Voluntary water intake during and following moderate exercise in the cold

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Running head: Voluntary water intake in the cold

Abstract

Exercising in cold environments results in water losses, yet examination of resultant voluntary water intake has focussed on warm conditions. The purpose of the study was to assess voluntary water intake during and following exercise in a cold compared to a warm environment. Ten healthy males (22±2 years, 67.8±7.0 kg, 1.77±0.06 m, $\dot{V}O_{2peak}$ 60.5±8.9 $ml.kg^{-1}.min^{-1}$) completed two trials (7-8d). In each trial subjects sat for 30 minutes before cycling at 70% $\dot{V}O_{2peak}$ (162±27W) for 60 minutes in 25.0±0.1°C, 50.8±1.5% relative humidity (RH) (warm) or 0.4±1.0°C, 68.8±7.5% RH (cold). Subjects then sat for 120 minutes at 22.2±1.2°C, 50.5±8.0% RH. *Ad libitum* drinking was allowed during the exercise and recovery periods. Urine volume, body mass, serum osmolality and sensations of thirst were measured at baseline, post-exercise and after 60 and 120 minutes of the recovery period. Sweat loss was greater in the warm trial (0.96±0.18 l v 0.48±0.15 l) ($p<0.0001$) but body mass losses over the trials were similar (1.15±0.34% (cold) v 1.03±0.26% (warm)). More water was consumed throughout the duration of the warm trial (0.81±0.42 l v 0.50±0.49 l; $p=0.001$). Cumulative urine output was greater in the cold trial (0.81±0.46 v 0.54±0.31 l) ($p=0.036$). Post-exercise serum osmolality was higher compared to baseline in the cold (292±2 v 287±3 $mOsm.kg^{-1}$, $p<0.0001$) and warm trials (288±5 v 285±4 $mOsm.kg^{-1}$; $p=0.048$). Thirst sensations were similar between trials ($p>0.05$). *Ad libitum* water intake adjusted so that similar body mass losses occurred in both trials. In the cold there appeared to a blunted thirst response.

Key Words: water intake, cold, thirst, osmolality

41 Introduction
42
43 It is well documented in the literature that dehydration during and resulting from endurance
44 exercise can impair performance, particularly when exercise is conducted in temperate or hot
45 conditions (Cheuvront, Carter, & Sawka, 2003; Murray 1995; Wendt, van Loon, &
46 Lichtenbelt, 2007). Dehydration resulting in body mass losses of greater than 2% body mass
47 loss have been shown to have a negative impact on performance both physically (Sawka et
48 al., 2007) and cognitively (Grandjean & Grandjean, 2007). One of the main mechanisms of
49 dehydration is sweat loss which is increased by exercise in the heat (Galloway & Maughan,
50 1997). Although often to a lesser extent than in warm and humid conditions, dehydration is
51 still apparent in the cold. This is in part due to many athletes wearing several layers of
52 clothing thus creating a warm microenvironment for them to exercise in. In the cold, water
53 losses can occur through sweating, cold induced diuresis and respiratory losses and in
54 addition to this there is a reduction in voluntary water intake (Freund & Sawka, 1995). Cold
55 environments, in relation to exercise studies, are often described as less than 10°C with many
56 studies using temperatures of 0-7°C (Cheuvront, Carter, Castellani, & Sawka, 2005;
57 Kenefick, Hazzard, Mahood, & Castellani, 2004ab; Kenefick, St Pierre, Riel, Cheuvront, &
58 Castellani, 2008; O'Brien, Young, & Sawka, 1998).
59
60 Despite the sweat losses in cold environments, water intake is often reduced and is often
61 insufficient to replace the water losses that have occurred (Maughan, Shirreffs, Merson, &
62 Horswill, 2005). It has been shown that sweat losses during 90 minute football training
63 sessions were similar in a cold (Maughan et al., 2005) and hot (Shirreffs et al., 2005)
64 environment, however this could have been attributed to greater amounts of clothing worn in
65 the cold therefore creating a warm microclimate.
66
67 With many researchers concentrating on exercise performance and water intake in the heat,
68 literature examining thirst and voluntary dehydration in the cold is sparse. Research has
69 focussed on low to moderate intensity exercise (50% $\dot{V}O_{2max}$) (Kenefick et al., 2004ab),
70 temperatures that have not been very cold (~7-10°C) (O'Brien et al. 1998) and work in field
71 environments where hormonal responses have not been analysed and clothing induced warm
72 microclimates are often created (Maughan et al., 2005; Seifert, Burke, White, & Luetkemeier,
73 2006). Examining voluntary dehydration with inclusion of these factors will assist with

assessment of the prevalence or potential for performance influencing dehydration levels occurring and whether further investigation is warranted into a potential effect on performance following exercise in the cold. Through measurement of the associated physiological mechanisms, an improved understanding of thirst and voluntary dehydration can be determined and therefore greater knowledge of water intake requirements when exercising in the cold. The aim of this study was to assess voluntary water intake and the response to thirst following moderate intensity exercise in the cold through measurement of blood indices and observed behaviour. It was hypothesised that voluntary water intake would be less during and following exercise in a cold environment primarily due to reduced sweat losses.

Methods

Subjects

Ten healthy male subjects (age 22 ± 2 years, mass 67.8 ± 7.0 kg, height 1.77 ± 0.06 m, $\dot{V}O_{2peak}$ 60.5 ± 8.9 $ml.kg^{-1}.min^{-1}$) were recruited. They took part in two trials, undertaken in a counter-balanced design. All subjects had the experimental protocol explained to them in writing and verbally. Subjects were not acclimatised to the heat or cold (i.e. had not visited hot or cold climates in the month preceding the first trial and throughout the duration of the trial). Subjects provided written informed consent and the experiment was approved by the Loughborough University Ethical Advisory Committee.

