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Cooling During Endurance Cycling in the Heat: Blunted Core Temperature but Not Inflammatory Responses

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Purpose: This study assessed the effects of cooling during endurance cycling (percooling) on changes in core body temperature (Tcore), inflammatory, and metabolic responses. ***Methods:*** A total of 12 male cyclists (peak oxygen uptake 60 [4] $mL \cdot kg^{-1} \cdot min^{-1}$) completed a 60-minute constant workload trial (55% of peak power output and ambient temperature 30.4°C [0.6°C]) in a randomized order both with (ICE) and without (CON) an ice vest. An ingestible capsule was used to measure Tcore. Blood samples were collected immediately before and after each trial to determine concentrations of blood lactate, serum cortisol, interleukin-6, and reactive oxygen and nitrogen species. ***Results:*** Tcore increased statistically ($P < .001$) both in CON (7.0% [1.4%], effect size [ES] = 6.3) and ICE (5.1% [1.1%], ES = 5.7). The increase in CON was statistically larger compared with ICE ($P = .006$, ES = 1.4). Concentrations of blood lactate (CON: 163% [63%], ES = 1.3; ICE: 149% [91%], ES = 1.3), cortisol (CON: 138% [123%], ES = 1.7; ICE: 81% [102%], ES = 1.0), and interleukin-6 (CON: 661% [324%], ES = 2.1; ICE: 624% [368%], ES = 1.2) statistically increased in both conditions ($P < .01$) to a similar extent. In addition, reactive oxygen and nitrogen species statistically decreased in both conditions (CON: −19.2% [14.9%], $P = .002$, ES = 0.9; ICE: −15.1% [16.5%], $P = .02$, ES = 0.9). No correlations were found between the changes of Tcore and blood parameters across the conditions. ***Conclusions:*** Despite attenuated Tcore, similar inflammatory and metabolic responses were observed. Our findings suggest percooling to be a promising strategy to attenuate thermal strain without compromising physiological function.

Keywords: ice vest, percooling, interleukin-6, ROS, cortisol

During exercise in the heat, cardiovascular demands are increased due to enhanced blood supply to the working muscles as well as the skin arterioles, while blood volume is concomitantly decreased due to sweat loss.[1] Consequently, core body temperature (Tcore) dramatically rises, impairing exercise performance and causing excessive fatigue.[2] This seems to be the case especially in endurance performance, where impaired mean power outputs during cycling of as much as 15% have been observed.[3]

Although heat acclimatization is still considered as the most important countermeasure for heat-associated impairments, rigor implementation is not always feasible due to logistic requirements.[4] Thus, synergistic cooling strategies may be applied prior to (precooling), as well as during (percooling), or even after exercise (postcooling). Interestingly, previous research has indicated that different types (ie, ingestion of cold water or ice slurry and cooling packs or vests) and timing of cooling (ie, precooling and percooling) were similarly effective in reducing thermal strain and maintaining performance.[5,6]

However, despite potential effects on exercise performance, it is also known that at least severe cold exposure may alleviate inflammatory responses induced by exercise,[7,8] thereby attenuating physiological stimuli desired to induce training adaptation.[9,10] For example, 5 days of whole-body cryotherapy after exercise reduced pro-inflammatory cytokine levels (interleukin-2 and interleukin-8) in well-trained athletes.[7] Similarly, precooling by a 60-minute whole-body cold-water immersion mitigated pro-inflammatory responses after 90 minutes of running in the heat, potentially mediated by a reduction in thermal strain during the initial phase of exercise.[9]

Reduced metabolic and inflammatory responses induced by cooling might have negative consequences for prolonged training adaptations, as exercise-induced inflammation is associated with altered gene expression,[10] such as stimulation of satellite cell proliferation.[11] Moreover, both cortisol and interleukin-6 (IL-6) are important mediators of metabolic adaptations, such as reduced dependence on plasma glucose and muscle glycogen.[12,13] Similarly, reactive oxygen and nitrogen species (RONS) are also involved in regulating adaptive responses and are linked to enhanced muscle contractions,[14] to the expression of an oxidative phenotype of muscle fibers,[15] and to the induction of beneficial adaptations contributing to heat acclimatization.[16,17] Consequently, reduced metabolic and inflammatory responses induced by cooling may impair physiological adaptations, warranting caution when applying cooling strategies during training.

