Heliyon 9 (2023) e20288

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Effect of CO_2 and H_2 gas mixture in cold water immersion on recovery after eccentric loading

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ARTICLE INFO

Keywords:
Cold water immersion
Eccentric exercise
Hydrogen
Muscle damage

ABSTRACT

Background: The findings of previous studies support the efficacy of cold water immersion (CWI) with carbon dioxide (CO_2) in enhancing muscle blood flow and maintaining aerobic performance efficiency. We hypothesize that the addition of hydrogen gas (H_2), known for its antioxidant properties and role in inflammation regulation, to C-CWI can enhance recovery after eccentric exercise.
Subjects: and Methods: Thirty-four healthy subjects performed a knee-extensor eccentric exercise. They were randomly allocated into four groups: control, CWI, CO_2-rich CWI (C-CWI), and CO_2 + H_2 gas mixture CWI (CH-CWI). In the three CWI groups, all subjects were immersed in the appropriate bath at 20 °C for 20 min immediately after 60 repetitions of eccentric exercise. Before exercise and after 48 h of recovery, the subjects' maximal voluntary isometric contraction torque (MVC-ISO), maximal voluntary concentric (MVC-CON) contraction torque, countermovement jump (CMJ) height, knee flexion range of motion (ROM), muscle soreness, and muscle thickness were measured.
Results: In the CH-CWI group only, the MVC-ISO, CMJ height, and ROM did not decrease significantly post-exercise, whereas all of these decreased in the other three groups. Muscle soreness at palpation, contraction, and stretching significantly increased post-exercise in all groups. Echo intensity and tissue hardness did not increase significantly in the CH-CWI group.
Conclusions: CH-CWI stimulated recovery from impairments in MVC-ISO torque, CMJ height, knee-flexion ROM, tissue hardness, and echo intensity. These findings indicate that CH-CWI can promote recovery after eccentric exercise.

1. Introduction

Cold water immersion (CWI) is the most commonly used cold modality to enhance the recovery of team sport athletes [1–3]. CWI

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https://doi.org/10.1016/j.heliyon.2023.e20288
Received 21 May 2023; Received in revised form 15 September 2023; Accepted 18 September 2023

generally involves immersion to the waist or mid-torso for 5–20 min in water chilled within the range of 8 °C–15 °C [3–5]. CWI's reported recovery enhancement is measured by decreases in markers of muscle damage such as creatine kinase [6,7] and by perceived levels of muscle soreness and fatigue [6,8].

Delayed-onset muscle soreness (DOMS), which is the feeling of muscle soreness that occurs some length of time after exercise, is characterized by heightened muscle stiffness, reduced muscle strength, a lower pain threshold, and limited range of motion (ROM) [9,10]. DOMS is reportedly caused especially by eccentric contractions. Eccentric contractions have been reported to impede movement and induce muscle fiber damage [11]. It is desirable to minimize the negative aspects of DOMS because they can hinder motivation for further exercise. Although CWI's potential to prevent or lessen DOMS could be very helpful to athletes, there is no consensus regarding its recovery effects [1].

The acute advantages of cold modalities for recovery have been documented, with several studies suggesting that CWI can reduce both immediate anabolic signaling and long-term adaptations after resistance exercise [12–14]. In a 2020 meta-analysis of male athletes, CWI following resistance exercise sessions attenuated improvements in muscular strength [15]. Another study suggested that the cooling stimulus provided by CWI may induce vasoconstriction, thus reducing blood flow [5]. Although there is not yet a consensus on the immediate and long-term impacts of CWI, it is evident that effective CWI treatment for injured muscles must address the challenge of cold-induced decreases in blood flow.

Our research group has paid particular attention to the vasodilatory effect of carbon dioxide (CO_2) gas; our 2020 investigation revealed that CO_2-rich CWI increased both skin and muscle blood flow [16]. Our later study confirmed that, compared to the use of CWI alone, CO_2-rich CWI accelerated both the post-exercise drop in deep-body temperature and the lactate elimination rate [17]. Our hypothesis thus postulated that the use of CO_2-rich CWI could promote recovery from DOMS better than CWI without CO_2 enhancement.

The anti-inflammatory effect of hydrogen (H_2) gas after acute exercise has also attracted attention [18]. H_2 can be delivered by various routes, including an drinking it or soaking in it. H_2 is thought to neutralize hydroxyl radicals and peroxynitrite selectively within cells, thus exerting cytoprotective effects against oxidative stress [19]. For instance, Kawamura et al. [20] evaluated the pain associated with DOMS using a visual analog scale (VAS). They noted that this pain level could be alleviated through H_2 soaking even though such soaking did not impact inflammatory responses.

The present study was conducted to assess the potential superiority of CWI including the addition of $CO_2 + H_2$ gas over other CWI modalities in accelerating recovery from DOMS. The study hypothesis was that the application of CO_2-rich + H_2 CWI would further decrease subjects' VAS ratings of DOMS caused by eccentric exercise and promote recovery from impairments in skeletal muscle function and performance.

2. Subjects and methods

2.1. Experimental design

We designed a randomized repeated measures experiment to compare the acute effects of four recovery strategies after eccentric loading: control vs. regular CWI vs. CO_2-rich CWI vs. CO_2-rich + H_2-rich CWI. The subjects (n = 34) described below performed 60 repetitions of an eccentric contraction of the knee extensor of the dominant leg (the preferred leg with which to kick a ball); they performed six sets of 10 repetitions each [21–23]. The subjects were randomly allocated into four groups: control, CWI alone, CO_2-rich CWI (C-CWI), and a CO_2 and H_2 gas mixture CWI (CH-CWI).