Experimental protocol

Subjects visited the laboratory four times for a $\dot{V}O_{2peak}$ test, a familiarisation trial and two experimental trials; warm and cold (schematic of the trial is presented in Figure 1). A discontinuous incremental test to volitional fatigue on an electrically braked cycle ergometer (Lode Corival; Lode BV, Groningen, Netherlands) was performed on the first visit to measure $\dot{V}O_{2peak}$. Expired gas was collected in Douglas bags during the final minute of each four minute incremental stage and analysed for oxygen and carbon dioxide concentration (Servomex 1400 Oxygen and Carbon Dioxide Gas Analyser; Servomex, Crowborough, UK). Gas volumes and temperature were measured using a Harvard dry gas meter (Harvard Apparatus Ltd., Edenbridge, UK) and thermometer (Edale Digital Thermometer D515: Edale instruments Ltd., Cambridge, UK) and corrected to STPD (standard temperature and pressure, dry).

In the three following visits, subjects attended for a familiarisation trial and two main experimental trials. The familiarisation trial was identical to the warm trial. Pre-trial standardisation occurred before each main trial and involved consuming 500 ml of water two hours before arrival at the laboratory, to try and ensure subjects were in a euhydrated state, and to arrive following an overnight fast. Subjects were asked to record their dietary intake (food and drink consumed, amount and method of preparation), refrain from strenuous physical activity and consumption of alcohol in the 24 hours prior to arriving at the laboratory for the first experimental trial. Subjects were asked to repeat this prior the second experimental trial. Dietary intake was not analysed, but recorded to allow for replication.

Separated by a period of seven or eight days, the experimental trials began in the morning at the same time. Trials were identical apart from the environmental conditions exercise was performed in. When arriving at the laboratory for the first trial, subjects did not know which trial they were participating in. Using incomplete Latin square design, experimental trial order was randomised. Exercise in the warm trial was performed at ~25°C, whilst the cold trial was performed at ~0°C. In each trial, on arrival, subjects voided and the whole urine volume measured and a 5 ml sample retained for later analysis and had nude body mass measured. A rectal thermistor 10 cm past the anal sphincter, skin thermistors were attached at the chest, tricep, thigh and calf and a heart rate (HR) monitor was positioned (Polar Vantage; Kempele, Finland). Core (T_c) and skin temperature (T_{sk}) were measured continuously throughout the trials (BIOPAC MP100 System; BIOPAC, Santa Barbara, CA, USA). Using the formula outlined by Ramanathan (1964) mean skin temperature was calculated. To allow for postural alterations in blood flow, subjects sat for 30 minutes at 21.4 ± 1.0°C and 52.4 ± 7.6% relative humidity (RH). Baseline heart rate values every 10 minutes were recorded. At the completion of the 30 minutes seated rest a 100 mm visual analogue subjective feelings questionnaire comprising of thirst and dry mouth scales was administered (0 mm = not all thirsty/mouth not at all dry, 100 mm = very thirsty/mouth very dry). A baseline (B) blood sample (5.5 ml) was collected without stasis from an antecubital vein in the arm.

Subjects cycled at 70% $\dot{V}O_{2max}$ (162 ± 27W) for 60 minutes in either 25.0 ± 0.1°C and 50.8 ± 1.5% RH (warm) or 0.4 ± 1.0°C and 68.8 ± 7.5% RH (cold). Every 10 minutes heart rate was recorded and subjects were asked to provide a rating of their perceived exertion (RPE) and thermal sensation. Subjects had free access to tap water maintained at a temperature of 11 ± 3°C throughout the duration of the exercise. The amount of water consumed was measured but the subject was not made aware of the volume or that the volume was being measured. Subjects were informed at the start that they could drink as they wanted and that the bottle would be refilled if necessary and were provided with no external cues to drink. During the familiarisation trial expired gas was collected between 14-15 minutes and 29-30 minutes to confirm the correct workload was being performed. Immediately following completion of exercise (post-exercise, PE), a blood sample (5.5 ml) was collected without stasis from an antecubital vein and thirst and dry mouth subjective feelings questionnaires were completed.

Subjects voided, the volume was measured and a 5ml sample was retained for later analysis and had body mass measured. Body mass was measured in clothing (trainers, socks and shorts) and with thermistors still attached. The mass of the thermistors and clothing were subtracted from the body mass recorded. Subjects rested for 120 minutes in 22.2 ± 1.2°C and 50.5 ± 8.0% RH with *ad libitum* water (11 ± 3°C) intake measured during each 30 minute period. As during the exercise period, subjects were unaware of this. Heart rate and thermal sensation were measured every 10 minutes. At 60 and 120 minutes a blood sample (5.5 ml) was collected without stasis from an antecubital vein in the arm and thirst and dry mouth subjective feelings questionnaires were completed. Following this, subjects voided, the urine volume was measured and a 5 ml sample was retained for later analysis and they then had body mass measured. After completion of the body mass measurement, subjects were allowed to leave the laboratory. At 10 minute intervals throughout the trials, temperature and relative humidity were measured (RH85 Digital Thermo-Hygrometer; Omega, Manchester, UK). To prevent the development and influence microclimates, during each trial subjects wore only shorts, socks and trainers.

