THE EFFECTS OF A NOVEL "FLUID-LOADING" STRATEGY COMBINING CREATINE AND GLYCEROL ON FLUID RETENTION AND DISTRIBUTION IN HUMANS

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ABSTRACT

The effects of three "fluid-loading" strategies combining creatine (Cr) and glycerol (Gly) on fluid retention and distribution were examined. Three 7-day Cr and Gly loading protocols (LP) were examined: LP1 - 10 g Cr and 75 g of glucose in 1 litre (L) of water (x 2 daily) and a bolus of 1 g Gly·kg^{-1} 2.5 hours prior to testing (n = 6); LP2 - 10 g Cr, 1 g Gly·kg^{-1} and 75 g of glucose in 1 L of water (x 2 daily) and final supplement consumed 3 hours prior to testing (n = 6); LP3 - as LP2 but final supplement consumed 5 hours prior to testing (n = 23). Only body mass increased (mean ± s.d.) by 1.59 ± 0.21 kg (P < 0.01) post-LP1 and by 1.20 ± 0.37 kg (P < 0.01) post-LP2. Post-LP3, body mass increased by 0.97 ± 0.28 kg (P < 0.01) with significant increases in total body water (0.87 ± 0.21 L; P < 0.01), intra-cellular and extra-cellular water and plasma volume. Combining Cr and Gly is potentially a clinically useful "fluid-loading" strategy which merits further evaluation for application in spaceflight.

1. INTRODUCTION

Space travel leads to severe physiological stresses that have profound effects on the wellbeing of astronauts [1]. Many studies have attempted to quantify the exact nature of the problems encountered by astronauts during and on return from prolonged space travel, though few practical solutions have been offered. Astronauts routinely experience a reduction in body mass of 1-3 kg on return from space flight [2,3] with losses up to 10 kg reported after long-duration missions [4]. The cause of this loss in body mass is believed to be multi-factorial, with insufficient fluid and energy intake and losses in musculo-skeletal mass considered the main contributors [1]. Several studies have reported a significant reduction in total body water (TBW) on return from space travel [4-6]. This water loss may largely be accounted for by loss of body mass [4] but also reflects water loss unrelated to muscle atrophy. Microgravity has been shown to remove all hydrostatic gradients within the body [7], resulting

in a cephalad fluid shift of approximately 1-2 L and a further shift of fluid from extra-cellular to intra-cellular water compartments [2]. In addition, there is indirect evidence to suggest that the thirst response is reduced in space to such an extent that fluid lost through respiration and sweating is not adequately replaced, resulting in a reduction in TBW and plasma volume by up to 17% [2,3,8]. The extent of plasma volume reduction incurred during space travel may be further exacerbated by the practice of "voluntary dehydration" undertaken by some astronauts prior to both take-off and re-entry to avoid the need to urinate [9]. The effects of a significant reduction in plasma volume may be profound, for example decreasing the thermoregulatory capacity of the body, resulting in an increased heart rate and core temperature during exercise and a reduced exercise tolerance [10]. Additionally, up to two thirds of astronauts suffer from orthostatic intolerance (inability to maintain a standing posture) on return to earth [11] due to the combined effects of reduced central venous pressure [12] and skeletal muscle atrophy that ensues from a lack of static exercise associated with prolonged exposure to microgravity [13].

One experimental approach to maintaining plasma volume during space travel has been to infuse isotonic saline. In a terrestrial environment this maintained central blood volume, resulting in an increased skin blood flow and associated convective heat loss [14]. The medical expertise required to insert and maintain a venous infusion and the restricted mobility that would result, makes this method of rehydration impractical during space travel. Currently, dehydration prior to re-entry into the earth's atmosphere is treated by oral intake of sodium chloride and water [15]. However, this treatment has limited efficacy and may be detrimental in astronauts who have dehydration-induced hypernatremia. While these current fluid strategies may be partially successful in replacing some of the fluid loss after dehydration has occurred, it may be more desirable to "fluid-load" prior to take-off to minimise subsequent dehydration during flight. Pre-hydrating prior to space flight by ingesting water or electrolyte solutions is ineffective at acutely expanding plasma volume as "excess" fluid ingested is rapidly filtered and excreted by the kidneys [16]. Two hydrating agents have shown promise in promoting water retention, glycerol (1,2,3-propanetriol) (Gly) and creatine (Cr). Supplementation with Gly or Cr has consistently produced a modest fluid retention of 400 to 800 ml [e.g. 17,18]. Seifert et al. [19] reported a 710 ml increase in mean TBW after Gly ingestion, including a 385 ml increase in interstitial fluid and 225 ml increase in intra-cellular fluid, a plasma expansion of 100 ml. In addition, Gly has been shown to effectively minimise an exercise-induced reduction in plasma volume [20] and a reduction in thermal and cardiovascular strain during heat stress [17,21,22]. Ingestion of Cr has also been shown to have substantial hydrating effects [18], although the exact mechanisms of this remain uncertain. However, unlike the whole body hydrating effects of Gly, Cr retains fluid predominantly in the intra-cellular fluid compartments [18]. Like Gly, oral Cr supplementation attenuates the rise in heart rate and core temperature during exercise, effects that have been

