Heat-acclimatisation and pre-cooling: a further boost for endurance performance?

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Short title: Heat-acclimatisation and Pre-cooling

Abstract

The purpose of this study was to determine if pre-cooling (PC) following a period of heat-acclimatisation (HA) can further improve self-paced endurance performance in the heat. 13 male triathletes performed two 20-km cycling time-trials (TT) at 35°C, 50% relative humidity, before and after an 8-day training camp, each time with (PC) or without (control) ice-vest PC. Pacing strategies, physiological and perceptual responses were assessed during each TT. PC and HA induced *moderate* (+10 ± 18 W; effect size [ES] 4.4 ± 4.6%) and *very large* (+28 ± 19 W; ES 11.7 ± 4.1%) increases in power output (PO), respectively. The overall PC effect became *unclear* after HA (+4 ± 14 W; ES 1.4 ± 3.0%). However, pacing analysis revealed that PC remained transiently beneficial post-HA, i.e. during the first half of the TT. Both HA and PC pre-HA were characterized by an enhanced PO without increased cardio-thermoregulatory (*unclear* changes in heart rate and core temperature) or perceptual (*unclear* changes in rating of perceived exertion) disturbances, while post-HA PC only improved thermal comfort. PC improved 20-km TT performance in unacclimatised athletes, but an 8-day HA period attenuated the magnitude of this effect. The respective converging physiological responses to HA and PC may explain the blunting of PC effectiveness. However, perceptual benefits from PC can still account for the small alterations to pacing noted post-HA.

Key words: heat-dissipating strategies; tropical climate; time-trial; pacing; cycling.

INTRODUCTION

Self-paced endurance exercise is reported to be compromised in the heat, with important implications for competitive endurance events (e.g., World Triathlon Series, Athletics Championships). As evidence, the -0.3% to -0.9% performance decrement per 1°C increase in ambient temperature above 10°C is well noted (Racinais et al., 2015) and these negative effects on performance are increased with greater exercise duration (~2% for ~6.5min, Altareki et al., 2009; ~7% for 30min, Tatterson et al., 2000; ~16% for ~70min, Racinais et al., 2015). To improve both performance and athlete health, various strategies have been developed to cope with exercise in the heat (Coris et al., 2004). In particular, heat-acclimatisation (HA) and pre-cooling (PC) procedures have both been shown to increase work capacity in the heat (e.g., Garrett et al., 2011; Ross et al., 2013). However, it is currently unknown if when combined, these strategies provide additional physiological benefits or ergogenic effects on endurance performance.

Medium-term heat-acclimation, i.e. 7-14 training days in the heat, improves endurance performance at high ambient temperatures for both fixed- (e.g., Nielsen et al., 1993) and self-paced (e.g., Lorenzo et al., 2010; Racinais et al., 2015) cycling. Systemic protective adaptations against heat stress have been suggested to underpin these benefits. For example, decreases in sweat electrolyte (e.g., sodium), and increases in plasma volume (PV) and sweat rate have been reported to provide cardiovascular and thermoregulatory benefits (Nielsen et al., 1993). In addition to these physiological adaptations, lower perception of effort and reduced feelings of heat stress have also been reported (Daanen et al., 2011), although these have received less attention as to their ergogenic benefits. Given the role of passive exposure (i.e., free living) in addition to training in the heat during HA to maximise these adaptations (Shido et al., 1999), there has been increased interest in the efficacy of using heat training camps to prepare athlete for competition (e.g., Racinais et al., 2013; 2015).

Cooling the body prior to exercise in the heat has been shown to protect athletes from the negative effects of the heat through delaying the rise in endogenous temperature and limiting the decrement in endurance performance (Bongers et al., 2015). Reduced skin temperature (Tskin) following PC has been proposed an important factor determining aerobic performance during submaximal exercise in the heat, via improved core-to-skin temperature gradients and thermal comfort (TC) (Cuddy et al., 2014). A greater core-to-skin gradient is rationalized to be ergogenic by improving heat transfer from the core to the periphery, and heart rate by reducing skin perfusion (Cuddy et al., 2014). Given their low level of invasiveness on athletes preparation or competition routines, and avoidance of cooling active musculature, cooling vests have become a popular form of PC. Worn either at rest or during warm-up, ice vests enable improved steady- and self-paced endurance cycling tests in the heat (Johnson et al., 2008; Bogerd et al., 2010). In part, this improvement may occur as a result of augmented perception of TC (Schlader et al., 2011), though high variability between athletes in perceived thermal comfort may subsequently influence the magnitude of performance changes.

It is presently unknown if there is additional benefit of combining PC following HA to performance in hot conditions for endurance athletes. Indeed, it is possible that the HA-related thermoregulatory adjustments (e.g. lower increase in core temperature [Tcore] for sweat onset) could be further improved during exercise by the addition of a favourable core-to-skin gradient achieved with PC. The improved metabolic efficiency achieved with HA (Febbraio et al., 1994) might also add to the lowered energy expenditure reported with PC. In contrast, the PV expansion following HA may not allow PC to provide the same magnitude of benefit to cardiovascular function (i.e., preserved blood volume) to exercise in the heat that has been reported in unacclimatised athletes (Quod et al., 2008), and this may reduce the expected performance effects. Similarly, it is presently unknown whether perceptual improvements from HA interact with the PC-related increased TC of unacclimatised athletes.

