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The field study about the effects of artificial CO_2-rich cool-water immersion after outdoor sports activity in a hot environment

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A R T I C L E I N F O

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Background/objective: In our previous laboratory experiment (room temperature of 25 °C), CO_2-rich cool-water immersion (CCWI) suppressed subjects' core body temperature even during repeated exercise. It is unclear whether the suppression of body temperature elevation would also continue after CCWI in a hot outdoor environment. Herein we investigated the thermal effects of CCWI after regular exercise training in heat on subjects' core temperature (T_{core}), three skin temperatures (T_{skin}), heart rate (HR), and the rate of perceived ice (RPI).

Methods: Thirty-six subjects (25 males, 11 females) were randomly allocated into three groups (CCWI, CWI, and control). After training at their competitive clubs, each subject was immersed up to the chest in CCWI or CWI at 20 °C for 20 min, followed by a 60-min recovery period. T_{core}, T_{skin}, HR, and RPI were measured at the initial rest, the end of immersion, and every 10 min during the recovery period.

Results: Compared to the control, the CCWI subjects' T_{core} was significantly lower at 50–60 min after the end of immersion ($p < 0.05$). T_{skin} at abdominal and lower-leg regions during the recovery period was maintained at significantly lower values in the CWI and CCWI groups versus control ($p < 0.05$). The CCWI subjects maintained lower T_{skin} for a longer time than the CWI subjects.

Conclusions: These findings indicate that CCWI suppresses the rise in body temperatures more than CWI, even in a hot environment, suggesting that CCWI may be a more effective countermeasure against increasing body temperature in a hot outdoor environment.

1. Introduction

Many athletes train intensely to improve their performance all year round. They may also compete multiple times a day or on consecutive days, which often results in severe stress on their bodies. Athletes thus need to manage their post-practice conditions and explore ways to accelerate their recovery from heavy training loads. It is also known that athletic training in a hot environment can induce heat stroke, and countermeasures against heat stroke have been a longstanding issue.[1]

In recent years, cold water immersion (CWI) has been used as a method of recovery from fatigue and heat stroke.[2–4] CWI is a cold therapy in which all or part of the body is immersed in cold water at mostly below 15 °C. Some studies have shown that CWI has no effect on recovery from post-exercise fatigue,[5,6] whereas other studies have shown it to be effective.[7,8] It was also reported that CWI appeared to improve recovery from high-intensity cycling, allowing athletes to maintain better performance over a 5-day period.[9]

A potential reason for the discrepant findings regarding the effectiveness of CWI may be related to the decrease in muscle blood flow, which has been considered to affect the recovery of damaged cells negatively. The removal of blood lactate was significantly related to muscle blood flow.[10,11] CWI after exercise may inhibit lactate removal because a cooling stimulus induces vasoconstriction and decreases blood flow. It is necessary to overcome CWI's disadvantage of reduced blood flow to establish a CWI method with

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which athletes can recover efficiently from a fatigued state and perform well.

Our research has focused on the vasodilating effect of carbon dioxide (CO_2) gas. We designed a new cooling method that uses CO_2 and eliminates the obstruction of blood flow, which has been a problem with the standard CWI method at a room temperature of 25 °C. Traditional studies demonstrated that acidosis might reduce the contractility of the vascular smooth muscles, leading to vasodilation.[12,13] We have succeeded in increasing the blood flow in skin and muscle by applying CO_2-rich cold water immersion (CCWI).[14] One of our investigations confirmed that CCWI also prevents heat stroke because it promotes heat dissipation.[15] Our laboratory evaluations of athletes' performance demonstrated that with CCWI, the athletes' core temperature (T_{core}) decreased, and the elimination of lactate was accelerated. We also observed that the T_{core} and lactate values were highly correlated with cycling work efficiency[15] and peak pedal repetitions in a high-intensity intermittent exercise test.[16]

We hypothesized that the positive thermal effects of CCWI would also be exerted in a hot outdoor environment and would be maintained relatively longer after exercise in such an environment. We conducted the present field study to compare the effects of CCWI, CWI, and passive recovery (control) on T_{core}, skin temperature (T_{skin}), heart rate (HR), and the rate of perceived ice (RPI) on athletes after regular exercise training of the participating disciplines in a hot outdoor environment.