In the three CWI groups, each subject underwent a 20-min session of CWI immediately after completing all 60 repetitions of the contractions. The dependent variables were as follows: knee-flexion ROM; maximal voluntary isometric contraction (MVC-ISO); maximal voluntary concentric contraction (MVC-CON) torque of the knee extensor; countermovement jump (CMJ) height; pain pressure threshold (PPT); muscle soreness during muscle contraction, stretching, and palpation; and muscle thickness immediately before (Pre) and at 48 h after (Post) maximal eccentric contraction. Our earlier investigation confirmed the high reliability of the outcome variables [21].

For each subject, all Pre and Post measurements were taken at the same time of day. Before the baseline measurements of the subject's measurement (dominant) leg, the subject was familiarized with the eccentric exercise and all of the measurement variables. The experiments were conducted between 9 a.m. and 6 p.m. Although the timing varied among subjects, the experiments for the same subject on the first day and 2 days later were conducted at the same time.

2.2. Subjects

Thirty-four healthy young men volunteered to participate in the study. Each subject reported no habitual exercise for more than 6 months before the study. Subjects were excluded if they reported a medical history of either lower-extremity neuromuscular disease or lower-extremity musculoskeletal injury. None of the subjects had participated in regular resistance or flexibility training.

The 34 subjects were randomly allocated into four groups. Control: n = 10, age 21.1 ± 0.6 yrs; height 175.4 ± 6.3 cm; weight 68.3 ± 8.4 kg (mean ± SD). CWI: n = 8, age 21.4 ± 0.8 yrs; height 171.7 ± 7.9 cm; weight 64.5 ± 7.6 kg. C-CWI: n = 8, age 21.0 ± 0.6 yrs; height 172.3 ± 6.5 cm; weight 66.5 ± 9.3 kg. CH-CWI: n = 8, age 21.0 ± 0.6 yrs; height 170.1 ± 2.0 cm; weight 63.2 ± 7.6 kg. The body mass index was 22.1 ± 2.1 for all subjects, and their anthropometric characteristics were consistent with the standards for the young male population in Japan. Each subject's height was self-reported, whereas weight was measured on a scale (HBF-212, Omron, Kyoto, Japan). According to a one-way analysis of variance (ANOVA) there were no significant differences among the four groups in age, height, or weight.

Each subject was fully informed of the study procedures and purpose, and each provided written informed consent. The study was approved by the Ethics Committee of Niigata University of Health and Welfare (#18560), Niigata, Japan, and conformed with the requirements of the Declaration of Helsinki.

2.3. *Eccentric exercise*

Each subject completed 60 repetitions of maximal eccentric contraction of the quadriceps femoris by performing six sets of 10 repetitions per set with the use of a dynamometer (Biodex System 3.0, Biodex Medical Systems, Shirley, NY, USA) [21–24]. To perform the exercise, the subject sat on the dynamometer chair (hip flexion angle 80°) and Velcro straps secured the trunk, pelvis, and thigh of the exercised side. The knee joint was aligned with the dynamometer's rotation axis. The subject was instructed to perform the maximal eccentric contraction from 20° to 110° (knee-flexion ROM, 90°) at 60°/s, as described [24]. After completion of each maximal eccentric contraction, the dynamometer's lever arm passively returned the subject's knee joint to 20° at 10°/s, providing a 9-sec rest between repetitions. This means that the 9 s time to return the lever was a continuous passive mode.

Each subject performed six sets of 10 repetitions with a 100-s rest between sets [22,25]. An observer strongly encouraged each subject to exert maximum force during each set.

2.4. *CWI, C-CWI, and CH-CWI*

After completing the eccentric exercise on the Biodex, the subject either sat passively in a chair for 20 min (control group) or immersed himself for 20 min in a half-body (from navel to toe) CWI, C-CWI, or CH-CWI recovery bath in 150 L of 20 °C water. In the case of the C-CWI treatment, approx. 830 ppm of CO_2 water was prepared by dissolving CO_2 in 20 °C tap water using a customized CO_2 and H_2 gas dissolution system (Iwatani, Kobe, Japan). For the CH-CWI treatment, the same gas dissolution system was used to create a $CO_2 + H_2$ gas mixture water bath (with a CO_2 concentration of 830 ppm and an H_2 concentration of 0.7 ppm) by dissolving CO_2 and H_2 in 20 °C tap water.

2.5. *MVC-ISO and MVC-CON contraction torque*

Each subject's MVC-ISO and MVC-CON torques were measured in the same position as that used for the eccentric contractions. Prior to measurement, the subject performed MVC-ISO warm-ups at intensities of 30%, 60%, and 90%. The same Biodex dynamometer was used to measure MVC-ISO torque at knee-flexion angles of 20° and 70°. To obtain the measurements, the subject was instructed to perform a maximal isometric contraction for 3-sec twice at each knee-flexion angle with a 60-sec rest between MVC-ISO measurements; the average was used in further analyses. MVC-CON torque was measured at 60°/s for 20°–90° knee angles (knee-flexion ROM was 70°) for three continuous MVC-CON in the direction of knee extension. Further analysis used the highest value obtained from the three contractions. An observed verbally encouraged each subject consistently throughout all trials.

2.6. *Countermovement jump (CMJ) height*

Each subject's CMJ height was measured by using a jump mat system (4Assist, Tokyo) to calculate the flight time. At the beginning of the CMJ measurement, the subject placed the foot of his dominant leg on the mat and placed his palms of his hands in front of his chest to prevent arm swinging. The subject was instructed to quickly perform a dip and reach a self-selected depth before jumping as high as possible in the subsequent concentric phase, all while using a single leg. Upon landing, the subject's uninvolved leg maintained a flexed knee position of approx. 90° [24]. The subject practiced the task several times to become familiar with it, then performed the CMJ three times. The greatest jump height achieved was selected for subsequent analysis.

