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Anaerobic performance after 3-day consecutive CO_2-rich cold-water immersion in physically active males

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A B S T R A C T

Background Objective: We investigated the effects of a 3-day consecutive CO_2-rich cold (20 °C) water immersion (CCWI) following a high-intensity intermittent test (HIIT) on subjects' sublingual temperature (T_{sub}), blood lactate ([La]b), and heart rate (HR) compared to cold (20 °C) tap-water immersion (CWI) or passive recovery (PAS).
Methods: Thirty-two subjects were randomly allocated into three groups (CCWI, CWI, and PAS), each of which completed 4 consecutive days of cycling experiments. HR, T_{sub}, and [La]b were recorded on each day of exercise testing (immersion from Day 1 to Day 3 and Day 4). HIIT consisted of 8 sets of 20-sec maximum exercise at an intensity of 120% of VO_2max with 10-sec passive rest. The mean and peak power, and peak pedal repetitions (PPR) within HIIT were averaged and the decline in PPR (ΔPPR) from Day 1 to Day 4 was measured.
Results: In CCWI and CWI, HR declined significantly following each immersion, with CCWI showing the larger reduction ($p < 0.001$). At Day 2, CCWI showed a significantly lower [La]b compared to PAS ($p < 0.01$). The changes in mean and peak power from Day 1 to Day 4 did not differ among the groups ($p = 0.302$). ΔPPR of HIIT was significantly correlated with the HR and [La]b values after immersions (ΔPPR-HR: $r^2 = 0.938$, $p < 0.001$, ΔPPR-[La]b: $r^2 = 0.999$, $p < 0.001$).
Conclusions: These findings indicate that CCWI is a promising intervention for maintaining peak performance in high-intensity intermittent exercise, which is associated with a reduction in [La]b and HR.

1. Introduction

Cold-water immersion (CWI) is a well-known post-exercise recovery method that is widely utilized among athletes who seek to minimize fatigue and accelerate recovery processes.[1] CWI may benefit recovery by reducing core and tissue temperatures,[2–6] alleviating acute inflammation and perceived pain,[7] and increasing parasympathetic activity.[1] CWI may also contribute to maintain physical performance in successive matches as reducing the perception of general fatigue and leg soreness,[8] improve peak and average power output during cycling exercise,[9] and provide small and moderate performance gains in basketball performance.[10] Moreover, it has been reported that consecutive use of CWI provides a better maintenance of sprint performance during consecutive days of training.[11] Besides CWI's beneficial effects on recovery and performance, it also has the disadvantage of inducing vasoconstriction in arterial and venous capillaries, which causes a reduction of peripheral blood flow.[1]

However, since carbon dioxide (CO_2) provokes both cutaneous and muscle vasodilation while being absorbed through the skin layers into the subcutaneous tissues, this disadvantage of cold exposure can be solved by dissolving CO_2 into the water,[12] which is attributed to effects of body heat removal and vasodilation, rather than vasoconstriction. Our group recently tested the acute effect of CO_2-rich cold-water immersion (CCWI) following intense exercise and concluded that CCWI is an effective intervention for maintaining repeated cycling work efficiency compared to CWI or passive recovery. This performance benefit is associated with

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reduction in blood lactate concentration ([La]b) and heart rate (HR), which may contribute to both cutaneous and muscle vasodilation by CO_2, and consequent improvement of the central circulation and delivery of blood to working muscles. On the other hand, water immersion with CO_2 may not benefit anaerobic performance, possibly due to the reduction in muscle temperature.[13] In addition, long-term recovery strategy of CWI following training seemed to improve the day-to-day training performance[11] and parasympathetic reactivation, as assessed by HR variability indices.[14] Our previous inconsistent findings and two limited reports led us to consider the consecutive use of CCWI. As with single intervention, we considered that CCWI would promote heat dissipation, lactate removal, and parasympathetic dominance due to heart rate attenuation,[13] and that the repeated CCWI would be more conductive to post-exercise CCWI recovery and maintain aerobic and possibly anaerobic performance.

We thus conducted the present study to compare the effectiveness of two post-exercise interventions (CCWI, and CWI), and passive recovery (PAS) on 4-day consecutive high-intensity intermittent cycling performance, blood lactate concentration, heart rate, and core temperature.

2. Methods

2.1. Participants

A total of 32 trained male athletes (mean ± SE: age, 20.3 ± 0.2 years; body mass, 68.4 ± 1.4 kg; height, 172.7 ± 1.1 cm, and VO_2max 53.8 ± 1.0 ml kg^{-1}•min^{-1}) volunteered to participate in the study. All subjects were members of college athletics teams and had been training for track and field short-distance events, rugby, soccer, or baseball for ≥2 years. The physical training status of subjects depended on their individual sports activities. They were all consistently training in their sports 4–5 days a week, and usually had matches or a day off on the weekend. Before their participation, all subjects were informed of the potential risks and requirements of the study, and each subject provided informed written consent. The study was approved by the human research ethics committee of Doshisha University (No. 15085).

