

Research article

A Mixed-Method Approach of Pre-Cooling Enhances High-Intensity Running Performance in the Heat

Minxiao Xu^{1,2}, Zhaozhao Wu^{2,6}, Yanan Dong⁴, Chaoyi Qu^{2,3}, Yaoduo Xu⁷, Fei Qin^{2,5}, Zhongwei Wang^{1,2}, George P. Nassis^{8,9} and Jiexiu Zhao²✉

¹School of Kinesiology, Shanghai University of Sports, Shanghai, China; ²Exercise Biological Center, China Institute of Sport Science, Beijing, China; ³School of Sport Science, Beijing Sport University, Beijing, China; ⁴Beijing Institute of Sport Science, Beijing, China; ⁵School of Physical Education, Jinan University, Guangzhou, China; ⁶Physical Education Department, Northwest University, Xi'an, China; ⁷Physical Education Department, Northwestern Poly-technical University, Xi'an, China; ⁸Physical Education Department-(CEDU), United Arab Emirates University, Al Ain, Abu Dhabi, United Arab Emirates; ⁹Department of Sports Science and Clinical Biomechanics, SDU Sport and Health Sciences Cluster), University of Southern Denmark, Odense, Denmark

Abstract

We investigated whether single or combined methods of pre-cooling could affect high-intensity exercise performance in a hot environment. Seven male athletes were subjected to four experimental conditions for 30 min in a randomised order. The four experimental conditions were: 1) wearing a vest cooled to a temperature of 4 °C (Vest), 2) consuming a beverage cooled to a temperature of 4 °C (Beverage), 3) simultaneous usage of vest and consumption of beverage (Mix), and 4) the control trial without pre-cooling (CON). Following those experimental conditions, they exercised at a speed of 80% VO₂max until exhaustion in the heat (38.1 ± 0.6 °C, 55.3 ± 0.3% RH). Heart rate (HR), rectal temperature (T_{core}), skin temperature (T_{skin}), sweat loss (SL), urine specific gravity (USG), levels of sodium (Na⁺) and potassium (K⁺), rating of perceived exertion (RPE), thermal sensation (TS), and levels of blood lactic acid ([Bla]) were monitored. Performance was improved using the mixed pre-cooling strategy (648.43 ± 77.53 s, *p* = 0.016) compared to CON (509.14 ± 54.57 s). T_{core} after pre-cooling was not different (Mix: 37.01 ± 0.27 °C, Vest: 37.19 ± 0.33 °C, Beverage: 37.03 ± 0.35 °C) in all cooling conditions compared to those of CON (37.31 ± 0.29 °C). A similar T_{core} values was achieved at exhaustion in all trials (from 38.10 °C to 39.00 °C). No difference in the level of USG was observed between the conditions. Our findings suggest that pre-cooling with a combination of cold vest usage and cold fluid intake can improve performance in the heat.

Key words: Cooling prior to exercise, external and internal cooling, hyperthermia, thermoregulation, high-intensity aerobic exercise.

Introduction

The Tokyo 2021 Summer Olympics (Tokyo 2020, postponed to 2021) was expected to be held on one of the hottest days recorded thus far (ambient temperature 27–31 °C, 60–80% relative humidity in July and August) (Gerrett et al., 2019; Notley et al., 2020). These hot-humid environmental conditions pose a major challenge to the physiological status of athletes (Vanos et al., 2018). As shown previously, exposure to hot climatic conditions could cause a decrease in athletic performance and potentially compromise safety during the competition (Choo et al., 2017; Wendt et al., 2007) perhaps by through elevated: thermoregulatory strain (Havenith and Fiala, 2016), cardiovas-

cular strain (Cheung and Sleivert, 2004), thermal perception (Schlader et al., 2010), and by altering the function of the central nervous system (Nybo, 2011). During exercise, hyperthermia could distribute more blood for peripheral convective cooling and augment sweat loss for evaporative cooling (Reilly et al., 2006), which impaired exercise capacity (Nybo, 2008). Furthermore, the augmented sweat loss could induce a progressive decrease in body fluids and lead to dehydration (Nassis and Geladas, 2003a; Nassis and Geladas, 2003b; Quod et al., 2006). A low degree of dehydration could impair thermoregulatory function and aggravate cardiovascular strain (Kenefick, 2018). Once the water deficit of body mass exceeds ~2%, endurance exercise performance declines under hot conditions (Cheuvront et al., 2003).

Athletes may use practical and effective pre-cooling strategies and devices to alleviate the adverse effects of hot conditions, and improve their exercise performance and capacity (Gibson et al., 2019; Périard et al., 2016). These strategies aim at reducing the body temperature before exercising in a hot environment (Bongers et al., 2014; Cotter et al., 2001; Marino, 2002; Montain and Coyle, 1992; Wohlfert and Miller, 2019). Pre-cooling strategies can decrease thermoregulatory strain, cardiovascular strain, improve thermal perception (Cheuvront et al., 2003; Siegel et al., 2011), and subsequently reduce the level of hyperthermia and dehydration (Montain and Coyle, 1992). Pre-cooling can attenuate the total sweat loss under hot conditions (Faulkner et al., 2019; Wilson et al., 2002). However, was there an unequivocal conclusion that pre-cooling could improve hydration when the total sweat loss decreased. Hence, the effects of pre-cooling on the hydration require further research.

Pre-cooling strategies are classified according to the application of the cooling technique used, that is, whether applied externally (the surface of the body) or internally (into the body through the mouth and/or nose) (Ross et al., 2013). Internal cooling can increase body heat storage capacity and reduce heart rate by decreasing the core temperature (Marino, 2002; Quod et al., 2006; Wegmann et al., 2012). External cooling can reduce blood flow in the skin (James et al., 2015) via superficial blood vessel constriction and increase the heat gradient from the core to the skin, thereby facilitating heat loss (Cheuvront et al., 2010).

When compared with non-cooling strategies, the use of internal pre-cooling strategies showed increase in time to exhaustion (19–31.9%) in highly trained athletes (>55 mL/kg/min of VO_2max), while external cooling elicited increase in distance (3.6–13.1%) covered by highly trained athletes (Rodríguez et al., 2020). However, large volumes of fluid consumed before high-intensity exercise could result in a high risk of gastrointestinal upset or discomfort (Ross et al., 2011), which could lead to shivering and increase glycogen utilization (Jacobs et al., 1994). Besides that, the stronger cooling power of pre-cooling protocol (exposure more part of body, longer period, and lower temperature) could induce muscle stiffness and uncomfortable sensations of coldness (Yeargin et al., 2006). Additionally, the mixed-method cooling (combination of internal and external strategies) vs. internal or external strategies did not exhibited any significant benefit, although a mixed-method approach showed a favourable trend to reduce thermal and improve performance in highly trained athletes (Rodríguez et al., 2020). To date, none of the studies on this topic have focussed on the effect of mixed-method cooling on the time to exhaustion under heat stress conditions in highly trained athletes (Aldous et al., 2019; Munoz et al., 2012; Ross et al., 2011).

