Effect of whey protein isolate on rehydration after exercise.

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Abstract

Studies have examined adding protein to carbohydrate-electrolyte rehydration drinks, but the effects of protein in isolation remain unknown. Ten subjects completed two trials in which they were dehydrated (~2% of pre-exercise body mass) by intermittent cycling in the heat . Subjects then rehydrated (150% total mass loss) over 1 h with mineral water (W) or mineral water plus 20 $g \cdot L^{-1}$ whey protein isolate (WP) and remained in the laboratory for a further 4 h. Blood and urine samples were provided pre-exercise, post-exercise, post-rehydration and every hour thereafter. From blood samples, serum osmolality, change in plasma volume and plasma albumin content was determined, whilst the volume and osmolality of urine samples were determined. There was no difference between trials for total urine volume (W: 1234 (358) mL; WP: 1306 (268) mL; P=0.409), drink retention (W: 40 (14) %; WP: 37 (14) %; P=0.322) or net fluid balance (W: -605 (318) mL; WP: -660 (274) mL; P=0.792) 4 h post-rehdyration . Plasma volume was greater 3 and 4 h post-drinking during WP and plasma albumin content relative to pre-exercise was increased 1-4 h post-drinking in WP only. These results suggest addition of 20 $g \cdot L^{-1}$ whey protein isolate neither enhances nor inhibits post-exercise rehydration, when a volume equivalent to 150% of sweat losses is ingested in 1 h. As post-exercise nutritional requirements are multifactorial (rehydration, glycogen resynthesis, myofibrillar/ mitochondrial protein synthesis), these data demonstrate that when post-exercise protein intake might benefit recovery or adaptation, this can be achieved without compromising rehydration.

Key Words: Water, Dehydration, Rehydration, Plasma Albumin, Milk Protein.

Introduction

During prolonged exercise, sweat losses generally exceed fluid intake resulting in hypohydration (Sawka et al. 2007), and in situations where sweat losses are significant, specific post-exercise rehydration might be necessary (Shirreffs et al. 2004). Many athletes train more than once a day and commencing exercise hypohydrated has been shown to impair endurance (Kennefick et al. 2010) and strength performance (Minshull and James 2013), making rehydration an important part of the post-exercise recovery process.

Ingesting sufficient protein after resistance (Moore et al. 2009) and endurance (Wilkinson et al. 2008) exercise has been shown to acutely enhance post-exercise protein synthesis. This potentially leads to beneficial adaptations during chronic exercise training (Hartman et al, 2007; Robinson et al. 2011; Ferguson-Stegall et al. 2011). Despite this, little is known about the effects of protein on post-exercise rehydration. Shirreffs et al. (2007b) demonstrated that after exercise-induced dehydration, ingestion of skimmed milk resulted in greater drink retention than when a traditional carbohydrate-electrolyte sports drink was ingested, a finding confirmed by Watson et al. (2008). There are a number of compositional differences between skimmed milk and a carbohydrate-electrolyte sports drink (*i.e.* energy density, electrolyte content, carbohydrate content and type) that might have accounted for the observed difference in drink retention. However, James et al. (2011; 2013) demonstrated that the protein contained in milk accounts for at least some of the enhanced drink retention observed, although the mechanisms by which milk and/ or milk protein increases drink retention are not fully understood.

Milk protein is comprised of ~80% casein proteins and ~20% whey proteins and whilst the post-exercise rehydration effects of casein are presently unknown, two studies have examined the effects of whey protein (Seifert et al. 2006; James et al. 2012). Seifert et al. (2006) reported that after exercise in the heat, a greater proportion of a commercially available 60 $g \cdot L^{-1}$ carbohydrate, 15 $g \cdot L^{-1}$ whey protein drink was retained compared to a commercially available 60 $g \cdot L^{-1}$ carbohydrate drink or bottled water. In contrast, James et al. (2012) reported no difference in drink retention between a 65 $g \cdot L^{-1}$ carbohydrate drink and a 50 $g \cdot L^{-1}$ carbohydrate, 15 $g \cdot L^{-1}$ whey protein isolate drink, when drinks were ingested in a volume equivalent to 150% of sweat lost during exercise and matched for energy density and electrolyte concentrations. The difference in drink volume ingested or the fact that energy

density was either matched or unmatched, might account for the divergent findings of these two studies.

The ingestion of protein post-exercise has been shown to increase plasma albumin content (Okazaki et al. 2009), which as the main plasma protein is the primary contributor to oncotic pressure and may play a role in post-exercise fluid balance, although this has not yet been examined in a post-exercise rehydration context.

The purpose of the present study was to investigate the rehydration effects of adding whey protein isolate to bottled water ingested after exercise-induced dehydration and to examine whether this was related to any changes in plasma albumin content.