2.7. *Knee-flexion ROM*

To measure knee-flexion ROM, the subject positioned himself in a side-lying position on a massage table, with the hip and knee of the nondominant leg flexed 90° to prevent any pelvic movement during the measurement [24]. The investigator passively flexed the knee of the dominant leg to its maximum extent while keeping the hip joint in a neutral position. The degree of knee flexion was measured twice with a goniometer (MMI universal goniometer Todai 300 mm, Muranaka Medical Instruments, Co., Ltd., Osaka, Japan). The two measurements were averaged for use in subsequent analyses.

2.8. *Muscle soreness*

The levels of knee-extensor muscle soreness during muscle contraction, stretching, and palpation were assessed on the basis of the subject's completion of a visual analog scale (VAS). This scale consisted of a 100-mm continuous line with the endpoints labeled as "not sore at all" (0 mm) and "very, very sore" (100 mm) [24,26]. Muscle soreness during contraction was evaluated during both the MVC-ISO and MVC-CON torque measurements, and the average value was utilized for further analysis.To assess muscle soreness during palpation, the subject lay supine on a massage table as the investigator palpated the proximal, middle, and distal points of the vastus medialis, vastus lateralis, and rectus femoris muscles [24,27]. The average value of each knee-extensor palpation point was employed in the subsequent analyses. Muscle soreness during the ROM measurements was assessed for each subject, and the resulting average value was utilized.

2.9. Pain pressure threshold (PPT)

The PPT was measured using an algometer (NEUTONE TAM-22, BT10; TRY ALL, Chiba, Japan) with the subject positioned supine on a massage table (Fig. 1). The measurement was taken at the midpoint between the anterior superior iliac spine and the upper end of the patella on the subject's dominant side. A metal rod algometer was used to apply gradually increasing pressure to the soft tissue at that point. The subject was instructed to press a trigger upon first feeling pain, not just pressure. The PPT was defined as the algometer's reading (in kg per cm^2) at the time point at which the subject said that he felt pain. Three such measurements were taken at 30-sec intervals, and the mean value (in kg per cm^2) was used for the data analyses as described [28,29].

2.10. Tissue hardness

Tissue hardness was measured by a portable tissue-hardness meter (NEUTONE TDM-N1; TRY-ALL) with the subject in the same-measurement position and posture as in the PPT measurements. This meter measured the penetration distance until 1.5 kg-force (kgf) pressure was reached [30]. The subject was instructed to relax while the tissue hardness was measured three times. A 30-sec rest period was provided between measurements. The mean of the measurements was used in further analysis.

2.11. Muscle thickness and echo intensity measurements

Each subject's quadriceps femoris thickness was measured by B-mode ultrasonography using an 8-MHz linear probe (LOGIQ e V2; GE Healthcare Japan, Tokyo) with the subject in the same position as in the PPT and tissue hardness measurements [31]. The transverse images were analyzed using ImageJ software (National Institutes of Health, Bethesda, MD, USA), and the muscle thickness was determined as the combined thickness of the rectus femoris and vastus intermedius muscles. The average echo intensity, represented on a grayscale from 0 (black) to 256 (white) histogram, was also computed for the rectus femoris muscle.

2.12. Statistical analyses

All data are presented as means ± standard deviation (SD) and were analyzed using SPSS ver. 24.0 software (SPSS Japan, Tokyo, Japan). To estimate the necessary sample size, a split-plot analysis of variance was conducted with an error probability of 0.05 (α), a power ($1-\beta$) exceeding 0.80, and an effect size of 0.68 [32] using G Power 3.1 analysis software [33]. The necessary sample size exceeded 10 in each group. Despite conducting a normality assessment of the data using the Shapiro–Wilk test, the results indicated that none of the data followed a normal distribution. Therefore, we performed the Wilcoxon signed-rank test to compare each subject's Pre and Post values. The effect size (ES) was determined as Cohen's d, measuring the percentage change from Pre to Post between the trials to elucidate the practical significance of the CH−CWI intervention. The criteria for interpreting the magnitude were defined as follows: A Cohen's d effect size of 0–0.2 was considered trivial, 0.2 to 0.6 was considered small, 0.6 to 1.2 was deemed moderate, 1.2 to 2.0 was categorized as large, and an effect size greater than 2 was considered very large [34]. A significance level of $p < 0.05$ was used to indicate a significant difference.

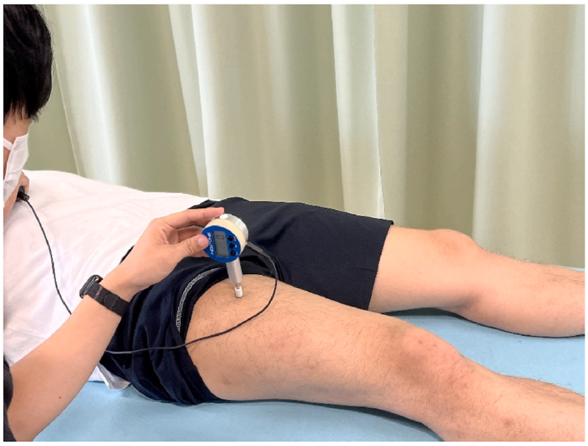


Fig. 1. Photograph of the point of measurement for the pain pressure threshold (PPT).