Thus, in practice, athletes and coaches are challenged to attenuate excessive increases in thermal strain for performance maintenance, but at the same time need to avoid blunting responses required for training adaptations or heat acclimatization.[9] Consequently, the question arises as to whether more practical percooling strategies with a moderate cooling stimulus, such as ice vest applications, will induce detrimental physiological responses. Therefore, the aim of the present study was to examine the effects of a percooling strategy with high practical relevance on Tcore and physiological responses to endurance cycling in the heat. It was hypothesized that the cooling stimulus induced by percooling with an ice vest would be sufficient to reduce thermal strain but would not blunt desirable inflammatory and metabolic responses.

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Methods

Experimental Design

In this randomized crossover trial, participants underwent 3 separate test sessions: a preliminary ramp test to determine peak power output (PPO) and peak oxygen uptake ($\dot{V}O_2$peak), as well as two 60-minute constant workload trials at an ambient temperature of 30°C and with a humidity of 38%. This environmental setting was chosen to realistically simulate tolerable heat stress commonly present during training and competition, similar to previous studies.[5] Participants performed 2 trials while wearing an ice vest (ICE) and the same vest without ice packs (CON). Baseline hydration was assessed by urine specific gravity prior to the experimental trials. An ingestible telemetric capsule was used to assess Tcore. In addition, capillary and venous blood samples were collected both prior to and immediately after the trials, in order to assess blood lactate concentrations and inflammatory responses.

Participants

A total of 12 male cyclists or triathletes (mean [SD]; age = 26 [5] y, height = 181 [7] cm, body mass = 75.0 [4.8] kg, $\dot{V}O_2$peak = 60.3 [4.0] $mL \cdot kg^{-1} \cdot min^{-1}$, PPO = 419 [40] W, previous training volume 8 [3] $h \cdot wk^{-1}$) participated in the study. Their performance level was classified as trained, based on the assessed $\dot{V}O_2$peak.[18] All testing was performed in the summer months of the northern hemisphere with daytime temperatures reaching values ≥25°C, in order to expect partial heat acclimatization of all participants.[19] This was considered a prerequisite to avoid potentially exaggerated cooling effects,[20] as outlined by current evidence-based recommendations regarding exercise in the heat.[4] The medical history of all participants was assessed through a standardized questionnaire. Prior to all testing, participants provided a written informed consent. The study was performed according to the Declaration of Helsinki and approved by the ethical committee of the German Sport University.

Procedures

Prior to their first visit, athletes were advised in written form to abstain from alcohol, caffeine, dietary supplements, and strenuous exercise 24 hours preceding each test. In addition, participants were required to keep the dietary intake constant before each test, which was controlled by means of a 24-hour food record. Laboratory visits were separated by at least 48 hours, and all tests were performed within a maximum of 4 weeks. In addition, testing was carried out at the same time of the day (±1h).

Performance Testing. To assess PPO and $\dot{V}O_2$peak, a ramp test was performed on a cycle ergometer (Schoberer Rad Meßtechnik SRM GmbH, Jülich, Germany) in thermoneutral ambient conditions (21.0°C [0.3°C] and 49% [9%] humidity). After a 2-minute resting measurement, the protocol commenced with a 4-minute familiarization phase at 100 W and increased by 30 W every minute. Breathing gases were recorded every second (Metalyzer® 3B; Cortex Biophysik GmbH, Leipzig, Germany). The spirometer was calibrated weekly with a reference gas (5% CO_2 and 15% O_2) and before each test with ambient air and with a 3-L syringe, according to the manufacturer's specifications. Participants were encouraged verbally to reach voluntary exhaustion. Correction of PPO was made for the time spent at the last increment, and $\dot{V}O_2$peak was defined as the highest 30-second average oxygen uptake.

