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Creatine and Glycerol Hyperhydration in Trained Subjects Before Exercise in the Heat

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The authors examined the effects of combined creatine (Cr) and glycerol (Gly) supplementation on responses to exercise in the heat. Subjects (N = 24) were matched for body mass and assigned to either a Cr or placebo (Pl) group. Twice daily during two 7-d supplementation regimens, the Cr group received 11.4 g of $Cr{\cdot}H_2O$ and the Pl group received 11.4 g of glucose. Subjects in both groups also ingested 1 g of Gly/kg body mass (twice daily) in either the first or the second supplementation regimen. This design allowed 4 possible combinations of supplements to be examined (Pl/Pl, Pl/Gly, Cr/Pl, and Cr/Gly). Exercise trials were conducted pre- and postsupplementation at 30 °C and 70% relative humidity. In the Pl group, total body water (TBW) increased by 0.50 ± 0.28 L after Gly and in the Cr group by 0.63 ± 0.33 L after Pl and by 0.87 ± 0.21 L after Gly. Both Cr/Pl and Cr/Gly resulted in significantly attenuated heart rate, rectal temperature, and perceived effort during exercise, although no regimen had any effect on performance. The addition of Gly to Cr significantly increased TBW more than Cr alone (P = 0.02) but did not further enhance the attenuation in HR, T_{re}, and RPE during exercise. These data suggest that combined Cr and Gly is an effective method of hyperhydration capable of reducing thermal and cardiovascular responses.

Key Words: thermoregulation, water loading, dehydration, hyperthermia

The possible advantages of hyperhydration over euhydration during exercise in the heat have been extensively debated without clear consensus (e.g., 1, 23, 25, 26, 32, 33). The rationale for hyperhydration stems from the traditionally accepted "cardiovascular model" of dehydration, which contends that fluid loss during exercise reduces plasma volume and consequently stroke volume and increases heart rate ("cardiac drift") in order to maintain cardiac output (9). During strenuous exercise in the heat the increase in heart rate might at times be insufficient to compensate for the decrease in stroke volume, and consequently maximal cardiac output is reduced (14). A significant linear relationship has been reported between reduction in skin blood flow and level of dehydration (31). Therefore, according to the "cardiovascular model" of dehydration, a reduction in cutaneous blood flow

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can impede heat exchange and reduce the temperature-regulating capacity of the body (10). These physiological responses that occur in response to dehydration of over 2% of body mass have also been associated with a reduction in 30-min cycling time-trial performance, even in a temperate climate (5). If the cardiovascular model holds true, maintaining blood volume and/or expanding plasma volume should result in the preservation of cardiovascular and thermoregulatory function and improvement of exercise performance in the heat, a matter of much research interest (e.g., 45). One approach has been to maintain plasma volume during exercise in the heat by infusion of isotonic saline (11). Using this method, Fortney et al. (11) found an attenuated rise in core temperature during exercise in the heat that they attributed to a maintenance of central blood volume resulting in an increase in skin blood flow and associated convective heat loss. Several other studies using acute plasma-volume expansion with either saline or dextran infusions reported an attenuation in the rise in heart rate and core temperature (e.g., 7). However, the finding of similar core temperatures during hypervolemia in the study by Grant et al. (15) led these authors to conclude that acute plasma-volume expansion did not directly enhance thermoregulation. Other studies have also failed to show any effect of plasma-volume expansion on heart rate, core temperature, skin blood flow, or indeed cycling time-trial performance during exercise in the heat (e.g., 45).

Hyperhydration before exercise by means of ingesting water or carbohydrate/electrolyte solutions is less effective than infusion methods at acutely expanding plasma volume because most "excess" fluid ingested is rapidly filtered and excreted by the kidneys (12). On the other hand, hydrating agents such as glycerol (1,2,3-propanetriol; Gly) have been shown to effectively expand plasma volume (33). Seifert et al. (40) reported a 701-mL increase in mean total body water (TBW) after subjects ingested 1.5 g Gly/kg body mass dissolved in 3.3 mL orange juice/kg body mass and 21 mL water/kg body mass, including a 385-mL increase in interstitial fluid and a 225-mL increase in intracellular fluid, with the remainder distributed within the plasma. Several studies have now concluded that ingesting 1.0–1.5 g Gly/kg body mass dissolved in 1.4–2.0 L of fluid and consumed 2.5–4 h before exercise reduces thermal and cardiovascular strain during exercise in the heat (1, 26, 35) and argue that these effects are caused by a preservation of blood volume and cutaneous blood flow (26). Not all studies, however, have shown such effects of Gly on thermoregulation during exercise in the heat (e.g., 25, 33). Methodological differences, including the amount of Gly and timing of ingestion before exercise, the exercise protocol, ambient conditions, and methods used to assess hydration status and core temperature are all likely to have contributed to the conflicting results.

Ingestion of creatine (Cr) has also been shown to have substantial hydrating effects (21, 23), although the exact mechanisms remain uncertain. Unlike the whole-body-hydrating effects of Gly, however, Cr retains fluid predominantly in the intracellular fluid compartments (23). Like Gly, ingestion of 20 g/d of Cr dissolved in 500 mL of water for 7 d has been shown to be effective in attenuating the rise in heart rate and core temperature during exercise in the heat (23). These effects have been attributed to an increase in intracellular water (ICW), resulting in an increased specific heat capacity of the body (21, 23). Supplementation with hydrating agents such as Gly or Cr has consistently produced modest fluid retention of 400–700 mL (27, 38) and 400–800 mL (21, 44), respectively. It seems plausible,

however, that a Gly-induced increase in extracellular water (ECW) coupled with a Cr-mediated increase in ICW could have synergistic effects resulting in a much larger increase in TBW than if either supplement were consumed alone. Therefore, the primary aim of this study was to examine whether combining Cr and Gly can induce a greater hyperhydration than either Cr or Gly alone. If successful, a secondary aim of this study was to assess the effects of this novel water-loading strategy on thermoregulation and performance during exercise in the heat.

Methods

Subjects

Twenty-four endurance-trained men (Table 1) gave their written informed consent to take part in the present study, which was approved by the local ethics committee and was performed according to the code of ethics of the World Medical Association (Declaration of Helsinki). One subject withdrew from the study because of an injury unrelated to this project. Subjects were questioned as to their training practices, and it was determined that no subject was acclimatized to exercise in the heat at the time of study. This interview also confirmed that all subjects had been Cr free for at least 8 wk prior to the study. The investigators did not reveal before the interview that subjects would be excluded if they had supplemented with Cr in the preceding 8 weeks. The subjects were fully informed of any risks and discomforts associated with the experiments before giving their written informed consent to participate.

