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Effects of carbohydrate-electrolyte dissolved alkaline electrolyzed water on physiological responses during exercise under heat stress in physically active men

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ABSTRACT

Purpose: This study investigated the effects of 1400 mL intake of alkaline electrolyzed water (AEW) or purified water (PW) into which carbohydrate-electrolyte (CE) was dissolved on improving physiological responses during exercise under heat stress.

Methods: This double-blinded, crossover randomized controlled trial included 10 male participants who completed two exercise trials in a hot environment (35 °C, ambient temperature, and 50% relative humidity) after consuming CE-dissolved PW (P-CE) or CE-dissolved AEW (A-CE). The exercise trial consisted of running for 30 min on a treadmill (at an intensity corresponding to 65% of heart rate reserve adjusted for heat stress conditions) and repeated sprint cycling (10 × 7-s maximal sprint cycling), with a 35-min rest interval between the two exercises, followed by a 30-min post-exercise recovery period. Before and after running, and after cycling, the participants drank P-CE (hydrogen concentration of 0 ppm, pH 3.8) or A-CE (0.3 ppm, pH 4.1). Blood samples were obtained before, during (rest interval between running and cycling), and post-exercise.

Results: Repeated sprint performance and oxidative stress response did not differ between the P-CE and A-CE trials. A-CE consumption significantly attenuated the increase in blood lactate concentration during the running exercise but not during repeated sprint cycling under heat stress conditions.

Conclusion: Our findings suggested that A-CE did not significantly affect repeated sprint performance; however, the attenuated elevation in blood lactate by A-CE ingestion implies a partial enhancement of endurance performance during submaximal exercise under heat stress.

1. Introduction

Athletic competitions in hot environments (i.e., the Summer Olympic and Paralympic games held in the Northern Hemisphere) are associated with severe heat stress for athletes and their supporters. Exercise under heat stress induces physiological (e.g., hyperthermia, dehydration, and cardiovascular load) and perceptual (e.g., increased ratings of perceived exertion) strain (Willmott et al., 2019).

In addition, oxidative stress may also affect physiological responses and athletic performance during exercise under heat stress. Exercise in the heat increased systemic oxidative damage compared to that in a thermoneutral environment (McAnulty et al., 2005). Generally, intensive exercise disturbs redox homeostasis, leading to oxidative stress. In turn, excessive oxidative stress can cause muscle fatigue, inflammation, and diseases in various tissues (Powers and Jackson, 2008; Koyama, 2014; Reid, 2016). Moreover, increased oxidative stress due to exercising under heat stress conditions impaired muscular performance compared to thermoneutral conditions (Hillman et al., 2011).

This study focused on alkaline electrolyzed water (AEW). During the electrolysis of tap water, AEW is generated on the cathode side and molecular hydrogen (H_2) is generated and dissolved in the AEW

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Abbreviations

8-OHdG	8-Hydroxy deoxyguanosine
A-CE	Carbohydrate-electrolyte dissolved alkaline electrolyzed water
AEW	Alkaline electrolyzed water
ALD	Aldolase
ANOVA	Analysis of variance
ATP	Adenosine triphosphate
BAP	Biological antioxidant potential
CE	Carbohydrate-electrolyte
CPK	Creatine phosphokinase
d-ROMs	Diacron-reactive oxygen metabolites
ELISA	Enzyme-linked immune sorbent assay
ES	Effect size
GSH	Reduced glutathione
H_2	Molecular hydrogen
HR	Heart rate
HRR	Heart rate reserve
HRrest	Heart rate during resting state
LDH	lactate dehydrogenase
P-CE	Carbohydrate-electrolyte dissolved purified water
PW	Purified water
RPE	Ratings of perceived exertion
SpO_2	Percutaneous arterial oxygen saturation
V_E	Minute ventilation
VO_2	Oxygen uptake

(Tanaka, 2017). Recent years have reported the beneficial antioxidant effects of dissolved H_2 on various diseases (Ohta, 2014). Moreover, a growing body of evidence suggests that H_2 attenuates exercise-induced oxidative stress (Ostojic, 2015; LeBaron et al., 2019; Kawamura et al., 2020). Our previous studies demonstrated that H_2 attenuated the intensive exercise-induced elevation in oxidative damage or the reduction in antioxidant capacity in humans (Koyama et al., 2008; Dobashi et al., 2020; Shibayama et al., 2020) and thoroughbred horses (Yamazaki et al., 2015). Moreover, H_2-rich water improved muscle fatigue (Aoki et al., 2012; Botek et al., 2021, 2022) and attenuated an increase in blood lactate concentrations during exercise (Drid et al., 2016; Botek et al., 2019, 2021; Mikami et al., 2019), as well as inflammatory responses (Ara et al., 2018; Nogueira et al., 2018, 2021). Furthermore, a recent study reported that AEW ingestion improved energy expenditure during submaximal endurance cycling in a heated environment (Ito et al., 2020). However, fluid and sodium loss through sweating during exercise in hot environments can reduce plasma volume, leading to cardiovascular drift, hyperthermia, and decreased exercise performance (Scrivin Rachel, 2018). Hence, athletes often consume carbohydrate-electrolyte (CE) beverages (i.e., sports drinks) before and during exercise in heated environments. Indeed, CE beverage ingestion in a hot environment improved endurance running performance (Millard-Stafford et al., 1997) and maximal cycling power output (Fritzsche et al., 2000) compared to water ingestion. Hence, the ergogenic effect of CE-dissolved AEW on exercise performance under heat stress conditions requires examination from a practical perspective. Especially, since various team sports require high levels of repeated sprint ability (Bishop et al., 2011) and heat stress impairs the repeated sprint performance (Girard et al., 2015), it is more important to investigate the effect of CE-dissolved AEW on repeated sprint performance in a hot environment. From the previous results, we hypothesized that CE-dissolved AEW attenuated the increases in blood lactate concentrations and oxidative stress during submaximal endurance exercise, thereby improved subsequent repeated sprint performance under heat stress condition. To test this, we determined the impact of CE-dissolved AEW in physically active men who performed prolonged running and repeated sprint cycling exercise under heat stress condition.

