Title: Duration-dependant response of mixed-method pre-cooling for intermittent-sprint exercise in the heat.

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Abstract

This study examined the effects of pre-cooling duration on performance and neuromuscular function for self-paced intermittent-sprint shuttle running in the heat. Eight male, team-sport athletes completed two 35-min bouts of intermittent-sprint shuttle running separated by a 15-min recovery on three separate occasions (33°C, 34% relative humidity). Mixed-method pre-cooling was completed for 20-min (COOL20), 10-min (COOL10) or no cooling (CONT) and reapplied for 5-min mid-exercise. Performance was assessed via sprint times, % decline and shuttle-running distance covered. Maximal voluntary contractions (MVC), voluntary activation (VA) and evoked twitch properties were recorded pre- and post-intervention and mid- and post-exercise. Core temperature ($T_c$), skin temperature, heart rate, capillary blood metabolites, sweat losses, perceptual exertion and thermal stress were monitored throughout. Venous blood draws pre- and post-exercise were analyzed for muscle damage and inflammation markers. Shuttle-running distances covered were increased $5.2\pm3.3\%$ following COOL20 ($P<0.05$), with no differences observed between COOL10 and CONT ($P>0.05$). COOL20 aided in the maintenance of mid- and post-exercise MVC ($P<0.05$; $d>0.80$), despite no conditional differences in VA ($P>0.05$). Pre-exercise $T_c$ was reduced by $0.15\pm0.13°C$ with COOL20 ($P<0.05$; $d>1.10$), and remained lower throughout both COOL20 and COOL10 compared to CONT ($P<0.05$; $d>0.80$). Pre-cooling reduced sweat losses by $0.4\pm0.3$ kg ($P<0.02$; $d>1.15$), with COOL20 $0.2\pm0.4$ kg less than COOL10 ($P=0.19$; $d=1.01$). Increased pre-cooling duration lowered physiological demands during exercise heat stress and facilitated the maintenance of self-paced intermittent-sprint performance in the heat. Importantly, the dose-response interaction of pre-cooling and sustained neuromuscular responses may explain the improved exercise performance in hot conditions.

Key words: thermoregulation, team-sports, heat-stress, fatigue, performance, ice-vest
Introduction

High ambient temperatures impair heat loss mechanisms, requiring an alteration in physiological and behavioural processes to balance rising internal body temperatures with the maintenance of exercise performance (Wendt et al. 2007). Pre-cooling induced improvements to heat storage may reduce these challenges, resulting in augmented work rates and extended exercise time (Duffield 2008; Marino 2002; Quod et al. 2006). Evidence from pre-cooling studies demonstrate performance benefits to be associated with suppressed skin and/or core temperature, cardiovascular, metabolic and perceptual loads (Arngrimsson et al. 2004; Castle et al. 2006; Duffield and Marino 2007; Hasegawa 2005; Lee and Haymes 1995). However, evidence against critical limiting temperatures (Ely et al. 2009), and study of self-paced exercise modes (Duffield et al. 2010; Kay et al. 1999), may suggest the integration of higher central regulation to control skeletal muscle recruitment in anticipation or response to increased thermal loads (Marino 2004; Nybo and Nielsen 2001; Tucker et al. 2004).

Heat stress ultimately disrupts central motor output, down-regulating skeletal muscle recruitment, voluntary activation and force output (Kay et al. 2001; Morrison et al. 2004; Nybo and Nielsen 2001; Todd et al. 2005). In spite of potential afferent or efferent origins (Marino 2004), thermal advantages obtainable with pre-cooling may safeguard neuromuscular pathways, protecting exercise performance in the heat and assisting to explain the maintenance of higher exercise intensities (Duffield et al. 2010; Kay et al. 1999). Previous research provides evidence for a dose-response relationship with pre-cooling (Castle et al. 2006; Daanen et al. 2006; Duffield and Marino 2007; Minett et al. 2011), whereby the greater the cooling stimulus to improve thermoregulatory efficiency, the lower the rate of heat storage and the better the ensuing performance outcome (González-Alonso et al. 1999). This ergogenic assistance has traditionally been linked with observed reductions in core temperature (Marino 2002), although cooler skin temperatures independent of core temperature change also appear to have some regulation of exercise intensity (Kay et al. 1999; Schlader et al. 2011). These findings have practical implications for most athletic disciplines, but might be of particular importance for team-sport athletes who experience higher internal body temperatures during intermittent activity compared with steady state exercise modes (Ekblom 1971).
The benefits of whole-body cold-water immersion are widely acknowledged and potentially relate to the volume of cold exposure provided by such a method (Duffield 2008; Marino 2002; Quod et al. 2006). However, issues of practicality surrounding its application in competitive situations provide difficulties to implementation and pre-event routines (Marino 2002; Quod et al. 2006). Whilst logistical concerns in the field may be eased through the manipulation and combination of multiple cooling techniques (Duffield and Marino 2007; Duffield et al. 2009; Minett et al. 2011; Quod et al. 2008; Ross et al. 2011), the influence of pre-cooling duration on ensuing physiological and performance responses is unknown. These data may prove important to the effective implementation of pre-cooling techniques, particularly given the potential for extended physiological benefits and subsequent performance gains with more extensive cooling exposure (Minett et al. 2011). Alternatively, any excessive dose of cooling duration resulting in pronounced reduction in skeletal muscle temperature (Peiffer et al. 2009) may be detrimental to motor unit recruitment and force output (Racinais and Oksa 2010). Accordingly, understanding the balance between reduced thermal loads and retaining muscle function is of value in optimising performance outcomes following pre-cooling.