Experimental Design

All subjects had their maximal oxygen uptake (VO_{2max}) and maximal work rate (WR_{max}) measured during an initial continuous incremental test to volitional exhaustion at standard room temperature (20–21 °C) and relative humidity (30–40%). After a 5-min warm-up at 20 W, the work rate was gradually increased at a rate of 15 W/min using an electrically braked cycle ergometer (Excalibur Sport, Lode, The Netherlands) until cadence could no longer be maintained above 50 revolutions/min. Respired volumes were measured with a bidirectional turbine transducer

Table 1 Physical Characteristics, Maximal Oxygen Uptake, (VO_{2max}) and Maximal Work Rate (WR) of the 2 Groups of Subjects, Mean ± SD

	Placebo group (*n* = 11)	Creatine group (*n* = 12)
Age (y)	31 ± 8	31 ± 6
Height (cm)	177 ± 6	177 ± 5
Weight (kg)	75.2 ± 7	75.0 ± 6
VO_{2max} (L/min)	4.3 ± 0.5	4.2 ± 0.4
WR_{max} (W)	361 ± 28	357 ± 28

(VMM, Alpha Technologies, Laguna Niguel, CA) calibrated with a 3-L syringe using a range of different flow profiles (Hans Rudolph, Kansas City, MO). Respired gas concentrations were measured every 20 ms by a quadruple mass spectrometer (QP9000, Morgan Medical, Gillingham, Kent, UK) that was calibrated against precision-analyzed gas mixtures. Barometric pressure was measured using a standard mercury barometer.

The experimental design by necessity is complicated and best understood by reference to Figure 1. The study consisted of 2 supplementation regimens, each lasting 7 d and encompassing 2 cycle performance trials consisting of 40 min of constant-load exercise at 63% WR_{max} followed by a 16.1-km (10-mile) time trial. Before the first of these experimental trials, familiarization trials were completed until the variability of 2 consecutive trials was within 5%; only 1 subject had to complete a third familiarization trial to achieve less than 5% variability. After this familiarization period, subjects were matched for body mass and were randomized in a double-blind fashion to either a Cr or a placebo (Pl) group. Subjects were separated into 2 groups because of the long washout period associated with oral Cr supplementation (43). Subjects in both groups performed an exercise trial pre- and postsupplementation for both supplementation regimens (i.e., a total of 4 experimental exercise trials; see Figure 1). The first test was conducted at least 48 h after each subject's final familiarization trial. Each supplementation period started on the day after the first test and finished on the day of the second test.

Cr supplementation consisted of 11.4 g of $Cr{\cdot}H_2O$ (Creatine 6000-ES, Iovate Health Sciences Research Inc., Canada; equivalent to 10 g Cr) and 70 g of glucose polymer (Maxim Energy Drink, Maxim Europe, The Netherlands) mixed in 1 L of warm water (approximately 50 °C) and allowed to cool to room temperature before being consumed twice daily (once in the afternoon and once in the evening) for 6 d and once more on the day of the experimental exercise trial. This protocol has been shown to increase resting muscle-phosphocreatine levels within 5 d (17). Each supplement was made fresh before consumption in order to prevent any degradation of Cr to creatinine. The Pl supplement consisted of 85 g of glucose polymer mixed in 1 L of warm water and consumed twice daily for 6 d and once more on the day of the experimental exercise trial. During the 2 supplementation regimens, subjects in both groups also received either 1 g Gly/kg body mass

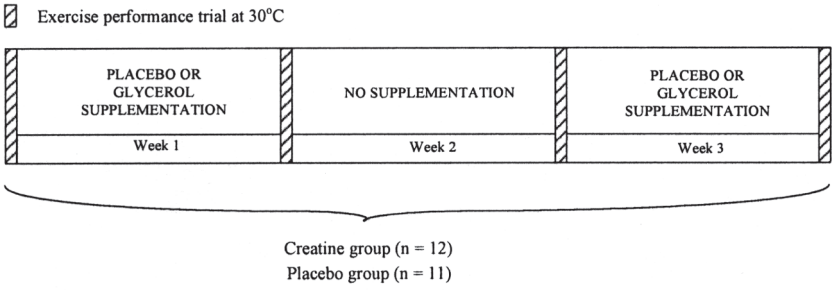

Figure 1 — Schematic representation of the experimental design.

(Aldrich-Chemical, Milwaukee, WI) or an equivalent amount of Pl (i.e., sucrose) diluted in each 1-L supplement. Therefore, 4 possible combinations of supplements were administered: Pl group: Pl/Pl and Pl/Gly; Cr Group: Cr/Pl and Cr/Gly. For the 2 postsupplementation trials, subjects began consuming the final supplement 5 h before the exercise-performance trial (with instructions to complete ingestion within 1 h; Figure 2). Hypertonic solutions such as the Cr/Gly combination (965 ± 61 mosmol/kg) cause an initial net secretion of water into the intestinal lumen (13), resulting in an effective loss of body water, albeit temporary. Unpublished work from our laboratory has indicated that a period of 5 h from commencement of ingestion results in a larger volume of fluid absorbed than during a 3-h period. All supplements had similar taste, texture, and appearance and were placed in generic water bottles to ensure double-blind administration. On each of the experimental test days, subjects ingested 500 mL of water 3 h before exercise and a further 500 mL of water 1 h before exercise in an attempt to ensure that they were euhydrated before all exercise trials (6) (see Figure 2).

Subjects otherwise followed their normal diet and weighed all food and drink consumed during each supplementation period using digital weighing scales readable to 1 g (Denver Instruments XP-300, CO). The diet was analyzed for energy intake and macronutrient content using computerized food-composition tables (19) (Food Meter U.K., Medimatica s.r.l., Benedetto, Italy). Subjects were asked to minimize caffeine intake to 1 cup of tea or coffee per day to lessen any possible confounding effects of caffeine on Cr (43).

Procedures

Subjects reported to the laboratory on each of the 4 d of exercise testing after a 3-h fast and having refrained from alcohol, caffeine, and strenuous exercise the day before. On arrival at the laboratory, height and nude body mass were measured and

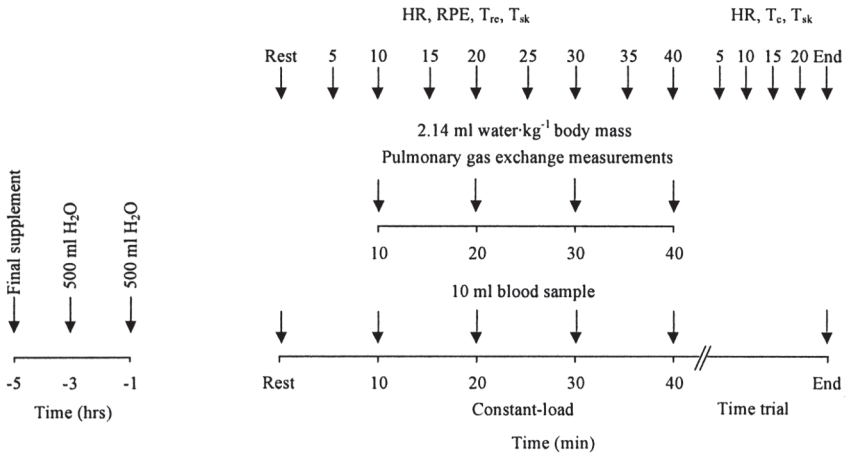

Figure 2 — Schematic representation of the experimental protocol.