2. Methods and materials

2.1. Participants

This study recruited 10 healthy male university students (age, 19.9 ± 0.4 years; height, 171.6 ± 1.6 cm; body weight, 67.4 ± 2.8 kg; body mass index, 22.8 ± 0.8 kg/m^2; body fat, 18.9 ± 1.5%; values are means ± standard error of the mean) belonging to sports clubs (soccer, baseball, basketball, volleyball, and dance). None of the participants had any history of cardiovascular or respiratory disease. After receiving a detailed explanation of the experimental procedure, each participant signed an informed consent form. All experimental procedures were approved by the Human Research Ethics Committee of the University of Yamanashi and performed in accordance with the guidelines of the Declaration of Helsinki (approval number: R001-005).

2.2. Overview of the study design

All participants visited our laboratory at the University of Yamanashi, Kofu, Yamanashi, Japan three times throughout the experiment. On the day of the first visit, a preliminary exercise test consisting of prolonged running on the treadmill and repeated cycling sprint was performed in a hot environment (35 °C ambient temperature at approximately 50% relative humidity) to determine the workload and to familiarize the participants with the exercise mode using a cycling ergometer.

More than 72 h after the first visit, the participants visited the laboratory twice (main experiments) on different days with an interval of more than 1 week between the visits. In the main experiment, the participants performed two exercise trials under heat stress conditions after consuming two different types of solvents in which CE was dissolved; namely, purified water (PW, P-CE trial) or AEW (A-CE trial). The participants were instructed not to take antioxidant supplements and alcohol, not to perform strenuous exercise, and not to receive any specific recovery treatments from 48 h before each experimental trial. A previous study reported seasonal variation in sweat responses in Japanese men between summer and winter (Nakamura and Okamura, 1998); thus, all experiments were conducted in a double-blinded and counter-balanced manner in September 2019 (25.2 °C mean atmospheric temperature and 70.6% mean relative humidity).

2.3. Determination of running speed

Preliminary tests were conducted to determine each participant's running speed for the prolonged exercises in the experimental trials; these tests used a modified American College of Sports Medicine protocol for multistage submaximal running testing on a treadmill (T7000; Johnson Co., Ltd., Tokyo, Japan) (Marsh, 2012), under the experimental heat conditions (35 °C, ambient temperature and approximately 50% relative humidity). Briefly, the participants performed 4–5 stages of treadmill running; the running speed was increased between stages while maintaining a constant gradient of zero. The running speeds in the incremental test varied from 3.0 to 10.2 km/h depending on the participants' fitness level. The participants' heart rate (HR) was recorded throughout the incremental test using a wireless monitor (RS800CX, Polar Electro, Tokyo, Japan). Each stage lasted for a minimum of 3 min until a steady-state in HR was achieved, defined as a change in HR of <2 bpm/min between 2 consecutive minutes of each stage (Marsh, 2012).

When the participants' HR increased to >65% of their HR reserve (HRR) (calculated as age-predicted HR max (220-age) - HR during resting state (HRrest)), the incremental running test was terminated.

2.4. Experimental procedures

On the morning of the main experiment, the participants urinated to empty their bladder voluntarily and recorded this time to calculate the urine flow rate 3 h before the start of the trials. They visited the laboratory after an overnight fast, except for the ingestion of 340 mL of commercial mineral water immediately after urination in the morning.

Fig. 1 presents an overview of the experimental procedure. Before exposure to heat stress conditions (defined as "Pre"), venous blood and urine samples were collected and body weight was measured as the baseline values. Thereafter, the participants rested in a seated position for 5 min under thermoneutral conditions (25 °C ambient temperature). Five minutes after consuming the experimental drinks, the participants entered the heated climatic environment. Following a 5-min standing rest on the treadmill, the participants ran at a speed corresponding to 65% of their HRR adjusted for a hot environment for 30 min (defined as "Running"). This exercise protocol is based on our previous study that a 30-min treadmill running at the intensity of 70% HRR under thermoneutral condition significantly increased systemic oxidative damage markers (Koyama et al., 2008). However, in our preliminary experiment, the running speed corresponding to 70% HRR was too fast to continue running for 30 min under heat stress condition. Collectively, we set 65% HRR as the intensity of a 30-min endurance running in this study. Our preliminary experiment also revealed that approximately 2% of body weight was lost after the 30-min running at the intensity of 65% HRR without any rehydration under the same heated condition.

After the completion of the running test, they stayed in a sitting position for the rest period in the hot environment for 35 min (defined as "Rest"). Thereafter, the participants performed a repeated sprint cycling test consisting of 10 × 7-s maximal sprints with 30-s rest periods between sprints on a cycle ergometer (defined as "Cycling"; Wattbike Pro, Nipponsykes Ltd., Tokyo, Japan) (Kasai et al., 2015). The Wattbike is an air-braked ergometer that allows the pedal resistance to be set between levels 1 and 10. Although the absolute pedaling load is constantly altered during cycling because of the air-brake system influenced by the forces applied to the chain over a load cell and angular velocity of the crank, the validity and reliability of the power output from the Wattbike was previously confirmed (Hopker et al., 2010). In this study, we set the pedaling resistance to level 3, based on previous repeated sprint exercise protocols (Hamlin et al., 2017; Dobashi et al., 2021). Since two of the 10 participants were completely exhausted mid-way Cycling and were unable to complete all sprints in the first experimental trial, they performed the same number of sprints in the second trial. Following completion of Cycling, the participants remained under heat stress conditions during the 30-min recovery period (defined as "Recovery"). When they returned to a thermoneutral environment after the recovery phase (defined as "Post"), venous blood and urine samples were collected and their body weights were measured.