Using this underlying rationale, the aim of this study was to determine whether a mixed-method cooling would exert potential ergogenic effects on the time to exhaustion (a measure of exercise capacity or endurance) under heat stress conditions in highly trained athletes. The other objective was to investigate whether the modified pre-cooling intervention, which did not cause gastrointestinal discomfort or shivering (Daries et al., 2000), could improve exercise performance while exercising in the heat. We hypothesised that pre-cooling approaches, including internal, external and mixed-method cooling strategies, could prolong the duration of exercise at a speed of 80% of VO_2max under heat stress conditions in highly trained athletes.

Methods

Participants

Seven male college athletes volunteered to participate in this study. No participant is acclimatised to the heat conditions. The mean age, stature, mass, body fat, and VO_2max were 20.3 ± 1.3 years old, 1.83 ± 0.04 m, 84.3 ± 4.7 kg, $12.7 \pm 4.5\%$, and 60.71 ± 4.07 mL·kg⁻¹·min⁻¹ (55–66 mL·kg⁻¹·min⁻¹), respectively. All participants were rugby players who regularly trained 5 times per week (20 hours per week). Their training history was 15.1 ± 2.7 years (10–18 years) of training. All procedures complied with the guidelines prescribed by the Declaration of Helsinki, 2013. Permission was obtained from the ethical committee of the Chinese Institute of Sport Science (the Fundamental Research Foundation of the China Institute of Sport Science (20-06) and the National Natural Science Foundation of China (11775059)). All volunteers provided verbal and written informed consent prior to this investigation.

Experimental procedures

All experimental procedures were conducted in the laboratory during the spring season (March to April) in Beijing, China. Athletes were in a fully hydrated state before arrival to the laboratory and refrained from strenuous exercise, caffeine intake, and alcohol consumption for 24 h prior to the experiments. Every volunteer was advised to replicate food and fluid supplementation for 24 h before the experiments. Trials were performed at 7-day intervals and were conducted at the same time of the day to eliminate the effects of circadian rhythm. The integrated experimental plan is shown in Figure 1.

All volunteers completed a graded exercise test (GXT) on a treadmill (Pulsar, H/P/Cosmos Sports & Medical, Nussdorf Traunstein, Germany) to get the VO_2max , which is the maximal rate of whole-organism oxygen consumption during the GXT (Daries et al., 2000; Millet et al., 2003), at normal temperature (27 °C, 40% RH) prior to the commencement of the experimental trials. The GXT included a continuous protocol on the treadmill as per previously described methods (Metaxas et al., 2005). Briefly, the participants began running at 8.0 km/h and 1.0% grade for 3 min. The speed was increased by 2.0 km/h every 3 min. When the treadmill speed reached 14.0 km/h, the grade increased to 2.0%. Thereafter, the speed continued to increase by 2.0 km/h every 3 min until a stage with a speed of 20.0 km/h was reached. Once this stage was reached (20.0 km/h and 2.0% grade), the treadmill speed and grade remained unchanged until volitional exhaustion. The criteria for reaching VO_2max were coincided with at least 2 as follows: 1) HR recorded a value 220-age; 2) the respiratory exchange ratio was higher than 1.05; 3) the RPE reached 19–20; 4) the participant could not keep up with the exercise intensity (Katica et al., 2017; Krishnan et al., 2017). Following the GXT, the participants rested in the laboratory. After the resting period, they had a short run at a speed of 80% VO_2max (3 min) in order to familiarise with the speed.

All experimental procedures (control and pre-cooling conditions) were conducted at the Special Environment Laboratory (CNRO Science Technology, Tianjin, China) at the Emphasis Laboratory of the General Administration of Sport of China. The experimental procedures were performed in a hot and humid environment (38.1 ± 0.6 °C, $55.3 \pm 0.3\%$ RH), in a randomised order. At the beginning of each experimental session, in a 27 °C, and 40% RH environment, a 30-min warm-up protocol at 60% VO_2max intensity was performed by each volunteer. After this warm-up, all physiological, psychological, and biochemical parameters were recorded for 5 min before pre-cooling (*before*). Thereafter, the 30 min of control procedure and the pre-cooling protocol were performed in a hot and humid environment (38.1 ± 0.6 °C; $55.3 \pm 0.3\%$ RH) under four separate conditions, as follows: 1) volunteers sat with no pre-cooling and ingesting nothing (CON); 2) volunteers wore a vest cooled to a temperature of 4 °C (Vest) as the external cooling method without ingesting any fluid; 3) volunteers ingested a beverage cooled to a temperature of 4 °C at a dose of 2.3 mL/kg body mass (Beverage) as the internal cooling method; and 4) the external and internal cooling methods were performed simultaneously (Mix).

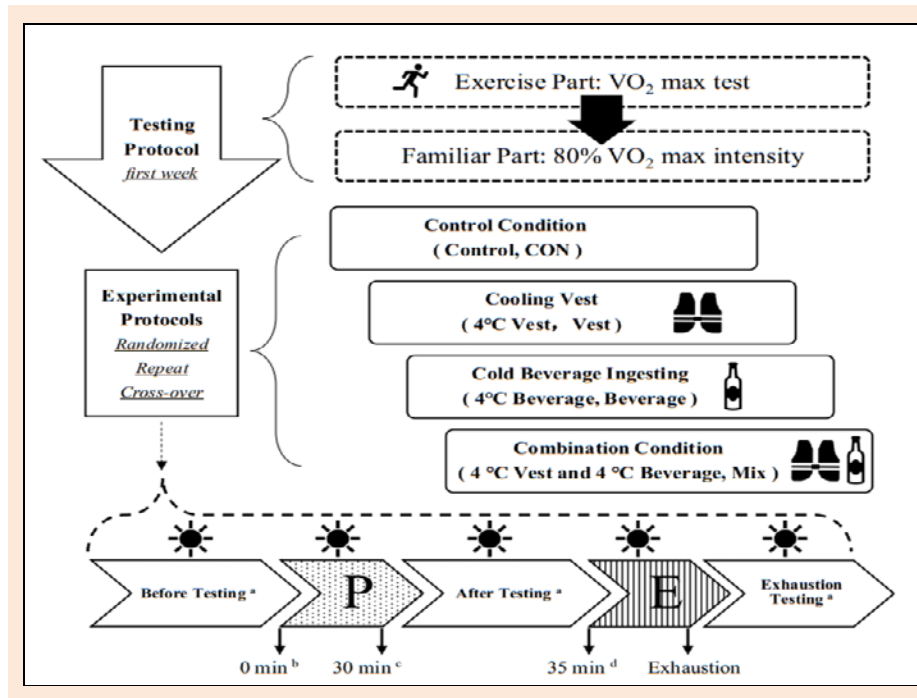


Figure 1. Flow-process diagram of the entire precooling research, which includes the procedure and measurements ($n = 7$). **P**; The 30-min precooling maneuvers include different interventions and one control. **E**; The exercise protocol would be terminated when the subject was exhausted. a. All data recorded within 5 min. b. Precooling protocols started. c. Precooling protocols terminated. d. Exercise protocol began.