To date, few studies have combined these strategies. Recently, after a 10-day heat-acclimation period, Castle et al. (2011) reported no further ergogenic effect of thigh PC in moderately trained subjects during an intermittent-sprint exercise protocol in the heat. However, this type of performance may be impaired by muscle PC due to loss in metabolic and neuromuscular efficiency (e.g., Sleivert et al., 2001), and thus not adequately reflect the HA-PC interaction for endurance performance. Brade et al. (2013) reported the same absence of cumulative effect in moderately trained participants using ice-slushie and cooling jackets after a five-session heat-acclimation exposure. However, in this study, no effect of PC on performance was reported pre-acclimation, making interpretation of the PC-HA interaction difficult. Accordingly, the present study investigated the effects of PC prior to, and following a period of HA on self-paced endurance performance in the heat. At the end of the training camp, we hypothesised ice vest PC would absorb excess body heat and increase TC prior to exercise, and HA would further reduce heat storage during exercise, resulting in greater performance improvement than each method separately.

METHODS

Participants

Thirteen well-trained national-level males triathletes (age 31 ± 4 y, height 179.5 ± 4 cm, body mass 71.7 ± 5.6 kg, maximal oxygen consumption [$\dot{V}O_{2max}$] 64.9 ± 6.9 ml·kg^{-1}·min^{-1}, maximal power output [MPO] 406 ± 34W) volunteered to participate in this study. All subjects had competed at the national level for at least 3 y and trained ≥7 sessions per week. Prior to inclusion in the study, participants were examined by a cardiologist to obtain a medical clearance. The experimental design of the study was approved by the Ethics

Committee of *Hôtel Dieu*, Paris (acceptance no. 2013-A00824-41) and the protocol was performed in accordance with the Declaration of Helsinki. After comprehensive verbal and written explanations of the study, all subjects gave their written informed consent for participation.

Experimental design

An outline of the full protocol is presented in Figure 1. All participants performed repeated testing with and without PC in a randomized fashion, before and after a period of HA. Specifically, at the commencement of the study, all athletes resided in Paris (France) and had no heat exposure in the previous six months. Each participant attended five pre- and three post-HA sessions (all at the same time of day), either at INSEP (Paris, France) or at the Centre of Sports Resources, Expertise and Performance (CREPS) of Pointe-à-Pitre (Guadeloupe). Performance testing was conducted on two consecutive days before travelling (7-hours) to Pointe-à-Pitre (departure at 10:00, west direction) or to Paris (departure at 17:00, east direction). Further, on the day prior to departure for Guadeloupe and on the day of return to Paris, PV was determined and a heat tolerance test (HTT) was completed. At both testing locations, the research equipment and procedures were standardised and overseen by the same research team. To note, as pre-HA sessions were performed in a climate chamber, at least 7 days were allowed between the familiarization and the experimental sessions to avoid initiating heat adaptations prior to the training camp.

<Insert Figure 1 about here>

During the first pre-HA session, a graded exercise test was performed in normothermic conditions (21°C, 40% RH) using an electronically-braked cycle ergometer (Excalibur Sport, Lode®, Groningen, The Netherlands). Subjects wore a facemask covering their mouth and nose to collect all expired breath (Hans Rudolph, Kansas City, MO). The exercise protocol started with a 5-min warm-up at a workload of 100 W, and then increased by 20 W per minute until voluntary exhaustion to estimate $\dot{V}O_{2max}$; Quark, Cosmed®, Rome, Italy) and MPO.

To limit learning-induced changes in pacing strategy, all participants were familiarised with the exercise protocol (time-trial) in the heat during the second session. The protocol commenced with a 10 min passive seated period, followed by a 15-min warm-up involving 10 min at a workload of 100 W and 5 min at 50% of the individual's MPO in a climate chamber (Thermo Training Room, Paris, France) at 35°C, 50% relative humidity (RH). After the warm-up, a 20-km time-trial (TT) was performed. Each participant performed both the warm-up and the TT on their own bike mounted on a braked Cyclus2 ergometer (RBM GmbH, Leipzig, Germany). To control for fluid intake between sessions, the participants were instructed that they could drink *ad libitum* during the passive phase, warm-up and TT, with the volume of water ingested measured, and then replicated for the ensuing experimental sessions.

For the two experimental pre-HA sessions, participants completed the protocol in the above laboratory conditions, either with (PC) or without (NC) a pre-cooling intervention, in a randomized and counterbalanced order (7 participants completed PC session in the first session). During the NC condition, the participants performed the same protocol as the familiarisation session. During the PC session, an ice vest (CryoVest®, CryoInnov, Saint Grégoire, France) was used that included four anterior and posterior pockets equipped with sealed packs of FirstIce® (150 x 150 mm, 120 g, EzyWrap, USA; body surface cooling = 0.18 m^2; total weight with the compresses ~1.9 kg). Ten minutes before the participant entered the room, the ice packs were removed from a -18°C freezer and placed inside the pockets to allow cold transfer to the vest. The vest was worn during both the passive phase and the warm-up, though removed before the TT.