3. Results

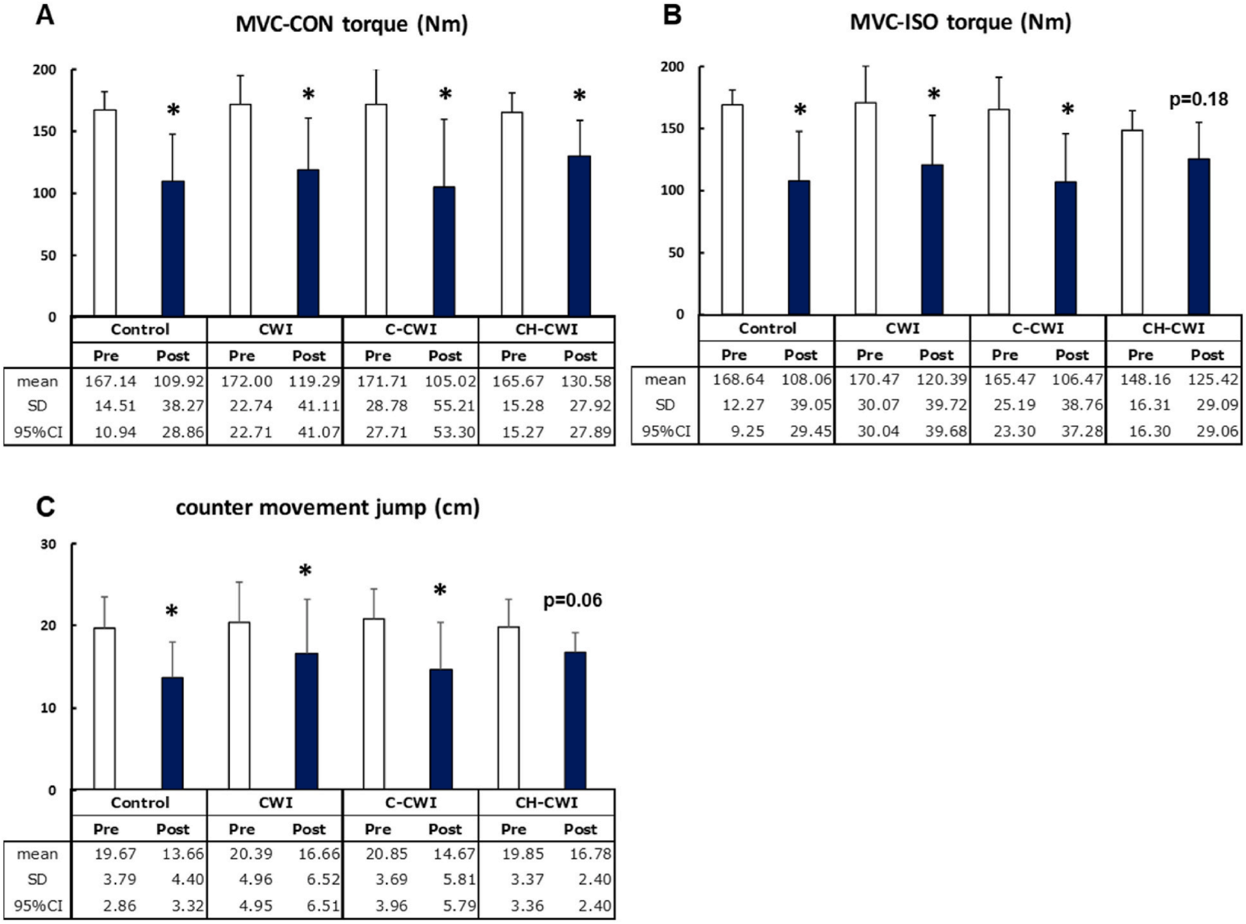
Fig. 2 depicts the differences in the subjects' MVC-ISO, MVC-CON, and CMJ height values between Pre and Post as indices of exercise performance. The Wilcoxon signed-rank test showed significant decreases in MVC-ISO torque, MVC-CON, and CMJ height in the Control, CWI, and C-CWI groups ($p < 0.05$). On the other hand, there were no significant differences in MVC-ISO ($ES = 1.00$) or CMJ height ($ES = 1.06$) in the CH-CWI group.

All groups exhibited significant decreases in PPT ($p < 0.05$). In addtion, in the three groups other than CH-CWI, knee-flexion ROM showed significant decreases ($p < 0.05$) and tissue hardness ($p < 0.05$) significant increases, as illustrated in Fig. 3. In the CH-CWI group, no significant differences were found in knee-flexion ROM with moderate ES ($ES = 0.97$) or in tissue hardness with moderate ES ($ES = 0.64$).

Our analyses also revealed changes in muscle soreness at palpation, contraction, and stretching as pain indices (Fig. 4), as well as changes in the subjects' muscle thickness and echo intensity (Fig. 5). Muscle soreness at palpation, contraction, stretching and muscle thickness increased significantly in all four groups ($p < 0.05$). Echo intensity and tissue hardness exhibited statistically significant increases in all groups, except for the CH-CWI group, in which the effect size (ES) was small ($ES = 0.31$).

4. Discussion

To our knowledge, this study represents the first endeavor to explore and compare the recovery effects of CO_2-rich (C-CWI) and $CO_2 + H_2$ gas (CH-CWI) following eccentric loading. Our primary objective was to comprehensively evaluate CWI's effects on the muscle damage incurred during eccentric exercise, with a particular focus on the recovery-promoting effects of CO_2-rich and $CO_2 + H_2$ gas-infused water. The results of our examination of 34 healthy male adults revealed that neither plain CWI nor C-CWI significantly improved recovery after eccentric loading, whereas CH-CWI enhanced recuperation from impairments in knee-flexion ROM, tissue hardness, MVC-ISO torque, CMJ height, and echo intensity.