2. Methods

2.1. Participants

The subjects were 25 male and 11 female athletes (mean ± SD: males' age 20.4 ± 1.2 years, height 172.8 ± 7.5 cm, weight 63.0 ± 9.2 kg, body mass index [BMI] 21.0 ± 2.2 kg·m^2; females' age 21.0 ± 1.3 years, height 157.2 ± 3.1 cm, weight 50.6 ± 4.2 kg, BMI 20.5 ± 1.4 kg·m^2) who volunteered to participate in the study. All the subjects were members of college athletic teams and had been training for long-distance running events or lacrosse for ≥1 year.

As a field study, we had no control over the content, duration, or intensity of the training program of each athletic team because coaches were not allowed to request training menus or experimental conditions. We can only ask the athletes to participate in our field experiment after regular exercise training.

We did not consider the female menstrual cycle (e.g., the follicular and the luteal phases) other than menstruation because female athletes play sports regardless of whether they are in their luteal or follicular phase. Before their participation, all subjects were fully informed of the experiment's purpose, content, and risks, and their consent to participate was obtained.

2.2. Experimental design

Fig. 1 presents an overview of the experimental design. The subjects trained in their regular outdoor workouts for approximately 2.5 h before the CCWI experiment. During regular training in a hot environment, the coaches encouraged athletes to take breaks and drink water to avoid heat-stroke. The three recovery conditions were CCWI and CWI at 20 °C and passive recovery (control). The 36 subjects were randomly allocated into three intervention groups (CCWI, CWI, and control). The subjects engaged in 5 min of rest (BL), 20 min of water immersion (WI20), and a 60-min recovery period (Rec60). The subjects in the CWI and CCWI groups were then immersed in a 140-L water bath maintained at 20 °C. The CO_2-rich (>1000 ppm) water was prepared by dissolving CO_2 in 20 °C tap-water with the use of a dual chamber/dual-vortex high-speed rotation system (type 1 S; Taikougiken, Kumamoto, Japan) and a gas regulator (N24; NIPPON TANSANGAS, Tokyo) in a bathtub (model KF-400S, Earth, Tokyo).[14] The depth of immersion was up to the subject's thoracic xiphoid process holding the subject's arms out. The subjects were immersed outdoors in the shade, and during the recovery period, they were in a room without air conditioning in order to avoid a temperature decrease due to wind. In addition, there was a short meeting after regular exercise training was over, and we simply waited for them to come to the eaves of the clubhouse adjacent to the training ground after they have finished changing out of their training clothes. The experiment was started after the regular exercise training (approx. 30 min later).

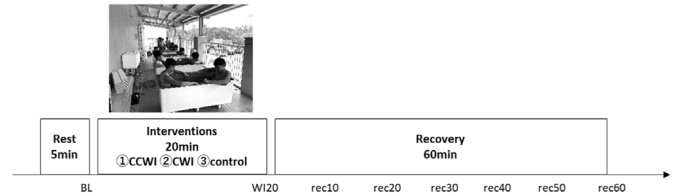

Fig. 1. Overview of the experimental design. After subjects trained in a heat environment at their club, they spent 5 min of rest (BL), 20 min of immersion (WI20), and 60 min of the recovery period (Rec60). CCWI: cold-water immersion with CO_2, CWI: cold-water immersion, and control: passive recovery.

In order to ensure a double-blind design, the subjects were not informed about whether the water was cool water alone or cool water with CO_2. The water temperature during CWI and CCWI was maintained at 20 °C with ice. In the control condition, the subject was seated and resting.

2.3. Room conditions during the recovery period in summer

The experiment was conducted on two separate days. On each day, the subjects trained in the regular practice program under a hot outdoor environment at their respective sports clubs: on day 1 at a temperature of 30.0 °C and 55.8% humidity, and on day 2 at 32.8 °C and 54.3% humidity in summer. The temperature was >30 °C on both days, which means that the experimental conditions were at the 'severe' alert level for the WetBulb Globe Temperature (WBGT) index, which is a measure of the heat stress in direct sunlight. The 60-min passive recovery room was located in the eaves of the clubhouse adjacent to the training ground. As all the windows were open, the room air temperature was the same as the outside air temperature.