body-water compartments estimated using a Bodystat Multiscan 5000 bioimpedance analyzer (Bodystat Ltd., Isle of Man). This method allows TBW and ECW to be estimated; from these measurements ICW can also be deduced. The bioimpedance measurements were taken while the subjects lay comfortably in a supine position for 10 min on a nonconductive surface with their arms and legs slightly abducted. There is good evidence to suggest that the estimation of TBW by bioimpedance is reliable and valid when subjects are euhydrated (36). To date, several studies have successfully used this technique in order to estimate hyperhydration-induced changes in TBW (e.g., 21, 23). Furthermore, the change in body mass from pre- to postsupplementation was determined to provide a further indirect measurement of the volume of fluid retained. After the bioimpedance measurement, a flexible rectal thermistor was inserted 10 cm beyond the anal sphincter to measure rectal temperature (T_{re}), an index of core temperature, and a heart-rate monitor (Polar Sports Tester, Polar Electro Oy, Kempele, Finland) was attached. The subject's right hand and forearm were immersed in water at 42–44 °C for 15 min in order to allow for arterialization of the venous blood. After this, a 21G cannula was introduced into a superficial vein on the dorsal surface of the heated hand. The subject was transferred to the climatic chamber (ambient temperature 30 ± 1 °C with a relative humidity of 70% ± 3% and air velocity of approximately 1.8 m/s) and seated on the cycle ergometer for 5 min. During this period, thermistors (C8600 10-channel microprocessor, Comark, Hertfordshire, UK) were attached to the subject's chest, triceps, thigh, and calf to determine weighted mean skin temperature (T_{skin}) (37).

The subject remained seated on the cycle ergometer for a further 1 min while resting heart rate, T_{re}, and T_{skin} were determined and a blood sample (10 mL) obtained (Figure 2). The venous cannula was kept patent by a 10-mL infusion of isotonic saline between samples. Subjects were then instructed to begin 5 min of unloaded cycling before the work rate was increased in a "single step" to the predetermined 63% WR_{max} (Pl group 227 ± 18 W, Cr group 225 ± 20 W). Subjects were required to maintain a pedal cadence of 70–100 revolutions/min for 40 min. Measurements of heart rate, T_{re}, and T_{skin} were obtained at 5-min intervals throughout the 40 min period and the time trial. Blood samples (10 mL) were obtained every 10 min during the constant-load exercise and on completion of the time trial. One-minute expired-gas collections were taken every 10 min of the constant-load exercise and analyzed within 5 min to determine oxygen uptake (VO_2), carbon-dioxide production (VCO_2), and minute ventilation (V_E).Subjects were required to consume 2.14 mL water/kg body mass (e.g., 150 mL for a 70-kg subject) every 10 min throughout the 40-min constant-load exercise (6). Ratings of perceived leg fatigue and dyspnea (RPE) were recorded every 5 min of the constant-load exercise and at the end of the time trial using the Borg category scale (3). On completion of the 40-min period, work rate was decreased to 20 W and the subject asked to maintain cadence for 1 min. After a further 4-min rest period, the subject was instructed to complete a 16.1-km (10-mile) self-paced time trial on a road-mounted cycle (King Cycle Indoor Trainer, Buckinghamshire, UK). After exercise, nude body mass was measured, and the difference before and after exercise was calculated and subsequently used to estimate sweat rate and sweat loss, after correcting for respiratory water loss and substrate oxidation (30). The time-trial completion time was recorded but withheld from the subject until all exercise tests had been completed.

Blood Treatment and Analysis

Blood was drawn into dry syringes and 6 mL dispensed into a tube containing K_3EDTA and the remaining 4 mL dispensed into plain tubes. Duplicate aliquots (400 μL) of whole blood from the K_3EDTA tube were rapidly deproteinized in 800 μL of ice-cold 0.3-mol/L perchloric acid, centrifuged, and the supernatant used to measure glucose and lactate using standard enzymatic methods with spectrophotometric detection (Mira Plus, ABX Diagnostics, Montpellier, France). Another aliquot of blood was centrifuged, and the plasma obtained was separated and used to measure Gly (2) (Mira Plus, ABX Diagnostics). The blood in tubes without anticoagulant was allowed to coagulate and then centrifuged; the serum collected was used to measure osmolality by freezing-point depression (Micro-osmometer 3300, Vitech Scientific, West Sussex, UK). The blood from the K_3EDTA tubes was also analyzed for hemoglobin (cyanmethemoglobin method, Sigma, Chemical Company Ltd., Dorset, UK) and packed-cell volume (conventional microhematocrit method). All blood analyses were carried out in duplicate, with the exception of packed-cell volume, which was carried out in triplicate. Plasma-volume changes were calculated from changes in hemoglobin and packed-cell volume relative to initial baseline values (8).

Data Analysis

Data were expressed as mean ± SD after a test for the normality of distribution. Statistical analysis was carried out using 3-factor mixed-model ANOVA with repeated measures, followed by a simple main-effects analysis for significant 3-way interactions (i.e., pre- vs. postsupplementation at each combination of time point and treatment) and simple main-effects analysis for 2-way interactions. In addition, the magnitude of change (Δ) between experimental trials (Pl/Pl, Pl/Gly, Cr/Pl, and Cr/Gly) was examined using either a 2-sample or a paired *t*-test when significance was identified using the simple main-effects analysis. All statistical procedures were completed using SPSS for Windows version 8.0. Statistical significance was set at $P \leq 0.05$.

Results

Body Mass and Water Compartments

The physical characteristics of the 2 groups were similar before supplementation (Table 1). In the Pl group, body mass increased significantly after Gly supplementation, with no change during the Pl regimen (Δ body mass was greater after Gly supplementation; Table 2, Figure 3). In the Cr group, body mass increased significantly during both the Pl and Gly regimens (Table 3, Figure 3). Furthermore, the increase in body mass was significantly greater when Gly was consumed in combination with Cr than when Cr was consumed alone ($P = 0.02$; Table 2, Figure 3). There was no difference presupplementation in TBW, ICW, and ECW between groups. In the Pl group, TBW and ECW increased significantly after Gly supplementation, whereas TBW and ECW were unaltered in the Pl regimen (Figure 3). There was a significant increase in ICW in the Pl group after Gly supplementation ($P = 0.01$) but not during the Pl regimen ($P = 0.10$). In the Cr group, TBW, ICW,

and ECW increased significantly during both supplementation regimens (Figure 3). In addition, the increase in TBW and ECW in the Cr group was significantly greater after Gly supplementation than after Pl ($P = 0.02$ and $P < 0.01$, respectively; Figure 3). There were no significant differences in the daily diet between the 2 groups or between Pl and Gly regimens (Table 3).

Cardiopulmonary Variables

There was no significant change in VO_2, VCO_2, or V_E during constant-load exercise, and no differences were found between groups before or as a result of supplementation (data not shown). There was no difference in resting heart rate between the 2 groups or after supplementation (Figure 4). During exercise, heart rate increased during all trials. In the Pl group, heart rate during exercise was significantly lower after Gly supplementation than it had been presupplementation ($P < 0.01$; Figure 4). No such difference was found in the Pl trial (Figure 4). In the Cr group, heart rate was significantly lower after both Cr/Pl and Cr/Gly supplementation regimens than it had been presupplementation (Figure 4). There was no difference in the Δ heart rate pre- and postsupplementation between the placebo and Gly supplementation regimens in the Cr group ($P = 0.65$).