Evidence supports the use of pre-cooling for intermittent-sprint exercise performance in the heat (Castle et al. 2006; Duffield and Marino 2007; Duffield 2009; Minett et al. 2011). Pre-cooling induced enhancements in heat storage capacity may reduce thermoregulatory strain, facilitating the maintenance of neuromuscular function and subsequent exercise performance (Duffield et al. 2010). Nevertheless, understanding pre-cooling duration required for ergogenic effects is lacking. Further, the relationship between pre-cooling duration, voluntary force and evoked twitch properties and self-paced exercise performance in the heat warrants further investigation. Hence, the aim of the present study is to investigate the effects of pre-cooling duration on self-paced intermittent-sprint exercise performance, physiological responses and neuromuscular function in hot environmental conditions.

Methods

Participants

Eight, moderate- to well-trained, male team-sport athletes were recruited for this study (mean ± SD: age 21.5 ± 2.7 yr; height 184.1 ± 9.7 cm; body mass 78.9 ± 8.2 kg). Participants were club and regional level athletes who reported completing 3-5 sports specific and conditioning training sessions per week and competition on a
weekly basis. All participants gave verbal and written consent before engaging in testing procedures and ethical clearance was given by the Ethics in Human Research Committee of the University.

Experimental Design

Participants reported to the laboratory for testing sessions on four separate occasions. Following an initial equipment and procedural familiarisation session that included completion of the exercise protocol in its entirety, the remaining three visits were conducted in a randomized, repeat measures cross-over fashion. The data reported in this paper were collected as part of a succession of cricket related investigations focused on fast bowling related intermittent-sprint exercise in the heat. In accordance with previous reports (Minett et al. 2011), fast bowling workload data (Petersen et al. 2010) were utilized to provide for a non-specific team-sport protocol to reflect previous research (Duffield and Marino 2007). All respective testing sessions were conducted on an enclosed 20 m synthetic running track in mean ± SD environmental conditions of 33.0 ± 0.9°C and 33.9 ± 5.9% relative humidity. Temperatures were controlled using a customized gas heating system and four electronic 2,000 W room heaters (Kambrook, Australia) positioned at 5 m increments alongside the running track. All testing sessions were identical, with only pre-cooling duration variable throughout. Participants performed three conditional trials including a control session (no pre-cooling), 10 min pre-cooling session and a 20 min pre-cooling session. All participants were required to refrain from strenuous exercise and alcohol 24 h before and all caffeine and food substances 3 h before each testing session.

Exercise protocol

During all sessions, participants performed a standardized 5 min warm-up followed by 2 x 35 min bouts (Bout 1 and 2) of intermittent-sprint activity, separated by a 15 min mid-exercise recovery interval. Warm-up procedures involved progressively increasing continuous 20 m shuttle run speeds and six repeated 15 m maximal sprints. Each identical bout consisted of a set pattern of intermittent-sprint, hard running, jogging and walking activities to reflect cricket fast bowling requirements (Petersen et al. 2010). Specifically, the exercise protocol involved 10 (2 x 5) sets of 6 x 15 m maximal sprints with a 30 s recovery to emulate a 6-ball cricket over. Further, 5 min periods between sprint sets were comprised of minute by minute periods of self-paced, sub-maximal exercise intensities as per Duffield and Marino (2007). Hard running, jogging and walking activity was performed in a 15 m shuttle run fashion, with participants resuming their starting position at 50 s of each self-paced minute to begin the following exercise intensity. Participants were offered verbal support throughout and instructed to
cover the greatest distance possible during hard run efforts, while jogging and walking were completed at self-paced intensities. To limit potentially confounding effects of any fluid intake, all consumption was restricted throughout each session. The reliability of mean sprint times, self-paced distances and hard running distances covered demonstrate the Pearson Product-Moment Correlation (r) as 0.94 – 0.98, Technical Error of Measurement as 0.02 – 1.5% and Co-efficient of Variation (CV) as 0.6 – 1.1% (Minett et al. 2011). A schematic representation of the exercise protocol is presented in Figure 1.

Pre-cooling intervention

Cooling apparatus were applied pre-exercise and for the final 5 min of the 15 min mid-exercise recovery interval in the two treatment trials. Durational effects of pre-cooling were determined by the comparison of control (CONT), 10 min (COOL10) and 20 min (COOL20) pre-cooling interventions. As per Minett et al. (2011) all pre-cooling procedures involved a mixed-method approach, whereby participants were cooled with an iced towel soaked in water (5.0 ± 0.5°C) covering the head, neck and shoulders, hands immersed to the wrist in cold water (9.0 ± 0.5°C), ice-vest covering the torso (Arctic Heat, Brisbane, Australia) and frozen ice-packs applied to the quadriceps (Techni Ice, Frankston, Australia). Ice-vests and ice-packs were stored at -20°C before and after application. No cooling stimuli were applied during the 20 min CONT trial. All treatments were completed as participants rested passively in a seated position in controlled laboratory conditions of 33°C and 34% relative humidity. Mixed-method pre-cooling presents a practical and ecologically valid alternative to cold water immersion (Duffield et al. 2009), with the larger surface area coverage, the greater the ergogenic effect (Minett et al. 2011).

Measures

Performance

Intermittent-sprint running performance was assessed via 15 m sprint time measured with an infra-red timing system (Speed-light, Swift, Australia). Self-paced distances accumulated were calculated using 1 m markings along the 15 m running track. Percentage decline in sprint times ((total time/(fastest time × sprint n) × 100)) are reported as an indicator of performance maintenance. Self-paced, sub-maximal exercise bouts are reported as an individual mean or total value for each exercise mode (walk, jog, hard run).

