Title:

Ischaemic preconditioning does not alter the determinants of endurance running performance in the heat.

Running title:

Ischaemic preconditioning in the heat

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Key words

Ischaemic preconditioning; occlusion; hyperthermia; endurance; lactate threshold; $\dot{V}O_{2max}$

Number of figures

7 (seven)

Number of tables

1 (one)

Word Count

4,997

Abbreviations:

ANOVA	Analysis of variance
CON	Control group
GXT	Graded exercise test
HR	Heart rate
IP	Ischaemic preconditioning
LTP	Lactate turnpoint
PSI	Physiological strain index
RE	Running economy
RER	Respiratory exchange ration
RPE	Rating of perceived exertion
T_{CORE}	Core temperature
T_{SKIN}	Skin temperature
TS	Thermal sensation
V_E	Ventilation rate
$\dot{V}O_{2max}$	Maximum oxygen consumption
$v\dot{V}O_{2max}$	Velocity at $\dot{V}O_{2max}$

Abstract

Purpose: Ischaemic preconditioning (IP) has been shown to be ergogenic for endurance performance in normothermic conditions and alleviate physiological strain under hypoxia, potentially through haemodynamic and/or metabolic mechanisms. Exertional hyperthermia is characterised by competition for blood flow between the muscles and skin, an enhanced metabolic strain and impaired endurance performance. This study investigated the effect of IP on the determinants of endurance performance, through an incremental exercise test in the heat.

Method: Eleven males completed two graded exercise tests in the heat (32°C, 62% RH) until volitional exhaustion, preceded by IP (4x5 min 220 mmHg bilateral upper leg occlusion) or a control (CON) condition (4x5-min 50 mmHg bilateral).

Result: IP did not improve running speeds at fixed blood lactate concentrations of 2 and 4 $mMol.L^{-1}$ (p=0.828), or affect blood glucose concentration throughout the trial (mean [±SD]; CON 5.03 [0.94] $mMol.L^{-1}$, IP 5.47[1.38] $mMol.L^{-1}$, p=0.260). There was no difference in $\dot{V}O_{2max}$ (CON-55.5 [3.7] $mL.kg^{-1}min^{-1}$, IP 56.0 [2.6] $mL.kg^{-1}min^{-1}$, p=0.436), average running economy (CON 222.3 [18.0] $mL.kg^{-1}.km^{-1}$, IP 218.9 [16.5] $mL.kg^{-1}.km^{-1}$, p=0.125), or total running time during graded exercise (CON 347 [42] s, IP 379 [68] s, p=0.166). The IP procedure did not change muscle temperature (CON Δ= 0.55 [0.57]°C, IP Δ=0.78 [0.85]°C, p=0.568), but did reduce T_{CORE} during exercise (~-0.1°C, p=0.001).

Conclusion: The novel application of IP prior to exercise in the heat, does not enhance the determinants of endurance performance. For events where IP appears ergogenic, muscle warming strategies are unnecessary as IP does not influence deep muscle temperature.

Introduction

Repeated bouts of occlusion and reperfusion, termed ischaemic preconditioning (IP), are established within clinical practice to prepare cardiac muscle for subsequent stresses arising from surgically induced hypoxia, infarction and reperfusion (Hausenloy & Yellon 2008). Skeletal muscle may also be exposed to modest exercise-induced arterial hypoxemia and ischaemic threat (Noakes 2000). Therefore, this simple, acute intervention has been utilised prior to a range of exercise protocols and shown to offer meaningful improvements in both high intensity and endurance exercise (Salvador et al. 2015).

Of note, Crisafulli et al. (2011) reported a 4% increase in maximal power output and a ~40 s increase in total exercise time during an incremental cycling test to exhaustion immediately following IP, whilst de Groot et al (2010) observed a 3% increase in $\dot{V}O_{2max}$, alongside a 1.6% increase in maximal power output during incremental cycling. Few studies have considered endurance running, however Bailey, Jones, et al. (2012) reported lower blood lactate concentration (mean difference -1.07 mMol.L^{-1}) throughout an incremental submaximal running test, although no subsequent effect on maximum oxygen consumption ($\dot{V}O_{2max}$) was observed. This was followed by improved 5 km running time with a mean improvement of 34 s (2.5%, p=0.03), highlighting the potential for use prior to endurance running. In swimming, IP may provide a particular benefit by mitigating against the relative exercise-induced hypoxia that occurs as a result of specified breathing patterns (Noakes 2000), that elicit a decreased arterial partial pressure of oxygen and decreased blood pH (Craig 1986; Sharp et al. 1991). Accordingly, Jean-St-Michel et al. (2011) reported a meaningful and statistically significant 0.7 s improvement in 100m swim time (-1.1%, p=0.02) following IP, improving personal best times within a highly trained cohort. Exercising in extreme environments may also elicit a heightened physiological strain and as such, IP has been shown to be beneficial under hypoxia (Foster et al. 2011; Foster et al. 2014), but has yet to be adopted under heat stress.

Bushell and Klenerman (2002) have mimicked the protective effects of IP through adenosine infusion. Adenosine may proffer beneficial clinical and exertional effects by acting as a vasodilator (Gustafsson et al. 1993), and/or opening potassium (K_{ATP}) channels, which in turn preserves energy (Pang et al. 1997; Cohen et al. 2000). This may occur reduced mitochondrial oxygen consumption (Cooper & Brown 2008), although a reduced oxygen consumption for a given submaximal exercise intensity is not uniformly observed (De Groot et al. 2010). There is also likely an effect of IP in promoting endothelial nitric oxide biosynthesis (Kimura et al. 2007), which appears to maintain vascular function that may be impaired following high intensity exercise (Bailey, Birk, et al. 2012). In

turn, such changes will serve to maintain the supply of oxygen and energy substrates, whilst removing metabolites during exercise. Collectively, these haemodynamic and/or metabolic ischaemic responses may present a more efficient muscular action, facilitating greater work to be completed for the same energy expenditure (Crisafulli et al. 2011; Pang et al. 1995). Clevidence et al. (2012) speculated that IP appears to be effective when an ischaemic event is most likely and may require a minimum metabolic stress to be achieved during the exercise bout to be effective during exercise. Thus, extreme environmental conditions, higher intensity exercise (Crisafulli et al. 2011) and/or activities such as running (Bailey, Jones, et al. 2012) and swimming (Jean-St-Michel et al. 2011), that elicit significant physiological strain, warrant investigation.