2.4. Measurements

The subjects' HR and blood pressure were measured by a medical electronic blood pressure monitor (HBP-1300, OMURON, Kyoto, Japan). The average mean arterial pressure (MAP) was calculated based on systolic and diastolic blood pressure values. The core temperature (T_{core}) was measured using an ear infrared thermometer (MC-510, OMURON, Kyoto, Japan). The subject self-reported the RPI on a 7-point scale based on the evaluation sheet in Table 1. The T_{skin} was measured at the forehead (T_{head}: mid-frontal bone), abdomen ($T_{abdomen}$: umbilical region), and lower leg ($T_{lowerlimb}$: mid-left tibialis anterior muscle) with a handheld thermograph (TVS-200EX, Nippon Avionics, Yokohama, Japan). Three skin temperatures were estimated by the change from the subject's baseline value to each time period; they were measured at pre-immersion (BL), post-immersion (WI20), and every 10 min during the recovery period (rec10~60).

Table 1
The rate of perceived ice (RPI) was rated by the subject based on a conventional seven-point scale comprising cold (−3), cool (−2), slightly cool (−1), neutral (0), slightly warm (+1), warm (+2), and hot (+3). The subject's RPI was reported 5 min before immersion, after immersion, and every 10 min during the 60-min recovery period.

score	Thermal Sensations
+3	Hot
+2	Warm
+1	Slightly Warm
0	Neutral
−1	Slightly Cool
−2	Cool
−3	Cold

2.5. Statistical analysis

All values are presented as the mean ± standard error (SE). The data distribution was assessed using the Shapiro–Wilk test, and the data were normally distributed. Therefore, the significance of differences in each variable was determined by a two-way analysis of variance (ANOVA) comparing water conditions (CCWI, CWI, control) × time periods (BL, WI20, and recovery at rec10~60). When there was a significant main effect or interaction, differences were identified with the Bonferroni test. We also performed a one-way ANOVA with no correspondence and Bonferroni test to compare the integrated values of the results of the control, CWI, and CCWI groups. All analyses were performed using SPSS software (Abacus Concepts, Berkeley, CA) with significance in all cases set at the 5% level.

3. Results

3.1. HR

A decreasing trend was observed in the control group (Time effect: $F\ (7, 231) = 4.583$, $p < 0.001$, $\eta^2 = 0.122$). In the recovery period, the CCWI subjects' HR values were slightly lower than those of the CWI subjects, but there was no interaction between the water and time factors. The sum of the change in HR from BL was negative for each condition, with CCWI significantly lower than the control group ($p < 0.05$) (Fig. 3A). A negative cumulative value of changes in HR from BL means that the recovery HR was continuously below the BL.

3.2. MAP

The MAP values for all three groups of athletes showed small changes (Fig. 2B), and the cumulative value of the changes in MAP was negative in all three conditions (Fig. 3B). In contrast, no significant differences in MAP were identified between the time factor and water conditions (Time × Condition: $F\ (14, 231) = 1.360$, $p = 0.221$, $\eta^2 = 0.076$).

3.3. T_{core}

The T_{core} values of the CWI and CCWI groups did not increase after the water immersion, and they continued to slight decrease during the recovery period; otherwise, the T_{core} values of the control group tended to rise slightly during the recovery period (Time effect: $F\ (7, 231) = 4.583$, $p = 0.04$, $\eta^2 = 0.122$) (Fig. 2C). T_{core} was significantly lower in the CCWI group at only 50 and 60 min of the recovery period compared to the control group ($p < 0.05$), but the CWI and control subjects' T_{core} values were not significant lower at any time points. The sum of the changes in T_{core} during the recovery period was negative in the CWI and CCWI groups, and the values for both of these groups were significantly lower compared to the control group ($p < 0.01$) (Fig. 3C).