2.2. Experimental design

Before participation, each subject completed a maximal cycling test on a cycling ergometer (75XLIII, AEROBIKE, Konami, Tokyo, Japan), which commenced at 30 W and increased by 30 W every 1 min at 60 rpm until exhaustion. The criteria for exhaustion were as follows: (1) a score of 19 on the rating of perceived exertion (RPE); and (2) failure to maintain 60 revolutions/min despite strong verbal encouragement.[15] The test was terminated when either two criterion was met. Each subject's peak power output and maximal oxygen uptake ($\dot{V}O_{2max}$) were determined with the use of a metabolic chart (AE-310s; Minato Medical, Osaka, Japan).

Fig. 1 presents an overview of the experimental design. Before each exercise testing session, subjects performed a 10-min warm-up exercise with resistance at 50% of the individual subject's maximal oxygen uptake. On Day 1, 8 sets of high-intensity intermittent test (HIIT),[16,17] consisting of 20 s of all-out cycling at maximum speed against a given resistance at 120% of the individual subject's maximal oxygen uptake and 10 s of passive rest, were conducted. After the 8 sets, the subject completed a 20 min recovery period with CCWI, CWI, or PAS, depending on the group to which he had been assigned. On Days 2 and 3, each subject carried out 8 sets of HIIT, then performed the same intervention according to the same protocol as on Day 1. The last experimental testing session on Day 4 was completed with HIIT, and no recovery interventions were conducted.

All tests were completed in a temperature-controlled laboratory (25 ± 0.1 °C, relative humidity 50 ± 0.8%). All subjects were required to maintain a food diary for 24 h before testing to ensure that their food intake was consistent for 4 consecutive days. In addition, the subjects were required to refrain from alcohol (48 h), caffeine (24 h), and strenuous exercise (24 h) before testing.[18]

2.3. CWI, CCWI, and PAS interventions

Each subject performed either passive sitting on the seat of a cycling ergometer (PAS) for 20 min or a whole-body (excluding head and neck) CWI or CCWI recovery on Day 1 to Day 3, after completion of HIIT. The subject was vertically immersed in a 140 L, 20 °C water bath for 20 min. CO_2-rich (>1,000 ppm) water was prepared by dissolving CO_2 in 20 °C tap water using a dual-chamber/dual-vortex high-speed rotation system (type 1S; Taikougiken, Kumamoto, Japan).[12,13]

Each subject completed experiments designed for 4 consecutive days and performed one experimental testing session on each day for a total of four sessions over 4 days. Every session except the one on Day 4 was followed by either of two interventions (CCWI or CWI), or PAS. The Excel RAND function was used to randomly assign subjects to one of the conditions. The physical characteristics of the three groups are summarized in Table 1. In the PAS condition, the subject was seated and resting. The subjects would not have been able to tell the difference between CCWI and CWI even after immersion, as CO_2 microbubbles are invisible to the human eye. However, the skin becomes red after 10 min in CCWI, and it is thus possible that the subjects could tell that the water that reddened their skin was CCWI at that point.

2.4. Outcome measures

(1) Performance

We determined the effectiveness of each intervention in maintaining anaerobic performance by comparing the subjects' HIIT on Day 1 and Day 4. The peak and mean power (watts) and the peak pedal repetition (rpm) in the HIIT were determined when the subject performed a set-time of 20-sec pedaling at maximum speed against a given resistance at 120% of the individual subject's VO_2max (Power MaxVII; Konami, Tokyo, Japan). The peak power refers to the highest 20-sec pedaling power during 8 sets of HIIT. The mean power refers to the average of all 8 sets of HIIT. The peak pedal repetition (PPR) refers to the highest 20-sec pedaling revolution during 8 sets of HIIT.

(2) Sublingual temperature

Each subject's core temperature, measured as the sublingual temperature (T_{sub}), was recorded (MC-652LC; Omron, Kyoto, Japan) at baseline, throughout the exercise bout (end HIIT), and after the 20-min recovery period (WI20; end of CCWI, CWI, or PAS).[13] Because T_{sub} is influenced even by breathing, the subjects were instructed to breathe through the nose with their mouths closed. Even though the measurements at baseline and WI20 were unlikely to be affected by mouth breathing, HIIT measurements were taken for 30-sec after the completion of the exercise, so that the subjects' hyperventilation had subsided, and they were able to breathe through the nose easily with a closed mouth.