Ratings of Perceived Exertion During Exercise

There was a progressive increase in RPE both for dyspnea and perceived leg fatigue during exercise reaching near-maximum ratings at the end of the time trial (Figure 5). A significant 3-way interaction ($P = 0.04$) was observed in RPE for perceived leg

Table 2 Change in Body Mass From Pre- to Postsupplementation in Each Supplementation Regimen

Subject	Pl/Pl	Pl/Gly	Subject	Cr/Pl	Cr/Gly
1	0.10	0.34	12	0.51	1.10
2	–0.09	0.82	13	0.85	1.03
3	–0.23	1.00	14	1.03	1.37
4	0.11	0.03	15	0.06	0.37
5	–0.16	0.80	16	0.90	1.00
6	0.03	0.45	17	1.37	1.14
7	0.45	0.85	18	0.35	0.84
8	0.45	0.40	19	0.88	0.95
9	0.42	0.63	20	1.35	1.42
10	0.00	0.52	21	0.39	0.87
11	0.00	0.45	22	0.97	0.75
			23	0.13	0.79
Mean ± SD	0.10 ± 0.24	0.57 ± 0.28[a,b]		0.73 ± 0.44[a]	0.97 ± 0.28[a,b]

[a]Significant difference pre- vs postsupplementation.

[b]Significantly greater change (Δ) in the Gly supplementation regimen than in the placebo supplementation regimen.

Pl indicates placebo; Gly, glycerol; and Cr, creatine.

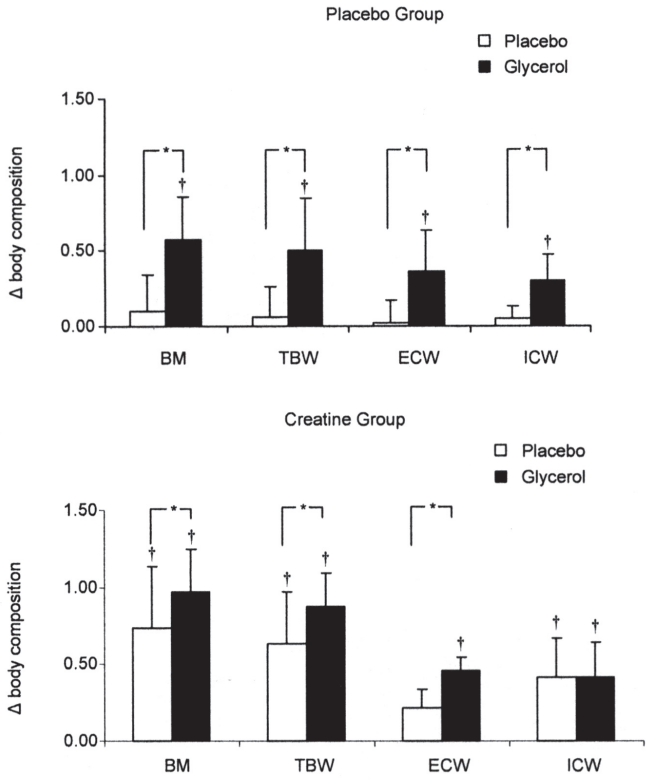

Figure 3 — Changes in body mass (BM), total body water (TBW), extracellular water (ECW), and intracellular water (ICW) in the 2 groups. Data presented as mean ± SD. †Significant difference pre- vs. postsupplementation. *Significantly greater change (Δ) in the Gly supplementation regimen than in the placebo supplementation regimen.

fatigue (Figure 5). Significantly lower ratings of perceived leg fatigue were found in the Cr group after both supplementation regimens; no such effect was found in the Pl group (Figure 5). There was also a significant 3-way interaction in RPE for dyspnea ($P = 0.05$; Figure 5). Significantly lower ratings of dyspnea were found after Cr/Gly supplementation ($P = 0.02$) but not after Cr/Pl ($P = 0.10$); no such effect was found in the Pl group in either supplementation regimen (Figure 5). There was no difference in Δ RPE for perceived leg fatigue or dyspnea pre- and postsupplementation between Pl and Gly supplementation regimens in either group.

Rectal- and Skin-Temperature Responses

Throughout the exercise period, T_{re} increased significantly during all trials (Figure 6). A simple main-effects analysis revealed that T_{re} during exercise was not significantly different in the Pl group during either the Pl ($P = 0.71$) or the Gly

Table 3 Composition of the Average Daily Diet in Each Supplementation Regimen, Mean ± SD

Group	Regimen	Energy (MJ/d)	Carbohydrate (%)	Fat (%)	Protein (%)
Pl	Pl	12.3 ± 2.7	64 ± 8	24 ± 5	12 ± 3
Pl	Gly	12.9 ± 3.2	65 ± 6	22 ± 4	13 ± 3
Cr	Pl	13.5 ± 2.6	67 ± 4	21 ± 5	12 ± 2
Cr	Gly	13.6 ± 2.8	67 ± 5	22 ± 5	11 ± 3

Pl indicates placebo; Gly, glycerol; and Cr, creatine.

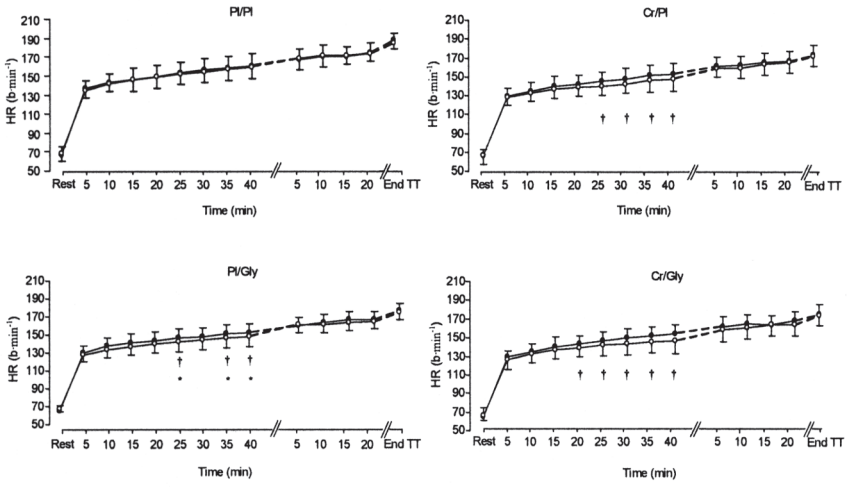

Figure 4 — Heart rate during exercise before (black circles) and after (white circles) supplementation in the 2 groups. Data presented as mean ± SD. †Significant difference pre- vs. postsupplementation. *Significantly greater change (Δ) in the Gly supplementation regimen than in the placebo supplementation regimen.