Constant Workload Trials. Participants reported to the lab 30 minutes prior to each constant workload trial. First, participants were provided with 6 $mL \cdot kg^{-1}$ of tap water to warrant proper hydration.[4] In addition, participants ingested a telemetric capsule for Tcore assessment (*e-Celsius*™; Bodycap, Caen, France), transmitting Tcore to a wireless data receiver (e-Viewer; Bodycap). The device has recently been tested in water bath experiments showing excellent validity (intraclass correlation coefficient = 1.00), test–retest reliability (intraclass correlation coefficient = 1.00), and inertia between 36°C and 44°C.[21]

Subsequently, participants passed a midstream urine sample to determine baseline hydration by urine specific gravity, using a digital handheld refractometer (DR-303; Index Instruments Ltd, Cambridgeshire, United Kingdom). Euhydration was defined by urine specific gravity ≤1.025 $g \cdot mL^{-1}$. In addition, body mass was assessed by means of a commercial scale (Silver Crest Diagnostic Scale; TARGA GmbH, Soest, Germany) with a resolution of ±0.1 kg. Both preexercise and postexercise measurements were performed nude and after drying.

After entering the hot chamber, 5 minutes of familiarization at 100 W were warranted for each athlete to adjust the cycle ergometer (ergoselect 50; ergoline GmbH, Bitz, Germany). Following a 60-second measurement for the assessment of baseline Tcore, the load was set to 55% of the individual PPO. Participants completed all sessions with their own pedals, shoes, and cycling suits. Saddle height was maintained in both loading conditions. Throughout the test, participants were required to refrain from water intake. In addition, ambient conditions were constantly monitored by means of a customary weather station (Technoline WS 9632-IT; Techno-Trade Import-Export GmbH, Wildau, Germany) with a temperature resolution of ±0.1°C and a humidity resolution of ±1% and were kept constant throughout all sessions (Table 1). For end-exercise Tcore assessment, the average value over the last 60 seconds of exercise was recorded.

Cooling Application. During each constant workload trial, participants wore the same lightweight vest (285 g) made of softshell fabric (Regatta Professional, Manchester, United Kingdom), without wearing a shirt underneath. While in the CON trial no cooling medium was added, in the ICE trial 10 cooling pads comprising a graphite-water-suspension (Active. Pad 3 × 6; EMCOOLS Sports GmbH, Traiskirchen, Austria) were inserted into the provided pockets. Mass and size of each pad equaled 173 g and 19.5 × 9.7 cm, respectively, so that an overall area of 1891.5 cm^2 was covered by a total of 1730 g of the coolant. Ice pads were stored in a

Table 1 Exercise Conditions (Mean [SD]) During the Constant Workload Trials With and Without Percooling

Variable	CON	ICE
Ambient temperature, °C	30.4 (0.8)	30.5 (0.5)
Humidity, %	38 (7)	38 (7)
Intensity, W	230 (22)	230 (22)
Intensity, %$\dot{V}O_2$peak	68 (6)	68 (6)
Baseline urine specific gravity, $g \cdot mL^{-1}$	1.009 (0.007)	1.011 (0.008)
Body mass loss, kg	−2.1 (0.6)	−1.8 (0.6)

Abbreviations: CON, without ice vest; ICE, with ice vest; $\dot{V}O_2$peak, peak oxygen uptake.

freezer at −21°C and were inserted into the vest 5 minutes prior to each constant workload trial.

Blood Sampling and Analysis

Capillary Blood Sampling. Capillary blood samples (20 µL) were drawn from the earlobe into hemolyzing solution cups (EKF Diagnostic Sales, Magdeburg, Germany). Blood lactate concentrations were analyzed using a *Biosen C-line* (EKF Diagnostic Sales) with a coefficient of variation of ≤1.5%.