3.4. RPI

The RPI values reported by the CWI and CCWI subjects were lower at WI20 than at BL and gradually moved toward BL during the recovery period (Time effect: $F\ (7, 231) = 10.148$, $p < 0.001$, $\eta^2 = 0.241$) (Fig. 2D). The control subjects' RPI values showed almost no change and no significant difference. There was a significant interaction between the water conditions and time factors (Time × Condition: $F\ (14, 231) = 4.033$, $p = 0.001$, $\eta^2 = 0.201$), and the RPI values of the CWI and CCWI groups were significantly lower compared to those of the control group at the end of the WI and at rec10 ($p < 0.05$, $p < 0.01$). The cumulative values of changes in RPI in the CWI and CCWI groups were significantly lower compared to that of the control group ($p < 0.05$, and $p < 0.01$, respectively) (Fig. 3D).

3.5. $T_{abdomen}$

The $T_{abdomen}$ values in the CWI and CCWI groups decreased significantly due to the water immersion and showed an uptrend after WI20 (Time effect: $F\ (7, 231) = 120.324$, $p < 0.001$, $\eta^2 = 0.785$) (Fig. 4A). An increase in $T_{abdomen}$ was also observed in the control subjects, with significantly higher values at rec20–60 compared to BL ($p < 0.01$). As a significant interaction between water and time was identified (Time × Condition: $F\ (14, 231) = 17.5$, $p < 0.001$, $\eta^2 = 0.515$), the $T_{abdomen}$ values of the CWI subjects were significantly lower from WI20 to rec10 compared to the control group ($p < 0.01$), and the CCWI subjects' $T_{abdomen}$ values were significantly lower from WI20 to rec40 ($p < 0.05$).

In the CWI group, the $T_{abdomen}$ values had returned to BL at rec20, whereas those of the CCWI group had returned to BL at rec 30. The cumulative value of changes in $T_{abdomen}$ in the CCWI subjects was also significantly lower than that of the control group ($p < 0.01$) (Fig. 5A). However, there was no significant difference in this parameter between the CWI and control subjects.

3.6. $T_{lowerlimb}$

The $T_{lowerlimb}$ values also showed a transient but significant decrease after the water immersion, and they continued to increase during the recovery period (Time effect: $F\ (7, 231) = 42.735$, $p < 0.001$, $\eta^2 = 0.564$) (Fig. 4B). The $T_{lowerlimb}$ values in the control subjects remained unchanged from BL, whereas a significant interaction occurred between water and time (Time × Condition: $F\ (14, 231) = 5.335$, $p < 0.001$, $\eta^2 = 0.244$). Significantly lower $T_{lowerlimb}$ values compared to the control data were observed in the CWI group from WI20 to rec40 ($p < 0.01$), and the CCWI group's $T_{lowerlimb}$ values were significantly lower from WI20 to rec60 compared to the control data ($p < 0.01$). The cumulative value of changes in $T_{lowerlimb}$ was significantly lower in both the CWI and CCWI groups compared to the control group's value ($p < 0.01$) (Fig. 5B). As was observed for their $T_{abdomen}$ values, the CCWI group's $T_{lowerlimb}$ values continued to decrease markedly longer compared to those of the CWI group.

3.7. T_{head}

As a value from a non-immersed site, the T_{head} tended to increase in all three conditions (Time effect: $F\ (7, 231) = 18.466$, $p < 0.001$, $\eta^2 = 0.359$) (Fig. 4C). Although the T_{head} values of the CWI