Neuromuscular function
Evaluation of maximal voluntary contractions (MVC) and evoked twitch properties of the right knee extensors were recorded pre-intervention, post-intervention, mid-exercise and post-exercise with an isokinetic dynamometer (Kin-Com, Model 125, Chattanooga Group Inc., Hixon, TN, USA) and customized computer software (v8.0, LabVIEW; National Instruments, North Ryde, NSW, Australia). The axis of rotation of the dynamometer was visually aligned with the lateral femoral epicondyle. Participants were fastened to the dynamometer chair with knee and hip positioned at 90° (0° represents full extension) using conventional shoulder and waist straps and the distal right leg fixed to the lever arm 1 cm above lateral malleolus. Supra-maximal activation of the femoral nerve was achieved via a single square-wave pulse with a width of 200 µs (400 V with a current of 100-450 mA) delivered by a Digitimer DS7 stimulator (Digitimer Ltd., Welwyn Garden City, Hertfordshire, England) linked to a BNC2100 terminal block and signal acquisition system (PXI1024; National Instruments, Austin, TX, USA). Muscle activation was achieved with reusable self-adhesive gel electrode cathode positioned on the anterior thigh 3 cm below the inguinal fold (diameter 10 mm; MEDI-TRACE™ Mini 100 Pediatric Foam Electrodes, Covidien, Mansfield, MA, USA). A 90 x 50 mm reusable self-adhesive gel electrode anode was located on the medio-posterior aspect of the upper thigh below the gluteal fold (Verity Medical Ltd., Stockbridge, Hampshire, England). Peak twitch force was identified through incremental increases in stimulus intensity and then increased by 10% to ensure supra-maximal stimulation. Baseline evoked twitch properties were determined through five pulses separated by 20 s delivered in a rested state. Assessment of muscle function involved a MVC protocol involving 5 x 5 s isometric trials with a superimposed twitch following attainment of MVC plateau to the resting muscle immediately post-contraction. Individual MVC efforts were separated by a 30 s recovery period. MVC was defined as the peak torque (Pt) value attained during voluntary contractions. Voluntary activation (VA) was calculated according to the twitch interpolation technique (Allen et al. 1995). Time to peak torque (TPt) was defined as the time from evoke force onset to peak potentiated twitch torque. Data was processed using MATLAB version 7.9.0.529 (R2009b, The Mathworks Inc., Natick, MA, USA).

**Physiological variables**

A mid-stream urine sample was collected on arrival to the laboratory to determine urine specific gravity (USG; Refractometer 503, Now. Nippon Optical, Works Co, Tokyo, Japan). Changes in nude body mass were recorded pre- and post-exercise using calibrated scales (HW 150 K, A & D, Thebarton, Australia) to estimate total body sweat loss. Heart rate (HR) was determined with a chest transmitter and wristwatch receiver (FS1; Polar Electro
Core temperature ($T_c$) was measured using a telemetric temperature capsule (VitalSense, Mini Mitter, Bend, USA) ingested 5 h pre-exercise to allow for passing into the gastrointestinal tract. HR and $T_c$ was recorded every 5 min during the intervention and mid-exercise rest period, and at 10 min intervals during the exercise protocol. Skin temperature ($T_{sk}$) was measured at four sites (sternum, mid-forearm, mid-quadriceps and medial calf) with an infra-red thermometer (ThermoScan 3000, Braun, Kronberg, Germany) as per Burnham et al. (2006) (ICC= 0.96; r= 0.92). $T_{sk}$ was recorded at 5 min increments during the pre-cooling intervention, mid-protocol break and post-exercise. Mean $T_{sk}$ was calculated using the Ramanathan (1964) formula and body heat storage was estimated according to the equation of Havenith et al. (1995).

**Blood collection and biochemical analysis**

Resting blood draws were collected to determine the effect of pre-cooling duration on anaerobic metabolites, muscle damage, inflammation and stress responses. Capillary blood samples were drawn from a hyperaemic earlobe to analyze pH, glucose, lactate [La'] and bicarbonate (HCO$_3$) (ABL825 Radiometer, Copenhagen, Denmark). Further capillary blood draws mid- and post-exercise were collected within 30 s of exercise completion. Venous blood samples were drawn from an antecubital vein with an evacuated venipuncture assembly and serum separator tubes (Monovette, Sarstedt, Numbrecht, Germany). Serum was obtained through centrifugation (4000 rpm for 10 min) and stored at -20ºC until analysis. Serum concentrations of creatine kinase (CK), C-reactive protein (CRP), testosterone (TEST), cortisol (CORT) and insulin (INS) were determined pre- and 30 min post-exercise. All serum samples were analyzed according to manufacturer’s instructions provided in the respective assay kits. Analysis of CK was completed using enzymatic and bichromatic rate procedures and for CRP with the particle enhanced turbidimetric immunoassay technique (Dimension Xpand spectrophotometer, Dade Bearing, USA). INS, TEST and CORT were calculated using a solid-phase, competitive chemiluminescent enzyme immunoassay (Immulite 2000, Diagnostic Products Corp., Los Angeles, CA). Statistical analyses were performed on measured circulating concentrations as corrections for plasma and blood volume changes were not performed. All samples for each subject were analyzed in the same assay run and intra-assay CV were < 5% for all venous blood analyses.

**Perceptual measures**

Rating of perceived exertion (RPE) and thermal sensation scale (TSS) were recorded every 5 min during pre-cooling and exercise protocols. RPE was determined according to the Borg CR-10 scale, where ranking ranged
from 0 (nothing at all) to 10 (maximal). TSS was assessed using an 8-point Likert scale, ranging from 0 (unbearably cold) to 8 (unbearably hot).

Statistical analysis

Data are reported as mean ± standard deviation (SD). A two-way (condition × time) repeated-measures ANOVA was performed to detect differences between cooling durations (0 min vs. 10 min vs. 20 min). Unprotected pairwise comparisons (Protected Fisher’s LSD) were applied to determine the source of significance, which was accepted when $P < 0.05$. Analysis was performed using the Statistical Package for Social Sciences (SPSS v 16.0, Chicago, IL). Standardised effect sizes (ES; Cohen’s d) analyses were used in interpreting the magnitude of differences between conditions. An ES was classified as trivial (<0.20), small (0.20–0.49), moderate (0.50–0.79) or large (>0.80) as expressed by dividing the mean difference by the between-subject SD.