Sample analysis

For each 5.5 ml venous blood sample, 2.5 ml was aliquoted and mixed with anticoagulant (K^+ EDTA; 1.5 $mg.ml^{-1}$). From this, plasma was separated and part was refrigerated for subsequent osmolality analysis by freezing point depression (Gonotec Osmomat auto Cryoscopic Osmometer; Gonotec, Berlin, Germany), and the remainder was frozen at -80°C for later analysis of hormone concentration. A further 1.0 ml was aliquoted and mixed with anticoagulant (K^+ EDTA; 1.5 $mg.ml^{-1}$) for analysis of haemoglobin concentration, haematocrit and glucose concentration. Serum was removed from the remaining blood (~2.0 ml) which was allowed to clot and was centrifuged at 3000rpm and 4°C for 15 minutes. Serum was later analysed for potassium and sodium concentration by flame photometry (Corning Clinical Flame Photometer 410C; Corning Ltd., Halstead, Essex, UK) and osmolality analysis by freezing point depression (Gonotec Osmomat auto Cryoscopic Osmometer; Gonotec, Berlin, Germany). Haemoglobin concentration was measured in duplicate using the cyanmethaemoglobin method. Haematocrit was measured in triplicate and determined by micro-centrifugation. Using haemoglobin concentrations and haematocrit values blood, plasma and red blood cell volume changes were calculated (method of Dill & Costill, 1974). A 100 µl sample of anticoagulated blood was pipetted into 0.3M perchloric

acid in a ratio of 1:10 in duplicate for analysis of glucose by the GOD-PAP method (Randox Laboratories Ltd., Crumbin, UK).

Following measurement of total sample volume and retention of a 5 ml sample, urine was analysed for osmolality through freezing point depression (Gonotec Osmomat auto Cryoscopic Osmometer; Gonotec, Berlin, Germany) and for potassium and sodium concentration by flame photometry (Corning Clinical Flame Photometer 410C; Corning Ltd., Halstead, Essex, UK). All urine analysis was carried out in duplicate.

Statistical analysis

Data were checked for normality of distribution using Shapiro-Wilks tests. All samples were normally distributed and subsequently either paired samples t-tests or repeated measures ANOVA was performed. If a significant main or interaction effect was found, a paired samples t-tests with Bonferroni correction were performed to identify where the statistical differences occurred and also used on significant and non-significant interaction effects. Pearson's product moment correlation coefficients were calculated between physiological and behavioural variables closely related to water balance. Statistical significance was accepted when $p<0.05$. Data expressed as mean ± SD.

Results

Baseline measures

Baseline measures of body mass (67.96 ± 6.33 v 67.69 ± 6.42 kg), serum osmolality (287 ± 3 v 285 ± 4 mOsmol.kg^{-1}), urine osmolality (320 ± 205 v 432 ± 228 mOsmol.kg^{-1}) and sensations of thirst (39 ± 23 v 42 ± 18) and mouth dryness (36 ± 23 v 38 ± 23) were similar between cold and warm trials respectively ($p>0.05$). The results indicate that subjects arrived in a similar state of euhydration (Sawka et al., 2007).

Body mass

Body mass losses over the trials were similar ($p>0.05$) (Table 1) but body mass had decreased from baseline values in both trials ($p<0.05$). After exercise, one subject in the cold trial and two in the warm trial had consumed more water than they had lost and thus, had gained weight.

Water balance and subjective feeling questionnaires

Sweat losses during exercise were lower in the cold trial compared to the warm trial ($p<0.0001$), whilst cumulative urine output over the duration of the trials was greater in the cold ($p=0.036$) Table 1).

More water was consumed throughout the duration of the warm trial compared to the cold trial ($p=0.001$) (Table 1). During the exercise period more water was consumed in the warm trial ($p<0.05$). Greater breakdown of the drinking periods showed that in the warm trial, more water was consumed during the first 30 minutes of the recovery period compared to 60-90 minutes and 90-120 minutes ($p<0.05$) (Figure 2). Similar volumes of water were consumed during the exercise period and each 30 minute period during the recovery in the cold trial ($p>0.05$). During the exercise period subjects consumed water to replace 44 ± 57% and 57 ± 39% of water losses in the cold and warm trials respectively ($p=0.259$).

Reported feelings of thirst were similar between cold and warm trials at baseline, post-exercise and after one and two hours of recovery ($p>0.05$) (Figure 4a). No difference between sample points was observed in the cold trial, however in the warm trial reported sensations of thirst were higher post-exercise compared to one ($p=0.012$) and two hours of

recovery ($p=0.006$). Reported sensations of mouth dryness were not different between trials and sample points (Figure 4b).

The amount of total sodium excreted was similar at post-exercise (13 ± 14 v 8 ± 2 mmol), and one (58 ± 45 v 30 ± 11 mmol) and two hours of the recovery (129 ± 86 v 87 ± 39 mmol) between cold and warm trials ($p>0.05$) whilst the total amount of potassium excreted was similar post-exercise (16 ± 9 v 11 ± 4 mmol) but greater in the cold trial after one (70 ± 25 v 44 ± 14 mmol) and two hours of the recovery period (156 ± 44 v 122 ± 47 mmol) ($p<0.05$).

