Interleukin-6 Responses to Water Immersion Therapy After Acute Exercise Heat Stress: A Pilot Investigation

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Context: Cold-water immersion is the criterion standard for treatment of exertional heat illness. Cryotherapy and water immersion also have been explored as ergogenic or recovery aids. The kinetics of inflammatory markers, such as interleukin-6 (IL-6), during cold-water immersion have not been characterized.

Objective: To characterize serum IL-6 responses to water immersion at 2 temperatures and, therefore, to initiate further research into the multidimensional benefits of immersion and the evidence-based selection of specific, optimal immersion conditions by athletic trainers.

Design: Controlled laboratory study.

Setting: Human performance laboratory

Patients or Other Participants: Eight college-aged men (age = 22 ± 3 years, height = 1.76 ± 0.08 m, mass = 77.14 ± 9.77 kg, body fat = $10\% \pm 3\%$, and maximal oxygen consumption = 50.48 ± 4.75 mL·kg⁻¹·min⁻¹).

Main Outcome Measures: Participants were assigned randomly to receive either cold ($11.70^\circ\text{C} \pm 2.02^\circ\text{C}$, $n = 4$) or warm ($23.50^\circ\text{C} \pm 1.00^\circ\text{C}$, $n = 4$) water-bath conditions after

exercise in the heat (temperature = 37°C , relative humidity = 52%) for 90 minutes or until volitional cessation.

Results: Whole-body cooling rates were greater in the cold water-bath condition for the first 6 minutes of water immersion, but during the 90-minute, postexercise recovery, participants in the warm and cold water-bath conditions experienced similar overall whole-body cooling. Heart rate responses were similar for both groups. Participants in the cold water-bath condition experienced an overall slight increase ($30.54\% \pm 77.37\%$) in IL-6 concentration, and participants in the warm water-bath condition experienced an overall decrease ($-69.76\% \pm 15.23\%$).

Conclusions: We have provided seed evidence that cold-water immersion is related to subtle IL-6 increases from postexercise values and that warmer water-bath temperatures might dampen this increase. Further research will elucidate any anti-inflammatory benefit associated with water-immersion treatment and possible multidimensional uses of cooling therapies.

Key Words: interleukins, cytokines, inflammation, exertional heat illness, hyperthermia

Key Points

- Changes in interleukin-6 were modest with immersion in cold and warm water baths.
- The 2 water-bath temperatures had different initial cooling efficiencies, but over a 90-minute, postexercise recovery, participants in both groups experienced similar whole-body cooling.
- Interleukin-6 might be a marker of subtle differences between water temperatures that otherwise affect physiology in similar ways.

Exercise heat stress has been associated with muscle cell damage,^{1,2} mild endotoxemia,^{3,4} and increased risk for exertional heat illness.⁵ The compound stress of physical activity in hot and humid environments is known to instigate inflammation,^{6–8} which might be implicated in heat-stroke pathophysiology.^{9,10} The inflammatory response to exercise involves acute-phase protein and cytokine responses, both of which are independent of infection-triggered activity in the immune system.^{11–13} Cytokines are of particular interest due to their roles in the balance of immune responses between beneficial and harmful physiologic effects.^{14,15} In particular, interleukin-6

(IL-6) has proinflammatory and anti-inflammatory properties, so it is relevant to exercise physiology due to the observed relationship with muscle regeneration and recovery.^{16,17} In addition, IL-6 responds to many different modes, durations, and intensities of exercise or training and to endogenous and environmental stressors.¹³ It increases measurably with exercise and heat stress and might be a valuable marker of stress and possibly of recovery.

Recovery from exertional heat illness is best ensured with cold-water immersion, which is the fastest way to reduce hyperthermic core temperature.^{5,18} Proulx et al¹⁹ identified maximal cooling of hyperthermic participants when water

baths at 2°C were compared with 8°C, 14°C, and 20°C. They found no differences in cooling rates among the 8°C, 14°C, and 20°C water baths, but cooling was effective in all cases. The relationship between cooling and immune function, or IL-6 specifically, was first studied by Brenner et al.²⁰ They demonstrated that exposure to cold without previous exercise leads to circulating IL-6 increases. Peake et al.²¹ measured serum IL-6 concentrations before and after exercise and during recovery; during the recovery period, participants rested for 20 minutes after 90 minutes of cycling exercise in warm (32°C) environments and were immersed in cold water (14°C) for 15 to 20 minutes. The researchers found decreases that were not different in serum IL-6 concentrations due to water immersion.