A

	Control		CWI		C-CWI		CH-CWI	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post
mean	167.14	109.92	172.00	119.29	171.71	105.02	165.67	130.58
SD	14.51	38.27	22.74	41.11	28.78	55.21	15.28	27.92
95%CI	10.94	28.86	22.71	41.07	27.71	53.30	15.27	27.89

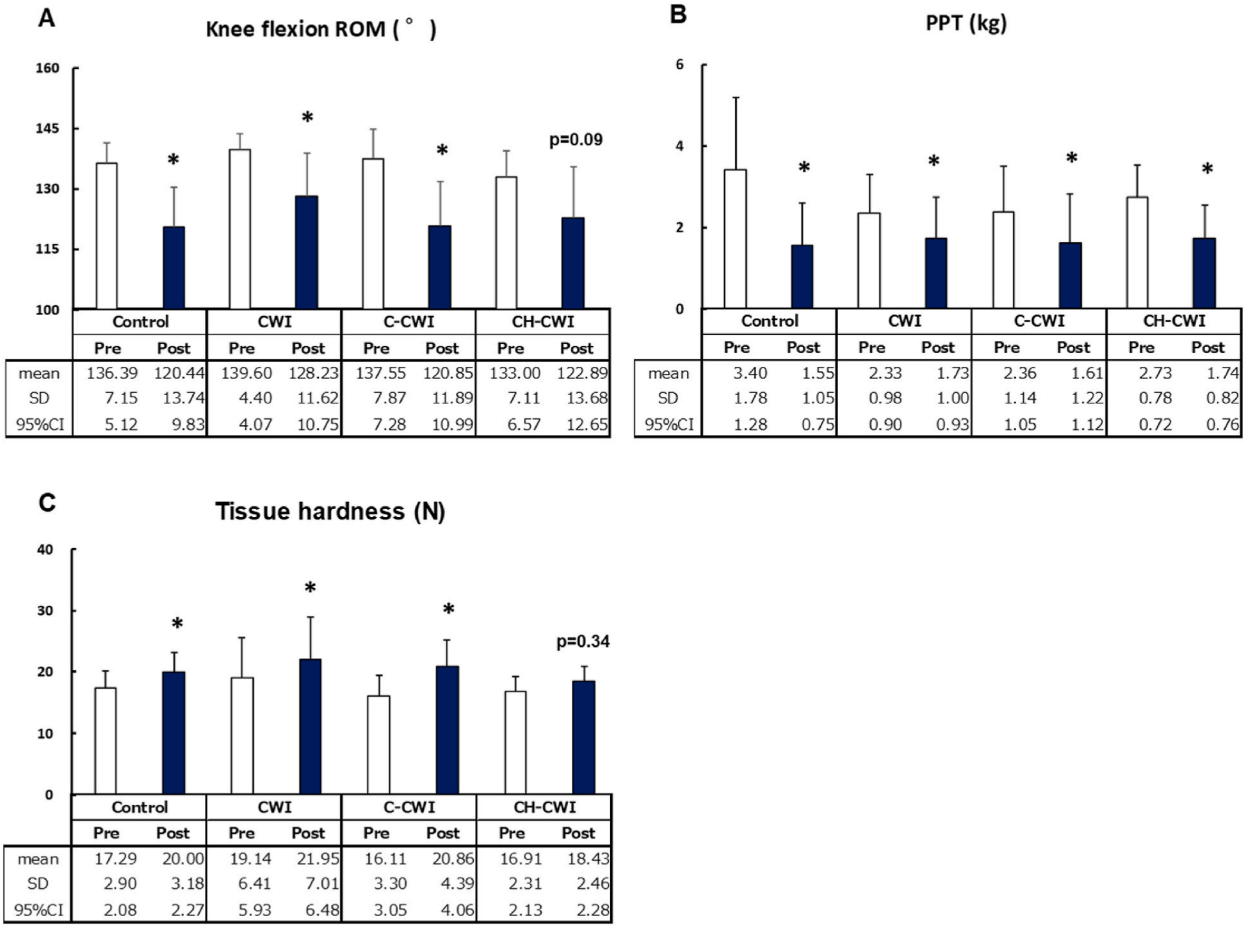
B

	Control		CWI		C-CWI		CH-CWI	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post
mean	168.64	108.06	170.47	120.39	165.47	106.47	148.16	125.42
SD	12.27	39.05	30.07	39.72	25.19	38.76	16.31	29.09
95%CI	9.25	29.45	30.04	39.68	23.30	37.28	16.30	29.06

C

	Control		CWI		C-CWI		CH-CWI	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post
mean	19.67	13.66	20.39	16.66	20.85	14.67	19.85	16.78
SD	3.79	4.40	4.96	6.52	3.69	5.81	3.37	2.40
95%CI	2.86	3.32	4.95	6.51	3.96	5.79	3.36	2.40

Fig. 2. Changes in maximal voluntary concentric (MVC-CON:A) and isometric (MVC-ISO:B) contraction torque and countermovement jump (CMJ: C) height before (Pre) and 48 h after (Post) teccentric exercise. CWI: cold water immersion, C-CWI: CO_2-rich CWI, CH-CWI: CO_2 and H_2 gas mixture CWI. Error bars: SD. *$p < 0.05$ compared to Pre.



