Evaluating the Effects of a Proprietary Water Formulation on Hydration and Physiological Responses During Exercise-Heat Stress in Active Adults

Original Research

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Abstract

Introduction: Hypohydration can impair physical and cognitive performance, particularly in hot and humid conditions or after intense activity. Hydration solutions aim to maintain euhydration and minimize electrolyte loss, yet the efficacy of a proprietary water (PW) formulation remains unclear. This study compared its effects on hydration status and performance to a carbohydrate-electrolyte drink (CE) and distilled water (DW), hypothesizing that CE would enhance hydration markers, perceptual responses, and cognitive and physiological outcomes.

Methods: Thirty-seven physically active males and females (25±7 years, 19 females) were randomized into PW (n=12), CE (n=13), or DW (n=12). Participants consumed 3.7 L (males) or 2.7 L (females) of their assigned fluid for five days before completing two 45-minute treadmill protocols simulating soccer play (28-33°C, 40-50% RH). Fluids lost were replenished at halftime. Cognitive assessments and subjective questionnaires were administered. A repeated measures linear mixed effects model analyzed group differences (α=0.05).

Results: Time significantly affected serum osmolality ($p=0.009$), lactate ($p<0.001$), vasopressin ($p=0.003$), and cortisol ($p<0.001$), but no significant group effects were found ($p>0.05$). PW participants reported a higher RPE post-exercise than DW ($p=0.041$), with no other significant cognitive or perceptual differences.

Conclusion: None of the fluids significantly impacted hydration status or performance variables. Future research may examine longer exercise durations or more extreme conditions to reveal potential differences.

Key Words: Thermoregulation, cognition, rehydration

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Introduction

Hydration status in humans may be classified along a spectrum of hypohydration, euhydration, and hyperhydration.[1] If severe enough, both hypohydration and hyperhydration can negatively impact physical and cognitive performance. However, hypohydration in particular is more likely to occur in hot and humid conditions or during strenuous exercise.[2]

Despite debate regarding the precise impact of hypohydration on physical and cognitive performance, even a 1-2% reduction in body mass can lead to observable performance decrements.[3-5] Therefore, although they often fail to do so,[6] active individuals should aim to remain in a euhydrated state to maintain physiological function and optimal performance.

In an effort to mitigate losses in total body water (TBW), perspiration and skin blood flow are reduced, which impairs thermoregulation.[7, 8] Consequently, hypohydration is associated with central fatigue, resulting in decreased neural drive to active musculature.[9] In addition, hypohydration during exercise reduces cerebral blood flow, potentially compromising substrate delivery to brain tissue and accelerating central fatigue.[10] Indeed, even 2% dehydration can impair cognitive performance, particularly in tasks that require attention, psychomotor, and immediate memory skills.[4, 11, 12] Furthermore, core body temperature increases by approximately 0.1 °C for every 1% dehydration and can lead to perceptions of fatigue, thirst, and thermal sensing.[11, 13] If left unaddressed, states of hypohydration can greatly impair physical and cognitive performance, especially during exercise.

To avoid hypohydration, active individuals may wish to focus on fluid delivery methods, timing of fluid intake, and fluid composition.[14] Previous research has been conducted on the topic of fluid composition, comparing the effects of carbohydrate-electrolyte (CE) drinks to water when assessing hydration status, perceptual measures, thermoregulatory variables, cardiovascular efficiency, cognition, and exercise performance.[13, 15] Mixed CE drinks have repeatedly been demonstrated to be superior to water for restoring fluid balance and rehydration[16] by aiding in cellular fluid uptake.[17] These benefits are largely a result of the mitigation of glycogen and electrolyte losses as well as the potential increase in volumes ingested due to flavoring.[18, 19] Not only do CE drinks assist in the preservation of liver glycogen, which plays a critical role in blood glucose homeostasis, they also assist in the replenishment of muscle glycogen, the primary energy substrate used during intense exercise.[16, 20]

As fluids intended to mitigate fluid losses are highly sought after by active individuals, beverage companies have developed hydration products that aim to minimize electrolyte losses and reduce hypohydration-related perceptual, cognitive, and physiological impairments. Importantly, absorption rates of various fluids can be rate-limiting for fluid replacement,[21] resulting in prolonged impairment of cardiovascular function, thermoregulation, and performance.[22] Recently, a proprietary water (PW) formulation was developed to improve fluid absorption rates. Specific energy inputs impact the behavior of the hydrogen bonds between the water molecules, altering their bond angle and length. The structural changes are purported to enhance the water molecule's ability to transfer energy (stored in the electrons of the treated water molecules), promoting cellular hydration. However, it has not yet been determined if PW can influence measures of dehydration and rehydration, and thereby attenuate hypohydration-related perceptual, cognitive, and physiological impairments. Therefore, the purpose of this study was to investigate the effects of PW on hydration status and perceptual, cognitive, and physiological variables in comparison to a common CE drink and distilled water (DW; control), hypothesizing that ingestion of a PW, CE, and DW would differentially influence markers of hydration status, as well as perceptual, cognitive, and physiological variables.

Methods

Participants

Thirty-seven physically active males and females (age 25 ± 7 years; N = 19 females) completed the study. Each individual completed five free-living days and one exercise day, with the exercise completed in an environmental chamber (Figure 1). An a priori power analysis (power = 0.80, $\alpha = 0.05$) revealed a minimum sample size of 36 (12 in each drink group) was necessary to obtain adequate power for plasma volume change comparisons between groups.[23] This research was approved, and written consent was waived, by the University of South Carolina Institutional Review Board for the Protection of Human Subjects (IRB# Pro00112790). All procedures performed were in accordance with the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards.

Inclusion/Exclusion Criteria

Individuals were considered eligible for the study if they regularly performed aerobic exercise at least 3 times per week for at least 150 minutes per week. Exclusion criteria for temperature pill use included a weight of less than 80 pounds, an obstruction condition of the gastrointestinal tract, hypomotility disorder of the gastrointestinal tract, an implanted electromagnetic device such as a pacemaker, a Magnetic Resonance Imaging (MRI) planned during the study procedures, and felinization, or transverse folds of the esophagus. If a potential subject had a contraindication to the temperature pill and still wished to participate, a rectal probe was used instead. Inclusion and exclusion criteria were confirmed by having participants fill out a detailed screening form online prior to their baseline visit.

Groups

Participants were randomized using a random number generator into one of the three groups. Even though actual blinding was not possible due to taste, participants were provided fluids blinded by opaque, unmarked containers and never verbally told which group they were assigned. Fluid groups included a CE drink, DW, and PW. A commercially available CE drink (Gatorade ©, standard formula) was used. DW was purchased from a commercial entity (Great Value, Walmart ©) and served as the control fluid. PW (H2PlusHoldings, LLC) in which the bonds between hydrogen and oxygen are angled and lengthened, by proprietary means, was used for the final group. According to the manufacturer, PW was produced in accordance with current good manufacturing practices pretreated with National Sanitation Foundation approved equipment, and the process stream was disinfected continuously with UV light. Process tanks were disinfected after each production run. The water was analyzed by a certified independent third-party lab (ALS Environmental, Fort Collins, CO, USA).