The two post-HA sessions were performed in a heat chamber at CREPS (Guadeloupe) at the end of the training camp and in the same order as the pre-HA sessions. To reproduce pre-HA conditions, participants were asked to rest in a temperate room at the same temperature as the pre-HA environment (~21°C) for 20 minutes before entering the heat chamber. Ambient conditions (temperature and RH) from the heat chambers of the two laboratories were controlled (Kestrel 4500, Nielsen-Kellerman Co, Boothwyn, USA) every 5 minutes throughout experimental sessions.

To ensure that any variations in performance during the TT's were due to experimental procedures and not to the previous training load, subjects were required to avoid heavy training or fatiguing activities during the 20 h prior to each laboratory session. During the TT, convective airflow from a fan set to a standard speed (750 mm, 1450 ± 5 rpm, ~8.5 m.s) facing the participant was used to mimic field conditions. The main measurements performed during the TT were the time required to complete the 20 km and the power output (PO) and speed (km·h^{-1}) recorded by the Cyclus2 software at a sampling rate of 2 Hz. No feedback was provided to the subjects during TT's except for the distance remaining, and they were not informed of their performance until the end of the study. To show the participant's mean pacing strategy, PO values obtained for each TT were reported per km of the TT.

Experimental measurements for the time-trial protocol

Six and a half hours (Lee et al., 2010) before arriving at the laboratory, the participants were instructed to swallow an ingestible radio telemetry capsule (VitalSense, Mini Mitter, USA) to measure Tcore via an external sensor (HQI Inc, Coretemp$^{®}$, Sarasota, FL). The participants were also instructed to consume 1L of water in the 2 hours prior to visiting the laboratory. Upon arrival at the laboratory, the subjects provided a urine sample as an indicator of hydration status based on urine specific gravity (USG) measured using a clinical refractometer (PAL-10S, Atago Co. Ltd, Tokyo, Japan). Following provision of a urine sample, participants then filled out a questionnaire assessing fatigue, motivation and delayed onset muscle soreness (DOMS; Vaile et al., 2008).

Tskin was measured in temperate conditions (~21°C) before the passive phase, immediately after the warm-up and the TT (~20 s) using a Thermo Vision SC 640 Thermal imaging camera (Flir Systems, Danderyd, Sweden) with the corresponding software (Thermacam Researcher Pro 2.10, Flir Systems, Danderyd, Sweden). Thermograms of the body (torso, abdominal, right and left forearms, arms, thighs, and legs, and respective posterior regions all towel dried) were obtained with the camera placed 4 m from the participant.

Immediately after each Tskin measurement, towel dried nude body mass (BM) was measured using a digital platform scale (ED3300; Sauter Multi-Range; Ebingen; West Germany, ± 100g) to estimate sweat loss (pre – post BM + fluid ingested).

During the TT, RPE was assessed every 4 km from the start to the end of the TT (Borg, 1998). At the same time, Tcore values were recorded, and TC assessed using a 10-point scale, with -5 as “very uncomfortable” and +5 as “very comfortable”. Heart rate (HR) was continuously sampled every 5 s (Polar, Kempele, Finland) during the TT.

Heat-acclimatisation measurements

Plasma volume

Plasma volume was determined before (on the day before departure to Guadeloupe), during (on the day before experimental sessions) and after (upon arrival in Paris) the training camp. Before and after the camp, PV was derived from the measurement of total hemoglobin mass, performed with a carbon monoxide (CO) rebreathing technique, as previously described (Robach et al., 2014). Briefly, after 20 min of rest, the subject breathed 100% O_2 for 4 min, before rebreathing chemically pure CO (CO N47; Air Liquide, Paris, France) for 10 min. After rest and immediately at the end of the rebreathing period, 1.5mL of blood was obtained for percent carboxyhemoglobin, hemoglobin concentration (hemoximeter ABL800; Radiometer, Copenhagen, Denmark) and hematocrit to derive PV (Robach et al., 2014). During the camp, hematocrit percentage (micromethod, 4 min at 13,500 rpm) and hemoglobin concentration (Dill & Costill, 1974) were analysed in quadruplicate and used to estimate percent changes in PV. All tests were performed by the same operator.

Heat Tolerance Test

The HTT was performed to examine thermoregulatory responses to given thermal and exercise stresses. Participants were instructed to standardise their hydration prior to each TT, and were not allowed to consume fluid during the HTT. The test consisted of a 10-min rest period and 30 min of cycling exercise at 50% of MPO (same ergometer and adjustments as the MPO test) in a climate chamber set at 35°C, 50% RH. Every 5 min during the cycling test, Tcore and perceived TC sensations were recorded.