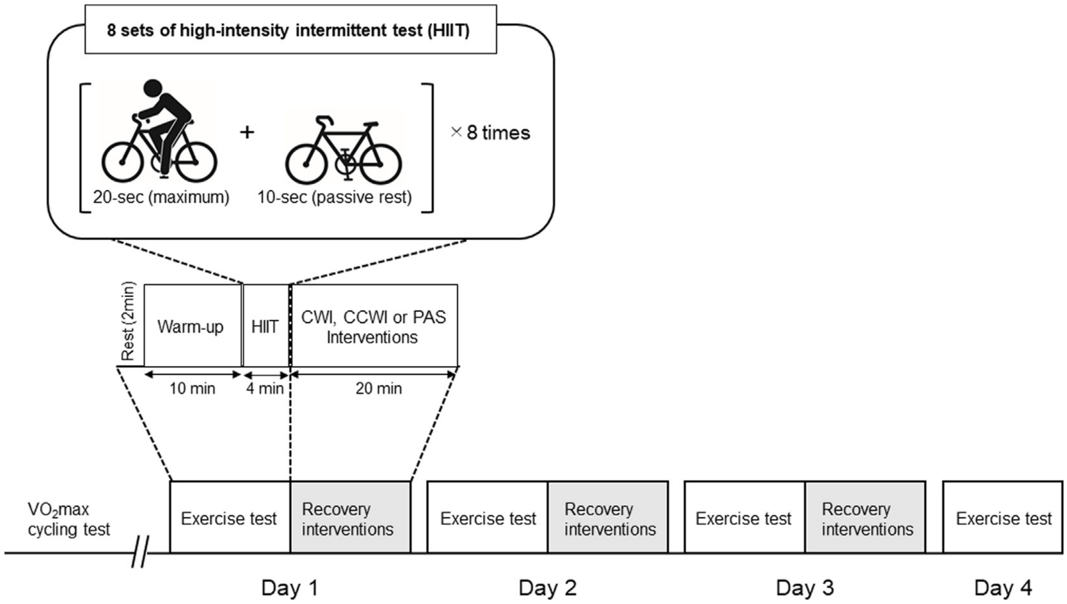

Fig. 1. Overview of the experimental design. Note: The high-intensity intermittent test (HIIT) consists of 20-sec all-out cycling at maximum speed against a given resistance at 120% of the individual subject's maximal oxygen uptake and 10 s of passive rest. Each participant completed experiments designed for 4 consecutive days and performed one experimental testing session on each day for a total of four sessions over 4 days. Every session except the one on Day 4 was followed by either of two interventions (CCWI or CWI), or PAS. CCWI: cold-water immersion with CO_2, CWI: cold-water immersion, PAS: passive recovery.

Table 1
Physical Characteristics and maximal oxygen uptake in all groups.

Groups	N	Age (year)	Height (cm)	Weight (kg)	VO_{2max} (ml·kg^{-1}·min^{-1})
PAS	11	20.5 ± 0.3	174.2 ± 1.9	68.3 ± 2.9	52.0 ± 1.5
CCWI	10	20.3 ± 0.4	174.7 ± 2.0	68.6 ± 1.6	56.4 ± 1.7
CWI	11	20.2 ± 0.3	169.4 ± 1.5	68.5 ± 2.6	53.3 ± 1.6

Data are shown as means ± SE.

(3) Lactate

The subjects' blood lactate concentrations ([La]b) were measured using an earlobe sample and analyzed with a portable analyzer (Lactate-Pro2; KDK Corp., Shiga, Japan). Since lactate accumulation following HIIT correlates with the RPE, the [La]b was recorded at baseline, throughout the exercise (end HIIT), and after the 20-min recovery period (end of CCWI, CWI, or PAS). To avoid the influence of lactate concentration at baseline, all subjects were required to maintain a food diary for 24 h before testing to ensure that their food intake was consistent.[18]

(4) Heart rate

Heart rate (HR) was monitored using a heart rate monitor (Polar Electro, RS800CX; Kempele, Finland) fitted to the subject before testing. HR was recorded at baseline and every 15-sec throughout the testing sessions. We focused on several periods at Baseline, the end of the HIIT, and after the 20-min recovery period (WI20).

2.5. Statistical analysis

We performed a two-way repeated measures analysis of variance (ANOVA) on all data to evaluate the effects of the two interventions (CCWI and CWI), and PAS as well as the interactions between days and these three interventions. Where there was a significant main effect or interaction, we identified differences by using the Bonferroni test. The averaged HR from Day 1 to Day 3 at WI20 and the decrements in PPR from Day 1 to Day 4 (ΔPPR) were examined by a one-way ANOVA, and a post hoc-comparison was performed with Tukey's test.

The effect size (ES) was calculated as Cohen's d between the trials' percentage change from Day 1 to Day 4 to elucidate the practical significance of the CCWI. The criteria to interpret the magnitude were as follows: 0–0.2 = trial, 0.2–0.6 = small, 0.6–1.2 = moderate, 1.2–2.0 = large and >2 = very large.[19] We assessed the strength of the associations between variables by determining the Pearson's correlation coefficient between the average of the 4 consecutive days of decline in PPR and the mean values of the [La]b or HR after interventions or PAS. Significance was set at $p < 0.05$. The data are presented as mean ± standard error (SE). The SE, which represents a within-participant deviation,[20,21] is also presented for the HR, [La] b, and T_{sub} values as indicators of the range of the "true" mean value.

3. Results

3.1. Heart rate

Fig. 2a shows the mean HR values in the PAS, CWI, and CCWI groups. In the first 3 days, HR decreased rapidly during the recovery period, but no significant differences were found among the three groups. However, HR at WI20 decreased significantly over the 3 consecutive days (intervention effect: $F(2,29) = 13.404$, $p < 0.001$, day effect: $F(1,29) = 17.041$, $p < 0.001$) (Fig. 2a), and the averaged HR from Day 1 to Day 3 at WI20 was significantly lower in CCWI than in PAS ($F(2,8) = 14.441$, $p < 0.01$) or in CWI ($F(2,8) = 14.441$, $p < 0.05$).