	Control		CWI		C-CWI		CH-CWI	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post
mean	136.39	120.44	139.60	128.23	137.55	120.85	133.00	122.89
SD	7.15	13.74	4.40	11.62	7.87	11.89	7.11	13.68
95%CI	5.12	9.83	4.07	10.75	7.28	10.99	6.57	12.65

	Control		CWI		C-CWI		CH-CWI	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post
mean	3.40	1.55	2.33	1.73	2.36	1.61	2.73	1.74
SD	1.78	1.05	0.98	1.00	1.14	1.22	0.78	0.82
95%CI	1.28	0.75	0.90	0.93	1.05	1.12	0.72	0.76

	Control		CWI		C-CWI		CH-CWI	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post
mean	17.29	20.00	19.14	21.95	16.11	20.86	16.91	18.43
SD	2.90	3.18	6.41	7.01	3.30	4.39	2.31	2.46
95%CI	2.08	2.27	5.93	6.48	3.05	4.06	2.13	2.28

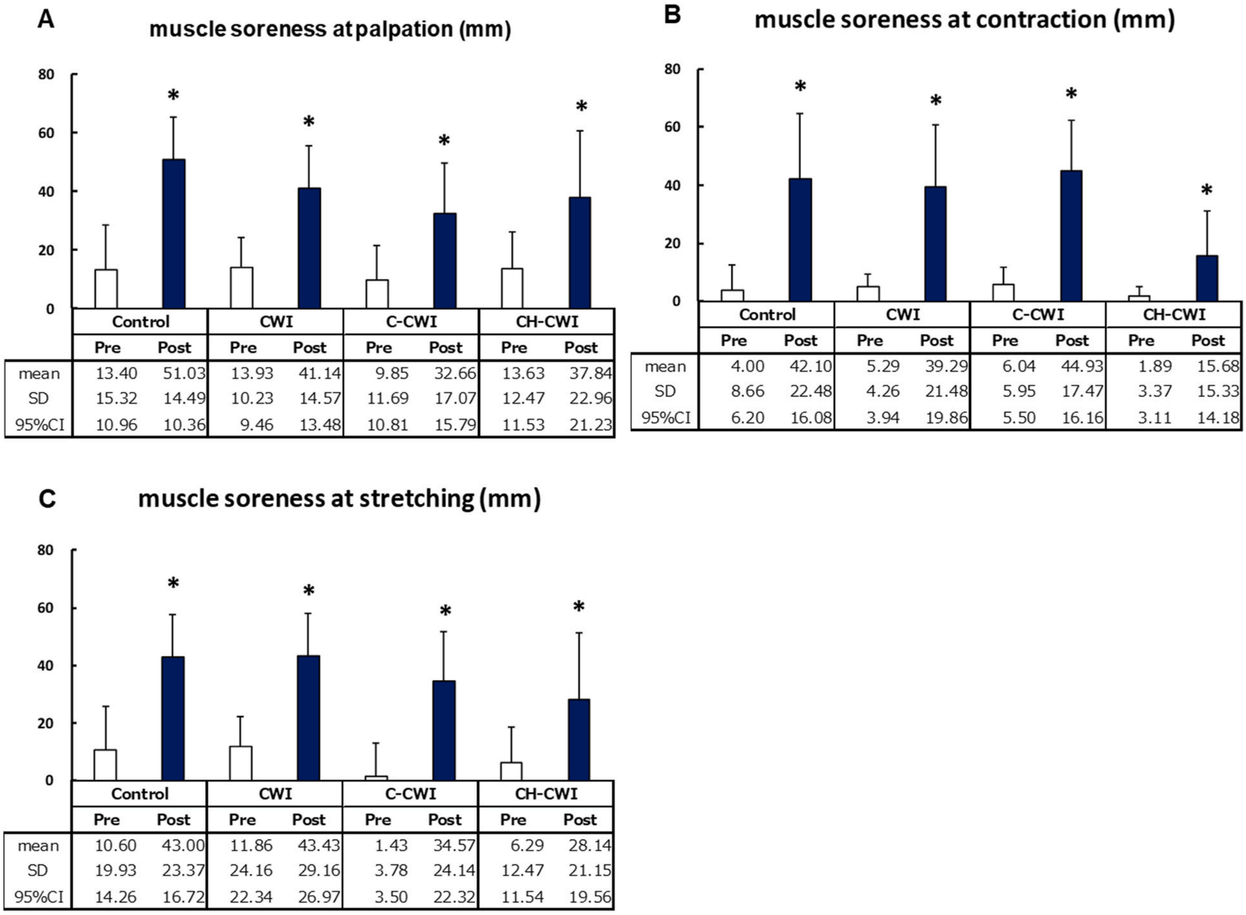
Fig. 3. Changes in knee-flexion range of motion (ROM:A), pain pressure threshold (PPT:B), and tissue hardness(C) from Pre to Post eccentric exercise. Error bars: SD. $*p < 0.05$ compared to Pre.

No practical recovery effects were observed after the application of CWI or C-CWI. In earlier investigations, however, CWI was associated with enhanced recovery, as indicated by decreased values of muscle damage markers [6,7] and decreased perceived levels of fatigue and muscle soreness [6,8]. Our present findings are inconsistent with those reports. Other investigations of CWI's effects on eccentrically damaged muscle did not identify any favorable responses of eccentrically damaged muscle to CWI, with results similar to those of our present study [35,36].

Our findings indicate that plain CWI may not have a beneficial recovery impact on muscle damage resulting from resistance training, particularly training that emphasizes eccentric contractions. A disadvantage of CWI is that it induces vasoconstriction in arterial and venous capillaries, thereby reducing peripheral blood flow [5], and this may be related to our findings. The study explored the potential recovery effects of C-CWI, which does not induce vasoconstriction [16], following eccentric loading. Our findings indicated that neither C-CWI with CO_2-rich water nor regular CWI with tap water yielded favorable responses. In two earlier studies, C-CWI after aerobic exercise provided a greater recovery effect than CWI [17,37], whereas in the present study C-CWI did not result in any favorable responses after eccentric loading. Differences in the exercise task may thus influence the recovery effect. It is possible that C-CWI is effective for recovery after aerobic exercise but not for repairing muscles damaged by eccentric contractions.