Results

Self-paced intermittent-sprint exercise performance

No significant differences and trivial to moderate ES ($P = 0.45–1.00; d = 0.00–0.40$) were present between all conditions for mean peak sprint times and % decline during Bout 1 (Table 1). However, significantly faster mean peak sprint times were observed in Bout 2 for COOL20 compared with CONT ($P = 0.02$; Figure 2A). Significant differences and large ES data indicated a smaller % decline during Bout 2 for COOL20 compared with CONT ($P = 0.04; d = 0.91$). No significant differences and trivial to moderate ES were apparent between cooling durations (10 v 20 min) for all sprint time variables ($P = 0.09–0.97; d = 0.01–0.38$).

Overall mean total distances covered were significantly greater following COOL20 (4801 ± 375 m) compared with COOL10 cooling (4584 ± 373 m; $P = 0.03; d = 0.82$) and CONT (4584 ± 411 m; $P = 0.01$). No significant difference and a trivial ES was observed for overall mean total distance accumulated between COOL10 cooling and CONT ($P = 0.90; d = 0.01$). Mean and total hard running distances completed in Bout 1 were significantly greater in COOL20 than in COOL10 ($P = 0.02; d = 1.14$) and CONT ($P = 0.03$) (Table 1; Figure 2B). Mean and total hard running distances were significantly increased for COOL20 compared with CONT in Bout 2 ($P = 0.01$). Mean and total jogging distances accumulated following COOL20 were also significantly greater than COOL10 in Bout 1 ($P = 0.04; d = 1.15$) and Bout 2 ($P = 0.01$) respectively. No
significant differences and trivial to moderate ES between conditions were observed for all mean and total distances covered for any walking measures ($P= 0.22 - 0.89; d= 0.04 - 0.48$).

[Insert Figure 2]

[Insert Table 1]

**Neuromuscular function**

Significant reductions in post-intervention mean peak torque were apparent during COOL20 sessions compared with COOL10 ($P=0.03; d=0.92$) and CONT ($P= 0.04$; Table 2). In contrast, mid-exercise mean peak torque were significantly greater in COOL20 compared with COOL10 ($P= 0.04; d= 0.83$) and respectively greater than CONT ($P= 0.01; d= 1.74$). Similarly, large ES demonstrate greater mid-exercise mean peak torque following COOL10 as opposed to CONT ($d= 1.03$). Mean peak torque post-exercise was significantly higher in COOL20 compared with COOL10 ($P= 0.03; d= 1.48$) and CONT ($P= 0.05; d= 1.44$). No significant differences and trivial to moderate ES ($P= 0.08 - 0.92; d= 0.07 - 0.76$) were evident between respective cooling conditions for VA pre-intervention, mid-exercise and post-exercise. However, COOL20 post-intervention VA tended to be reduced compared with COOL10 ($d= 0.88$). No significant differences and trivial to moderate ES were apparent for Tpt between conditions at all time points ($P= 0.08 - 0.54; d= 0.05 - 0.69$).

[Insert Table 2]

**Physiological variables**

No significant differences and small to moderate ES ($P= 0.53 - 0.64; d= 0.17 - 0.56$) were present in HR values between all conditions pre- or post-intervention (Figure 3A). Whilst there were no significant differences between all conditions for mean Bout 1 HR responses ($P= 0.19 - 0.44$), COOL20 values tended to be reduced compared with COOL10 ($d= 0.88$) and CONT ($d= 0.99$). No significant differences in HR values were evident between cooling durations during the mid-protocol recovery period ($P= 0.08 - 0.14$); though large ES indicated reduced values in the COOL20 condition compared with COOL10 ($d= 1.27$) and CONT ($d= 1.59$). No significant differences and small to moderate ES ($P= 0.30 - 0.80; d= 0.26 - 0.54$) were apparent between conditions for mean Bout 2 HR responses.
Significant differences and large ES indicated lower $T_c$ values following COOL20 compared with COOL10 ($P= 0.02; d= 1.32$) and CONT ($P= 0.03; d= 1.16$) immediately post intervention (Figure 3B). $T_c$ remained lower for the entirety of the exercise protocol during COOL20 compared with CONT ($P= 0.003 – 0.04; d= 0.82 – 1.20$). Moreover, large ES also indicated lower $T_c$ values during the COOL10 sessions than CONT ($d= 0.80 – 1.10$). No significant differences in $T_c$ were evident between cooling durations throughout the exercise protocol ($P= 0.18 – 0.95$). Significant differences and large ES indicate lower $T_{sk}$ throughout the intervention period following COOL20 cooling compared with CONT ($P= 0.001; d= 3.89 – 7.47$) (Figure 3C). Similarly, large ES was observed for lower $T_{sk}$ for the entirety of the cooling application within COOL10 sessions compared with CONT ($P= 0.01 – 0.03; d= 1.72 – 7.60$). Both pre-cooling durations significantly reduced post-intervention heat storage compared with CONT ($P= 0.000 – 0.001; d= 3.66 – 8.18$) (Figure 3D). Further, durational effects were evident with a large ES indicating a greater decrease in heat storage post-intervention following COOL20 compared with COOL10 ($P= 0.64; d= 2.44$). Heat storage remained significantly reduced at 35 min in COOL20 trials ($P= 0.03$), with large ES demonstrating lower heat storage with both pre-cooling conditions compared with control during the mid-exercise rest period ($d= 1.12 – 1.64$). Following the reapplication of cooling stimulus, heat storage was significantly lower with both pre-cooling durations at 50 min ($P= 0.001 – 0.004; d= 3.34 – 3.37$) compared with CONT. COOL20 displayed a reduced heat storage compared with COOL10 ($P= 0.07; d= 1.41$) and CONT at 85 min ($P= 0.0001; d= 2.48$). Finally, a large ES indicates a reduced heat storage with COOL10 compared with CONT immediately post-exercise ($P= 0.12; d= 0.96$).