Environmental heat stress provides an additional stressor to exercise performance (Galloway & Maughan 1997). Indeed, endurance running in the heat is characterised by competition for blood flow between the muscles and skin as individuals become hyperthermic (Gonzalez-Alonso et al. 2008) and the potential for hyperthermia-induced central fatigue (Nybo & Nielsen 2001), alongside metabolic alterations that include increased blood lactate production, carbohydrate oxidation and glycogen depletion (Hargreaves 2008). Collectively, these alterations contribute towards the widely reduced exercise duration and intensity during exercise under heat stress (Nybo et al. 2014), whilst an increase in glycogenolysis may have implications for prolonged exercise or regular training in the heat (Hargreaves 2008). Mechanisms underpinning the shift towards carbohydrate metabolism may relate to compromised blood flow due to enhanced skin blood flow for thermoregulation, a Q10 effect upon glycolysis enzymes and/or elevated plasma adrenaline levels (Febbraio 2001). Alongside these changes, the lactate turnpoint (Lorenzo et al. 2011), the exercise intensity that elicits an exponential increase in blood lactate concentration (Jones 2006), and $\dot{V}O_{2max}$ (Nybo et al. 2014) are impaired in the heat. These markers help determine endurance performance, when combined with running economy (Bassett & Howley 2000). These changes are significant given that the lactate turnpoint (LTP) remains a strong predictor of time trial performance in hot environments (Lorenzo et al. 2011) and appears sensitive to the effects of IP (Bailey, Jones, et al. 2012), whilst $\dot{V}O_{2max}$ is considered the best individual predictor of performance, explaining 90.2% of the total variance in a 16-km run (McLaughlin et al. 2010). Furthermore, evidence exists that occlusion may modify thermoregulatory responses, through the muscle metaboreflex, independently of body temperature (Kondo et al. 1999). Thus, through effecting a change in the peripheral vasculature, muscle metaboreflex and/or influence mitochondrial function, IP may ameliorate some of the metabolic changes and thermoregulatory responses under heat stress, in turn influencing markers such as LTP and $\dot{V}O_{2max}$, thereby reducing the fractional utilisation of $\dot{V}O_{2max}$. Theoretically therefore, IP may be a beneficial acute intervention for athletes to consider, in addition to traditional techniques such as

precooling (James et al. 2015; Randall et al. 2015) and heat acclimation (Gibson et al. 2015; Mee et al. 2015; Willmott et al. 2016), that seek to alleviate the significant impairment to endurance performance afforded by heat stress (Guy et al. 2015).

This study investigated the effect of IP across a range of submaximal and maximal physiological markers during incremental exercise in the heat. It was hypothesised that IP would ameliorate the decline in the lactate turnpoint and $\dot{V}O_{2max}$ during endurance running in the heat, providing potential as a simple, acute ergogenic technique for use prior to competition.

1.1 Methods

1.1.1 Participants

Eleven male, recreational club runners volunteered as participants (mean [±SD]): age 37 (12) years, stature 178.3 (5.9) cm, mass 74.4 (6.1) kg, sum of four skinfolds 25.1 (3.3) mm, $\dot{V}O_{2max}$ 57.6 (3.2) mL.kg^{-1}.min^{-1}. All participants trained at least three times per week and had run a sub 22 min 5 km in the previous month (mean [±SD] 20:06 [1.0] min). Each participant provided written informed consent and institutional ethical approval was issued in accordance with the Declaration of Helsinki 1964, as revised in 2013. Participants arrived hydrated, having refrained from intense exercise for 48 hours preceding trials, as well as avoiding alcohol, caffeine and replicating their diet for 24 hours prior to each test, as is common in similar trials (James et al. 2014; Hayes et al. 2014). Finally, participants were asked to prepare for each trial as they would a competition.

1.1.2 Experimental Design

A repeated measures, crossover design was used to investigate the effect of a brief period of IP on the determinants of endurance performance in the heat, with two conditions; IP and control (CON). All participants undertook a prior, cooler familiarisation trial (24°C, 50% relative humidity [RH]), containing no treatment, before two experimental trials in the heat (32°C, 60% RH). Testing occurred during the UK spring (mean ambient temperature of 12°C) therefore, participants had been absent from repeated external heat exposure across during previous months. All trials were separated by 7-10 days to prevent an acclimation effect from repeated heat exposures (Barnett & Maughan 1993), and occurred at the same time of the day (±2 hours) to minimise fluctuation in thermoregulatory responses as a result of circadian variation (Reilly & Waterhouse 2009).

1.1.3 Ischaemic Preconditioning

In both IP and CON trials, whilst in the supine position, participants were fitted with automated tourniquet thigh cuffs (10 x 30 cm), positioned around the upper thigh of both legs. Each