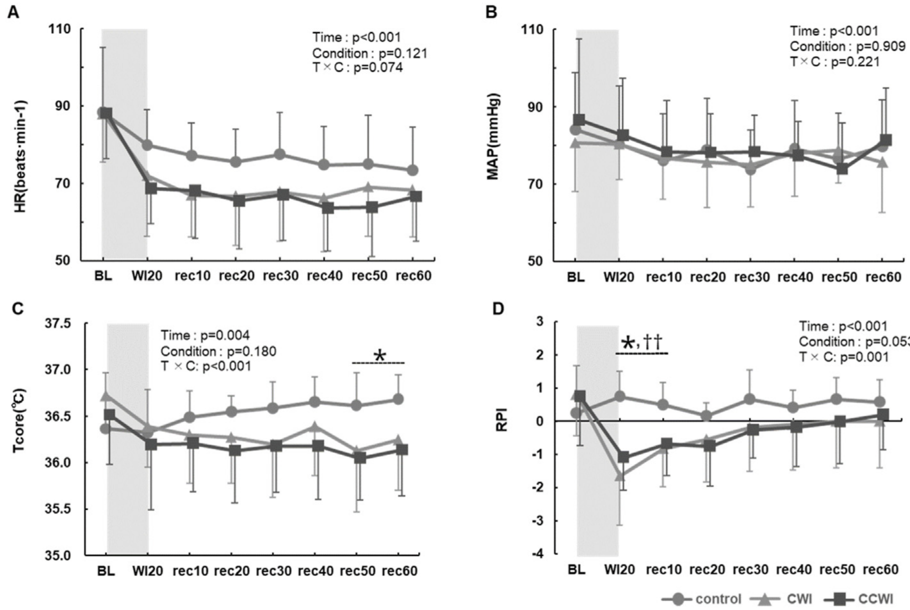

Fig. 2. The subjects' heart rate (HR: A), mean arterial pressure (MAP: B), core temperature (Tcore: C), and rate of perceived ice (RPI: D) values in the control, CWI, and CCWI groups. Values are mean ± standard error (SE). T_{core} was significantly lower in CCWI at rec 50 to rec60 compared to the control ($p < 0.05$). *p < 0.05 vs. CCWI, ##p < 0.01 vs. CWI.

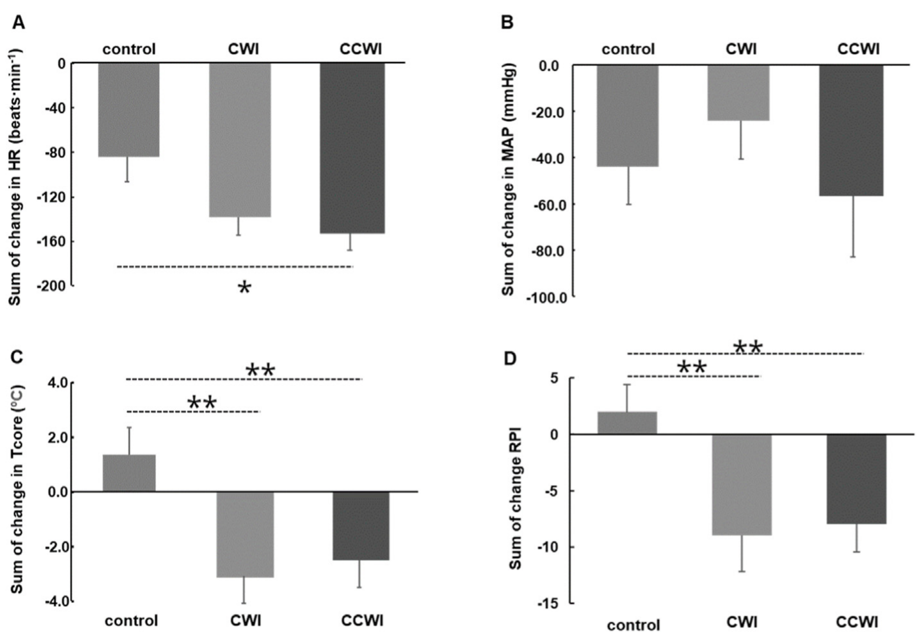

Fig. 3. Sum of changes in HR (A), MAP (B), T_{core} (C), and RPI (D) in control, CWI, and CCWI. Total amount of change from BL ± SE. The sum of the changes in HR was negative for each condition, with CCWI significantly lower than the control. The sum of the changes in T_{core} and RPI in CWI and CCWI were both significantly lower compared to the control. *p < 0.05, **p < 0.01 vs. control.

and CCWI groups were somewhat lower than those of the control subjects during the recovery period, there was no significant difference among the three conditions (condition effect: $F(14, 231) = 0.715$, $p = 0.698$, $\eta^2 = 0.042$). The cumulative value of changes in T_{head} did not show any significant differences among the conditions (Fig. 5C).