Methods

Participants and Ethical Approval

After ethical approval from the Nottingham Trent University Ethical Advisory Committee and in accordance with the guidelines of Harriss and Atkinson (2011), ten subjects (7 male, 3 female; age: 22 (2) y, height: 1.71 (0.12) m, weight: 70.58 (10.03) kg) gave their informed consent and completed a medical screening questionnaire. Female subjects also completed a menstrual cycle questionnaire to determine the length of their menstrual cycle. All subjects then completed a familiarisation trial and two experimental trials. Experimental trials were separated by at least 7 days for male subjects and exactly 1 menstrual cycle for female subjects and completed in a randomised counterbalanced order. The familiarisation trial replicated the experimental trials (described below), with a shortened 1 h monitoring period. Using G*Power 3.1.6 (Faul et al., 2009) and the data of Seifert et al. (2006), an α of 0.05 and statistical power of 0.8, it was determined that nine subjects would be required to reject the null hypothesis.

In the 24 h preceding the first experimental trial subjects recorded their diet and physical activity, replicating these patterns before the second trial. Subjects were also instructed to refrain from any strenuous exercise or alcohol ingestion in the 24 h before experimental trials.

Protocol

Trials commenced in the morning after an overnight fast, with the exception of 500 ml water ingested 1.5 h before arriving at the laboratory. Upon arrival at the laboratory a venous blood sample (7.5 mL) was taken by venepuncture of an antecubital vein. Subjects then provided a total void urine sample, before their body mass (in underwear only) was measured to the nearest 0.01 kg (Adam CFW 150 scale; Adam Equipment Co Ltd, Milton Keynes, UK). Subjects then exercised on a cycle ergometer (Monark Ergomedic 874E, Cranlea, Birmingham, UK) in a temperature (35°C) and humidity (60% relative humidity) controlled environment (Design Environmental Ltd., Ebbw Vale, UK). Subjects exercised in 10 min blocks, with initial exercise intensity ~2 W·kg body mass^{-1}. Exercise blocks were separated by 5 min rest periods, during which subjects were re-weighed (in underwear only) and this continued until subjects had lost 1.7% of their pre-exercise body mass. Target toal body mass loss was 2% body mass and it was anticipated subjects would lose the additional 0.3% in the 15 min post-exercise. Subjects then showered and towel dried, before being re-weighed (in dry underwear only) to determine their total body mass loss. A 20g plastic cannula was then inserted into an antecubital vein and a blood sample (7.5 mL) was drawn, after which subjects provided another total void urine sample (-1 h).

Subjects were then rehydrated with mineral water (W) (Volvic, Danone UK Ltd., London, UK) or mineral water with the addition of 20 g·L^{-1} whey protein isolate (WP) (Volactive Hydrapro, Volac International Ltd. Orwell, UK) over a period of 1 h. The composition of the protein powder per 100 g powder was: 89 g protein, 0.1 g carbohydrate, 0.2 g fat, 20 mg sodium, 10 mg potassium, 10 mg chloride (data supplied by the manufacturer). The volume of drink ingested was 150% of the total body mass loss and was ingested in four aliquots of equal volume at 15 min intervals. The final composition of the drinks is presented in table 1. Subjects rated the drinks for sweetness, saltiness, bitterness and pleasantness at the end of the 1 h rehydration period using a 100 mm visual analogue scale. Subjects then rested in the laboratory for the next 4 h, with further blood (7.5 mL) and total void urine samples obtained at the end of the rehydration period (0 h) and every hour thereafter (1 h, 2 h, 3 h and 4 h). Subjects body mass was again measured at the end of the trial. Between samples subjects were free to move around the laboratory, although movement was kept to a minimum and all blood samples were taken after at least 15 min in an upright seated position.

Blood Handling and Analysis

Of each 7.5 mL blood sample, 1.3 mL was dispensed into a pre-chilled tube containing 1.75 $mg \cdot L^{-1}$ EDTA and was placed in ice, before centrifugation (3000 g, 10 min, 3°C). The resultant plasma was stored at -80°C and analysed for albumin concentration by the bromcresol green method (Pentra ABX400; HORIBA Medical, Northampton, UK). A further 1.3 mL of blood was mixed with EDTA (1.75 $mg \cdot L^{-1}$) and was used for analysis of haemoglobin by the cyanmethaemoglobin method and haematocrit by microcentrifugation. Haemoglobin and haematocrit values were used to estimate changes in plasma volume, relative to pre-exercise (Dill and Costill, 1974). The remainder of the blood sample was dispensed into a plain tube and allowed to clot, before serum was separated by centrifugation (3000 g, 10 min, 3°C). Serum was analysed for osmolality by freezing point depression (Gonotec Osmomat 030 Cryoscopic Osmomter; Gonotec, Berlin, Germany). The volume of each urine sample was measured before an aliquot was retained and analysed for osmolality. Drink samples were also analysed for osmolality, as well sodium and potassium concentration by flame photometry (Corning Clinical Flame Photometer 410C; Corning Limited, Essex, UK).