Sweat concentration analysis

Before participants entered the heat chamber, dermal patches (5 x 9 cm, Tegaderm, HP, 3M®, Neuss, Germany) were applied inferiorly to the participants' *scapula* to collect sweat samples. At the end of the HTT, the absorbent tissue contained in the patch was carefully separated from the adhesive tape using sterile tweezers, before being inserted into the tube of a single-use syringe (Terumo syringe SS+10ES1 10mL, Terumo Europe, Belgium) for sweat extraction. The sweat sample obtained was then stored frozen at -18°C in aliquots (Eppendorf type, 2000 µL per sample) until analysis.

Sweat samples from the HTT's were analysed for sodium concentration using Inductively Coupled Plasma Atomic Spectrometry (ICP-AES) on a ICAP® 6300 DV simultaneous spectrometer (Thermo Scientific, Les Ulis, France). Samples were diluted 1:10 in ultrapure water (MilliQ®, Millipore, Guyancourt, France). Calibration curves were made with NaCl 0.09 % in place of sweat and spiked with 0.1 g/L multielements standard solution (CCS-4, Inorganic™ Ventures, distributed by Analab, Hœnheim, France). Final standard concentrations were: 0; 62.5; 125; 250 mg/L. Utak® urine normal and high ranges (Utak Laboratories) and Seronorm urine Level 2 (Sero) both distributed by Ingen-Biosciences (Chilli-Mazarin, France) were used as internal QC control.

Heat-acclimatisation procedure

During the 8-day training camp in Guadeloupe, from breakfast to dinner, participants were instructed to remain outdoors and asked to return to their accommodation only to shower after training sessions. Meals, recovery periods and social activities were completed outdoors. Running, cycling and swimming sessions were all performed outdoors (30 ± 5°C, 74 ± 15% RH) while strength and conditioning training was performed in a weight room (26 ± 3°C, 43 ± 16% RH). The participants reproduced their habitual weekly training program so that training distribution, activities and content were kept constant from Paris to the training camp (see next section and Table 1 for details).

Training monitoring

The participant's internal and external training loads were monitored throughout the study. Before the training camp, subjects continuously recorded their usual training program over a 3-week period. For each training session, they were equipped with a Global Positioning System (GPS) monitor (Garmin Forerunner 305 GPS®, Garmin International, Inc., Kansas, MO, USA) to measure training distance and speed. Based on these measures, a typical training week was calculated so that participants could reproduce it during the training camp (i.e. matched for weekly training distribution, activities and content). To ensure that the training completed in Guadeloupe was similar to those applied in France, the external training loads were also monitored. Additionally, all training sessions were monitored using the session-RPE method (Foster et al., 2001) using Borg's category-ratio 15 scale (1998).

Data analysis

As previously recommended by Hopkins et al. (2009) for studies in sports medicine and exercise sciences, magnitude-based inference analyses were performed on each aforementioned dependent variable. Accordingly, we calculated the between-trial standardized differences or effect sizes (ES, 90% CI) using the pooled standard deviation (Cohen, 1988). Threshold values for ES statistics were 0.2, 0.6, 1.2, 2.0 and 4.0 of the within-athlete variation, as thresholds for *small*, *moderate*, *large*, *very large* and *extremely large* differences in the changes observed between trials (Hopkins et al., 2009). The smallest worthwhile change (SWC) was defined as 1) 0.2 x 1.3 for TT's performance (Paton & Hopkins, 2006), 2) 0.2 x 1.3 x 2.5 for PO values (Bonetti & Hopkins, 2009), and 3) a small standardized effect based on Cohen's effect size principle (0.2 x between-athletes standard deviation [Hopkins et al., 2009]) for other parameters. Accordingly, the SWC was determined to be 0.3% in performance time, and 0.7% in PO. Quantitative chances of higher or lower differences were qualitatively evaluated as follows: <1%, *almost certainly not*; 1-5%, *very unlikely*; 5-25%, *unlikely*; 25-75%, *possible*; 75-95%, *likely*; 95-99%, *very likely*; >99%, *almost certain*. If the chance of higher or lower differences was >5% when considering the proportion of positive vs. negative effects of the intervention on the variable of interest (e.g. 50/25/25), the true difference was deemed *unclear*. Otherwise, we interpreted that change as the observed chance. All values are presented as means ± standard deviation (SD).

Since the effects of PC progressively dissipate as exercise in the heat continues, we investigated whether the pattern of PC-related effects on performance at Post (Guadeloupe) differed from these at Pre (Paris). To examine this, a Pearson correlation was performed between PC-induced differences in PO before and after HA on a 5 km-block basis. In addition, as individual responses may be observed relative to PC-induced TC, and that this may affect performance, associations between individual perceptual responses to PC and PO were also explored.

RESULTS

Participant, environmental and training characteristics

Characteristics of each parameter are shown in Table 1. Differences in DOMS, fatigue, motivation, USG levels and thermal environment between the four conditions, as well as differences in the weekly training characteristics (volume, distance or frequency) between Paris and Guadeloupe were *unclear*. In contrast, as compared to Paris, the training camp induced a *likely* (76/23/1, ES ± 90% CI, 0.33 ± 0.32) increase in internal training load.