($P = 0.10$) supplementation regimen (Figure 6). In the Cr group, T_{re} was significantly lower after both Pl ($P < 0.01$) and Gly ($P < 0.01$) supplementation regimens than it had been presupplementation (Figure 6). There was no difference, however, in the ΔT_{re} pre- and postsupplementation between the Pl and Gly supplementation regimens in the Cr group ($P = 0.29$). There was a significant increase in mean T_{skin} from rest, with no significant differences between groups or after supplementation (Figure 6).

Sweat Rates and Total Sweat Loss During Exercise

There was a significant increase in sweat rate after Gly supplementation in both the Pl (1.4 ± 0.3 L/h vs. 1.6 ± 0.4 L/h, $P = 0.02$) and the Cr (1.3 ± 0.4 L/h vs.

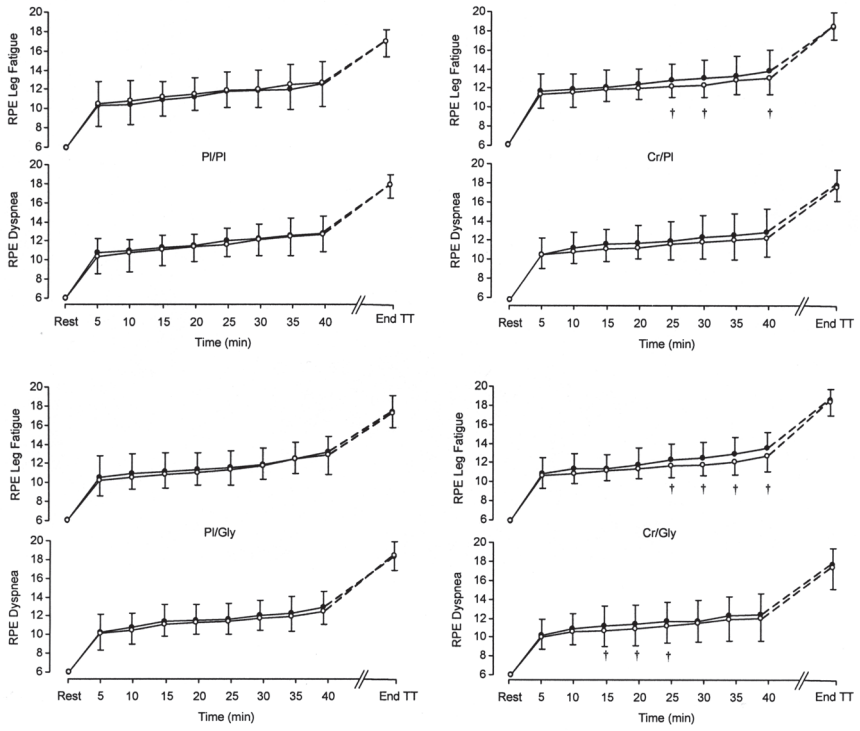

Figure 5 — Rating of perceived exertion (RPE) for leg fatigue and dyspnea during exercise before (black circles) and after (white circles) supplementation in the 2 groups. Data presented as mean ± SD. †Significant difference pre- vs. postsupplementation. *Significantly greater change (Δ) in the Gly supplementation regimen than in the placebo supplementation regimen.

1.5 ± 0.4 L/h, $P < 0.01$) groups. No such increase was observed during the Pl supplementation regimen in either the Pl or the Cr group. Furthermore, total sweat loss increased significantly after Gly supplementation in both the Pl (1.5 ± 0.3 L vs. 1.7 ± 0.4 L, $P = 0.02$) and the Cr (1.4 ± 0.4 L vs. 1.5 ± 0.4 L, $P = 0.02$) groups. No such increase was observed during the Pl supplementation regimen in either the Pl or the Cr group.

Blood Metabolite Concentrations and Plasma-Volume Changes

Resting blood glucose and lactate were not different between groups or after Gly supplementation (data not shown). Briefly, blood glucose decreased significantly from approximately 4.8 mmol/L at rest to 4.4 mmol/L after initiation of exercise before rising gradually throughout the constant-load exercise and peaking at

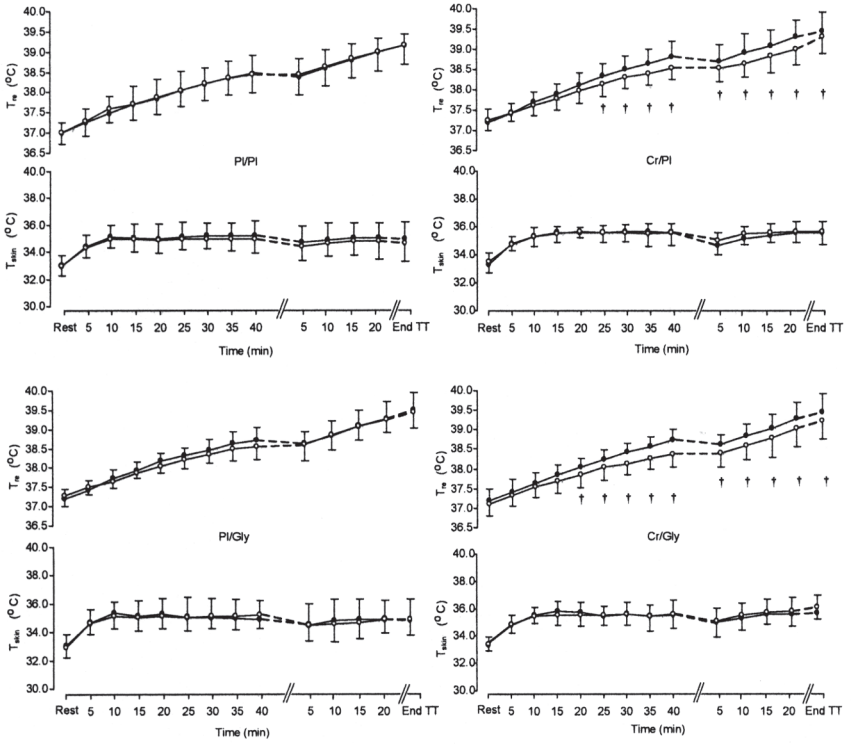

Figure 6 — Rectal (T_{re}) and mean skin (T_{skin}) temperature during exercise before (black circles) and after (white circles) supplementation in the 2 groups. Data presented as mean ± SD. †Significant difference pre- vs. postsupplementation. *Significantly greater change (Δ) in the Gly supplementation regimen than in the placebo supplementation regimen.

approximately 5.5 mmol/L at the end of the time trial. The initial increase in blood lactate from approximately 0.7 to 2.3 mmol/L during exercise was maintained until the end of the constant-load exercise. There was a further significant increase in blood lactate to approximately 7.4 mmol/L between the constant-load exercise and the end of the time trial. Resting plasma Gly was significantly higher after Gly supplementation than presupplementation in both the Pl ($P < 0.01$) and Cr ($P < 0.01$) groups (Table 4). Plasma Gly remained significantly higher throughout exercise after Gly supplementation than in the presupplementation trial in both the Cr and the Pl group. There was no difference in resting plasma Gly or during exercise between pre- and postsupplementation during the Pl supplementation regimen in either the Pl or the Cr group (Table 4). Plasma Gly was not correlated to the increase in TBW after either Pl/Gly ($r = 0.37$, $P = 0.48$) or Cr/Gly ($r = 0.51$, $P = 0.23$) supplementation. Plasma volume was reduced by approximately 8% after 40 min of constant-load exercise and 12% after the 16.1-km time trial, with no significant differences between groups or after supplementation (Figure 7).