Blood analysis

Haemoglobin concentrations increased in both trials following exercise ($p<0.05$) and remained elevated in the cold trial ($p<0.05$) (Figure 5a). In the warm trial concentrations returned to baseline after one hour of recovery but were elevated above baseline values following two hours of recovery ($p=0.018$). Haematocrit values were similar between trials ($p>0.05$) but within trials were elevated at all sample points compared to baseline in the cold trial and post-exercise and after one hour of recovery in the warm trial ($p<0.05$) (Figure 5b). Following one and two hours of recovery in the cold trial, although values did not return to baseline, they were lower than post-exercise samples ($p<0.05$). Plasma volume change from baseline was similar at post-exercise (-11.3 ± 2.0 v -9.9 ± 5.5% for the cold and warm trial respectively; $p>0.05$) and after one (-5.9 ± 3.2 v -2.0 ± 1.6% for the cold and warm trial respectively; $p>0.05$) and two hours of the recovery period (-6.6 ± 3.8 v -5.5 ± 2.7% for the cold and warm trial respectively; $p>0.05$). Blood volume decrease from baseline was greater in the cold trial compared to the warm trial after one hour of the recovery period (-3.3 ± 2.2 v -0.6 ± 1.0%) ($p<0.05$) but was similar at post-exercise (-6.5 ± 1.4 v -6.3 ± 3.3%) and after two hours (-3.9 ± 2.6 v -3.1 ± 1.7%) of the recovery period ($p>0.05$). Red blood cell volume change from baseline was similar between trials at post-exercise (-0.3 ± 1.3 v -1.3 ± 1.1%) and after one (0.1 ± 1.6 v 1.2 ± 1.2%) and two hours of the recovery (-0.4 ± 2.1 v 0.0 ± 0.5%) ($p>0.05$). In the cold trial, blood glucose concentrations did not change from baseline ($p>0.05$) (Figure 5c). In the warm trial, post-exercise concentrations were higher than baseline ($p<0.0001$) and compared to post-exercise concentrations in the cold trial ($p=0.016$). During recovery, blood glucose concentrations returned to baseline ($p>0.05$).

In the cold trial serum osmolality was greater post-exercise compared to baseline ($p<0.0001$) and there was a tendency to be greater compared to one ($p=0.054$) and two hours of recovery

(p=0.054) (Figure 3). In the warm trial post-exercise values were greater than baseline values (p=0.048) but returned to baseline values during the recovery period.

There was no difference in serum sodium concentrations between trials or over the duration of the study ($p<0.05$). Serum sodium concentrations were 142 ± 1 v 142 ± 1 $mmol.l^{-1}$ at baseline, 142 ± 1 v 141 ± 2 $mmol.l^{-1}$ post exercise, 142 ± 1 v 142 ± 1 $mmol.l^{-1}$ after one hour of recovery and 142 ± 1 v 141 ± 1 $mmol.l^{-1}$ after two hours of recovery in the cold and warm trials respectively ($p>0.05$). Serum potassium concentrations were higher post-exercise (5.0 ± 0.4 and 5.0 ± 0.3 $mmol.l^{-1}$; cold and warm trial respectively) and after one hour of recovery (5.1 ± 0.4 and 4.9 ± 0.2 $mmol.l^{-1}$; cold and warm trial respectively) compared to baseline (4.6 ± 0.3 and 4.5 ± 0.3 $mmol.l^{-1}$; cold and warm trial respectively) ($p<0.05$). No differences were observed between trials at each sample point.

Correlations

Total water intake was positively related to cumulative urine output in both the cold ($r=0.851$, $p=0.002$) and the warm trials ($r=0.949$, $p<0.0001$), however, water intake during each hour of the trial was not related to corresponding urine output volume in both trials (cold, $r=0.218$, $p=0.246$; warm, $r=0.130$, $p=0.492$). No relationship was observed between serum osmolality, subjective feelings of thirst and mouth dryness and the subsequent water intake in the following monitored time period. Serum osmolality was positively related to feelings of thirst ($r=0.429$, $p=0.011$) and mouth dryness ($r=0.470$, $p=0.005$) in the cold trial but there was no relationship with feelings of thirst ($r=0.267$, $p=0.127$) and mouth dryness ($r=0.145$, $p=0.412$) in the warm trial.

Core and skin temperature

Core temperatures were similar between trials ($p>0.05$) (Figure 6a). In both trials, during exercise, core temperature rose ($p<0.05$) before returning to baseline values during the recovery period. Mean weighted skin temperature was similar throughout the warm trial ($p>0.05$). During the cold trial, skin temperature decreased during the exercise period ($p<0.05$) but returned to baseline values on exiting the environmental chamber ($p>0.05$) (Figure 6b).

Rating of perceived exertion, heart rate and thermal sensation

During exercise, RPE values were similar between trials, however after 30 minutes RPE values were lower in the cold trial (14 ± 1) compared to the warm trial (15 ± 1) ($p<0.0001$). Heart rate values were lower after 20 (65 ± 11v 72 ± 12 beats.min^{-1}; $p<0.0001$) and 40 minutes (61 ± 10 v 72 ± 11 beats.min^{-1}; $p=0.006$) of the recovery period in the cold trial. No difference was observed during other time points ($p>0.05$). During exercise, mean heart rate values were 144 ± 11 beats.min^{-1} and 154 ± 13 beats.min^{-1} in the cold and warm trials respectively. During exercise thermal sensation was lower in the cold after 10 (-4 ± 2 v 3 ± 1; $p<0.0001$), 20 (-4 ± 2 v 4 ± 1; $p<0.0001$), 30 (-4 ± 2 v 4 ± 2; $p<0.0001$), 40 (-4 ± 2 v 5 ± 1; $p<0.0001$), 50 (-4 ± 2 v 5 ± 2; $p<0.0001$) and 60 minutes (-4 ± 2 v 5 ± 1; $p<0.0001$).

Discussion

The aim of this study was to assess voluntary water intake during and following moderate exercise in either a warm or cold environment. In both trials, indication of hydration status through body mass change showed similar body mass losses despite reduced voluntary water intake in the cold.

