Physiological responses to incremental exercise in the heat following internal and external precooling

C.A. James, A.J. Richardson, P.W. Watt and N.S. Maxwell

Exercise in Extreme Environments Research Group University of Brighton Welkin House, Denton Road Eastbourne BN20 7SN

Mr Carl James Dr Alan Richardson Dr Peter Watt Dr Neil Maxwell C.James2@brighton.ac.uk A.J.Richardson@brighton.ac.uk P.Watt@brighton.ac.uk N.Maxwell@brighton.ac.uk

Running head

Comparison of internal and external cooling

Abstract

Twelve males completed three incremental, discontinuous treadmill tests in the heat (31.9[1.0]°C, 61.9[8.9]%) to determine speed at two fixed blood lactate concentrations (2 & 3.5 mmol.L⁻¹), running economy (RE) and maximum oxygen uptake (\dot{VO}_{2max}). Trials involved 20 min of either internal cooling (ICE, 7.5 g.kg⁻¹ ice slurry ingestion) or mixed-methods external cooling (EXT, cold towels, forearm immersion, ice vest and cooling shorts), alongside no intervention (CON). Following precooling, participants ran 0.3 km.h⁻¹ faster at 2 mmol.L⁻¹ and 0.2 km.h⁻¹ faster at 3.5 mmol.L⁻¹ (p=0.04, partial₁₂=0.27). Statistical differences were observed versus CON for ICE (p=0.03, d=0.15), but not EXT (p=0.12, d=0.15). There was no effect of cooling on RE (p=0.81, partial $\eta^2=0.02$), nor on $\dot{V}O_{2max}$ (p=0.69, partialn²=0.04). An effect for cooling on physiological strain index was observed (p < 0.01, partial $\eta^2 = 0.41$), with differences versus CON for EXT (p=0.02, d=0.36), but not ICE (p=0.06, d=0.36). Precooling reduced thermal sensation (p < 0.01, partial₁₂=0.66) in both cooling groups (p < 0.01). Results indicate ICE and EXT provide similar physiological responses for exercise up to 30 min duration in the heat. Differing thermoregulatory responses are suggestive of specific event characteristics determining the choice of cooling. Precooling appears to reduce blood lactate accumulation and reduce thermoregulatory and perceptual strain during incremental exercise.

Key words: precooling, endurance, ice slurry, external cooling, lactate threshold, thermoregulation.

Introduction

Endurance exercise is underpinned by the ability to transfer chemical energy into a given exercise velocity (Coyle 1999). The status of this biological process can be assessed using physiological markers such as the lactate thresholds, running economy (RE) and maximum oxygen uptake (\dot{VO}_{2max}). Under normothermic conditions, when combined with the peak treadmill velocity, these markers have been shown to account for 97.8% of the variation in 16 km run time (McLaughlin et al. 2010). McLaughlin et al. (2010) highlighted that \dot{VO}_{2max} accounted for 90.2% of variation in running time in a group with hetergenous \dot{VO}_{2max} values. Furthermore, Lorenzo et al. (2011) has shown the lactate turn-point (LTP) to be a strong predictor of time trial performance in both cold (r=0.89) and hot (r=0.87) environments.

The addition of heat stress during endurance running is characterised by an enhanced metabolic (Parkin et al. 1999), cardiovascular (González-Alonso et al. 2008) and sensory strain (Villanova et al. 1997) as core temperature (T_{CORE}) increases. At moderate levels of heat strain, such alterations are associated with reductions of the LTP (Lorenzo et al. 2011) and \dot{VO}_{2max} (Nybo et al. 2014). The reduction in LTP in the heat is of particular importance given it remains a valid predictor of endurance performance in hot environments (Lorenzo et al. 2011). This decline may be associated with the shift towards carbohydrate oxidation (Fink et al. 1975; Parkin et al. 1999) and the increased blood lactate accumulation observed during heat strain (Hargreaves 2008). At maximal exercise intensities, $\dot{V}O_{2max}$ is attenuated due to increased skin blood flow required for heat dissipation, which leads to a reduction in stroke volume as a consequence of cutaneous pooling, and ultimately limits muscular blood flow and oxygen delivery (González-Alonso et al. 2008). Enhanced oxygen consumption has also been reported during heat strain (Consolazio et al. 1973), although not all studies observed this effect (González-Alonso et al. 1999). Furthermore, during prolonged or intense endurance exercise, a protective reduction in central nervous system (CNS) motor output may be observed as core temperature approaches 40°C (Cheung 2007). Thus, thermal interventions such as precooling that reduce body temperature thereby increasing heat storage capacity or reducing the rate of heat storage, have been shown to benefit endurance exercise in the heat.

A dichotomous approach towards precooling is apparent, with interventions either cooling externally or internally, eliciting different skin, core and muscle temperatures and therefore potentially different physiological responses. The attenuated LTP in the heat may be in part a consequence of increased muscular glycolysis (Fink et al., 1975; Parkin et al., 1999), an alteration in muscle metabolism that external body cooling has previously been shown to mediate (Kozłowski et al. 1985). Therefore, external cooling may help reduce plasma lactate accumulation and could have an effect on LTP. Similarly, by reducing T_{CORE} , internal cooling may reduce the cutaneous circulation that can inhibit cardiac filling, thereby ameliorating cardiovascular strain that ultimately causes a reduction in \dot{VO}_{2max} . Therefore, accurately quantifying any differences in the responses to different types of cooling is important to optimise cooling strategies.