3.2. Sublingual temperature

There were no differences in the T_{sub} values among the three groups over 4 days (day effect: $F(1,29) = 3.596$, $p = 0.068$). However, the T_{sub} values after WI20 decreased from Day 1 to Day 4 in each group, and this reduction was smallest in CWI compared to

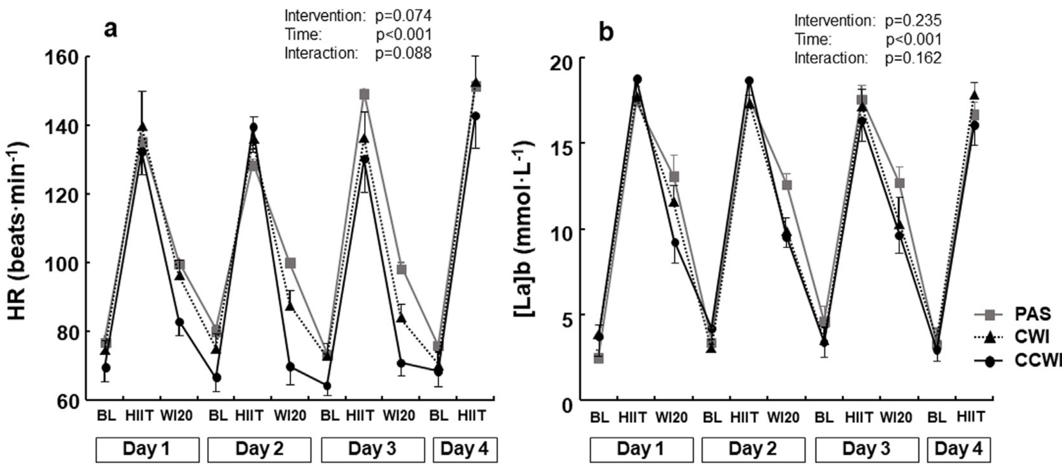

Fig. 2. Mean values of heart rate (HR) **(a)** and blood lactate concentration ([La]b) **(b)** in the three recovery groups (PAS, CCWI, CWI) over 4 consecutive days of experiments. HR and [La]b were measured at baseline (BL), end of HIIT, and after the 20-min recovery period (WI20, end of CCWI, CWI, or PAS). During the recovery periods, the mean values of HR and [La]b was lower for CCWI than for CWI or PAS. Values are means ± standard error (SE).

Table 2
Changes in sublingual temperature (T_{sub}) at WI20 from Day 1 to Day 4.

			Two-way analysis of variance		
	Day 1	Day 4	Intervention	Day	Interaction
T_{sub} (°C)					
PAS	36.7 ± 0.2	36.5 ± 0.1			
CCWI	36.5 ± 0.1	36.2 ± 0.2	p = 0.472	p = 0.068	p = 0.650
CWI	36.5 ± 0.1	36.5 ± 0.1			

Data are shown as means ± SE.

PAS with a small ES (ES = 0.35), or in CCWI with a small ES (ES = 0.38) (Table 2).

3.3. Lactate

The [La]b values decreased during the recovery period (Fig. 1b), and the CCWI maintained the lower [La]b value at WI20, whereas no significant interaction effect between WI and day was observed. Note that a significant difference in [La]b at WI20 on Day 2 was observed between PAS and CCWI (Intervention effect: $F(2,29) = 6.67$, $p < 0.01$) and between PAS and CWI (Intervention effect: $F(2,29) = 6.67$, $p = 0.019$). (Fig. 2b).

3.4. Performance

The peak power of HIIT from Day 1 to Day 4 did not significantly differ among the three groups (Interaction effect: $F(2,20) = 1.271$, $p = 0.302$). Similarly, the mean power did not significantly differ among the groups (Interaction effect: $F(2,21) = 1.717$, $p = 0.204$) (Table 3).

On the other hand, PPR declined within 8 consecutive sets of HIIT in each group. The decrements in PPR from Day 1 to Day 4 (ΔPPR) was likely smaller for CCWI (−1.5 ± 3.4 rpm) than for PAS (−8.8 ± 3.3 rpm) or CWI (−4.1 ± 5.3 rpm), whereas no significant differences were found among the three groups ($F(2,20) = 0.761$, $p = 0.48$). In addition, the ΔPPR of HIIT was significantly correlated with the mean values of HR after both interventions and the PAS at Day 1, Day 2, and Day 3 (Fig. 3a, $r^2 = 0.938$, $p < 0.001$). Similarly, the ΔPPR was also significantly correlated with the mean values of [La]b after interventions (Fig. 3b, $r^2 = 0.999$, $p < 0.001$).

Table 3
Changes in the peak and mean power of HIIT from Day 1 to Day 4.

			Two-way analysis of variance		
	Day 1	Day 4	Intervention	Day	Interaction
Peak power (watts·kg^{-1})					
PAS	6.97 ± 1.15	7.01 ± 1.24	p = 0.779	p = 0.112	p = 0.302
CCWI	6.69 ± 1.07	6.47 ± 1.13			
CWI	7.58 ± 3.71	7.07 ± 2.92			
Mean power (watts·kg^{-1})					
PAS	6.31 ± 1.00	6.37 ± 1.01	p = 0.192	p = 0.704	p = 0.204
CCWI	6.35 ± 0.68	6.21 ± 0.81			
CWI	5.52 ± 0.84	5.67 ± 0.94			

Data are shown as means ± SE.