The vest (2.4 kg, Raxwell RAX-201, SYSBEL, Shanghai, China) could deposit four sealed cooling packs containing phase change material (0.4 kg, Raxwell RAX-2011, Sysbel, China) in two anterior and posterior pockets and was tested using an infrared thermometer. The cooling beverage (6% carbohydrate, Gatorade, PepsiCo, New York, USA) was ingested at the beginning of pre-cooling, at 10 min and 20 min of pre-cooling period. When the 30-min pre-cooling procedures were completed, the physiological parameters were recorded for a 5-min (*after*). Thereafter, the exercise protocol was performed at a speed of 80% VO_{2max} on a treadmill until volitional exhaustion (Figure 1). When the exercise regimen was completed, the physiological parameters were recorded once again (*exhaustion*).

Measurement

Height (GMCS-IV stadiometer, shanghai, China, accuracy of 0.01 m) and weight (RCS-2 electronic scales, shanghai, China, accuracy of 0.01 kg) of each participant were measured in the laboratory. Fat mass was determined using dual-energy X-ray absorptiometry scan (DXA, Lunar Prodigy; General Electric, Madison, WI, USA). Expired air samples were collected via a metabolic cart (Cortex Biophysik Metalyzer 3B, Leipzig, Germany) to determine VO_{2max} , which was calculated as the average of the VO_{2} values measured over the final 30 s.

Urine samples were collected before pre-cooling and after exercise to determine the urine specific gravity (USG) by using a hand-held refractometer (MASTER-URC/NM, ATAGO, Japan). After removing sweat with a towel, the body mass was determined using a weighting scale (In-Body 370, InBody, Korea). The whole body sweat loss (SL) was calculated using a modified equation corrected for fluid ingestion, urine excretion, and blood removal

(Stevens et al., 2016). Thus, the SL (L) can be estimated using Eq.1 mentioned below:

$$SL = W_0 - W + F - U - B \quad (1)$$

Where, W_0 is the initial body mass measurement of pre-cooling (W_{Before}). W is the final body mass measurement of exercise ($W_{Exhaustion}$). F indicates the fluid ingested, U is urine volume, and B is blood removed.

Heart rate (HR) was recorded with a chest HR sensor monitor (H10, Polar, Finland) and a sports watch (V800, Polar, Finland) throughout the pre-cooling and exercise protocol. T_{core} was measured with a thermometer (T12, DEDAKJ, Germany), self-inserted in the volunteer's rectum approximately 10 cm past the edge of the anal sphincter and securely positioned. T_{skin} was recorded with thermocouple thermometers (YHT309, ShenZhen YuanHeng-Tong Technology, China) at the following locations: forehead ($T_{forehead}$), left upper chest (T_{chest}), right scapula ($T_{scapula}$), right upper arm ($T_{upper-arm}$), left lower arm ($T_{lower-arm}$), left hand (T_{hand}), right anterior thigh (T_{thigh}), and left calf (T_{calf}). Mean T_{skin} was estimated as (Faulkner et al., 2019):

$$T_{skin} = 0.07T_{forehead} + 0.175T_{scapula} + 0.175T_{chest} + 0.07T_{upper-arm} + 0.07T_{lower-arm} + 0.05T_{hand} + 0.19T_{thigh} + 0.2T_{calf} \quad (2)$$

The rating of perceived exertion (RPE) and thermal sensations (TS) were recorded every 5 min during the pre-cooling period and exercise protocol with the 6–20 RPE scale (6: least effort, 20: maximum effort) and the 0–8 TS scale (0: unbearably cold, 8: unbearably hot) (Bedny and Seglin, 1997; Gagge et al., 1969). Blood was collected from the fingertip to determine the blood lactic acid ([Bla]) concentration using a Lactate Scout portable lactate test analyser (EKF Diagnostics, Berlin, Germany). Blood sample

(5 mL) were collected from the antecubital vein of the arm. A coagulant was added to all blood samples to separate the serum via centrifugation (X-15R, Beckman, American) at 4 °C for 15 min. The separated serum sample was stored at -80 °C until determination of sodium (Na⁺) and potassium (K⁺) concentrations. The concentrations of sodium and potassium were tested using an ion selective electrode (ISE, Roche Group).

Statistical analyses

The data were non-normally distributed. Consequently, the Kruskal-Wallis H test used to test the differences between variables, which served as the primary test for assessment of potential statistical differences. The Kruskal-Wallis H test was used to determine differences in HR, T_{core}, T_{skin}, Na⁺, K⁺, RPE, TS, and [Bla] values before and after pre-cooling and at the end of exercise, SL during pre-cooling and exercise, and time to exhaustion and USG at the point of exercise exhaustion, between the four pre-cooling trials. When a difference was detected, the Cohen’s d effect sizes (ES) were then verified to evaluate the level/degree of difference. The following criteria were used: an ES <0.2 was classified as ‘trivial’, ES = 0.2–0.49 as ‘small’, ES = 0.5–0.79 as ‘moderate’, and ES >0.8 as a ‘large’ effect (Hopkins et al., 2009). Data were analysed using Statistics SPSS 22.0 for Windows (Version 22, SPSS Inc., Chicago, Illinois, USA) with statistical significance set at p < 0.05 and high statistical significance set at p < 0.01. All data are presented as mean ± standard deviation (S.D.).

Results

Performance

The time to exhaustion was longer in Mix (648.43 ± 77.53 s) compared to that in CON (509.14 ± 54.57 s, H = 9.120, p = 0.016, ES = 0.72, Figure 2). There was no difference between other pre-cooling trials and CON (Vest: 582.43 ± 86.81 s, p = 0.81, Beverage: 591.57 ± 99.94 s, p = 0.493).

Physiological responses

No significant differences were observed in VO₂ at exhaustion among the four trials (CON: 56.71 ± 5.45 mL·kg⁻¹·min⁻¹, Vest: 57.29 ± 3.77 mL·kg⁻¹·min⁻¹, Beverage: 57.86 ± 5.96 mL·kg⁻¹·min⁻¹, Mix: 58.14 ± 3.53 mL·kg⁻¹·min⁻¹, H = 0.879, p = 0.831).