Ad libitum water intake appeared to prevent body mass losses greater than 2% occurring during and following exercise in cold and warm conditions. It has been shown that during exercise, *ad libitum* water intake, when compared to prescribed volumes of water replacement, can prevent body mass losses of greater than 2% (Dugas, Oosthuizen, Tucker, & Noakes, 2009). *Ad libitum* water intake is believed to be largely driven by sensations of thirst, and this has thought to be sufficient to replace water losses (Greenleaf, 1992). However, *ad libitum* water intake can be affected by inappropriate sensations and/or inappropriate interpretations of thirst (Maughan & Shirreffs, 2010). If *ad libitum* water intake results in too little water consumed then dehydration levels may be greater than a 2% body mass loss (Cheuvront & Haymes, 2001). If too much is consumed, so that there is a gain in body mass, then there is often an accompanying increase in urine output and increased water losses (Wong, Williams, Simpson, & Ogaki, 1998). In the current study all subjects prevented a 2% body mass loss from occurring, and with the exception of one subject in the cold trial and two in the warm trial, did not consume so much water for weight gain to occur during the exercise period (one subject in the cold and two in the warm trial gained weight following the exercise period and so consumed more water than they lost). By the completion of the trials all subjects were in negative water balance but not at a level that was likely to affect endurance performance (>2% body mass loss) (Sawka et al., 2007). When water intake was factored out, 4 subjects in the cold and 6 in the warm condition would have experienced body mass losses greater than 2%, but due to voluntary water intake were able to prevent this.

Despite the difference in water intake between the cold and warm trials, body mass loss was similar in both trials. This suggests that water intake was adjusted to suit the physiological responses to the environment and therefore was appropriate for the situation. Subjects were able to consume enough water to offset a sufficient volume of the water losses through sweating, respiration and urine output. Larger sweat losses in the warm trial were offset with

increased voluntary water intake. As environmental temperatures increase, heat loss by evaporation increases, with cooling by convection, conduction and radiation becoming less effective (Galloway & Maughan, 1997). In the warm trial, in an attempt to dissipate heat and prevent rises in core temperature, there was increased sweat losses and subsequent increased water intake to replace water losses. In addition to similar body mass losses, similar values for serum osmolality, urine and serum sodium and potassium concentrations and plasma volume changes between trials confirmed that water intake within each trial was sufficient to prevent large levels of dehydration.

Individual water intake patterns were varied within and between trials. In the cold trial two subjects consumed no water during the exercise period, whilst one subject consumed 0.959 l, equating to a 177% replacement of the water lost. Maughan et al. (2005) found large variation in individual water intake patterns during a 90 minute football training session in the cold (5.1 ± 0.7°C, 81 ± 6% RH) (mean intake 0.423 ± 0.215 l, range (0.044 – 0.951 l). In addition Maughan and Shirreffs (2008) have also recommended that athletes create individualised hydration strategies. In the present study, those that consumed smaller amounts relative to other subjects repeated the trend in the second trial. Translation to a practical setting would suggest that during exercise in the cold or in the warm it is important to cater to individual needs and identify those that may be consuming too much water. This could potentially have lead to unnecessary weight gain, which can often be conflicting for maximal sporting performance. Furthermore an increase in urine output can increase water losses (Wong et al., 1998) and may provide inconvenience through increased frequency of urination. It is also important to identify those that are not completely responding to thirst signals or have incorrect thirst signals and are not consuming sufficient water. However, identification of these individuals is difficult as it was not known whether subjects were drinking in response to sensations of thirst or perhaps due to a habitual response. Asking them this question may have influenced water consumption.

A greater cumulative urine output was observed in the cold trial and has been shown previously following cold exposure (O'Brien et al., 1998). However, O'Brien and colleagues found that this only occurred when the participant was in a euhydrated condition suggesting than in states of hypohydration urine output was reduced to prevent water loss. To increase reabsorption of water in the kidneys by increasing permeability to water of the collecting ducts and reduce urine output there is release of vasopressin to activate the V2R receptors in

the kidney (Bankir, 2001). In the cold, vasoconstriction of the peripheral blood vessels and redistribution of blood volume to the central areas of the body causes an increase in central blood pressure (Lennquist, Granberg, & Wedin, 1974). The increase in pressure is detected as increased extracellular water and therefore is removed resulting in increased urine production (Stricker & Verbalis, 1988). In the current study, the lack of difference in individual urine outputs between the trials at each timepoint may be attributed to the time spent in the different environmental conditions. Subjects did not rest in the cold environment and so once the exercise protocol had finished, the effect of the environment on causing cold-induced diuresis was diminished. Despite subjects not resting in the cold environment following the exercise period, it appeared that there was still a marginal effect of cold-induced diuresis. This was indicated by the greater cumulative urine output measured over the duration of the cold trial.

Serum osmolality values were greater post-exercise in both trials compared to baseline. In addition, the post-exercise serum osmolality values in the cold trial were greater than the threshold for thirst outlined by Phillips, Rolls, Ledingham, Forsling, and Morton (1985) (290 $mOsmol.kg^{-1}$). However, water intake was lower and reported sensations of thirst were similar, compared to values in the warm trial. Above this threshold value, it has been reported that the first sensation of thirst occurs, ultimately resulting in a desire to drink. In the cold it has also been suggested that there is a blunted thirst response which may affect water intake volumes (Kenefick et al., 2008). Following 30 min exposure to the cold, Kenefick and colleagues reported that the sensation of thirst was attenuated to a serum osmolality threshold of approximately 304 $mOsmol.kg^{-1}$. This attenuation of thirst, resulting from the cold-exposure, could be negated by an increase in plasma osmolality, in this instance, through sodium chloride ingestion. Yet, unlike in the current study the subsequent effect of thirst sensations on water intake behaviours was not examined. In the current study, the reduced water intake in the cold trial, despite similar rises in serum osmolality, would suggest that there was a blunting of the thirst response. Due to the relatively small duration of the exercise protocol, and thus cold exposure, there appeared not to be sufficient time for the blunted thirst response to have a greater impact on voluntary water intake.