2.5. Preparation of experimental drink

Commercially available CE powder was prepared and dissolved in two different solvents (PW and AEW) to serve as the experimental beverages in the present study. PW and AEW were generated from tap water using a home-used alkaline-ionized water apparatus. The structure and principles of the apparatus have been reported previously (Tanaka, 2017). As the molecular hydrogen (H_2) concentration dissolved in liquid decreases over time (Hasegawa et al., 2017; Mikami et al., 2019), we first dissolved the CE powder in PW to make a solution 20 times more concentrated than the target final concentration. Thereafter, the concentrated CE-dissolved solution was diluted 1:20 with PW or AEW in a sealed aluminum pack. Moreover, a previous systematic review demonstrated the beneficial impact of beverages with temperatures <5 °C on exercise performance under heat stress conditions (Burdon et al., 2010). Therefore, the experimental drinks were stored in a refrigerated chamber (4 °C) for approximately 10 h until consumption. Based on the participants' reports of palatability during exercise in the heat (Shirreffs et al., 2004), the final concentration of CE in the experimental drinks was set at half of the concentration recommended by its distributor. The nutritional contents per 100 mL of the experimental beverages were the same between P-CE and A-CE trials (9.3 kcal energy, 25 mg protein, 0 g fat, 2.3 g carbohydrate, 50 mg NaCl, 5 mg potassium, 13.8 mg arginine, 0.5 mg isoleucine, 0.5 mg valine, and 0.25 mg leucine), except for the solvent water compositions. The pH, H_2 concentration, and temperature of the beverages were measured using a portable pH meter (LAQUAtwin pH-33B; Horiba Ltd., Kyoto, Japan), a

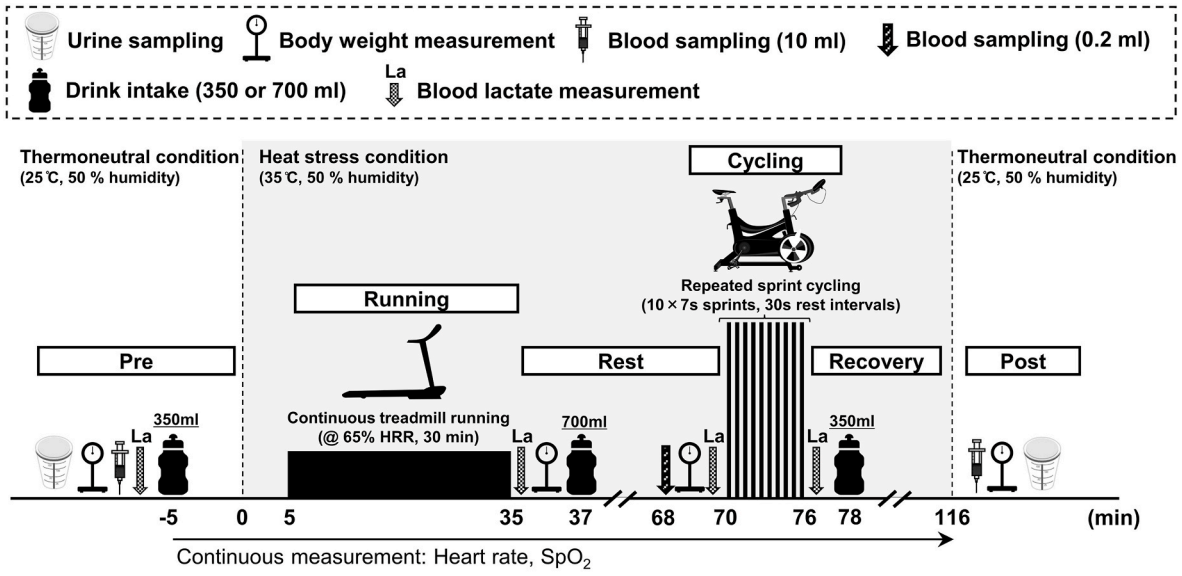

Fig. 1. Overview of the experimental procedure Pre, before exposure to heat stress conditions; Running, during continuous treadmill running; Rest, during 35-min resting interval between Running and Cycling; Cycling, during repeated sprint cycling; Recovery, during 30-min resting recovery after Cycling; Post, after exposure to heat stress conditions (after return to thermoneutral conditions); SpO_2, percutaneous arterial oxygen saturation; HRR, heart rate reserve.

Table 1
Properties of the solvent and the experimental drinks.

Property	P-CE	A-CE
pH		
Solvent (before mixture)	7.6	8.9
Experimental drink (post incubated)	3.8	4.1
H_2 concentration (ppm)		
Solvent (before mixture)	0.0	0.6
Experimental drink (post incubated)	0.0	0.3
Temperature (°C)		
Solvent (before mixture)	22	22
Experimental drink (post incubated)	4	4

CE, carbohydrate-electrolyte; P-CE, CE dissolved purified water; A-CE, CE dissolved alkaline electrolyzed water.

portable dissolved hydrogen meter (DH-35A; Toa DKK Ltd., Tokyo, Japan), and a digital thermo-hygrometer (5682; A&D Ltd., Tokyo, Japan), respectively. The properties of the solvents and the experimental drinks are presented in Table 1.

In this study, a total volume of 1400 mL of the experimental drinks (P-CE and A-CE) was administered in three doses; specifically, 350 mL 5 min before entering the heat stress conditions (within 2.5 min), 700 mL within 5 min after measuring body weight following running, and 350 mL within 2.5 min after cycling (Fig. 1). Generally, the drinking volume should be set based on body weight loss during exercise under heat stress condition (Periard et al., 2021). However, due to the methodological limitation, we could not prepare the experimental beverages corresponding to the participants' individual body weight loss. Instead, the amount of water that would induce dehydration (more than 2% of weight loss) based on the participants' average body weight was calculated to be approximately 1350 mL. Moreover, we considered the capacity of the sealed aluminum pack and referred the single ingestion volume and time, and total volume of rehydration in previous studies (Botek et al., 2019, 2021; Mikami et al., 2019; Dobashi et al., 2020). Collectively, we set the above drinking protocol in this study.

2.6. Analysis of cardiorespiratory and psychological responses

HR was monitored every 1 s using a wireless HR monitor (RS800CX). We averaged the HR values during Pre (5-min rest period before heat exposure), Running, Rest, Cycling, and Recovery, respectively. Every 5 min during Pre, Running, Rest, and Recovery, and immediately after Cycling, percutaneous arterial oxygen saturation (SpO2) was measured using a finger pulse oximeter of a vital sensor (TM-256; A&D Co., Ltd., Tokyo, Japan). We used the average SpO_2 values in each phase (Pre, Running, Rest, and Recovery) in further analysis.

The participants' subjective fatigue was evaluated using Borg's rating of perceived exertion (RPE) scale (Borg, 1982) in Pre, every 5 min during Running and Rest, and immediately after Cycling. For the analysis of RPE during the Running and Rest periods, we used the average values for each.