<Insert Table 1 about here>

Heat-acclimatisation

During each day of the training camp the athletes spent ~14 h in outdoor natural heat exposure and 121 ± 31 min per day training in the heat. Changes in PV were *almost certain* (100/0/0) both during (from 3603 ± 508 ml to 4190 ± 602 ml, 16.6 ± 8.5%, ES ± 90% CI, 0.98 ± 0.23) and after the camp (4068 ± 492 ml, 11.8 ± 6.9%, ES ± 90% CI, 0.71 ± 0.20). From pre- to post-training camp, smaller increases in Tcore during the HTT were *almost* *certain* (-0.2 ± 0.3°C, 0/2/97, ES ± 90% CI, -0.85 ± 0.54) (Figure 2a), although changes in TC were *unclear* (0.6 ± 1.3, 35/14/51, ES ± 90% CI, -0.23 ± 2.15) (Figure 2b). Increases in sweat loss from the HTT were *almost certain* (0.26 ± 0.25 l, 99/1/0, ES ± 90% CI, 0.95 ± 0.49) (Figure 2c), whilst the decrease in sweat sodium concentration was *likely* (221 ± 200 mg·l^{-1}, 1/12/87, ES ± 90% CI, -0.50 ± 0.47) (Figure 2d).

<Insert Figure 2 about here>

Time-trial performances

Power output. The temporal changes in PO for each TT are shown in Figure 3. An overall range of individual effects of *likely* and *trivial* to *very large* beneficial effects of PC on PO was observed Pre-HA (249 ± 38 W and 259 ± 35 W for the NC and PC conditions, respectively, 94/1/5, 4.4 ± 4.6%). Specifically, during each 5-km split of the TT, PC benefits were *likely* (0-5 km: 14 ± 29 W, 93/1/6, 6.5 ± 7.4%), *very likely* (5-10 km: 13 ± 23 W, 95/1/4, 6.1 ± 6.0%; 10-15 km: 11 ± 19 W, 95/1/4, 4.7 ± 4.6%) and *unclear* (15-20 km: 2 ± 22 W, 57/6/38, 0.6 ± 4.6%).

When comparing the pre to post-HA NC trials, the training camp induced an *almost certain very large* to *extremely large* improvement in PO (28 ± 19 W, 100/0/0, 11.7 ± 4.1%).

At the end of the training camp, PC induced an overall *unclear* effect on PO (from 277 ± 37 W to 281 ± 35 W, 76/7/17, 1.4 ± 3.0%). During each 5-km split of the TT, however, PC-related benefits were first *possible* (0-5 km: 5 ± 25 W, 70/5/25, 1.7 ± 5.0%) and *likely* (5-10 km: 5 ± 19 W, 80/4/16, 2.2 ± 4.9%), before becoming *unclear* (10-15 km, 3 ± 21 W, 65/6/29, 1.1 ± 4.2%; 15-20 km: 2 ± 16 W, 64/8/27, 0.8 ± 2.9%). As compared to pre-HA, wearing the ice-vest post-HA induced a *likely* (93/3/5) and *trivial* to *very large* (8.4 ± 9.3%) beneficial effect on PO.

Finally, correlational analyses revealed that PC-induced differences in PO before and after HA were positively correlated (r = 0.43 ± 20). Furthermore, individual differences in PC-related changes in TC between Post and Pre were related to individual changes in PC effects on PO between Post and Pre. In particular, this was evident at 4km (r = 0.54 ± 0.38) and 8km (r = 0.48 ± 0.39), then reducing in association for the rest of the TT (12km, r = 0.26 ± 39; 16km, r = 0.23 ± 35; 20km, r = 0.09 ± 32).

<Insert Figure 3 about here>

TT duration. Changes in individual performance for each TT are shown in Figure 4. Before HA, PC induced a *likely* benefit on TT duration (min:s; from 32:29 ± 01:39 to 32:04 ± 01:14 for NC and PC, respectively; 4/8/87, -1.3 ± 1.6%). The training camp had an *almost certain* positive effect on TT duration (-67 ± 58 s from the NC test at Pre to the NC test at Post; 100/0/0, -3.1 ± 1.7%). At the end of the training camp, the overall PC effect on TT duration was *unclear* (-6 ± 42 s, 14/30/56, -0.4 ± 1.1%). As compared to pre-HA, wearing the ice-vest post-HA induced a *likely* (94/2/4) beneficial effect on TT duration (-1.7 ± 1.5%)

<Insert Figure 4 about here>

Physiological and perceptual measurements during the TT

TT Core and Skin temperatures. Time-trials resulted in *almost certain* increases in Tcore (from 37.6 ± 0.5°C pre-TT to 39.5 ± 0.6°C post-TT, 100/0/0, ES ± 90% CI, 4.61 ± 0.52). However, the changes in Tcore within- and between pre- and post-HA TT’s were *unclear*.