Protocol

Free-Living Days

An observational cohort research design was employed in the participant's free-living environment for five days. They were given a daily standardized plan integrating their assigned fluid into daily hydration habits, and they were educated at the end of their baseline visit and given take-home instructions to keep their diet, exercise, and other hydration habits consistent. Participants completed a basic food log (MyFitnessPal, Inc) every evening of the free-living days on their mobile device. Additionally, they were asked to collect a urine sample from their first urination of the day for analysis of urine specific gravity (USG) which would be provided for daily USG testing. Following urine collection, perceptual measures were collected via an online survey. A reminder regarding the urine sample, survey, and food log were sent via text message, phone call, or email, as preferred by the participant. They were instructed to consume 3.7L (men) and 2.7L (women) of their study product throughout the day, per recommended daily fluid intakes.[1, 24-26] Specific instructions were provided for their last free-living day, including to refrain from exercise, caffeine, and alcohol the day prior to the heat chamber session and to fast overnight (12 hours). Additionally, participants were provided an additional 6ml/kg·bm of their assigned study product to consume both prior to sleep and upon arrival at the laboratory.[23, 27] The participant swallowed the telemetric capsule used to measure core body temperature between 5 and 12 hours prior to the experimental visit.

Experimental Visit

For baseline testing, participants completed consent procedures and a demographic questionnaire in addition to height, weight, resting heart rate, and perceptual and cognitive measures. Participants arrived at the laboratory for the experimental visit following an overnight fast. Participants completed a current health questionnaire to verify they were able to complete all experimental tasks safely. The participants then completed the experimental protocol (Figure 1). The heat chamber was set at 28-33 °C and 40-50% relative humidity, and a treadmill protocol previously established to mimic the physical demands of a soccer match in a laboratory setting was utilized.[28] The treadmill protocol included two 45-minute halves separated by a 15-minute halftime, or rest, with varying speeds over the two halves to represent in-game sprints and changes in speed at a constant 2% grade.[28] At the 15-minute break and during the recovery period, participants consumed 100% of fluids lost during the treadmill exercise (i.e., if a participant lost 0.5 kg of water during the exercise, they were instructed to consume 0.5 L of their assigned fluid).[29-31]

Objective Measures

Objective measures included participant anthropometrics (height and body mass) core body temperature, and heart rate. Height was obtained with socks and shoes removed using a stadiometer and was measured only at the baseline visit while body mass was measured using a digital scale. Participants wore only shorts and a sports bra (if relevant). Temperature and humidity were obtained every 30 minutes inside the heat chamber to ensure maintenance of the desired temperature. Core body temperature was measured via CorTemp ingestible telemetric capsules paired with a data logger.[32-34] If the capsule was erroneous (values outside normal values), a rectal probe was utilized continuously from the beginning of the first round to the end of the final round of cognitive assessments. Gastrointestinal temperature was measured at select timepoints (TPs) and every 10 minutes during the treadmill protocol. Heart rate was measured continuously throughout the visit via a chest strap (H10, Polar Electro Oy, Helsinki, Finland). Body mass measurements occurred at TPs 1 through 7. For sweat rate, the change in mass between each TP while accounting for fluid intake and urine output (if relevant) was calculated.

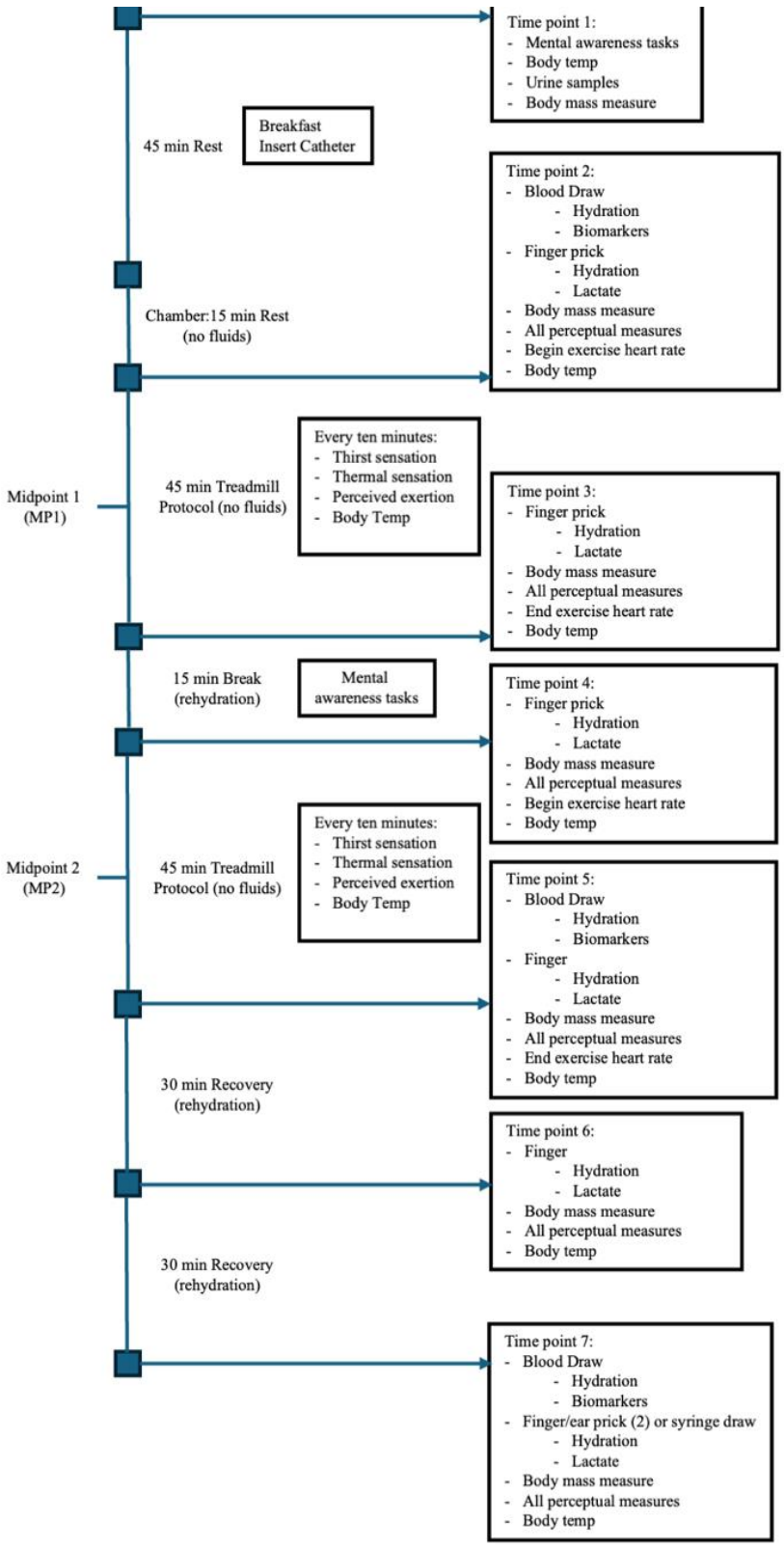

Figure 1. Outline of experimental visit protocol.