Water immersion is undoubtedly the criterion standard in treating hyperthermia in exertional heat illness¹⁸ and might have other recovery or performance benefits.^{21–28} Researchers have explored the possibility that cold-water immersion or cryotherapy might have beneficial effects in addition to rapid conductive cooling in hyperthermic athletes and patients^{25,29–36}; some of these benefits might be related to cold-induced or cooling-related regulation of inflammation after intense exercise and heat stress.^{21,23,28} Few researchers have systematically and clearly defined immune-response kinetics during cold-water immersion after exercise heat stress.

Our research aim was to provide a first-time kinetic analysis of IL-6, which is a cytokine that clearly is increased with exercise heat stress, during cold-water immersion. We sought to evaluate IL-6 responses during commonly used cold temperature (11.70°C ± 2.02°C) water baths (COLD)^{21,23,24,26,27,29,37,38} and during a more easily generated warm, or room, temperature (23.50°C ± 1.00°C) water bath (WARM), which might be more applicable or feasible in certain field or clinical settings.

Although we expected the 2 bath conditions to have similar cooling rates,¹⁹ we hypothesized that subtle molecular differences might support the multidimensional benefit of one versus the other. Therefore, our purpose was to provide novel information about serum IL-6 changes during water immersion with 2 different but comparably effective cooling temperatures. Our pilot data support future research into the possible mechanisms by which water immersion might be diversely beneficial. This future research will support mechanistic, evidence-based prescription of cold-water immersion, cryotherapies, and other cooling strategies.

METHODS

Participants

We recruited male participants in mid-April from the University of Connecticut. Nine college-aged men participated in this study. However, only 8 participants (age = 22 ± 3 years, height = 1.76 ± 0.08 m, mass = 77.14 ± 9.77 kg, body fat = 10% ± 3%, maximal oxygen consumption = 50.48 ± 4.75 mL·kg⁻¹·min⁻¹) were included in the analysis because 1 participant did not experience core temperature elevations during exercise heat stress and required no cold-water immersion therapy. This was not related to study design but likely due to an exceptional resilience of this participant's thermoregulatory capabilities. Female volun-

teers were not recruited to avoid possible confounding factors from diverse hormone therapy, contraceptive use, and menstrual cycles; no one has determined whether these variables might affect IL-6 via endocrine signaling^{35,39} and, if they do, how they affect it. Each potential participant completed a medical history questionnaire form, and volunteers were excluded if they indicated use of tobacco; use of medication or dietary supplements; recent exposure or acclimatization to heat; history of cardiovascular, metabolic, or respiratory disease; or history of exertional heat stroke or heat intolerance. All participants provided written, informed consent, and the University of Connecticut Institutional Review Board for Human Studies approved the study.

Design

Each participant underwent a familiarization day consisting of body-composition testing via skinfold measurements, maximal oxygen consumption measurement, and instruction in the exercise heat protocol. After this preliminary visit, they took part in a 1-day trial. Initiation of the exercise-immersion protocol was standardized to afternoon for all participants. They were randomized into either the COLD (11.70°C ± 2.02°C) or WARM (23.50°C ± 1.00°C) immersion group. For analysis and presentation of data, COLD group participants were numbered 1 through 4, and WARM group participants were numbered 5 through 8.

During the exercise heat and water-immersion protocols, blood samples were collected at the immediate postexercise time point: 5 minutes postexercise, when participants entered the water-immersion tubs; 10 minutes postexercise; 60 minutes postexercise; and 90 minutes postexercise. The final 2 blood samples were collected during a sitting equilibration because all 8 participants cooled to 38°C (core temperature) within the first 30 minutes; after their core temperatures reached 38°C, participants exited the immersion tub and sat for the remainder of the 90 minutes postexercise.