The vast number of cooling techniques reflects the challenge of providing a large cooling impulse through a technique that remains practical for use across a number of venues. Considerable growing evidence supports the use of internal cooling through ice slurry ingestion (ICE) as an ergogen for endurance exercise in the heat (Jones et al., 2012; Siegel & Laursen 2012; Wegmann et al. 2012). In addition to increasing heat storage capacity, direct cooling of sensitive thermoreceptors within the sphlanchic region may contribute to a reduced perceived thermal strain (Villanova et al. 1997). Further, visceral cooling may preserve sphlanchic flow that reduces during heat strain (Rowell et al. 1968) as well as prevent against endotoxin leakage that has been associated with impaired muscle force generation (Supinski et al. 2000) and exertional heat illness (Sawka et al. 2011). ICE typically elicits a substantial reduction in T_{CORE} of 0.3-0.6°C (Siegel et al. 2010; Siegel et al. 2012) and has been shown to aid time trial performance in the heat, improving a 40 km laboratory cycle by 6.5% compared with no cooling (Ihsan et al., 2010). ICE appears to permit similar running time to exhaustion as the gold standard technique of cold water immersion (Siegel et al. 2012), with recent systematic reviews advocating ICE to avoid impracticalities with water immersion (Jones et al. 2012; Siegel & Laursen 2012; Ross et al. 2013) Moreover, ICE is a simple strategy that may complement hydration and nutritional strategies during competition in the heat.

From an exogenous perspective, mixed method whole body external cooling (EXT) is gaining prominence following the apparent limited effectiveness of

individual cooling garments on endurance performance (Ranalli et al. 2010; Jones et al. 2012). Duffield et al. (2009) combined cooling garments to enhance the cooling volume and reported a blunted rise in T_{CORE} during exercise, resulting in increased work during 30 min of intermittent sprinting. External cooling may not always elicit a reduction in T_{CORE} prior to exercise (Minett et al. 2011; Minett, et al. 2012), but appears to permit a reduced rate of heat storage during exercise by enhancing heat dissipation through an increased core-to-skin gradient (Kay et al. 2010). The reduced T_{SKIN} will also lead to reduced vasodilation of peripheral capillary beds, potentially lowering the overall cardiovascular (CV) strain. Cooling of the skin may be an important mediator of thermal sensation (Schlader et al. 2011), and has been associated with a 6% increase in self-selected exercise intensity during a 30 min cycling trial when an initial reduction in T_{CORE} was not observed (Kay et al. 1999). Minett et al. (2011) has subsequently identified a dose-dependent response with skin cooling surface area coverage, the critical factor for exercise capacity and adopting a mixed methods approach to increase total work during intermittent sprinting in the heat, compared with no cooling (Minett et al. 2011, Minett et al. 2012). As with ICE, EXT is a simple and practical technique, however it has yet to be assessed prior to endurance running or on the physiological markers that are strongly associated with endurance exercise.

The aim of this study was to compare the physiological responses to practical and evidenced internal and external precooling techniques through the markers of lactate thresholds, RE and $\dot{V}O_{2max}$. Our first hypothesis stated both precooling techniques would increase lactate threshold, improve running economy and increase $\dot{V}O_{2max}$, relative to no cooling. Our second hypothesis stated internal cooling would elicit the greatest improvement within these markers due to the magnitude of T_{CORE} reduction, and the size of effects previously reported for this technique.

Method

Participants

Twelve male recreational club runners volunteered as participants (mean [SD]): age 38 (11) years, stature 177.8 (7) cm, mass 76.1 (5.7) kg, sum of four skinfolds 32.6 (7.1) mm, $\dot{V}O_{2max}$ 57.5 (4) mL.kg⁻¹.min⁻¹. All participants met the eligibility criteria of running a sub-21 min 5km or sub 43 min 10km race in the previous 2 months. Each participant provided written informed consent and institutional ethical approval was issued in accordance with the Helsinki declaration 1975 (revised 2008). Participants replicated their diet in the 12 hours prior to each session refraining from alcohol, caffeine and strenuous activity for 24 hours prior to the measurements in line with similar previous research (Gibson et al. 2014). Finally, participants were asked to prepare for each trial as they would a competition.

Experimental design

A randomised controlled design was used with each participant performing experimental trials under three conditions; control (no cooling, CON), internal cooling (ice slurry ingestion, ICE) and external cooling (mixed methods, EXT). Four trials were completed, involving two graded exercise tests during each visit and the first trial serving as a familiarisation. Participants were instrumented during the familiarisation. The subsequent three trials were completed in a randomised order, separated by 7-10 days. An overview of each trial is provided in Figure 1. Briefly, trials comprised four phases; 10 min rest, 20 min precooling, 5 min warm-up and then the graded exercise tests (GXT 1 and GXT 2), with the entire trial within a hot and humid environment (31.9 (1.0)°C, 61 (8.9)% relative humidity). In order to replicate a competition schedule, the exercise test began 15 min after cooling.

FIGURE 1 HERE.

Cooling interventions

During ICE, participants ingested 7.5 g.kg⁻¹ body mass of ice slurry (-1°C) during the cooling phase. Such a volume has previously been shown to elicit large reductions in T_{CORE} without inducing gastrointestinal distress (Siegel et al. 2012). Of this volume, the drink consisted of two thirds shaved ice using a snow cone

maker (JM Posner, Watford, UK) and one third diluted drinking cordial (High Juice, 7.3 g carbohydrate per 100 ml of diluted drink). This cordial is a non-carbonated syrup made from fruit juice, water and carbohydrate, that was diluted one part cordial to four parts water. Slurry was dispensed in equal amounts every five min over the 20 min precooling period to prevent gastrointestinal discomfort, with total drink volumes typically between 500-600 ml.

During EXT, participants were cooled using the manoeuvre adopted by Minett et al. (2011). This involved wet, iced towels covering the head and neck, forearm and hand immersion in cold water (9°C), an ice vest on the torso (Artic Heat, Queensland, Australia) and ice packs affixed to the quadriceps using cooling shorts. Towels were swapped after 10 min and hand immersion water temperature was actively maintained throughout. The same volume of squash was provided to match sugar intake between all conditions, with the drink delivered to the hot environment from an ambient laboratory temperature (21°C) during EXT and CON.