Physiological Biomarkers

To assess hydration status, serum osmolality (S_{osm}),[35-39] urine specific gravity (USG),[40] plasma volume changes, and body mass changes were analyzed. S_{osm} was measured from blood samples taken at TPs 2, 5 and 7. Approximately 500 microliters of serum were needed for each TP. Plasma volume and Sosm were selected as primary hydration markers due to their sensitivity to acute fluid shifts and their established use in hydration research.

For USG, a clinical refractometer with a prism was used to compare the urine sample particulates to water (Atago USA, Inc., Bellevue, Wa, USA). Measurements were determined instantaneously. Urine sample testing occurred daily after participants provided their daily urine sample during each of the five days of free-living, and upon arrival to the lab to ensure euhydration (USG <1.024). Changes in percent plasma volume were determined by assessing changes in hematocrit concentrations and hemoglobin.[37, 39, 41] The equation used plasma volume (PV) obtained from hemoglobin before (PVB) and after (PVA) each TP to determine the PV change [(Δ PV, % = 100 (PVA – PVB)/PVB)].[42] Hemoglobin was determined with a HemoPoint H2 Hemoglobin Meter and ~8 microliters of blood.[43] Lactate was used as an indicator of relative exercise intensity. A lancet and lactate analyzer, the Lactate Plus by Nova Biomedical, was used to measure blood lactate from a capillary finger stick at TPs 2, 3, 4, 5, 6, and 7.[44] Other biomarkers included arginine vasopressin (AVP), and cortisol which were sampled at TPs 2, 5, and 7. About 1000 microliters were needed at each TP. Blood samples were obtained from the antecubital vein (antecubital fossa, 21G, BD Vacutainer, Safety-Lok) into tubes consisting of anticoagulant (EDTA) to collect plasma, or serum tubes. Plasma and serum samples were frozen and shipped to a CLIA-certified laboratory (Bio-Reference Laboratories, Inc.; Elmwood Park, NJ, USA) on dry ice for analysis.

Perceptual Measures

Perceptual measures were reported each morning of the free-living days, at TPs 2 through 7 in the heat chamber, and at the halfway point during exercise. Perceived sensations of thirst, thermal stress, and stomach 'slosh' were each rated on 10-point Likert scales. A score of 10 indicated the highest intensity of perceived sensation for each respective domain. The Rating of Perceived Exertion (RPE)[45] is a scale from 6-20 in which participants choose the number that best corresponds to their perceived effort from 'no exertion at all' to 'maximal exertion'.

Profile of Mood State

The Profile of Mood State (POMS) is a measure of mood that assesses a multitude of domains: tension, depression, fatigue, confusion, anger, vigor, and esteem-related affect.[46] The current study used an abbreviated 40-item version of the POMS answered on a 5-point Likert scale (0 = not at all - 4 = extremely).[47] Each mood subscale was calculated by summation of individual scores for questions relative to that specific mood. Total Mood Disturbance (TMD) was calculated by summing the negative subscales (tension, depression, fatigue, confusion, anger) and then subtracting the positive subscales (vigor and esteem-related affect). A higher positive TMD value is indicative of a greater disturbance in mood, with a lower score indicative of a more stable mood profile. The POMS was completed each morning of the free-living days and at TP 2, 5, and 7.

Cognitive Assessments

Cognitive assessments were conducted using the BKIN End-Point Lab (BKIN Technologies, Kingston, ON, Canada). Participants were seated in a custom-built chair set on floor-mounted tracks and a hydraulic lift. They used two independent handles linked to robotic motors and could freely move either handle within a horizontal two-dimensional plane. Handle movements were linked to an augmented reality display that allowed participants to interact with projected objects within the same horizontal plane. This system used a high-resolution camera mounted at the rear of the workspace and was able to compensate for any head movements that occurred, allowing the participants to complete tasks without the use of a head restraint. Three tasks were used to assess cognition: the Object Hit and Avoid, the Trail Making Task, and the 2-Back Task.

Object Hit and Avoid

The Object Hit and Avoid was designed to assess rapid response execution and inhibition during a complex, continuous task. Several objects of different shapes moved from the top of a horizontal workspace toward the individual. The participant used virtual paddles, located over the hand, to hit away salient objects whilst avoiding all others. Task difficulty progressively increased by increasing the number of objects falling and the speed at which the objects fall.[48] Total number of targets hit, distractors avoided (%), and average hand speed (cm/s) were assessed.

Trail Making Task
The Trail Making Task employed was an augmented reality variant of two classic pencil and paper assessments.[49] In the alpha version (TMA), subjects moved their hands in an alphabetical order between lettered targets (A, B, C…). In the alphanumeric version (TMB), subjects moved their hands in an ascending alphabetical-serial order between lettered and numbered targets (A, 1, B, 2, C, 3…). The total number of errors during the TMB (n), and trail making score (TMS) were assessed. Time to completion for each version was calculated in seconds (TMAs and TMBs, respectively). The TMS was calculated with the equation ((TMBs - TMAs)/(TMBs + TMAs)). A greater positive TMS is indicative of a poorer ability to adapt to an increase in cognitive load.

2-Back Task
The 2-Back Task was developed to systematically measure an individual's working memory capacity.[50] A series of stimuli (red shapes) appeared one at a time onscreen and the participant was required to indicate with a yes or no button press whether the current shape was the same as the shape two stimuli before. Overall performance accuracy (%), target reaction time (RT; ms), and non-target RT were reported as metrics of performance.

Statistical Analysis
Descriptive statistics were calculated for all variables. PV and BM are reported as percent change from baseline, while the remaining variables are presented as mean ± standard deviation for the absolute change from baseline. Absolute changes in BM are given in Table 1 for descriptive purposes. A repeated measures linear mixed effects model was used to evaluate the effects of each group over time. Post hoc pairwise comparisons with the Holm method of correction were then conducted to investigate statistically significant differences. The Holm method of correction controls for multiple comparisons while minimizing error rate.[51] Effect sizes were calculated using Cohen's *d* and are presented in tabular form. An alpha level of 0.05 was used to determine statistical significance.