leg was exposed to 5 min of occlusion with the cuff inflated to 220 mmHg (IP) or 50 mmHg (CON), followed by 5 min of reperfusion and rest for 4 consecutive cycles, as has previously been shown to be effective (Jean-St-Michel et al. 2011; Bailey, Jones, et al. 2012). An overview of the preconditioning protocol and subsequent graded exercise test (GXT) is shown in Figure 1. The occlusion pressure of 220 mmHg was chosen following pilot testing to identify the limb occlusion pressure when blood flow traces within the popliteal artery ceased, utilising Doppler ultrasound (Shenzhen Delicate Electronics Co. Ltd., Shenzhen, China). Occlusion pressure for the group was between 160-170 mmHg, therefore 220 mmHg ensured the pressure was at least 50 mmHg above systolic blood pressure. This pressure is supported by similar IP literature (Bailey, Jones, et al. 2012; de Groot et al. 2010; Sharma et al. 2015), however recent near infrared spectroscopy data indicates arterial pulses may be observed up to 300 mmHg (Kido et al. 2015). Pilot data, collected using Doppler ultrasound, as well as published literature (Gibson et al. 2013), identified 50 mmHg as an appropriate control pressure, as it provides a sensation of pressure, without impairing arterial flow. As IP cannot be truly blinded, participants were informed both conditions had the potential to be equally effective. Participants completed an extended rest period during the familiarisation trial, prior to exercise. As shown in Figure 1, participants began a low intensity five min warm-up ten min after IP. Following the warm-up there was a 5 min break before the exercise test began.
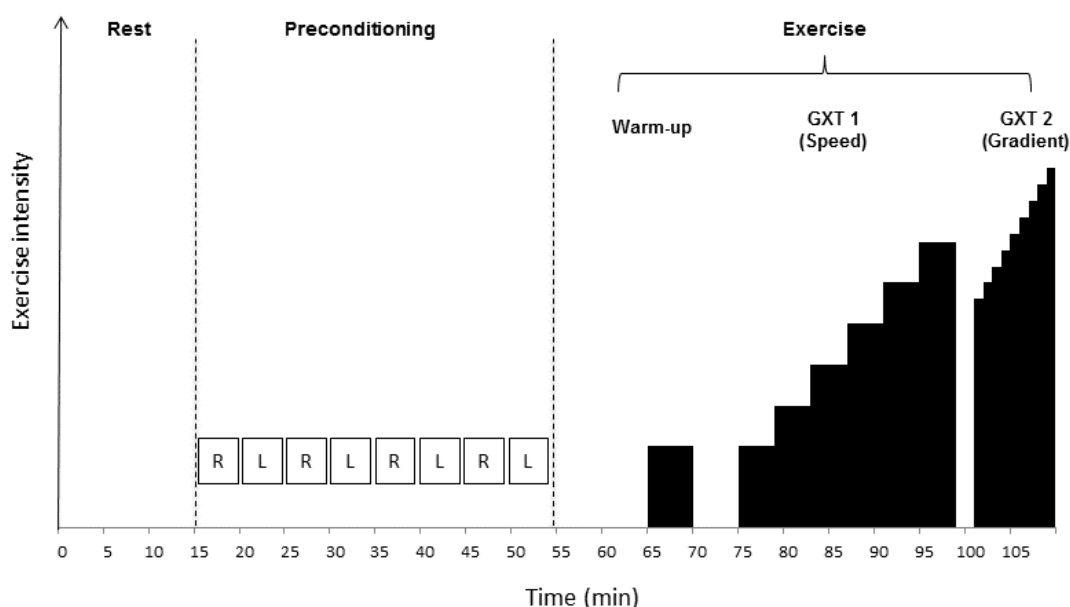

Figure 1: Protocol overview. The entire protocol was completed in a hot environment. 'R' and 'L' represent occlusion of right and left legs, respectively. 'GXT 1' denotes 3 min exercise stages with increments of 1km.h^{-1}. 'GXT 2' denotes gradient based test to exhaustion, incorporating 1 min stages with increments of 1%.

1.1.4 Graded exercise tests

A graded exercise test, split into two parts; GXT 1 and GXT 2, was adopted as displayed in Figure 1. Participants entered the thermostatically controlled environmental chamber (WatFlow control system TISS, Hampshire, UK) within which conditions were continuously monitored throughout the trial using a heat stress meter (HT30, Extech Instruments, USA). GXT 1 was similar to that adopted by James et al. (2015), initially a submaximal incremental speed protocol followed by GXT 2, an incremental gradient protocol to volitional exhaustion. Starting speeds were between 8–11 $km.h^{-1}$ (1% gradient, Jones & Doust [1996]) depending upon 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 Glucose and Lactate analyser (YSI, Hampshire, UK. 2% typical error at 2 $mMol.L^{-1}$, 1.4% typical error at 4 $mMol.L^{-1}$). Exercise continued until blood lactate concentration exceeded 4 $mMol.L^{-1}$. The same number of exercise stages and running speeds completed during the familiarisation trial were replicated during all subsequent trials. Following a 10 min rest, GXT 2 began at a speed 2 $km.h^{-1}$ below the previous GXT 1 final speed, with gradient increasing by 1% each min and continuing until volitional exhaustion. Participants were not permitted to drink and were blinded to all forms of feedback throughout the duration of the trial.

1.1.5 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 provided upon arrival for assessment of hydration status. Euhydration was achieved when urine osmolality and urine specific gravity were below 700 $mOsmol.kg^{-1}$ H_2O 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 core temperature (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) as per James et al. (2014). Heart rate was monitored continuously using a Polar 810i heart rate monitor (Kempele, Finland). Pre and post the IP protocol, muscle temperature was measured through a needle thermistor probe (Type MLA connected to DM 852 Medical Precision Thermometer, Ellab A/S,

Hilleroad, Denmark) inserted to a depth of two centimetres into the right thigh at the mid-point between the greater trochanter and tibial tuberosity, in the vastus lateralis.

Heart rate (HR), T_{CORE}, T_{SKIN}, rating of perceived exertion (RPE, Borg, 1998) and thermal sensation (TS, 0=unbearably cold to 8=unbearably hot, Gagge et al. 1969) were noted every 5 min during rest and the IP protocol, and at the end of each stage during exercise. The following physiological responses were calculated; lactate thresholds, running economy (RE), $\dot{V}O_{2max}$ and velocity at $\dot{V}O_{2max}$ ($v\dot{V}O_{2max}$). Fixed blood lactate concentrations of 2 and 4 $mMol.L^{-1}$ were used to denote the lactate threshold and lactate turn-point respectively by solving the polynomial regression equation for blood lactate concentration versus speed at 2 and 4 $mMol.L^{-1}$ as per Saunders & Green (2013) and previously adopted (James et al. 2015). This approach accounted for differences in the number of stages completed, removed subjectivity of experimenter identification and provided precision to less than 1 $km.h^{-1}$. Ventilatory gases were measured using 30 s averaging from a Metalyzer 3B 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 five exercise stages is presented, although the data from each individual stage was used for analysis. During the $\dot{V}O_{2max}$ test, the highest 30 s moving average represented $\dot{V}O_{2max}$. A $\dot{V}O_{2max}$, not $\dot{V}O_{2peak}$, was accepted when a $\dot{V}O_2$ plateau (<2 $mL.kg^{-1}.min^{-1}$ across two successive 30 s fixed-time averages) was observed. Whilst a subsequent verification phase is recommended for the robust assessment of $\dot{V}O_{2max}$ (Midgley & Carroll 2009), the precise consequences of heat strain cannot not be accurately replicated, which would be necessary given the strong relationship between heat strain and $\dot{V}O_{2max}$ decrement (Nybo et al. 2014). Therefore, in the absence of a plateau, a test was deemed maximal if three out of the following four criteria were met; blood lactate concentration >8 $mMol.L^{-1}$, HR within 10 beats of age predicted maximum, respiratory exchange ratio >1.1, and RPE at or above 19 (Edvardsen et al. 2014). 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 dividing by the mean running economy ($mL\ O_2.kg^{-1}.km^{-1}$) determined during the first five 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.