In this study, we emphasized the novel anti-inflammatory potential of hydrogen and explored its effects in combination with carbon dioxide CWI. The results showed that CH-CWI enhanced MVC-ISO torque, CMJ height, knee-flexion ROM, tissue hardness, and echo intensity. These results indicated that CH-CWI can be considered a new physical intervention to help muscles recover after eccentric loading. In the protocol used herein, the CO_2 and H_2 gas were simultaneously dissolved in water, and CO_2-induced vasodilation may have effectively increased the uptake of H_2. The anti-inflammatory reaction of hydrogen thus appears to have suppressed inflammation in the muscle [18] and led to a nonsignificant increase in echo intensity (Fig. 5). This anti-inflammatory H_2 reaction may also have prevented the decrease in tissue hardness as muscle swelling and the decrease in the subjects' knee-flexion ROM (Fig. 3).

It is also speculated that the muscle damage recovery from the CH-CWI bath could have accelerated the recovery of MVC-ISO torque and CMJ height values as indicators of physical performance (Fig. 2). Our observation that CH-CWI can mitigate the deterioration of physical performance and muscle soreness is a novel finding. Bathing in cold water with a $CO_2 + H_2$ gas mixture may be different from



A muscle soreness at palpation (mm)

	Control		CWI		C-CWI		CH-CWI	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post
mean	13.40	51.03	13.93	41.14	9.85	32.66	13.63	37.84
SD	15.32	14.49	10.23	14.57	11.69	17.07	12.47	22.96
95%CI	10.96	10.36	9.46	13.48	10.81	15.79	11.53	21.23

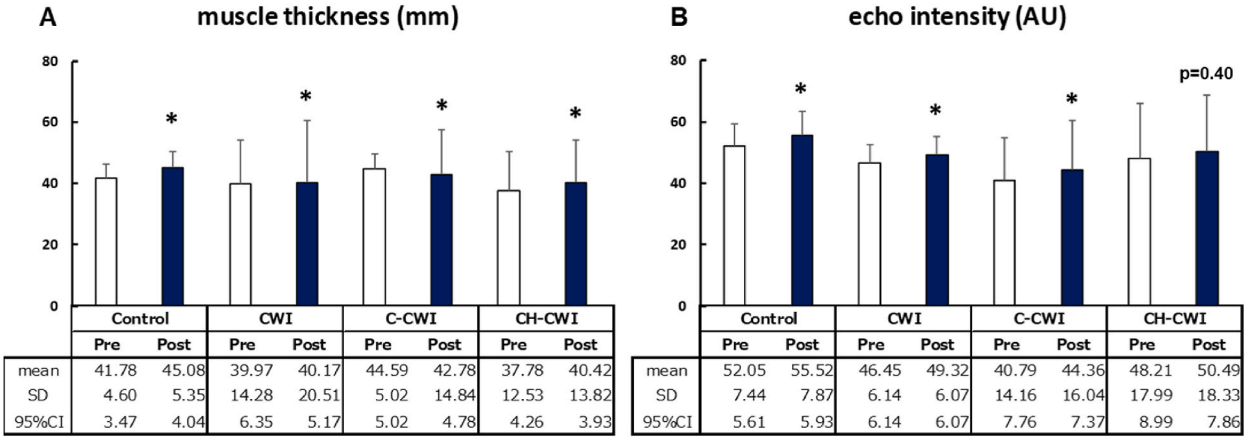
B muscle soreness at contraction (mm)

	Control		CWI		C-CWI		CH-CWI	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post
mean	4.00	42.10	5.29	39.29	6.04	44.93	1.89	15.68
SD	8.66	22.48	4.26	21.48	5.95	17.47	3.37	15.33
95%CI	6.20	16.08	3.94	19.86	5.50	16.16	3.11	14.18

C muscle soreness at stretching (mm)

	Control		CWI		C-CWI		CH-CWI	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post
mean	10.60	43.00	11.86	43.43	1.43	34.57	6.29	28.14
SD	19.93	23.37	24.16	29.16	3.78	24.14	12.47	21.15
95%CI	14.26	16.72	22.34	26.97	3.50	22.32	11.54	19.56

Fig. 4. Changes in muscle soreness at palpation (A), contraction (B), and stretching (C) from Pre to Post eccentric exercise. Error bars: SD. *$p < 0.05$ compared to Pre.



A muscle thickness (mm)

	Control		CWI		C-CWI		CH-CWI	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post
mean	41.78	45.08	39.97	40.17	44.59	42.78	37.78	40.42
SD	4.60	5.35	14.28	20.51	5.02	14.84	12.53	13.82
95%CI	3.47	4.04	6.35	5.17	5.02	4.78	4.26	3.93

B echo intensity (AU)

	Control		CWI		C-CWI		CH-CWI	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post
mean	52.05	55.52	46.45	49.32	40.79	44.36	48.21	50.49
SD	7.44	7.87	6.14	6.07	14.16	16.04	17.99	18.33
95%CI	5.61	5.93	6.14	6.07	7.76	7.37	8.99	7.86

Fig. 5. Changes in muscle thickness (A) and echo intensity (B) from Pre to Post eccentric exercise. Error bars: SD. *$p < 0.05$ compared to Pre.