Results
Hydration and Physiological Measures
Descriptive statistics for all outcome measures can be found in Table 1. For estimated PV changes, there was no significant group x time interaction ($p=0.168$) or main effect for group ($p=0.222$). However, there was a main effect of time ($p<0.001$), indicating that compared to baseline value before exercise (TP2), PV was decreased during (TP3=-6.3±10.4%, TP4=-2.1±8.5%, TP5=-7.6±6.7%) and immediately following (TP6=2.8±8.6%, TP7=1.6±9.5%) exercise.

For percent changes in body mass, there was a significant group x time interaction ($p=0.033$), a significant main effect for group ($p=0.012$), and a significant main effect of time ($p<0.001$). In the PW group, body mass, compared to baseline, was decreased following the first bout of exercise ($TP3_{PW}$=-1.0±0.4%) and remained decreased for the remainder of timepoints ($TP4_{PW}$=-0.7±0.6% ; $TP5_{PW}$=-1.6±0.5%; $TP6_{PW}$= -1.6±0.5%; $TP7_{PW}$=-0.9±0.5%). The DW and CE group, however, saw temporary restoration after the first recovery period at the second timepoint following the first bout of exercise ($TP4_{DW}$=-0.1±0.4%; $TP4_{CE}$=-0.3±0.6%), but demonstrated decreases immediately following the first bout of exercise ($TP3_{DW}$=-0.5±0.4%, $TP3_{CE}$=-1.0±0.4%) and at all other timepoints ($TP5_{DW}$=-1.0±0.4%, $TP6_{DW}$=-1.0±0.4%, $TP7_{DW}$=-0.6±0.5% $TP5_{CE}$=-1.1±0.6%, $TP6_{CE}$=-1.1±0.6%, $TP7_{CE}$=-0.6±0.6%)

For blood lactate, there was no significant group x time interaction ($p=0.312$) or main effect for group ($p=0.138$). However, there was a significant main effect of time ($p<0.001$). Compared to baseline measurements (TP2=1.4±0.5mM) lactate concentrations were elevated immediately following exercise (TP3=1.6±1.2mM, TP5=1.7±1.1mM), but there was no longer a statistically significant difference after a period of recovery (TP4=1.2±0.5mM, TP7=1.2±0.7mM).

For S_{osm}, there was no significant group x time interaction ($p=0.146$) or main effect for group ($p=0.765$). However, there was a significant main effect of time ($p=0.009$), indicating that S_{osm} decrease after recovery from the second bout of exercise (TP5=289±5mOsm/kg, TP7=286±5mOsm/kg when compared to baseline (TP2=289±5mOsm/kg).

For AVP, there was no significant group x time interaction ($p=0.101$) or main effect for group ($p=0.833$). However, there was a significant main effect of time ($p=0.003$), indicating that AVP concentrations were elevated following exercise (TP5=2.5±2.5pg/mL; TP7=1.4±2.6pg/mL) as compared to before exercise (TP2=0.8±0.9pg/mL). For total cortisol, there was no significant group x time interaction ($p=0.547$) or main effect for group ($p=0.471$). However, there was a significant main effect of time ($p<0.001$), indicating that cortisol concentrations were decreased following

the second bout of exercise (TP5=10.3±4.1μg/dL) and the final hour of recovery (TP7=10.3±4.4μg/dL) compared to baseline (TP2=14.2±4.3μg/dL).

Table 1. Hydration and physiological measures.

	TP1			**TP2**		
	DW	**CE**	**PW**	**DW**	**CE**	**PW**
USG (g/ml)	1.015±0.007	1.012±0.008	1.010±0.007			
PV (%)						
AVP (pg/mL)				0.8±0.9	1.1±1.0	0.7±1.0
Cortisol (μg/dL)				15.8±5.3	13.9±3.4	12.8±3.6
S_{osm} (mOsm/kg)				291±6	289±4	288±4
Lactate (mM)				1.3±0.5	1.5±0.4	1.4±0.5
BM (Kg)				69.5±13.7	73.7±10.2	73.4±17.6
BM (%)						
CBT(°C)	37.1±0.3	37.4±0.9	37.1±0.5	37.2±0.3(0.37)	37.1±0.3(-0.36)	37.3±0.5(0.32)
	TP3			**TP4**		
	DW	**CE**	**PW**	**DW**	**CE**	**PW**
USG (g/ml)						
PV (%)	-8.8±9.0	-2.5±14.8	-6.8±6.6	-2.2±11.4(0.73)	-4.8±7.2(-0.16)	0.4±4.6(1.09)
AVP (pg/mL)						
Cortisol *(μg/dL)*						
S_{osm} (mOsm/kg)						
Lactate (mM)	1.7±0.9(0.31)	2.0±1.7(0.34)	1.6±0.9(0.33)	1.1±0.3(-0.61)	1.5±0.8(0.04)	1.1±0.4(-0.34)
BM (Kg)	69.1±13.6(-1.35)	73.1±10.1(-2.18)	72.9±17.4(-1.50)	69.4±13.8(-0.28)	73.5±10.1(-0.45)	73.2±17.5(-1.04)
BM (%)	**-0.5±0.4#**	**-1.0±0.4#**	**-1.0±0.4#**	-0.1±0.4(1.00)	-0.3±0.6(1.75)	**-0.7±0.6(0.75)#**
CBT(°C)	38.2±0.3(2.00)	38.2±0.7(0.70)	38.2±0.6(1.44)	37.7±0.2(1.26)	37.7±0.5(0.36)	37.7±0.3(1.20)
	TP5			**TP6**		
	DW	**CE**	**PW**	**DW**	**CE**	**PW**
USG (g/ml)						