PC-induced decreases in whole body Tskin pre-TT were *almost certain* both pre- and post-HA (-1.5 ± 0.9°C, 100/0/0, ES ± 90% CI, 3.23 ± 0.56 and -1.2 ± 0.7°C, 100/0/0, ES ± 90% CI, 2.43 ± 0.55, respectively) (Figure 5). Before the training camp, during the NC condition, the increase in Tskin was *likely* during the TT (0.9 ± 1.5°C W, 90/9/1, ES ± 90% CI, 0.59 ± 0.51). In contrast, during the NC condition post-HA, changes in Tskin were *unclear* during the TT (0.2 ± 1.1°C, 41/24/35, ES ± 90% CI, 0.06 ± 1.15).

Accordingly, the increase in the gradient of temperature during the TT's post-HA was *likely* higher as compared to pre-HA (1.0 ± 0.3°C, 82/15/4, ES ± 90% CI, 0.53 ± 0.65). Moreover, regardless of the HA status, changes in the core-to-skin gradient were *almost certain* during the TT in the NC condition (1.2 ± 0.3°C, 100/0/0, ES ± 90% CI, 3.44 ± 0.47) though *unclear* using the ice vest (0.0 ± 0.3°C, 31/28/41, ES ± 90% CI, -0.07 ± 0.99).

<Insert Figure 5 about here>

TT Sweat loss and Heart rate. Sweat losses during the TT were *likely* increased due to HA (0.28 ± 0.32 l, 93/6/1, ES ± 90% CI, -0.75 ± 0.60), and *likely* reduced by PC pre-HA (-0.05 ± 0.23 l, 3/22/75, ES ± 90% CI, -0.40 ± 0.52), but not post-HA (*unclear*, -0.03 ± 0.23 l, 8/52/39, CI ± 90%, -0.14 ± 0.41). Changes in HR due to HA (-2 ± 10 bpm, 17/35/48, ES ± 90% CI, -0.18 ± 0.69), PC pre-HA (-1 ± 5 bpm, 8/83/8, ES ± 90% CI, 0.00 ± 0.24) or PC post-HA (1 ± 7 bpm, 25/64/10, ES ± 90% CI, 0.06 ± 0.36) were *unclear* (table 1).

Thermal comfort and RPE. Pre- and post-HA, TC was *almost certainly* (0.7 ± 0.8 UA, 98/2/, CI ± 90%, 0.52 ± 0.26) and *likely* (0.4 ± 0.8 UA, 77/22/0, CI ± 90%, 0.32 ± 0.28) improved due to PC, respectively. HA-related changes in TC were *unclear* (0.0 ± 1.2 UA, 20/58/22, CI ± 90%, -0.01 ± 0.43). Changes in RPE values due to HA (0.4 ± 0.8 UA, 49/44/8, ES ± 90% CI, 0.19 ± 0.46), PC pre-HA (-0.2 ± 1.1 UA, 6/36/58, ES ± 90% CI, -0.26 ± 0.48) or PC post-HA (0.2 ± 1.0 UA, 20/64/16, ES ± 90% CI, 0.01 ± 0.38) were *unclear*.

DISCUSSION

The primary aim of this study was to test the hypothesis that heat-acclimatisation (HA) and pre-cooling (PC) cumulate to improve self-paced endurance performance in the heat. In contrast to our hypothesis, the results showed that the combination of HA and PC using an ice vest did not further improve 20-km TT performance in the heat as compared to HA alone. Nonetheless, analysis of pre- and post-HA pacing strategies based on presence of PC revealed a small effect of PC on PO during the initial stages of the TT post-HA. Given that both PC and HA improved TT performance without exacerbated physiological responses, it is likely that the HA-induced cardiovascular and thermoregulatory adaptations reduced the ergogenic effects of PC. Despite the blunted effects on the overall performance, PC following HA improved perceptions of thermal tolerance, which appeared to be related to pacing.

Acute precooling for endurance performance in the heat

In agreement with some (Johnson et al., 2008; Bogerd et al., 2010), but not all (Quod et al., 2008; Stannard et al., 2011) studies, the present results showed that ice-vest PC improved 20-km TT performance in the heat. While the 4.4% increase in PO observed in the current study is similar to the 5.2% improvement during a 20 km TT reported by Johnson et al. (2008), Quod et al. (2008) only reported a 1.5% improvement in similar environmental and self-paced exercise conditions to the present study. However, in their study, Quod et al. (2008) removed the cooling vest before the 20 min warm-up, which may have reduced the efficacy of PC on the TT performance. Also, despite comparable durations of PC during the warm-up, our results differ from those of Stannard et al. (2011), who reported no effect of wearing a cooling vest before 40min running exercise in warm conditions. The greater thermal stress imposed in our study (35°C v ~25°C in Stannard et al. [2011]), as well as the ice vests' cooling–efficiency may explain this difference (Ross et al., 2013). Notably, the

CryoVest® (body surface cooling = 0.18 m^2; weight <2 kg) enhanced TT performance despite fan-related airflow – which restricts PC benefits in laboratory settings (Morrison et al., 2014). This type of cooling might thus be relevant for outdoor competitions in the heat of ~30 min, as the effects of PC on PO progressively decreased with exercise duration.