2.7. Analysis of lactate, oxidative stress, and inflammatory responses

Lactate concentrations in capillary blood samples were measured before and immediately after Running and Cycling. Before collecting the sample, the fingertip was cleaned using an alcohol wipe to remove sweat. The fingertip was then punctured with a lancet and the first blood drop was wiped away. The second drop was analyzed using an automated lactate analyzer (Lactate Pro2; Arkray, Tokyo, Japan). To compare the rate of change in blood lactate concentrations before and after exercise, we analyzed the data separately between Running and Cycling. Additionally, we also calculated the rate of change in blood lactate concentration immediately after the two exercises from the respective pre-exercise values (immediately before each exercise).

Venous blood samples from the forearm vein and urine samples were collected at Pre and Post in thermoneutral conditions to analyze markers of oxidative stress and inflammatory responses. Moreover, 2 min before Cycling (at Rest), blood samples from the fingertip (200 μL) were collected for the analysis of serum oxidative stress markers. After blood collection, serum samples were obtained after 10 min of centrifugation (3000 rpm, 4 °C) and stored at −80 °C until analysis.

We analyzed levels of serum diacron-reactive oxygen metabolites (d-ROMs), biological antioxidant potential (BAP), and urinary 8-hydroxy deoxyguanosine (8-OHdG) excretion rate as oxidative stress markers, which were altered by drinking H_2-rich water according to previous reports in humans (Koyama et al., 2008; Dobashi et al., 2020) and horses (Yamazaki et al., 2015). The principles and methods of assessment of d-ROMs and BAP have been described previously (Carratelli et al., 2001). The relative total antioxidant capacity in serum was expressed as BAP/d-ROMs. We assessed these blood oxidative stress markers in Pre and Post, and at the end of Rest. The 8-OHdG excretion rate was assessed from urine samples obtained in Pre and Post. To calculate the urinary 8-OHdG excretion rate, urinary 8-OHdG concentrations were determined by enzyme-linked immunosorbent assay (ELISA) and normalized to the urine flow rate at a clinical laboratory (Nikken Seil Co., Ltd., Shizuoka, Japan) (Shibayama et al., 2020).

Serum creatine phosphokinase (CPK) activity, serum aldolase (ALD) activity, serum lactate dehydrogenase (LDH) activity, and leukocyte counts were assessed as markers of muscular damage and inflammation in the laboratory (Kofu Medical Association). These markers were assayed by the enzymatic and Japan Society of Clinical Chemistry standardized methods, respectively.

2.8. Analysis of exercise performances

The peak and mean power outputs were recorded every 7 s of maximal sprint during Cycling. The peak and mean power output values were corrected for body weight measured immediately before Cycling (Dobashi et al., 2021). As mentioned above, two participants failed to complete all sprints; therefore, we used the averaged values of the peak and mean power outputs during their completed sprints in the further analysis. In addition, the power output decrement of the mean power output during Cycling was calculated as [(sprint 1- the last sprint of each participant)/sprint 1] × 100, as a fatigue index (Kasai et al., 2015).

2.9. Assessment of hydration status

Body weight was measured using a body composition analyzer four times during the experiment (DC-320; Tanita Co., Ltd., Tokyo, Japan). Additionally, whole blood protein, hemoglobin, erythrocyte, and serum electrolyte (albumin, uric acid, sodium, potassium, chloride, and calcium) levels were analyzed (Kofu Medical Association, Kofu, Japan) to assess the hydration status.

2.10. Data analysis and statistical analysis

Values are presented as means ± standard deviation. To compare the time-course changes in oxidative stress responses, blood lactate

Table 2
Performance variables during repeated sprint cycling under a heat stress conditions.

	P-CE			A-CE			*P*-values [ES]
Mean power output (W/kg)	9.0	±	1.4	8.8	±	1.3	$P = 0.55$ [0.19]
Peak power output (W/kg)	11.5	±	1.8	11.3	±	1.6	$P = 0.67$ [0.14]
Power decrement (%)	21.9	±	20.1	28.0	±	16.1	$P = 0.29$ [0.35]

Values are represented by means ± standard deviation. CE, carbohydrate-electrolyte; P-CE, CE dissolved purified water; A-CE, CE dissolved alkaline electrolyzed water.

responses, cardiorespiratory and psychological responses, inflammatory responses, and hydration status, two-way repeated measures analysis of variance (ANOVA) was applied to detect the main effects (solvent and time) and the interaction (solvent × time). Regarding multiple comparisons, we adjusted the *p*-value based on Bonferroni's procedure and reported the adjusted *p*-values. For comparisons of exercise performance and relative changes in blood lactate responses during each exercise, paired t-tests were performed for each variable. $P < 0.05$ or adjusted $p < 0.05$ were considered statistically significant. Effect sizes (ES) were also calculated based on partial eta squared (η_p^2) for the two-way repeated-measures ANOVA and Cohen's *d* for the paired t-tests. All analyses were performed using Prism v. 9.0 (GraphPad Inc., La Jolla, CA) and R ver. 4.0.3.

3. Results

3.1. Exercise performances during repeated sprint cycling

As shown in Table 2, the mean and peak power outputs during cycling normalized according to body weight did not differ significantly between the P-CE and A-CE trials (both $p > 0.05$). The difference in the power decrement ratio between the trials was also not significant ($p > 0.05$).

3.2. Oxidative stress responses

Fig. 2 shows the changes in oxidative stress markers; that is, serum d-ROMs, BAP, BAP/d-ROMs, and urinary 8-OHdG excretion rate. Serum d-ROMs and urinary 8-OHdG excretion rates were significantly higher in