PV (%)	-10.2±8.9(-0.16)	-6.0±3.5(-0.24)	-5.7±5.2(0.17)	1.0±12.2(1.09)	3.5±5.1(0.41)	0.3±6.6(1.08)
AVP (pg/mL)	2.4±0.5(2.76)	3.7±2.6(0.92)	1.9±0.1(0.21)			
Cortisol *(μg/dL)*	10.4±4.1(-1.23)	10.5±2.8(-0.83)	10.0±5.3(-0.58)			
S_{osm} (mOsm/kg)	289±6(-0.22)	290±3(0.20)	288±6(0.13)			
Lactate (mM)	1.6±0.8(0.26)	1.9±1.5(0.29)	1.7±1.1(0.51)	0.9±0.3(-1.00)	1.8±0.8(0.46)	1.0±0.3(-0.70)
BM (Kg)	68.8±13.6(-1.95)	72.9±9.9(-1.53)	72.5±17.4(-2.21)	69.1±13.7(-1.05)	73.3±10.0(-0.80)	73.0±17.6(-1.60)
BM (%)	**-1.0±0.4(-1.25)#**	**-1.1±0.6(-0.25)#**	**-1.5±0.5(-1.25)#**	**-0.6±0.5(-0.25)#**	**-0.5±0.6(1.25)#**	**-1.0±0.5(0.00)#**
CBT(°C)	38.2±0.4(1.88)	38.4±0.7(0.93)	38.2±0.5(1.63)	37.5±0.4(0.96)	37.8±0.8(0.32)	37.5±0.3(0.55)
	TP7			**ANOVA (p-value)**		
	DW	**CE**	**PW**	**DW**	**CE**	**PW**
USG (g/ml)						
PV (%)	-4.4±9.3(0.49)	4.7±6.1(0.49)	6.1±9.5(1.95)	0.222	<0.001	0.168
AVP (pg/mL)	7.0±7.7(0.68)	2.3±1.3(0.51)	3.1±0.9(1.59)	0.833	0.003	0.101
Cortisol *(μg/dL)*	10.6±5.1(-1.77)	10.8±3.0(-0.60)	9.2±5.0(-0.80)	0.471	0.001	0.547
S_{osm} (mOsm/kg)	284±4(-1.37)	288±4(-0.14)	286±7(-0.22)	0.765	0.009	0.146
Lactate (mM)	1.1±1.0(-0.34)	1.6±0.6(0.16)	1.0±0.3(-0.57)	0.138	<0.001	0.312
BM (Kg)	69.1±13.7(-1.05)	73.3±10.1(-0.84)	73.5±18.3(-1.30)			
BM (%)	**-0.6±0.5(-0.25)#**	**-0.6±0.6(1.00)#**	**-0.9±0.5(0.25)#**	**0.012**	**<0.001**	**0.033**
CBT(°C)	37.3±0.4(0.46)	37.5±0.8(0.06)	37.2±0.3(0.46)	0.649	<0.001	0.972

Data are reported as mean ± SD (*d*) to the precision of the measurement tool. All effect sizes (*d*) are compared to initial values. * represents statistical significance $p \leq 0.05$ in change from TP2; # represents statistical significance $p \leq 0.001$ in change from TP2. USG, urine specific gravity; PV, plasma volume; AVP, arginine vasopressin; S_{osm}, serum osmolality; BM, body mass; CBT, core body temperature; TP, time point; DW, distilled water; CE, carbohydrate-electrolyte drink; PW, proprietary water.

Group average and maximum heart rate values for each bout of exercise and for the combined time exercising are presented in Table 5. There were no differences found in average heart rates between groups for the first bout of exercise (p=0.797), the second bout of exercise (p=0.678), or for all exercise combined (p=0.729).

Table 2. Heart rate values

HR Average (BPM)	DW	CE	PW	HR Max (BPM)	DW	CE	PW	ANOVA (p-value)
1st Half	137±19	139±21	134±20	**1st Half**	176±15	174±19	171±16	0.797
2nd Half	138±24	137±24	133±22	**2nd Half**	173±21	174±16	170±16	0.678
Total	137±21	138±22	134±21	**Total**	175±20	177±15	172±16	0.729

Data are reported as mean ± SD to the precision of the measurement tool. HR, heart rate; BPM, beats per minute.

Perceptual Measures

Descriptive statistics for perceptual measures can be found in Table 3. Sensation of thirst showed no significant group x time interaction ($p=0.365$) or main effect for group ($p=0.512$). However, there was a significant main effect of time ($p<0.001$) indicating that in comparison to TP2 (3.1 ± 0.3) participants thirst significantly increased following exercise at TP3 (5.0 ± 0.3; $p<0.001$) and TP5 (4.7 ± 0.3; $p<0.001$), and half-way through the first bout of exercise (MP1; 4.2 ± 0.3; $P<0.001$), but significantly decreased following rehydration at TP4 (2.3 ± 0.3; $P=0.009$) and TP7 (2.3 ± 0.3; $P=0.008$). Perceived thermal sensation showed no significant group x time interaction ($p=0.081$) or main effect for group ($p=0.890$). However, there was a significant main effect of time ($p<0.001$) indicating that compared to TP2 (4.5 ± 0.2) participant thermal ratings significantly increased after exercise at TP3 (5.4 ± 0.2; $p<0.001$) and TP5 (5.4 ± 0.2; $p<0.001$), and halfway through each bout of exercise (MP1=5.5 ± 0.2, $p<0.001$; MP2=5.6 ± 0.2, $p<0.001$), but significantly decreased after recovery and rehydration at TP6 (3.6 ± 0.2; $p<0.001$) and TP7 (3.1 ± 0.2; $p<0.001$). Perceived sensation of slosh showed no significant group x time interaction ($p=0.139$) or main effect for group ($p=0.764$). However, there was a significant main effect of time ($p<0.001$) indicating that in comparison to TP2 (2.1 ± 0.2) participants slosh significantly increased following the first instance of rehydration at TP4 (3.0 ± 0.2; $p<0.001$). For RPE there was a significant group x time interaction ($p=0.041$), indicating that participants receiving PW had higher RPE compared to those in the DW group after exercise.

POMS

Descriptive statistics for POMS can be found in Table 4. For the rating of tension there was no significant group x time interaction ($p=0.058$) or main effect for group ($p=0.774$). However, there was a significant main effect of time ($p<0.001$) indicating that in comparison to TP2 (1.7 ± 0.3) participants' tension significantly decreased at TP3 (0.8 ± 0.3; $p=0.007$), TP4 (0.8 ± 0.3; $p=0.007$), TP5 (0.7 ± 0.3; $p=0.002$), and TP7 (0.4 ± 0.3; $p<0.001$). For the rating of anger there was no significant group x time interaction ($p=0.161$) or main effects for group ($p=0.054$) or time ($p=0.206$). For the rating of fatigue there was no significant group x time interaction ($p=0.301$) or main effect for group ($p=0.615$). However, there was a significant main effect of time ($p<0.001$), indicating that in comparison to TP2 (0.7 ± 0.5) participants' fatigue significantly increased at TP3 (2.6 ± 0.5; $p<0.001$), TP4 (2.0 ± 0.5; $p=0.004$), TP5 (4.2 ± 0.5; $p<0.001$), and TP7 (3.1 ± 0.5; $p<0.001$). For the rating of depression there was no significant group x time interaction ($p=0.703$), or main effects for group ($p=0.119$) or time ($p=0.322$). For the rating of esteem-related affect (ERA) there was no significant group x time interaction ($p=0.077$) or main effect for group ($p=0.051$). However, there was a significant main effect of time ($p=0.010$), indicating that in comparison to TP2 (6.21 ± 0.5) participant ERA significantly increased at TP3 (7.2 ± 0.5; $p=0.031$). For the rating of vigor there was no significant group x time interaction ($p=0.201$) or main effect for group ($p=0.315$). However, there was a significant main effect of time ($p<0.001$), indicating that in comparison to TP2 (3.3 ± 0.3) participants' vigor significantly increased at TP3 (5.0 ± 0.3; $p<0.001$), but significantly decreased at TP7 (2.5 ± 0.3; $p=0.032$). For the rating of confusion there was no significant group x time interaction ($p=0.263$), or main effects for group ($p=0.497$) or time ($p=0.315$). For the composite score of total-mood disturbance there was a significant group x time interaction ($p=0.002$). Compared to TP2, TMD decreased in the DW group but increased in groups CE and PW following exercise.