Venous Blood Sampling. Venous blood samples were drawn from the antecubital vein by experienced medical staff. Blood was collected into sterile vacutainers, either dipotassium ethylenediaminetetraacetic acid (K_2-EDTA) tubes for blood cell counts or serum separation tubes (BD Vacutainer Systems, Plymouth, United Kingdom), for the assessment of inflammatory markers. The K_2-EDTA tubes were analyzed by fluorescent flow cytometry (Sysmex KX-21 N; Sysmex Corporation, Kobe, Japan), to determine hemoglobin and hematocrit for fluid loss correction. The serum separation tubes were stored for 15 minutes at room temperature, after which they were centrifuged at 3500 revolutions per minute for 10 minutes at 4°C (Heraeus® Multifuge® 3 L-R; Kendro Laboratory Products, Newton, MA). Immediately after centrifugation, serum was separated into 1-mL aliquots (SARSTEDT AG & Co. KG, Nümbrecht, Germany) and stored at −80°C for further analysis.

Enzyme-Linked Immunosorbent Assays. Serum concentrations of cortisol were assessed using human enzyme-linked immunosorbent assay (ELISA) kits (Cortisol ELISA EIA-1887; DRG Instruments GmbH, Marburg, Germany). The serum concentrations of IL-6 were measured using human *Quantikine® High Sensitivity ELISA kits* (Human IL-6 Immunoassay HS600C; R&D Systems, Inc, Minneapolis, MN). Serum levels of RONS were assessed using *OxiSelect™ In Vitro ROS/RNS Assay Kits* (STA-347; Cell Biolabs Inc, San Diego, CA). All samples were analyzed in duplicates using a microplate reader (Multiscan™ FC; Thermo Scientific™, Waltham, MA), and the mean was used for statistical analysis.

Correction for Fluid Loss. Concentrations of all inflammatory markers were corrected for fluid loss, as calculated from hemoglobin and hematocrit according to Dill and Costill.[22] Conversely, blood lactate concentrations were corrected according to the technique proposed by Matomäki et al.[23]

Statistical Analysis

Prior to statistical analysis, homoscedasticity and normality were checked by visual inspection of residual histograms, residual plots, and Q–Q plots. As deviations were observed for blood lactate and IL-6, log transformations were performed. As baseline data did not statistically differ between conditions, data were analyzed by 2-way repeated-measures analyses of variance using the *afex* package in R.[24] Main and interaction effects were explored using Tukey honestly significant difference test from the *emmeans* package. Consequently, *P* values were reported along with Cohen effect sizes (ES) and 95% confidence intervals determined by means of the *effsize* package, using Hedge correction for small sample sizes. In addition, associations between the changes in the dependent variables within as well as across both conditions were assessed by means of the Pearson product–moment correlation coefficient *r* (95% confidence intervals) using the *stats* package. For all tests, statistical significance was accepted at $P < .05$. All data are presented as mean (SD).

Results

Exercise Conditions and Hydration Status

Exercise intensity, ambient temperature, humidity, baseline urine specific gravity, and body mass loss were similar in both exercise conditions (Table 1).

Core Temperature

For Tcore, a statistical main effect was observed for time ($P < .001$) and interaction ($P = .004$). Tcore statistically increased in ICE by +5.1% (1.1%) ($P < .001$; ES = 5.74 [2.56–8.92]) and CON by +7.0% (1.4%) ($P < .001$; ES = 6.29 [2.88–9.71]). The increase in Tcore was statistically larger in CON compared with ICE ($P = .004$; ES = 1.37 [0.29–2.45]) (Figure 1).