Previous studies have examined the response to cold exposure without periods of exercise (Kenefick et al., 2008: O'Brien et al., 1998: O'Brien, Freund, Young, & Sawka, 2005) and have not combined this with a recovery period allowing *ad libitum* water rehydration to be

monitored. Although in this study, the recovery period was at a temperature of approximately 22.2°C; therefore not causing continual exposure to the cold environment, this situation was felt to occur more readily in a sporting situation. Often, following completion of exercise, individuals retreat to warmer environments and only remain exposed to the cold when a warmer environment is not accessible. It is possible that longer exposure to the cold, or exposure without the heat generating effect of exercise, would have also exacerbated the blunted thirst response and increased urine output.

Conclusion

Voluntary water intake was less in the cold environment, however in both the warm and cold environment, *ad libitum* water intake was sufficient to ensure an appropriate state of hydration. In the cold there appeared to a blunted thirst response, however the severity and length of the cold exposure was not enough to exacerbate this problem in relation to hydration status. In a practical setting, it appears the body adjusts to the magnitude of physiological and behavioural cues in different environments to ensure large water deficit do not accrue. Despite a blunted thirst response in the cold, water intake was regulated to an appropriate level that resulted in similar body mass losses in both environmental conditions. Due to reduced sweat losses, those exercising in a cold environment will find that despite a blunted thirst response, desire and necessity to drink will be sufficient to prevent large water losses.

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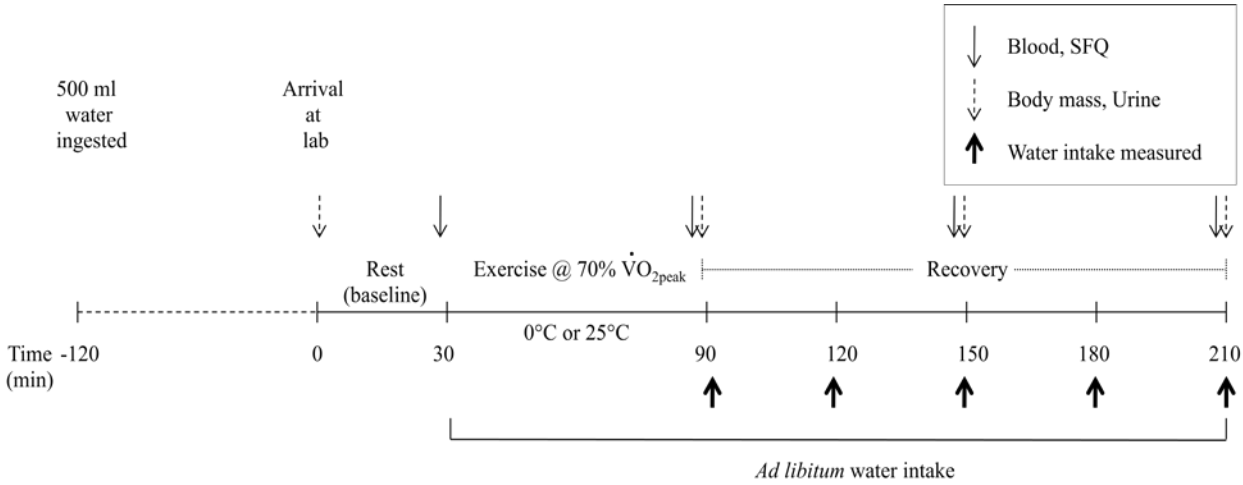
519 List of figures

520 **Figure 1** Schematic diagram indicating the testing protocol. Arrows represent sampling
521 points. SFQ denotes subjective feelings questionnaire
522 **Figure 2** Voluntary water intake (l) during each trial. * different to exercise period ($p<0.05$). #
523 different to 0-30 min ($p<0.05$). † different between trials ($p<0.05$). Mean ± SD
524 **Figure 3** Serum osmolality over the duration of each trial ($mOsmol.kg^{-1}$). * different to
525 baseline ($p<0.05$). B denotes baseline sample, PE denotes post-exercise sample. Mean ± SD
526 **Figure 4** Subjective feelings of thirst (a) and mouth dryness (b) over the duration of each
527 trial. 0mm = not at all thirsty / mouth not at all dry, 100mm = very thirsty / mouth very dry. #
528 different to post-exercise in the warm trial ($p<0.05$). B denotes baseline sample, PE denotes
529 post-exercise sample. Mean ± SD
530 **Figure 5** Haemoglobin concentration (a), haematocrit (b) and blood glucose concentration (c)
531 over the duration of each trial. * different to baseline ($p<0.05$). # different to post-exercise
532 ($p<0.05$). ^ different to one hour of recovery ($p<0.05$). † different between trials ($p<0.05$). B
533 denotes baseline sample, PE denotes post-exercise sample. Mean ± SD
534 **Figure 6** Core (T_c) temperature (a) and mean weighted skin (T_{sk}) temperatures (b) over the
535 duration of each trial. * Different to baseline, † different between trials ($p<0.05$). At 30 and
536 150 min the decreases in core and skin temperature were caused when the Biopac connection
537 was interrupted to allow for body mass measurement. Mean ± SD
538



539
540 Figure 1

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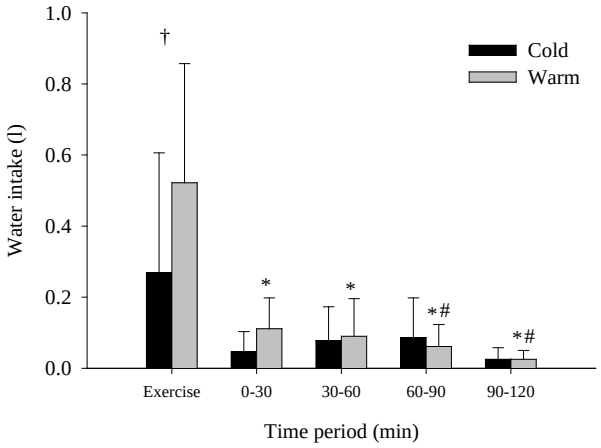
543 Figure 2