attributed to an increase in intra-cellular water (ICW) resulting in an increased specific heat capacity of the body [18,23].

It seems plausible that a Gly-induced increase in extra-cellular water (ECW) coupled with a Cr-mediated increase in ICW could have a synergistic effect resulting in a much larger increase in TBW than if either supplement was consumed alone. However, the potential implications for fluid balance of simultaneously ingesting two fluid retaining agents are unknown. Therefore, this present series of studies were carried out to examine the effects of three "fluid-loading" strategies that combined Cr and Gly on fluid retention and distribution. The overall aim was to develop an effective "fluid-loading" strategy for use during conditions of restricted water access, such as during space travel.

2. MATERIALS AND METHODS

2.1. Subjects
35 endurance-trained males (Table 1) were invited to take part in the present studies that were approved by the local Ethics Committee and performed according to the code of ethics of the World Medical Association (Declaration of Helsinki). Subjects were interviewed to ensure that they had not taken Cr for at least 8 weeks prior to each study. The investigators did not reveal prior to the interview that subjects would be excluded if they had supplemented with Cr in the previous 8 weeks. The subjects were fully informed of any risks and discomforts associated with the experiments before giving their written informed consent to participate.

2.2. Experimental design
Each study design consisted of two 7 day supplementation periods with measurements obtained pre- and post-supplementation. Subjects followed their normal diet during each supplementation period. Subjects were asked to minimise caffeine intake to lessen any possible confounding effect(s) of caffeine on Cr supplementation [24]. Each subject was required to follow one of three loading protocols (i.e. LP1, LP2, LP3).

2.3. Loading protocols

2.3.1. Loading protocol 1 (LP1)
Subjects (n=6) underwent a preliminary baseline test before receiving either combined Cr and Gly or placebo for 7 days followed by a post-supplementation test. Subsequent to this, subjects who received Cr/Gly in the first regimen received placebo and vice versa during a second 7 day supplementation regimen followed by a repeat test (i.e. a total of 3 tests). Cr/Gly supplementation consisted of 11.4 g of Cr·H_2O (equivalent to 10 g Cr) and 70 g of glucose polymer made up in 1 L of warm water and consumed twice daily for 6 days and a bolus of 1 g Gly·kg^{-1} body mass diluted in 500 ml of water with 125 ml unsweetened diluting juice 2 hours prior to the start of the test. This Cr supplementation protocol has been shown to increase resting muscle phosphocreatine levels within 5 days [25]. Each supplement was made immediately prior to consumption in order to prevent degradation of Cr to creatinine. The placebo consisted of 85 g of glucose polymer made up in 1 L of warm water and consumed twice daily for 6 days and a bolus of 500 ml of water with 125 ml unsweetened diluting juice 2 hours prior to testing. All supplements

LP1	(n=6)
Age (yr)	29 ± 5
Height (cm)	180 ± 6
Weight (kg)	79.2 ± 13

LP2	Pl Group (n=3)	Cr Group (n=3)
Age (yr)	23 ± 5	25 ± 9
Height (cm)	171 ± 14	174 ± 9
Weight (kg)	72.7 ± 12	74.1 ± 12

LP3	Pl Group (n=11)	Cr Group (n=12)
Age (yr)	31 ± 8	31 ± 6
Height (cm)	177 ± 6	177 ± 5
Weight (kg)	75.2 ± 7	75.0 ± 6

Table 1. The physical characteristics of subjects in each loading protocol. Data presented as the mean ± s.d.

had similar taste, texture and appearance and were placed in generic water bottles to ensure double blind administration. On each of the test days subjects ingested a further 500 ml of water 1 hour prior to the test in an attempt to ensure subjects were euhydrated [26].