4. Discussion

In our previous studies, we investigated the effects of cold carbonated water immresion in a room controlled for room temperature and humidity.[14–16] In the present study, we conducted a field experiment in a real-world hot environment to test our laboratory findings in an athletic context. It has been shown that the HR decreases during water immersion due to water pressure and water temperature.[17,18] In the present study, the HR of the subjects who underwent CWI or CCWI remained lower than the HR of the control subjects after the end of water immersion until the 60-min recovery time point. In general, when CO_2 molecules enter the blood percutaneously, extracellular acidosis occurs, reducing the vascular smooth muscles' contractility and dilating the vessels.[12,13] Decreased smooth muscle contractility has been attributed

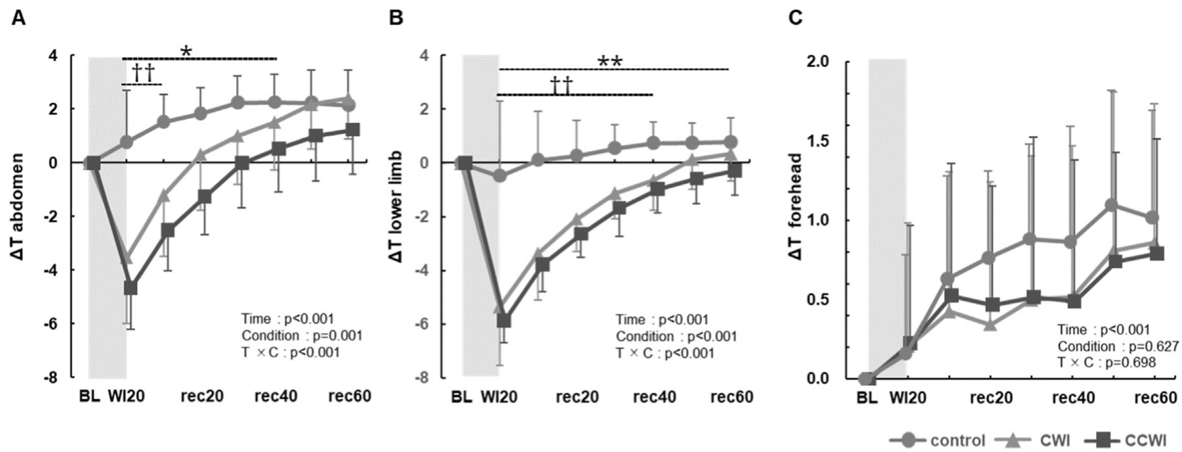

Fig. 4. Changes in temperature at the abdomen (A), lower limb (B), and forehead (C) from BL. Values are mean ± SE. $T_{abdomen}$ and $T_{lowerlimb}$ in CCWI and CWI were significantly lower than in the control. *p < 0.05, **p < 0.01 vs. CCWI. #p < 0.05, ##p < 0.01 vs. CWI.