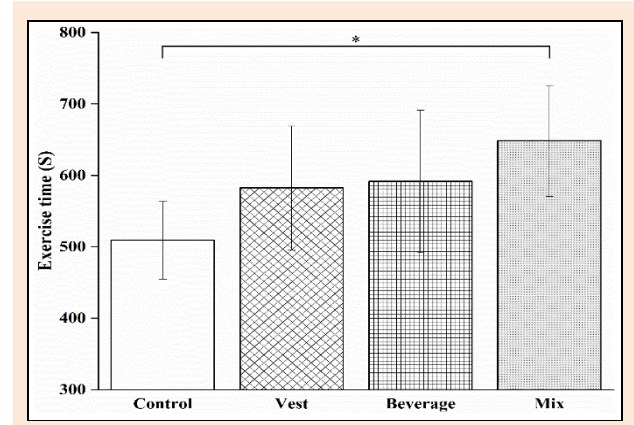


Figure 2. Time to exhaustion at 80% of VO₂max in a hot environment (38.1 ± 0.6 °C, 55.3 ± 0.3 % relative humidity, mean ± S.D., n = 7). * p < 0.05 vs. CON

In terms of the HR responses, there were no significant differences (H = 4.680, p = 0.197, Table 1) between Vest and CON, Beverage and CON, Mix and CON and among the pre-cooling trials at the end of the exercise protocol.

Thermoregulatory responses

T_{core} did not differ among the conditions before pre-cooling (H = 0.392, p = 0.942, Table 2) There were no significant differences among the four different conditions (H = 5.159, p = 0.161) after pre-cooling. There were no significant differences between the trials (H = 0.046, p = 0.997) at the time of exhaustion.

Table 1. Heart rate (HR) before and after pre-cooling and at the end of exercise in a hot environment. Data are means ±SD, n = 7.

Variable	Control	Vest	Beverage	Mix
HR <i>before</i> (bpm)	68 ± 7	69 ± 3	69 ± 10	67 ± 7
HR <i>after</i> (bpm)	82 ± 7	79 ± 5	77 ± 7	76 ± 8
HR <i>exhaustion</i> (bpm)	197 ± 5	192 ± 8	190 ± 7	187 ± 9

Table 2. Rectal (T_{core}) and skin temperature (T_{skin}) before and after pre-cooling and at the end of exercise in a hot environment. Data are means ±SD, n = 7.

Variable	Control	Vest	Beverage	Mix
T _{core} <i>before</i> (°C)	36.93 ± 0.29	36.94 ± 0.14	36.91 ± 0.32	37.01 ± 0.24
T _{core} <i>after</i> (°C)	37.31 ± 0.29	37.19 ± 0.33	37.03 ± 0.35	37.01 ± 0.27
T _{core} <i>exhaustion</i> (°C)	38.63 ± 0.27	38.63 ± 0.15	38.61 ± 0.22	38.60 ± 0.26
T _{skin} <i>before</i> (°C)	34.67 ± 0.33	34.48 ± 0.59	34.59 ± 0.46	34.53 ± 0.89
T _{skin} <i>after</i> (°C)	35.62 ± 0.20	35.16 ± 0.40	35.37 ± 0.39	35.09 ± 0.24
T _{skin} <i>exhaustion</i> (°C)	36.64 ± 0.38	36.56 ± 0.40	36.60 ± 0.48	36.45 ± 0.43

Table 3. Whole body sweat loss (SL) during pre-cooling and exercise at 80% of VO₂max in a hot environment. Data are means ±SD, n = 7.

Variable	Control	Vest	Beverage	Mix
SL <i>pre-cooling</i> (L)	0.44 ± 0.18	0.36 ± 0.15	0.27 ± 0.13	0.14 ± 0.10**
SL <i>exercise</i> (L)	0.70 ± 0.17	0.67 ± 0.16	0.66 ± 0.19	0.63 ± 0.11

** p < 0.01 vs. Control.

Table 4. Na⁺ and K⁺ concentration before and after pre-cooling and at the end of exercise in a hot environment. Data are means ±SD, n = 7.

Variable	Control	Vest	Beverage	Mix
Na ⁺ before (mmol/L)	137.71±1.38	137.86±1.35	137.43±1.81	137.43±1.51
Na ⁺ after (mmol/L)	138.14±3.24	137.86±1.95	137.57±2.07	137.14±1.21
Na ⁺ exhaustion (mmol/L)	140.14±1.68	139.14±2.61	138.43±2.37	138.14±1.86
K ⁺ before (mmol/L)	3.72±0.17	3.69±0.08	3.71±0.11	3.71±0.16
K ⁺ after (mmol/L)	3.89±0.16	3.85±0.24	3.85±0.17	3.82±0.09
K ⁺ exhaustion (mmol/L)	4.00±0.18	3.93±0.23	3.90±0.17	3.87±0.20

T_{skin} was similar in all conditions before pre-cooling (H = 1.033, p = 0.793, Table 2). After pre-cooling, there were no significant differences between each trial with T_{skin} (H = 9.574, p = 0.691, Table 2). At the time of exhaustion, there were no significant differences between the trials (H = 1.189, p = 0.756).

SL was lower in Mix as compared to that in CON at the end of pre-cooling (H = 11.667, p = 0.009, ES = 0.67, Table 3). At the same time, there were no significant differences between Vest and CON (p = 1.000), Beverage and CON (p = 0.579), Mix and Vest (p = 0.072) and Mix and Beverage (p = 0.706). At the exhaustion of exercise, there was no difference in SL among the four experimental (H = 0.824, p = 0.844, Table 3).

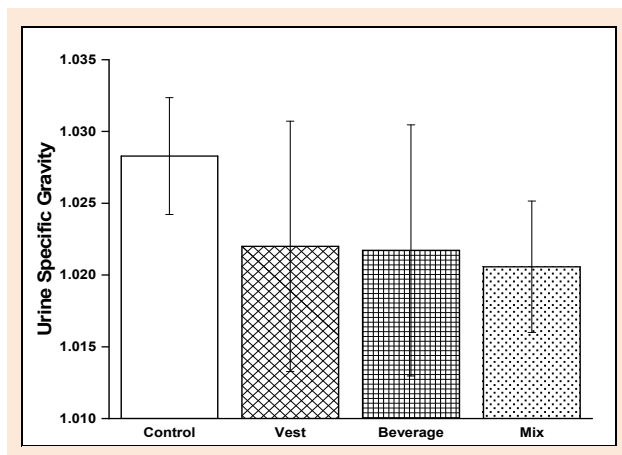


Figure 3. Urine specific gravity (USG) change at the point of exercise exhaustion. Data are means ±SD, n = 7.

Hydration status

At the time of exhaustion, no significant differences

among the trials were observed in USG (H = 1.877, p = 0.202, Figure 3).

Before pre-cooling, the differences in Na⁺ concentrations among the four trials were not significant (H = 0.629, p = 0.890, Table 4). Even after pre-cooling the difference among all trials was not significant (H = 0.306, p = 0.959). At the time of exhaustion, no differences were found (H = 3.587, p = 0.310) among the four different conditions as well.