Osmolality

Resting serum osmolality was significantly higher after Gly supplementation than presupplementation in both the Pl ($P = 0.02$) and the Cr ($P < 0.01$) groups (Table 5). Serum osmolality remained significantly higher throughout exercise after Gly supplementation than in the presupplementation trial in both the Cr and the Pl groups. There were no other differences in serum osmolality (Table 5).

Time-Trial Performance

Time-trial performance was not significantly different between the groups presupplementation ($P = 0.62$). Time-trial performance did not differ significantly pre- to postsupplementation in either the Pl group (Pl/Pl, pre vs. post, 23.1 ±1.0 min vs. 22.9 ± 1.1 min; Pl/Gly, pre vs. post, 23.1 ± 1.3 min vs. 22.9 ± 1.0 min) or Cr group (Cr/Pl, pre vs. post, 23.4 ± 1.5 min vs. 23.2 ± 1.2 min; Cr/Gly, pre vs. post, 23.4 ± 1.3 min vs. 23.0 ± 1.2 min) in either supplementation regimen.

Side Effects

In general, subjects tolerated the supplementation protocol well, with only 1 report of gastrointestinal distress after Gly supplementation. Three subjects from each group correctly identified the subject group, and 7 subjects correctly identified the Gly supplementation regimen, while all other subjects were unsure of the treatment they received.

Discussion

This study has demonstrated that supplementation with either Cr or a combination of Cr and Gly significantly increased TBW by up to 1.4 L before exercise (Figure 3) and reduced perception of effort (Figure 5) and cardiovascular (Figure 4) and thermoregulatory (Figure 6) responses during exercise in the heat. Furthermore, combining Gly with a standard Cr supplementation regimen (17) resulted in a significantly greater increase in TBW (0.87 ± 0.21 L) than either supplement alone (Figure 3). Gly supplementation alone resulted in a significant increase in TBW of 0.57 ± 0.28 L and attenuated heart rate during exercise without significantly influencing core temperature or RPE. Despite the significant increase in TBW and consequently improved thermoregulatory responses during exercise, no hydration intervention had any effect on exercise performance.

In the present study, subjects experienced, on average, a TBW increase of 500 ± 240 mL (range 200–1000 mL) over the 7-d supplementation period when Gly alone was ingested (Pl/Gly), an average increase that falls within the range (400–800 mL) previously reported using a similar Gly dose (e.g., 32). Previous studies, however, used a single Gly dose combined with a bolus of water consumed between 2 and 3 h before measurement, whereas in the present study Gly was administered for a period of 7 d with the final Gly dose administered 4–5 h before exercise. The average peak Gly in the present study after Gly supplementation was 7.6 ± 1.36 mmol/L (range 5.6–9.9) L and 7.1 ± 1.41 mmol/L (range 5.7–9.8) in the Pl and Cr groups, respectively (Table 3), which is lower than the peak concentration reported

Table 4 Plasma Gly (mmol/L) During Exercise Before and After Supplementation in the 2 Groups, Mean ± SD

				Exercise Time (min)				
Group	Regimen	Trial	Rest	10	20	30	40	End TT
Pl	Pl	Pre	0.05 ± 0.02	0.14 ± 0.04	0.19 ± 0.05	0.23 ± 0.04	0.26 ± 0.06	0.38 ± 0.09
Pl	Pl	Post	0.06 ± 0.03	0.16 ± 0.02	0.21 ± 0.05	0.25 ± 0.06	0.28 ± 0.06	0.40 ± 0.10
Pl	Gly	Pre	0.04 ± 0.02	0.16 ± 0.04	0.19 ± 0.05	0.25 ± 0.06	0.28 ± 0.08	0.37 ± 0.11
Pl	Gly	Post	7.60 ± 1.36[a]	6.92 ± 1.38[a]	6.62 ± 1.35[a]	6.28 ± 1.39[a]	6.06 ± 1.33[a]	5.26 ± 1.22[a]
Cr	Pl	Pre	0.06 ± 0.02	0.15 ± 0.04	0.20 ± 0.05	0.27 ± 0.06	0.29 ± 0.09	0.40 ± 0.10
Cr	Pl	Post	0.03 ± 0.02	0.13 ± 0.04	0.18 ± 0.05	0.22 ± 0.07	0.25 ± 0.06	0.36 ± 0.04
Cr	Gly	Pre	0.04 ± 0.02	0.16 ± 0.04	0.21 ± 0.05	0.25 ± 0.06	0.28 ± 0.07	0.42 ± 0.12
Cr	Gly	Post	7.12 ± 1.41[a]	6.55 ± 1.43[a]	6.32 ± 1.39[a]	5.98 ± 1.38[a]	5.76 ± 1.37[a]	4.89 ± 1.40[a]

[a]Significant difference pre- vs. postsupplementation and significantly greater change (Δ) in the Gly supplementation regimen than in the placebo supplementation regimen.

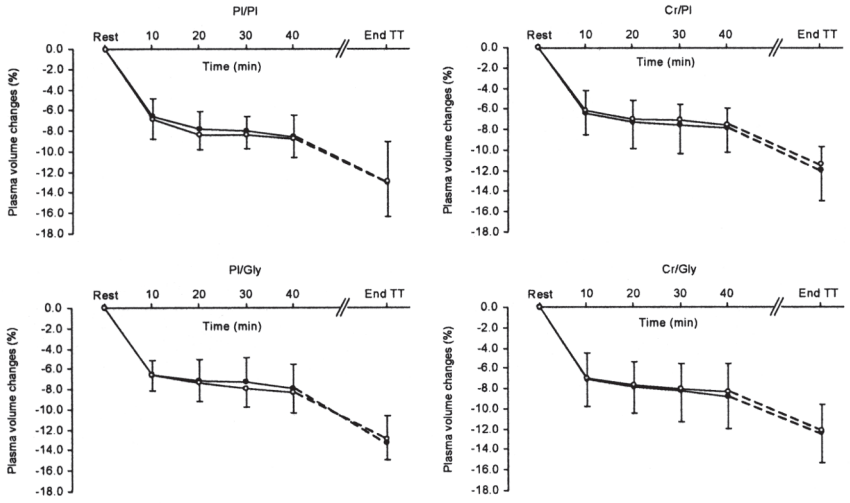

Figure 7 — Changes in plasma volume during exercise before (black circles) and after (white circles) supplementation in the 2 groups. Data presented as mean ± SD. †Significant difference pre- vs. postsupplementation. *Significantly greater change (Δ) in the Gly supplementation regimen than in the placebo supplementation regimen.