Statistical Analysis and Calculations

Data was analysed using IBM SPSS Statistics 20 (v) (Chicago, IL, USA). All data were checked for normality of distribution using a Shapiro-Wilk test. Data containing two factors were then analysed using a two-way repeated measures ANOVA. The Mauchly test was used, and where it indicated that the assumption of sphericity had been violated, the degrees of freedom for the data set were corrected using the Greenhouse-Geisser estimate. Significant differences were located using Bonferroni adjusted paired t-tests for normally distributed data or Bonferroni-adjusted Wilcoxon signed-ranked tests for non-normally distributed data. Variables containing one factor (*i.e.* drink perception) were analysed using paired t-tests or Wilcoxon signed-ranks tests as appropriate. $P \leq 0.05$ was used to determine statistical significance. Data are presented as means (1 SD).

Net fluid balance (NFB) was calculated relative to pre-exercise, at which time subjects were assumed to be in NFB. NFB at each time point was determined using fluid lost through sweating during exercise (estimated from total body mass loss during exercise) and cumulative urine production, and fluid gained through drink ingestion.

Albumin content was determined using plasma albumin concentration and the change in plasma volume. At pre-exercise subjects were assumed to have a plasma volume (in L)

equivalent to 5% of body mass (in kg) and plasma volume at each time point was determined using this pre-exercise plasma volume and the relative change in plasma volume.

Results

Pre-trial, exercise and rehydration variables

Pre-exercise, there was no difference between trials for body mass (P=0.122), urine osmolality (P=0.286), serum osmolality (P=0.351) or plasma albumin concentration (P=0.536), indicating subjects started each trial in a similar state of hydration. The exercise protocol elicited a similar degree of body mass loss during trials (W: 1.37 (0.18) kg, WP: 1.38 (0.15) kg; P=0.929), representing a loss of 1.96 (0.21) % of initial body mass. This meant the volume of drink ingested during rehydration was also not different between trials (W: 2058 (272) ml, WP: 2074 (221) ml; P=0.865). During trial WP subjects ingested 41 (4) g of protein during the rehydration period. The exercise produced a mean work load of 137 (23) W over both trials and was not different between trials (P=0.303). Similarly, exercise duration (P=0.161) and total heat exposure (P=0.143) were not different between trials and over both trials were 55 (9) min and 84 (14) min, respectively.

Urine markers

Whilst there was a main effect of time (P<0.001), there was no main effect of trial (P=0.510) or interaction effect (P=0.913) for the volume of urine produced each hour after drinking (Fig 1.). Compared to -1 h, urine volume was increased at 1 h, 2 h and 3 h for both W and WP (P<0.05). Consequently, total urine volume after drinking (W: 1234 (358) mL; WP: 1306 (268) mL; P=0.409) and the fraction of the ingested drink retained (W: 40 (14) %; WP: 37 (14) %; P=0.322) were not different between trials. For NFB (Fig 2.), there was a main effect of time (P<0.001), but no main effect of trial (P=0.792) or interaction effect (P=0.620). Compared to pre-exercise, net fluid balance was negative at -1 h during both trials (P<0.001), was positive at 0 h during both trials (P<0.001) and was again negative from 2 h onwards during both trials (P<0.05).

Whilst there was a main effect of time (P<0.001) for urine osmolality (Fig 3.) there was no main effects of trial (P=0.436) or interaction effect (P=0.509). Compared to pre-exercise,

urine osmolality was increased at -1 h during both trials (P<0.05) and tended to be decreased at 2 h during W (P=0.084).

Blood markers

There was a main effect of time for serum osmolality (P<0.001) (Fig 4.) and a tendency for a main effect of trial (P=0.062), but no interaction effect (P=0.402). Compared to pre-exercise, serum osmolality was increased at -1 h during both trials, but was not different from pre-exercise at any other time point. For plasma albumin content (Fig. 5.) there was a main effect of time (P<0.001) and a tendency for an interaction effect (P=0.090), but no main effect of trial (P=0.330). Compared to pre-exercise, plasma albumin content was increased from 1 h onwards during WP (P<0.05), but was not different at any time point during W (P>0.198). There was a main effect of time (P<0.001), but no main effect of trial (P=0.941) or interaction (P=0.247) for plasma albumin concentration (data not shown). For the estimated change in plasma volume (Fig 6.), there was a main effect of time (P<0.001) and an interaction effect (P=0.05), as well as a tendency for a main effect of trial (P=0.059). Compared to pre-exercise, plasma volume was decreased at -1 h during both trials (P<0.05) and was greater during WP than W at 3 h and 4 h (P<0.05).