[Insert Figure 3]

No significant differences and trivial to small ES were evident for pre-exercise USG values during COOL20 cooling (1.015 ± 0.006), COOL10 cooling (1.015 ± 0.007) and CONT trials (1.016 ± 0.004; $P= 0.66 – 0.89; d= 0.10 – 0.29$). Mean changes in pre- to post-exercise body mass were significantly less following pre-cooling (COOL20= 1.8 ± 0.3; COOL10= 2.0 ± 0.3) compared with CONT (2.3 ± 0.4; $P= 0.003 – 0.013; d= 1.17 – 2.12$). Although not significant ($P= 0.19$), a large ES indicates sweat loss induced changes in body mass to be less with COOL20 than COOL10 ($d= 1.01$).
Venous and capillary blood variables

No significant differences ($P= 0.06 – 1.00$) were detected in capillary blood measures for pH, glucose, [La$^-$], HCO$_3^-$ pre- and post-exercise (Figure 4). Mid-exercise [La$^-$] concentrations were decreased with COOL20 compared with COOL10 ($d= 1.07$) and CONT ($d= 1.43$). This trend continued throughout, with [La$^-$] values higher in CONT than COOL10 ($d= 0.82$) and COOL20 ($d= 2.13$) post-exercise. No significant differences and trivial to moderate ES ($d= 0.04 – 0.53$; Table 3) were evident for pre-exercise CK, CRP, TEST, INS and CORT concentration. Significant differences and large ES indicate reduced CK post-exercise with COOL20 compared with COOL10 ($P= 0.03$; $d= 1.02$) and CONT ($P= 0.04$; $d= 1.49$). Large ES denote lesser CK post-exercise under COOL10 than CONT conditions ($d= 1.13$). Further, pre- to post-exercise change in CK was attenuated with COOL 20 ($P= 0.03$; $d= 1.51$) and COOL10 ($P= 0.21$; $d= 1.14$) compared with CONT. Similarly, relative to total shuttle run distance completed, pre- to post-exercise changes in CK were lower following COOL20 (0.02 ± 0.01; $P= 0.05$; $d= 1.66$) and COOL10 (0.03 ± 0.03; $P= 0.09$; $d= 1.19$) than CONT (0.08 ± 0.07). Although not significant, a large ES indicated increased post-exercise CORT concentrations in the COOL20 trial compared with CONT ($P= 0.25$; $d= 0.88$), with this large trend maintained when compared relative to shuttle running workload completed in COOL20 (0.07 ± 0.04) and CONT (0.04 ± 0.04; $P= 0.27$; $d= 1.02$). No significant differences and trivial to moderate trends were observed in all remaining venous blood variables ($P= 0.10 – 0.97$; $d= 0.03 – 0.64$).

[Insert Figure 4]
[Insert Table 3]

Perceptual measures

No significant differences and trivial to small ES ($P= 0.29 – 0.96$; $d= 0.001 – 0.38$) were apparent for mean RPE values between COOL20 (4.8 ± 0.6), COOL10 (4.8 ± 0.6) and CONT (5.0 ± 0.9). Significant differences and large ES represent a reduced mean TSS value with COOL20 (5.2 ± 0.5) compared with COOL10 (6.0 ± 0.4; $P= 0.02$; $d= 2.29$) and CONT (6.3 ± 0.6; $P= 0.00$; $d= 3.14$). Mean TSS ratings during COOL10 sessions were largely reduced compared with CONT ($d= 1.16$).

DISCUSSION
Findings from the present study indicate the possible existence of a duration effect of mixed-method pre-cooling on ensuing exercise performance and physiological responses. Increasing evidence demonstrates dose-specific effects of pre-cooling (surface area coverage or temperature) on both physiological and performance outcomes of exercise in the heat (Bogerd et al. 2010; Castle et al. 2006; Daanen et al. 2006; Minett et al. 2011). Similarly, these results highlight the benefits of a longer pre-cooling duration (up to 20 min) when using mixed-method techniques by providing greater augmentation of performance and blunting of physiological loads. An apparent maintenance of post-exercise MVC following pre-cooling in the heat may demonstrate sustained neuromuscular function, despite higher shuttle-running distances completed, and a similar change in VA. Accordingly, the observed performance improvements and greater physiological changes apparent with the longer cooling duration (COOL20 > COOL10 > CONT) may implicate greater maintenance of endogenous thermal control, with an associated preservation of neuromuscular force production possibly facilitating subsequent exercise performance benefits.

Effective pre-cooling increases heat storage reserve (Figure 3D), allowing athletes to better accommodate high levels of metabolic and environmental heat stress, elongating periods of higher exercise intensity (Duffield 2008; Marino 2002; Quod et al. 2006). This is demonstrated in the current data with higher self-paced, sub-maximal running distances maintained throughout the exercise protocol. In accordance with previous investigations (Duffield and Marino 2007; Duffield et al. 2009; Minett et al. 2011), greater shuttle-running distances were covered during the longest exposure trial (COOL20; Table 1; Figure 2B). Further, COOL20 aided repeat-sprint ability and maintenance of sprint times during Bout 2 (Table 1; Figure 2A). Whilst these findings support previously documented benefits of pre-cooling for self-paced intermittent-sprint performance in the heat (Castle et al. 2006; Duffield and Marino 2007; Duffield et al. 2009; Minett et al. 2011), minimal differences between COOL10 and CONT (Table 1; Figure 2A; Figure 2B) suggest the importance of cooling duration with this method for attaining ergogenic effects. Accordingly, the reduced cooling exposure of the COOL10 condition (10 min), and to a lesser extent the 5 min mid-exercise reapplication, may have been of insufficient duration to achieve explicit performance benefits. Similarly beneficial effects of increasing dose by either surface area coverage (Minett et al. 2011), temperature (Bogerd et al. 2010) or cooling duration as shown here highlight the requirement of mixed-method pre-cooling intervals greater than 10 and up to 20 minutes to evoke beneficial performance outcomes (Castle et al. 2006; Duffield and Marino 2007; Duffield et al. 2009).
Despite the growing consensus over the benefits of pre-cooling for intermittent-sprint exercise in the heat, care is required to ensure desirable dose-responses are achieved. Given the relationship between reduced muscle temperatures and suppressed neuromuscular recruitment and contractile properties (Racinai and Oksa 2010), it could be suggested that the durational effects of cooling application may account for the observed reduction in MVC immediately post COOL20 application (Table 2). However, this does not explain the observed slower Tpt post-intervention under COOL10 conditions otherwise absent following COOL20 cooling (Table 2). Interestingly, Peiffer et al. (2009) report no difference in muscle function between post-exercise cooling durations regardless of changes in muscle temperature. Methodological discrepancies prevent direct comparison with the current data, yet findings of acute impairment in muscle function immediately following pre-cooling is not surprising and may explain previously reported detrimental effects of cooling in short-duration, high-intensity exercise (Duffield 2008; Marino 2002; Quod et al. 2006). Nevertheless, such a finding emphasizes the importance of post-cooling warm-up procedures to avoid possible initial ergolytic effects of temperature inhibited voluntary force production and ensuing exercise performance as observed by Skein et al. (2012). Accordingly, the combined effects of pre-cooling followed by an adequate warm-up may improve neuromuscular contractile function and perceptual readiness for exercise, while still attenuating cardiovascular and thermoregulatory strain associated with exercise in the heat.