Graded exercise tests

Participants entered the environmental chamber (TISS, Hampshire, UK) within which conditions were continuously monitored throughout the trial using a heat stress meter (HT30, Extech Instruments, USA). Following rest and precooling phases, a five min warm-up was completed at 9 km.h⁻¹ on a motorised treadmill (Woodway ELG2, Weil am Rhein, Germany). GXT 1 was similar to that described by Jones (2006), initially a submaximal incremental speed protocol followed by GXT 2, an incremental gradient protocol to volitional exhaustion. Starting speeds were between 8–10 km.h⁻¹ (1% gradient) depending on recent running performance, with each participant completing a minimum of six stages, using speed increments of 1 km.h⁻¹. Each stage was 4 min, consisting of 3 min running and 1 min for capillary blood sampling, analysed using a YSI 2300 lactate analyser (YSI, Hampshire, UK). Exercise continued until an exponential increase in blood lactate was observed, or the participant felt unable to complete the subsequent stage. The first capillary sample was taken 18 min after hand immersion ceased, of which 8 min was exercise. The same number of exercise stages and running speeds completed during the familiarisation trial were replicated during all subsequent trials. Following a 2 min rest, GXT 2 began at a speed 2 km. h^{-1} below the previous final speed with gradient increasing by 1% each min and continuing until volitional exhaustion (Jones, 2006).

Physiological measures

During the familiarisation trial, anthropometric data were collected for stature, body mass and a four site skin fold calliper assessment (Harpenden, Burgess Hill, UK) across iliac crest, subscapular, triceps and biceps (Durnin & Womersley 1974).

During all trials a urine sample was requested upon arrival for assessment of hydration status. Euhydration was achieved when urine osmolality and urine specific gravity were below 700 mOsmol.kg⁻¹ H₂O and 1.020, respectively (Sawka et al. 2007). Single-use rectal probes (Henleys Medical, UK, Meter logger Model 401, Yellow Springs Instruments, Missouri, USA) were inserted 10 cm beyond the anal sphincter for T_{CORE} measurement. Telemetry thermistors (U-Type connected to Gen II GD38 transmitter, Eltek, UK) were attached to the mid-belly of the pectoralis major, biceps brachii, rectus femoris and gastrocnemius for measurement of skin temperature (T_{SKIN}) with data transmitted wirelessly to a datalogger (RX250AL 1000 series Wireless Squirrel Logger, Eltek). Heart rate was monitored continuously using a Polar 810i heart rate monitor (Kempele, Finland). Heart rate (HR), T_{CORE} , T_{SKIN} , rating of perceived exertion (RPE,) and thermal sensation (TS, 0=unbearably cold to 8=unbearably hot, Gagge et al. 1969) were noted every five min during rest and precooling and at the end of each stage during exercise. The following physiological responses were calculated; lactate thresholds, running economy (RE), maximum oxygen consumption ($\dot{V}O_{2max}$) and velocity at $\dot{V}O_{2max}$ ($v\dot{V}O_{2max}$). Fixed blood lactate concentrations of 2 and 3.5 mmol.L⁻¹ were used to denote the lactate threshold and lactate turn-point respectively by solving the polynomial regression equation for blood lactate versus speed at 2 and 3.5 mmol.L⁻¹ as per Saunders & Green (2013). Ventilatory gases were measured using 30 s averaging from a Metalyzer Sport analyser (Cortex, Leipzig, Germany) and the two values from the final min of each stage used for measuring RE, ventilation (V_E) and respiratory exchange ratio (RER). Average running economy across the first 6 exercise stages is presented, although the data from each individual stage was used for analysis. During the $\dot{V}O_{2max}$ test, the highest 15 s moving average recorded represented $\dot{V}O_{2max}$. A different data averaging approach was adopted due to the short recovery period between the two parts of the test to attenuate a potential effect on blunted $\dot{V}O_{2max}$ values. Recovery was minimal in order to help ensure both physiological and perceptual effects of cooling would still be present whilst testing VO_{2max}.

Velocity at $\dot{V}O_{2max}$ ($v\dot{V}O_{2max}$) was calculated by multiplying $\dot{V}O_{2max}$ (mL.kg⁻¹.min⁻¹) by 60 and divided by the mean running economy (mL O_2 .kg⁻¹.km⁻¹) determined during the first 6 stages of the treadmill test as per Jones (2006). Sweat rate (L.hr⁻¹) was calculated from the difference in pre and post nude body mass divided by the individual exercise duration.

Statistical Analysis and Derivative Calculations

The following derivative calculations were completed for: Mean skin temperature (T_{SKIN}) (Ramanathan 1964):

 $Mean T_{SKIN} = 0.3(T_{CHEST}+T_{ARM}) + 0.2(T_{THIGH}+T_{CALF})$

Physiological Strain Index (PSI) (Moran et al. 1998) whereby T_{CORE0} and HR_0 denote baseline and T_{CORE1} and HR_1 denotes measurement taken at the respective time:

 $\mathsf{PSI} = (5 * (\mathsf{T}_{\mathsf{CORE}^1} - \mathsf{T}_{\mathsf{CORE}^0})) / ((39.5 - \mathsf{T}_{\mathsf{CORE}^0}) + (5 * (\mathsf{HR}^1 - \mathsf{HR}^\circ) * (180 - \mathsf{HR}^0)).$

Sweat rate (L.hr⁻¹) was calculated from the difference in pre and post nude body mass divided by the individual exercise duration. All outcome variables were assessed for normality and sphericity prior to further analysis. Data were analysed in three phases: rest, cooling and exercise. Two way, repeated measures ANOVA (cooling type*time) were used to test for differences in blood lactate indices, respiratory responses, T_{CORE} , T_{SKIN} , PSI, RPE and TS. One way, repeated measures ANOVA were used to detect differences between running time until $\dot{V}O_{2max}$ during GXT 2, $v\dot{V}O_{2max}$, thermoregulatory variables at rest, sweat rate, absolute change during cooling and change during exercise. Where appropriate, Bonferroni adjusted pairwise comparisons revealed where differences occurred. Data were analysed using SPSS (Version 20, SPSS Inc, Illinois, USA) with significance set at p<0.05 and the data are presented as means and SD. Effect sizes for main effects and interactions are presented as partial eta squared (η^2), whilst differences between two related samples were evaluated through Cohen's d_{av} in accordance with Lakens (2013).