Drink perception

Subjects perceived drink W to be more pleasant than drink WP (P<0.001) and drink WP to be more bitter than drink W (P<0.01), but perceived no difference between drinks in terms of sweetness (P=0.740) or saltiness (P=0.101).

Discussion

The main findings of this study are that following exercise-induced dehydration equivalent to ~2% initial body mass, the addition of 20 $g \cdot L^{-1}$ whey protein isolate to a rehydration drink neither enhanced nor inhibited rehydration. With the exception of plasma volume, there was no difference in any of the measured fluid balance variables between bottled mineral water and bottled mineral water plus 20 $g \cdot L^{-1}$ whey protein isolate when the rehydration drink was ingested in a volume equivalent to 150% of sweat losses, over 1 h.

Despite ingesting a volume of rehydration drink equivalent to 150% of their sweat losses, subject's NFB at the end of the trials was -605 (318) mL and -660 (274) mL during the W and

WP trials, respectively. Whilst it is clear that a sufficient volume of drink must be ingested to facilitate complete rehydration, these results demonstrate that the composition of the drink is of paramount importance for drink retention. These findings are consistent with numerous previous studies examining the post-exercise rehydration effects of different drinks (Shirreffs et al. 2007a; Shirreffs et al. 2007b; Evans et al. 2009; James et al. 2012). It seems that when rehydration drinks are ingested in a volume equivalent to 150% of sweat losses over 1 h, the addition of carbohydrate (Evans et al. 2009), protein (James et al. 2012) or potassium (Shirreffs et al. 2007a) might not sufficiently reduce urine production to prevent fluid balance from becoming negative in the hours after drinking. In contrast, the addition of sodium to a rehydration drink has been shown to consistently influence urine production in a dose-dependent manner (Maughan and Leiper 1995; Shirreffs et al. 1996; Merson et al. 2008).

There are a number of studies that have investigated the post-exercise rehydration effects of protein containing drinks (Seifert et al. 2006; Shirreffs et al. 2007b; Watson et al. 2008; James et al. 2011; James et al. 2012; James et al. 2013). It appears that ingestion of skimmed milk or a drink containing milk protein enhances rehydration after exercise-induced dehydration (Shirreffs et al. 2007b; Watson et al. 2008; James et al. 2011; James et al. 2013), but the effect of whey protein on post-exercise rehydration appears to be less consistent (Seifert et al. 2006; James et al. 2012).

Seifert et al. (2006) reported that a 60 $g \cdot L^{-1}$ carbohydrate, 15 $g \cdot L^{-1}$ whey protein drink was better retained than a 60 $g \cdot L^{-1}$ carbohydrate drink or mineral water, with drinks ingested in a volume equivalent to sweat losses. In contrast, James et al. (2012) reported that there was no difference in drink retention between a 50 $g \cdot L^{-1}$ carbohydrate, 15 $g \cdot L^{-1}$ whey protein drink and a 65 $g \cdot L^{-1}$ carbohydrate drink, with drinks ingested in a volume equivalent to 150% of sweat losses.

The difference in findings between the studies of Seifert et al. (2006) and James et al. (2012) might be explained by the difference in rehydration drink volume ingested (100% vs. 150%) or the fact that the energy density of the rehydration drinks was either matched (James et al. 2012) or unmatched (Seifert et al. 2006). In the present study, the rehydration drinks were not matched for energy density and were ingested in a volume equivalent to 150% of subjects sweat losses, which is in line with current recommendations (Sawka et al. 2007). In contrast to the study of Seifert et al. (2006), the additional energy provided as protein in the rehydration drink in the present study did not augment any increase in drink retention. This

suggests that the increased drink retention observed by Seifert et al. (2006) might be related to the lower drink volume ingested. Ingesting a lower drink volume produces a less pronounced dieresis after drink ingestion (Shirreffs et al. 1996) and it is possible that if the effects of whey protein on rehydration are only subtle, the volume induced diuresis caused by ingestion of 150% of sweat losses over 1 h in the present study and that of James et al. (2012) might have masked any beneficial effects of the added whey protein. Furthermore, the very low energy density of the drinks used in the present study would lead to rapid gastric emptying (Vist and Maughan 1994) and consequently a rapid appearance in the peripheral circulation and a larger diuresis compared to if carbohydrate was included in the drink (Osterberg et al. 2010). In a practical setting, where drinks are generally ingested more slowly after exercise, the rise in plasma albumin content 2-3 h after whey protein ingestion might benefit post-exercise rehydration, although this remains to be tested.

The mechanism by which whey protein might enhance post-exercise rehydration is currently unknown. When the volume of drink ingested is fixed, oral rehydration after exercise involves three interrelated processes. The drink must first empty from the stomach into the small intestine, then be absorbed from the small intestine into the peripheral circulation and finally must be retained within the body. The nutrients contained within a rehydration drink interact with one or more of these processes to affect whole body retention of the ingested drink. It is likely there are two main mechanisms by which nutrients might exert their effects on rehydration. Firstly by reducing the rate at which a drink moves through the gastrointestinal system, thus slowing the delivery of water to the circulation and reducing the hemodilution that occurs when a large volume of fluid is ingested; or secondly, through the inclusion of osmotically active nutrients that increase the amount of water retained once the drink reaches the circulation.