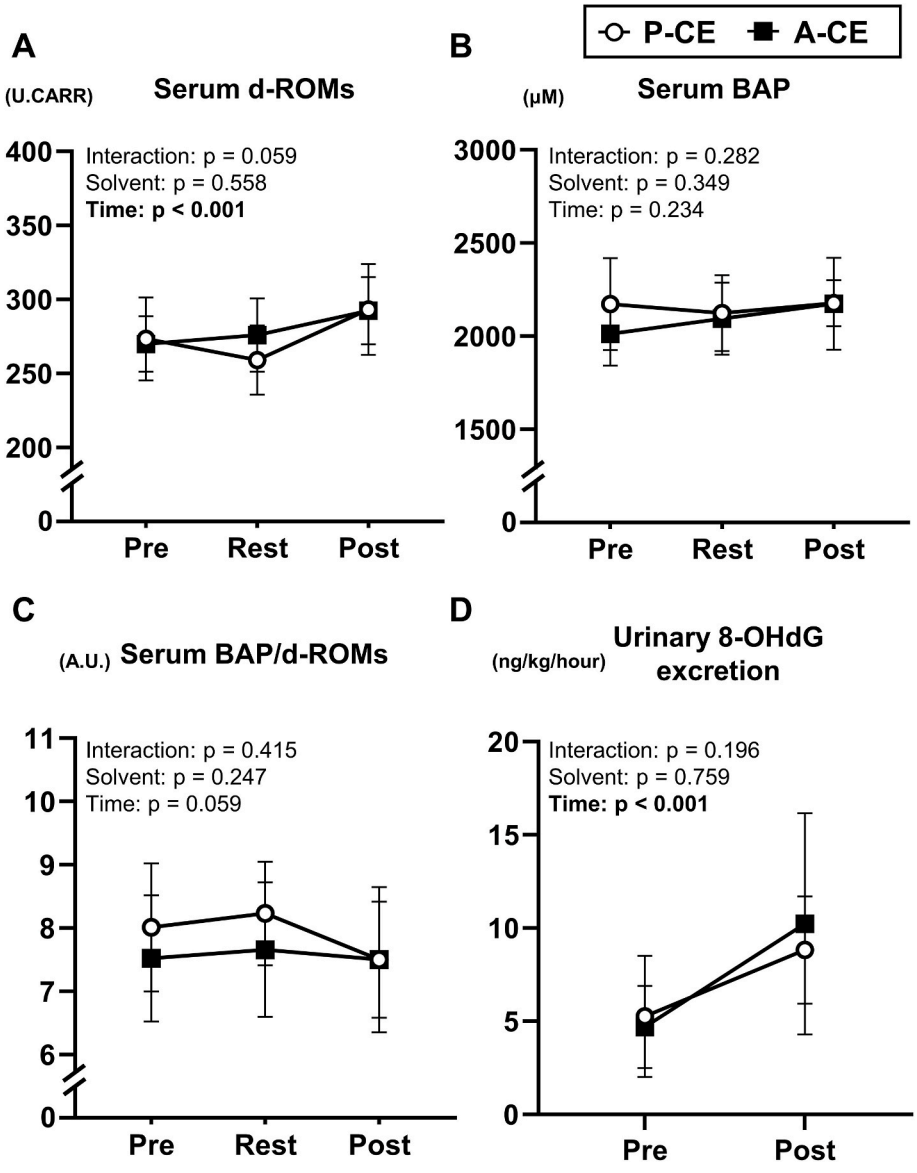

Fig. 2. Oxidative stress responses to exercise under heat stress (A) Serum diacron-reactive oxygen metabolites (d-ROMs), (B) Serum biological antioxidant potential (BAP), (C) Serum relative total antioxidant capacity (BAP/d-ROMs), and (D) Urinary 8-hydroxy-deoxyguanosine (8-OHdG) excretion rate. Values are represented as means ± standard deviation. Two-way repeated measures analysis of variance was performed. P-CE, carbohydrate-electrolyte dissolved in purified water; A-CE, carbohydrate-electrolyte dissolved in alkaline electrolyzed water; *n.s.*, not significant; Pre, before exposure to heat stress conditions; Rest, during 35-min resting interval between running and cycling; Post, after exposure to heat stress conditions (after return to thermoneutral conditions).

Table 3
Skeletal muscle damage and inflammatory responses to exercise under heat stress conditions.

							Two-way ANOVA [ES]		
	Pre			Post			Interaction	Solvent	Time
Serum CPK activity (U/L)									
P-CE	257.3	±	53.4	287.3	±	55.2	$P = 0.35$ [0.10]	$P = 0.21$ [0.17]	$P < 0.01$ [0.84]
A-CE	187.3	±	44.4	221.3	±	50.3			
Serum ALD activity (U/L)									
P-CE	6.2	±	1.0	6.6	±	1.0	$P = 0.17$ [0.20]	$P = 0.14$ [0.23]	$P < 0.01$ [0.63]
A-CE	4.8	±	0.4	5.4	±	0.4			
Serum LDH activity (U/L)									
P-CE	196.3	±	7.1	230.5	±	9.7	$P = 0.47$ [0.06]	$P = 0.08$ [0.30]	$P < 0.01$ [0.91]
A-CE	184.8	±	9.0	225.2	±	7.6			
Leukocytes (μL)									
P–CE	5674.0	±	269.7	7432.0	±	797.0	$P = 0.99$ [0.002]	$P = 0.71$ [0.01]	$P < 0.01$ [0.61]
A-CE	5754.0	±	427.5	7515.0	±	574.1			

Values are presented as means ± standard error of mean. Pre, before exposure to heat stress conditions; Post, after exposure to heat stress conditions (after return to thermoneutral conditions); CPK, creatine phosphokinase; ALD, aldolase; LDH, lactate dehydrogenase; CE, carbohydrate-electrolyte; P-CE, CE dissolved purified water; A-CE, CE dissolved alkaline electrolyzed water; ANOVA, analysis of variance.