2.3.2. Loading protocol 2 (LP2)
Subjects (n=6) were matched for body mass then randomised in a double blind fashion to either a Cr or a placebo group. Subjects in both groups were assessed pre- and post-testing during two separate supplementation regimens, with one week free from any supplementation between each regimen (i.e. a total of 4 tests). There was no crossover between subject groups. Each supplementation period started on the day after the pre-supplementation test and finished on the day of the post-supplementation test. Cr supplementation consisted of 11.4 g of $Cr \cdot H_2O$ (equivalent to 10 g Cr) and 70 g of glucose polymer made up in 1 L of warm water and consumed twice daily for 6 days and once more 3 hours prior to testing. The placebo supplement consisted of 85 g of glucose polymer in 1 L of warm water and consumed twice daily for 6 days and once more 3 hours prior to testing. During the first supplementation regimen, subjects in both groups also received either 1 g $Gly \cdot kg^{-1}$ body mass or an equivalent amount of placebo (i.e. sucrose) diluted in each 1 L supplement before receiving the opposite during the second supplementation regimen. Therefore, four possible combinations of supplements were administered: Placebo group - placebo/placebo and placebo/Gly; Cr Group - Cr/placebo and Cr/Gly. On each of the four test days, subjects ingested a further 500 ml of water 1 hour prior to testing.

2.3.3. Loading protocol 3 (LP3)
Subjects (n=23) completed LP3 which was identical to the LP2 protocol outlined above with the exception that subjects began consuming their final supplement 5 hours prior to each test with instructions to complete ingestion within one hour.

2.4. Procedures
Subjects reported to the laboratory on the day of testing following a 3 hour fast

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and having refrained from alcohol, caffeine and strenuous exercise the day before. Upon arrival at the laboratory, height and nude body mass were measured and body water compartments estimated using a Bodystat Multiscan 5000 Bioimpedance analyser (Bodystat Ltd, Isle of Man). This method allows TBW and ECW to be estimated, and ICW to be deduced. The bioimpedance measurements were taken after the subjects had lain comfortably in a supine position for 10 min on a non-conductive surface with their arms and legs slightly abducted. Estimation of TBW by bioimpedance is reliable and valid when subjects are euhydrated [27]. Several studies have successfully utilised this technique in order to estimate hyperhydration induced changes in TBW [18,23]. Furthermore, the change in body mass from pre- to post-supplementation was determined to provide a further indirect measurement of the volume of fluid retained. Blood was obtained in all subjects from a superficial vein on the dorsal surface of the hand after standardising for postural changes (i.e. seated position for 15 min).

2.5. 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 was rapidly deproteinised in 800 µL of ice cold 0.3 mol·l^{-1} perchloric acid, centrifuged and the supernatant used for the measurement of glucose using standard enzymatic methods with spectrophotometric detection (Mira Plus, ABX Diagnostics, Montpellier, France). A further aliquot of blood was centrifuged and the plasma obtained was separated and used for the measurement of Gly [28]. The blood in tubes without anticoagulant was allowed to coagulate and then centrifuged; the serum collected was used for the measurement of osmolality by freezing point depression (Micro-osmometer 3300, Vitech Scientific, West Sussex, UK) (LP2 and LP3 only). The blood from the K_3EDTA tubes was also analysed for haemoglobin (Hb) (cyanmethaemoglobin method, Sigma, Chemical Company Ltd., Dorset, UK) and haematocrit (Hct) (conventional microhaematocrit method). All blood analyses were carried out in duplicate with the exception of Hct, which was carried out in triplicate. Plasma volume changes were calculated from changes in Hb and Hct relative to initial pre-supplementation values, assuming no change in red cell mass during the 7 day supplementation regimen [29].

2.6. Data analysis

Data were expressed as the mean ± s.d. following a test for the normality of distribution. Statistical analysis was carried out using three factor mixed model ANOVA with repeated measures, followed by a simple main effects analysis for significant three way interactions (i.e. pre- vs. post-supplementation for each subject group and treatment) and simple main effects analysis for two way interactions. In addition, the magnitudes of change (Δ) in the four supplement regimens (placebo/placebo, placebo/Gly, Cr/placebo and Cr/Gly) were examined using either a two-sample or a paired t-test when significance was identified using the simple main effects analysis. Statistical significance was declared at $P<0.05$.

3. RESULTS

3.1 Body mass and water compartments

3.1.1 LP1
There was no significant change in the body mass of subjects following placebo supplementation (P=0.43) but increased significantly following Cr/Gly supplementation (1.59 ± 0.21 kg) (P<0.01) (Fig. 1). There were no significant changes in TBW, ECW or ICW following either placebo or Cr/Gly supplementation compared to pre-supplementation values (Fig. 1).

