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Respiratory and Aerobic Responses Associated with Improved Endurance Exercise Performance Following Cold Drink Pre-Cooling in the Heat

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Abstract

This study investigated the physiological responses associated with improved endurance exercise performance following cold drink pre-cooling in the heat, with particular emphasis on respiratory and aerobic responses. Twenty trained male athletes completed three randomized trials: one trial under normothermic conditions with ingestion of a 7.5 mL/kg body weight (BW) ambient-temperature sports drink (Con), and two trials within a hot environment involving administration of either a 7.5 mL/kg BW normothermic sports drink (Non) or a 4 °C pre-cooled sports drink (Pre). Endurance exercise duration, cardiorespiratory function, heart rate (HR), core temperature (T_{core}), and perceptual responses were measured during incremental cycling tests. Endurance exercise duration was significantly shorter in the Non condition than in the Con condition ($p = 0.011$), whereas no significant difference was observed between the Pre and Con conditions ($p = 0.576$). Within the hot environment, exercise duration was significantly longer in the Pre condition than in the Non condition ($p = 0.009$). At 10% of total exercise duration, BF ($p = 0.028$) and HR ($p = 0.044$) were significantly lower in the Pre condition than in the Non condition. At exhaustion, VE_{peak} ($p = 0.013$), BF_{peak} ($p = 0.019$), $PetO_2$ ($p = 0.005$), and HR_{peak} ($p = 0.014$) were significantly higher in the Pre condition. Pre-cooling significantly reduced T_{core} ($p = 0.001$) and thermal sensation ($p < 0.001$) at exercise onset, but these differences were no longer evident at exhaustion. In conclusion, pre-exercise ingestion of a 4 °C sport drink at 7.5 mL/kg BW mitigates heat-induced impairment in endurance exercise performance. These findings indicate that the ergogenic benefits of cold drink pre-cooling are primarily associated with reductions in initial thermal and cardiovascular strain, whereas the higher peak cardiorespiratory values observed at exhaustion likely represent terminal physiological responses rather than underlying mechanisms mediating the performance benefits.

Keywords: pre-cooling; endurance exercise performance; respiratory function; aerobic capacity; hot environments

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1. Introduction

With ongoing global climate change, exercise in hot environments is becoming increasingly unavoidable. Heat stress can substantially impair endurance exercise performance

through a combination of elevated core temperature (T_{core}), increased metabolic strain, hypohydration, and electrolyte disturbances [1,2]. Thermal discomfort is also an important perceptual component of heat-related performance impairment and is commonly assessed using thermal sensation (TS) [3]. Kroesen et al. [4] reported that TS was significantly elevated during exercise in hot compared with temperate environments. However, because TS is inherently subjective, T_{core} is frequently used as an objective physiological indicator of thermal strain [5]. T_{core} increases progressively during exercise and may approach 40 °C, a level at which central thermoregulatory mechanisms have been proposed to limit skeletal muscle power output and exercise capacity [6]. Nevertheless, the existence of a universal critical core temperature that triggers exercise termination remains a matter of debate. In addition, heat-induced increases in skin blood flow may compromise cerebral perfusion, potentially contributing to central fatigue and reduced motor unit recruitment [7], although this proposed pathway has yet to be directly established. Heat stress may also impair aerobic function, with maximal oxygen consumption (VO_{2max}) and peak oxygen uptake (VO_{2peak}) progressively declining as thermal strain increases [1].

Pre-cooling strategies have therefore been widely investigated as an approach to attenuate heat-induced reductions in endurance exercise performance. By lowering pre-exercise T_{core} and increasing the body's capacity to store heat before reaching a critical thermal threshold, pre-cooling may delay the development of hyperthermia and associated fatigue [8]. Pre-cooling can be achieved through internal strategies, such as ingestion of cold fluid or ice slurry [9,10], or external strategies, including exposure to cold air, use of cooling vests, head cooling, and cold-water immersion [11–13]. Internal pre-cooling has been reported to improve ventilatory efficiency and thermal comfort [14], whereas external cooling strategies may attenuate central fatigue and reduce thermal strain [15,16]. Among these approaches, cold-fluid and ice-slurry ingestion are particularly attractive because they are relatively simple, practical, and readily applicable to athletic settings. For example, ingestion of approximately 7.5 g/kg body weight (BW) of ice slurry or cold fluids 30 min before exercise has been reported to improve endurance exercise performance in the heat [17]. However, ice slurry may be associated with gastrointestinal discomfort [14] and can be less convenient to consume, particularly during high-intensity exercise [18]. By comparison, cold beverages are easier to consume and may facilitate heat dissipation. Proposed mechanisms include reductions in brain temperature, increased heat-storage capacity, and attenuation of perceived fatigue, potentially enabling athletes to sustain higher exercise intensities [19,20]. Nevertheless, these proposed physiological mechanisms have yet to be directly established.

Although the thermoregulatory and perceptual effects of internal pre-cooling have been relatively well characterized, its potential influence on respiratory function during endurance exercise in the heat remains insufficiently understood. Heat exposure can induce thermal tachypnea, characterized by an increase in breathing frequency accompanied by reductions in tidal volume, which may compromise pulmonary gas exchange and contribute to reductions in VO_{2max} [21]. Because adequate pulmonary ventilation is essential for maintaining carbon dioxide elimination and oxygen availability during high-intensity exercise, alterations in respiratory function may represent an additional pathway through which pre-cooling influences endurance exercise capacity. Nevertheless, whether cold drink pre-cooling can attenuate heat-induced respiratory limitations and thereby contribute to improved endurance performance has not been adequately established.

The effectiveness of pre-cooling strategies under heat stress has also varied considerably across studies. Several investigations have demonstrated beneficial effects of internal cooling. For example, ingestion of crushed ice or ice slurry has been shown to reduce T_{core} and improve exercise performance under hot conditions [22], while external head cooling initiated 20 min before a 5 km time trial (TT) has also been reported to improve

performance [12]. In contrast, other studies have reported limited or no ergogenic effects of individual cooling modalities. Crushed-ice ingestion 30 min before cycling, for instance, did not significantly affect skin temperature, TS, or exercise performance [22], while 6 min of cold-water immersion failed to attenuate neuromuscular fatigue [23]. Xu et al. [24] similarly reported that isolated pre-cooling using either a cold drink or cooling vest did not improve high-intensity performance, whereas combined different cooling modalities prolonged exercise time and reduced ratings of perceived exertion (RPE) without compromising performance [25]. These inconsistent findings may partly reflect differences in cooling modality, temperature, volume, timing, exercise protocol, and participant characteristics.

Importantly, previous research has primarily examined whether pre-cooling improves endurance performance or alters thermoregulatory and perceptual responses, whereas the respiratory and aerobic mechanisms potentially underlying these performance benefits have received comparatively limited attention. In particular, the responses of minute ventilation (VE), breathing frequency (BF), end-tidal partial pressure of oxygen ($PetO_2$), and VO_2 during progressive exercise may provide important insights into how internal pre-cooling influences cardiopulmonary function under thermal stress. A more comprehensive examination of these responses may help clarify whether the ergogenic effect of cold drink pre-cooling is attributable solely to reduced thermal strain or also involves preservation or enhancement of respiratory and aerobic function.

Therefore, the present study investigated the physiological mechanisms underlying the effects of pre-exercise cold drink ingestion on endurance exercise performance in a hot environment. Specifically, we examined the effects of ingesting a 4 °C sports drink at a dose of 7.5 mL/kg BW on endurance exercise performance, respiratory responses (VE and BF), aerobic responses (VO_2 and $PetO_2$), cardiovascular responses [heart rate (HR)], T_{core}, and perceptual responses during incremental cycling exercise to exhaustion. We hypothesized that cold drink pre-cooling would attenuate the heat-induced decline in endurance exercise performance, partly through reductions in pre-exercise thermal strain and attenuation of heat-related alterations in respiratory and aerobic responses during exercise. In this study, the term "respiratory responses" refers specifically to the ventilatory and gas-exchange variables measured during exercise (VE, BF, and $PetO_2$) and is used to describe physiological responses to exercise rather than direct evidence of changes in respiratory function or capacity.