Despite initial reduction in MVC, longer pre-cooling duration maintained voluntary force during and following exercise. Previous research suggests a suppression of neuromuscular drive under heat stress may be attributable to a centrally mediated impairment of VA (Morrison et al. 2004; Nybo and Nielsen 2001; Thomas et al. 2006; Todd et al. 2005). This response was not present following COOL20, with greater heat removal possibly facilitating the maintenance of MVC mid- and post-exercise (Table 2). However, similar Tc irrespective of pre-cooling duration mid- and post-exercise (Figure 3B), contradict the demonstrated association between a reduced MVC and elevated thermal loads as previously shown with passive heating/cooling techniques (Morrison et al. 2004; Thomas et al. 2006). Further, the lack of inter-trial differences in VA, even though enhanced MVC and self-paced running workloads were achieved with more extensive pre-cooling, make it difficult to distinguish performance alterations as of CNS modulation alone. Whilst a higher force production following COOL20, without conditional differences in VA, suggests the interaction of peripheral mechanisms, similar Tpt between conditions post-exercise demonstrates the maintenance of contractile function below the neuromuscular junction. Alternatively, it could be postulated that dose-dependent responses of mixed-method
pre-cooling duration result in sustained reduction of $T_{\text{sk}}$, TSS and the same absolute $T_{c}$ for a greater workload highlighting the maintenance of voluntary force in the heat (Schlader et al. 2011). Nevertheless, such a response remains speculative given the inconclusive relationship between cooling duration, physiological and perceptual responses and the maintenance of MVC for a similar change in VA.

To explain reduced exercise performance in the heat, CNS fatigue in hot conditions has been hypothesized to engage a complex interaction of feedback and/or feed-forward controls (Marino 2004; Nybo and Nielsen 2001; Nybo 2008; Tucker et al. 2004). The suppression of coexisting central, peripheral and perceptual strain with longer pre-cooling duration may have culminated in the retention of neuromuscular function and subsequent preservation of MVC mid- and post-exercise, despite increased work performed (Duffield et al. 2010; Tucker et al. 2004). Thus, it is possible that pre-cooling may aid in the sustained recruitment of exercising musculature in the heat (Kay et al. 2001; Tucker et al. 2004), presenting the potential for attainment of higher self-paced running workloads and preventing the reduction in repeat-sprint activity (Duffield 2008). Regardless of the specific mechanism/s, discrepancies in voluntary force production and exercise performance outcomes demonstrated using this step-wise approach to cooling highlight possible alterations in feedback and/or feed-forward processes that may be altered with sufficient mixed-method pre-cooling duration. Hence it is possible that performance benefits presented here may owe to the duration specific application of the cooling stimulus, and subsequent reductions in physiological (HR, $T_c$ and $T_{\text{sk}}$) and perceptual loads (TSS) assisting to maintain neuromuscular function (MVC) and extend the period of desired exercise intensity in hot conditions (Duffield 2008).

Both pre-cooling durations attenuated thermoregulatory demands to exercise-induced heat stress, with reductions in $T_c$ and $T_{\text{sk}}$ providing increased heat storage reserve. Nevertheless, the longer the mixed-method pre-cooling was applied (COOL20 > COOL10 > CONT), the greater the reduction in thermal stress remained throughout the exercise protocol (Figure 3). Alterations to the thermal gradient may have precluded concurrent demands for blood flow to the active musculature as well as the periphery for heat dissipation (González-Alonso et al. 1997). Accordingly, centralized blood volume is maintained, easing the cardiovascular challenges associated with exercise in the heat (Wendt et al. 2007). Further, greater heat removal with more extensive pre-cooling reduced sweat loss alterations in blood volume, preventing cardiovascular drift and leaving any work related increases in HR significantly attenuated (Marino 2002). Differences between inter-trial sweat loss (~500
mL) are comparable to previous finding (Arngrímsson et al. 2004; Duffield et al. 2010; Kay et al. 1999; Minett et al. 2011) and it is unknown if such volumes are sufficient to explain these performance outcomes alone.

Given the dose-specific physiological responses to cooling duration and increasing self-paced work rates, consideration of acute biochemical reactions may prove beneficial. The lack of conditional discrepancies in anaerobic metabolite and muscle damage markers previously reported are largely reiterated here (Castle et al. 2006; Duffield and Marino 2007). However, the reduction in [La−] mid- and post-exercise with longer cooling duration suggests a lower dependence on glycolytic energy sources as heat stress is reduced throughout COOL20 trials (Young et al. 1985). Interestingly, marked alteration in biochemical responses to muscle damage and stress demonstrate divergent reactions to mixed-method cooling durations relative to shuttle running distances covered. Whilst elevated CORT post-exercise with COOL20 may reflect a compensatory stress response to the increased work performed (Minett et al. 2011), CK were attenuated with longer mixed-method cooling applications (Table 3). Considering the higher workloads completed with mixed-method pre-cooling, it is unlikely that a lesser CK concentration could be attributed to a reduction in exercise-induced muscle damage. Rather, it is possible that lessening thermal demands with COOL10 and COOL20 may facilitate a greater maintenance of cellular integrity, resulting in a reduced CK efflux and lower circulatory concentrations (Alzeer et al. 1997). Although the acute performance effects of pre-cooling in these data are clear, the potential effects of a higher catabolic state on subsequent adaptation and recovery present an area for future study.