Table 3. Metrics for perceptual measures.

	Evaluation					
Measure	**TP2**			**MP1**		
	DW	**CE**	**PW**	**DW**	**CE**	**PW**
Thirst	3±1	4±1	3±2	4±1(1.20)	4±1(0.79)	4±2(1.17)
Thermal	5±1	4±1	5±0	6±1(1.31)	5±1(1.78)	6±1(1.78)
Slosh	2±1	2±1	2±1	2±1(0.00)	2±1(0.00)	2±1(-0.86)
RPE	6±1	7±1	7±1	12±1(4.17)	12±2(4.92)	12±2(2.59)
Measure	**TP3**			**TP4**		
	DW	**CE**	**PW**	**DW**	**CE**	**PW**
Thirst	5±2(2.25)	5±2(1.04)	5±2(2.42)	2±1(-0.12)	2±1(-0.69)	2±1(-0.85)
Thermal	6±1(1.00)	5±1(1.00)	6±1(0.81)	4±1(-0.23)	4±0(0.00)	4±1(-0.94)
Slosh	2±1(-0.45)	2±1(-0.68)	2±1(-0.62)	3±2(0.83)	3±2(0.92)	3±1(0.07)
RPE	10±2(1.68)	11±3(1.49)	10±4(1.07)	8±2(0.77)	8±2(0.84)	8±2(0.91)
Measure	**MP2**			**TP5**		
	DW	**CE**	**PW**	**DW**	**CE**	**PW**
Thirst	3±2(0.58)	4±2(0.19)	4±2(0.50)	4±2(1.02)	5±2(0.37)	5±2(1.41)
Thermal	6±1(1.96)	5±1(1.66)	6±1(1.24)	5±1(0.33)	5±1(1.30)	6±1(1.63)
Slosh	2±2(0.42)	2±1(-0.09)	2±1(-0.50)	2±1(0.16)	2±1(-0.25)	2±1(-0.85)
RPE	12±1(3.63)	12±2(2.23)	13±3(2.43)	9±2(1.27)	10±2(2.04)	12±4(1.55)#
Measure	**TP6**			**TP7**		
	DW	**CE**	**PW**	**DW**	**CE**	**PW**
Thirst	2±2(-0.06)	3±1(-0.53)	3±1(-0.62)	2±2(-0.11)	3±1(-0.57)	2±1(-0.75)
Thermal	4±1(-0.33)	4±1(-0.86)	3±1(-1.66)	3±1(-1.13)	3±1(-1.02)	3±1(-3.18)
Slosh	2±1(0.32)	2±1(-0.29)	2±1(-0.31)	2±1(-0.30)	2±1(-0.42)	2±1(0.00)
RPE	6±2(0.00)	7±1(0.50)	8±2(0.79)	6±1(-0.30)	7±1(0.00)	6±2(-0.29)
	ANOVA (p-value)					
	Group	**Time**	**Group x Time**			
Thirst	0.512	<0.001	0.365			
Thermal	0.89	<0.001	0.081			
Slosh	0.764	<0.001	0.139			
RPE	0.291	<0.001	0.041			

Data are reported as mean ± SD (*d*) to the precision of the measurement tool. All effect sizes (*d*) are compared to initial values. * represents statistical significance $p \leq 0.05$; # represents statistical significance $p \leq 0.001$. RPE, rating of perceived exertion; TP, time point; MP1, half-way through first bout of exercise; MP2, half-way through second bout of exercise; DW, distilled water; CE, carbohydrate-electrolyte drink; PW, proprietary water.

Table 4. Metrics for profile of mood states

	Evaluation					
Measure	**TP2**			**TP3**		
	DW	**CE**	**PW**	**DW**	**CE**	**PW**
Tension	2±3	1±2	2±2	1±2(-0.97)	1±2(0.13)	1±1(-0.48)
Anger	0±0	0±1	0±0	0±0(0.00)	0±1(0.23)	0±0(0.00)
Fatigue	1±1	1±1	0±1	2±2(0.95)	3±2(1.07)	3±3(0.79)
Depression	0±0	0±1	0±0	0±0(0.30)	1±1(0.38)	0±0(0.30)
ERA	6±4	7±3	6±2	8±2(0.79)	8±3(0.46)	5±2(-0.15)
Vigor	3±2	3±2	3±2	6±2(-1.17)	4±1(-0.67)	5±2(-0.96)
Confusion	1±2	0±1	0±1	0±1(0.45)	0±1(0.00)	0±1(0.00)
TMD	0±5	-2±5	-1±4	1±4(0.28)	**1±5(0.88)***	**2±4(0.59)***
Measure	**TP4**			**TP5**		
	DW	**CE**	**PW**	**DW**	**CE**	**PW**
Tension	1±2(-1.01)	1±2(0.09)	1±1(-0.42)	0±1(-0.79)	1±2(0.13)	1±2(-0.33)
Anger	0±0(0.00)	1±2(0.42)	0±0(0.00)	0±0(0.00)	1±2(0.42)	0±0(0.00)
Fatigue	2±2(0.60)	3±2(0.96)	2±3(0.61)	4±5(0.67)	4±3(1.30)	5±4(1.13)
Depression	0±0(0.00)	1±1(0.40)	0±0(0.00)	0±0(0.00)	1±2(0.30)	0±1(0.30)
ERA	7±3(0.69)	7±3(-0.10)	5±2(-0.62)	8±3(0.71)	7±3(-0.10)	6±3(0.04)
Vigor	4±2(-0.56)	4±2(-0.09)	3±2(0.26)	4±2(-0.51)	3±2(-0.27)	4±2(-0.62)
Confusion	0±1(0.35)	1±1(-0.40)	0±1(0.32)	0±1(0.39)	1±1(0.00)	0±1(0.30)
TMD	0±4(-0.15)	1±6(0.92)*	1±5(0.18)	0±5(-0.33)	3±7(1.43)#	4±7(0.58)#
				ANOVA (p-value)		
Measure	**TP7**			**Group**	**Time**	**Group x Time**
	DW	**CE**	**PW**			
Tension	0±0(-0.80)	1±2(-0.13)	0±0(-0.71)	0.774	<0.001	0.058
Anger	0±0(0.00)	0±2(0.30)	0±0(0.00)	0.054	0.206	0.161
Fatigue	2±3(0.53)	3±2(1.31)	4±4(0.96)	0.615	<0.001	0.301
Depression	0±0(0.00)	1±2(0.30)	0±0(0.00)	0.119	0.322	0.703
ERA	8±3(0.65)	7±4(0.04)	5±2(-0.41)	0.051	0.01	0.077
Vigor	3±3(0.36)	3±2(0.26)	2±2(0.77)	0.315	<0.001	0.201
Confusion	0±1(0.37)	0±1(-0.30)	0±0(0.45)	0.497	0.315	0.263
TMD	-3±3 (-0.80)*	0±7(0.73)*	1±4(0.39)	0.497	<0.001	0.002

Data are reported as mean ± SD (*d*) to the precision of the measurement tool. All effect sizes (*d*) are compared to initial values. * represents statistical significance $p \leq 0.05$ in change from TP2; # represents statistical significance $p \leq 0.001$ in change from TP2. ERA, esteem-related affect; TMD, total-mood disturbance; TP, time point; DW, distilled water; CE, carbohydrate-electrolyte drink; PW, proprietary water.