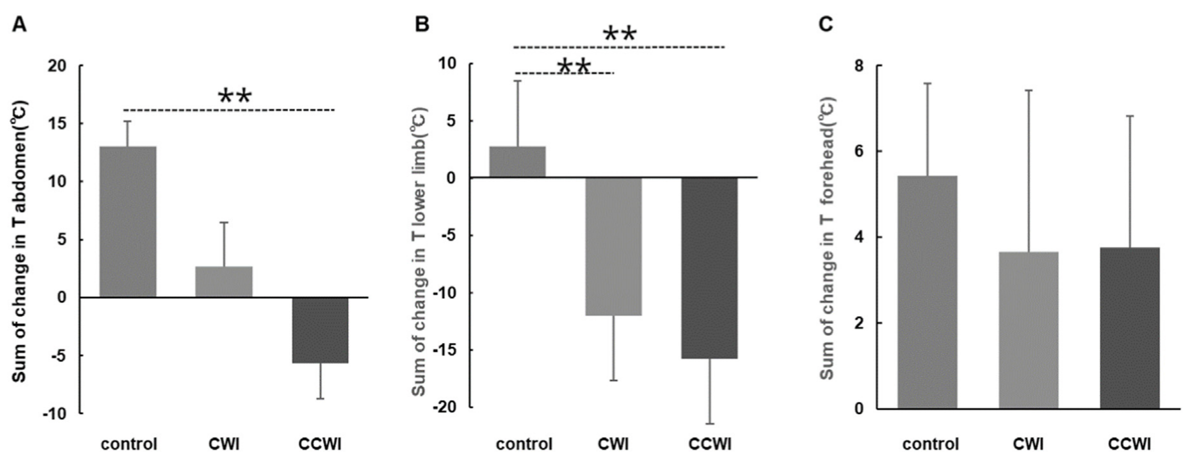

Fig. 5. The sum of changes in $T_{abdomen}$ (A), $T_{lowerlimb}$ (B), and $T_{forehead}$ (C) in control, CWI, and CCWI. The total amount of change from BL ± SE. $T_{abdomen}$ in CWI and CCWI was significantly lower than control, and $T_{lowerlimb}$ in CCWI was also significantly lower than control. **p < 0.01 vs. control.

to decreased calcium influx and to the suppression of muscle fiber contractility,[19,20] and consequently, blood inflow into the arteries is increased. We thus speculate that bradycardia persisted into the recovery phase due to the present application of CCWI even after exercise training in a hot environment.

The effect of CCWI was observed not only in the reduction of HR but also in the reduction of T_{core} (Fig. 3A, C). Although no significant differences in T_{core} were observed between the control and CWI groups, the T_{core} of the subjects who underwent CCWI was significantly lower than that of the control group during the 50- to 60-min recovery period (Fig. 2 C). Our earlier study demonstrated that CCWI resulted in lower skin temperature and greater superficial skin blood flow compared to the CWI group.[14] The cooled blood flow due to the cool-water immersion returned inside the body, causing a significant decrease in the core temperature in the present CCWI group compared to the CWI group. In addition, judging from the T_{skin} response, the increased skin blood flow and greater heat dissipation in the CCWI group continued over the 60-min recovery period, which might also be related to the decrease in T_{core}.

On the other hand, the T_{core} in the control group increased by ~0.4 °C due to heat transfer from outside to inside the body under the influence of the higher ambient temperatures (30.0 °C and 32.8 °C). Interestingly, both CWI and CCWI prevented the increase in T_{core}, even though all of the subjects stayed in the same room during the 60-min recovery phase. The decrease in T_{core} in the CWI and CCWI subjects was maintained for a relatively long time during the recovery period. Still, the decrease in the RPI disappeared within about 10 min of the recovery period. Since the RPI is sensitive to environmental temperature, it recovered quickly after the water immersion.

According to Tipton et al. the cooling effect of cold-water immersion can be divided into three phases depending on the duration of immersion[21] cold-water immersion for <3 min cools the skin, and immersion for >3 min cools the superficial muscles. Cold-water immersion for >30 min cools the deep muscle tissues; hypothermia may be caused, depending on the water temperature. In CWI studies, water temperatures up to 14 °C and immersion periods of 15 min or less are the most common CWI prescription.[22] Referring to the CWI data in paper Choo et al.,[22] there were no differences in thigh skin temperature, mean body temperature, and skin vascular resistance between the two temperatures (9 and 15 °C) conditions at the 60-min recovery period after CWI. We chose 20 °C with a longer immersion time (15–20 min),[14–16] and our CCWI conditions in those studies resulted in temperature decreases of −4.7 °C in the abdomen and −5.9 °C in the lower legs at the end of immersion. Therefore, our CCWI might not be much different as a thermal effect compared to Choo's results.[22]

In the present study, significantly lower $T_{abdomen}$ values continued for 10 min in the CWI subjects and for 40 min in the CCWI subjects following the water immersion compared to the control values. Similarly, significantly lower $T_{lowerlimb}$ values continued for 40 min in the CWI group and for 60 min in the CCWI group compared to the control. These results indicate that the cooling effect continued for 20–30 min longer in the CCWI compared to the CWI at both body sites. Considering that a relaxation of smooth muscles induced superficial vasodilation due to intravascular acidosis caused by CO_2 gas as well as a decrease in the core body temperature,[14] we speculate that in the present experiment active heat dissipation continued after rec20 and consequently caused a significant decrease in skin temperature.