The rate at which a drink empties from the stomach after ingestion is linearly related to its energy density (Calbet and MacLean 1997) and thus increasing the energy content of a rehydration drink by adding energy containing nutrients will slow gastric emptying and delay the delivery of water to the peripheral circulation (Evans et al. 2011; Clayton et al. 2013). This delayed delivery of water to the circulation attenuates the decline in serum osmolality that occurs when a large volume of drink is ingested, reducing urine production after drinking (Osterberg et al. 2010; Evans et al 2011; Clayton et al. 2013). In contrast to this, the addition of sodium (the major cation in the extracellular space) to a rehydration drink prevents the decline in serum sodium concentration and osmolality that occurs with the ingestion of a low

sodium drink (Nose et al. 1988) and consequently reduces urine production (Nose et al. 1988; Maughan and Leiper 1995; Shirreffs and Maughan 1998; Merson et al. 2008).

In theory, as an energy containing macronutrient, the addition of whey protein to a rehydration drink should delay the rate at which the drink empties from the stomach (Calbet and MacLean 1997), thus slowing the delivery of water to the circulation. It is however unlikely that the addition of 20 $g \cdot L^{-1}$ whey protein isolate is a large enough increase in energy density to significantly alter gastric emptying rate or fluid delivery (Vist and Maughan 1994). In line with this and as observed by Seifert et al. (2006), recovery of plasma volume in the hours immediately after drinking was not different between trials in the present study, suggesting a similar rate of water delivery to the circulation. In contrast, in situations where gastric emptying is delayed, the recovery of plasma volume is also delayed (Evans et al. 2009; Evans et al. 2011; Clayton et al. 2013).

Another potential mechanism by which whey protein might increase drink retention is via an increase in osmotic/ oncotic pressure after drinking, which would be expected to decrease urine production. Both Seifert et al. (2006) and Watson et al. (2008) observed that plasma/ serum osmolality was greater after ingestion of a drink containing protein. Whilst there was no significant difference in serum osmolality between the W and WP trials in the present study, there was a tendency for a main effect of trial (P=0.062) and mean values were higher in the 3 h after drinking during WP. Ingestion of a protein containing drink increases circulating amino acid concentrations and Hall et al. (2003) reported that ingestion of 48 g whey protein increased total plasma amino acid concentrations by 1-2 $mmol \cdot L^{-1}$ for the 3 h after ingestion. Protein ingestion during WP of the present study was 43 (7) g, similar to that of Hall et al. (2003) and although not measured, it is reasonable to expect a similar increase in total plasma amino acid concentrations to that observed by Hall et al. (2003). It appears from the results of the present study that such an increase in plasma amino acid concentrations might not result in a large enough change in serum osmolality to influence urine production. As the main plasma protein, albumin is the major contributor to oncotic pressure and plasma albumin content is known to influence plasma volume (Francessconi et al. 1983). Carbohydrate-protein feeding (0.55 $g \cdot kg^{-1}$ carbohydrate, 0.18 $g \cdot kg^{-1}$ protein) immediately after a bout of high intensity exercise has been shown to influence plasma albumin content and plasma volume over a 23 h recovery period (Okazaki et al. 2009). Similarly, in the present study plasma albumin content was increased from 1 h after drinking during WP, a

difference that was not evident during W and this change in plasma albumin content likely explains why plasma volume was greater at 3 h and 4 h during WP compared to W.

Ingestion of a dilute, low-sodium rehydration drink in a volume equivalent to 150% of sweat losses over a 1 h period results in a rapid and pronounced diuresis in the 2 h after drinking (Fig 1.). Whilst the addition of whey protein in the present study increased plasma albumin content from 1 h onwards, this did not result in any change in urine production or fluid balance. It seems that the diuresis caused by the ingestion of such a large volume of drink (~2 L) over a short time period (*i.e.* 1 h) negates any effect that the rise in plasma amino acid concentrations or plasma albumin content might have on urine production. Ingesting a rehydration drink more slowly after dehydrating exercise has been shown to increase drink retention (Jones et al. 2010) and it is possible in situations where a rehydration drink is ingested in this manner, that the addition of whey protein might further augment post-exercise rehydration, but this remains to be investigated.

In conclusion, the present study provides novel data demonstrating that the addition of whey protein isolate to a drink (20 $g \cdot L^{-1}$) neither enhances nor inhibits the rehydration process after exercise when a volume equivalent to 150% of sweat losses is ingested in 1 h. This information is of relevance to the athlete or recreational exerciser as post-exercise nutritional requirements are often multifactorial (rehydration, glycogen resynthesis, myofibrillar/ mitochondrial protein synthesis) and the present data demonstrates that when post-exercise protein intake might benefit recovery or adaptation, this can be achieved without compromising rehydration.

