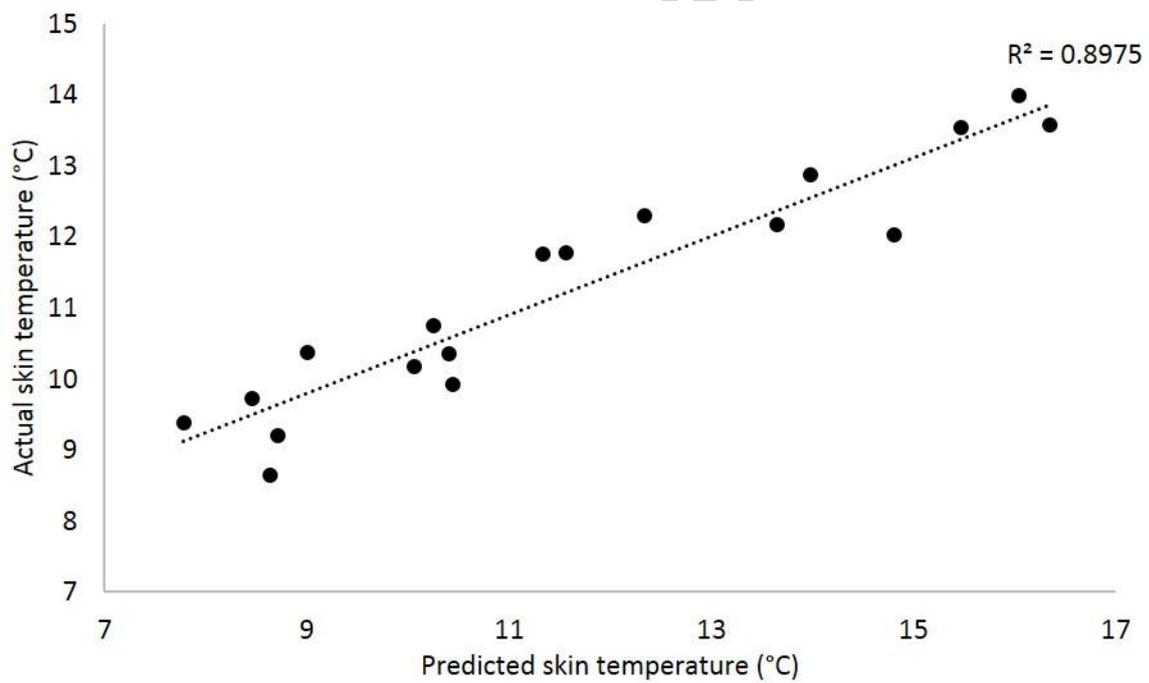
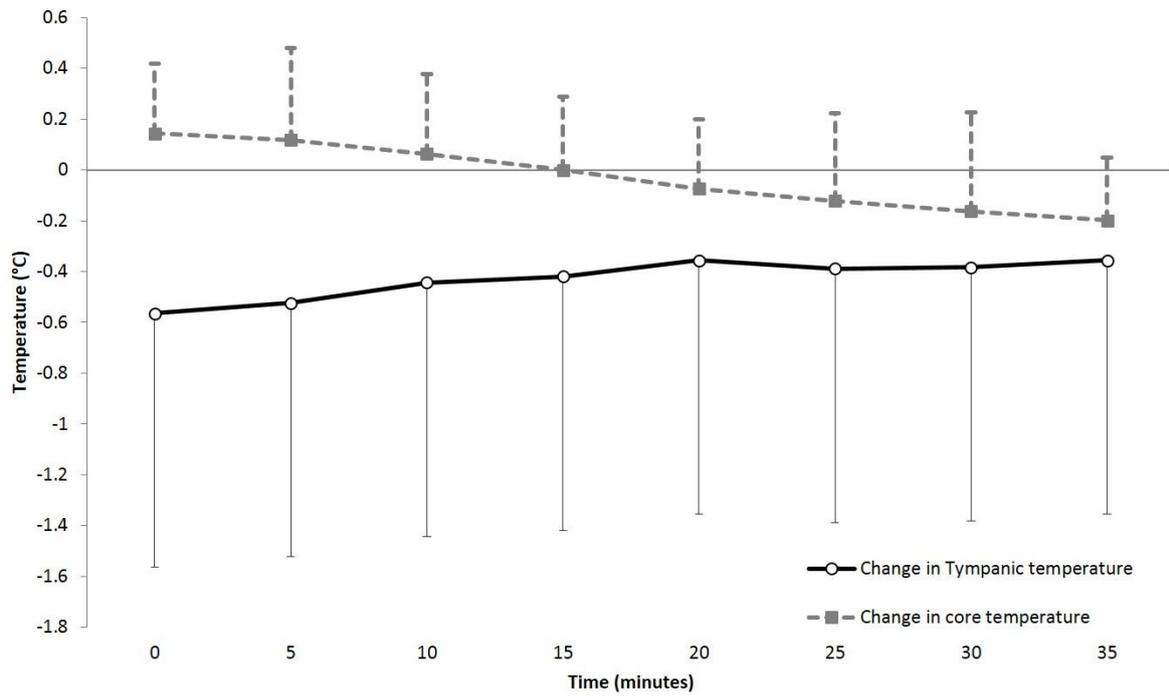
\*indicates a significant difference ( $P \leq 0.05$ ).