Cognitive Measures

Object Hit and Avoid

Descriptive statistics for OHA can be found in Table 5. There was no significant group x time interaction ($p=0.617$) or main effect for group ($p=0.966$) for targets hit. However, there was a significant main effect of time ($p<0.001$), indicating in comparison to PRE (86.1 ± 0.98%), the number of targets hit significantly increased at MID (88.7 ± 0.98%; $p<0.001$) and POST (89.9 ± 0.98%; $p<0.001$). There was no significant group x time interaction ($p=0.423$), or main effects for group ($p=0.086$) or time ($p=0.275$) for distractors avoided. There was no significant group x time interaction ($p=0.147$) or main effect for group ($p=0.882$) for average hand speed. However, there was a significant main effect of time ($p=0.011$) indicating in comparison to PRE (16.3 ± 0.8cm/s), average hand speed significantly increased at MID (17.5 ± 0.8cm/s; $p=0.030$) and at POST (17.6 ± 0.8cm/s; $p=0.016$).

Trail Making Task

Descriptive statistics for TMT can be found in Table 4. There was no significant group x time interaction ($p=0.993$), or main effects for group ($p=0.170$) or time ($p=0.798$) for trail making score. There was no significant group x time interaction ($p=0.775$) or main effect of time ($p=0.563$) for TMB errors. However, there was a significant main effect for group ($p=0.002$). DW had significantly fewer errors during TMB compared to CE (0.44 ± 0.18 vs 1.39 ± 0.19; $p=0.001$). There were no significant differences in the number of errors made for PW (0.89 ± 0.19) vs DW ($p=0.122$), or CE ($p=0.122$).

2-Back Task

Descriptive statistics for 2-Back can be found in Table 4. There was no significant group x time interaction ($p=0.746$) or main effect for group ($p=0.707$) for accuracy. However, there was a significant main effect of time ($p<0.001$) indicating that in comparison to pre-exercise (PRE) (75.4 ± 1.99%) participant accuracy significantly improved at half time (MID) (80.2 ± 1.99%; $p<0.001$) and post-exercise (POST) (80.7 ± 1.99%; $p<0.001$). There was a significant group x time interaction for target RT ($p=0.017$), indicating that RT decreased following exercise in participants receiving DW and CW. There was no significant group x time interaction ($p=0.548$) or main effect for group ($p=0.737$) for non-target RT. However, there was a significant main effect of time ($p<0.001$) indicating that in comparison to PRE (667 ± 22.4ms), participant non-target RT significantly decreased at MID (612 ± 22.4ms; $p<0.001$) and at POST (604 ± 22.4ms; $p<0.001$).

Table 5. Metrics for cognitive assessments.

	Evaluation					
Measure	**PRE**			**MID**		
	DW	**CE**	**PW**	**DW**	**CE**	**PW**
OHA						
Targets Hit (%)	86±5	86±5	86±7	89±6(0.60)	88±7(0.40)	89±6(0.43)
Distractors Avoided (%)	93±4	90±5	94±4	93±3(0)	90±7(0)	92±4(-0.50)
Avg.Hand Speed (cm/s)	15.5±4.6	16.9±4.9	16.6±3.8	17±5.5(0.33)	17.2±5.2(0.06)	18.2±4.2(0.42)
TMT						
Trail Making Score	0.16±0.08	0.19±0.10	0.15±0.07	0.15±0.08(-0.13)	0.19±0.12(0)	0.14±0.16(-0.14)

TMB Errors (n)	0±0	1±1	1±1	1±1(0)	2±2(1.00)	1±1(0)
2-Back						
Accuracy (%)	72±9	78±11	76±15	78±12(0.67)	81±10(0.27)	81±14(0.33)
Target RT (ms)	668.0±160.5	707.9±107.0	598.9±112.4	623.3±189.0 (-0.28)	634.8±115.3 (-0.68)*	543.2±90.0(-0.50)
Non-Target RT (ms)	678.4±154.1	675.5±147.7	647.3±109.8	626.1±167.5 (-0.34)	634.9±119.6 (-0.27)	576.1±106.5 (-0.65)
				ANOVA (p-value)		
Measure	**POST**			**Group**	**Time**	**Group x Time**
	DW	**CE**	**PW**			
OHA						
Targets Hit (%)	90±4(0.80)	90±6(0.80)	89±7(0.43)	0.966	<0.001	0.617
Distractors Avoided (%)	94±3(0.25)	88±10(-0.40)	91±6(-0.75)	0.086	0.275	0.423
Avg.Hand Speed (cm/s)	17.5±5.5(0.43)	18.5±6.0(0.33)	16.9±3.8(0.08)	0.882	0.011	0.147
TMT						
Trail Making Score	0.14±0.1 (-0.25)	0.19±0.15(0)	0.12±0.11(-0.43)	0.17	0.798	0.993
TMB Errors (n)	1±1(0)	1±1(0)	1±1(0)	0.002	0.563	0.775
2-Back						
Accuracy (%)	80±11(0.89)	82±11(0.36)	80±15(0.27)	0.707	<0.001	0.746
Target RT (ms)	596.5±161.2 (-0.45)*	580.6±101.8 (-1.19)#	578.6±134.1 (-0.18)	0.388	<0.001	0.017
Non-Target RT (ms)	602.3±158.9 (-0.49)	620.8±120.2(-0.37)	588.9±115.4 (-0.53)	0.737	<0.001	0.548

Data are reported as mean ± SD (*d*) to the precision of the measurement tool. All effect sizes (*d*) are compared to initial values. *represents statistical significance $p \leq 0.05$; # represents statistical significance $p \leq 0.001$. OHA, object hit & avoid; TMT, trail making task; TMB, alphanumeric version of trail making task; RT, reaction time; cm/s, centre

meters per second; ms, milliseconds; PRE, pre-exercise; MID, half-way through total exercise; POST, post-exercise; DW, distilled water; CE, carbohydrate-electrolyte drink; PW, proprietary water.

Discussion

The present study investigated the effects of a CE solution, DW, and a PW on markers of hydration status as well as perceptual, cognitive, and physiological variables. Contrary to the present study's hypothesis, there were few if any differences between the three groups, with remarkably similar effects over time in response to the protocol.