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Table 1. Final composition of the rehydration drinks. Values are Mean (SD).

	W drink	WP drink
Energy ($kJ \cdot L^{-1}$)	0	342
Protein ($g \cdot L^{-1}$)	0	20
Carbohydrate ($g \cdot L^{-1}$)	0.02	0.02
Fat ($g \cdot L^{-1}$)	0.04	0.04
Sodium ($mmol \cdot L^{-1}$)	0.4 (0.0)	0.5 (0.0)
Potassium ($mmol \cdot L^{-1}$)	0.1 (0.0)	0.1 (0.0)
Osmolality ($mosmol \cdot kg^{-1}$)	2 (0)	14 (1)

Figure legends

Fig 1. Urine volume produced (mL) each hour after exercise for W (□) and WP (▲) trials. Points are mean values. Error bars represent SD. * point significantly different from -1 h.

Fig 2. Whole body net fluid balance (mL) for W (□) and WP (▲) trials. Points are mean values. Error bars represent SD. * point significantly different from pre-exercise.

Fig 3. Urine osmolality (mosmol·kg^{-1}) for W (□) and WP (▲) trials. Points are mean values. Error bars represent SD. * point significantly different from pre-exercise.

Fig 4. Serum osmolality (mosmol·kg^{-1}) for W (□) and WP (▲) trials. Points are mean values. Error bars represent SD. * point significantly different from pre-exercise.

Fig 5. Plasma albumin content (g·kg^{-1}) for W (□) and WP (▲) trials. Points are mean values. Error bars represent SD. * point significantly different from pre-exercise.

Fig 6. Change in plasma volume relative to pre-exercise (%) for W (□) and WP (▲) trials. Points are mean values. Error bars represent SD. * Point significantly different from pre-exercise. # Point significantly different from W trial.

Fig 1.

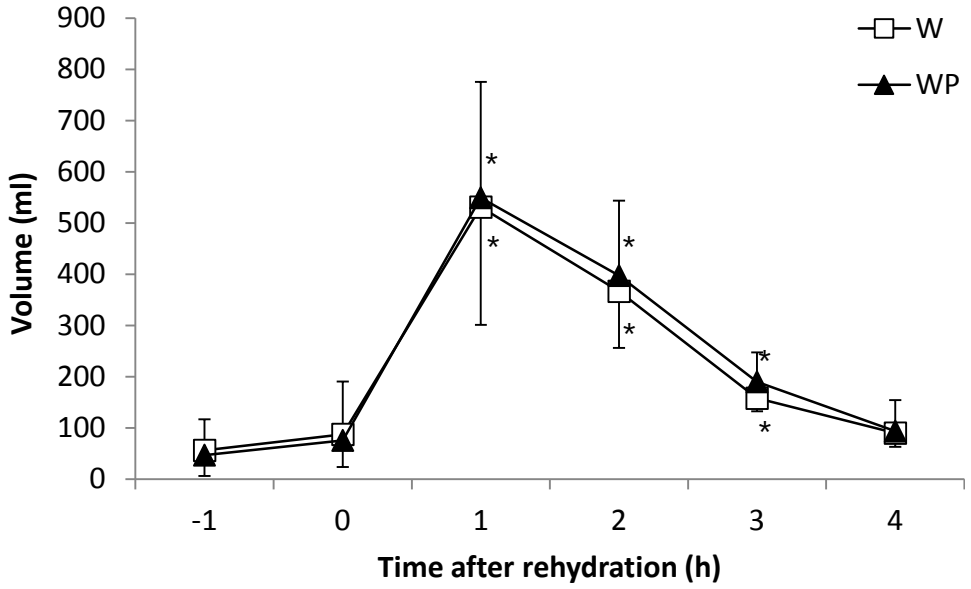

900
800
700
600
500
400
300
200
100
0
Volume (ml)
-1
0
1
2
3
4
Time after rehydration (h)
W
WP
*
*
*
*
*
*

Fig 2.