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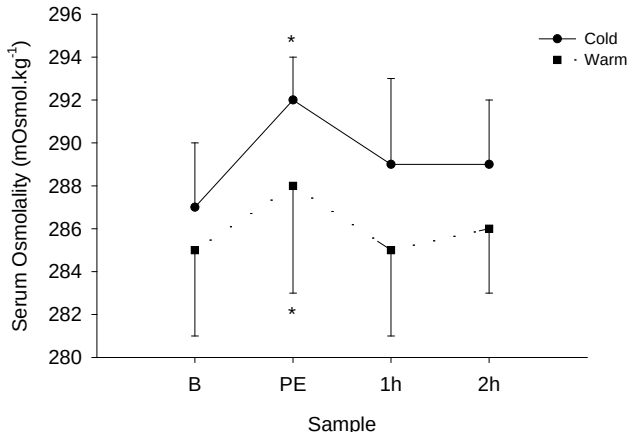

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548 Figure 3

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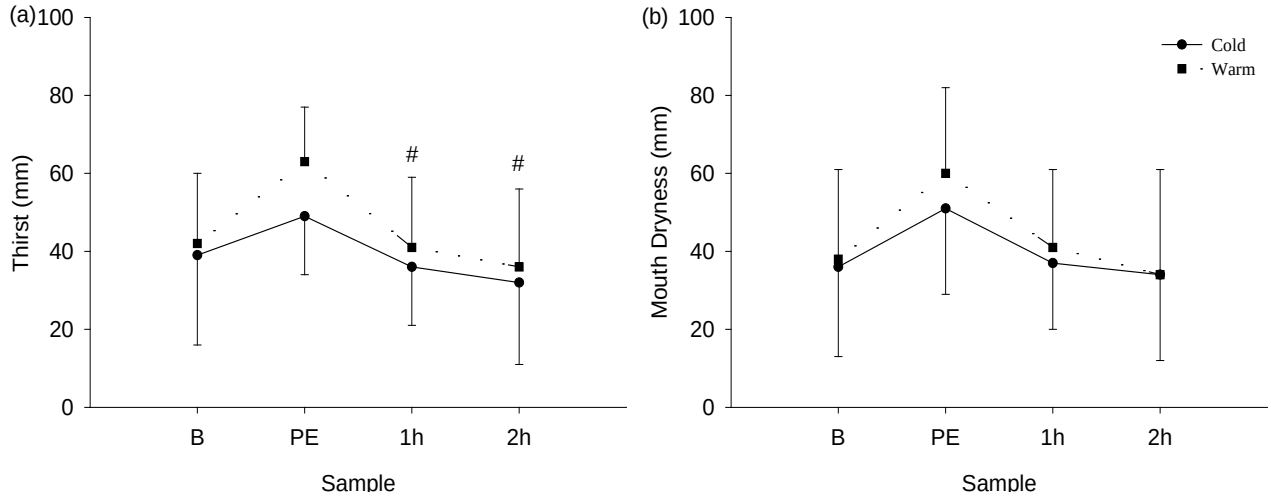
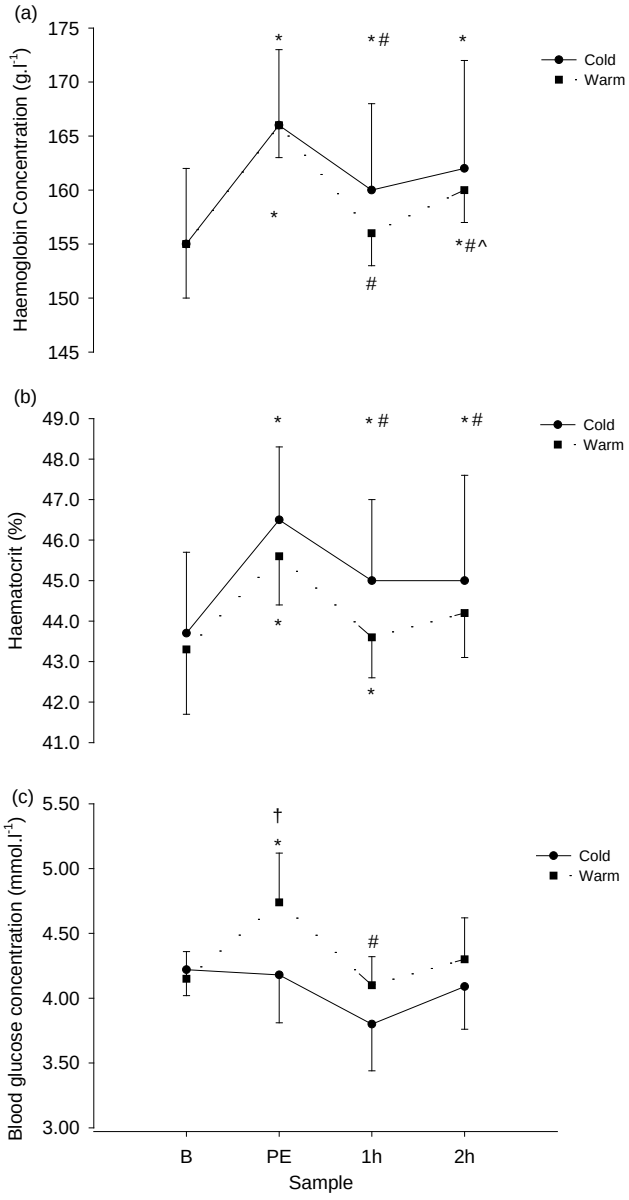