Blood Parameters

For hematocrit and hemoglobin, statistical main effects were observed for time ($P < .001$) but not interaction. Both hematocrit and hemoglobin statistically increased in ICE by +4.9% (4.9%) ($P = .003$; ES = 0.53 [0.20–0.86]) and +5.0% (3.8%) ($P < .001$; ES = 0.56 [0.29–0.83]), respectively, and in CON by +4.2% (3.4%) ($P = .012$; ES = 0.44 [0.22–0.66]) and +5.2% (3.7%) ($P < .001$; ES = 0.61 [0.33–0.90]), respectively. Plasma volume changes were similar in both ICE (–7.79% [6.15%]) and CON (–7.56% [4.88%]).

Similarly, for concentrations of blood lactate, a statistical main effect was observed for time ($P < .001$) but not interaction. Blood lactate concentrations statistically increased in ICE by +149% (91%) ($P < .001$; ES = 1.31 [0.65–1.96]) and CON by +163% (63%) ($P < .001$; ES = 1.32 [0.68–1.95]).

Statistical main effects for time but not interaction were also observed for cortisol, IL-6, and RONS ($P < .001$), IL-6 ($P < .001$), and RONS ($P = .006$). Concentrations of cortisol statistically increased from pre to post by +80.5% (102.0%) in ICE ($P = .007$; ES = 1.04 [0.18–1.90]) and by +137.8% (123.3%) in CON ($P < .001$; ES = 1.72 [0.93–2.51]) (Figure 2A). Concentrations of

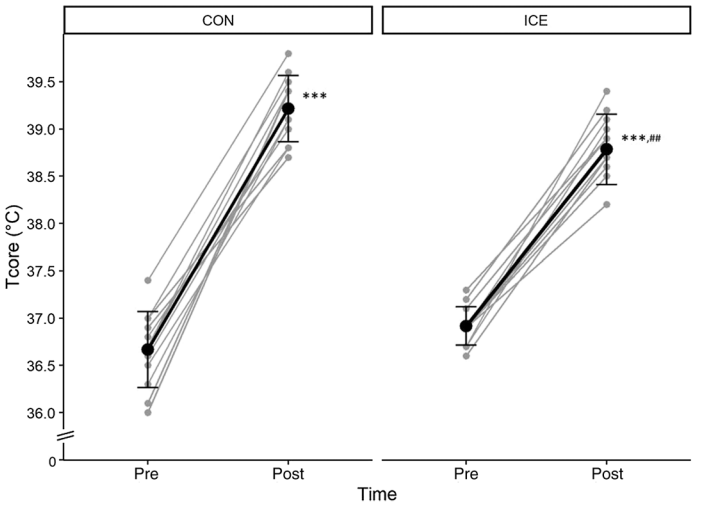

Figure 1 — Responses of Tcore to the constant workload trials with ice vest (ICE) and without cooling (CON). Individual responses are depicted as small dots combined with gray lines and mean values are shown in black and bold. Statistically significant differences to pre are denoted by $^{***}P < .001$ and to CON by $^{\#\#}P < .01$. CON indicates without ice vest; ICE, with ice vest; Tcore, core body temperature.