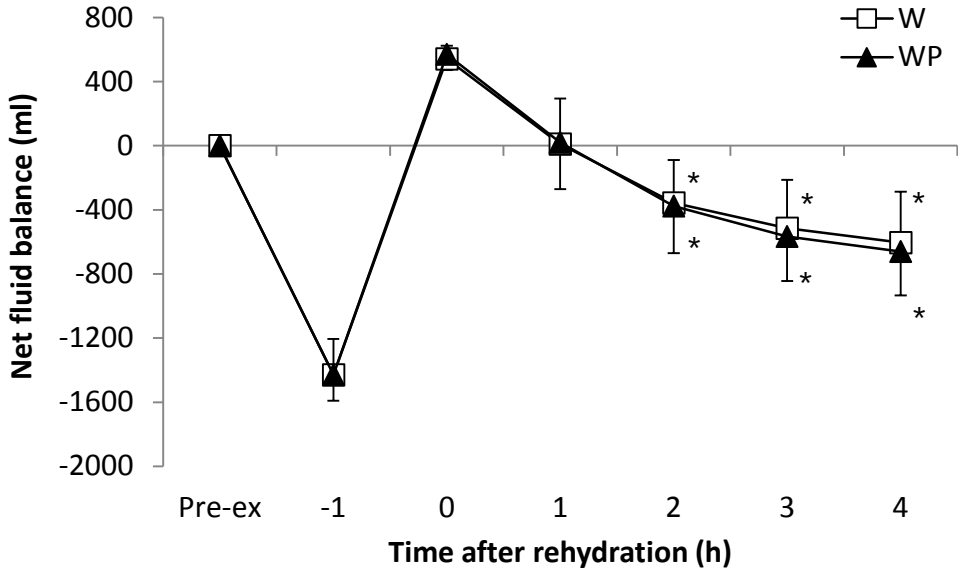

800
400
0
-400
-800
-1200
-1600
-2000
Net fluid balance (ml)
W
WP
*
*
*
*
*
*
Pre-ex
-1
0
1
2
3
4
Time after rehydration (h)

Fig 3.

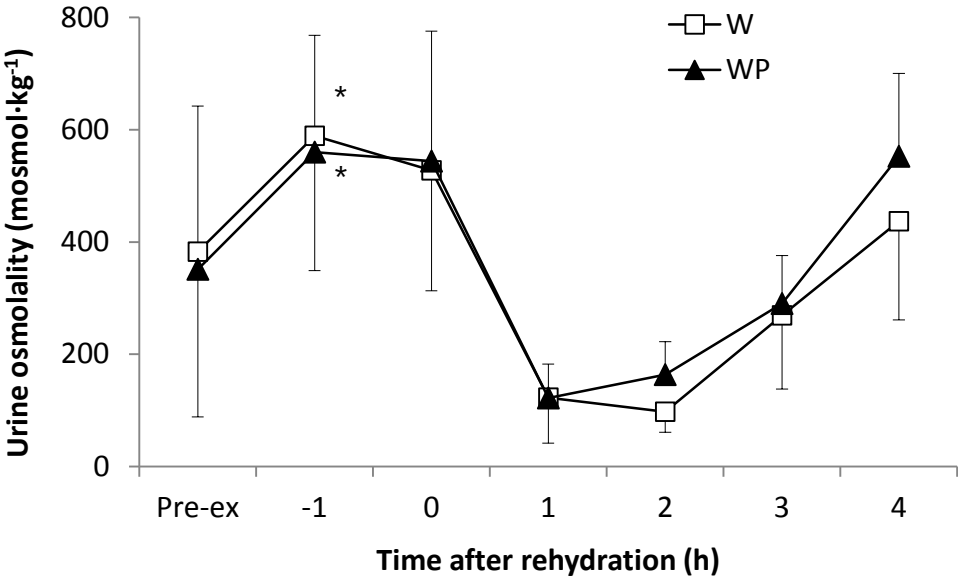

800
600
400
200
0
Urine osmolality (mosmol·kg-1)
W
WP
*
*
Pre-ex
-1
0
1
2
3
4
Time after rehydration (h)

Fig 4.

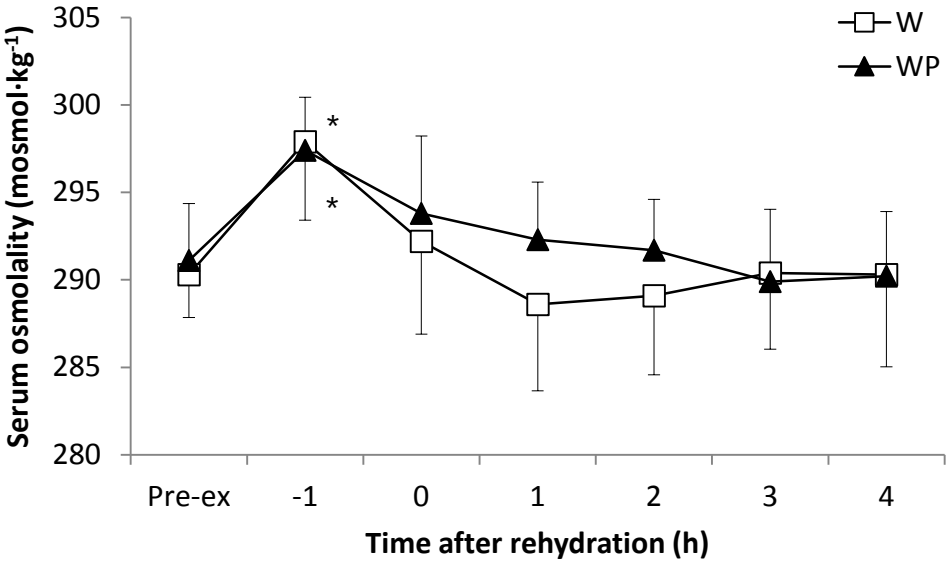

W
WP
305
300
295
290
285
280
Serum osmolality (mosmol·kg-1)
*
*
Pre-ex
-1
0
1
2
3
4
Time after rehydration (h)

Fig 5.