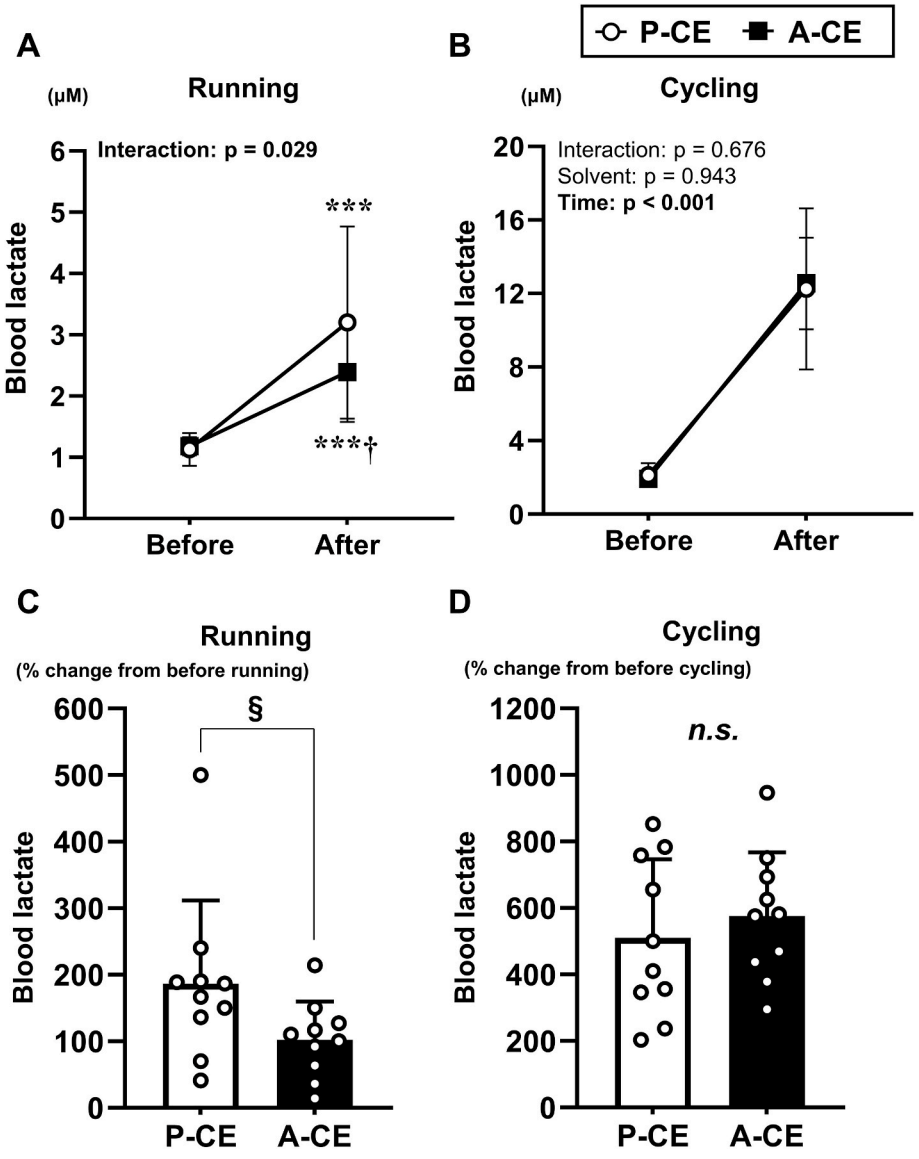

Fig. 3. Blood lactate responses to exercise under heat stress Blood lactate concentrations before and after Running (A) and Cycling (B) during the experiment. Values are represented as means ± standard deviation. Two-way repeated measures analysis of variance was performed. ***adjusted $p < 0.01$ vs. before each exercise, †adjusted $p < 0.05$ vs. after Running in P-CE. The change ratio of blood lactate concentrations during running (C) and cycling (D). The scatter plots indicate individual values. A paired *t*-test was conducted for each variable. P-CE, carbohydrate-electrolyte dissolved in purified water; A-CE, carbohydrate-electrolyte dissolved in alkaline electrolyzed water. §$p < 0.05$.

Table 4
HR, SpO_2, and RPE responses to exercise under heat stress conditions.

	Pre			Running			Rest			Cycling			Recovery			Two-way ANOVA [ES]		
																Interaction	Solvent	Time
HR (beats/min)																		
P-CE	70.2	±	10.3	156.6	±	6.7*	118.7	±	13.3*†	162.0	±	9.8*†§	118.3	±	11.0*†#	$P = 0.91$ [0.02]	$P = 0.35$ [0.10]	$P < 0.01$ [0.97]
A-CE	70.4	±	8.3	160.8	±	11.4*	121.8	±	15.1*†	165.2	±	14.5*†§	119.3	±	19.1*†#			
SpO_2 (%)																		
P-CE	97.5	±	1.6	96.0	±	0.6*	96.8	±	0.7*†	96.6	±	1.1*	96.5	±	0.9*	$P = 0.46$ [0.10]	$P = 0.81$ [0.01]	$P < 0.01$ [0.48]
A-CE	97.9	±	1.7	96.3	±	0.8*	96.8	±	0.7*†	96.3	±	1.1*	96.5	±	0.8*			
RPE																		
P-CE	6.2	±	0.4	16.3	±	2.2*	9.7	±	2.4*†	18.7	±	1.6*†§	–			$P = 0.83$ [0.04]	$P = 0.08$ [0.30]	$P < 0.01$ [0.91]
A-CE	6.0	±	0.0	15.1	±	3.5*	9.2	±	2.4*†	17.7	±	2.5*†§	–					

Values are expressed as means ± standard deviation. Pre, before exposure to heat stress conditions; Running, during or immediately after treadmill running exercise; Rest, during 35-min resting interval between Running and Cycling; Cycling, during or immediately after repeated sprint cycling; Recovery, during 30-min resting recovery after Cycling; SpO_2, percutaneous arterial oxygen saturation; RPE, ratings of perceived exertion; CE, carbohydrate-electrolyte; P-CE, CE dissolved purified water; A-CE, CE dissolved alkaline electrolyzed water; ANOVA, analysis of variance.
*adjusted $P < 0.05$ vs. Pre.
†adjusted $P < 0.05$ vs. Running.
§adjusted $P < 0.05$ vs. Rest.
#adjusted $P < 0.05$ vs. Cycling.

Post than Pre ($p < 0.01$, serum d-ROMs; Fig. 2A; $p < 0.01$ for urinary 8-OHdG excretion rate, Fig. 2D); however, there was no significant difference between the trials (both $p > 0.05$). Serum BAP and BAP/d-ROMs were unchanged by solvent or time (Fig. 2B and C).

3.3. Skeletal muscle damage and inflammatory responses

Table 3 shows the time course changes in muscle damage and inflammatory responses; that is, serum CPK, ALD, and LDH activities, and leukocyte counts. None of these factors revealed a significant difference between the two experimental drinks; however, all of them were significantly increased in Post compared with values in Pre (all $p < 0.01$).

3.4. Blood lactate responses

Fig. 3 illustrates the blood lactate responses in the two exercise trials under heat conditions. Both Running and Cycling showed significantly increased blood lactate concentrations compared to each pre-exercise value, irrespective of the solvent type (adjusted $p < 0.001$). Multiple comparisons revealed that blood lactate concentrations after Running were significantly lower in the A-CE than in the P-CE trials (adjusted $p < 0.05$, Fig. 3A), while those after Cycling were comparable between the two trials (adjusted $p > 0.05$, Fig. 3B).