Results

Physiological responses

An effect on running speed was observed across both fixed lactate concentrations (F=3.78, p=0.04, partial $\eta^2=0.27$). Mean values at 2 mmol.L⁻¹ were ICE 12.3(1.1)km.h⁻¹, EXT 12.3(1.1)km.h⁻¹, CON 12.0(1.1)km.h⁻¹ and at 3.5 mmol.L⁻¹, ICE 13.8(1.0)km.h⁻¹, EXT 13.8(1.0)km.h⁻¹, CON 13.6(1.0)km.h⁻¹. Bonferroni comparisons identified a difference between ICE and CON (p=0.03, d=0.15), but not between EXT and CON (p=0.12, d=0.15), or between ICE and EXT (p=1.00, d<0.001). The mean blood lactate response is displayed in Figure 2, whilst lactate and oxygen uptake during GXT 1 are plotted in Figure 3. There was no effect of cooling on RE (ICE 230[18]mL.kg⁻¹.km⁻¹, EXT 230[17]mL.kg⁻¹.km⁻¹ ¹, CON 227[13] mL.kg⁻¹.km⁻¹, p=0.82, partial $\eta^2=0.02$), nor for $\dot{V}O_{2max}$ (ICE 57.5[5.6]mL.kg⁻¹.min⁻¹, EXT 58.4[4.7]mL.kg⁻¹.min⁻¹, CON 57.3[4.9]mL.kg⁻¹.min⁻¹, p=0.69, partial $\eta^2=0.04$). No statistical difference in running time until $\dot{V}O_{2max}$ during GXT 2 was observed (p=0.707, partial $n^2=0.03$). However, the mean of both precooling groups was greater than CON (368(79) s, ICE 375(57) s, EXT 381(73) s) which equated to a 2% (d=0.11) difference following INT and 3.4% (d=0.17) difference following EXT. Times for EXT were 1.5% (d=0.08) greater than ICE. No statistical difference was found in $v\dot{V}O_{2max}$ (p=0.49, partial η^2 =0.08), although speed after EXT (15.4[1.3]km.h⁻¹, ICE 15.0[1.4]km.h⁻¹, CON 15.0[1.7]km.h⁻¹) equated to a 2.5% difference versus CON (d=0.26) and a 2.8 % (d=0.31) difference versus ICE. Speed following ICE was marginally below that of CON (-0.2%, d=0.02). No differences in heart rate (p=0.81, partial $\eta^2=0.20$) were observed between groups; CON 146(16) b.min⁻¹, ICE 145(15) b.min⁻¹, EXT 143(15) b.min⁻¹.

FIGURE 2

FIGURE 3

No differences were observed throughout the submaximal exercise test in either V_E (mean across exercise; CON 85.6[12.5]L.min⁻¹, ICE 85.0[12.5] L.min⁻¹, EXT 85.0[11.6] L.min⁻¹, p=0.90, partial $\eta^2=0.01$) or RER (mean across exercise; CON 0.97[0.03], ICE 0.97[0.03], EXT 0.98[0.06], p=0.44, partial $\eta^2=0.08$) were observed across six exercise stages. Similarly, no interaction effects were observed for V_E (p=0.149, partial $\eta^2=0.131$) or RER (p=0.11, partial $\eta^2=0.16$).

Thermoregulatory responses

Figure 3A illustrates mean T_{CORE} data for each condition. After the 10 min rest period there were no differences between conditions (CON 37.13[0.23]°C, ICE

37.18[0.25]°C, EXT 37.13[0.31]°C, p=0.43, partial $n^2=0.08$). During the cooling phase, an interaction between cooling type and time was observed (F=25.86, p<0.001, partial $n^2=0.70$). Ice slurry ingestion resulted in a greater reduction in T_{CORE} (-0.32[0.11]°C) than EXT (-0.05[0.08]°C, p<0.001, d=2.97) and CON (-0.05[0.07]°C, p<0.001, d=3.03). No main effect for cooling type during exercise was found (p=0.13, partial $n^2=0.17$), although a trend towards a higher T_{CORE} for CON is apparent. The failure to detect an effect for cooling type may be explained by the presence of a cooling type*time interaction (F=4.38, p=0.01, partial $n^2=0.29$). As shown in Table 1 the overall change in T_{CORE} across the exercise phase was greatest during ICE (1.34[0.27]°C) compared with EXT (1.01[0.25]°C, p=0.001 d=1.29). However, there was no statistical difference vs CON (1.11[0.29]°C, p=0.10, d=0.82) and between CON and EXT (p=0.44, d=0.40). Finishing T_{CORE} for each group at \dot{VO}_{2max} were CON 39.03(0.45)°C, ICE 38.96(0.55)°C and EXT 38.88(0.38)°C.

Figure 3B illustrates mean T_{SKIN} data for each condition. A difference was observed between conditions at rest (CON 33.70 [0.5]°C, ICE 33.94[0.39]°C, EXT 33.48[0.60]°C, p=0.04 partial $n^2=0.28$), however this effect was not detected by Bonferroni post hoc. ANOVA revealed a main effect for cooling type (F=230.53, p<0.001, partial η^2 =0.96) and a cooling type*time interaction (F=5.74, p<0.001, partial $\eta^2 = 0.37$) during the cooling phase with EXT displaying the lowest mean T_{SKIN} temperatures throughout. EXT also resulted in a greater reduction in T_{SKIN} (-6.64[1.46]°C) than ICE (-0.17[0.52] °C, p<0.001, d=6.90) and CON (-0.40[0.39]°C, p<0.001, d=7.62). There was no difference between CON and ICE (p=0.51, d=0.52). An effect for cooling type was apparent during exercise $(F=44.20, p<0.001, partial n^2=0.82)$ with EXT (pre-post; 32.32[0.6]-35.01[0.59]°C) lower than CON (pre-post; 34.56[0.55]-35.27[0.67]°C, p<0.001, *d*=1.03) and ICE (pre-post; 34.94[0.39]-35.28[0.68]°C, *p*<0.001, *d*=1.29). A cooling type*time interaction was observed (F=44.14, p<0.001, partial n²=0.72), with a greater rate of increase within EXT resulting in no differences versus CON after stage 4 (p=0.058) and versus ICE after stage 5 (p=0.07). Consequently, EXT also displayed the largest overall change in T_{SKIN} during exercise (2.69[0.61]°C) with post hoc analysis revealing differences versus CON (0.71[0.42], p<0.001, d=3.86) and ICE (0.69[0.46], p<0.001, d=3.73) as shown in Table 1.