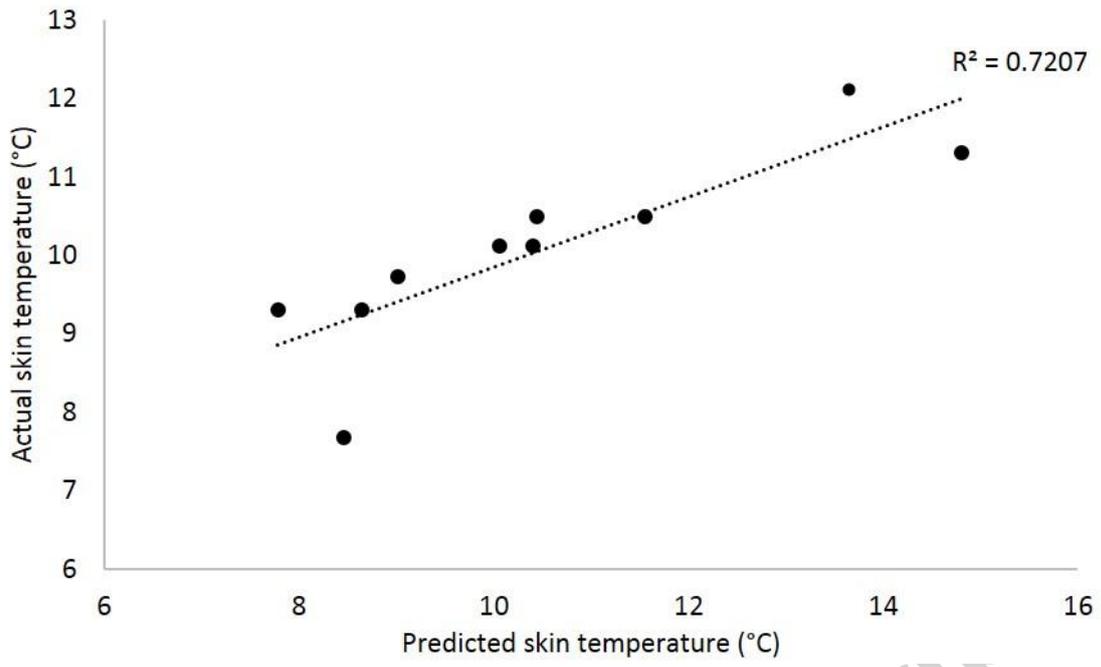
## Highlights

- Mean body temperature and local site specific Tsk were different between sexes.
- Females have a tendency for a greater insulative response to whole body cryotherapy.
- Sexually dimorphism is not accounted for when attempting to optimise WBC treatments.
- The use of tympanic temperature measurements should not be used with whole body cryotherapy treatments.









Accepted manuscript