Before pre-cooling, the differences in K⁺ concentrations among the four trials were not significant (H = 0.122, p = 0.989, Table 4). After pre-cooling, and at the time of exhaustion, no significant differences among the trials were observed in K⁺ concentrations (H = 0.448, p = 0.398 and H = 1.514, p = 0.679).

Rating of perceived exertion and thermal sensations

No significant difference was observed in RPE among the trials at the time of exhaustion (H = 1.381, p = 0.710, Table 5).

No significant differences were observed in the TS among the four trials before and after pre-cooling (H = 0.269, p = 0.966 and H = 7.013, p = 0.071, respectively, Table 5). At the time of exhaustion, there were no significant differences in TS in any condition (H = 2.250, p = 0.522, Table 5).

Blood lactic acid levels

Before pre-cooling, the differences in [Bla] levels among the four trials were also not significant (H = 3.392, p = 0.862). After pre-cooling, [Bla] level in the mixed trial was significantly lower than that in CON (H = 13.396, p = 0.005, ES = 0.87; Table 6). At the end of exhaustion, [Bla] level in the mixed trial was significantly lower than that in CON (H = 16.932, p = 0.003, ES = 0.89; Table 6).

Table 5. Rating of perceived exertion (RPE) and thermal sensations (TS) before and after pre-cooling and at the end of exercise in a hot environment. Data are means ±SD, n = 7.

Variable	Control	Vest	Beverage	Mix
RPE before	9.4±2.0	9.6±1.0	9.4±1.6	9.4±1.4
RPE after	11.0±1.5	10.9±2.3	10.9±1.4	10.7±0.8
RPE exhaustion	17.9±1.1	17.6±1.9	17.6±1.5	17.1±0.9
TS before	5.0±0.6	4.9±0.9	4.9±0.9	4.9±0.7
TS after	6.0±0.6	5.9±0.4	5.6±0.5	5.3±0.5
TS exhaustion	6.9±0.4	6.9±0.4	6.9±0.4	6.9±0.4

Table 6. Blood lactic acid ([Bla]) concentration before and after pre-cooling and at the end of exercise in a hot environment. Data are means ±SD, n = 7.

Variable	Control	Vest	Beverage	Mix
[Bla] before (mmol/L)	1.74±0.48	1.80±0.22	1.60±0.08	1.61±0.22
[Bla] after (mmol/L)	2.87±0.22	2.27±0.80	1.89±0.29	1.61±0.18**
[Bla] exhaustion (mmol/L)	8.71±0.61	7.59±1.20	6.64±1.36	5.18±1.13**

** p < 0.01 vs. CON

Discussion

Our primary findings show that 30 min pre-cooling strategies with a mixed-method improved the high-intensity running capacity in a hot and humid environment. The mixed-method strategy that combines external and internal cooling may be more effective than the others in improving performance.

This finding is consistent with previous studies that used a mixed pre-cooling strategy. Aldous and colleagues (2019) reported that exercise performance increased by simultaneous ingestion of 7.5 g/kg body mass of $-1\text{ }^{\circ}\text{C}$ ice slurry and usage of $-14.0 \pm 4.6\text{ }^{\circ}\text{C}$ ice packs. Brade and colleagues (2014) concluded that after 30 min of combined body-cooling techniques (internal cooling: jacket; external cooling: 7.5 g/kg ice slurry), the repeated sprint cycling performance improved compared with a single cooling method. The effects of internal (ingested 7.5 g/kg body mass of $-1\text{ }^{\circ}\text{C}$ ice slurry) and external (immersed in $24\text{ }^{\circ}\text{C}$ cold water) pre-cooling measures on exercise performance in the study conducted by Siegel et al. (2011) were identical to those in the current study. In the study of Thomas and colleagues (2019) reported that a combination of internal and external cooling techniques did not offer benefits to high-intensity exercise despite the fact that this pre-cooling method reduced HR, T_{core} , and TS. This contradiction among the studies may be due to differences in exercise protocols. In the study by Thomas and colleagues, a self-paced exercise protocol was adopted, as opposed to our study, where in exercise at a fixed intensity was considered.

Evidence regarding combination of external and internal pre-cooling strategies confirmed that mix-method could augment the reduction in body temperature compared with the application of a single method (Bongers et al., 2014). It is logical that external pre-cooling would lower the core temperature and thus may benefit subsequent exercise performance in the heat (Castle et al., 2006). Internal pre-cooling (ice slurry) could contribute to a reduction in heat storage in the body and an increase in the capacity of heat storage (Jay and Morris, 2018). Therefore, combining external and internal cooling methods provided cooling effect through thermoregulatory factors (Gibson et al., 2019), thereby favourably affecting exercise performance during intense exercise in a hot and humid environment.

Wearing a vest cooled to a temperature of $4\text{ }^{\circ}\text{C}$, as utilized in our study, can transfer and dissipate excessive heat by increasing conduction and evaporation to temporarily offset the thermal equilibrium (Ross et al., 2013). A mixed-method approach may ameliorate thermoregulation stress to maintain homeostasis for enhancement of exercise performance. The high T_{core} temperature is a major factor to limit to the exercise performance (González-Alonso et al., 1999; Gregson et al., 2002), which supports the experimental result that the T_{core} was same at the exhaustion state. Even though no difference occurred in T_{core} at the end of exhaustion, the rate of the T_{core} rise during exhaustion exercise was lower in this research. The mix pre-cooling

could forbid the T_{core} to rising too fast, which could maintain T_{core} in a relative lower level and delay the fatigue happened during exercise. Therefore, mix pre-cooling could improve the ability of attenuating the arising of T_{core} drastically without changing, which maintain the homeostasis in a dynamic stable state. Besides that, whether the mix pre-cooling could prolong the time of exercise through increasing critical T_{core} during exercise need further research.

Our findings of improved performance in the mix condition were observed despite similar rectal and skin temperatures after pre-cooling as well as at the point of exhaustion. Previous research has shown that external pre-cooling does not always result in a reduction in the core temperature (James et al., 2015). Duffield et al. (2010) reported performance benefits following external cooling techniques that did not generate reduction in T_{core} . During exercise, increasing of the T_{skin} prematurely would impede the heat dissipation (Cotter et al., 2001). In this study, eight locations were used for the calculation of T_{skin} (Faulkner et al., 2019), which was more precise than a 4 site T_{skin} equation (Aldous et al., 2019). In this research, the same pre-cooling duration and the same temperature of the environment contribute the result of T_{skin} , which were no difference between each one. The difference in T_{skin} was similar to the study of Griggs and Havenith (Griggs et al., 2017).