3.1.2 LP2
The physical characteristics of the placebo and Cr groups were similar before supplementation (Table 1). In the placebo group, body mass increased significantly following Gly supplementation, with no change following placebo (Δ body mass was greater following Gly supplementation; Fig. 1). In the Cr group, body mass increased significantly during both the placebo and Gly regimens (Fig. 1) with no difference in Δ body mass between regimens. TBW, ICW and ECW did not differ significantly pre- to post-supplementation in either the placebo or Cr groups in either supplementation regimen (Fig. 1).

3.1.3 LP3
The physical characteristics of the placebo and Cr groups were similar before supplementation (Table 1). In the placebo group, body mass increased significantly following Gly supplementation, with no change after placebo (Δ body mass was greater following Gly supplementation; Fig. 1). In the Cr group, body mass increased significantly during both the placebo and Gly regimens (Fig. 1). The combination of Cr and Gly lead to a significantly greater increase in body mass than when either was used alone (P=0.02) (Fig. 1). There was no difference pre-supplementation in TBW, ICW and ECW between groups. In the placebo group, TBW and ECW increased significantly following Gly supplementation, whereas TBW and ECW were unaltered after the placebo regimen (Fig. 1). There was a significant increase in ICW in the placebo group following Gly supplementation (P=0.01) but not during the placebo regimen (P=0.10). In the Cr group, TBW, ICW and ECW increased significantly during both supplementation regimens (Fig. 1). Additionally, the increase in TBW and ECW in the Cr group was significantly greater following Gly supplementation than following placebo (P=0.02 and P<0.01, respectively) (Fig. 1).

3.2 Blood metabolite concentrations, osmolality and plasma volume changes

3.2.1 LP1
Plasma [glucose] was not different between pre- and post-supplementation measurements following either placebo or Cr/Gly supplementation (Table 2). Plasma [Gly] increased significantly following Cr/Gly supplementation compared to pre-supplementation with no change following placebo supplementation (Table 2). [Hb] and [Hct] remained unchanged from pre- to post-placebo supplementation. There was an increase in [Hb] and [Hct] following Cr/Gly supplementation that did not reach statistical significance (P=0.13 and P=0.08, respectively) (Table 2). Plasma volume was not significantly altered by either supplementation regimen, although there

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was a tendency (P=0.07) for plasma volume to be reduced after Cr/Gly supplementation (Fig. 2).

3.2.2 LP2

Plasma [glucose] was not different between pre- and post-supplementation measurements following either placebo or Gly supplementation in both the placebo and the Cr groups (Table 2). Plasma [Gly] increased significantly following placebo/Gly and Cr/Gly supplementation compared to pre-supplementation with no change following placebo/placebo or Cr/placebo supplementation (Table 2). Serum osmolality was also significantly higher post Gly supplementation compared to pre-supplementation in both the placebo (P<0.01) and Cr (P<0.01) groups (Table 2). [Hb] and [Hct] remained unchanged from pre- to post-placebo/placebo and Cr/placebo supplementation. The small increases in [Hb] and [Hct] following placebo/Gly (P=0.21 and P=0.17) and Cr/Gly (P=0.18 and P=0.11) supplementation did not reach statistical significance (Table 2). Plasma volume was not significantly altered following either placebo/placebo or Cr/placebo supplementation (Fig. 2), whereas both placebo/Gly and Cr/Gly supplementation resulted in a small (2-3%) non-significant reduction in plasma volume (P=0.11 and P=0.09 respectively) (Fig. 2).

3.2.3 LP3

Plasma [glucose] was not different between groups or following Gly supplementation (Table 2). Plasma [Gly] was significantly higher post Gly supplementation compared to pre-supplementation in both the placebo (P<0.01) and Cr (P<0.01) groups (Table 2). Additionally, serum osmolality was

significantly higher post Gly supplementation compared to pre-supplementation in both the placebo (P=0.02) and Cr (P<0.01) groups (Table 2). [Hb] and [Hct] remained unchanged from pre-to post-supplementation after both placebo/placebo and Cr/placebo supplementation (Table 2). The increase in [Hb] and [Hct] was small and non-significant following placebo/Gly supplementation (P=0.18 and P=0.06 respectively) but statistically significant following Cr/Gly supplementation (P=0.01 and P=0.04 respectively) (Table 2). Plasma volume was not significantly altered following either placebo/placebo or Cr/placebo supplementation (Fig. 2). Both placebo/Gly and Cr/Gly supplementation resulted in an increase in plasma volume, although only after the Cr/Gly supplementation did this increase reach statistical significance (P=0.06 and P=0.01, respectively) (Fig. 2).

3.4 Side effects

In general, subjects tolerated the supplementation protocol well with only one report of gastrointestinal distress after Gly supplementation during LP3.