FIGURE 4 TABLE 1

An effect for cooling on PSI during exercise was observed (F=6.91, p=0.005, partial η_2 =0.41), with differences between CON (CON 5.2[1.6]) and EXT (4.6[1.6], p=0.02, d=0.36), and a non-significant trend for ICE (4.58[1.8], p=0.058, d=0.36). A cooling type*time interaction was also discovered (F=3.98, p=0.01, partial η^2 =0.26) due to different rates of increase between groups, with trends displayed in Figure 4. Sweat rates did not differ between groups (F=2.00, p=0.16, partial η^2 =1.66) with groups means as follows; (mean [SD], percentage of body mass); CON 1.4(0.7) L.hr⁻¹ (1.2%), ICE 1.6(0.6) L.hr⁻¹ (1.4%) and EXT 1.6(0.5) L.hr⁻¹ (1.4%).

FIGURE 5

Perceptual measures

Precooling reduced thermal sensation during exercise (F=20.98, p<0.01, partial $\eta_2 = 0.66$) with CON (6.2 [0.8]) higher than ICE (5.7 [0.9], p=0.005, d=0.50) and EXT (5.4 [0.8], p<0.001, d=0.98). However this reduction did not remain throughout exercise, evidenced by a cooling type*time interaction effect (F=4.98, p<0.001, partial η_2 =0.31). No differences in RPE (F=1.96, p=0.54, partial η^2 =0.06) were observed between groups.

Discussion

The aims of this study were to compare the physiological responses from internal and external precooling methods during graded exercise tests in the heat. In accordance with our first hypothesis, both precooling interventions resulted in greater running speeds at fixed blood lactate of 2 and 3.5 mmol.L⁻¹ compared with no cooling. However, in contrast to our second hypothesis, no difference in running economy or $\dot{V}O_{2max}$ was observed. Furthermore, no difference in the physiological responses between techniques was found despite a larger pre-exercise reduction in T_{CORE} following ICE. Finally, there were different thermoregulatory responses from the cooling techniques, suggesting specific event characteristics will determine the choice of cooling.

Effects of cooling

In this study, fixed blood lactate concentrations of 2 and 3.5 mmol.L⁻¹ were used to represent the lactate threshold and lactate turn-point, respectively (Saunders & Green 2013). This approach accounted for differences in the number of stages completed, removed subjectivity of experimenter identification and provided precision to less than 1 km.h⁻¹. ICE displayed a statistically significant greater running speed across both markers relative to CON, whilst a trend was observed for EXT. Such differences may be important given LTP remains a valid predictor of endurance performance in the heat (Lorenzo et al. 2011). Both precooling techniques displayed the same mean difference to CON at 2 (+0.3 km.h⁻¹) and 3.5 mmol.L⁻¹ (+0.2 km.h⁻¹), as well as the same overall effect size. As a result, a magnitude based inference statistical approach may conclude that both interventions had the same effect on lactate indices (Hopkins et al., 2009). Such changes in blood lactate response following precooling are small and likely fall at the upper end of what may be considered day to day variation of 0.2 mmol.L⁻¹ for this type of test (Saunders & Green 2013). However, the modest differences observed were consistent throughout the 23 minute trial and constitute a 2% improvement in running speed at the lactate threshold, which may be meaningful as it exceeds the 1.5% coefficient of variation for speed at lactate threshold (Hopkins et al., 2001). For this participant cohort, when running at lactate turn-point pace, such a difference would equate to 31 seconds over 5 km. At elite level, 10 km is typically completed in under 28 minutes and such a change could equate to an improvement of approximately 16 seconds. Figure 3 illustrates the lactate $\dot{V}O_2$ relationship during GXT 1 and provides some further evidence of a trend towards precooling eliciting a modest effect on the lactate thresholds during this test. The lack of difference in ventilation or RER suggests that under this level of heat strain cooling directly elicits an effect on lactate production or clearance rather than an altered $\dot{V}O_2$ in the muscle. This is supported by the apparent mediated metabolic strain not transferring to a reduced energetic cost of running in the heat. Despite competing demands for cardiac output for both exercising skeletal muscle and cutaneous vasodilation, it is apparent that muscular blood flow is maintained at submaximal intensities (Nybo et al. 2014; González-Alonso et al. 2008). Therefore, any changes in lactate are unlikely to be explained by cooling eliciting alterations in muscular blood flow. Rather, as thermoregulatory strain leads to a reduction in visceral circulation during exercise (Rowell et al. 1968), it more likely that by directly cooling T_{CORE} . ICE may elicit a heat sink away from the skin and maintain the rate of lactate-pyruvate conversion in the liver. Similarly, by substantially lowering T_{SKIN}, EXT may also reduce demands for skin blood flow, preserving splanchnic circulation and enhancing lactate clearance. An increase in splanchnic blood flow during exercise has previously been suggested as a mechanism that explains the enhancement in LTP following heat acclimation (Lorenzo et al. 2010). It should be noted that the alterations in the lactate response occurred at moderately elevated levels of T_{CORE} with mean T_{CORE} 38.3°C in CON after 23 minutes of graded exercise. Thus these results warrant an investigation into whether a greater effect of cooling on lactate occurs when individuals are hotter, and where metabolic alterations would be expected to be more pronounced.

Running economy incorporates both biomechanical as well as physiological parameters and is thought to account for differences between elite athletes who display similar $\dot{V}O_{2max}$ values (Bassett & Howley 2000). Despite indications that precooling may benefit some determinants of RE such as oxygen uptake (Lee & Haymes 1995), stride length (Folland et al. 2006) and neuromuscular function (Siegel et al. 2011), no differences were found, supporting the results of Winke and Yates (2008) who examined RE following ice slurry precooling. Thus, beneficial effects of precooling do not appear to present through improved RE whilst exercising for relatively short durations in the heat.