2. Materials and Methods

2.1. Participants

The required sample size was estimated using G*power (version 3.1; Düsseldorf, Germany). An F-test for repeated-measures ANOVA within factors was selected, with three experimental conditions (Con, Pre, and Non). The calculation was based on an effect size of $f = 0.25$, a Type I error rate of 0.05 ($\alpha = 0.05$), and a statistical power of 0.80. The effect size was adopted from a previous study [26]. The assumed correlation among repeated measures was 0.50, and the nonsphericity correction factor (ε) was set to 1.0. Based on these parameters, the estimated minimum required sample size was 15 participants. To account for potential participant attrition or exclusion, 20 trained male athletes were recruited (age: 21.1 ± 1.6 years; height: 182.7 ± 5.4 cm; BW: 76.7 ± 8.6 kg; training experience: 3.0 ± 1.1 years; Table 1).

Participants were eligible if they met all of the following criteria: (1) good overall health status; (2) competitive status equivalent to a national second-class athlete or higher; (3) no history of skeletal muscle or lower-limb joint injury within the preceding 6 months; and (4) no known cardiovascular or gastrointestinal disorders. All participants were informed of the experimental procedures, potential risks, and study requirements before providing

written informed consent. The study protocol was approved by the Ethics Committee of Sports Science Experiment at Beijing Sport University (protocol code 2023180H) and was conducted in accordance with the principles of the Declaration of Helsinki.

Table 1. Participants' physical characteristics ($n = 20$).

	Mean ± SD
Age (years)	21.1 ± 1.6
Height (cm)	182.7 ± 5.4
Body weight (kg)	76.7 ± 8.6
Training experience (years)	3.0 ± 1.1

2.2. Study Design

A randomized crossover design was employed (Figure 1). Following a familiarization session, each participants completed three experimental conditions: one control condition in a temperate environment with a normothermic sports drink (Con), one hot-environment condition with a normothermic sports drink (Non), and one hot-environment condition with a pre-cooled sports drink (Pre). Thus, each participant served as his own control across experimental conditions.

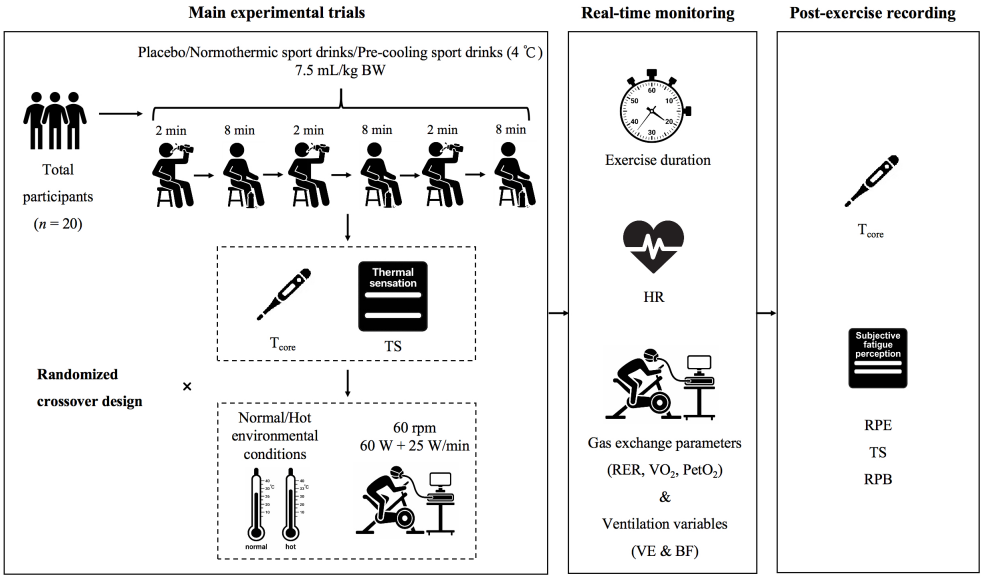


Figure 1. The experimental design. BF, breathing frequency; BW, body weight; HR, heart rate; $PetO_2$, end-tidal partial pressure of oxygen; RER, respiratory exchange rate; RPB, rating of perceived breathlessness; RPE, rating of perceived exertion; rpm, revolutions per minute; T_{core}, core temperature; TS, thermal sensation; VE, minute ventilation; VO_2, oxygen uptake.

The temperate condition was conducted at an ambient temperature of 26.4 ± 0.7 °C, whereas the hot-environment conditions were conducted at 33.8 ± 0.9 °C. Relative humidity was maintained between 40% and 60% across all experimental sessions. A 7-day washout period was implemented between consecutive sessions to minimize potential carryover effects. The hot-environment threshold of >33 °C was selected based on previous evidence indicating that passive heat exposure at this temperature substantially increases T_{core}, skin temperature, and sweating rate, thereby imposing considerable physiological strain and increasing the potential for accumulated fatigue [27,28].

Ambient temperature and humidity were monitored using a handheld environmental detector (OHR-WS60, Fujian Shunchang Hongrun Precision Instrument Co., Ltd., Nanping,

China). To minimize potential confounding effects, participants were instructed to avoid strenuous exercise, alcohol, and caffeine for 24 h before each experimental session, maintain their usual dietary habits throughout the study, and refrain from food intake for at least 2 h before testing. Participants were instructed to maintain their habitual dietary intake and avoid dietary deviations. A 24 h abstinence from caffeine and alcohol, together with a 2 h fasting period, was enforced consistently across all trials, and all testing sessions were scheduled at the same time of day to minimize dietary confounding factors.

During the hot-environment trials, participants consumed either a normothermic sports drink (Non) or a pre-cooling sports drink maintained at 4 °C (Pre). In the temperate control condition, participants consumed the same normothermic sports drink (Con). The sports drink was commercially available (Gatorade, PepsiCo, New York, NY, USA), and all beverages were identical in flavor and nutritional composition. Each 100 g of beverage contained 6 g of carbohydrates, 43 mg of sodium, and 13 mg of potassium. The total beverage volume was standardized according to individual BW at 7.5 mL/kg BW.

2.3. Experimental Procedure

Participants attended the laboratory on four occasions. The first session was used for familiarization with the experimental procedures and incremental cycling protocol. The subsequent three sessions consisted of the randomized experimental conditions described above. Before each experimental trial, anthropometric measurements, including height and BW, were obtained.

Endurance exercise was performed on an electronically braked cycle ergometer (Model No. 20110481, LODE B.V. Medical Technology, Groningen, The Netherlands). Participants maintained a cadence of 60 revolutions per minute (rpm) throughout the incremental test. Exercise began at 60 W, which was increased by 25 W every minute until volitional exhaustion [28]. The saddle and handlebars positions were individually adjusted during the familiarization session and maintained consistently across all subsequent trials. Volitional exhaustion was determined when the first of the following criteria were met [29]: (1) participants reached volitional fatigue despite standardized verbal encouragement, corresponding to a RPE of 19–20; (2) participants were unable to maintain the required cadence of 60 rpm despite repeated attempts; (3) the respiratory exchange rate (RER) reached ≥1.15; (4) a plateau in VO_2 was observed despite a further increase in workload. Among these criteria, (1) and (2) served as test termination endpoints, whereas criteria (3) and (4) were assessed post hoc to verify achievement of maximal exertion and were not used as independent termination triggers. Exercise duration was recorded as the primary performance outcome.

Cardiopulmonary responses were continuously monitored using a metabolic cart (MetalLyzer 3B, CORTEX, Isernhagen, Germany). HR was continuously recorded using a chest-strap monitor (Polar Team pro, Polar Electro Oy, Helsinki, Finland). The electrodes were moistened before application to ensure stable electrical contact throughout exercise. Respiratory and metabolic data were acquired and processed using Cortex MetaSoft® Studio (Version 5.12.0; CORTEX Biophysik GmbH, Leipzig, Germany).