4. DISCUSSION

Supplementation with combined Cr and Gly using the LP3 hyperhydration regimen significantly increased body mass, TBW and plasma volume (Figs. 1 and 2). Despite no significant increase in TBW and plasma volume after Cr/Gly ingestion during LP1 and LP2 (Figs. 1 and 2), there was an increase in body mass, suggesting a significant retention of fluid. This may be explained by water requiring greater than 3 hours from ingestion of the final Cr/Gly supplement to disperse throughout the body water
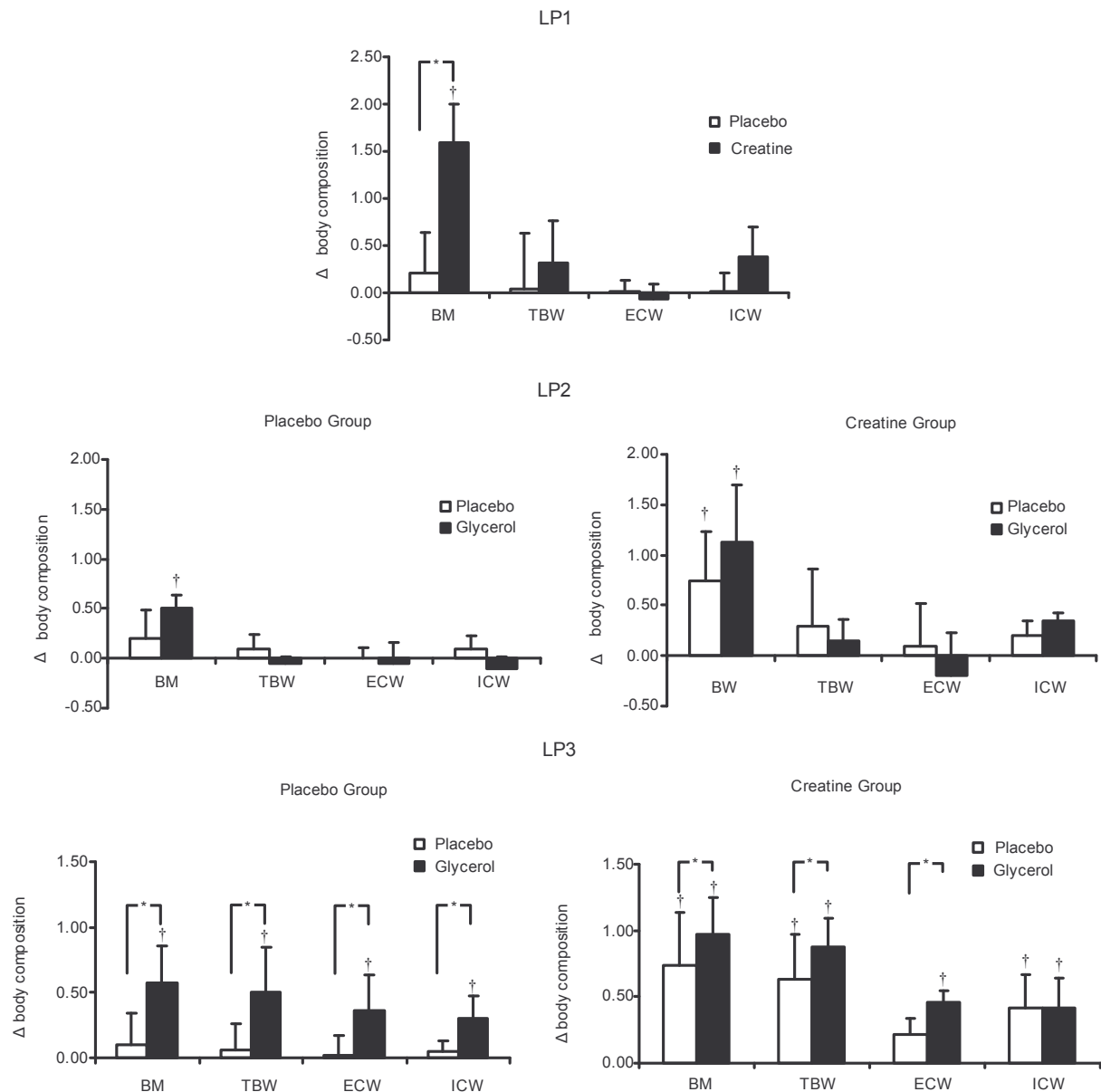
Figure 1. Changes in body mass (BM), total body water (TBW), intra-cellular water (ICW) and extra-cellular water (ECW) in the two groups. Data presented as the mean ± s.d. †: indicates a significant difference pre- vs. post-supplementation. *: indicates a significant greater change (Δ) in the Gly supplementation regimen compared to the placebo supplementation regimen.