The increase in skin temperature in the CWI group after the water immersion was more rapid than that in the CCWI group during the recovery period (Fig. 4). We surmise that the difference in superficial blood flow caused this. As a characteristic of each body part, the abdomen recuperates more quickly, while the lower legs maintain a cooler state. Since heat dissipation occurs at the arteriovenous anastomoses at the extremities, heat dissipation was likely more active in the lower legs than in the abdomen. In addition, acidosis induced by CO_2 gas during CCWI may have further accelerated heat dissipation.

On the other hand, the abdomen, which is closer to the center of the body, is less susceptible to temperature drops than the lower legs, and there is thicker subcutaneous fat in the abdomen than the lower legs. The insulation effect of subcutaneous fat against external cooling should also be considered,[23] and the difference in the recuperation of the subcutaneous fat thickness between the abdomen and lower legs may be reflected by the reduction of the skin temperature after immersion (abdomen: −4.7 °C, lower leg: −5.9 °C). Since the decrease in $T_{abdomen}$ was small, it is natural that the temperature recuperation in the abdomen was rapid. It is also interesting that the skin temperature at a non-cooled site was reduced during partial cooling.[24] In the present study, the temperatures at the non-immersed site (i.e., the forehead) in the CWI and CCWI groups tended to be lower than those in the control group.

There were some limitations in this study. The experiment was designed for actual use in the field, and it was impossible to carry it out under fully controlled conditions, such as in laboratory tests. The coaches were not required to have a special exercise training menu for the experiment to create hyperthermic condition and ensured that training sessions were carried out as usual. Each subjects did not have the same training sessions or fatigue levels. The subjects were heat adapted before the start of the experiment. We believe any differences in exercise load between subjects were small due to random allocation.

Subjects were not in the hyperthermic range. Hence, the evaluation of CCWI in heat-stroke recovery is limited. It will be tested in other sports and at higher intensities and temperatures in the future. As mentioned above, the observed below 37 °C body temperature in BL was also a limitation of field studies. Therefore, it is necessary to confirm the useful thermal effect of CCWI under condition of exercise-induced hyperthermia in a hot outdoor environment.

Moreover, if a large number of subjects are to be studied at the same time in the filed, it is unavoidable to measure tympanic temperature as T_{core}. This is why we challenged the issues by 1) differences in body temperature during recovery, 2) increasing the sample size, and 3) setting up an unintended sporting discipline.

Although the subjects' skinfold adipose tissue thickness (ATT) was not measured in this study, it is generally known that the skinfold ATT of the lower legs is approx. 3–5 mm, and that of the abdomen is approx. 5–10 mm in young male and female adults.[25] We suspect that the subcutaneous ATT's insulation effect also affected the present study's change in skin temperature.[26] Since two subjects were immersed in a bathtub at the same time, there was a rise in water temperature of ~0.5 °C during the 20-min CWI and CCWI. Although ice was added to the bathtub at appropriate times to prevent the rise in the water's temperature, it was difficult to control the water temperature at exactly 20 °C (Fig. 1). In addition, to maximize the effect of CCWI, the immersion conditions should be adjusted to consider the outside/ambient temperature and wind speed.

5. Conclusion

We examined the effects of 20 °C cool carbonated water immersion (CCWI) after regular exercise training in a hot environment. Significant reductions in heart rate, core temperature, and skin temperature were observed in the subjects who underwent the CCWI. These reductions were sustained up to 60 min after the end of the immersion. The results demonstrated that compared to CWI, CCWI at 20 °C for 20 min would be more effective for preventing the increase of body temperature in a hot outdoor environment.

Author contributions

MY and YF were responsible for the design and conception of the work. MY, MN, AA, and YF were responsible for the data acquisition and analysis, and all authors were responsible for the interpretation of the data. TH, as a clinician, managed the subjects' condition before and after they perform the experiments. MY and YF drafted the work and all authors revised it critically for important intellectual content, approved the final version to be published, and agreed to be accountable for all aspects of the work. All authors have read and agreed to the published version of the manuscript.

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Ethical approval

This study was approved by the human research ethics committee of Doshisha University (approval no. 15085).

Declaration of competing interest

The authors declare that there are no conflicts of interest regarding the publication of this article.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jesf.2023.05.001.

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