T_{core} was assessed at the tympanic membrane using an infrared thermometer (YHT100, Jiangsu Yuyue Medical Equipment & Supply Co., Ltd., Danyang, China), following standardized procedures previously described in studies involving exercise in the heat [30]. Tympanic temperature is an accepted site for estimating core temperature because the tympanic membrane shares its blood supply with the hypothalamus through the carotid circulation and can provide an estimate of central temperature [30]. Previous research has reported that infrared tympanic thermometry can track changes in body temperature during exercise in the heat with acceptable validity and repeatability when measurements are

standardized [30]. Before each measurement, the left external auditory canal was visually inspected and cleared of cerumen. The thermometer probe was then gently positioned to seal the ear canal, and the device was allowed to equilibrate before each reading. A single reading was obtained at each time point. All measurements were performed on the left ear by the same investigator across all trials and time points under controlled ambient conditions to minimize procedural and environmental variability. Participants were not provided with feedback regarding their exercise performance or physiological responses during any experimental trial. They were informed only when the exercise test had been completed. This approach was adopted to minimize potential expectancy and pacing effects.

The three experimental conditions were defined as follows: (1) the Con condition, in which participants exercised in the temperate environment while consuming the normothermic sports drink; (2) the Pre condition, in which participants exercised in the hot environment after consuming the 4 °C pre-cooled sports drink; and (3) the Non condition, in which participants exercised in the hot environment after consuming the normothermic sports drink. All participants consumed the same beverage volume of 7.5 mL/kg BW in each condition.

Throughout the incremental exercise test, the following variables were assessed: exercise duration; ventilation variables, including VE and BF; gas exchange variables, including RER, VO_2, and $PetO_2$; HR; TS; RPE; and rating of perceived breathlessness (RPB). TS, HR, T_{core}, RPE, and RPB were measured at two points: before exercise onset (pre-exercise baseline) and at volitional exhaustion. RPE and RPB were also assessed every 2 min during exercise. For the matched-time comparison between the two hot-environment conditions, ten time points corresponding to 10% increments of exercise duration were established using the shorter exercise duration across the two hot trials as the reference [31].

The total beverage volume was consumed over a 30 min pre-exercise period in three equal portions. Each portion was consumed over 2 min, with an 8 min interval between consecutive portions, consistent with a previously described protocol [14]. The selected dose of 7.5 mL/kg BW was based on previous evidence indicating that fluid ingestion within the range of approximately 7–14 mL/kg BM may support thermoregulation and exercise performance in hot environments [10].

2.4. Cardiopulmonary and Perceptual Measurements

During the incremental cycling test, VE, BF, VO_2, RER, $PetO_2$, and HR were recorded continuously on a breath-by-breath basis and processed using Cortex MetaSoft® Studio with the software's default settings. To minimize the influence of short-term physiological fluctuations and signal noise, respiratory, metabolic, and HR variables were averaged over the final 10 s of each 60s exercise stage. Specifically, all available valid measurements within this 10 s window were used to calculate the mean value for each variable at each stage. This averaging window was selected to capture responses during the latter part of each stage, after the initial transient response to the preceding 25 W workload increment had largely subsided.

The individual isochronous method was subsequently used to compare physiological responses at matched absolute time points, corresponding to equal percentages of total exercise duration (10% increments), between the two hot-environment conditions [31]. Because cardiopulmonary variables were recorded continuously, values at each 10% time point were extracted directly from the continuous data record by averaging measurements over a ±5 s window centered on the corresponding time point. Thus, time points that did not coincide exactly with the end of a 1 min exercise stage did not require interpolation or substitution with values from an unrelated stage endpoint. For each participant, the shortest exercise duration observed across the Pre and Non conditions were used as the reference

duration. This approach ensured that physiological responses were compared at identical absolute time points during the period in which both conditions were still being performed.

Specifically, the reference exercise duration was divided into ten equal intervals corresponding to 10% increments of total exercise duration. For example, if a participant's shortest exercise duration was 500 s, physiological responses were extracted at 50, 100, 150, 200, 250, 300, 350, 400, 450, and 500 s from both hot-environment trials. This procedure enabled direct comparison of physiological responses at matched time points while avoiding bias arising from the longer exercise duration observed following pre-cooling.

2.5. Statistical Analysis

All statistical analyses were performed using IBM SPSS Statistics (version 25.0; IBM, Chicago, IL, USA). Data are presented as mean ± standard deviation (SD). The Shapiro–Wilk test was used to assess the normality of data distributions.

Endurance exercise duration was designated as the prespecified primary outcome, whereas all respiratory, metabolic, cardiovascular, thermal, and perceptual responses were treated as secondary exploratory outcomes. A repeated-measures ANOVA, with Greenhouse–Geisser correction where appropriate, was used to compare exercise duration across the three experimental conditions. Pairwise comparisons were performed when appropriate.

For comparisons between the two hot-environment conditions (Pre and Non), continuously measured variables (VE, BF, VO_2, $PetO_2$, HR, T_{core}, RPE, RPB, and TS) were analyzed across the isochronous (matched-time) period using a two-factor repeated-measures ANOVA, with condition (Pre and Non) and time (the matched absolute time points) as within-subject factors. This model was used to examine overall differences in physiological responses between conditions and changes in these responses over time. Between-condition comparisons at the two prespecified time points, namely 10% of the reference exercise duration and volitional exhaustion, were further examined using paired-samples t-tests. These time points were defined a priori as the principal comparison points rather than selected post hoc from the repeated-measures series.

As multiple secondary outcomes were examined without formal adjustment for multiplicity, corresponding findings were interpreted cautiously and considered exploratory. Exact *p*-values and Cohen's d were reported for all comparisons. Findings with *p*-values close to the conventional significance threshold, such as those for BF and HR at 10% of exercise duration ($p = 0.028$ and 0.043, respectively), were interpreted with particular caution. All statistical tests were two-sided. Statistical significance for the primary outcome was set at $p < 0.05$, whereas findings for secondary outcomes were considered hypothesis-generating. Effect sizes were calculated using Cohen's d and interpreted as trivial (<0.2), small (0.2–0.6), moderate (0.6–1.2), large (1.2–2.0), and very large (>2.0) [32]. For paired comparisons, Cohen's d was calculated as $d = t/\sqrt{n}$, where n represents the number of paired observations. Figures were generated using GraphPad Prism (version 9.5; GraphPad Software Inc., San Diego, CA, USA).

3. Results

3.1. Effects of Pre-Cooling on Endurance Exercise Performance in the Heat

A significant main effect of condition was observed for endurance exercise duration ($F = 14.054$, $p = 0.001$). As shown in Figure 2, endurance exercise duration was significantly shorter in the Non condition than in the Con condition ($p = 0.011$, Cohen's $d = -0.645$), indicating that exposure to the hot environment impaired endurance exercise performance. In contrast, no significant difference in exercise duration was observed between the Pre and Con conditions ($p = 0.576$, Cohen's $d = -0.238$). Within the hot-environment conditions,

exercise duration was significantly longer in the Pre condition than in the Non condition ($p = 0.009$, Cohen's d = 0.410), indicating that pre-exercise ingestion of the 4 °C sports drink may help mitigate the heat-related reduction in endurance exercise performance.

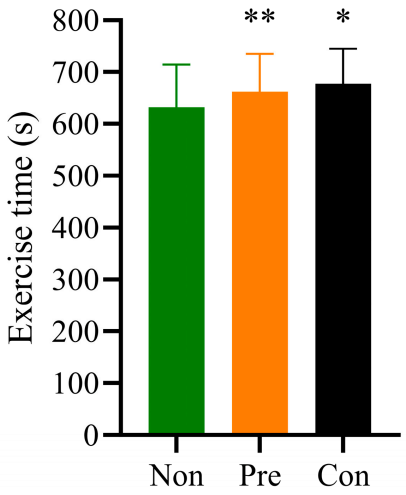


Figure 2. Effects of pre-cooling on endurance exercise performance in the heat. Non, hot environment + normothermic sports drink condition; Pre, hot environment + pre-cooling sports drink (4 °C) condition; Con, normal environment + normothermic sports drink condition. Compared with Non, * $p < 0.05$; ** $p < 0.01$.