1.1.6   Statistical Analyses and Derivative Calculations

Mean skin temperature (T_{SKIN}) (Ramanathan 1964):

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

Physiological Strain Index (PSI) (Moran et al. 1998):

$$PSI = (5 * (T_{CORE}^1 - T_{CORE}^0)) / ((39.5 - T_{CORE}^0) + (5 * (HR^1 - HR^o) * (180 - HR^0)).$$

T_{CORE}^{0} and HR^{0} denote baseline and T_{CORE}^{1} and HR^{1} denotes measurement taken at the respective time.

All outcome variables were assessed for normality and sphericity prior to further analysis. Where assumptions of normality were not met, data was log transformed as appropriate. Two way, repeated measures ANOVA (treatment*time) were used to test for differences in blood lactate indices, expired metabolic gases, T_{CORE}, T_{SKIN}, PSI, RPE and TS. Where appropriate, Bonferroni adjusted pairwise comparisons revealed where differences occurred. Paired samples t-tests were used to detect differences between running time until $\dot{V}O_{2max}$ during GXT 2, $v\dot{V}O_{2max}$ sweat rate, environmental conditions and muscle temperature. Data were analysed using SPSS (Version 21, SPSS Inc, Illinois, USA) with statistical significance set at $p<0.05$ and data presented as means and standard deviation (SD). Effect sizes for main effects and interaction effects are presented as partial eta squared ($\eta2$), whilst differences between two related samples were evaluated through Cohen's d_{av} (d) in accordance with Lakens (2013).

1.2 Results

Environmental conditions did not differ between conditions; CON 32.6 (0.3)°C, 61.8 (3.1)% RH, IP 32.4 (0.3)°C, 60.8 (3.6)% RH (Wet bulb globe temperature CON 28.2 [0.5]°C, IP 27.8 [0.6]°C, t=1.531, p=0.157). Similarly, comparisons of physiological variables between CON and IP, at rest prior to cuff inflation, did not reveal differences between conditions (Table 1).

Table 1: Comparisons of physiological variables at rest during CON and IP, prior to cuff inflation (mean ±SD).

	CON	IP	t	p	d
Hydration (mOsmol.kg^{-1} H$_2$O)	247 (191)	322 (208)	0.842	0.419	0.37
Blood lactate (mMol.L^{-1})	0.85 (0.38)	0.80 (0.29)	0.533	0.605	0.11
Blood glucose (mMol.L^{-1})	5.17 (0.94)	5.08 (1.26)	0.432	0.675	0.11
Heart rate (b.min^{-1})	55 (6)	52 (8)	2.138	0.058	0.52
T_{CORE}(°C)	37.00 (0.28)	37.04 (0.36)	0.463	0.654	0.11
T_{SKIN} (°C)	34.15 (0.74)	34.13 (0.36)	0.107	0.918	0.03
T_{THIGH} (°C)	33.90 (0.69)	33.84 (0.57)	0.274	0.792	0.10
T_{CALF} (°C)	33.69 (0.74)	33.54 (0.58)	0.605	0.564	0.23
T_{MUSC} (°C)	34.82 (0.69)	34.50 (0.96)	0.732	0.483	0.39

T_{CORE} = core temperature, T_{SKIN} = skin temperature, T_{THIGH} = thigh temperature, T_{CALF} = calf temperature, T_{MUSC} = muscle temperature, t = t-test value, p = significance value, d = Cohen's d effect size.

Table 1

During GXT 1, IP did not improve running speeds at fixed blood lactate concentrations of 2 and 4 $mMol.L^{-1}$ as shown in Figure 2 and Figure 3. At 2 $mMol.L^{-1}$, the mean running speed in CON was 11.6 (1.2) $km.h^{-1}$ and 11.7 (1.4) $km.h^{-1}$ in IP. At 4 $mMol.L^{-1}$ running speed in CON was 13.6 (1.0) $km.h^{-1}$ and 13.5 (1.2) $km.h^{-1}$ in IP. There were no main effects between conditions ($F=0.050$, $p=0.828$, partial $\eta2= 0.005$), or interaction effects across the levels of blood lactate concentration ($F=1.319$, $p=0.277$, partial $\eta2= 0.117$). Similarly, during exercise, no main effect of treatment on blood glucose concentration was observed (CON − 5.03 [0.94] $mMol.L^{-1}$, IP − 5.47 [1.38] $mMol.L^{-1}$, $F=1.423$, $p=0.260$, partial $\eta2= 0.125$), nor an interaction effect ($F=2.056$, $p=0.087$, partial $\eta2= 0.171$).
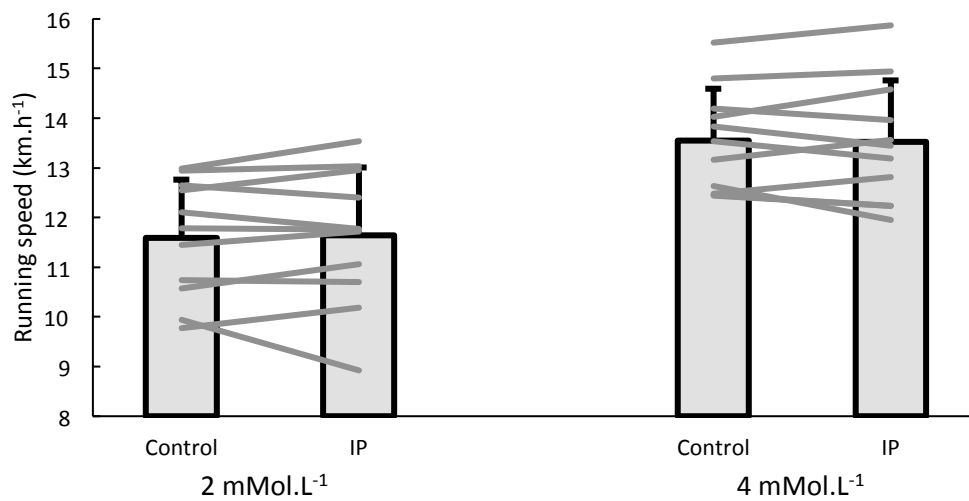