As expected, PC reduced Tskin at the beginning of the TT, which likely enabled participants to adopt a higher PO (+10 ± 18 W) while maintaining similar cardiovascular and thermoregulatory responses to the NC condition. This absence of increases in HR, Tcore and sweat loss despite the greater PO may be due to cooling-related reduced cutaneous vasodilatation and higher heat loss via tissue conduction (transfer from the core to the skin) and subsequent fan-based convection (transfer from the skin to the environment), respectively (Saunders et al., 2005). Tskin reached similar temperatures at the end of the TT's in both NC and PC conditions, suggesting the PC-induced benefits on PO paralleled the ephemeral PC effect on the core-to-skin gradient. Either due to, or alongside the reduced relative physiological loads, PC up-regulated the pacing pattern while maintaining the same perceptual exertion as in NC. This may result from the central integration of cutaneous afferences, as TC *per se* is able to influence PO without modifying RPE (Schlader et al., 2011).

Heat-acclimatisation and endurance performance in the heat

As determined from the HTT, the participant's exhibited symptoms of HA such as reduced heat gain (-0.2 ± 0.3°C) and improved sweating rate (0.26 ± 0.25l). PV also increased (16.6 ± 8.5% on day 7, 11.8 ± 6.9% on day 11) and sweat sodium concentrations were reduced during the TT (-17 ± 19%). Comparatively, these changes are similar to those observed in well-trained cyclists after a two-week training camp (Karlsen et al., 2015), but greater than those previously reported using training camp models in professional football players (see Buchheit et al., 2011; Racinais et al., 2013). Such discrepancies may be as a result of the effective training volume of the present triathlete population and/or the large daily passive phases spent outdoors (Garrett et al., 2011).

The HA exhibited a *very large* effect on PO in the NC condition (ES ± 90% CI, 11.7 ± 4.1%). Such extent of performance improvement has not previously been reported on self-paced cycling exercise after heat-acclimation of similar duration (e.g., Lorenzo et al., 2010). Hence, it may be the combination of daily passive heat exposure with the heat training that amplified the performance improvement (Racinais et al., 2015; Shido et al., 1999). Moreover, the magnitude of physiological (i.e. cardiovascular and thermoregulatory) changes and perceptual adaptations may explain this improvement. Specifically, it is possible PV expansion and Tskin reduction enabled increased PO whilst limiting homeostasis disturbance (e.g. Tcore and cardiac strain, muscle metabolism). As evidence, PV expansion is suggested to offset increased HR responses by facilitating improved blood flow distribution during exercise (Nielsen et al., 1993). In parallel, as supported by our results, increased sweat losses provided greater evaporative cooling and core-to-skin gradient, especially in the presence of airflow (Saunders et al., 2005). Additionally, as a result of, or alongside these improved thermoregulatory adaptations, the athletes TC during the 20-km TT was similar to pre-HA despite the higher metabolic rate, and this may also allowed for increased PO (Schlader et al., 2011; Schulze et al., 2015).

Effect of pre-cooling after heat-acclimatisation

In contrast to the initial hypothesis, PC did not provide further benefits on the overall performance once athletes were heat-acclimatised (4 ± 14W). These findings extend previous studies (Castle et al., 2011; Brade et al., 2013) that examined the combined effects of PC with HA during repeated intermittent sprints performed in the heat. Accordingly, it is possible that the HA from the training camp led to a “ceiling effect” (i.e. saturation) of physiological adaptations (Castle et al., 2011), or blunted the PC-related physiological effects (i.e. converging effects), thus reducing the likelihood of further notable PC benefits on performance. Crucially, although our results do not enable to distinguish between these two hypotheses, both strategies demonstrated similar influence on the physiological responses to the TT. Specifically, while post-HA PC decreased Tskin to a similar extent as pre-HA, the sudomotor adjustments resulting from the heat exposure also promoted a lowering of Tskin during the TT. It is therefore possible that earlier and larger sweat losses resulting from HA (Shido et al., 1999) may have subsequently reduced the effectiveness of PC by inducing a larger core-to-skin temperature gradient than pre-HA. Similarly, PC improved cardiovascular efficiency during the unacclimatized TT’s, which was also reflected in the post-HA responses in regards to PV expansion. However, the extent of PV increase (16.6 ± 8.5%) may have minimised the cardiovascular effect of PC observed Pre-HA by facilitating both cutaneous and muscle blood flow distribution (Nielsen et al., 1993). Together with the absence of any changes in psychometric variables pre-TT’s, these results suggest that HA did not allow PC-related initial effects on PO to be as evident post-HA as pre-HA due to consubstantial physiological (i.e. cardiovascular and thermoregulatory) adaptations.

Although this study shows the advantage of PC is minimised by HA, the analysis of pacing suggests that PC still may have some role in the *acute* protection of exercise performance in the heat, regardless of acclimatisation status. Indeed, at post-HA, PC temporarily induced *small* to *moderate* benefits in the TT, though only during the initial 10km. This observation is consistent with the fact that PC- and post-HA PC-pacing profiles demonstrated comparable relationships (r = 0.43) for the trend of diminished benefit throughout the TT’s (from 14 ± 29 W to 2 ± 22 W at Pre, and from 5 ± 25 W to 2 ± 16 W at

Post). Such similarity in the temporal profiles, albeit with a smaller magnitude at Post-HA, suggests that the effectiveness of PC be related to individual athletes HA level – and it could further be speculated that the lesser the extent of HA the more effective PC would be.