Despite evidence that maximum oxygen consumption is attenuated in the heat (Lorenzo et al. 2011), cooling had no effect on subsequent $\dot{V}O_{2max}$. González-Alonso and Calbet (2003) have demonstrated that under heat stress, cardiac

output and mean arterial pressure reduce at maximal exercise intensities, leading to a reduction in skeletal muscle blood flow. Thus, a cooler body would be expected to mediate the decline in \dot{VO}_{2max} under heat stress through reducing competition for blood flow and maintaining cardiac output and mean arterial pressure for longer. Such a mechanism is supported by the maintenance of skeletal muscle blood flow and \dot{VO}_{2max} in thermoneutral, relative to hot, conditions (Périard et al. 2011). However, it would appear that thermometric effects of both cooling techniques were no longer present at the point of \dot{VO}_{2max} , as evidenced by the similar finishing T_{CORE} between cooling techniques. The time-course of the current protocol shares similarities with many athletic events whereby a precooling intervention must occur prior to a warmup, with individuals completing 24-32 min of exercise, which may culminate in an all-out end-spurt. It remains plausible however, that cooling may still elicit an effect on \dot{VO}_{2max} if a greater cooling impulse is provided or \dot{VO}_{2max} is measured during a shorter duration protocol.

Interestingly, despite reductions in body temperature and thermal sensation, RPE remained unchanged following both cooling methods. This may indicate that enhanced performance in the heat and associated pacing adjustments following precooling are closer linked to thermal sensation rather than overall perceived exertion. Such a relationship could have implications for future cooling techniques more aggressively targeting locations that determine thermal sensation.

Responses to internal and external precooling

The physiological responses from each cooling technique were similar, with neither ICE nor EXT having an effect on \dot{VO}_{2max} or RE, and the magnitude of the effect on lactate similar between techniques. Although a small mean difference suggests towards enhanced $v\dot{VO}_{2max}$ following EXT, no statistical difference between treatments was observed. Further, whilst a change of 2% (0.4 km.h⁻¹) in EXT compared with ICE and CON could be interpreted as meaningful, this change is below what has been suggested to constitute a meaningful difference of 0.5 km.h⁻¹ (Billat & Koralsztein 1996). Therefore, it would appear that the estimate of the maximum speed that can be maintained by oxidative phosphorylation is similar, irrespective of cooling method.

Although both precooling manoeuvres produced a similar, lowered PSI throughout the trial, there were markedly different thermoregulatory responses

which may determine application. Whilst ICE resulted in a 0.3°C reduction in T_{CORE}, in keeping with other literature (Ihsan et al. 2010; Ross et al. 2011; Siegel et al. 2012), EXT did not elicit a reduction in T_{CORE}. This is not uncommon following external cooling techniques, as an 'after-drop' may be observed whereby T_{CORE} remains unchanged during cooling, before falling at the start of exercise as vasoconstriction dissipates and warm blood from the core is subsequently cooled in the periphery. Whilst an after-drop was not observed through a reduction in T_{CORE} , the rate of increase in T_{CORE} during exercise following EXT was smaller than both ICE and CON. The lack of an after-drop may be attributable to differences in the time-course and intensity of exercise following cooling compared with other research (Kay et al. 1999). Similarly, Duffield (2009) did not report a reduction in T_{CORE} following EXT and subsequently observed a reduced T_{CORE} throughout exercise. Both Uckert & Joch (2007) and Duffield et al. (2010) have reported performance benefits following external cooling techniques that did not elicit an initial reduction in T_{CORE} , as greater pace may be achieved through a reduced rate of heat storage or T_{SKIN} . Such a lower T_{SKIN} may be associated with reduced thermal discomfort (Gagge & Gonzalez 1974) and appears to be a key mediator of behavioural thermoregulation, contributing towards a greater selected intensity during self-paced prolonged exercise (Schlader et al. 2011). Indeed, the size of reduction in thermal strain relative to CON was greatest following EXT, in accordance with a reduced T_{SKIN} . ICE is thought to affect performance through an enhanced absolute heat storage capacity that prevents or delays CNS motor drive reduction, again permitting greater exercise intensity. In addition to an enhanced capacity for storing heat, functional magnetic resonance imaging has indicated that thermoreceptors within the splanchnic region may activate pleasure centres of the brain, possibly leading to a deceptive effect concerning overall thermal strain (Guest et al. 2007). The current results **may** suggest splanchnic thermoreceptors are less sensitive than those on the skin. However, the lack of a self-paced performance test within the current study limits the extrapolation of findings to endurance performance, therefore future research should investigate how a reduced thermal sensation following EXT transfers into running speed during self-paced protocols relative to ICE.

Similar trends in the T_{CORE} response were observed by Siegel et al. (2012) during fixed intensity exercise, whereby ICE produced a faster rise in T_{CORE} relative to cold water immersion, the rise from which was in turn slower than a no cooling

control condition. Sawka et al. (2012) suggested that cooling the core without a concurrent reduction in T_{SKIN} decreases the core: skin gradient, impairing the ability to dissipate heat to environment. Such an interaction between the rates of rise in T_{CORE} indicates ICE would be better suited to shorter duration endurance events, with the rate of heat storage during EXT making it also appropriate for longer duration exercise. However, future research should consider combining ICE and EXT which may provide an increased heat storage capacity alongside a reduced rate of heat storage during exercise. Techniques may be utilised sequentially in order not to impair heat dissipation through a heightened core: skin gradient, however time constraints within competition may necessitate concurrent use if such an additive effect exists.

Perspectives

In conclusion, internal and external precooling induced similar improvements in the lactate thresholds, whilst neither altered RE or $\dot{V}O_{2max}$ during incremental treadmill exercise up to 30 minutes. However, different thermoregulatory responses from the cooling techniques are suggestive of specific event characteristics determining the choice of cooling. Internal cooling may be limited to shorter duration events whilst external cooling may remain appropriate for exercise over 30 min due to the reduced rate of heat storage. Future research should consider the potential benefits of combining INT and EXT techniques. Finally, precooling appears to reduce blood lactate accumulation in the heat and reduce thermoregulatory and perceptual strain during incremental exercise.