Figure 2: Running speed at 2 & 4 $mMol.L^{-1}$ respectively. Grey columns represent group means (±SD), with individual data shown by grey lines.
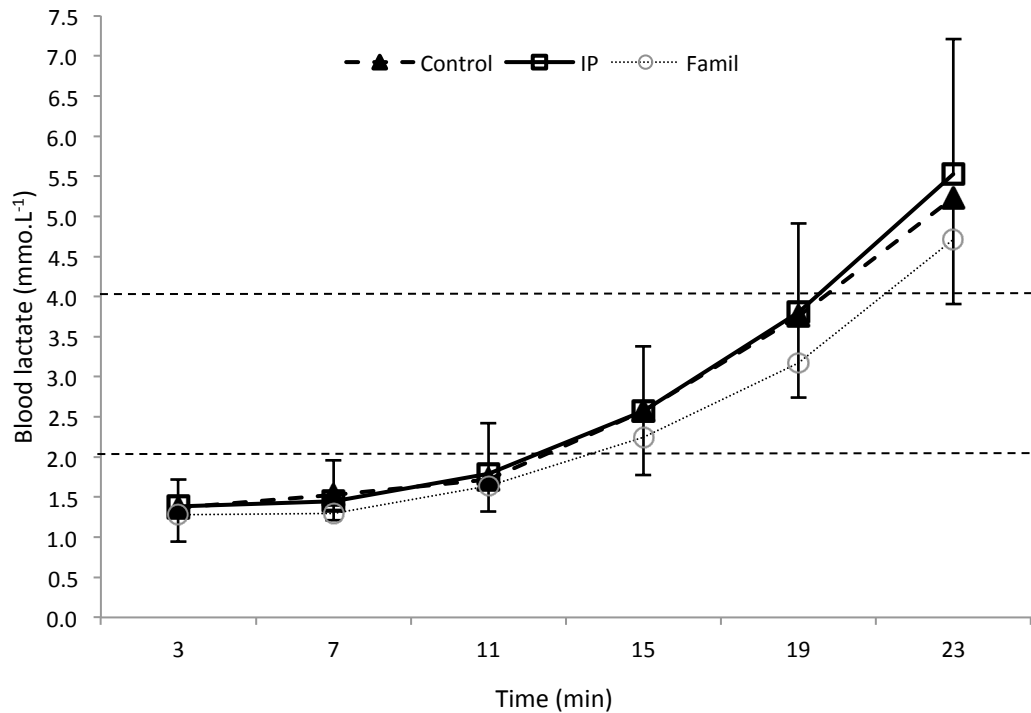
Figure 3: Mean (±SD) blood lactate concentration during GXT 1 for IP and CON. 'Famil' represents cooler familiarisation, and is presented in grey, without error bars for clarity.

There was no main effect for treatment in RE (CON – 222.3 [18.0] mL.kg^{-1}.km^{-1}, IP –218.9 [16.5] mL.kg^{-1}.km^{-1}, F=2.806, p=0.125, partial η2= 0.219), nor an interaction effect (F=0.446, p=0.774, partial η2= 0.043). No main effect was observed on V_E for treatment (F=0.013, p=0.910, partial η2= 0.001), nor an interaction effect (F=0.843, p=0.477, partial η2= 0.078). Similarly, there was no main effect for RER (F=1.593, p=0.236, partial η2= 0.137), or an interaction effect (F=1.318, p=0.272, partial η2= 0.116).

$\dot{V}O_{2max}$, as defined by specified criteria, was achieved in 16 trials, with the remaining 6 trials demonstrating $\dot{V}O_{2peak}$. There were 7 $\dot{V}O_{2max}$ and 4 $\dot{V}O_{2peak}$ in CON, and 9 $\dot{V}O_{2max}$ and 2 $\dot{V}O_{2peak}$ following IP. There was no difference in $\dot{V}O_{2max}$ (CON - 55.5 (3.7) mL.kg^{-1}.min^{-1}, IP 56.0 (2.6) mL.kg^{-1}.min^{-1}, t=-0.812, p=0.436, d=0.17), as shown in Figure 4, nor in total running time during GXT 2 (CON – 347 [42] s, IP – 379 [68] s, t=1.496, p=0.166, d=0.58) (Figure 5). However, differences in v$\dot{V}O_{2max}$ were observed (Figure 6), with IP demonstrating a greater predicted running speed (CON 15.0 [1.2] km.h^{-1}, 15.4 [1.2] km.h^{-1}, t=2.727, p=0.021, d=0.33).

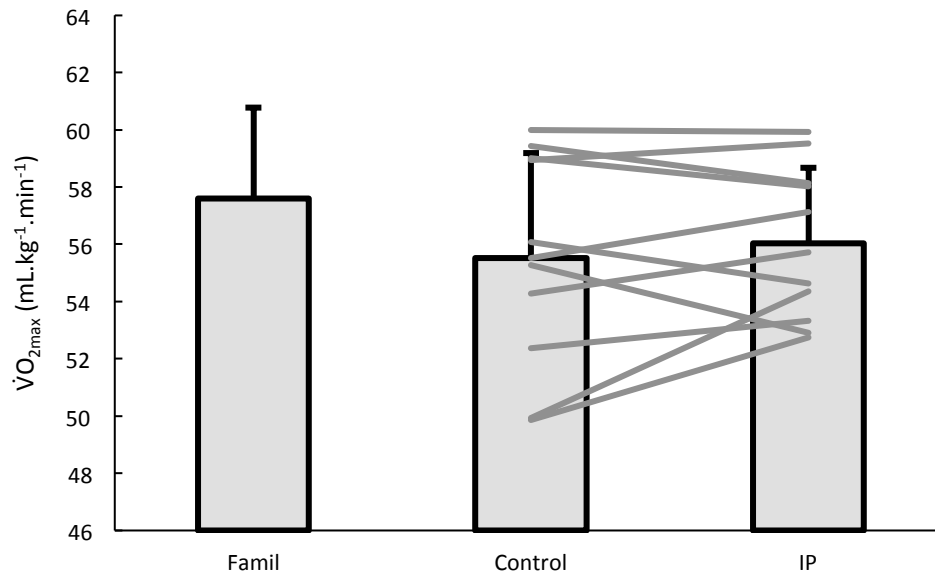Figure 4: V̇O$_{2max}$ during familiarisation, Control and IP. Grey columns represent group means (±SD), with individual data shown by grey lines. 'Famil' represents normothermic familiarisation.
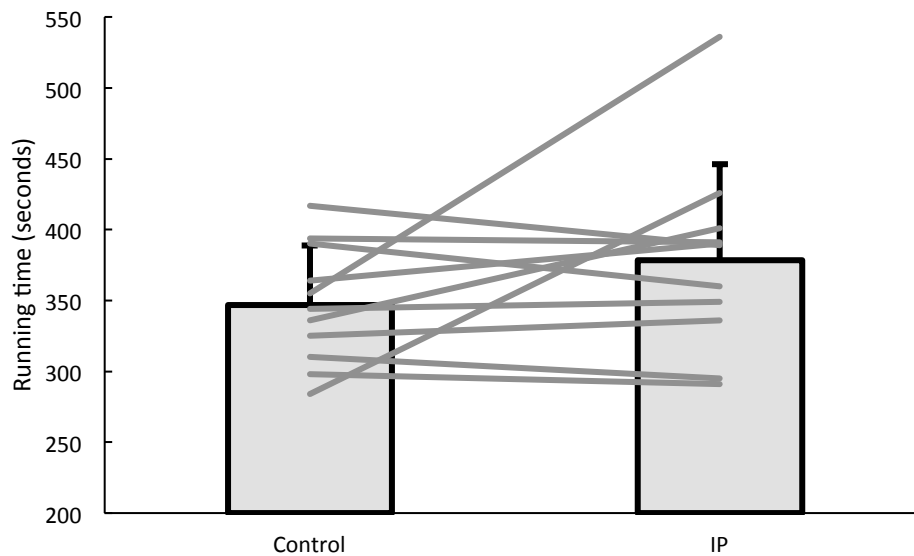