Our study demonstrates improved performance in hot conditions with the mixed-method approach pre-cooling. Attenuating increases in skin temperature may facilitate the transfer of heat from the body's core to the periphery, although this was not translated into a lower core temperature in our study. A lower skin temperature may also contribute to a better cardiovascular function. Indeed, the HR at exhaustion was lower in the mixed method compared to that observed in the control in the trend (CON: 197.3 ± 5.0 bpm v.s. Mix: 187.3 ± 8.8 bpm) in our study, which is indicative of a possibility of a better cardiovascular function. It appears that T_{skin} and skin wetness are important mediators of behavioural thermoregulation and may explain the extension of exercise time in our subjects. In a previous study (Schlader et al., 2011), thermal sensation was reported to be associated with skin temperature.

Wohlfert and Miller (2018) also reported the same results that there was no clinically meaningful differences between pre-cooling modalities in RPE and TS. Pre-cooling could create a heat sink to store more heat and blunt the body temperature to rise, which could attenuate the adverse effects, the RPE, and the TS (Rasmussen et al., 2010). During exercise, the heat stress improving lead the beta band cortical oscillation declined which damage the cerebral blood flow and metabolism through the cortical deactivation, finally, all those influence and change were verified to contribute to a higher RPE and TS (Nybo et al., 2014). Researches also suggested that thermoregulatory behavior is regulated by the perceived exertion before pronounced elevation in the thermo-physiological parameter during exercise (Rasmussen et al., 2010). As such, cool water consumed intermittently may alter the sensory feedback and attenuate the inhibitory effect on neural drive, thus improving perceptual measures and exercise performance without

an obvious cooling effect (Watkins et al., 2018).

One of the limitations of our study is that only included 7 subjects. There is no doubt that a higher number of participants would strengthen our findings. Nevertheless, our participants were well-trained, and our findings could be used by competitive athletes. Another one of the limitations is that the fixed intensity exercise protocol in laboratory, even exhaustion protocol could measure the exercise capacity, still limits the practice application in real field. Another limitation is that we only recruited male participants. The extension of these research questions to the female population would increase the significance and impact of the results. The strength of our study relies on the fact that we tested our participants in an environment that was more stressful than that expected during the Tokyo Summer Olympic Games. Indeed, previous data on climatic conditions of Tokyo in July and August indicate a WBGT of 27 ± 2.5 °C, which is lower than the estimated WBGT of 34 °C recorded in our study. Therefore, our findings may be beneficial to the athletes preparing for the Olympic Games in 2021.

Conclusion

In the current study, 30-min pre-cooling strategies, simultaneous (Mix) could enhance the duration of 80% $\dot{V}O_2\text{max}$ high-intensity aerobic exercise in hot and humid conditions. It could be inferred that mix pre-cooling could suppress the increase in temperature during exercise in the hot conditions. Concurrently, mixed pre-cooling could decrease the sweat loss to delay the water loss during the exercise in the heat. Additionally, pre-cooling could ameliorate the cardiovascular strain during exercise. Finally, combination of external and internal pre-cooling provided a cooling effect through thermoregulatory factors (forbid the indexes to rising too fast), thereby enhancing exercise performance favourably in a hot and humid environment.

Furthermore, combination of beverage consumption and vest wearing, which are portable and inexpensive approaches, may be recommended to players and practitioners prior to training sessions and match play events to counter the challenge of heat stress.

Acknowledgements

The authors would like to thank the players of the school team of China Agricultural University, who volunteered to participate in this research, and the Emphasis Laboratory of General Administration of Sport of China at the Chinese Institute of Sport Science and its staff for allowing the use of its laboratory and facilities. We extend our gratitude to the students of the Chinese Institute of Sport Science as well. The study complied with the laws of the country of the authors' affiliation. The authors have no conflicts of interest to declare.

References

- Aldous, J.W.F., Christmas, B.C.R., Akubat, I., Stringer, C.A., Abt, G. and Taylor, L. (2019) Mixed-methods pre-match cooling improves simulated soccer performance in the heat. *European Journal of Sport Science* **19**, 156-165.
- Bedny, G. and Seglin, M. (1997) The Use of Pulse Rate to Evaluate Physical Work Load in Russian Ergonomics. *American Industrial Hygiene Association Journal* **58**, 375-379.
- Bongers, C., Thijssen, D., Veltmeijer, M., Hopman, M. and Eijssvogels, T. (2014) Precooling and percooling (cooling during exercise) both improve performance in the heat: a meta-analytical review. *British Journal of Sports Medicine* **49**, 377-384.
- Brade, C., Dawson, B. and Wallman, K. (2014) Effects of different precooling techniques on repeat sprint ability in team sport athletes. *European Journal of Sport Science* **14**, S84-S91.
- Castle, P., Macdonald, A., Philp, A., Webborn, N., Watt, P. and Maxwell, N. (2006) Precooling leg muscle improves intermittent sprint exercise performance in hot, humid conditions. *Journal of Applied Physiology* **100**, 1377-1384.
- Cheung, S. and Sleivert, G. (2004) Multiple Triggers for Hyperthermic Fatigue and Exhaustion. *Exercise and Sport Sciences Reviews* **32**, 100-106.
- Cheuvront, S., Kenefick, R., Montain, S. and Sawka, M. (2010) Mechanisms of aerobic performance impairment with heat stress and dehydration. *Journal of Applied Physiology* **109**, 1989-1995.
- Cheuvront, S., Carter, R. and Sawka, M. (2003) Fluid balance and endurance exercise performance. *Current Sports Medicine Reports* **2**, 202-208.
- Choo, H., Nosaka, K., Peiffer, J., Ihsan, M. and Abbiss, C. (2017) Ergogenic effects of precooling with cold water immersion and ice ingestion: A meta-analysis. *European Journal of Sport Science* **18**, 170-181.
- Cotter, J., Sleivert, G., Roberts, W. and Febbraio, M. (2001) Effect of pre-cooling, with and without thigh cooling, on strain and endurance exercise performance in the heat. *Comparative Biochemistry and Physiology. Part A, Molecular & Integrative Physiology* **128**, 667-677.
- Daries, H.N., Noakes, T.D. and Dennis, S.C. (2000) Effect of fluid intake volume on 2-h running performances in a 25 degrees C environment. *Medicine and Science in Sports and Exercise* **32**, 1783-1789.
- Duffield, R., Green, R., Castle, P. and Maxwell, N. (2010) Precooling can prevent the reduction of self-paced exercise intensity in the heat. *Medicine and Science in Sports and Exercise* **42**, 577-584.
- Faulkner, S.H., Broekhuijzen, I., Raccuglia, M., Hupperets, M., Hodder, S.G. and Havenith, G. (2019) The Threshold Ambient Temperature for the Use of Precooling to Improve Cycling Time-Trial Performance. *International Journal of Sports Physiology and Performance* **14**, 323-330.
- Gagge, A.P., Stolwijk, J.A.J. and Saltin, B. (1969) Comfort and thermal sensations and associated physiological responses during exercise at various ambient temperatures. *Environmental Research* **2**, 209-229.
- Gerrett, N., Kingma, B., Sluijter, R. and Daanen, H. (2019) Ambient Conditions Prior to Tokyo 2020 Olympic and Paralympic Games: Considerations for Acclimation or Acclimatization Strategies. *Frontiers in Physiology* **10**, 414-426.
- Gibson, O., James, C., Mee, J., Willmott, A., Turner, G., Hayes, M. and Maxwell, N. (2019) Heat alleviation strategies for athletic performance: A review and practitioner guidelines. *Temperature* **7**, 3-36.
- González-Alonso, J., Teller, C., Andersen, S.L., Jensen, F.B., Hyldig, T., and Nielsen, B. (1999) Influence of body temperature on the development of fatigue during prolonged exercise in the heat. *Journal of Applied Physiology* **86**, 1032-1039.
- Gregson, W., Drust, B., Batterham, A. and Cable, N. (2002) The effects of pre-warming on the metabolic and thermoregulatory responses to prolonged submaximal exercise in moderate ambient temperatures. *European Journal of Applied Physiology* **86**, 526-533.
- Griggs, K.E., Havenith, G., Paulson, T.A.W., Price, M.J. and Goosey-Tolfrey, V.L. (2017) Effects of cooling before and during simulated match play on thermoregulatory responses of athletes with tetraplegia. *Journal of Science and Medicine in Sport* **20**, 819-824.
- Havenith, G. and Fiala, D. (2016) Thermal Indices and Thermophysiological Modeling for Heat Stress. *Comprehensive Physiology* **6**, 255-302.
- Hopkins, W., Marshall, S., Batterham, A. and Hanin, Y. (2009) Progressive Statistics for Studies in Sports Medicine and Exercise Science. *Medicine and Science in Sports and Exercise* **41**, 3-13.
- Jacobs, I., Martineau, L. and Vallerand, A. (1994) Thermoregulatory Thermogenesis in Humans during Cold Stress. *Exercise and Sport Sciences Reviews* **22**, 221-250.
- James, C., Richardson, A., Watt, P., Gibson, O. and Maxwell, N. (2015) Physiological responses to incremental exercise in the heat following internal and external precooling: Comparison of internal and external cooling. *Scandinavian Journal of Medicine & Science in Sports* **25**, 190-199.