4. Discussion

We focused on the 3-day consecutive use of CO_2-rich cold-water on the physiological parameters of T_{sub}, HR, [La]b, and anaerobic performance. The 3-day consecutive CCWI led to some significant reductions in HR and [La]b compared to CWI or PAS. With the repeated use of CCWI, the reduction in the peak pedal repetitions within 8 consecutive sets of HIIT was lower in CCWI relative to PAS or CWI.

Our present findings demonstrated that, compared to PAS, the administration of CCWI and that of CWI were both effective for reducing the subjects' HR during the recovery period. Even at WI20 of Day 1, HR in CCWI was significantly reduced and continued to decrease until Day 3 compared to PAS or CWI. CWI also gradually reduced HR at WI20, inducing a significant reduction in HR at Day 3. A previous study found that cold-water immersion reduces blood flow to the skin through cutaneous vasoconstriction and redirects blood back into the central circulation, leading to bradycardia.[1] It should be noted that the increases in hydrostatic pressure and vasoconstriction following CCWI increase central blood volume and venous return, thereby improving cardiac efficiency, the delivery of blood to the working muscles, and muscle performance during subsequent exercise.[22] The greater reduction in HR after WI20 of CCWI may be at consequence of a more rapid rise in parasympathetic activity, which may or may not be independent of blood redistribution.[23]

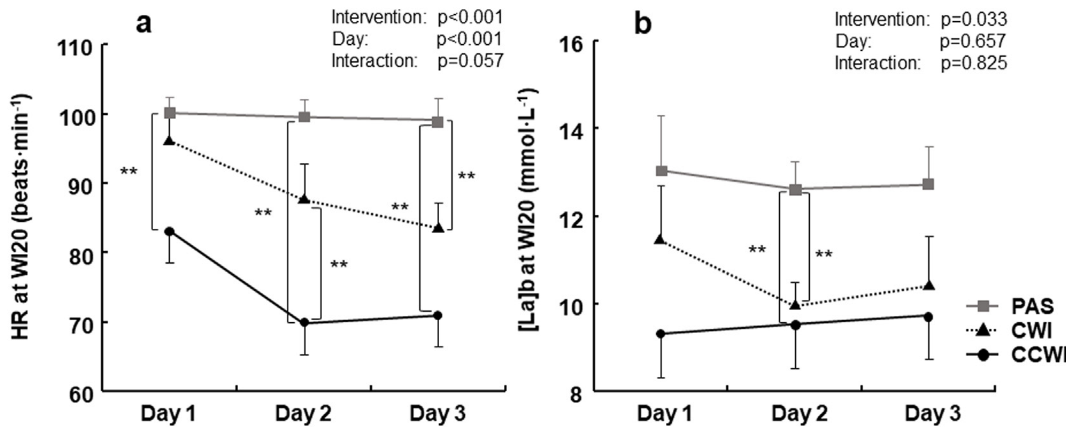

Fig. 3. Mean values of HR at WI20 **(a)** and [La]b at WI20 **(b)** in PAS, CCWI, and CWI. Over 3 consecutive days, HR and [La]b during the recovery periods stayed lowest in CCWI. **$p < 0.01$ compared to CCWI.

At WI20 the [La]b in CCWI was constantly lower, and significant differences at WI20 on Day 2 were observed between PAS and both CCWI and CWI. Our findings regarding both CWI and CCWI suggest the possibility that cold treatment accelerates recovery by stimulating blood circulation as mentioned above, releasing metabolic waste,[24] stimulating the central nervous system, and reducing the thermal and cardiovascular loads.[1,25] Therefore it seems reasonable to use cold treatment as a mechanism for enhancing the removal of metabolites.[26] Our present results indicate that combining cold water and CO_2, i.e. CCWI, accelerated the clearance of [La]b after this intervention (Fig. 2b) and resulted in a remarkable depression of HR kinetics compared to CWI and PAS (Fig. 2a). The physiological mechanism by which CCWI influences recovery is not entirely clear,[27] but in our previous study,[12] the local oxygenation/deoxygenation (which reflects the blood flow) at two leg-muscle sites in subjects at rest were measured, and the cutaneous and muscular blood flows were significantly increased during CCWI compared to CWI. In addition, during the recovery period after CCWI, a significantly lower skin temperature continued until the end of the recovery period compared with CWI, which may be attributed to vasodilation by CO_2. These findings and the mechanism (which may be related to vasodilation by CO_2, the augmented heat transfer from the body, and hydrostatic pressure effects) may explain our present observation of a great reduction in HR and [La]b in CCWI, indicating that CCWI is more practical intervention than CWI.