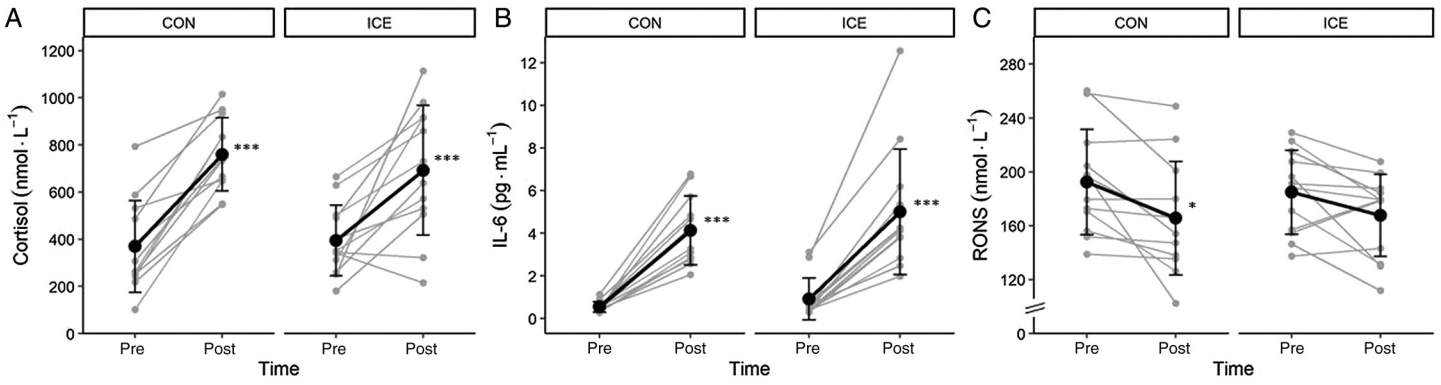

Figure 2 — Responses of (A) cortisol, (B) IL-6, and (C) RONS to the constant workload trials with ice vest (ICE) and without cooling (CON). Individual responses are depicted as small dots combined with gray lines and mean values are shown in black and bold. Statistically significant differences to pre are denoted by $^{*}P < .05$, $^{**}P < .01$, and $^{***}P < .001$. IL-6 indicates interleukin-6; RONS, reactive oxygen and nitrogen species.

IL-6 statistically increased in ICE by +624.3% (367.9%) ($P < .001$; ES = 1.16 [0.66–1.65]) and by +661.1% (323.7%) ($P < .001$; ES = 2.08 [1.13–3.02]) in CON (Figure 2B). RONS statistically decreased by −15.1% (16.5%) in ICE ($P = .02$; ES = 0.88 [0.22–1.54]) and by −19.2% (14.9%) ($P = .002$; ES = 0.94 [0.40–1.47]) in CON (Figure 2C).

No statistical associations between the changes in blood parameters (concentrations of blood lactate, cortisol, IL-6, and RONS) and the rise in Tcore were found for within condition or pooled analysis. However, the rise in IL-6 was statistically associated with that of blood lactate levels across both conditions ($r = .42$ [.01–.70], $P = .04$).

Discussion

This study aimed to examine the effects of percooling on Tcore as well as inflammatory and metabolic responses during prolonged exercise in the heat. Consistent with our hypotheses, a marked increase in Tcore was observed in both conditions, but the absolute elevation was lower in ICE compared with CON. Notably, irrespective of the exercise condition, statistical increases of blood lactate, cortisol, and IL-6 were observed, while concentrations of RONS decreased. Changes in Tcore were not associated with changes in inflammatory and metabolic markers.

Previous research has provided a clear consensus that both precooling and percooling strategies appear to be effective when vigorous cold stimuli are applied to large areas of the body.[5,6] Likewise, in the present study, the rise in Tcore was significantly attenuated when ICE was applied. However, although Tcore has previously been described as an important modulator of inflammatory responses to exercise,[25,26] the increase in cortisol and IL-6 concentrations was similar in both conditions. Our data, thus, indicate that percooling may reduce thermal strain, while not compromising physiological responses, supporting the use of percooling by ice vests not only during competitive situations but also during regular training sessions.

During prolonged exercise in the heat, Tcore elevations are typically accompanied by elevations in perceived exercise strain, due to reduced maximal aerobic power (ie, reduction in maximal oxygen uptake by about 11%).[2] Consequently, exercise intensities calculated based on a percentage of PPO or $\dot{V}O_2$peak assessed in thermoneutral conditions are often lower in hot as compared with thermoneutral conditions. Reduced exercise intensities or workloads during training might, in turn, attenuate beneficial adaptive responses.[10] As our findings indicate reduced thermal strain without affecting metabolic and inflammatory responses, percooling through an ice vest may be a strategy to maintain or even increase absolute exercise intensities, thereby enhancing the stimulus required for desirable adaptations.