- Jay, O. and Morris, N. (2018) Does Cold Water or Ice Slurry Ingestion During Exercise Elicit a Net Body Cooling Effect in the Heat? *Sports Medicine* **48**, 17-29.
- Katica, C., Wingo, J., Herron, R., Ryan, G., Bishop, S. and Richardson, M. (2017) Impact of upper body precooling during warm-up on subsequent time trial paced cycling in the heat. *Journal of Science and Medicine in Sport* **21**, 621-625.
- Kenefick, R. (2018) Drinking Strategies: Planned Drinking Versus Drinking to Thirst. *Sports Medicine* **48**, 31-37.
- Krishnan, A., Singh, K., Sharma, D., Upadhyay, V. and Singh, A. (2017) Effect of wrist cooling on aerobic and anaerobic performance in elite sportsmen. *Medical Journal Armed Forces India* **74**, 38-43.
- Marino, F. (2002) Methods, advantages, and limitations of body cooling for exercise performance. *British Journal of Sports Medicine* **36**, 89-94.
- Metaxas, T., Koutlianos, N., Kouidi, E. and Deligiannis, A. (2005) Comparative Study of Field and Laboratory Tests for the Evaluation of Aerobic Capacity in Soccer Players. *Journal of Strength and Conditioning Research* **19**, 79-84.
- Millet, G., Libicz, S., Borrani, F., Fattori, P., Bignet, F. and Candau, R. (2003) Effects of increased intensity of intermittent training in runners with differing VO_2 kinetics. *European Journal of Applied Physiology* **90**, 50-57.
- Montain, S. and Coyle, E. (1992) Influence of graded dehydration on hyperthermia and cardiovascular drift during exercise. *Journal of Applied Physiology* **73**, 1340-1350.
- Munoz, C., Carney, K., Schick, M., Coburn, J., Becker, A. and Judelson, D. (2012) Effects of oral rehydration and external cooling on physiology, perception, and performance in hot, dry climates. *Scandinavian Journal of Medicine & Science in Sports* **22**, e115-e124.
- Nassis, G. and Geladas, N. (2003a) Cardiac output decline in prolonged dynamic exercise is affected by the exercise mode. *Pflügers Archiv: European Journal of Physiology* **445**, 398-404.
- Nassis, G. and Geladas, N. (2003b) Effect of water ingestion on cardiovascular and thermal responses to prolonged cycling and running in humans: a comparison. *European Journal of Applied Physiology* **88**, 227-234.
- Notley, S.R., Racinais, S. and Kenny, G.P. (2020) Do sex differences in thermoregulation pose a concern for female athletes preparing for the Tokyo Olympics? *British Journal of Sports Medicine*. 1-2. Online ahead of print.
- Nybo, L. (2008) Hyperthermia and fatigue. *Journal of Applied Physiology* **104**, 871-878.
- Nybo, L. (2011) Brain temperature and exercise performance. *Experimental Physiology* **97**, 333-339.
- Nybo, L., Rasmussen, P. and Sawka, M.N. (2014) Performance in the Heat-Physiological Factors of Importance for Hyperthermia-Induced Fatigue. *Comprehensive Physiology* **4**, 657-689.
- Périard, J., Racinais, S., Timpka, T., Dahlstrom, O., Spreco, A., Jacobsson, J., Bargoria, V., Halje, K. and Alonso, J. (2016) Strategies and factors associated with preparing for competing in the heat: a cohort study at the 2015 IAAF World Athletics Championships. *British Journal of Sports Medicine* **51**, 264-270.
- Quod, M., Martin, D. and Laursen, P. (2006) Cooling athletes before competition in the heat: comparison of techniques and practical considerations. *Sports Medicine* **36**, 671-682.
- Rasmussen, P., Nielsen, J., Overgaard, M., Krogh-Madsen, R., Gjedde, A., Secher, N.H. and Petersen, N.C. (2010) Reduced muscle activation during exercise related to brain oxygenation and metabolism in humans. *The Journal of Physiology* **588**, 1985-1995.
- Reilly, T., Drust, B. and Gregson, W. (2006) Thermoregulation in elite athletes. *Current Opinion in Clinical Nutrition and Metabolic Care* **9**, 666-671.
- Rodríguez, M.A., Piedra, J.V., Sanchez Fernandez, M., Del Valle, M., Crespo, I. and Olmedillas, H. (2020) A Matter of Degrees: A Systematic Review of the Ergogenic Effect of Pre-Cooling in Highly Trained Athletes. *International Journal of Environmental Research and Public Health* **17**, 2952-2966.
- Ross, M., Abbiss, C., Laursen, P., Martin, D. and Burke, L. (2013) Precooling Methods and Their Effects on Athletic Performance: A Systematic Review and Practical Applications. *Sports Medicine* **43**, 207-225.
- Ross, M., Garvican-Lewis, L., Jeacocke, N., Laursen, P., Abbiss, C., Martin, D. and Burke, L. (2011) Novel Precooling Strategy Enhances Time Trial Cycling in the Heat. *Medicine and Science in Sports and Exercise* **43**, 123-133.
- Schlader, Z., Stannard, S. and Mundel, T. (2010) Human thermoregulatory behavior during rest and exercise - a prospective review. *Physiology & Behavior* **99**, 269-275.
- Schlader, Z.J., Simmons, S.E., Stannard, S.R. and Mündel, T. (2011) Skin temperature as a thermal controller of exercise intensity. *European Journal of Applied Physiology* **111**, 1631-1639.
- Siegel, R., Maté, J., Watson, G., Nosaka, K. and Laursen, P. (2011) Pre-cooling with ice slurry ingestion leads to similar run times to exhaustion in the heat as cold water immersion. *Journal of Sports Sciences* **30**, 155-165.
- Stevens, C., Kittel, A., Sculley, D., Callister, R., Taylor, L. and Dascombe, B. (2016) Running performance in the heat is improved by similar magnitude with pre-exercise cold-water immersion and mid-exercise facial water spray. *Journal of Sports Sciences* **35**, 1-8.
- Thomas, G., Cullen, T., Davies, M., Hetheron, C., Duncan, B. and Gerrett, N. (2019) Independent or simultaneous lowering of core and skin temperature has no impact on self-paced intermittent running performance in hot conditions. *European Journal of Applied Physiology* **119**, 1-13.
- Vanos, J., Kosaka, E., Iida, A., Yokohari, M., Middel, A., Scott-Fleming, I. and Brown, R. (2018) Planning for spectator thermal comfort and health in the face of extreme heat: The Tokyo 2020 Olympic marathons. *The Science of The Total Environment* **657**, 904-917.
- Watkins, E.R., Hayes, M., Watt, P. and Richardson, A.J. (2018) Practical pre-cooling methods for occupational heat exposure. *Applied Ergonomics* **70**, 26-33.
- Wegmann, Melissa, Faude, Oliver, Poppendieck and Medicine, W.J.S. (2012) Pre-Cooling and Sports Performance: A Meta-Analytical Review. *Sports Medicine* **42**, 545-564.
- Wendt, D., Loon, L. and van Marken Lichtenbelt, W. (2007) Thermoregulation during exercise in the heat: strategies for maintaining health and performance. *Sports Medicine* **37**, 669-682.
- Wilson, T., Johnson, S., Petajan, J., Davis, S., Gappmaier, E., Luetkemeier, M. and White, A. (2002) Thermal regulatory responses to submaximal cycling following lower-body cooling in humans. *European Journal of Applied Physiology* **88**, 67-75.
- Wohlfert, T. and Miller, K. (2019) Precooling, Exertional Heatstroke Risk Factors, and Postexercise Cooling Rates. *Aerospace Medicine and Human Performance* **90**, 12-17.
- Wohlfert, T.M. and Miller, K.C. (2018) Does Precooling With Whole-Body Immersion Affect Thermal Sensation or Perceived Exertion? A Critically Appraised Topic. *Journal of Sport Rehabilitation* **28**, 517-521.
- Yeargin, S.W., Casa, D.J., McClung, J.M., Knight, J.C., Healey, J.C., Goss, P.J., Harvard, W.R. and Hipp, G.R. (2006) Body cooling between two bouts of exercise in the heat enhances subsequent performance. *Journal of Strength and Conditioning Research* **20**, 383-389.