The peak and mean power of HIIT from Day 1 to Day 4 did not differ among the three groups, and the decrements of peak and mean power in anaerobic cycling performance in both water immersions were very small and thus cannot be taken as significant alterations (Table 2). These findings were similar with those of previous studies, which reported that CWI decreased sprint swimming or cycling performance.[28,29] By contrast, our present results demonstrated that the ΔPPR from Day 1 to Day 4 was likely small in CCWI. Interestingly, there was a negative correlation between [La]b after the recovery trials and the ΔPPR of HIIT ($r^2 = 0.999$ $p < 0.001$). There was also a negative correlation between the depression of HR values following CCWI and the reduction in the ΔPPR ($r^2 = 0.938$, $p < 0.001$)(Fig. 4). These results demonstrated that significantly lower [La]b and HR values following CCWI would be associated with the maintenance of anaerobic performance after consecutive days of bouts of high-intensity intermittent exercise. Note that any learning effect was minimized by the warm-up exercise conducted before each HIIT sessions.

Previous investigators have reported that oxygen consumption during cycling exercise increases linearly or curvilinearly with the increase in pedaling rate.[30–32] Similarly, heart rate could be curvilinearly related to pedaling rate.[31,32] Moreover, it was previously reported that cycling at a cadence higher than 95 rpm induces a significant increase in oxygen consumption, ventilation, and lactate concentration after 30 min of exercise in triathletes.[33] These previous results suggest that the metabolic demands could be high when the pedaling rate is high. In our present study, a higher pedaling rate (91.5 ± 0.54 rpm; average of 3 groups over 4 days) with maximal power output was performed by subjects during HIIT, suggesting that subjects were required to endure a high metabolic demand to maintain their pedaling rate. We observed a smaller reduction in PPR (ΔPPR) after CCWI compared to CWI or PAS. This can be explained by the fact that the efficacy of CCWI, which stimulates blood circulation and the release of metabolic waste, compensates for the higher oxygen consumption or increase in heart rate during higher pedaling rate, and minimizes muscle fatigue, thus enabling subjects to maintain their pedaling rate over consecutive days of exercise.

However, a careful consideration is necessary, because our present study regarding the peak and mean power in HIIT resulted that there was no significant difference among the three groups, whereas several studies demonstrated that the implementation of CWI improves repeated cycling performance[2,34] and may have a positive effect on sprint cycling performance compared to passive recovery.[11] This conflicting result remains a debate whether the cold-water immersion provide the beneficial effects on sprint performance. However, our current observation, that a small reduction in PPR (ΔPPR) following CCWI is associated with great reductions in HR and clearance of [La]b, indicated that combining cold water and CO_2 may be useful intervention for maintaining high intensity cycling performance. Several studies demonstrated that the immersion in CO_2-enriched water increases both cutaneous blood flow and oxygen partial pressure,[35–37] causing changes in oxygen dissociation and increased oxygen utilization, which attributed to effect of vasodilation resulting from CO_2 application. These previous studies may help to provide an explanation for the results in our present study, that the maintained PPR following CCWI, suggesting that combining cold water and CO_2 accelerates even more the blood circulation and the release of metabolic waste, thus enabling subjects to maintain their consecutive days of high intensity cycling performance.

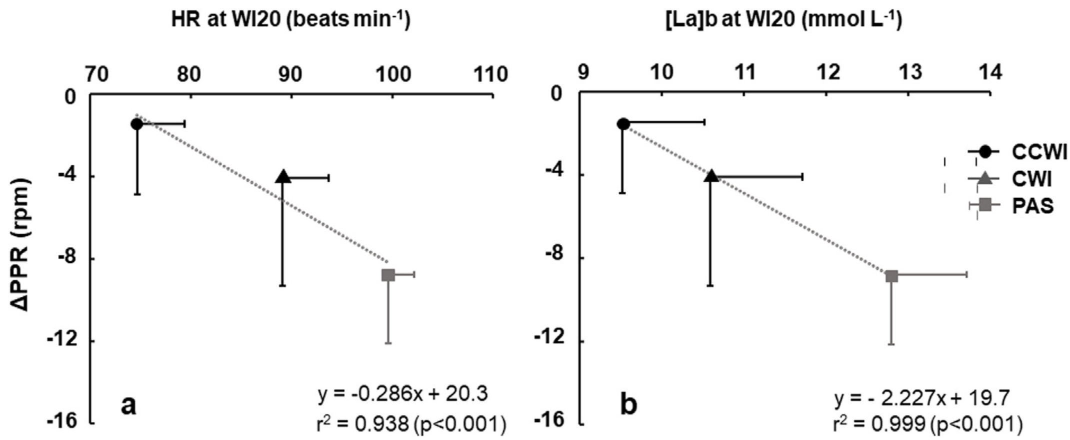

Fig. 4. Relationship between the decrease in the peak pedal repetitions (ΔPPR) within 8 consecutive sets of HIIT from Day 1 to Day 4 and the mean values of HR **(a)** and [La]b **(b)** after the recovery interventions or PAS. ΔPPR was associated with both the reduction of HR and the clearance of [La]b after recovery.

4.1. Study limitation

The assessors were unable to conduct the experiments without knowing the intervention assignment (i.e., which subjects were assigned to which intervention) because they prepared the CO_2-rich cold water and cold tap water interventions. This may be a possible limitation of the present study, as the assessors' bias may potentially have influenced the results. However, the subjects were not informed whether the water was CWI or CCWI to ensure a single-blind design. The assessors were aware of the risk of their conscious and unconscious bias, and made an effort to reduce the risk by carefully monitoring their behavior when addressing participants during experiments.