We also analyzed the magnitude of increase in blood lactate concentrations during Running and Cycling; that is, pre- and post-exercise changes. A significant difference was observed in the changes in blood lactate concentration during Running ($p = 0.02$, ES = 0.94, Fig. 3C), but not during Cycling ($p = 0.50$, ES = 0.22, Fig. 3D) between the two trials.

3.5. HR, SpO_2, and subjective variables

Table 4 shows the HR, SpO_2, and RPE responses for the two exercise trials. Compared to HR during the Pre and Recovery time points, the values were significantly higher during Running, Rest, and Cycling (adjusted $p < 0.01$). Moreover, the HR during Cycling was significantly higher than that during Running (adjusted $p < 0.05$). Nevertheless, the HRs did not differ between the P-CE and A-CE trials throughout the experiment.

SpO_2 levels under heat stress conditions were significantly lower than those in Pre, and this trend was maintained after the termination of heat exposure (adjusted $p < 0.05$). Nevertheless, no significant differences in SpO_2 were detected between the two beverages during the experiment.

Both Running and Cycling trials showed significantly increased RPE compared to Pre (adjusted $p < 0.001$). Additionally, the RPE immediately after Cycling was significantly higher than that immediately after Running (adjusted $p < 0.001$). However, the time course changes in RPE did not differ between the two experimental drinks.

While we also measured HR, SpO_2, and RPE responses every 5 min during the Running trial, none were significantly different between the two trials (data not shown).

3.6. Hydration status

Changes in body weight immediately after the Running, before Cycling, and in Post time points relative to the baseline values (Pre) did not differ between the two trials: (P-CE vs. A-CE; after Running, −0.7 ± 0.1% vs. −0.8 ± 0.1%; before Cycling, −0.3 ± 0.2% vs. −0.3 ± 0.1%; Post, −0.8 ± 0.3% vs. −0.9 ± 0.2%). Table A1 shows the time course changes in blood parameters reflecting hydration status. Blood total protein, hemoglobin, erythrocyte counts and serum albumin, uric acid, chloride, and calcium concentrations in Post were significantly higher than those in Pre (all $p < 0.05$); however, these variables did not differ significantly between the P-CE and A-CE trials. Serum sodium and potassium concentrations were not affected by the solvent or time.

4. Discussion

To our knowledge, this study is the first to investigate whether CE-dissolved AEW (i.e., A-CE) ingestion attenuated the elevation of blood lactate concentrations during submaximal exercise and improved

subsequent maximal exercise performance by reducing muscle fatigue, oxidative damage, and inflammation under heat stress. We found that A-CE attenuated the elevation in blood lactate concentrations during submaximal endurance running but it did not improve repeated sprint cycling performance, oxidative damage, inflammation, nor hydration status.

As expected, the increase in blood lactate concentration during Running was attenuated by drinking the A-CE compared to the P-CE; nevertheless, no significant differences in HR, SpO_2, and RPE were observed between the two trials throughout the experiments. This result is consistent with previous reports that the ingestion of H_2-rich water attenuated the endurance exercise-induced elevation in blood lactate levels in men (Ostojic et al., 2011; Aoki et al., 2012; Botek et al., 2019; Mikami et al., 2019) and women (Drid et al., 2016). A clinical study demonstrated that H_2-rich water decreased the lactate/pyruvate ratio in patients with mitochondrial myopathy, indicating that H_2-rich water may improve mitochondrial function and oxidative metabolism (Ito et al., 2011). Moreover, recent studies demonstrated that the ingestion of H_2-rich water attenuated the increase in ventilatory equivalent for oxygen (V_E/VO_2) during incremental exercise under thermoneutral conditions (Botek et al., 2019) and energy expenditure during endurance exercise under heat stress conditions (Ito et al., 2020). Thus, H_2-rich water may enhance mitochondrial oxidative respiration, thereby improving maximal endurance exercise capacity by delaying the lactate threshold (Ghosh, 2004). However, these explanations are speculative without further investigation, as we did not measure mitochondrial metabolism.

In contrast, repeated sprint cycling performance, oxidative stress, and blood lactate responses during Cycling did not differ between the two solvent trials. Although the precise mechanisms remain unknown, the H_2 concentration (0.3 ppm) in the A-CE may be too small to enhance sprint performance. Our recent study reported that the ingestion of high concentrations of H_2 in saturated water (approximately 5.0 ppm) attenuated the reduction in systemic antioxidant capacity after three consecutive days of sprint cycling exercise (Dobashi et al., 2020). Moreover, H_2 concentrations of approximately 1.0 ppm improved muscular performance in a previous study (Aoki et al., 2012; Botek et al., 2021, 2022). Since there is no clearly defined dose-response curve of H_2 to induce physiological changes (Ostojic, 2015), it is uncertain whether an H_2 concentration of 0.3 ppm is enough to improve various exercise performances and redox responses. In this study, we stored the experimental drinks in a refrigerated chamber (4 °C) for approximately 10 h until the time of consumption, based on previous results demonstrating the beneficial impact of lower beverage temperatures on exercise performance in a hot environment (Burdon et al., 2010). However, H_2 concentration in water decreases over time (Hasegawa et al., 2017; Mikami et al., 2019); thus, the concentration of H_2 in A-CE gradually fell to 0.3 ppm during the 10-h incubation in the refrigerator. Additionally, drinking H_2-rich water at a neutral temperature (22 °C) reportedly improved muscular performance (Botek et al., 2021). Based on these properties of H_2-dissolved water, if the experimental drink was consumed immediately after preparation (i.e., without reducing the H_2 concentration and temperature of the beverage), the physiological responses would have differed compared to the present results. Future studies should examine the ergogenic effects of various temperature and H_2 concentrations in the experimental drink.