Table 5 Serum Osmolality (mosmol/kg) During Exercise Before and After Supplementation in the 2 Groups, Mean ± SD

				Exercise Time (min)				
Group	Regimen	Trial	Rest	10	20	30	40	End TT
Pl	Pl	Pre	283 ± 2	286 ± 3	290 ± 2	290 ± 3	290 ± 3	294 ± 3
Pl	Pl	Post	283 ± 2	287 ± 2	290 ± 2	290 ± 3	290 ± 3	295 ± 3
Pl	Gly	Pre	284 ± 3	287 ± 3	289 ± 2	289 ± 2	290 ± 2	294 ± 3
Pl	Gly	Post	291 ± 3[a]	294 ± 2[a]	295 ± 3[a]	296 ± 2[a]	295 ± 2[a]	299 ± 4[a]
Cr	Pl	Pre	285 ± 2	289 ± 3	290 ± 3	289 ± 2	290 ± 2	294 ± 2
Cr	Pl	Post	281 ± 2	286 ± 3	288 ± 2	288 ± 2	289 ± 3	292 ± 2
Cr	Gly	Pre	284 ± 3	288 ± 4	289 ± 3	288 ± 3	289 ± 3	293 ± 2
Cr	Gly	Post	292 ± 2[a]	294 ± 2[a]	294 ± 3[a]	293 ± 2[a]	293 ± 2	298 ± 2[a]

[a]Significant difference pre- vs. postsupplementation and significantly greater change (Δ) in the Gly supplementation regimen than in the placebo supplementation regimen.

by Montner et al. (32) (11.4 mmol/L) and Freund et al. (12) (13.0 mmol/L) but higher than the concentration reported by Murray et al. (33) (2.8 mmol/L). Differences in the size of the Gly dose and time between ingestion and measurement are likely to account for the noted differences in Gly. Gly supplementation resulted in a similar distribution of the retained fluid between intra- and extracellular water compartments (Figure 3) because of the free distribution of Gly in all body-water compartments with the exception of cerebral spinal fluid and aqueous humor (12, 40, 42).

In previous Gly hyperhydration studies, water retention was quantified by measuring the volume of urine produced (e.g., 1, 12), which gives no indication as to where the retained water was distributed. This Gly-induced water retention has been attributed to an increased concentration of antidiuretic hormone (ADH) (12). Although ADH was not measured in the present study, previously reported differences in ADH between Gly and water interventions were small and only approached statistical significance (12). Although an ADH mechanism cannot be ruled out, it is more likely that this Gly-induced water retention is mediated by the action of Gly on the kidneys. When blood Gly is at normal physiological levels, almost all filtered Gly is passively reabsorbed by the proximal and distal renal tubules of the kidneys (41). When blood Gly is increased with exogenous Gly ingestion, there is an increase in Gly and associated water reabsorption (24). In the present study, Gly supplementation also induced a significant elevation in serum osmolality (Table 4), which is directly attributable to the increased plasma Gly as previously described (12, 33). Cr supplementation alone, on the other hand, significantly increased ICW, with only a minor, nonsignificant increase in ECW, resulting in a TBW increase of approximately 630 ± 330 mL on average (range 100–1200 mL; Figure 3). The increases in body mass and TBW after Cr supplementation in the present study were of magnitude similar to those of a previous study from this laboratory (23) and the study by Kern et al. (21). Several studies have now confirmed that the increase in TBW associated with Cr supplementation is confined predominantly to the intracellular compartments of skeletal muscle (e.g., 23). It has been suggested that an increase in body mass of greater than 0.2 kg identifies a "responder" to Cr supplementation (22). The individual increases in body mass after Cr supplementation in the present study (Table 2) would suggest that only 2 subjects were nonresponders to Cr.

The present study is the first to show that the volume of water retained by ingesting either Cr or Gly can be significantly enhanced by combining these 2 hyperhydrating agents. This novel water-loading strategy that combines Cr and Gly resulted in a mean TBW increase of approximately 870 ± 210 mL (range 600–1400 mL), a significantly larger volume than either Cr or Gly alone (Figure 3). In addition, the retained water was dispersed equally between the intra- and extracellular water compartments. It seems plausible that the water retained by combining the ingestion of Cr and Gly was mediated via a Cr-induced increase in ICW and an increase in ECW as a consequence of the added Gly. This hyperhydration induced by combined Cr and Gly supplementation is the highest directly measured increase in hydration reported in the literature to date (e.g., 23, 32). Therefore, these data would suggest that combined Cr and Gly supplementation is potentially the most effective method of hyperhydrating before exercise.

Furthermore, this innovative water-loading strategy includes 2 agents that specifically target both intra- and extracellular body-water compartments and in doing so overcomes the limitations of previous hyperhydration strategies. For example, Gly has been investigated as a potential hyperhydrating agent for a number of decades and continues to be of great interest as evidenced by a number of recently published studies (e.g., 1, 28). The benefits of Gly hyperhydration are equivocal, however, with at least 23 original articles published on Gly hyperhydration to date providing conflicting results. In instances when hyperhydration was induced, the hydrating effects of Gly were transient because of the metabolism of Gly by the liver and kidneys. Of greater significance however, a major problem associated with Gly ingestion is the fact that it permeates the blood–brain barrier extremely slowly and thus causes cerebral dehydration and associated headaches (e.g., 42). Combining Gly with Cr overcomes this major problem because in contrast to Gly, Cr is taken up by the brain (29) and in the process counteracts the negative effects associated with Gly ingestion, increasing the level of initial hydration, but will also potentially prolong the period for which hyperhydration will last. It is currently unknown, however, whether Gly ingestion or infusion for prolonged periods of time might cause cerebral edema. A single bolus of oral Gly supplementation is unlikely to be harmful because of delayed absorption by the brain and rapid metabolism by the liver and urinary excretion before Gly reaches cerebral circulation (41, 42). Although Gly was ingested for 7 d in the present study without incident, further research is required to examine the effects of Gly ingestion for prolonged periods of time on intracranial pressure.

The mean TBW increase of 870 ± 210 mL produced by the combined ingestion is approximately 20% lower than the sum of the mean increase in TBW produced by Cr and Gly (i.e., 1130 mL), suggesting that the level achieved with the combined ingestion might represent the upper limit of hyperhydration. Under normal physiological conditions, water balance is controlled by sensitive osmoreceptors located in the hypothalamus, possibly via ADH-mediated changes in water excretion in the urine and thirst-mediated changes in water ingestion (4). For example, Freund et al. (12) reported that ingestion of a bolus of water resulted in a significant reduction in ADH and a subsequent increase in free-water clearance. Ingestion of a bolus of water combined with Gly also resulted in a significant reduction in plasma ADH, although the decrease tended to be attenuated. The decrease in ADH occurred despite a Gly-induced increase in plasma osmolality, which the authors attributed to the diluting effect of hyperhydration on plasma Na^+. Therefore, attempting to increase water retention further by hyperhydrating with combined Cr and Gly is likely to have a similar diluting effect on plasma Na^+, resulting in a further reduction of ADH and increased urine production, thus limiting the volume of water that can be retained. Furthermore, 5-d supplementation with 20 g of Cr has been reported to elevate skeletal muscle Cr stores by a margin dependent on the initial muscle Cr (16). Continued supplementation with Cr after this period will not result in any appreciable further increases in the skeletal-muscle Cr pool (20), and subsequently there will be no further increase in ICW retention.