4.2. Perspective

It would be interesting to know whether there exists an optimal cooling threshold.[12] Future research should test the effects of differences in water temperature on sprint performance, with a view towards establishing individualized cooling strategies that could even consider different sports activities. The relatively high cooling temperature of 20 °C and 3 consecutive days of CO_2-rich water immersion water can provide a positive effect that may lead to a redistribution of blood flow (as evidenced by the reductions in [La] b and HR) and a small deterioration in peak pedal repetitions. Our findings also indicate that CO_2-rich cold-water immersion for 3 consecutive days may be useful when the maintenance of anaerobic high intensity performance is desired.

5. Conclusion

Compared with traditional recovery techniques such as passive recovery and cold tap water immersion, the 3-day consecutive use of CO_2-rich cold-water immersion may be effective for maintaining high intensity performance for peak pedal repetition, which is associated with a reduction in heart rate and enhanced clearance of blood lactate concentration. Combined with our previous findings that repeated use of CO_2-rich cold-water immersion is beneficial for cycling work efficiency,[13] the present findings suggest that CO_2-rich cold-water immersion is a promising intervention for aerobic (and possibly anaerobic) performance and can be adapted to different sports activities.

Ethical approval

The study was approved by the human research ethics committee of Doshisha University (No. 15085).

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Author contributions

MF, MY and YF were responsible for the design and conception of the work. MF, MY, MN, 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. MF, 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.

Declaration of competing interest

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

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References

1. Ihsan M, Watson G, Abbiss CR. What are the physiological mechanisms for post-exercise cold water immersion in the recovery from prolonged endurance and intermittent exercise? *Sports Med.* 2016;46:1095–1109. https://doi.org/10.1007/s40279-016-0483-3.
2. Vaile J, O'Hagan C, Stefanovic B, Walker M, Gill N, Askew CD. Effect of cold water immersion on repeated cycling performance and limb blood flow. *Br J Sports Med.* 2011;45:825–829. https://doi.org/10.1136/bjsm.2009.067272.
3. Enwemeka CS, Allen C, Avila P, Bina J, Konrade J, Munns S. Soft tissue thermodynamics before, during, and after cold pack therapy. *Med Sci Sports Exerc.* 2002;34:45–50. https://doi.org/10.1097/00005768-200201000-00008.
4. Lee DT, Toner MM, McArdle WD, Vrabas IS, Pandolf KB. Thermal and metabolic responses to cold-water immersion at knee, hip, and shoulder levels. *J Appl Physiol.* 1997;82:1523–1530. https://doi.org/10.1152/jappl.1997.82.5.1523.
5. Merrick MA, Jutte LS, Smith ME. Cold modalities with different thermodynamic properties produce different surface and intramuscular temperatures. *J Athl Train.* 2003;38:28–33.
6. Yanagisawa O, Homma T, Okuwaki T, Shimao D, Takahashi H. Effects of cooling on human skin and skeletal muscle. *Eur J Appl Physiol.* 2007;100:737–745. https://doi.org/10.1007/s00421-007-0470-3.