compartments. Subjects experienced a body mass increase of 1.59 ± 0.21 kg after 7 days of supplementation with Cr/Gly in LP1 and 1.20 ± 0.37 kg in LP2 (Fig. 1). This exceeds the 0.4-1.0 kg increase in body mass observed in other hyperhydration studies [17,18,21]. This increase in body mass was not accompanied by an increase in TBW indicating an increase in water retention that was not measured by bioimpedance analysis. These results are not in

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agreement with other hyperhydration studies that demonstrated an increase in fluid retention as reflected by a significant increase in TBW [18,19,23]. For example, subjects in the study by Kilduff et al. [18] experienced a 0.8 kg increase in body mass accompanied by a 0.6 L increase in TBW after 7 days of Cr supplementation. Conversely, combined Cr/Gly supplementation following LP3 resulted in a significant 0.97 ± 0.28 kg increase in body mass accompanied by a 0.87 ± 0.21 L increase in TBW. The results from LP3 also demonstrate that both the increase in body mass and the volume of water retained by ingesting either Cr or Gly can be significantly enhanced by combining these two hyperhydrating agents. The 870 ml (range 600-1400 ml) increase in TBW resulting from ingestion of this novel "water-loading" strategy that combines Cr and Gly, was significantly larger than the volume retained with either Cr or Gly alone (Fig. 1). Additionally, the retained water was dispersed equally between the intra- and extra-cellular water compartments (Fig. 1). It seems plausible that the water retained by combining Cr and Gly was mediated via a Cr-induced increase in ICW and a Gly-induced increase in ECW. This innovative "water-loading" strategy comprised of two agents that specifically target both ICW and ECW compartments and as such, may overcome the limitations of previous hyperhydration strategies. For example, Gly has been investigated as a potential hyperhydrating agent for a number of decades [17,22]. However, the benefits of Gly hyperhydration are equivocal with at least 23 original papers published on Gly hyperhydration providing conflicting results. Where
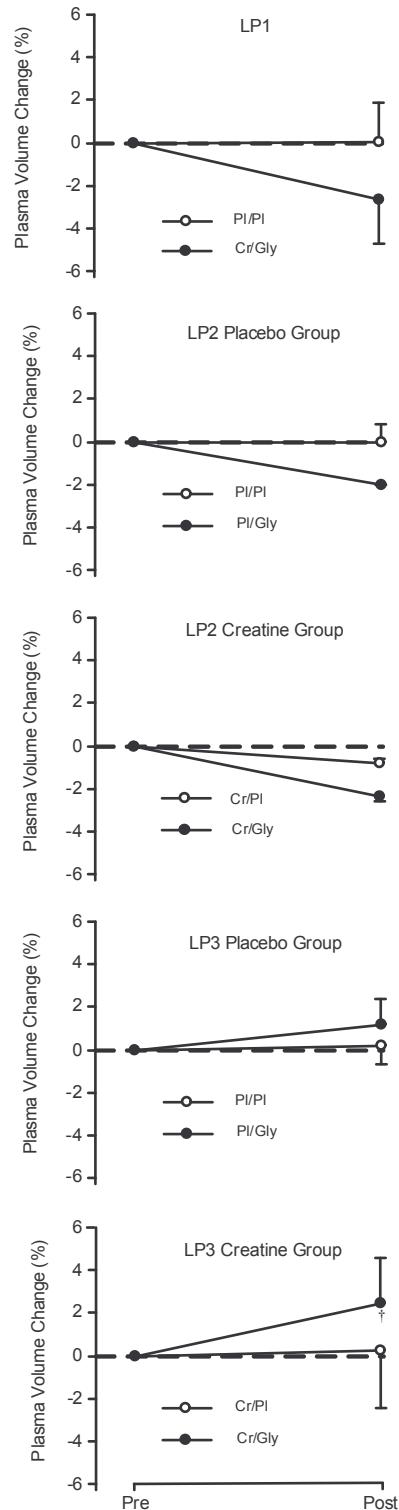

Figure 2. Changes in plasma volume between pre- and post supplementation in each subject group and loading protocol. Data presented as the mean ± s.d. †: indicates a significant difference pre- vs. post-supplementation.