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References

Bassett DR, Howley ET. Limiting factors for maximum oxygen uptake and determinants of endurance performance. Med Sci Sport Exerc. 2000;32:70-84.

Billat L, Koralsztein J. Significance of the velocity at VO2max and time to exhaustion at this velocity. Sport Med. 1996;22:90–108.

Borg G. Borg's Perceived Exertion and Pain Scales. Champaign, IL: Human Kinetics; 1998

Cheung SS. Neuropsychological determinants of exercise tolerance in the heat. Prog Brain Res. 2007;162:45–60.

Consolazio CF, Johnson HL, Daws TA, Nelson RA. Energy requirement and metabolism during exposure to extreme environments. World Rev Nutr Diet. 1973;18:177–94.

Coyle EF. Physiological determinants of endurance exercise performance. J Sci Med Sport. 1999;2:181-9.

Duffield R, Green R, Castle P, Maxwell N. Precooling can prevent the reduction of self-paced exercise intensity in the heat. Med Sci Sports Exerc. 2010;42:577–84.

Duffield R. The use of mixed-method, part-body pre-cooling procedures for teamsport athletes training in the heat. J Strength Cond Res. 2009;23:2524–2532.

Durnin J, Womersley J. Body fat assessed from total body density and its estimation from skinfold thickness: measurements on 481 men and women aged from 16 to 72 years. Br J Nutr. 1974;32:77–97.

Fink WJ, Costill DL, Handel PJ. Leg muscle metabolism during exercise in the heat and cold. Eur J Appl Physiol Occup Physiol. 1975;34:183–190.

Folland JP, Rowlands DS, Thorp R, Walmsley a. Leg heating and cooling influences running stride parameters but not running economy. Int J Sports Med. 2006;27:771-9.

Gagge A, Gonzalez R. Physiological and physical factors associated with warm discomfort in sedentary man. Environ Res. 1974;242:230–242.

Gagge A, Stolwijk J, Saltin B. Comfort and thermal sensations and associated physiological responses during exercise at various ambient temperatures. Environ Res. 1969;229:209–229.

Gibson OR, Dennis A, Parfitt T, Taylor L, Watt PW, Maxwell NS. Extracellular Hsp72 concentration relates to a minimum endogenous criteria during acute exercise-heat exposure. Cell Stress Chaperones. 2014;19:389–400.

González-Alonso J, Calbet J. Reductions in systemic and skeletal muscle blood flow and oxygen delivery limit maximal aerobic capacity in humans. Circulation. 2003;107:824–830.

González-Alonso J, Calbet JA, Nielsen B. Metabolic and thermodynamic responses to dehydration-induced reductions in muscle blood flow in exercising humans. J Physiol. 1999;520 Pt 2:577–89.

González-Alonso J, Crandall CG, Johnson JM. The cardiovascular challenge of exercising in the heat. J Physiol. 2008;586:45–53.

Guest S, Grabenhorst F, Essick G, Chen Y, Young M, McGlone F, de Araujo I, Rolls ET. Human cortical representation of oral temperature. Physiol Behav. 2007;92:975–84.

Hargreaves MA. Physiological limits to exercise performance in the heat. J Sci Med Sport. 2008;11:66-71.

Hopkins WG, Marshall SW, Batterham AM, Hanin J. Progressive statistics for studies in sports medicine and exercise science. Med Sci Sports Exerc. 2009;41:3-13.

Hopkins WG, Schabort EJ, Hawley JA. Reliability of power in physical performance tests. Sports Med. 2001;31:211–34.

Ihsan M, Landers G, Brearley M, Peeling P. Beneficial effects of ice ingestion as a precooling strategy on 40-km cycling time-trial performance. Int J Sports Physiol Perform. 2010;5:140-51.

Jay O, Kenny G. The determination of changes in body heat content during exercise using calorimetry and thermometry. J human-environmental Syst. 2007;10:19-29.

Jones AM. The Physiology of the World Record Holder for the Women's Marathon. Int J Sport Sci Coach. 2006;1:101–116.

Jones PR, Barton C, Morrissey D, Maffulli N, Hemmings S. Pre-cooling for endurance exercise performance in the heat: a systematic review. BMC Med. 2012;10:166.

Kay D, Taaffe D, Marino F. Whole-body pre-cooling and heat storage during selfpaced cycling performance in warm humid conditions. J Sports Sci. 1999;17:937– 944.

Kozłowski S, Brzezińska Z, Kruk B, Kaciuba-Uściłko H, Greenleaf JE, Nazar K. Exercise hyperthermia as a factor limiting physical performance: temperature effect on muscle metabolism. J Appl Physiol. 1985;59:766-73.

Lakens D. Calculating and reporting effect sizes to facilitate cumulative science: a practical primer for t-tests and ANOVAs. Front Psychol. 2013;4:863.

Lee DT, Haymes EM. Exercise duration and thermoregulatory responses after whole body precooling. J Appl Physiol. 1995;79:1971–1976.

Lorenzo S, Halliwill JR, Sawka MN, Minson CT. Heat acclimation improves exercise performance. J Appl Physiol. 2010;109:1140–7.

Lorenzo S, Minson CT, Babb TG, Halliwill JR. Lactate threshold predicting time-trial performance: impact of heat and acclimation. J Appl Physiol. 2011;111:221–7.

McLaughlin JE, Howley ET, Bassett DR, Thompson DL, Fitzhugh EC. Test of the classic model for predicting endurance running performance. Med Sci Sports Exerc. 2010;42:991–7.

Minett GM, Duffield R, Kellett A, Portus M. Mixed-method pre-cooling reduces physiological demand without improving performance of medium-fast bowling in the heat. J Sports Sci. 2012;30:907–15.

Minett GM, Duffield R, Marino FE, Portus M. Duration-dependant response of mixed-method pre-cooling for intermittent-sprint exercise in the heat. Eur J Appl Physiol. 2012;112:3655–66.