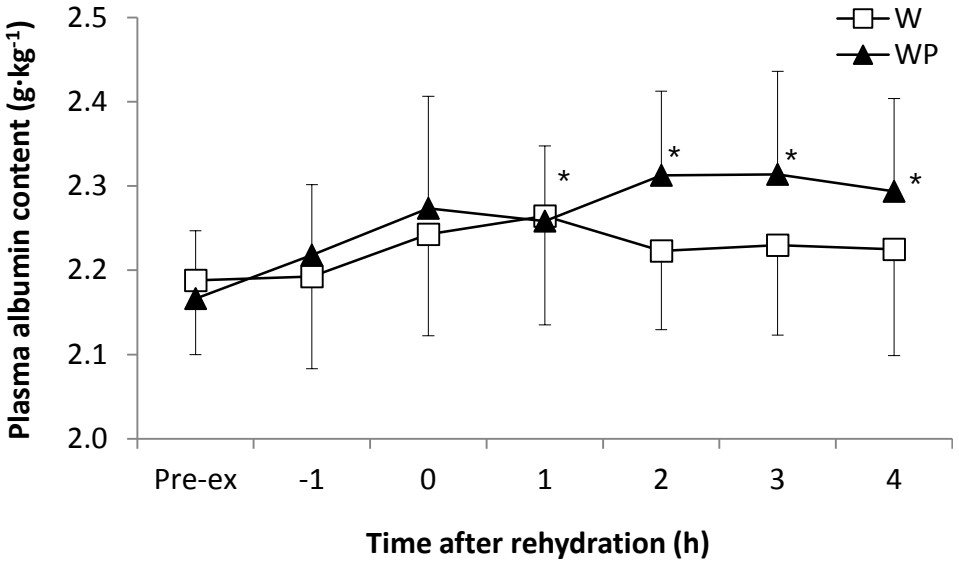
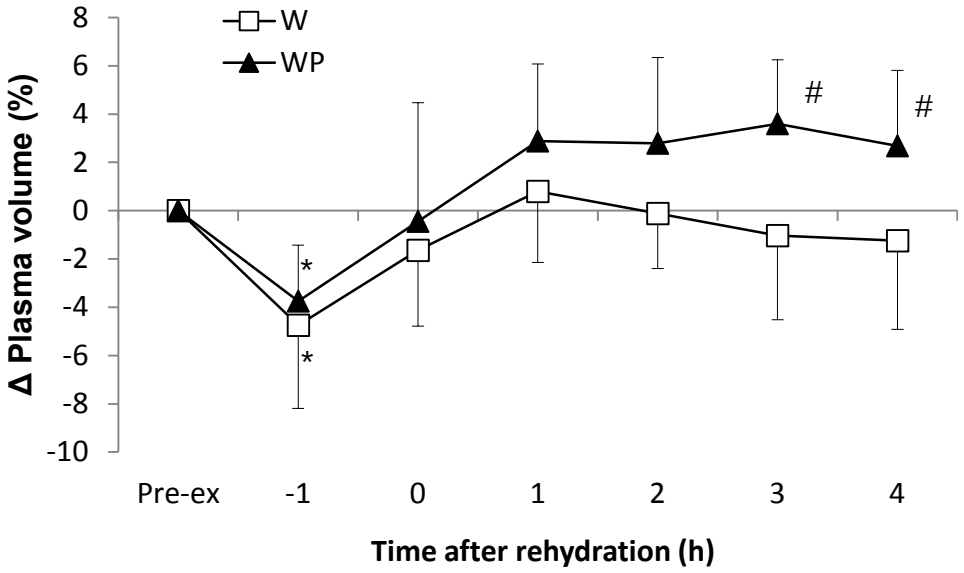

2.5
2.4
2.3
2.2
2.1
2.0
Plasma albumin content (g·kg-1)
W
WP
*
*
*
*
Pre-ex
-1
0
1
2
3
4
Time after rehydration (h)

Fig 6.


W
WP
8
6
4
2
0
-2
-4
-6
-8
-10
Δ Plasma volume (%)
*
*
#
#
Pre-ex
-1
0
1
2
3
4
Time after rehydration (h)