Exercise duration (mean ± SD) was 677.9 ± 67.0 s in the Con condition, 632.2 ± 82.4 s in the Non condition, and 662.2 ± 72.6 s in the Pre condition (Figure 2). Compared with the Con condition, exercise duration was reduced by 45.7 s in the Non condition (95% CI: 11.5 to 79.9; $p = 0.011$). Pre-cooling increased exercise duration by 30.0 s relative to the Non condition (95% CI: 8.4 to 51.6; $p = 0.009$). The difference between the Pre and Con conditions was −15.7 s (95% CI: −73.1 to 41.8; $p = 0.576$). Although exercise duration did not differ significantly between these two conditions, this finding should not be interpreted as evidence of equivalence between pre-cooled performance in the heat and performance in the temperate environment.

3.2. Effects of Pre-Cooling on Respiratory Function During Endurance Exercise in the Heat

3.2.1. Minute Ventilation (VE)

VE progressively increased with increasing exercise duration in both hot-environment conditions (Figure 3A). At exhaustion, VE_{peak} was significantly higher in Pre condition than in the Non condition (t = 2.745, $p = 0.013$, Cohen's d = 0.504; Figure 3B). These findings indicate that cold drink pre-cooling was associated with a greater maximal ventilatory response during incremental exercise to exhaustion.

3.2.2. Breathing Frequency (BF)

BF also increased progressively as exercise duration increased (Figure 3C). At 10% of the total exercise duration, BF was significantly lower in the Pre condition than in the Non condition (t = −2.410, $p = 0.028$, Cohen's d = −0.558, Figure 3C). However, at exhaustion, BF_{peak} was significantly higher in the Pre condition than in the Non condition (t = 2.561, $p = 0.019$, Cohen's d = 0.225, Figure 3D). Thus, pre-cooling was characterized by a lower breathing frequency during the early stage of exercise but a higher maximal breathing frequency at exhaustion.

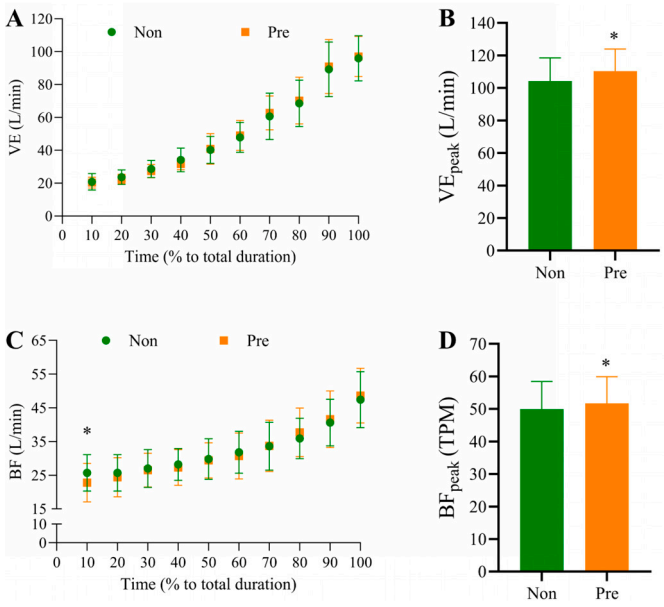


Figure 3. Effects of pre-cooling on respiratory function during endurance exercise in the heat. (**A**) Changes in VE during endurance exercise. (**B**) Differences in VE_{peak} at exhaustion. (**C**) Changes in BF during endurance exercise. (**D**) Differences in BF_{peak} at exhaustion. Non, hot environment + normothermic sports drink condition; Pre, hot environment + pre-cooling sports drink (4 °C) condition; VE, minute ventilation; BF, breathing frequency. Compared with Non, * $p < 0.05$.

3.3. Effects of Pre-Cooling on Aerobic Responses During Endurance Exercise in the Heat

3.3.1. Oxygen Consumption (VO_2)

VO_2 increased progressively with exercise duration in both hot-environment conditions (Figure 4A). No statistically significant difference in VO_{2peak} was observed between the Pre and Non conditions (t = 1.932, $p = 0.068$, Cohen's d = 0.206, Figure 4B). Although VO_{2peak} was numerically higher in the Pre condition, the difference did not reach statistical significance, providing no clear evidence that pre-cooling enhanced peak oxygen uptake.

3.3.2. End-Tidal Partial Pressure of Oxygen ($PetO_2$)

$PetO_2$ increased steadily during the incremental exercise test (Figure 4C). At exhaustion, $PetO_2$ was significantly higher in the Pre condition than in the Non condition (t = 3.218, $p = 0.005$, Cohen's d = 0.719; Figure 4D). Therefore, although cold drink pre-cooling did not significantly alter VO_{2peak}, it was associated with a significantly higher $PetO_2$ at exhaustion.

3.4. Effects of Pre-Cooling on Core Temperature During Endurance Exercise in the Heat

At the onset of exercise, T_{core} was significantly lower in the Pre condition than in the Non condition (t = −4.714, $p = 0.001$, Cohen's d = −1.092, Figure 5A). This finding indicates that ingestion of the 4 °C sports drink effectively reduced pre-exercise thermal strain. However, no significance between-group difference in T_{core} was observed at exhaustion (t = 0.237, $p = 0.815$, Cohen's d = 0.053, Figure 5B).

3.5. Effects of Pre-Cooling on Heart Rate and Perceptual Responses During Endurance Exercise in the Heat

3.5.1. Heart Rate (HR)

HR increased progressively throughout the incremental exercise test (Figure 6A). At 10% of total exercise duration, HR was significantly lower in the Pre condition than in the Non condition (t = −2.158, $p = 0.044$, Cohen's d = −0.328, Figure 6A). In contrast, HRpeak at

exhaustion was significantly higher in the Pre condition than in the Non condition (t = 2.708, $p = 0.014$, Cohen's d = 0.490, Figure 6B).

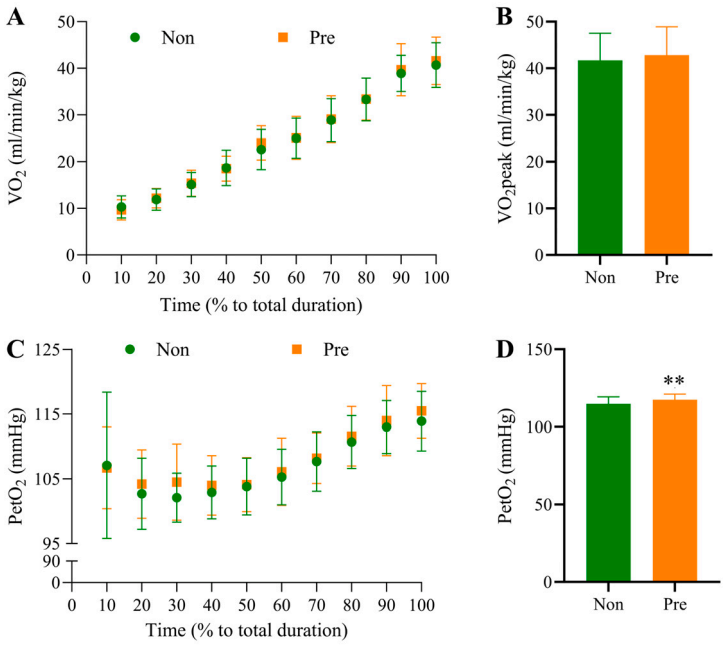


Figure 4. Effects of pre-cooling on aerobic capacity during endurance exercise in the heat. (**A**) Changes in VO_2 during endurance exercise. (**B**) Differences in VO_{2peak} at exhaustion. (**C**) Changes in $PetO_2$ during endurance exercise. (**D**) Differences in $PetO_2$ at exhaustion. Non, hot environment + normothermic sports drink condition; Pre, hot environment + pre-cooling sports drink (4 °C) condition; VO_2, oxygen consumption; $PetO_2$, end-tidal partial pressure of oxygen. Compared with Non, ** $p < 0.01$.