For further standardization of data results, all participants drank 500 mL of water the evening before testing and 500 mL on the morning of testing to ensure euhydration. Participants submitted activity logs (mode, duration, intensity) and diet record, following detailed written and oral instructions in recording procedures. Data were collected in 2 randomized iterations 10 days apart in late April and early May at the Human Performance Laboratory of the University of Connecticut. The mean daily temperatures in Storrs during this time were 9°C (range, 5°C–17°C) and 11°C (range, 6°C–17°C), respectively.

Testing Day Protocols

Familiarization Session. On arrival, participants were instructed to void their bladders before being weighed in shorts on an electronic scale (model 349KL; Healthometer Inc, Bridgeview, IL) to the nearest 50 g. We used calipers (Lange skinfold calipers; Cambridge Scientific Industries, Watertown, MA) to measure skinfold thickness at 7 sites on the right side of the body. Measurements were made in duplicate and included chest, triceps, subscapular, suprailiac, umbilical, midaxillary, and thigh sites. The Jackson and Pollock⁴⁰ equation was used to determine body

density, and the Siri equation³⁶ was used to calculate percentage of body fat. The maximal oxygen consumption test was performed using a motorized treadmill protocol in an environmental chamber (model 2000; Minus-Eleven, Inc, Malden, MA) at 22°C. Treadmill (Precor, Woodinville, WA) speed and grade started at 1.52 m/s (3.4 miles per hour) and 5%, respectively. After 3 minutes, the speed was increased to 1.78 m/s (4 miles per hour), and the grade was increased by 2% every 2 minutes until participants reached volitional cessation. Oxygen consumption was measured breath by breath (30-second averages) using on-line, open-circuit spirometry (model CPX-D; Medical Graphics, Inc, St Paul, MN) that was calibrated according to the manufacturer's guidelines before each test.

Morning Session. Morning protocols were scheduled to begin between 6:30 and 8:00 AM and were staggered to facilitate blood collection and processing. Upon arrival, participants urinated into a clean, inert, plastic container; urine was analyzed directly for specific gravity to verify euhydration (≤ 1.020). Wearing only underwear, participants then were weighed on an electronic scale (± 50 g). They returned later in the day for the exercise and immersion protocols.

Exercise Protocol. Upon return, participants cycled for approximately 20 minutes on a cycle ergometer (model 818E; Monark Ergomedic, Stockholm, Sweden) in the laboratory (temperature = $25^{\circ}\text{C} \pm 1^{\circ}\text{C}$, relative humidity = $45\% \pm 6\%$) at a workload equal to 1.5 W/kg body mass. End time was taken as the onset of sweating by visual and the participant's oral verification. Next, they consumed a standard meal consisting of a bagel, cream cheese, a banana, and 200 mL of water; this provided 312 kcal from carbohydrate, 126 kcal from fat, and 56 kcal from protein. We inserted a cannula (Critikon, Inc, Tampa, FL) into a superficial forearm vein, and participants entered the environmental chamber.

Participants stood quietly for 15 minutes inside the chamber to allow body fluids, plasma volume, and skin temperatures to stabilize. The environmental conditions in the chamber included a temperature of $37^{\circ}\text{C} \pm 1^{\circ}\text{C}$ and relative humidity of $52\% \pm 11\%$. Exercise was performed on a motorized treadmill at each participant's fastest walking pace (range among participants, 1.69–1.83 m/s [3.8–4.1 miles per hour]), which had been determined during the familiarization visit, at a 5% grade for 90 minutes or until volitional cessation. All participants completed 90 minutes of exercise and none required emergency interruptions. During exercise heat exposure, heart rate and rectal temperature were monitored every 15 minutes via telemetry (CorTemp; HQ Inc, Palmetto, FL) and a rectal thermistor (model YSI 401 rectal probe; Yellow Springs, Yellow Springs, OH), respectively. The rectal thermistor was inserted 10 cm beyond the external anal sphincter by the participant before entrance into the environmental chamber. Before and after heat exposure, participants were weighed on an electronic scale (± 50 g). During exercise, participants consumed 0.17% body mass of water every 15 minutes. Treadmill belt speed was verified during each use with a handheld digital tachometer (model 92-4059-20; Fisher Scientific, Pittsburgh, PA).