Key points

- Precooling strategies with 30 min independent or simultaneous precooling maneuvers could improve running performance of 80% VO_2max in hot and humid climatic conditions compared with the control condition.
- Wearing a 4°C vest might augment the capacity of heat dissipation by decreasing T_{skin} .
- Ingesting a 4°C beverage might increase the capacity of heat storage and maintain homeostasis by reducing T_{core} , HR and [Bla].
- The mix method-precooling maneuver seems to be more effective than others by maintaining core-to-skin thermal gradient and fortifying heat storage to relieve thermophysiological strain.

AUTHOR BIOGRAPHY



Minxiao XU
Employment
 Exercise Biological Center, China Institute of Sport Science. School of Kinesiology, Shanghai University of Sports
Degree
 PhD Student
Research interests
 Exercise physiology and biochemistry.
E-mail: minxiao@163.com



Zhaozhao WU
Employment
 Exercise Biological Center, China Institute of Sport Science. Physical Education Department, Northwest University
Degree
 Master Degree in Sport Science
Research interests
 Exercise physiology, Exercise biochemistry
E-mail: 290785412@qq.com



Yanan DONG
Employment
 Exercise Biological Center, China Institute of Sport Science. Beijing Institute of Sport Science
Degree
 Master Degree in Sport Science
Research interests
 Exercise physiology, Exercise biochemistry
E-mail: 15092692183@163.com



Chaoyi QU
Employment
 Exercise Biological Center, China Institute of Sport Science. School of sport science, Beijing Sport University
Degree
 PhD Student
Research interests
 Exercise physiology, Exercise biochemistry
E-mail: 1257216185@qq.com



Yaoduo XU
Employment
 Physical Education Department, Northwestern Poly-technical University
Degree
 Master Degree in Economics
Research interests
 Exercise training
E-mail: xuyaoduo@nwpu.edu.cn



Fei QIN
Employment
 Exercise Biological Center, China Institute of Sport Science. School of Physical Education, Jinan University
Degree
 PhD
Research interests
 Exercise physiology, Exercise biochemistry, Special environment with exercise
E-mail: qinfei@ciss.cn



Zhongwei WANG
Employment
 Exercise Biological Center, China Institute of Sport Science. School of Kinesiology, Shanghai University of Sports
Degree
 Master Degree in Sport Science
Research interests
 Exercise physiology, Exercise biochemistry
E-mail: wangzhongwei16@outlook.com



George P. NASSIS
Employment
 Physical Education Department–(CEDU), United Arab Emirates University, Al Ain, Abu Dhabi, United Arab Emirates
Degree
 PhD
Research interests
 Methods to enhance performance, football science
E-mail: georgenassis@gmail.com



Jiexiu ZHAO
Employment
 Exercise Biological Center, China Institute of Sport Science
Degree
 PhD
Research interests
 Exercise fatigue and physiology. Thermoregulation and athletic performance, Heat and special environment with exercise
E-mail: zhaojiexiu@ciss.cn

✉ **Dr Jiexiu Zhao**
 China Institute of Sport Science, No.11, Tiyuguan Road, Dongcheng District, Beijing, China