There are a few possible explanations for our finding of similar metabolic and inflammatory responses despite Tcore reductions. The absolute advantage of percooling in terms of Tcore reductions was smaller (ie, 0.4°C) than that observed in previous studies with mean reductions of 1.0°C (0.7°C).[6] Moreover, it was previously suggested that differences would have to equal at least 1°C to independently affect hormone and cytokine responses.[27] In line with this, attenuations of inflammatory responses were accompanied by Tcore differences of 1°C to 2.5°C due to exercise in different ambient temperatures or precooling.[9,25,26] However, the attenuation of 0.4°C in the present ICE condition is well in line with previous findings assessing changes in Tcore induced by percooling in well-trained athletes.[28] Thus, while the rise in Tcore may be sufficient to stimulate sympathoadrenal activation as evident from statistically increased cortisol concentrations,[29] percooling through ice vests might not blunt inflammatory responses.

The absolute rise in Tcore also needs to be considered when interpreting our findings. Irrespective of cooling, the authors observed maximal Tcore values of 39.2°C. As a threshold for Tcore elevations of ≥39°C has recently been related to a marked immune reaction due to increased intestinal permeability,[30] the thermal strain in the present study still appears tolerable. In fact, studies that reported major differences between immune responses to exercise in different environments observed Tcore values considerably exceeding those observed in our study. For example, elevations of up to 39.8°C accompanied by an increased lymphocyte protein oxidative damage have previously been reported.[25] Therefore, it remains to be investigated whether similar findings are observed when percooling is utilized in more extreme conditions.

In contrast, it seems reasonable that the rise in IL-6 concentrations in the present study mainly resulted from skeletal muscle release. Since percooling was applied to the upper body, the working muscles of the lower limbs likely did not benefit from cooling. In line with previous work showing enhanced reliance on glucose metabolism with rising temperatures,[31] our pooled correlation analysis

showed a strong association between blood lactate and IL-6, indicating that IL-6 may have acted as a glucoregulatory sensor.[13] The statistical correlation between both parameters further underlines the association of IL-6 levels with skeletal muscle rather than with immune cell release. However, reduced renal blood flow may also have contributed to the marked IL-6 elevations, due to a reduced clearance.[27]

Besides thermophysiological alterations, the participants' training status may also influence responses to exercise in the heat. Well-trained athletes were previously found to exhibit heat acclimatized phenotypes, likely as a result of regular Tcore elevations during their usual training.[19] This assumption is reinforced by our findings of RONS responses. The decrease in systemic concentrations of RONS across all trials likely resulted from an upregulation of endogenous antioxidant capacity that exceeded RONS production due to exercise in the heat. Such an upregulation has recently been shown in both trained[32] and acclimatized athletes,[16] potentially facilitated by an enhanced heat shock protein expression.[17] This, in turn, might also explain why the authors observed only slight mitigation of Tcore as a result of percooling as trained and acclimatized athletes already possess thermo-physiological adaptations to reduce thermal strain.[4]

Practical Applications

Our data indicate that percooling by an ice vest may mitigate exercise- and heat-induced thermal strain without compromising physiological signaling vital for beneficial training adaptations. Therefore, percooling may be a beneficial method when moderate elevations of inflammatory and metabolic parameters are desired but heat strain ought to be limited. Moreover, percooling-induced reductions in thermal strain may even facilitate a more pronounced training stimulus (eg, through maintained absolute exercise intensities) during exercise in the heat. Consequently, ice vests may be worn during competitions as well as during regular training sessions in hot environments, especially during nonweight-bearing exercise.

Conclusions

Our findings indicated that percooling with an ice vest statistically reduced thermal strain but not acute inflammatory and metabolic responses of trained athletes, as shown by similar levels of blood lactate, cortisol, IL-6, and RONS. Future studies should assess whether this remains true also for percooling utilized in more extreme environments, with even larger expected increases in Tcore.

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