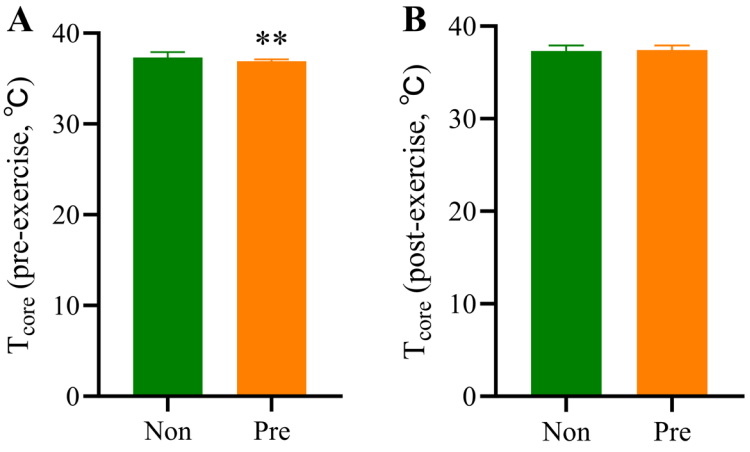


Figure 5. Effects of pre-cooling on T_{core} during endurance exercise in the heat. (**A**) T_{core} at exercise onset. (**B**) T_{core} at exhaustion. Non, hot environment + normothermic sports drink condition; Pre, hot environment + pre-cooling sports drink (4 °C) condition. Compared with Non, ** $p < 0.01$.

3.5.2. Rating of Perceived Exertion (RPE), Rating of Perceived Breathlessness (RPB), and Thermal Sensation (TS)

At exhaustion, no significant between-group differences were observed in RPB (t = −0.890, $p = 0.385$, Cohen's d = −0.360, Figure 7A) or RPE (t = −1.674, $p = 0.110$, Cohen's d = −0.654, Figure 7B). In contrast, TS was significantly lower in the Pre condition than in the Non condition at the beginning of exercise (t = −6.658, $p < 0.001$, Cohen's

d = −1.406, Figure 7C). No significant difference in TS was observed between the conditions at exhaustion (t = −0.698, $p = 0.494$, Cohen's d = −0.247, Figure 7D).

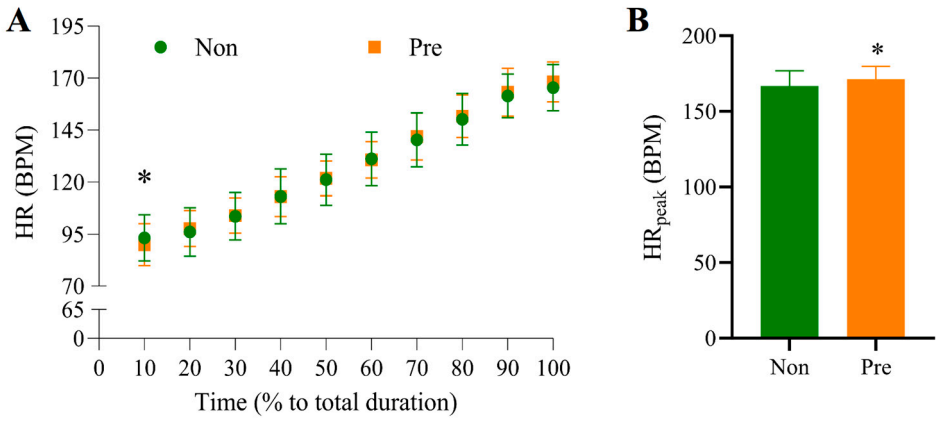


Figure 6. Effects of pre-cooling on HR during endurance exercise in the heat. (**A**) Changes in HR during endurance exercise. (**B**) Differences in HR_{peak} at exhaustion. Non, hot environment + normothermic sports drink condition; Pre, hot environment + pre-cooling sports drink (4 °C) condition. Compared with Non, * $p < 0.05$.

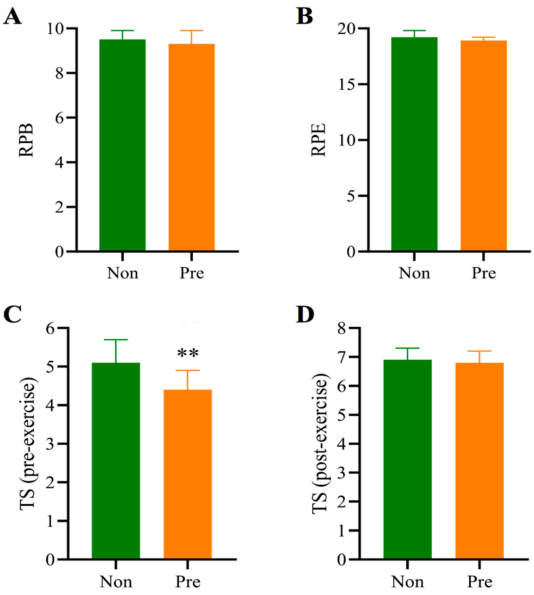


Figure 7. Effects of pre-cooling on RPB, RPE, and TS during endurance exercise in the heat. (**A**) Differences in RPB at exhaustion. (**B**) Differences in RPE at exhaustion. (**C**) Differences in TS at exercise onset. (**D**) Differences in TS at exhaustion. Non, hot environment + normothermic sports drink condition; Pre, hot environment + pre-cooling sports drink (4 °C) condition; RPB, rating of perceived breathlessness; RPE, rating of perceived exertion; TS, thermal sensation. Compared with Non, ** $p < 0.01$.

Overall, pre-exercise ingestion of the 4 °C sports drink improved endurance exercise duration in the heat and was accompanied by a lower initial T_{core} and TS, lower BF and HR during the early stage of exercise, and higher VE_{peak}, BF_{peak}, $PetO_2$, and HR_{peak} at exhaustion. VO_{2peak} did not differ significantly between the two hot-environment conditions.

4. Discussion

The present study investigated the effects of pre-exercise ingestion of a cooled sports drink (7.5 mL/kg BW, 4 °C) on endurance exercise performance and the physiological re-

sponses underlying this effect in a hot environment. The main findings were that cold drink pre-cooling attenuated the heat-induced reduction on endurance exercise performance, elicited higher peak ventilatory responses at exhaustion, and altered selected aerobic and cardiovascular responses during incremental cycling to exhaustion. Specifically, compared with the normothermic drink condition in the heat, pre-cooling increased VE_{peak}, BF_{peak}, $PetO_2$, and HR_{peak}, while reducing BF and HR during the early stage of exercise. Pre-cooling also lowered T_{core} and TS at the onset of exercise, although these differences were no longer evident at exhaustion. By contrast, no significant between-condition differences were observed in VO_{2peak}, RPE, or RPB at exhaustion. Collectively, the isochronous findings suggest that the performance benefit may be partly attributable to reduced initial thermal strain and attenuated cardiovascular and respiratory strain during the early stages of exercise. However, the higher VE_{peak}, BF_{peak}, $PetO_2$, and HR_{peak} values observed at exhaustion in the Pre condition were recorded after a longer exercise duration and at a higher terminal workload. Therefore, these values are more appropriately interpreted as terminal physiological responses associated with prolonged exercise tolerance rather than as mechanistic mediators of the performance benefit.

4.1. Pre-Cooling and Respiratory Function During Endurance Exercise in the Heat

The present study demonstrated that ingestion of 7.5 mL/kg BW of a 4 °C sports drink significantly increased VE_{peak} and BF_{peak} at exhaustion during endurance exercise in the heat, while no clear between-condition difference was observed in VE during the earlier stages of exercise. VE is a fundamental output variable of cardiopulmonary exercise testing (CPET) and an important indicator of the ventilatory response to increasing metabolic demand [33]. During progressive exercise, the ventilatory requirement increases substantially as metabolic activity increases. Particularly during the later stages of exercise, the progressive accumulation of metabolic by-products and associated acid–base disturbances can markedly stimulate ventilation.

As exercise intensity approaches maximal levels, RER generally increases as the contribution of anaerobic metabolism becomes progressively greater [34]. Increased lactate production is accompanied by bicarbonate buffering, which generates additional non-metabolic carbon dioxide (CO_2) [35]. The resulting increase in arterial CO_2 partial pressure provides a potent stimulus to the respiratory centers, thereby increasing VE to facilitate CO_2 elimination and maintain acid–base homeostasis [35]. The enhanced ventilatory response may, in turn, support pulmonary gas exchange and oxygen delivery to metabolically active tissues during the final stages of exercise. Consistent with this physiological framework, BF approaches its individual physiological maximum as exercise intensity increases toward exhaustion [36]. The significantly higher BF_{peak} observed in the Pre group was recorded at a later time point and under a higher terminal workload, reflecting the longer exercise duration in this condition. Therefore, this finding most likely reflects the greater absolute workload achieved rather than an intrinsically enhanced capacity to increase ventilation.