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LP 1

	Pre	Pl/Pl	Cr/Gly
[Hb] g·dL⁻¹	16.1 ± 1.6	16.2 ± 1.2	16.3 ± 1.5
Hct (%)	44.2 ± 3.8	44.0 ± 3.9	44.8 ± 3.4
[Gly] (mmol·l⁻¹)	0.05 ± 0.02	0.05 ± 0.03	11.04 ± 3.22
[Glucose] (mmol·l⁻¹)	4.7 ± 0.5	4.6 ± 0.5	4.6 ± 0.2

LP 2

	Pre Pl/Pl	Post Pl/Pl	Pre Pl/Gly	Post Pl/Gly	Pre Cr/Pl	Post Cr/Pl	Pre Cr/Gly	Post Cr/Gly
[Hb] g·dL⁻¹	14.9 ± 0.8	14.9 ± 0.7	14.9 ± 0.6	15.1 ± 0.4	14.7 ± 0.4	14.9 ± 0.2	15.6 ± 1.0	15.9 ± 1.1
Hct (%)	44.5 ± 1.4	44.8 ± 1.8	44.4 ± 0.5	44.8 ± 1.1	46 ± 2.1	46 ± 2.1	46.4 ± 1.2	46.8 ± 1.1
[Gly] (mmol·l⁻¹)	0.06 ± 0.03	0.05 ± 0.03	0.05 ± 0.02	10.92 ± 2.14 †	0.04 ± 0.03	0.03 ± 0.03	0.05 ± 0.02	10.18 ± 2.67 †
[Glucose] (mmol·l⁻¹)	4.9 ± 0.2	5.1 ± 0.6	5.1 ± 0.3	5.0 ± 0.1	4.6 ± 0.2	4.3 ± 0.8	4.4 ± 0.4	4.6 ± 0.2
Osmolality (mosmol·kg⁻¹)	285 ± 4	286 ± 2	284 ± 2	294 ± 4 †	287 ± 3	289 ± 3	287 ± 4	293 ± 2 †

LP 3

	Pre Pl/Pl	Post Pl/Pl	Pre Pl/Gly	Post Pl/Gly	Pre Cr/Pl	Post Cr/Pl	Pre Cr/Gly	Post Cr/Gly
[Hb] g·dL⁻¹	15.9 ± 0.9	15.9 ± 0.9	16.0 ± 0.8	15.8 ± 0.7	16.0 ± 1.0	15.6 ± 0.9	16.1 ± 1.0	15.7 ± 0.9 †
Hct (%)	46.6 ± 1.8	46.4 ± 1.8	46.8 ± 2.1	46.5 ± 2.3	47.0 ± 2.1	46.9 ± 2.2	47.0 ± 2.1	46.6 ± 2.8 †
[Gly] (mmol·l⁻¹)	0.05 ± 0.02	0.06 ± 0.03	0.04 ± 0.02	7.60 ± 1.36 †	0.06 ± 0.02	0.03 ± 0.02	0.04 ± 0.02	7.12 ± 1.41 †
[Glucose] (mmol·l⁻¹)	4.7 ± 0.5	4.9 ± 0.5	4.7 ± 0.4	4.8 ± 0.4	4.8 ± 0.5	4.7 ± 0.5	4.8 ± 0.5	4.7 ± 0.4
Osmolality (mosmol·kg⁻¹)	283 ± 2	283 ± 2	284 ± 3	291 ± 3 †	285 ± 2	281 ± 2	284 ± 3	292 ± 2 †

Table 2. Resting blood [haemoglobin] and haematocrit content, plasma [glycerol] and [glucose], and serum osmolality before and after supplementation in each loading protocol. Data presented as the mean ± s.d. †: indicates a significant difference pre- vs. post-supplementation.

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hyperhydration was induced, the hydrating effects were transient due to the metabolism of Gly by the liver and kidneys. Of greater concern is the potential side-effect of cerebral dehydration, due to the relatively impermeability of the blood-brain barrier to Gly, with resulting headaches [30]. Combining Gly with Cr may overcome this, as in contrast to Gly, Cr is taken up by the brain [31] and could counteract the effects of Gly.

A key finding of the present series of studies is the importance of methodology that led to the differences of changes in TBW between LP2 and LP3. The only difference between LP2 and LP3 was the length of time between the ingestion of the final supplement and testing. Thus, it would seem apparent that a period of time in excess of 3 hours is required for the retained water to be distributed within body water compartments. Previous studies examining the effectiveness of Gly supplementation as a hyperhydration method have consistently utilised a single Gly bolus mixed with water and ingested between 2-3 hours prior to analysis [17,21]. However, when Gly was delivered in a similar fashion alongside a Cr hyperhydration protocol previously shown to be successful in our lab [18], no increase in hydration was measured (Fig. 1). Previous Gly hyperhydration studies have quantified water retention by the volume of urine produced [16,22], which provides no information as to where the retained water was distributed. Body compartment analysis by multifrequency bioimpedance combined with changes in body mass used in the present study, provides data indicating fluid changes in both the intra- and extra-cellular water compartments. Furthermore, the mechanism by which bioimpedance