Water Immersion Protocol. Immediately after exercise, time of exercise was recorded, and a blood sample was collected while the participant stood, assisted if needed, on

the stopped treadmill belt. Participants were escorted to a nearby locker room where 2 plastic tubs full of water at either $11.70^{\circ}\text{C} \pm 2.02^{\circ}\text{C}$ or $23.50^{\circ}\text{C} \pm 1.00^{\circ}\text{C}$ had been prepared approximately 30 minutes earlier. Water temperature was measured with a fully submerged pool thermometer; to ensure that water temperature did not change, ice chips were prepared for cooling the water baths, and water was circulated continuously around participants while immersed. Before entering the tub, they removed all clothing except shorts and underwear, then sat in a chair beside the tub for 5 minutes. Participants were allowed to drink water ad libitum in this time after exercise. At 5 minutes postexercise, a blood sample was collected, and participants were assisted into the tubs where they sat with the water levels up to their sternums, legs stretched out and completely immersed, and upper extremities out of the water and resting on the sides of the tubs. Heart rate and rectal temperature were recorded every minute during immersion.

Participants were immersed only until core temperature had decreased to 38.0°C to prevent overshoot and possible mild hypothermia. When reaching 38.0°C , participants left the water and were allowed to towel off and change before being escorted to a nearby room (temperature = $25^{\circ}\text{C} \pm 1^{\circ}\text{C}$, relative humidity = $45\% \pm 6\%$) where they sat quietly in recliners before the 60-minute and 90-minute postexercise blood draws, thermal sensation reports, and heart rate measurements. At the final blood draw, we removed the cannula and ensured the well-being and safety of the participants before allowing them to depart the laboratory.

Blood Collection

All participants had a flexible, indwelling, 18-gauge Teflon cannula placed in a superficial forearm vein. A 15.2-cm extension tube fitted with a 3-way stopcock was attached to the cannula. Patency was maintained with saline; the tubing (2 mL of dead space) was flushed with 4 mL of blood before each sample was obtained. Each draw was 5 mL into a dry syringe, which was transferred into a chilled 5-mL endotoxin- and pyrogen-free blood-collection tube (BD Vacutainer; BD, Franklin Lakes, NJ). Blood was allowed to clot and immediately processed to ensure accurate measurement of interleukin. Samples were centrifuged at 3000 rpm for 20 minutes at 4°C before the resulting serum was transferred to 1.5-L microcentrifuge tubes and stored at -80°C for subsequent analysis of IL-6.

Analytical Methods

Diet records were analyzed for energy, macronutrient content, sodium content, potassium content, and fluid volume (Nutritionist Pro, version 1.3; First Databank, Inc, The Hearst Corporation, San Bruno, CA). Serum was analyzed for IL-6 concentration by enzyme immunoassay (IL-6 Ultrasensitive Human EIA; Alpco Diagnostics, American Laboratory Products Co, Windham, NH). The interassay and intra-assay coefficients of variation (CVs) were 11.1% and 3.0%, respectively. The theoretical sensitivity of the ultrasensitive IL-6 assay was 0.16 to 10.0 pg/mL. Interassay CVs were calculated from the average CV of each duplicate pair. The intra-assay CVs are the mean of the CV of the standards used to generate the

Figure 1. Heart rate postexercise. Water immersion began 5 minutes after the cessation of exercise. From the beginning of water immersion, heart rate in beats per minute was measured via a heart-rate monitor (telemetry) every minute. We found decreases in heart rate for the cold ($11.70^{\circ}\text{C} \pm 2.02^{\circ}\text{C}$) water-bath immersion and warm ($23.50^{\circ}\text{C} \pm 1.00^{\circ}\text{C}$) water-bath immersion groups during the recovery period postexercise, but the decreases were statistically similar for the 2 groups.

standard curve. Absorbance was read on a multilabel counter (VersaMax; Molecular Devices, Sunnyvale, CA).