Figure 5: Total running time during GXT 2. Grey columns represent group means (±SD), with individual data shown by grey lines.
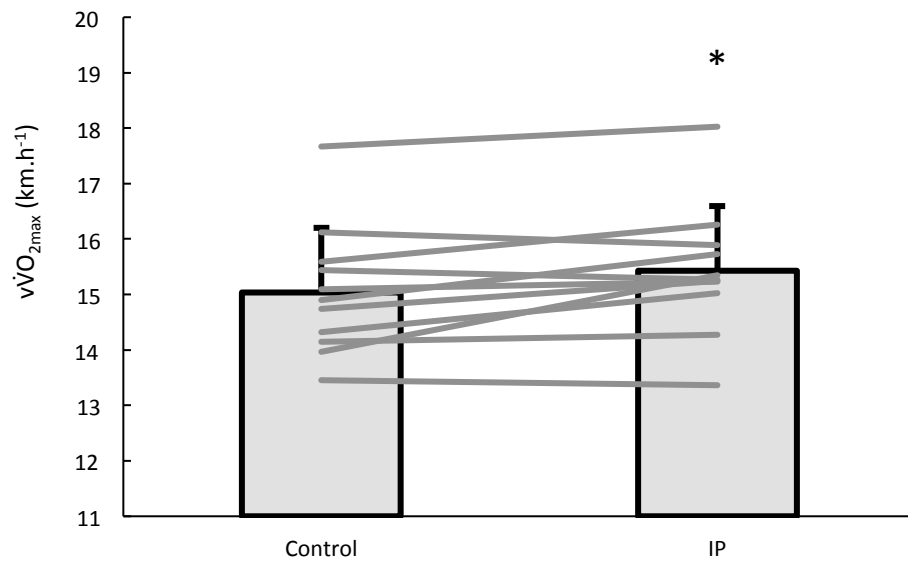
Figure 6: vV̇O$_{2max}$ during GXT 2 between conditions. Grey columns represent group means (±SD), with individual data shown by grey lines. * represents a statistical difference ($p<0.05$).
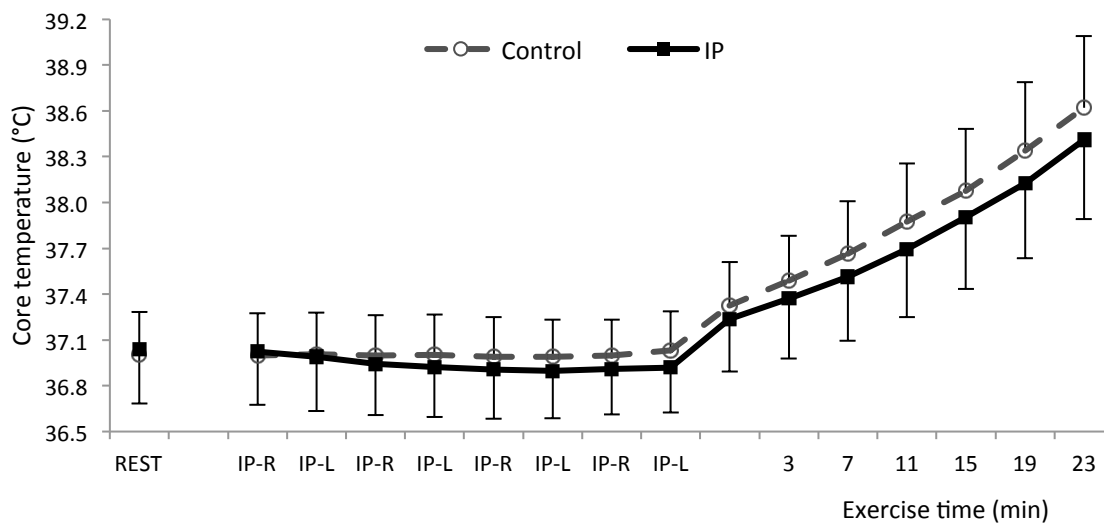

Figure 7: Mean (±SD) Core temperature throughout the protcocol. Exercise data is taken from GXT 1 only. Error bars represent one standard deviation. IP-R represents ichaemic preconditioning of the right leg, IP-L the left leg.