Although direct evidence regarding the specific respiratory effects of cold beverage pre-cooling remains limited, several potential physiological mechanisms may help explain the present findings. Ingestion of cold sports drinks can stimulate cold-sensitive ion channels, including transient receptor potential melastatin 8 (TRPM8), within the oral cavity and gastrointestinal tract [37]. Activation of these thermosensitive receptors can produce a rapid sensation of cooling and may alter autonomic and respiratory responses. Cold beverage ingestion has also been proposed to trigger rapid visceral reflexes that activate the sympathetic nervous system, potentially increasing respiratory center activity [38]. Such autonomic activation may increase respiratory muscle electromyographic activity, augment pleural and abdominal pressures during ventilation, and enhance respiratory

muscle contractility during exercise [38]. These responses could contribute to the greater ventilatory capacity observed at exhaustion following cold drink ingestion.

Nevertheless, the relationship between cold beverage ingestion and respiratory function is not unequivocal. Koehn et al. [39], for example, reported that ingestion of a cold beverage did not improve respiratory metabolism or cerebral cooling during exercise in the heat. The authors proposed that the anatomical proximity of the esophagus to the carotid artery might result in localized cooling of the carotid body, potentially altering cerebral blood flow and pulmonary ventilation and thereby limiting endurance performance [39]. In addition, the vagus nerve plays an important role in respiratory regulation, and cold stimuli may elicit vagally mediated defensive responses, including coughing, changes in airway resistance, and alterations in mucosal secretion [40]. In theory, these responses could counteract the facilitatory effects of cold stimulation and compromise ventilation.

Importantly, the present study did not observe a reduction in VE during submaximal exercise following cold drink ingestion. This finding suggests that any potential inhibitory effects of cold-induced vagal or airway reflexes were insufficient to impair the ventilatory response during exercise. Moreover, the significantly higher VE_{peak} and BF_{peak} observed at exhaustion indicate that cold drink pre-cooling was associated with greater terminal ventilatory output. However, given that these peak values were recorded after different exercise durations, they should be interpreted as terminal physiological responses rather than definitive evidence of enhanced maximal ventilatory capacity.

One possible explanation for the apparently contrasting findings across studies is the timing of cold beverage ingestion relative to exercise onset. In the present study, the beverage was consumed approximately 30 min before exercise, rather than immediately before or during exercise. The acute autonomic responses elicited by oral and gastrointestinal cold stimulation may therefore have diminished during the pre-exercise interval, potentially reducing transient defensive respiratory reflexes while retaining the thermoregulatory and perceptual benefits of pre-cooling. This temporal separation may help explain why cold drink ingestion did not suppress VE in the present study and may have allowed the beneficial effects of reduced thermal strain and altered autonomic regulation to become more apparent as exercise intensity approached exhaustion. However, this proposed mechanism remains speculative because respiratory muscle activity, autonomic activity, and airway responses were not directly measured in the present study.

4.2. Pre-Cooling-Induced Changes in Aerobic Responses

$PetO_2$ represents an important CPETvariable and can provide information regarding pulmonary gas exchange and the balance between oxygen delivery and utilization during exercise [41]. During the early stages of incremental exercise, $PetO_2$ typically decreases as exercise intensity increases because of the rapid increase in tissue oxygen extraction and metabolic oxygen consumption. As exercise progresses toward high intensity, metabolic acidosis stimulates ventilation disproportionately relative to oxygen consumption. The resulting hyperventilation lowers arterial and alveolar CO_2 partial pressure and increases alveolar oxygen tension, contributing to a subsequent elevation in $PetO_2$ [42].

In the present study, $PetO_2$ increased progressively throughout exercise and was significantly higher in the Pre condition than in the Non condition at exhaustion. This finding indicates that cold drink pre-cooling altered the pulmonary gas-exchange response during the final stage of exercise. The higher $PetO_2$ observed in the Pre condition may reflect an increased ventilatory drive and a greater ability to maintain alveolar oxygen availability at the highest exercise intensity. However, as with the other variables measured at exhaustion, this value was recorded after a longer exercise duration in the Pre condition and should therefore be interpreted as a terminal physiological response rather than evidence

of enhanced maximal physiological capacity. $PetO_2$ should not be interpreted as a direct measure of peripheral tissue oxygen extraction. Rather, it is influenced by ventilation, alveolar gas composition, metabolic rate, and pulmonary gas exchange. Therefore, the present findings provide evidence of altered aerobic and ventilatory responses but do not establish that pre-cooling directly enhanced peripheral oxygen extraction.

The VO_2 findings provide additional context. Although no statistically significant difference in VO_{2peak} was observed between the Pre and Con conditions, VO_2 was numerically higher the Pre condition at most matched time points, except at 10% of total exercise duration. The absence of a statistically significant difference in VO_{2peak} indicates that cold drink pre-cooling did not clearly increase maximal whole-body oxygen consumption under the present conditions. Nevertheless, the numerically higher VO_2, together with the higher $PetO_2$ and enhanced ventilatory responses, suggests that pre-cooling may have influenced cardiopulmonary responses during exercise in the heat. However, given that the difference in VO_{2peak} did not reach statistical significance ($p = 0.068$), these findings should be interpreted cautiously and do not provide conclusive evidence of enhanced aerobic capacity.

Cardiovascular responses may also have contributed to the observed pattern. Stadheim et al. [43] demonstrated that increases in HR_{peak} can contribute to improvements in VO_{2peak} and reported a positive association between HR_{peak} and VO_{2peak}. Consistent with this observation, the present study found a significantly higher HR_{peak} following pre-cooling. However, because this peak was attained at a higher terminal workload in the Pre condition, it is more appropriately interpreted as a terminal physiological response. Furthermore, cardiac output is determined by HR and stroke volume [44]. As neither stroke volume nor cardiac output was directly measured, the higher HR_{peak} cannot be considered evidence that pre-cooling preserved cardiovascular reserve during maximal exercise.

The present findings extend previous research by examining $PetO_2$ and ventilatory responses alongside VO_2 during incremental exercise. Although the ergogenic effects of pre-cooling on endurance performance are relatively well established, comparatively few studies have examined whether these benefits are accompanied by changes in specific cardiopulmonary indices. A meta-analysis demonstrated that internal pre-cooling can improve aerobic performance in the heat, including TT duration, time to exhaustion (TTE), and exercise speed [45]. Similarly, a recent meta-analysis suggested that external pre-cooling may improve aerobic exercise performance, particularly during running-based exercise [8]. Differences between these findings and those of the present cycling-based study may partly reflect differences in exercise modality, including energy expenditure, patterns of neuromuscular activation, muscle mass recruited, and mechanisms of heat production and dissipation [46,47].

Previous studies have also reported inconsistent effects of pre-cooling on VO_{2max}. Zimmermann et al. [48] found that internal pre-cooling did not significantly alter VO_{2max} in female athletes, whereas Ihsan et al. [49] reported that internal pre-cooling failed to increase VO_{2max} but improved submaximal aerobic performance. These findings, together with the present results, suggest that the ergogenic effects of pre-cooling may not necessarily involve an increase in maximal oxygen consumption. Instead, pre-cooling may preserve the ability to sustain a high level of physiological function under thermal stress, thereby delaying the development of limiting factors that ultimately terminate exercise. Differences among studies may also arise from variations in cooling modality, beverage temperature, dosage, timing of ingestion, exercise protocol, environmental conditions, sex, and training status. Further research using comprehensive measurements of cardiac output, stroke volume, oxygen saturation, and respiratory muscle function would help clarify the mechanisms linking pre-cooling with aerobic performance.