Statistical Analysis

The means $\pm$ standard deviations (SDs) are presented throughout the “Results” section, but for (nonnormal) IL-6 data, medians and interquartile range are presented as indicated. Fewer than 4% of the total (120 data points, 40 time points in triplicate) data points were outside the enzyme-linked immunosorbent assay sensitivity range and were ascribed to be the mean IL-6 concentrations of the group (cool-water or warm-water temperature) at the designated time point. The IL-6 data were not normally distributed but displayed equal variance between COLD and WARM. The IL-6 at time points 5, 10, 60, and 90 minutes postexercise and percentage changes from time 0 were analyzed with nonparametric Kruskal-Wallis tests to calculate differences across water-temperature treatments. Spearman ρ correlation analysis was performed to determine the relationship between IL-6 and core temperature. Comparison of means on all other normally distributed variables was performed using a univariate analysis of variance. We used SPSS (version 14.0, release 2005; SPSS

Inc, Chicago, IL) to analyze the statistics. Significance was established as $P \leq .05$.

RESULTS

Baseline Measures

All nutritional, hydration, and anthropometric variables between the COLD and WARM groups were similar. Daily caloric, macronutrient, sodium, and potassium intake were not different across days and participants, whereas water intake was greater on the testing day than the preacclimation day ($F_{9,63} = 4.852$, $P \leq .001$, $\eta^2 = 0.409$, observed power = 0.998). Urine specific gravity on familiarization and testing days indicated that all participants were euhydrated on both days (1.020 ± 0.005).

The demographic characteristics were not different between the COLD group ($n = 4$, age = 22 ± 1 years, height = 1.74 ± 0.07 m, mass = 72.4 ± 9.0 kg, maximal oxygen consumption = 47.3 ± 2.9 mL/kg $^{-1}$ /min $^{-1}$) and the WARM group ($n = 4$, age = 23 ± 3 years, height = 1.77 ± 0.08 m, mass = 81.9 ± 9.0 kg, maximal oxygen consumption = 52.9 ± 4.9 mL/kg $^{-1}$ /min $^{-1}$) ($F_{1,12}$ range,

Figure 2. A, Rectal temperature postexercise. Rectal temperatures at the end of exercise were statistically similar for cold water-bath immersion (COLD) and warm ($23.50^{\circ}\text{C} \pm 1.00^{\circ}\text{C}$) water-bath immersion (WARM) groups. The COLD group experienced a decrease in rectal temperature by 10 minutes postexercise, which was 5 minutes after water-immersion therapy began. The rectal temperatures for the COLD and WARM groups were similar at each time point throughout recovery. B, Rectal temperatures at 60 and 90 minutes postexercise. C, Overall cooling rate for whole cold-water immersion. Overall cooling rate from the beginning of water immersion through cooling and to the end of the 90-minute recovery period postexercise was not different between COLD and WARM conditions, but we found a trend for higher cooling rates in the COLD group ($P = .09$). D, The cooling rate for first 6 minutes of cold-water immersion. Water immersion began 5 minutes after the end of exercise, and during the first 6 minutes of cooling the rate of cooling was faster with the COLD than the WARM condition ($P < .05$). *Indicates a within-group difference.

Figure 3. A, Serum interleukin-6 (IL-6) concentrations at immediate postexercise time point. Whereas participants were randomized into warm ($23.50^{\circ}\text{C} \pm 1.00^{\circ}\text{C}$) water-bath immersion (WARM) and cold ($11.70^{\circ}\text{C} \pm 2.02^{\circ}\text{C}$) water-bath immersion (COLD) treatments, a difference existed in mean serum IL-6 concentrations postexercise, with participants who were randomized into the WARM condition having higher IL-6 ($P < .05$). B, Percentage change in serum IL-6 from baseline, normalized to baseline values for each participant over recovery time. At 60 minutes and 90 minutes postexercise, participants in the WARM and COLD groups were sitting at equilibrium after cooling treatments, but the COLD group had higher relative IL-6 increases from baseline for each respective group. C, Serum IL-6 concentrations versus rectal temperatures at various postexercise time points. We did not find a strong linear relationship between core temperature at each time point and concurrent serum IL-6 concentrations at each time after exercise for the WARM and COLD groups. ^aIndicates a difference at $P < .05$.