Resting HR was not different between groups (Table 1), however HR, as noted at the end of each 5 min occlusion period, was consistently lower whilst undergoing IP, compared with CON (CON 60 [10] b.min^{-1}, IP - 56 [10] b.min^{-1}, $F=9.977$, $p=0.010$, partial $\eta2= 0.499$). The lower pre-exercise heart rate did not continue during exercise, with HR similar throughout GXT 1 ($F=0.132$, $p=0.724$ partial $\eta2= 0.13$), and at maximum during GXT 2 (CON 186 [10] b.min^{-1}, 186 [11] b.min^{-1}, $t=0.341$, $p=0.740$, $d=0.03$). A main effect for PSI was observed, with PSI lower following IP ($F=5.548$, $p=0.043$, partial $\eta2= 0.381$), with no interaction effect ($F=1.380$, $p=0.250$, partial $\eta2= 0.133$). No differences were observed between IP and CON for RPE ($F=0.028$, $p=0.870$, partial $\eta2= 0.003$), or thermal sensation ($F=0.054$, $p=0.821$, partial $\eta2= 0.005$).

There were no differences in thermoregulatory variables at rest, as shown in Table 1. There was also no main effect for treatment on T_{CORE} during the preconditioning phase ($F=0.666$, $p=0.433$, partial $\eta2= 0.062$). However, there was a progressive decrease in T_{CORE} during this phase, which was not matched in CON, providing an interaction effect ($F=6.098$, $p=0.009$, partial $\eta2= 0.379$) as shown in Figure 7. However, Bonferroni post hoc did not detect differences at any time points. The IP phase did not elicit a change in muscle temperature (CON Δ = 0.55 [0.57]°C, IP Δ= 0.78 [0.85]°C, $t=-0.593$, $p=0.568$, $d=0.33$). There was also no main effect for treatment on mean T_{SKIN} throughout the IP phase ($F=2.177$, $p=0.184$, partial $\eta2= 0.237$) and no differences in localised skin temperatures of the legs at the calf ($F= 0.001$, $P= 0.971$, partial $\eta2= 0.000$) or thigh ($F=4.522$, $p=0.071$, partial $\eta2= 0.392$).

The reduced T_{CORE} following IP continued during GXT 1, with IP lower throughout ($F=27.886$, $p=0.001$, partial $\eta2= 0.756$), however the rate of increase was similar between the groups, so no interaction effect was seen ($F=3.308$, $p=0.05$, partial $\eta2= 0.269$). No difference in mean T_{SKIN} during exercise was observed between trials ($F=0.001$, $p=0.976$, partial $\eta2= 0.000$). Similarly, calf ($F=0.804$, $p=0.400$, partial $\eta2= 0.103$) and thigh ($F=0.475$, $p=0.513$, partial $\eta2= 0.064$) skin temperatures did not differ between conditions during exercise. Finally, sweat loss did not differ between conditions (CON – 2.76 [0.54], IP 2.87 [0.83] L.hr^{-1}, $t=-0.471$, $p=0.648$, $d=0.15$).

1.3 Discussion

This study investigated the effect of IP on the primary physiological determinants of endurance running, notably the lactate turn-point, $\dot{V}O_{2max}$ and running economy, under heat stress. Whilst an enhanced cardiovascular and metabolic strain are not limiting factors leading to the cessation of exercise during sub-maximal exercise in the heat (González-Alonso et al. 2008), the competition for blood flow between the skin and the muscles during exercise leads to impaired

performance in competition or training, relative to cooler conditions. It was hypothesised that the novel application of IP in the heat would alleviate some of the deleterious physiological consequences of heat strain, possibly by enhancing skeletal muscle vasodilation and/or influencing muscle metabolism, leading to a mediated metabolic strain, as has been demonstrated under normothermic conditions. Moreover, occlusion has previously been shown to initiate thermoregulatory responses independently of changes in body temperature (Kondo et al. 1999). However, these data indicate IP does not provide any benefit to the determinants of endurance performance in the heat.

The lack of difference in blood lactate accumulation during GXT 1 is distinctly different from the results of Bailey, Jones, et al. (2012). They reported a reduced blood lactate concentration throughout an incremental, submaximal run and attributed subsequent improved 5 km performance to improvements in vascular function, facilitating enhanced removal and transport of lactate for uptake and use. Bailey, Jones, et al. (2012) speculated that such changes may increase lactate oxidation in the mitochondria of working skeletal muscles, and/or reflect energy sparing, through augmented mitochondrial flux or increased excitation-contraction coupling efficiency, as has previously been observed in preconditioned pig muscle (Pang et al. 1995). Recently, observed improvements in endurance exercise of the forearm suggests such alterations in muscle metabolism may occur without concomitant haemodynamic changes (Barbosa et al. 2015). Previously, the improvements to vascular function following IP have been attributed to biochemical changes that follow ATP degradation during periods of occlusion. For example, the accumulation of intracellular adenosine interacts with adenosine receptors A_1 and A_2 (Marongiu & Crisafulli 2014), and ultimately mediates vasodilation (Riksen et al. 2008). Adenosine accumulation has also been suggested to maintain endothelium function, when it is normally impaired following strenuous exercise (Bailey, Birk, et al. 2012). Alongside adenosine, ischaemia stimulates increased endothelial nitric oxide (NO) release that attenuates sympathetic vasoconstriction, inducing smooth muscle relaxation as part of the secondary plateau phase of the bimodal cutaneous response to local hyperthermia (Kellogg et al. 1998). It is possible that under heat stress, any reduction in skeletal muscle energy consumption were too small for these measures to detect or, from a haemodynamic perspective, skeletal muscle may not be sensitive to further increases in NO following IP, due to the apparently greater role of prostaglandins, relative to NO for inducing vasodilation (Kellogg et al. 2005). Moreover, it is possible that blood vessels are already maximally dilated from heat stress (Lorenzo & Minson 2010).

Muscle temperature was measured pre and post IP to quantify the thermal effects of such large, cyclical alterations in major limb blood flow. This measurement would appear to be novel