Another interpretation of our results is that AEW might enhance only 'aerobic' ATP supply. Generally, since severe exercise rapidly requires large amounts of ATP, the ATP generating system shifts from aerobic (in the mitochondrial electron transport chain) to anaerobic phosphorylation (glycolysis). Hence, in situations in which the ATP demand from anaerobic phosphorylation is elevated during severe exercise, blood lactate concentrations are drastically increased, especially during short-term supramaximal exercise (Morales-Alamo and Calbet, 2014). Indeed, we observed increased concentrations of blood lactate and serum d-ROMs levels as well as urinary 8-OHdG excretion rates, after Cycling irrespective of the type of beverage, indicating that the workload of Cycling was physiologically severe. This might be one explanation for the significant impact of AEW on blood lactate levels after Running, rather than Cycling. A previous study showing that AEW reduced the increase in blood lactate concentration during submaximal endurance exercise, but not immediately after exhaustive exercise (Ostojic et al., 2011) supports this view. Additionally, a recent study reported that AEW attenuated the increase in energy expenditure during submaximal exercise, but did not improve the time to exhaustion during subsequent incremental cycling exercise under heat stress conditions (Ito et al., 2020). Although further investigation is warranted, the AEW-induced ergogenic effects may be relevant for submaximal endurance exercise rather than for extreme exercise performance under heat stress conditions.

This study has several limitations. First, we did not establish a 'no beverage' or 'only solvent water (PW and AEW without CE)' supplementation trial. Thus, it is unclear whether the ergogenic effects of CE and AEW contributed to the physiological responses during exercise under heat stress conditions. Second, the ingestion volume of the experimental drinks was the same among the participants due to the methodological limitations of beverage preparation. Third, we did not assess other physiological indices, such as blood pH, and core and skin temperature, which could affect exercise performance and physiological responses under heat stress conditions. Finally, for practicality reasons, we recruited a relatively small number of young physically active men and not elite athletes. Since all experiments had to been conducted within 4 weeks in September to avoid the seasonal variation in sweat responses (Nakamura and Okamura, 1998), female participants who have the menstrual cycle affecting exercise performance (McNulty et al., 2020) were not recruited in this study. Moreover, we acknowledge the possibility that older participants might show different physiological responses compared with younger during exercise under heat stress condition. Furthermore, a recent study suggested that the magnitude of the H_2-rich water-induced ergogenic effect is dependent on the subject's athletic performance levels (Botek et al., 2020). Future studies should examine larger populations.

5. Conclusions

Drinking A-CE did not affect exercise performance, redox status, or blood lactate accumulation during repeated sprint cycling under heat stress conditions. However, compared to P-CE, A-CE intake significantly attenuated the increase in blood lactate concentration during treadmill running exercise under heat stress conditions. Although we expected the ergogenic effect of A-CE that improves repeated sprint ability, the present results suggest that A-CE may be at least partly useful for athletes who perform submaximal endurance running in hot environments.

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Table A1
Time course changes in blood properties and serum electrolyte concentrations

	Pre			Post			Two-way ANOVA [ES]		
							Interaction	Solvent	Time
Blood total protein (g/dL)									
P-CE	7.4	±	0.3	7.9	±	0.4	$P = 0.22$ [0.16]	$P = 0.08$ [0.30]	$P < 0.01$ [0.91]
A-CE	7.5	±	0.3	8.2	±	0.4			
Blood hemoglobin (g/dL)									
P-CE	15.6	±	0.7	15.9	±	0.9	$P = 0.73$ [0.01]	$P = 0.08$ [0.31]	$P < 0.01$ [0.72]
A-CE	16.0	±	1.0	16.4	±	1.0			
Erythrocytes (10^4 counts/μL)									
P-CE	516.1	±	20.9	525.4	±	26.4	$P = 0.35$ [0.10]	$P = 0.09$ [0.29]	$P < 0.01$ [0.65]
A-CE	527.3	±	28.4	540.8	±	25.2			
Serum albumin (g/dL)									
P-CE	4.8	±	0.2	5.1	±	0.3	$P = 0.35$ [0.06]	$P = 0.10$ [0.28]	$P < 0.01$ [0.90]
A-CE	4.9	±	0.3	5.3	±	0.3			
Serum uric acid (g/dL)									
P-CE	6.2	±	1.7	8.2	±	1.7	$P = 0.34$ [0.10]	$P = 0.85$ [0.004]	$P < 0.01$ [0.92]
A-CE	6.1	±	1.3	8.2	±	1.7			
Serum sodium (mEq/L)									
P-CE	140.3	±	0.6	140.2	±	1.5	$P = 0.31$ [0.11]	$P = 0.78$ [0.01]	$P = 0.39$ [0.08]
A-CE	140.5	±	0.8	139.8	±	1.5			
Serum potassium (mEq/L)									
P-CE	4.3	±	0.3	4.3	±	0.3	$P = 0.26$ [0.14]	$P = 0.25$ [0.14]	$P = 0.32$ [0.11]
A-CE	4.5	±	0.3	4.3	±	0.3			
Serum chloride (mEq/L)									
P-CE	101.5	±	1.7	100.4	±	2.7	$P = 0.59$ [0.03]	$P = 0.85$ [0.004]	$P < 0.05$ [0.38]
A-CE	101.7	±	1.5	100.4	±	2.1			
Serum calcium (mEq/L)									
P-CE	9.5	±	0.2	10.2	±	0.4	$P = 0.37$ [0.09]	$P = 0.08$ [0.30]	$P < 0.01$ [0.87]
A-CE	9.7	±	0.2	10.4	±	0.2			

Values are represented as means ± standard deviation. Pre, before exposure to heat stress conditions; Post, after exposure to heat stress conditions (after return to thermoneutral conditions); CE, carbohydrate-electrolyte; P-CE, CE dissolved purified water; A-CE, CE dissolved alkaline electrolyzed water; ANOVA, analysis of variance.

CRediT authorship contribution statement

Shohei Dobashi: Conceptualization, Investigation, Validation, Data curation, Writing – original draft, contributed to the artwork, drafted the article. **Tomohiro Kobayashi:** Conceptualization, Investigation, Validation. **Yoshinori Tanaka:** Conceptualization, Preparing experimental apparatus, Data curation, Writing – review & editing. **Yudai Shibayama:** Investigation, Writing – review & editing. **Katsuhiro Koyama:** Conceptualization, Investigation, Validation, Data curation, Writing – review & editing, All authors approved the final version of the manuscript.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Katsuhiro Koyama reports financial support was provided by Panasonic Corporation. co-author Yoshinori Tanaka is an employee of the Appliances Company, Panasonic Corporation.

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