0.317–3.871; $P > .05$). Water temperature between the 2 groups was different (COLD = $11.7^{\circ}\text{C} \pm 2.0^{\circ}\text{C}$, WARM = $23.5^{\circ}\text{C} \pm 1^{\circ}\text{C}$; $F_{1,12} = 109.351$, $P < .001$).

Heart Rate

The COLD and WARM groups experienced similar heart rate changes during immersion and recovery. Thus, the WARM and COLD immersion baths did not result in different cardiovascular responses. Heart rates in beats per minute are given in Figure 1. We found no differences between the COLD and WARM groups at each time point ($F_{1,78} = 2.037$, $P > .05$). After entering the baths, both COLD and WARM groups experienced decreases in heart rate within the first 2 minutes ($P < .05$). This heart rate recovery continued through the 90-minute recovery period postexercise for both groups ($P < .001$).

Core Temperature

Absolute rectal temperatures were not different between the COLD and WARM groups. Relative change from the immediate postexercise temperatures, which were not different, indicated greater cooling for the COLD than WARM group in the first 6 minutes of immersion. Rectal temperatures for the COLD and WARM groups are depicted in Figure 2A and 2B. Postexercise rectal temperatures were $39.80^{\circ}\text{C} \pm 0.23^{\circ}\text{C}$ and $39.26^{\circ}\text{C} \pm 0.61^{\circ}\text{C}$ for COLD and WARM, respectively, and were not different from each other ($F_{1,12} = 2.702$, $P = .15$, $\eta^2 = 0.310$, observed power = 0.284). By the time participants entered the water baths 5 minutes later, rectal temperature had decreased to $39.75^{\circ}\text{C} \pm 0.22^{\circ}\text{C}$ in the COLD group and $39.21^{\circ}\text{C} \pm 0.63^{\circ}\text{C}$ in the WARM group ($F_{1,12} = 2.128$, $P = .20$, $\eta^2 = 0.262$, observed power = 0.234). We found no differences between COLD and WARM at each time point during cooling ($P > .05$).

Relative changes from immediate postexercise, within COLD and within WARM, indicated that differences existed in cooling in the first 6 minutes of immersion. Rectal temperature in the COLD group was lower 5 minutes into immersion (time point: 10 minutes) than immediately postexercise ($F_{1,12} = 4.811$, $P = .049$, $\eta^2 = 0.286$, observed power = 0.523). The WARM group did not exhibit rectal temperatures that were different from their immediate postexercise temperatures until 60 minutes postexercise. The COLD and WARM groups experienced different cooling rates for the first 6 minutes of immersion (Figure 2D).

The total cooling rate from entry into the immersion tub until 90 minutes postexercise for the COLD group ($0.62^{\circ}\text{C}/\text{min} \pm 0.61^{\circ}\text{C}/\text{min}$) was more than twice that of the WARM group ($0.25^{\circ}\text{C}/\text{min} \pm 0.59^{\circ}\text{C}/\text{min}$), but this difference was not statistically significant ($F_{1,12} = 3.77$, $P = .08$, $\eta^2 = 0.309$, observed power = 0.282). Similarly, the average immersion time was not different between the WARM (10 ± 5.48 minutes) and COLD (8 ± 6.68 minutes) groups.

Interleukin-6

Relative changes from postexercise values were different between the COLD and WARM groups. The COLD group experienced an increase in IL-6, whereas the WARM group experienced a decrease in IL-6. Postexercise IL-6 values

Table. Interleukin-6 Concentrations During Postexercise Recovery Period (Mean $\pm$ SD)

Time, min	Water-Bath Condition	
	COLD, pg/mL ^a	WARM, pg/mL ^b
0 (Immediate postexercise)	0.93 ± 1.78	5.24 ± 6.48
5 (Entrance into immersion tub)	1.21 ± 1.10	1.70 ± 3.64
10	0.94 ± 1.10	2.27 ± 4.05
60	1.05 ± 1.55	1.83 ± 1.67
90	1.30 ± 1.51	1.49 ± 1.40

^a Indicates cold water-bath immersion temperature was $11.70^{\circ}\text{C} \pm 2.02^{\circ}\text{C}$.