4.3. Subjective Fatigue and Perceptual Responses to Pre-Cooling

A substantial body of evidence indicates that internal pre-cooling can reduce TS and T_{core} during exercise in the heat [50–52]. Lower TS has frequently been associated with lower RPE and delayed development of subjective fatigue [17,53,54]. From a thermoregulatory perspective, the hypothalamus integrates thermal information and coordinates physiological responses to maintain homeostasis. During exercise in the heat, increasing T_{core} and associated thermal afferent input may increase inhibitory signals within the CNS, thereby constraining exercise intensity as a protective response against excessive hyperthermia [55]. Cold stimulation may also modify thermoregulatory and perceptual responses through activation of peripheral temperature-sensitive receptors, thereby altering the perception of thermal strain [37].

Consistent with this previous evidence, the present study demonstrated significantly lower TS and T_{core} in the Pre condition at the onset of exercise. These reductions occurred before the exercise-induced increase in thermal strain and were accompanied by a longer endurance exercise duration. The findings support the concept that lowering initial thermal strain may provide additional heat-storage capacity and delay the accumulation of thermal stress during subsequent exercise.

However, no significant differences in RPE, RPB, or TS were observed between the Pre and Non conditions at exhaustion. This finding should be interpreted in the context of the experimental design. All participants exercised to volitional exhaustion, meaning that the exercise endpoint was determined by the participant's inability or unwillingness to continue at the required workload. Consequently, perceptual responses at exhaustion may converge toward similarly high levels despite differences in the physiological trajectory leading to exhaustion. The absence of a between-group difference in T_{core} at exhaustion provides further support for this interpretation. Although T_{core} was lower before exercise in the Pre group, the difference had disappeared by exhaustion, suggesting that pre-cooling primarily altered the rate or timing at which thermal strain accumulated rather than the final thermal state reached at exercise termination.

The present findings are therefore consistent with the concept that pre-cooling may increase the amount of heat that can be stored before a critical level of thermal strain is reached. Rather than maintaining a lower T_{core} throughout the entire exercise bout, the pre-cooled condition may delay the attainment of a similar end-exercise thermal state. The commonly discussed critical T_{core} of approximately 40 °C has been proposed as one physiological factor associated with exercise limitation in the heat [6,56], although the relationship between T_{core} and exercise termination is multifactorial and should not be interpreted as a universal fixed threshold.

Notably, the lower TS observed before exercise provides an important perceptual component of the pre-cooling response. Lim et al. [56] suggested that RPE may provide a sensitive indicator of accumulating fatigue under conditions in which cardiovascular responses are altered or blunted. In the present study, however, RPE at exhaustion did not differ significantly between conditions. Therefore, the results do not support the conclusion that cold drink pre-cooling directly reduces perceived exertion at the point of exhaustion. Instead, the primary perceptual benefit appeared to occur before or during the early phase of exercise, when the reduced TS may have contributed to improved initial exercise tolerance. This distinction is relevant for endurance exercise because relatively small reductions in thermal discomfort at exercise onset may facilitate the maintenance of exercise intensity over a prolonged period, even when perceptual responses eventually converge at exhaustion.

4.4. Overall Effects of Pre-Cooling on Endurance Exercise Performance

The present findings are broadly consistent with previous evidence supporting the ergogenic potential of internal pre-cooling during exercise in the heat. Lee et al. [57] reported that ingestion of a cold beverage approximately 35 min before exercise in a 35 °C environment reduced heat accumulation and prolonged endurance exercise time. Similarly, Yeo et al. [58] found that internal pre-cooling improved 10 km outdoor running TT performance, while Burdon et al. [9] reported an approximately 5% increase in power output following cold beverage ingestion 15 min before endurance exercise. Nevertheless, beneficial effects have not been observed consistently across studies. Xu et al. [24] and Khlifi et al. [25], for example, reported limited effects of isolated pre-cooling strategies under certain experimental conditions, although combined cooling strategies may provide additional benefits for prolonging time to exhaustion. Such inconsistencies indicate that the effectiveness of pre-cooling depends on the interaction between the cooling stimulus and the specific characteristics of the exercise and environmental conditions.

The ergogenic effects of pre-cooling are likely mediated by a combination of central and peripheral mechanisms rather than by a single physiological pathway [59]. Ingestion of a cold beverage stimulates temperature-sensitive receptors in the oral cavity, esophagus, and gastrointestinal tract [54,60], producing an immediate perception of cooling that may increase thermal comfort and exercise tolerance [57,61]. At the physiological level, the ingestion of cold fluid introduces a thermal sink that can remove heat from the body and reduce pre-exercise T_{core} through heat transfer and associated circulatory mechanisms [62,63]. Lowering T_{core} before exercise increases the amount of heat that can be accumulated before reaching a high level of thermal strain and may consequently delay thermoregulatory limitations on exercise performance [25].

The temperature of the beverage may also influence the magnitude of these responses. The 4 °C sports drink used in the present study may have provided a sufficiently strong thermal stimulus to activate oral and upper gastrointestinal temperature-sensitive pathways. Previous research has suggested that cold stimulation around this temperature can activate oral sensory areas within the central nervous system and influence respiratory and metabolic responses [64]. This potential mechanism is consistent with the higher VE_{peak}, BF_{peak}, and $PetO_2$observed in the Pre condition, as well as the numerically higher, although not statistically significant, VO_{2peak}. However, because the present study did not directly assess neural activity or autonomic function, the involvement of specific sensory pathways remains to be established.

Pre-cooling also influenced the cardiovascular response to exercise. HR was significantly lower during the early stage of exercise in the Pre group than in the Non group, consistent with previous observations that cold beverage ingestion may attenuate the early cardiovascular strain associated with exercise in the heat [57]. A lower HR at a given early stage of exercise may indicate reduced cardiovascular stress and potentially greater circulatory efficiency under thermal strain. As exercise progressed, however, HR increased progressively, and the Pre condition achieved a significantly higher HR_{peak} at exhaustion. This pattern may indicate greater preservation of cardiac reserve during the final stage of exercise, although direct measurements of stroke volume and cardiac output would be required to confirm this interpretation [1,65].

Taken together, the present findings suggest that the performance benefit of cold drink pre-cooling cannot be attributed solely to a reduction in T_{core}. Instead, the intervention elicited a broader pattern of physiological responses, including lower initial thermal strain and TS, as well as attenuated cardiovascular and respiratory strain during the early stages of exercise at matched time points. In contrast, the higher ventilatory and cardiopulmonary peak values observed at exhaustion in the Pre condition were attained after a longer exercise

duration and at a higher terminal workload. These values should therefore be interpreted as terminal physiological correlates of prolonged exercise tolerance rather than as mechanisms directly responsible for the performance benefit. Among the secondary outcomes, several between-condition comparisons yielded *p*-values close to the conventional significance threshold of 0.05 (e.g., BF and HR at 10% of exercise duration: $p = 0.028$ and 0.044, respectively). As these physiological responses were examined as exploratory secondary outcomes without adjustment for multiple comparisons, the findings warrant cautious interpretation and should be considered hypothesis-generating rather than confirmatory.

Notably, BF was lower in the Pre condition at 10% of exercise duration but higher at exhaustion. These findings represent two distinct stages of the exercise trajectory: an early submaximal stage and the terminal stage reached after a longer exercise bout. Accordingly, they should not be interpreted as evidence of a progressive transition from early respiratory suppression to enhanced ventilatory capacity. Establishing such a transition would require longitudinal statistical evidence demonstrating changes across the matched-time exercise course, which cannot be inferred from the separate paired comparisons at individual time points performed in the present study.

Importantly, the present study showed that exercise performance in the Non condition was significantly lower than in the temperate Con condition, whereas performance in the Pre condition did not differ significantly from the Con condition. This pattern indicates that cold drink pre-cooling attenuated, but should not necessarily be interpreted as completely eliminating, the physiological burden imposed by the hot environment. Because the study was not designed to establish equivalence between the Pre and Con conditions, the absence of a statistically significant difference should not be interpreted as proof that performance was fully restored to temperate-environment levels. Nevertheless, the findings support the practical use of pre-cooled sports drinks as a strategy for reducing the performance impairment associated with endurance exercise in the heat.