Hyperhydration achieved through Gly and Cr supplementation in the present study was successful in attenuating the increase in heart rate by up to 5 and 7 beats/min, respectively, during constant-load exercise in the heat (Figure 4).

Despite a further increase in TBW when Cr and Gly were combined (compared with Cr alone), however, there was no further significant attenuation in heart rate (heart rate was reduced by up to 9 beats/min; Figure 4). Cr/Gly supplementation increased TBW by an average of 240 mL more than Cr/Pl, which might not be large enough to significantly alter the physiological responses to exercise in the conditions of the present study. Previous studies examining the effects of either Gly or Cr supplementation on cardiovascular responses during exercise in the heat have been equivocal, with some showing a reduction in heart rate (1, 23, 32) and others finding no such effect (21, 25, 33). Nonetheless, it is well established that dehydration results in an increased heart rate and reduced stroke volume and cardiac output during exercise (14). Therefore, it would be expected that as the magnitude of body-water loss increases through sweating, there would be an increase in core temperature during exercise in the heat (39). The fluid loss from sweat is obtained in varying proportions from both the intra- and extracellular water compartments of the body in order to maintain blood volume (39). Nose et al. (35) reported a strong association between the loss of water in sweat and urine and the decrease in intracellular fluid after prolonged exercise in the heat. In the present study, when Cr induced an increase in ICW, there was a significant attenuation in the rise in T_{re} by up to 0.35 °C during exercise in the heat (Figure 6). It is possible that this Cr-induced increase in ICW resulted in an increase in the specific heat capacity of the body, resulting in a greater capacity to store heat (23).

The potential physiological advantage from the hyperhydration-induced reductions in heart rate and core temperature are unclear from the results of the present study. Given the association between attainment of a critical core temperature and the development of fatigue (34), however, it is tempting to assume that hyperhydration would have resulted in an increased time to exhaustion had this experimental protocol been used. Furthermore, subjects who had supplemented with Cr had significantly lower ratings of perceived leg fatigue during the constant-load exercise (Figure 5), suggesting that subjects were able to discern the benefits of the reduction in thermal responses mediated by Cr supplementation.

There are 2 published reports to date that appear to confirm the reduction in core temperature during exercise in the heat after Cr supplementation (21, 23). Conversely, Gly supplementation, which increased ICW to a lesser extent, did not significantly reduce the rise in T_{re} during the exercise period (Figure 6). It is therefore unsurprising that there is considerable debate in the literature about whether Gly ingestion can reduce thermal responses during exercise in the heat, with several studies reporting a reduced core temperature during exercise (1, 26, 40) and numerous other studies finding no such effect (18, 25, 28, 33). This might also explain why several studies reported that plasma-volume expansion via saline or dextran infusion has no effect on heart rate, core temperature, skin blood flow, or performance during exercise in the heat (e.g., 15, 45). In the current study, Gly supplementation resulted in a significant increase in both sweat rate and total sweat loss in both the Pl and Cr groups. It might therefore be somewhat surprising that there was no reduction in T_{re} because of the expected increase in evaporative cooling in the Pl group after Gly supplementation. To date, the vast majority of studies have concluded that Gly has no influence on sweat loss (e.g., 18, 33), with Lyons and colleagues (26) the only authors to report both an

increased sweat loss and decreased core temperature during exercise in the heat as a direct result of Gly supplementation. Nonetheless, the mean increase in total sweat loss in the investigation by Lyons et al. (26) was significantly larger than in the present study (450 mL vs. 210 mL, respectively).

Despite the reduction in core temperature, heart rate, and perceived leg fatigue during constant-load exercise in the present study after supplementation with Cr/Pl and Cr/Gly, time-trial performance was not affected. Several studies have indicated that the increased cardiovascular and thermal responses resulting from dehydration can have a negative impact on exercise performance (e.g., 5). For example, Cheuvront et al. (5) determined that hypohydration was associated with an increased core temperature and heart rate and a significant reduction in work performed during a 30-min cycling time trial, even in a temperate (20 °C) environment. Therefore, if dehydration could be minimized there would potentially be less of an associated reduction in exercise performance. As such, several studies have concluded that hyperhydration is associated with a significant improvement in exercise performance in the heat (1, 18, 23, 32). Subjects in the studies by Kilduff et al. (23) and Montner et al. (32) were required to cycle submaximally until exhaustion, whereas the studies by Hitchins et al. (18) and Anderson et al. (1) used a self-paced time trial for 30 and 15 min, respectively, to quantify performance.

The findings of Hitchins et al. (18) seem particularly surprising given that cardiovascular and thermoregulatory responses during exercise were not different between Gly- and water-ingestion trials, meaning that the authors could provide no explanation for the observed ergogenic effect of Gly. Subjects in the study by Anderson et al. (1) were required to complete 90 min of steady-state exercise before commencing the time trial, more than twice as long as in the present study. The greater degree of dehydration that would occur during this prolonged submaximal exercise period might explain why preexercise hyperhydration resulted in a significant improvement in time-trial performance in the study by Anderson et al. (1). Therefore, it seems likely that the exercise trial in the present study was not of sufficient duration and therefore too high an exercise intensity for hyperhydration to have a significant effect on performance. Similarly, other studies found no effect of hyperhydration on exercise performance when compared with euhydration (25, 28). For example, Marino et al. (28) found Gly hyperhydration had no effect on a 60-min cycling time trial in hot and humid conditions compared with preexercise water ingestion. Latzka et al. (25) produced similar findings when subjects were asked to complete treadmill exercise at 55% VO_{2max} until exhaustion. These authors also reported, however, that after either Gly or water ingestion, exercise time to exhaustion was significantly greater than if no water had been consumed before exercise. Therefore, it would appear that commencing exercise in a hyperhydrated state might not confer any significant advantage in terms of exercise performance compared with euhydration or, indeed, modest dehydration (i.e., loss of 2–3% body mass). The results from the present study are compatible with such an idea, although further research is needed to determine the effects of hyperhydration on physiological responses and performance during a more prolonged exercise trial in which a more marked degree of dehydration would be expected.

Conclusion

In the present study, supplementation with both Cr and combined Cr and Gly for 7 d was effective in increasing TBW and reducing cardiovascular and thermal responses during prolonged exercise in the heat. The key finding of this study was that the increase in TBW after combined Cr and Gly supplementation was significantly greater than with Cr supplementation alone. Despite the increased hydration associated with combined Cr and Gly, there was no further attenuation in heart rate or T_{re} compared with Cr alone. Hyperhydrating before exercise through Cr, Gly, or a combination of the 2 did not result in any significant improvement in 16.1-km time-trial performance compared with euhydration. This might be because the time trial was too short to induce a degree of dehydration high enough to confer a significant improvement in exercise performance as a result of the altered hydration status. Furthermore, hyperhydration might not offer any significant advantage in terms of exercise performance compared with euhydration or, indeed, modest dehydration (i.e., loss of 2–3% body mass).

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