^b Indicates warm water-bath immersion temperature was $23.50^{\circ}\text{C} \pm 1.00^{\circ}\text{C}$.

were not different between the WARM (5.24 ± 6.48 pg/mL; median $\pm$ interquartile range) and COLD (0.93 ± 1.78 pg/mL) groups based on a nonparametric Kruskal-Wallis test ($N = 8$, $\chi^2_1 = 3.000$, $P = .08$). Absolute values for the IL-6 concentrations of both groups are shown in the Table.

Because a measurable qualitative difference existed between postexercise IL-6 for the COLD and WARM groups, absolute IL-6 concentrations and subsequent changes might not be accurately reflective of the standardized WARM and COLD effects (Figure 3A). Thus, we used the percentage change in IL-6 from baseline as a measure of IL-6 change (Figure 3B). We found no differences in percentage change in IL-6 between the COLD and WARM groups until 90 minutes postexercise. By 90 minutes postexercise, IL-6 in the COLD group had increased by 30.54%, and IL-6 in the WARM group had decreased slightly by 69.76% ($N = 8$, $\chi^2_1 = 4.083$, $P = .04$) (Figure 3C). Using within-group comparisons, we verified that absolute IL-6 concentrations in both groups were different at 60 minutes and 90 minutes from concentrations at postexercise ($P < .05$), supporting the percentage change data.

To explore the possibility that serum IL-6 might be related to core temperature at a given time point, we analyzed the relationship between serum IL-6 and core temperature during the COLD and WARM conditions. In the COLD condition, serum IL-6 remained fairly constant in relationship to core temperature ($P = .40$, $\rho = -0.202$) (Figure 3B). In the WARM condition, serum IL-6 seemed to decrease with core temperature ($P = .044$, $\rho = 0.454$). The IL-6 was not correlated with core temperature when pooling WARM and COLD condition data ($P = .28$, $\rho = 0.176$). The 2 groups differed by only 2 minutes in cooling time and reached the same cooling endpoint of 38°C core temperature.

DISCUSSION

We performed a pilot investigation to provide novel information about serum IL-6 kinetics after exercise heat stress during water immersion and recovery. We observed modest changes in IL-6 for both the COLD and WARM conditions. However, we also observed that the COLD condition was uniquely associated with percentage increases in serum IL-6 during postexercise cooling. This finding was in the context of similar cardiovascular (heart rate) and core temperature recovery during the 90 minutes postexercise. Given the initial, brief difference in cooling rate between the COLD and WARM conditions and the brevity (range, 8–10 minutes) of the cooling time relative to total recovery time

(90 minutes), we conclude that this result requires further research. Overall, we found that IL-6 might be one marker of subtle differences between water temperatures that otherwise affect physiology in similar ways. Further research will highlight mechanisms by which specific immersion conditions provide benefits related to inflammation in addition to general whole-body cooling. This type of research will allow athletic trainers to make decisions with the multidimensional aspects of recovery in mind.

The efficacy of the COLD and WARM conditions was comparable, but we noted a difference in cooling rate in the first 6 minutes of water immersion. Postexercise core temperature was not different between the COLD and WARM conditions. Rectal temperature continued to decrease to resting temperatures during the 90-minute recovery. Time to cool was not different between groups. However, despite a small sample size and only a 10°C difference in water-bath temperatures, we found an initial difference in cooling rates in the first 6 minutes of immersion. This early difference in cooling rates could have affected lagging changes in serum IL-6 concentrations, but further research is required. In the future, researchers also will clarify whether slight differences like this in cooling relate to notable differences in physiologic or immune-related benefits. Athletic trainers then will be able to rationalize certain immersion conditions as more standard, broadly beneficial prescriptions for recovery or treatment.