In summary, mixed-method pre-cooling duration appears important to subsequent suppression of physiological and perceptual responses to exercise induced heat stress. Accordingly, enhanced thermoregulatory control may facilitate the maintenance of sprint times and self-selected sub-maximal efforts. Most pertinent, however, was the dose effect demonstrated, with the incremental mixed-method pre-cooling durations resulting in different levels of physiological and performance responses. Consequently, the greater reduction in heat stress experienced during the COOL20 trial may have aided in the maintenance of MVC and improved running workloads completed. Whilst these findings provide evidence for the importance of duration of pre-exercise cooling for ensuing exercise performance in hot conditions, consideration for individual logistics and demands should be considered prior to field-based application.
Acknowledgements

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Conflict of Interest

MP is an employee of Cricket Australia. There are no conflicts of interest for any of the authors.
References


**Table Captions**

Table 1. Mean ± SD sprint time variables and sub-maximal running distances covered per session for 20min, 10 min and control conditions.  
* Significant difference compared to Control condition (*P* < 0.05).  
  b Significant difference compared to 10 min condition (*P* < 0.05).  
  1 Large ES compared to Control condition (d > 0.80).  
  2 Large ES compared to 10 min condition (d > 0.80).

Table 2. Mean ± SD mean peak torque, time to peak torque and voluntary activation (VA) level for pre-cooling methods pre-intervention, post-intervention, mid-exercise and post-exercise.  
* Significant difference compared to Control condition (*P* < 0.05).  
  b Significant difference compared to 10 min condition (*P* < 0.05).  
  1 Large ES compared to Control condition (d > 0.80).  
  2 Large ES compared to 10 min condition (d > 0.80).  
  * Significant difference compared to pre-intervention values (*P* < 0.05).  
  † Large ES compared to pre-intervention values (d > 0.80).

Table 3. Mean ± SD biochemical data comparison between cooling duration and time.  
* Significant difference compared to Control condition (*P* < 0.05).  
  b Significant difference compared to 10 min condition (*P* < 0.05).  
  1 Large ES compared to Control condition (d > 0.80).  
  2 Large ES compared to 10 min condition (d > 0.80).
Figure Captions

Figure 1. Schematic representation of the self-paced intermittent-sprint exercise protocol. MVC represents maximal voluntary contraction. VB represents venous blood sample. CB represents capillary blood sample. HR represents heart rate. $T_c$ represents core temperature. $T_{sk}$ represents skin temperature. RPE represents rating of perceived exertion. TSS represents thermal sensation scale.

Figure 2. A Mean ± SD individual 15-m sprint times (s) across all pre-cooling conditions. B Mean ± SD individual hard running distances (m) covered across all pre-cooling conditions.

Figure 3. A Mean ± SD core temperature, B mean ± SD skin temperature and C mean ± SD heart rate for COOL20, COOL10 and Control conditions. $^a$ represents a significant difference between COOL20 and Control conditions (P < 0.05). $^b$ represents a significant difference between COOL20 and COOL10 conditions (P < 0.05). $^c$ represents a significant difference between COOL10 and Control conditions (P < 0.05). $^1$ represents a large ES between COOL20 and Control conditions (d > 0.80). $^2$ represents a large ES between COOL20 and COOL10 conditions (d > 0.80). $^3$ represents a large ES between COOL10 and Control conditions (d > 0.80).

Figure 4. Mean ± SD capillary blood comparison of anaerobic metabolites between pre-cooling conditions. $^1$ represents a large ES between COOL20 and Control conditions (d > 0.80). $^2$ represents a large ES between COOL20 and COOL10 conditions (d > 0.80). $^3$ represents a large ES between COOL10 and Control conditions (d > 0.80).
Figure 1.
Figure 2.
Figure 3.
Figure 4.
Table 1.

<table>
<thead>
<tr>
<th>Activity</th>
<th>Control</th>
<th>10 min</th>
<th>20 min</th>
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<tbody>
<tr>
<td><strong>Sprint time variables</strong></td>
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<tr>
<td>Mean Bout 1 sprint (s)</td>
<td>2.59 ± 0.07</td>
<td>2.60 ± 0.15</td>
<td>2.61 ± 0.12</td>
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<td>Mean Bout 2 sprint (s)</td>
<td>2.71 ± 0.13</td>
<td>2.68 ± 0.16</td>
<td>2.66 ± 0.11 (^a)</td>
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<td>Bout 1 decline (%)</td>
<td>5.62 ± 1.95</td>
<td>4.92 ± 1.88</td>
<td>6.50 ± 3.69</td>
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<tr>
<td>Bout 2 decline (%)</td>
<td>8.82 ± 4.83</td>
<td>6.95 ± 5.00</td>
<td>6.09 ± 3.55 (^{a1})</td>
</tr>
</tbody>
</table>