Despite the smaller magnitude, the ephemeral beneficial effect of post-HA PC is likely related to perceptual improvements. For individual participants, an additional increase in TC from the vest at Post- compared to Pre-HA was associated with an additional increase in PO, notably during the first half of the TT (r = 0.54 and r = 0.48, at 4 km and 8 km, respectively) i.e. when PC-induced benefits were the most remarkable. This suggests that when pre-cooled, athletes who perceived a greater increase in TC following HA were those continuing to gain performance benefit from wearing the vest. This relationship reduces with increased TT duration. Nonetheless, these observations highlight the purported role of improvements in TC following PC to improve endurance performance beyond cardiovascular or thermoregulatory implications (Schulze et al., 2015).

Perspectives

This study strengthens previous findings that PC (Johnson et al., 2008) and HA (Lorenzo et al., 2010) independently improve self-paced endurance performance in the heat (Castle et al., 2011). However, given each of these heat-combating strategies are likely to induce convergent physiological effects, the combination of HA and ice vest PC provided no additional overall ergogenic effect to 20 km TT performance in the heat. In spite of this, when considering pacing adjustments, PC-induced ergogenic effects were still persistent post-HA during the first half of the TT, and thus remain of interest for HA athletes. Accordingly, we would still recommend that these combined strategies be encouraged when competing in the heat to ensure improved TC and assist performance benefits. Indeed, individual perceptual benefits from PC may potentially up-regulate pacing strategies – particularly if effective HA has already provided the athlete an improved physiological tolerance of the heat. In this perspective, the respective role of physiological (Bogerd et al., 2010; Bongers et al., 2015) *vs*. perceptive (Schlader et al., 2011; Schulze et al., 2015) pathways inherent to PC strategies and leading to endurance performance improvement in the heat remains to be fully elucidated.

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Table 1. Mean environmental and individual characteristics, and data from training monitoring for Paris and Guadeloupe.

Notes. NC = non-cooling; PC = pre-cooling; env = environmental; RH = relative humidity; DOMS = delayed onset muscle soreness; USG = urine specific gravity upon arriving at the laboratory. * *likely* increase compared to Paris (ES ± 90% CI, 0.33 ± 0.32)

Figure 1. Schematic representation of the full experimental protocol.

Notes. $\dot{V}O_{2max}$ = maximal oxygen consumption; PV = estimated plasma volume; HTT = heat tolerance test.

Figure 2. Individual changes to heat-acclimatisation-related HTT measurements pre- and post- training camp in **a**. Tcore; **b**. Thermal comfort; **c**. Body mass loss; **d**. Sodium concentration in sweat.

Notes. * *almost certain* decrease compared to pre (ES ± 90% CI, -0.85 ± 0.54); # *almost certain* decrease compared to pre (ES ± 90% CI, 0.95 ± 0.49); † *likely* decrease compared to pre (ES ± 90% CI, -0.50 ± 0.47).

Figure 3. Power output per kilometre for the four time-trials in the experimental protocol. Results are presented as the group mean ± SD.

Notes. HA = heat-acclimatisation; NC = non-cooling; PC = pre-cooling.

* changes at least *likely* between Pre-NC and Pre-PC; # changes at least *almost certain* between Pre-NC and Post-NC; † changes at least *possible* between Post-NC and Post-PC.

Figure 4. Individual time-trial duration for the four conditions of the experimental protocol.

Notes. HA = heat-acclimatisation; NC = non-cooling; PC = pre-cooling.

* *likely* decrease compared to Pre-HA NC (ES ± 90% CI, -1.3 ± 1.6%); # *almost certain* decrease compared to Pre-HA NC (ES ± 90% CI, -3.1 ± 1.7%).

Without the participant reporting drastic decreases in the two PC conditions (at the top of the graphs), results were as follow: *possible* between Pre-HA NC and Pre-HA PC (4/23/72; ES ± 90% CI, -0.6 ± 0.8%); *very likely* between Pre-HA NC and Post-HA NC (2/3/95; ES ± 90% CI, -2.5 ± 2.1%), and *unclear* between Post-HA NC and Post-HA PC (31/51/17; ES ± 90% CI, 0.1 ± 0.7%).

Figure 5. Changes in skin temperature at different times during the experiment for each condition Pre- and Post-training camp.

Results are presented as the group mean ± SD.

Notes. HA = heat-acclimatisation; NC = non-cooling; PC = pre-cooling; Pre-WU = before the passive phase; Pre-TT = immediately before the time-trial; Post-TT = immediately after the time-trial.

* *almost certain* decrease compared to NC conditions (ES ± 90% CI, 3.23 ± 0.56 and ES ± 90% CI, 2.43 ± 0.55 for Pre-HA and Post-HA, respectively); # *likely* increase compared to Pre-WU (ES ± 90% CI, 0.59 ± 0.51).