Minett GM, Duffield R, Marino FE, Portus M. Volume-dependent response of precooling for intermittent-sprint exercise in the heat. Med Sci Sports Exerc. 2011;43:1760–9.

Nybo L, Rasmussen P, Sawka MN. Performance in the heat-physiological factors of importance for hyperthermia-induced fatigue. Compr Physiol. 2014;4:657–89.

Parkin JM, Carey MF, Zhao S, Febbraio MA. Effect of ambient temperature on human skeletal muscle metabolism during fatiguing submaximal exercise. J Appl Physiol. 1999;86:902–8.

Périard JD, Cramer MN, Chapman PG, Caillaud C, Thompson MW. Cardiovascular strain impairs prolonged self-paced exercise in the heat. Exp Physiol. 2011;96:134-44.

Ramanathan NL. A New Weighting System for Mean Surface Temperature of the Human Body. J Appl Physiol. 1964;19:531–3.

Ranalli G, DeMartini J, Casa D, McDermott BP, Armstrong LE, Maresh CM. Effect of body cooling on subsequent aerobic and anaerobic exercise performance: a systematic review. J Strength Cond Res. 2010;24:3488–3496.

Ross M, Abbiss C, Laursen P, Martin D, Burke L. Precooling methods and their effects on athletic performance: a systematic review and practical applications. Sports Med. 2013;43:207–25.

Ross ML, Garvican LA, Jeacocke NA, Laursen PB, Abbiss CR, Martin DT, Burke LM. Novel precooling strategy enhances time trial cycling in the heat. Med Sci Sports Exerc. 2011;43:123–33.

Rowell LB, Brengelmann GL, Blackmon JR, Twiss RD, Kusumi F. Splanchnic blood flow and metabolism in heat-stressed man. J Appl Physiol. 1968;24:475–84.

Saunders, PU. and Green, DJ. Runners and Walkers. In Physiological tests for elite athletes. 2nd Ed. Edited by R.K. Tanner and C.J. Gore. Champaign, IL: Human Kinetics, 2013:397-410.

Sawka MN, Burke LM, Eichner ER, Maughan RJ, Montain SJ, Stachenfeld NS. American College of Sports Medicine position stand. Exercise and fluid replacement. Med Sci Sports Exerc. 2007;39:377–90.

Sawka MN, Cheuvront SN, Kenefick RW. High skin temperature and hypohydration impair aerobic performance. Exp Physiol. 2012;97:327–32.

Schlader ZJ, Simmons SE, Stannard SR, Mündel T. Skin temperature as a thermal controller of exercise intensity. Eur J Appl Physiol. 2011;111:1631–9.

Siegel R, Laursen PB. Keeping Your Cool Heat with Internal Cooling Methods. Sport Med. 2012;42:89–98.

Siegel R, Maté J, Watson G, Nosaka K, Laursen PB. Pre-cooling with ice slurry ingestion leads to similar run times to exhaustion in the heat as cold water immersion. J Sports Sci. 2012;30:155-65.

Siegel R, Maté J, Watson G, Nosaka K, Laursen PB. The influence of ice slurry ingestion on maximal voluntary contraction following exercise-induced hyperthermia. Eur J Appl Physiol. 2011;111:2517–24.

Uckert S, Joch W. Effects of warm-up and precooling on endurance performance in the heat. Br J Sports Med. 2007;41:380-4.

Villanova N, Azpiroz F, Malagelada JR. Perception and gut reflexes induced by stimulation of gastrointestinal thermoreceptors in humans. J Physiol. 1997;502 Pt 1:215-22.

Winke, M. and Yates, JW. Precooling and running economy. ACSM 55th Annual Meeting Indianapolis, Presentation number 800. 2008.

Tables

Table 1: Mean (SD) change in core temperature and mean skin temperature across six incremental exercise stages (left) and change per 5 min (right). Differences (p<0.05) against control are denoted by '*' and differences between internal and external cooling groups by '†'.

	∆ total			∆ per 5 min		
Cooling type	Control	lce	External	Control	lce	External
		slurry			slurry	
Δ T _{CORE} (°C)	1.11	1.38†	1.01†	0.23	0.29	0.21
(SD) A Mean Turr	(0.29)	(0.26)	(0.25)	(0.06)	(0.05)	(0.05)
(°C)	0.71	0.69*†	2.69*†	0.15	0.14*†	0.56*†
(SD)	(0.42)	(0.46)	(0.61)	(0.09)	(0.10)	(0.13)



Figure 1: Protocol overview. Entire protocol completed in hot environment. 'GXT 1' denotes 3 min exercise stages with increments of 1km.h⁻¹. 'GXT 2' denotes gradient based test to exhaustion incorporating 1 min stages with increments of 1%.

Figures



Figure 2 Mean lactate response over six incremental sub-maximal exercise stages. Total time is displayed with error bars displaying standard deviation. Each stage constituted 3 min exercise and 1 min blood sampling, with increments of 1 km.h⁻¹. Horizontal dotted line indicates blood lactate concentration of 2 mmol.L⁻¹ from which individual running speeds were calculated to represent lactate threshold. All participants completed a minimum of 6 stages, with some participants completing additional stages before displaying blood lactate concentrations exceeding 3.5 mmol.L⁻¹. A main effect for cooling type was observed (p=0.04, partial η^2 =0.27), with differences identified between CON and ICE.



Figure 3 Blood lactate versus oxygen uptake during GXT 1. Horizontal dotted line indicates blood lactate concentration of 2 mmol.L⁻¹ from which individual running speeds were calculated to represent lactate threshold. Error bars represent one standard deviation.



Figure 4A: Mean core temperature response, 4B: Mean skin temperature response across protocol. '*' denotes difference between Ice slurry and Control, '#' denotes difference between External and Control, '†' denotes difference between Ice slurry and External (p<0.05). Error bars represent one standard deviation.

GXT 1 Exercise time



Figure 5: Physiological strain index across 6 incremental exercise stages. '*' denotes difference (p<0.05) between internal vs control and '†' denotes difference between external vs control. Error bars represent one standard deviation.