4.5. Strength and Limitations

A major strength of the present study is its integrated assessment of the physiological responses potentially linking internal pre-cooling with endurance exercise performance in the heat. In addition to exercise duration, we simultaneously assessed respiratory function (VE and BF), aerobic responses (VO_2 and $PetO_2$), cardiovascular responses (HR), thermal strain (T_{core}), and perceptual responses (RPE, TS, and RPB). This multidimensional approach allowed the effects of cold drink ingestion to be considered from respiratory, aerobic, cardiovascular, thermoregulatory, and perceptual perspectives rather than relying on a single outcome. The randomized crossover design also allowed each participant to serve as his own control, thereby reducing between-participant variability and strengthening the internal validity of the comparisons between hot-environment conditions.

Several limitations should nevertheless be acknowledged. First, although VO_{2peak} and $PetO_2$ were used to characterize aerobic responses, peripheral oxygen saturation (SpO_2) and key hemodynamic variables, such as stroke volume and Q_{peak}, were not measured. Consequently, the mechanisms responsible for the observed changes in $PetO_2$ and HR_{peak} cannot be determined conclusively.

Second, because pre-cooling prolonged exercise duration in the Pre condition, the exhaustion-stage variables (VE_{peak}, BF_{peak}, $PetO_2$, and HR_{peak}) were measured at different terminal workloads and after different exercise durations across conditions. Therefore, between-condition differences in these variables cannot be interpreted as evidence of enhanced maximal physiological capacity following pre-cooling. Instead, they are more appropriately regarded as terminal physiological responses associated with the longer exercise duration. Accordingly, mechanistic interpretations in the present study are pri-

marily based on the isochronous (matched-time) comparisons. Nevertheless, although this comparison aligned the two hot conditions at equivalent percentages of total exercise duration, they matched absolute exercise time rather than relative exercise intensity or physiological demand. Given the difference in total exercise duration between the Pre and Non conditions, the same isochronous time point may represent different relative exercise intensities, such as different proportions of peak power or VO_{2peak}. Thus, differences in VE, BF, and VO_2 at these time points should be interpreted as differences under matched absolute exercise times rather than at equivalent relative exercise intensities. Furthermore, multiple respiratory, metabolic, cardiovascular, thermal, and perceptual outcomes were compared across several time points without adjustment for multiple comparisons. Therefore, findings with *p*-values close to the conventional significance threshold, such as those for BF and HR at 10% of exercise duration, should be interpreted cautiously and considered exploratory and hypothesis-generating rather than confirmatory.

Third, T_{core} was measured at the tympanic membrane using a handheld infrared thermometer, providing an indirect estimate of core temperature rather than a direct measurement obtained through gastrointestinal, esophageal, or rectal thermometry. Compared with these direct measurement methods, tympanic temperature measurements may be more susceptible to environmental and procedural variability during exercise in the heat. Therefore, the observed reduction in pre-exercise T_{core} should be interpreted with this methodological limitation in mind. Future studies incorporating direct core-temperature measurements would provide more robust evidence regarding the thermoregulatory effects of pre-cooling.

Fourth, the sports drink used in the present study contained carbohydrates and electrolytes, both of which may independently influence exercise performance. However, because the beverage composition and volume were identical between the Pre and Non conditions, both of which involved exercise in a hot environment, these components are unlikely to have confounded the within-heat comparison of beverage temperature. The only difference between these two conditions was the temperature of the beverage. Nevertheless, all three experimental conditions involved the same carbohydrate-electrolyte beverage rather than a temperature-controlled alternative, such as cold water or a carbohydrate-free beverage. Consequently, the specific contribution of the cooling stimulus cannot be fully separated from the potential effects of beverage ingestion itself when compared with plain water or no fluid intake.

Fifth, the beverage volume was standardized at 7.5 mL/kg BW based on previous evidence suggesting that approximately 7–14 mL/kg BW may be beneficial for thermoregulation and exercise performance in the heat [11]. However, the optimal dose within this range remains uncertain.

Sixth, the primary outcome was exercise duration during an incremental cycling test to volitional exhaustion, with workload increasing by 25 W/min. This protocol primarily assesses exercise tolerance rather than performance in a fixed-distance time trial or a self-paced competitive endurance event. Therefore, improvements in time to exhaustion may not translate directly into comparable improvements in competitive performance. The present findings should consequently not be generalized to competitive endurance performance without further validation using time-trial or self-paced exercise protocols.

Seventh, participants blinding to beverage temperature was not feasible because consuming the 4 °C pre-cooling drink produced an internal cold sensation in the oral cavity and esophagus that could not be masked by the external serving container. Nevertheless, all beverages were served in identical insulated, opaque containers and were matched in flavor, color, and composition. Participants were also not informed that beverage temperature was the primary intervention of interest and received no performance feedback during the trials,

which may have helped minimize expectancy-related and pacing effects. However, the potential influence of residual expectancy or placebo effects on time to exhaustion cannot be excluded and should be acknowledged.

Finally, the study included only trained male athletes. The findings may therefore not be directly generalizable to female athletes, recreationally active individuals, older adults, or individuals with different levels of heat acclimatization and training status. In addition, baseline hydration status and the degree of heat acclimatization were not formally assessed or standardized beyond participants' habitual training practices. Inter-individual differences in these factors may therefore have influenced the thermoregulatory and performance responses observed. Sex-related differences in thermoregulation, body composition, fluid regulation, and hormonal responses may influence the efficacy of internal pre-cooling. Future research should include more diverse populations and examine whether the respiratory, aerobic, and thermoregulatory responses observed in the present study are consistent across different athlete groups and exercise modalities.

5. Conclusions

Pre-exercise ingestion of a 4 °C sport drink at a dosage of 7.5 mL/kg BW improved exercise tolerance under the specific experimental conditions of the present study. However, these findings do not establish that improvements in respiratory function or aerobic capacity mediated this effect. Rather, the ergogenic benefit was associated with reduced pre-exercise thermal strain and attenuated cardiovascular and respiratory strain during the early stages of exercise. This benefit was not attributable to enhanced maximal aerobic capacity, as VO_{2peak} did not differ significantly between conditions. The higher peak ventilatory and cardiopulmonary values observed at exhaustion are more appropriately interpreted as terminal physiological responses following a longer exercise duration rather than as mechanistic mediators of the performance benefit. Perceptual benefits were limited to lower thermal sensation at exercise onset, whereas RPE did not differ significantly at exhaustion. Overall, these findings support pre-exercise ingestion of a 4 °C sports drink as a potential strategy for mitigating the heat-induced reduction in exercise tolerance under the specific experimental conditions investigated. Nevertheless, the accompanying respiratory, cardiovascular, and thermal responses should be regarded as potential physiological correlates rather than established mechanisms underlying the ergogenic effect.

The present findings further suggest that the ergogenic benefits of internal pre-cooling were accompanied by changes in selected respiratory, cardiovascular, and thermal responses, rather than being solely attributable to reduced thermal strain. Lower pre-exercise thermal strain may increase the capacity for subsequent heat storage and delay the development of excessive thermal stress. In contrast, the altered cardiopulmonary responses observed at exhaustion should be interpreted as terminal physiological responses associated with prolonged exercise duration, rather than as evidence of enhanced maximal ventilatory or aerobic capacity. The lack of intergroup differences in T_{core}, TS, RPE, or RPB at the point of exhaustion further implies that pre-cooling primarily modifies the physiological progression toward exhaustion, rather than preventing the high thermal and perceptual strain attained at maximal exertion.

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Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: The datasets generated during and/or analyzed during the present study are available from the corresponding author upon reasonable request.

Conflicts of Interest: The authors declare no conflicts of interest.

Abbreviations

The following abbreviations are used in this manuscript:

BF	Breathing frequency
BW	Body weight
CNS	Central nervous system
CPET	Cardiopulmonary exercise testing
EMG	Electromyographic
HR	Heart rate
$PetO_2$	End-tidal partial pressure of oxygen
Q_{peak}	Peak cardiac output
RER	Respiratory exchange rate
RPB	Rating of perceived breathlessness
RPE	Rating of perceived exertion
RPM	Revolutions per minute
SD	Standard deviation
SPO_2	Oxygen saturation
SV	Stroke volume
T_{core}	Core temperature
TS	Thermal sensation
TT	Time trial
TTE	Time to exhaustion
VE	Minute ventilation
VO_2	Oxygen uptake
VO_{2max}	Maximal oxygen consumption
VO_{2peak}	Peak oxygen uptake

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