bathing in H_2 alone, and the combination of CO_2 and H_2 can be expected to have some effect. Regrettably, this study did not delve into the mechanisms that underlie the effects of H_2 or explore its potential synergistic effects with CO_2. Generated H_2 can be delivered into the body orally or transcutaneously. Based on the concentration of H_2 in the breath, H_2 can be distributed throughout the human body after only 10 min of soaking in a H_2 bath. A 2016 report by Kawamura et al. showed that weekly H_2 soaking had not significant impact on redox homeostasis (measured by thiobarbituric acid reactive substances [TBARS], reactive oxygen metabolites [d-ROMs], or the biological antioxidant potential [BAP]), inflammatory responses (measured by interleukin [IL]-6, IL-17a, and myeloperoxidase [MPO]), or the levels of muscle damage markers (such as creatine kinase [CK] and myoglobin [Mb]) in the blood [20]. However, in the same study they found that weekly H_2 soaking did alleviate muscle soreness following downhill running. Using luminol-dependent chemiluminescence, Kawamura et al. [38] later demonstrated that H_2 soaking following downhill running did not have an impact on the peripheral neutrophil count or its functions, including migration activity or reactive oxygen species (ROS) production. As the ideal utilization of H_2 in the field of sports science is still not fully understood [39], further research is warranted to delve into the mechanism through which CH-CWI enhances ROS scavenging activity.

Our present experiment focused on cold water immersion's effects after eccentric loading. The results demonstrated that a 20-min CH-CWI soak promoted muscle recovery. This type of soak could therefore be adopted as a new recovery intervention for athletes after resistance training. It has also been pointed out that CWI attenuates long-term adaptations after resistance exercise [12–14,40]. Therefore, the effects of CH-CWI after resistance exercise on long-term adaptations should be investigated.

To ensure a single-blind design, the subjects who underwent the present study's CWI, C-CWI, and CH-CWI interventions were not told which type of CWI they were undergoing. However, after approximately 10 min of soaking in C-CWI or CH-CWI, the skin may become red due to the vasodilatory effect of CO_2 gas. This could have enabled the subjects to correctly guess which type of bath they were in. Dissolved CO_2 and H_2 gases in water are invisible to the human eye, so the subjects would have been unable to discern any differences between the C-CWI and CH-CWI baths, even after immersion. As it was not possible to differentiate between C-CWI and CH-CWI baths, there is a strong conviction that the effect of H_2 gas on these two intervention groups was completely blinded.

The differences in the outcomes between the C-CWI and CH-CWI groups were small, but since the study design only allowed for pre- to post-intervention comparisons, it was difficult to further analyze the results. There is a consideration to expand the CH-CWI study to increase the sample size and evaluate changes over a longer duration.

5. Study limitations

The hypothesis for this study is based on previous research suggesting that 20 °C C-CWI enables both prevention of reduced blood flow and body cooling [16]. However, measurements of muscle blood flow were not conducted in this study, and indicators such as MVC, CMJ, and muscle thickness were used instead.

The participants performed eccentric exercises, and the post easurements were conducted 48 h later, which is a potential peak time for muscle soreness. However, markers of muscle inflammation may reach their peak at different time points. This study could not evaluate the continuous inflammatory and recovery responses following exercise.

The body fat mass in the subject was not measured in this study, and the insulation effect of subcutaneous fat may have also affected the results.

6. Conclusions

The potential recovery effects of three different interventions were investigated in young males after eccentric exercise: cold water immersion (CWI), carbonated cold water immersion (C-CWI), and hydrogen-rich carbonated cold water immersion (CH-CWI). In contrast to the Control, CWI, and C-CWI protocols, CH-CWI promoted recovery from impairments in several key areas: knee-flexion range of motion, tissue hardness, maximum voluntary isometric contraction torque, countermovement jump height, and echo intensity. The results suggest that a CH-CWI bath has the potential to significantly promote recovery after eccentric exercise. In the future, we plan to investigate the mechanisms by which hydrogen gas in CH-CWI promoted ROS scavenging activity and suppressed the inflammation of muscle damage by direct measurement of ROS.

Ethics statement

This study was approved by the Ethics Committee of Niigata University of Health and Welfare, Niigata, Japan (Procedure #18560) and complied with the requirements of the Declaration of Helsinki.

Funding

This study was supported by Iwatani Corporation and by an Osaka City Innovation Creation Support Grant (2019). The funding sources had no role in the study design, data collection, data analysis, or manuscript preparation.

Informed consent statement

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

Author contribution statement

Conceived and designed the experiments; Miho Yoshimura, Tatsuya Hojo, Masatoshi Nakamura and Yoshiyuki Fukuoka. Performed the experiments; Masatoshi Nakamura, Kazuki Kasahara, Riku Yoshida, and Yuta Murakami. Analyzed and interpreted the data; Masatoshi Nakamura and Yoshiyuki Fukuoka. Contributed reagents, materials, analysis tools or data; Masatoshi Nakamura, Goich

Inoue, Naohisa Makihira and Yoshiyuki Fukuoka. Wrote the paper. Miho Yoshimura, Masatoshi Nakamura, Kazuki Kasahara, Riku Yoshida, Yuta Murakami, Tatsuya Hojo, and Yoshiyuki Fukuoka.

Data availability statement

Data will be made available on request.

Additional information

No additional information is available for this paper.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Masatoshi Nakamura reports financial support was provided by Iwatani Corporation. Yoshiyuki Fukuoka reports financial support was provided by Osaka City Innovation Creation Support Grant.

Acknowledgements

The authors gratefully acknowledge the study subjects and the support from Iwatani Corporation.

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