(a)
Thirst (mm)
100
80
60
40
20
0
#
#
B
PE
1h
2h
Sample
(b)
Mouth Dryness (mm)
100
80
60
40
20
0
Cold
Warm
B
PE
1h
2h
Sample

Figure 4

553



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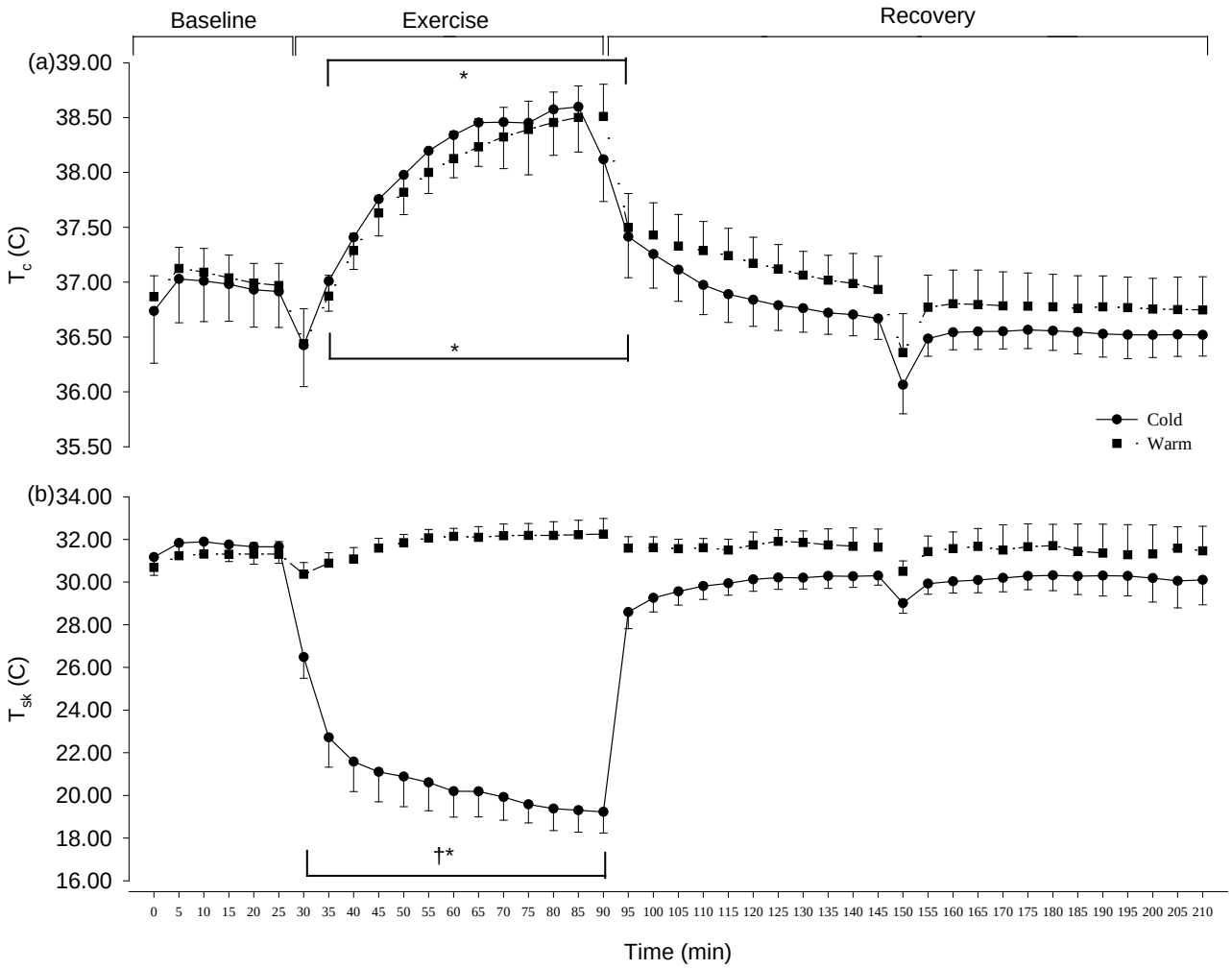

557
558 Figure 6

Table 1. Body mass changes and water balance between trials. * denotes different between trials ($p<0.05$), ^ denotes different between trials ($p<0.0001$), # denotes different to baseline in the same trial ($p<0.05$). Mean ± SD

	Timepoint							
	Baseline to Post-Exercise		0-1h recovery		1-2h recovery		Total	
	Warm	Cold	Warm	Cold	Warm	Cold	Warm	Cold
Body mass change (%)	-0.63 ± 0.56	-0.50 ± 0.50	0.00 ± 0.25	-0.32 ± 0.38	-0.40 ± 0.36	-0.33 ± 0.19	-1.03 ± 0.26#	-1.15 ± 0.34#
Sweat loss (l)	0.80 ± 0.17*	0.39 ± 0.13	0.10 ± 0.06	0.05 ± 0.03	0.06 ± 0.02	0.05 ± 0.02	0.96 ± 0.18^	0.48 ± 0.15
Water intake (l)	0.522 ± 0.335*	0.269 ± 0.337	0.200 ± 0.140*	0.124 ± 0.147	0.086 ± 0.076	0.111 ± 0.134	0.809 ± 0.420^	0.504 ± 0.487
Urine output (l)	0.13 ± 0.04	0.22 ± 0.19	0.11 ± 0.06	0.30 ± 0.24	0.30 ± 0.27	0.29 ± 0.20	0.54 ± 0.31	0.81 ± 0.46*
Respiratory loss (l)	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown

N.B. Respiratory water losses were not measured but have been included to indicate they were apparent