estimates body water provides insight into the confounding data from LP1 and LP2. Since hypertonic solutions such as the Cr/Gly combination (965 ± 61 $mosmol \cdot kg^{-1}$) cause an initial net movement of fluid into the intestinal lumen [32], there is a loss of ECW and thus TBW, which ultimately leads to dehydration, albeit temporarily. This is confirmed by the small reductions in plasma volume that occurred after supplementation with both Pl/Gly and Cr/Gly in LP1 and LP2. Interestingly, fluid changes in the trunk have little effect on bioimpedance measurements as the trunk only accounts for 5-12% of total body impedance [33]. This is confirmed by the relatively small impact on bioimpedance measurements of up to 2 L of fluid within the abdominal cavity [34]. Additionally, the profoundly high osmolality of the Cr/Gly solution may have inhibited gastric emptying [35], further contributing to the lack of increase in TBW 2-3 hours after Cr/Gly ingestion as demonstrated in LP1 and LP2. Although Cr/Gly supplementation results in significant water retention, a period of time greater than 3 hours is required after ingestion of the final Cr/Gly supplement before significant hydrating effects are discerned throughout the body water compartments. Furthermore, it is possible that a similar effect would have been observed after LP1 and LP2 had a longer period of time been left between ingestion of the final supplement and testing. This is in contrast to the majority of previous studies that utilised Cr or Gly alone to hyperhydrate, which concluded that there were no changes in plasma volume compared to water or a placebo [e.g. 16-18]. Similarly to these previous studies, there was no appreciable change in plasma volume in the present study in LP3 from pre- to post-supplementation

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when either Cr or Gly alone were ingested (Fig. 2). However, the fact that combined Cr and Gly resulted in a significantly greater retention of fluid than Cr or Gly alone and the unique way in which this novel hyperhydration strategy retains fluid, may account for these notable differences. Plasma volume can decrease during space travel by as much as 17% [2,3,8]. The 2.4% expansion induced by Cr and Gly in the present study may not be large enough to offset this deficit. Further research is required to examine whether plasma volume expansion via Cr and Gly hyperhydration can minimise the adverse effects of plasma volume reduction during space travel and orthostatic intolerance on return to earth.

Despite the relatively minor increases in plasma volume associated with combined creatine and glycerol ingestion, astronauts encounter several other physiological problems during space travel that this novel hyperhydration strategy may potentially counteract. For example, the effect of plasma volume loss in space may result in excessive heat strain for astronauts wearing protective garments during launch and landing. Dehydrated (> 3% body mass) unacclimatised individuals will exhibit excessive heat strain (core temperature exceeding 39°C) during the pre-launch and launch of the space shuttle [36]. Core temperature has been measured in crewmembers during landing and found to be significantly elevated to approximately 38°C despite the use of a liquid cooling garment [37]. Recent studies completed in this lab suggest that ingestion of Cr and Gly can significantly reduce cardiovascular and thermal strain during exercise induced heat stress [38]. Potentially this could improve physical and mental performance

during space travel and offer astronauts some protection from heat injury. Additionally, Cr supplementation prior to and during prolonged periods of immobilisation (hypokinesia) significantly attenuated skeletal muscle atrophy [39]. Hypokinesia encountered during microgravity has been shown to induce muscle atrophy, slow-to-fast twitch muscle fibre shift and a decrease in force generation capacity [39,40]. One of the proposed mechanisms for the protective effect of Cr on skeletal muscle includes higher mitotic activity in satellite cells [41]. In addition, it is also possible that Cr is able to activate signalling pathways, protecting skeletal muscle against proteolysis [42]. Alternatively, the increase in cellular hydration that occurs after Cr supplementation (Fig. 1) may also have a role in controlling protein turnover as this can act as a signal, stimulating protein synthesis and attenuating protein breakdown [43]. However, further research is required to examine the exact effects of Cr on skeletal muscular atrophy alongside a structured weight-training program during prolonged space travel.

5. CONCLUSIONS

This study has demonstrated that supplementation with combined Cr and Gly significantly increased body mass, TBW and plasma volume. The timing of ingestion is critical, with the final supplement requiring to be consumed approximately 5 hours prior to the need for hyperhydration (e.g. prior to take-off and landing). Although Cr/Gly appear to have a synergistic effect on hydration status and thermoregulation in a terrestrial environment, it remains to be determined whether this translates into

minimising the adverse physiological effects of microgravity.

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