within IP studies and the lack of change in muscle temperature suggests passive heating or rewarming of muscles to be unnecessary when IP is adopted, in order that muscle temperature is maintained for the start of an anaerobic exercise bout. While muscle temperature did not change following IP, interestingly IP appeared to elicit a small change in resting T_{CORE} (~0.1°C). This may reflect a redistribution of blood away from the core to the periphery as part of recovery for the previously occluded limb. Alternatively, this could be explained through the central integration and command of peripheral inputs, with changes in muscle metaboreflex having previously initiated thermoregulatory responses in the absence of changes in body temperature (Kondo et al. 1999). During exercise this difference was maintained, presenting a small, but consistent change in T_{CORE}, which in turn influenced PSI. However, the small magnitude of difference in T_{CORE} between conditions (~0.1°C) explains the lack of an independent effect of body temperature on performance measures such as $\dot{V}O_{2max}$ and blood lactate indices. Furthermore, the performance implications of this reduced T_{CORE} are likely negligible, given the subsidiary role of T_{CORE}, relative to T_{SKIN}, for influencing perceived thermal strain (Flouris & Schlader 2015), which was unchanged following IP. Alongside perceived thermal strain, cardiovascular strain may be considered a primary determinant of endurance performance in the heat (Schlader et al. 2011; Périard & Racinais 2015), however the small reduction in T_{CORE} did not elicit a change in HR. However, it should be acknowledged that modest changes in T_{CORE} have the potential to modify effector thresholds such as vasomotor and sudomotor sensitivity or sweat rate (Kondo et al. 2009), however this effect may necessitate sweating to have commenced, which is unlikely during IP at rest, and may explain why neither T_{SKIN} nor sweat rate subsequently differed. Whilst the observed reduction in T_{CORE} may be considered small in comparison to that following precooling techniques (Bongers et al. 2015; Tyler et al. 2015), future research may also consider how this could complement a cooling strategy prior to competing in the heat. Moreover, future research should investigate changes in whole-body blood flow in order to understand the observed change in T_{CORE}, that appears to be independent of changes in both muscle and skin temperatures.

Among other studies to have found no significant effect from IP during submaximal exercise, Clevidence et al (2012) suggested their incremental cycling exercise protocol may not have provided sufficient ischaemic challenge to realise the benefits of IP, although the authors also acknowledge they may not have achieved full occlusion during the IP procedure. Notwithstanding, the elevated physiological and metabolic strain observed during submaximal running in the heat, compared with familiarisation data collected under cooler conditions (Figure 3), is supportive of an ischaemic challenge, especially given the distinct improvements following IP observed by Bailey, Jones, et al. (2012) during a similar normothermic incremental running protocol. However, Febbraio (2001) has

highlighted that oxygen availability is not the main factor mediating the augmented glycogenolysis and elevated blood lactate concentration during moderate exercise under heat stress, although they did not rule out a role for decreased muscular blood flow influencing metabolic processes in an oxygen independent manner. Indeed, Febbraio et al. (1994) have previously highlighted a strong relationship between reduced body temperature and reduced plasma adrenaline levels in explaining metabolic changes following heat acclimation, notably a reduced RER, indicating greater fatty acid or ketone oxidation and less glycogenolysis. These findings were in the apparent absence of changes in fibre type recruitment and with changes far exceeding that expected from a Q10 effect on glycogenolytic and glycolytic processes. Therefore, despite potentially greater blood flow to the muscle following IP, metabolic alterations and metabolite production by the muscle were maintained. This is in keeping with elevated muscle temperature and adrenaline being the prominent drivers of increased intramuscular carbohydrate utilisation in the heat, rather than localised ischemia (Febbraio 2001), although it is acknowledged these were not directly measured in the current study.

$\dot{V}O_{2max}$ is attenuated in the heat (Nybo et al. 2014), as a consequence of cardiovascular strain and insufficient muscular blood flow at maximal intensities (Gonzalez-Alonso et al. 2008). Given that mean finishing T_{CORE} in both conditions was elevated (39.0°C CON, 38.9°C IP), and $\dot{V}O_{2max}$ was lower than the cooler familiarisation ($\sim$-2 mL.kg^{-1}min^{-1}, Figure 4), it is probable $\dot{V}O_{2max}$ was indeed impaired in this cohort. Notwithstanding that IP was applied under different environmental conditions, effects of IP on $\dot{V}O_{2max}$ appear equivocal (Marocolo et al. 2015). A potential explanation for such variability in $\dot{V}O_{2max}$ following IP are methodological differences between exercise protocols, time of exercise following IP, training status of participants, as well as data analysis techniques, such as $\dot{V}O_{2}$ averaging time. Furthermore, it is unclear if other studies adopted $\dot{V}O_{2max}$ criteria as in the present study, or may in fact be reporting $\dot{V}O_{2peak}$. Although the adoption of $\dot{V}O_{2max}$ criteria is not without criticism (Poole et al. 2008), verification phases are supported to reaffirm the attainment of $\dot{V}O_{2max}$ (Midgley & Carroll 2009), however this is not reported across the IP literature. In the current study, a verification phase would be confounded by the environmental conditions, given $\dot{V}O_{2max}$ impairments are determined by the progressive influence of heat strain (Gonzalez-Alonso et al. 2008). Interestingly, there was a statistical difference in $v\dot{V}O_{2max}$ (+0.4 km.h^{-1}), a composite measure of running economy and $\dot{V}O_{2max}$, which could indicate small improvements in both of these variables, which were not substantial enough to be seen individually, but is apparent from the combined calculation. However, the mean difference of 0.4 km.h^{-1}, does not exceed our calculated typical error of 0.5 km.h^{-1} established during pilot across a series of similar studies under the same conditions, indicating that changes may not be meaningful.

Crisafulli et al. (2011) has highlighted a role for altered fatigue perception to explain enhanced performance following IP, after observing no differences in physiological parameters including $\dot{V}O_{2max}$, maximal stroke volume and maximal cardiac output. These data cannot support, or refute this, as testing occurred under different environmental conditions. However, no differences were found in RPE and whilst a mean improvement in total running time of 32 s during GXT 2 could be considered meaningful, this was not statistically significant, nor did it far exceed our own laboratory typical measurement error of 27 s derived from a larger cohort of similar standard runners under the same environmental conditions. Recently, Tocco et al. (2014) did not observe an effect from IP with highly trained athletes during free paced 5 km time trials on a track. They also suggested altered fatigue sensations could account for some of the benefits observed by IP and that training status may modify the sensitivity to this alteration. Such a suggestion would help explain the difference between effects in sub-elite (Bailey, Jones, et al. 2012) and elite runners (Tocco et al. 2014), but does not appear to be universal, with Jean-St-Michel et al. (2011) having reported improvements in elite swimmers. Furthermore, the influence of a 'placebo effect' should be considered, as IP is difficult to blind. Although we tried to maintain participant nativity, the potential for a placebo effect to influence performance remains a limitation across all IP research.

In conclusion, this study investigated the novel application of IP during exercise in the heat. These data indicate IP does not provide any benefit to the determinants of endurance performance in the heat. Furthermore, four cycles of IP did not influence deep muscle temperature, but elicited a modest influence on T_{CORE} during subsequent exercise.

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