When hyperthermic individuals were immersed in water baths immediately after exercise heat stress, serum IL-6 concentrations did not change acutely. However, 90 minutes into recovery, once core temperature was restored to normal, serum IL-6 appeared to increase slightly (from postexercise values) with colder bath temperatures in comparison with warmer temperatures (+10°C). We observed slightly different, albeit statistically nonsignificant, postexercise serum IL-6 values for our 2 treatment groups. In considering this, we present results that represent relative percentage changes in serum IL-6 from postexercise levels. Larger sample sizes in future studies might eliminate such random variation between experimental groups. Nevertheless, despite our small sample sizes and the smaller starting value of the COLD condition, the COLD group experienced an increase of approximately 31% by 90 minutes of recovery. During the 90 minutes, serum IL-6 concentrations consistently increased in the COLD condition but decreased slightly or reached a plateau in the WARM condition (Figure 3C). Putting our pilot findings in the context of a few other studies in which researchers have provided evidence that cold-water immersion or cold exposure might affect key inflammatory cytokines highlights the possibility that specific immersion conditions not only might promote cooling but also might benefit athletes and patients in other ways.

For example, Brenner et al²⁰ observed increased plasma IL-6 concentrations after 1 hour of exposure to cold air in resting participants and after 2 hours when the exposure to cold was preceded by exercise with a thermal clamp or passive heating. Without previous exercise or heat stress, cold exposure induced an increase in plasma IL-6 of approximately 53% after 1 hour and of approximately 85% by 2 hours of exposure to cold air. Adding exercise with a thermal clamp or having participants exercise in water that prevented increases in core temperature resulted in an increase in IL-6 of approximately

50% after 1 hour of exposure to cold and of approximately 75% after 2 hours. From these qualitative observations, exercise alone did not seem to contribute to any changes in IL-6 kinetics during exposure to cold.

In their passive-heating group, Brenner et al²⁰ found that IL-6 increased approximately 30% after 1 hour of exposure to cold and approximately 113% after 2 hours. From approximate qualitative observations such as these, increases in core temperature seem to contribute to perhaps a dampening of increases in IL-6 caused by 1 hour of exposure to cold. Supporting this speculation is the observation that combined increases in core temperature with exercise before exposure to cold resulted in an increase in IL-6 of approximately 45% after 1 hour of cold exposure and of approximately 60% after 2 hours. We did not investigate any functional implications of IL-6 responses, so future research is required to determine whether dampening or exacerbating IL-6 increases is more desirable in treating exercise heat stress. The decisions regarding water-immersion conditions, because they are based in inflammatory responses, could affect recovery or other treatments prescribed simultaneously for an athlete or patient.

Unlike Brenner et al,²⁰ Peake et al²¹ found no effect of cold-water immersion on circulating IL-6 after exercise heat stress. They speculated that their results were divergent because they introduced water immersion 20 minutes after the end of exercise rather than immediately after exercise. We hypothesize that the effects of delayed water immersion in the study by Peake et al²¹ support the idea that without rapid cooling, postexercise IL-6 might eventually return to resting levels, depending on the intensity of exercise heat stress and degree of hyperthermia experienced. Further research also will characterize the timing of water immersion and how that affects inflammation. This information will better arm health care providers with information to prescribe the best cooling therapy or recovery/ergogenic treatment in light of all the other factors that have a basis in inflammatory signaling and regulation.

CONCLUSIONS

Water immersion is the criterion standard for treating hyperthermia in exertional heat illness,¹⁸ and water immersion, body cooling, and cryotherapy all seem to have some mechanistic basis in regulating inflammation.^{25,29–34} Coaches, athletic trainers, and clinical professionals prescribe cold-water immersion for various reasons under diverse circumstances. Better understanding the effect of immersion therapy on inflammation will equip professionals with information about how best to prescribe such therapy in the context of other prophylactic and treatment modalities, ergogenic aids, and commonly used anti-inflammatory or pharmacologic remedies used in the course of training, competition, and recovery. Understanding the fundamental molecular and cellular effects of a therapy, such as water immersion, also allows health care providers to make informed decisions about the optimal conditions of a clearly beneficial modality, such as water immersion.

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