| **Sub-maximal running distances** |               |               |               |
| Mean Bout 1 hard run (m)        | 156.6 ± 17.2  | 153.0 ± 15.4  | 165.2 ± 15.4 \(^{ab2}\) |
| Mean Bout 2 hard run (m)        | 146.6 ± 18.3  | 151.3 ± 14.9  | 154.3 ± 17.4 \(^a\) |
| Mean Bout 1 jog (m)             | 107.2 ± 13.6  | 103.6 ± 7.5   | 110.4 ± 8.9 \(^{b2}\) |
| Mean Bout 2 jog (m)             | 101.4 ± 12.0  | 101.2 ± 9.6   | 105.8 ± 9.5 \(^b\) |
| Mean Bout 1 walk (m)            | 63.6 ± 4.5    | 62.9 ± 4.4    | 64.6 ± 5.4    |
| Mean Bout 2 walk (m)            | 60.7 ± 6.1    | 61.7 ± 5.6    | 61.5 ± 5.9    |
| Total Bout 1 hard run (m)       | 1252.9 ± 137.8| 1223.8 ± 123.6| 1321.8 ± 119.3 \(^{ab2}\) |
| Total Bout 2 hard run (m)       | 1172.9 ± 146.5| 1210.8 ± 122.9| 1234.0 ± 139.1 \(^a\) |
| Total Bout 1 jog (m)            | 857.9 ± 109.1 | 828.9 ± 60.2  | 882.9 ± 71.6 \(^{b2}\) |
| Total Bout 2 jog (m)            | 811.0 ± 96.0  | 809.4 ± 77.1  | 846.3 ± 75.8 \(^b\) |
| Total Bout 1 walk (m)           | 254.5 ± 18.1  | 251.6 ± 17.5  | 258.4 ± 21.6  |
| Total Bout 2 walk (m)           | 242.9 ± 24.6  | 246.6 ± 22.6  | 246.0 ± 23.6  |
Table 2.

<table>
<thead>
<tr>
<th>Variable</th>
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<tr>
<td><strong>Pre-Intervention</strong></td>
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<tr>
<td>Mean Peak Torque (Nm)</td>
<td>155.18 ± 28.17</td>
<td>152.6 ± 17.6</td>
<td>154.2 ± 24.8</td>
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<td>Time to Peak Torque (ms)</td>
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<td>92.6 ± 17.6</td>
<td>89.3 ± 9.1</td>
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<tr>
<td>VA Level (%)</td>
<td>75.63 ± 8.80</td>
<td>74.6 ± 6.6</td>
<td>78.3 ± 7.9</td>
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<tr>
<td><strong>Post-Intervention</strong></td>
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<tr>
<td>Mean Peak Torque (Nm)</td>
<td>146.89 ± 28.32</td>
<td>153.7 ± 18.5</td>
<td>139.9 ± 23.6 ab2</td>
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<td>Time to Peak Torque (ms)</td>
<td>91.85 ± 9.43</td>
<td>102.7 ± 14.9 a1</td>
<td>97.3 ± 13.5</td>
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<td>VA Level (%)</td>
<td>69.31 ± 17.66</td>
<td>77.2 ± 11.0</td>
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<td><strong>Mid-Exercise</strong></td>
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<tr>
<td>Mean Peak Torque (Nm)</td>
<td>125.69 ± 23.71</td>
<td>141.0 ± 17.8</td>
<td>151.4 ± 17.7 ab12</td>
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<td>Time to Peak Torque (ms)</td>
<td>92.07 ± 14.47</td>
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<tr>
<td>VA Level (%)</td>
<td>66.05 ± 8.91</td>
<td>68.7 ± 15.1</td>
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<td>Mean Peak Torque (Nm)</td>
<td>125.26 ± 37.03</td>
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<td>153.6 ± 19.72 ab12</td>
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<td>95.4 ± 10.2</td>
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<td>VA Level (%)</td>
<td>70.21 ± 16.82</td>
<td>70.9 ± 10.0</td>
<td>68.9 ± 11.8</td>
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Table 3.

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<tr>
<td>CK (U·L⁻¹)</td>
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<tr>
<td>Pre</td>
<td>238.3 ± 137.4</td>
<td>213.0 ± 76.0</td>
<td>200.5 ± 41.4</td>
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<tr>
<td>Post</td>
<td>585.1 ± 368.3</td>
<td>365.1 ± 127.9</td>
<td>305.8 ± 68.9</td>
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<tr>
<td>Δ</td>
<td>346.9 ± 312.0</td>
<td>152.1 ± 135.8</td>
<td>105.3 ± 72.1</td>
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<tr>
<td>CRP (U·L⁻¹)</td>
<td>Pre 3.00 ± 4.94</td>
<td>2.86 ± 4.75</td>
<td>2.43 ± 3.55</td>
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<td></td>
<td>Post 3.23 ± 5.01</td>
<td>3.04 ± 4.76</td>
<td>2.69 ± 3.85</td>
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<td>Δ 0.23 ± 0.44</td>
<td>0.18 ± 0.13</td>
<td>0.26 ± 0.41</td>
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<td>INS (µL·mL⁻¹)</td>
<td>Pre 5.94 ± 1.61</td>
<td>5.73 ± 1.14</td>
<td>6.24 ± 1.98</td>
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<td></td>
<td>Post 5.09 ± 1.82</td>
<td>5.33 ± 1.63</td>
<td>5.49 ± 1.51</td>
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<tr>
<td></td>
<td>Δ -0.74 ± 1.87</td>
<td>-0.35 ± 1.74</td>
<td>-1.25 ± 1.64</td>
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<td>TEST (ng·dL⁻¹)</td>
<td>Pre 379.1 ± 84.3</td>
<td>404.8 ± 63.3</td>
<td>383.5 ± 95.8</td>
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<tr>
<td></td>
<td>Post 494.0 ± 156.4</td>
<td>491.6 ± 59.3</td>
<td>514.9 ± 114.0</td>
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<td></td>
<td>Δ 215.0 ± 1.94.5</td>
<td>290.1 ± 201.0</td>
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<td>CORT (nmol·L⁻¹)</td>
<td>Pre 330.4 ± 98.4</td>
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<td></td>
<td>Post 545.4 ± 198.6</td>
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<tr>
<td></td>
<td>Δ 114.9 ± 110.0</td>
<td>86.9 ± 39.3</td>
<td>131.4 ± 99.0</td>
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