7. Washington LL, Gibson SJ, Helme RD. Age-related differences in the endogenous analgesic response to repeated cold water immersion in human volunteers. *Pain*. 2000;89:89–96. https://doi.org/10.1016/S0304-3959(00)00352-3.
8. Rowsell GJ, Coutts AJ, Reaburn P, Hill-Haas S. Effects of cold-water immersion on physical performance between successive matches in high-performance junior male soccer players. *J Sports Sci*. 2009;27:565–573.
9. Vaile J, Halson S, Gill N, Dawson B. Effect of hydrotherapy on recovery from fatigue. *Int J Sports Med*. 2008;29:539–544.
10. Montgomery PG, Pyne DB, Hopkins WG, Dorman JC, Cook K, Minahan CL. The effect of recovery strategies on physical performance and cumulative fatigue in competitive basketball. *J Sports Sci*. 2008;26:1135–1145.
11. Stanley J, Peake JM, Buchheit M. Consecutive days of cold water immersion: effects on cycling performance and heart rate variability. *Eur J Appl Physiol*. 2013;113:371–384. https://doi.org/10.1007/s00421-012-2445-2.
12. Yoshimura M, Hojo T, Yamamoto H, et al. Application of carbon dioxide microbubbles on the skin- and muscle- oxygenation of different muscle sites of lower limb during cold water immersion in human. *PeerJ*. 2020;8:e9785. https://doi.org/10.7717/peerj.9785.
13. Yoshimura M, Hojo T, Yamamoto H, Tachibana M, Nakamura M, Fukuoka Y. Effects of artificial CO_2-rich cold-water immersion on repeated-cycling work efficiency. *Res Sports Med*. 2020;10:1–12. https://doi.org/10.1080/15438627.2020.1860048.
14. Al Haddad H, Parouty J, Buchheit M. Effect of daily cold water immersion on heart rate variability and subjective ratings of well-being in highly trained swimmers. *Int J Sports Physiol Perform*. 2012;7:33–38.
15. Davies JA. Direct determination of aerobic power. Physiological assessment of human fitness. In: Maud PJ, Foster C, eds. *Human Kinetics*. 1995:9–16.
16. Tabata I, Nisimura K, Kouzaki M, et al. Effects of moderate-intensity endurance and high-intensity intermittent training on anaerobic capacity and VO_{2max}. *Med Sci Sports Exerc*. 1996;28:1327–1330.
17. Foster C, Farland CV, Guidotti F, et al. The effects of high intensity interval training vs steady state training on aerobic and anaerobic capacity. *J Sports Sci Med*. 2015;4:747–755.
18. Abdulkarim DA, Ebine N, Nakae S, Hojo T, Fukuoka Y. Application of molecular hydrogen as an antioxidant in responses to ventilatory and ergogenic adjustments during incremental exercise in humans. *Nutrients*. 2021;13:459. https://doi.org/10.3390/nu13020459.
19. Hopkins WG, Marshall SW, Batterham AM, Hanin J. Progressive statistics for studies in sports medicine and exercise science. *Med Sci Sports Exerc*. 2009;41:3–13. https://doi.org/10.1249/MSS.0b013e31818cb278.
20. Atkinson G, Nevill AM. Statistical methods for assessing measurement error (reliability) in variables relevant to sports medicine. *Sports Med*. 1998;26:217–238. https://doi.org/10.2165/00007256-199826040-00002 10.
21. Hopkins WG. Measures of reliability in sports medicine and science. *Sports Med*. 2000;30:1–15. https://doi.org/10.2165/00007256-200030010-00001.
22. Marsh D, Sleivert G. Effect of precooling on high intensity cycling performance. *Br J Sports Med*. 1999;33:393–397. https://doi.org/10.1136/bjsm.33.6.393.
23. Mourot L, Bouhaddi M, Gandelin E, et al. Cardiovascular autonomic control during short-term thermoneutral and cool head-out immersion. *Aviat Space Environ Med*. 2008;79:14–20. https://doi.org/10.3357/ASEM. 2147.2008.
24. Tomlin DL, Wenger HA. The relationship between aerobic fitness and recovery from high intensity intermittent exercise. *Sports Med*. 2001;31:1–11. https://doi.org/10.2165/00007256-200131010-00001.
25. Tipton MJ. Environmental extremes: origins, consequences and amelioration in humans. *Exp Physiol*. 2016;101:1–14. https://doi.org/10.1113/EP085362.
26. Cochrane DJ. Alternating hot and cold water immersion for athlete recovery. *Rev Phys Ther Sport*. 2004;5:26–32. https://doi.org/10.1016/j.ptsp.2003.10.002.
27. White GE, Wells GD. Cold-water immersion and other forms of cryotherapy: physiological changes potentially affecting recovery from high-intensity exercise. *Extreme Physiol Med*. 2013;2:26. https://doi.org/10.1186/2046-7648-2-26.
28. Parouty J, Al Haddad H, Quod M, Leprêtre PM, Ahmaidi S, Buchheit M. Effect of cold water immersion on 100-m sprint performance in well-trained swimmers. *Eur J Appl Physiol*. 2010;109:483–490. https://doi.org/10.1007/s00421-010-1381-2.Epub2010Feb17.PMID:20162301.
29. Crowe MJ, O'Connor D, Rudd D. Cold water recovery reduces anaerobic performance. *Int J Sports Med*. 2007;28:994–998. https://doi.org/10.1055/s-2007-965118.Epub2007May29.PMID:17534786.
30. Takaishi T, Yasuda Y, Moritani T. Neuromuscular fatigue during prolonged pedalling exercise at different pedalling rates. *Eur J Appl Physiol Occup Physiol*. 1994;69:15–48.
31. Chavarren J, Calbet JA. Cycling efficiency and pedalling frequency in road cyclists. *Eur J Appl Physiol*. 1999;80:555–563.
32. Coast JR, Welch HG. Linear increase in optimal pedal rate with increased power output in cycle ergometry. *Eur J Appl Physiol*. 1985;53:339–342.
33. Brisswalter J, Hausswirth C, Smith D, Vercruyssen F, Vallier JM. Energetically optimal cadence vs. freely-chosen cadence during cycling: effect of exercise duration. Int J Sport Med. 200,21:60–64.
34. Peiffer JJ, Abbiss CR, Watson G, Nosaka K, Laursen PB. Effect of a 5-min cold-water immersion recovery on exercise performance in the heat. *Br J Sports Med*. 2010;44:461–465.
35. Hartmann BR, Drews B, Bassenge E. CO_2-induced acral blood flow and the oxygen partial pressure in arterial occlusive disease. *DMW (Dtsch Med Wochenschr)*. 1991;116(43):1617–1621.
36. Hartmann BR, Drews B, Burnus C, Bassenge E. Increase in skin blood circulation and transcutaneous oxygen partial pressure of the top of the foot in lower leg immersion in water containing carbon dioxide in patients with arterial occlusive disease. results of a controlled study compared with fresh water. *VASA. Zeitschrift Fur Gefasskrankheiten*. 1991;20(4):382–387.
37. Hartmann BR, Bassenge E, Pittler M. Effect of carbon dioxide-enriched water and fresh water on the cutaneous microcirculation and oxygen tension in the skin of the foot. *Angiology*. 1997;48(4):337–343.