Hydration and Physiological Measures

BM was decreased in all groups following exercise and remained significantly below baseline at all TPs between all groups, with the only exception being in those receiving DW and CE at TP4, following the first bout of exercise and halftime rehydration period. Considering all participants were rehydrated relative to changes in BM, and no significant differences were found between groups for CBT, it is speculated that these discrepancies were the result of increased perspiration and urination during the rehydration periods following each bout of exercise, as replacing only 100% of BM losses does not account for continued fluid losses post-exercise. When fluids are consumed post-exercise, not all ingested water is retained due to increased diuresis, particularly in the absence of sufficient sodium intake. 150% fluid replacement may be favorable for fully restoring fluid balance because it compensates for urine and sweat production. Ingesting only 100% of lost fluids may lead to an initial restoration of BM, but subsequent fluid losses through sweating, urination, and even respiratory water loss can contribute to a continued deficit.

In addition, PV was reduced when assessed after each bout of exercise, but differences were similar across the conditions, indicating that CE did not have a pronounced advantage over PW or DW in mitigating PV losses or altering the rate of PV replacement. This observation stands in contrast with prior research suggesting improved recovery of hydration status with CE drinks.[52, 53] Furthermore, there were similar reductions across groups in S_{osm} during recovery from the second bout of exercise. However, the findings observed here align with studies which propose analogous effects on PV and S_{osm} between plain water and CE solutions during exercise-heat stress.[54] Hypohydration is accompanied by impaired physical and cognitive performance, decreased neural drive, and strain on the cardiovascular and thermoregulatory systems[7] with hypovolemia likely playing a critical role in these effects.[55] Thus, elucidating the mechanisms for maintaining PV are of the utmost importance.

Total cortisol concentrations were reduced from pre- to post-exercise, with an even greater reduction following the 1-hour rehydration period. This finding also stands in contrast to previous work demonstrating that moderate-to-high intensity exercise elicits increases in circulating cortisol.[56] However, it should be noted that the baseline blood draw occurred in the morning when cortisol levels were likely already elevated.[57] Thus, the observed pattern may be a function of normal diurnal changes. While these findings are unexpected, the increased blood lactate and AVP concentrations observed pre- to post-exercise suggest that the exercise intensity employed was sufficient to induce a stress response.

Perceptual and Cognitive Measures

There were no differences between the groups for perceived rating of stomach 'slosh', which supports PW could be consumed as an alternative to common beverages during sporting events that may be susceptible to this type of somatic discomfort.[58] However, participants receiving PW reported significantly higher RPE compared to those in the DW group at TP5, suggesting the PW group exhibited greater perceived effort during exercise, though no differences in objective exercise performance were observed between the groups. Particularly given that the effect was only seen at a singular TP, this observation may have been anomalous. Alternatively, the increase in RPE could be attributed to psychological factors, such as expectancy effects, given the lack of corresponding physiological differences. Despite PW and DW being identical in taste, participants may have perceived PW as distinct in its effects, leading to altered effort perception. In addition, there were no differences between the fluid groups for all mood domains on the POMS. Comparing within group patterns for change indicated that participants who consumed PW or CE reported a greater disturbance in mood compared to respective baseline scores at TP2 versus a decrease in DW. The fact CE had a negative effect on mood contrasts with previous research, which also used the POMS to assess the differential effects of fluid types on mood during exercise.[59, 60] Furthermore, negative subscales along with vigor tended to have very low absolute scores, highlighting that the driving factor in TMD differences was moderate changes in ERA throughout the protocol. One possible explanation for the greater mood disturbance in the CE group is the potential for expectancy effects, as participants consuming CE may have anticipated suboptimal performance or rehydration compared to PW,

leading to varying psychological responses. In light of this, it is likely that the observed results of this study bear little clinical meaningfulness.

Similar to the physical performance metrics, outcomes of cognitive performance did not support the proposed theoretical advantage of consuming PW. Indeed, there were no differences between beverages for the majority of outcome measures in this category. The DW group exhibited significantly fewer errors during TMB compared to CE, which adds to the body of inconclusive evidence for CE drinks to improve cognitive processing during and after exercise.[61, 62] Participants receiving either DW or CE significantly improved target RT as the exercise bout progressed whereas no such effect was seen in those who consumed PW. Taken together, this indicates that PW was unable to improve a participant's working memory capacity to the level demonstrated by the other fluids.

Limitations

Despite the present study implementing a 5-day fluid intake prior to the experimental visit, the manufacturer claims a longer duration of intake may be necessary to observe differences between the fluid groups within the evaluated parameters. However, the lack of differences seen in baseline levels of S_{osm} or USG after 5 days suggests that continued ingestion of PW might not confer any noticeable differences associated with benefits. It is acknowledged that the benefits of the proposed mechanisms (e.g., altered molecular bonds) for improved absorption with PW may be speculative. Additionally, differences in fluid taste likely limited true blinding, introducing potential expectancy bias. However, DW was seemingly indistinguishable from PW. Future studies should consider implementing additional strategies to improve blinding. Other limitations revolve around the workload and variability in the degree of hypohydration among the participants during the protocol. While all subjects completed the same absolute workload during the protocol, differences in fitness and physiology may have significantly impacted the relative workloads of the participants. This seems unlikely, however, as average heart rates throughout were comparable between groups (Table 5). Finally, there may have been discrepancies in the extent to which participants were truly dehydrated.

Conclusions

The importance of hydration status in influencing physical and cognitive performance is well-understood. While the current investigation delved into the comparative impacts of PW, CE, and DW on measures of hydration status and an array of physiological, cognitive, and perceptual outcomes, there were no significant differences between the three fluid groups. Indeed, this investigation revealed a lack of statistical or practical distinctions concerning hydration status, physiological parameters, perceptual assessments, and cognitive metrics between fluid groups. Importantly, these findings suggest that active individuals possess the flexibility to effectively employ any of the three fluids for the purpose of maintaining euhydration before and during exercise. Although this study was conducted in a controlled laboratory setting, the treadmill protocol was based on GPS data from high-level soccer match play. However, differences in environmental conditions, playing surfaces, and movement patterns in competitive, real-world settings may influence hydration dynamics and fluid requirements. Furthermore, while some measures reached statistical significance, the absolute differences between groups were small, limiting their practical impact on performance or hydration status. Given that all groups maintained euhydration, the observed differences may not translate into meaningful advantages in applied settings. Although the primary focus of this study did not center on hydration interventions utilized in the days preceding and during exercise per se, these results underscore the inadvertent effectiveness of these interventions in maintaining euhydration, facilitating rehydration during exercise, and positively influencing perceptual and cognitive measures. Thus, further investigation of the effects of PW, DW, and CE across a broader spectrum of conditions and criteria is warranted.

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