The addition of whey protein to a carbohydrate-electrolyte drink does not influence post-exercise rehydration.

Running Title: Carbohydrate, Protein and Rehydration

Abstract

The addition of whey protein to a carbohydrate-electrolyte drink has been shown to enhance post-exercise rehydration when a volume below that recommended for full fluid balance restoration is provided. We investigated if this held true when volumes sufficient to restore fluid balance were consumed, and if differences might be explained by changes in plasma albumin content. Sixteen participants lost ~1.9% of their pre-exercise body mass by cycling in the heat and rehydrated with 150% of body mass lost with either a 60 $g \cdot L^{-1}$ carbohydrate drink (CHO) or a 60 $g \cdot L^{-1}$ carbohydrate, 20 $g \cdot L^{-1}$ whey protein isolate drink (CHO-P). Urine and blood samples were collected pre-exercise, post-exercise, post-rehydration and every hour for 4 h post-rehydration. There was no difference between trials for total urine production (CHO 1057±319 mL; CHO-P 970±334 mL; P=0.209), drink retention (CHO 51±12%; CHO-P 55±15%; P=0.195) or net fluid balance (CHO -393±272 mL; CHO-P -307±331 mL; P=0.284). Plasma albumin content relative to pre-exercise was increased from 2-4 h during CHO-P only. These results demonstrate that the addition of whey protein isolate to a carbohydrate-electrolyte drink neither enhances nor inhibits rehydration. Therefore, where post-exercise protein ingestion might benefit recovery, this can be consumed without effecting rehydration.

Key Words: Fluid balance; Macronutrients; Hydration; Dehydration; Hypohydration; Plasma albumin**.**

Introduction

During prolonged exercise in a warm environment sweat losses generally exceed fluid intake, resulting in hypohydration (Shirreffs, Armstrong & Cheuvront, 2004). This makes post-exercise rehydration an important consideration for the training athlete, particularly when the time between exercise bouts is short, since incomplete rehydration may lead to a decrement in subsequent exercise performance (Judelson et al., 2007).

Rehydration can be separated into three main physiological phases: gastric emptying, intestinal absorption, and fluid retention. Several factors have been shown to influence the rate of gastric emptying for a drink including volume, osmolality and energy density (Vist & Maughan 1994; Vist & Maughan 1995). Intestinal absorption is also influenced by a number of interrelated factors such as availability/ efficiency of transporters and osmotic gradients between the intestine and the blood (Leiper & Maughan 1986; Shi et al., 1994). Finally, fluid retention is influenced by hormonal secretion, serum osmolality and osmotic/ oncotic pressures (Nose, Mack, Shi & Nadel, 1988).

The addition of protein to a rehydration drink has the potential to influence each physiological phase of the rehydration process. The rate of gastric emptying and thus delivery of fluid to the intestine is similar for isoenergetic protein and carbohydrate solutions (Maughan, Leiper & Vist, 2004). Protein is co-transported out of the intestine with sodium (Stevens, Kaunitz & Wright, 1984) and since protein and carbohydrate use non-

competing active sodium co-transporters across the intestinal wall, ingestion of both these macronutrients together may increase sodium uptake and enhance water absorption due to the greater osmotic gradient created (Seifert, Harmon & DeClercq, 2006). Finally, fluid retention might be enhanced with the addition of protein to a rehydration drink as it might prevent the rapid drop in blood osmolality and reduce urine output compared to a carbohydrate drink or water (Seifert et al., 2006).

While studies have shown that the addition of whey protein (Seifert et al., 2006) or milk protein (James, Clayton & Evans, 2011) to a carbohydrate rehydration drink might decrease urine production, the possible mechanisms of action remain unclear. Proposed potential mechanisms include proteins assisting in water and sodium absorption from the intestine (Wapnir, Wintertzahn & Teichberg, 1997), increased plasma protein synthesis resulting in higher oncotic pressure (Okazaki et al., 2009) or a slowing of gastric emptying. Increased water and sodium absorption will assist in the restoration of plasma volume and osmolality, while increased synthesis of plasma albumin, which is the main plasma protein, draws fluid into the vascular space. Both these effects will increase plasma volume, which might enhance the restoration of fluid balance after exercise. If the rate of gastric emptying is slowed, then the rate of water delivery to the circulation might be reduced and the diuresis attenuated (Clayton, Evans & James, In press).

Seifert et al. (2006) reported that adding 15 $g \cdot L^{-1}$ of whey protein to a 60 $g \cdot L^{-1}$ carbohydrate-electrolyte drink consumed in a volume equal to 100% of body mass loss after dehydrating exercise increased drink retention. In contrast, James, Gingell and Evans (2012) observed no difference in post-exercise rehydration between a 65 $g \cdot L^{-1}$ carbohydrate drink and a drink containing 50 $g \cdot L^{-1}$ carbohydrate plus 15 $g \cdot L^{-1}$ whey protein isolate when the volume of drink consumed was equivalent to 150% body mass loss. The difference in findings between these two studies might be related to differences in the volume of drink ingested or the energy density of the drinks.

For complete and rapid rehydration, current recommendations are to ingest a volume of drink equivalent to 150% of fluid lost during exercise. Post-exercise nutritional requirements are often multifactorial in nature, and frequently carbohydrate to stimulate glycogen resynthesis and protein to stimulate protein synthesis, as well as water for rehydration will be required. Therefore the purpose present study was to investigate whether whey protein isolate added to a carbohydrate-electrolyte drink affects the retention of a rehydration drink when ingested in a volume equal to 150% of fluid lost during exercise and if this was via an increase in plasma albumin content.

Methods

Participants

Sixteen participants (13 male, 3 female; age 24±6 y; height 1.75±0.08 m; body mass 75.8±13.5 kg) gave their written informed consent to participate in this study, which was approved by the Nottingham Trent University Ethical Advisory Committee. Participants completed a medical screening questionnaire and female participants also completed a menstrual cycle questionnaire to determine the length of their menstrual cycle. Participants completed a familiarisation trial and two experimental trials, separated by at least 1 week for males and an appropriate amount of time to standardise menstrual cycle phase for females. The familiarisation trial followed the same protocol as the experimental trials (described below), with a shortened (1 h) monitoring period.

Participants recorded their diet and physical activity for the 24 h preceding the first experimental trial and replicated these conditions before the second trial. Participants were instructed to refrain from any strenuous exercise or alcohol in the 24 h before experimental trials.

Protocol

Experimental trials commenced in the morning after an overnight fast (~10 h), with the exception of 500 mL water ingested 1.5 h before arrival at the laboratory. Upon arrival participants assumed a seated position and after 15 min a 7.5 mL venous blood sample was taken by venepuncture of an antecubital vein. A urine sample was then provided, before body mass (in underwear only) was measured to the nearest 0.01 kg (Adam CFW 150 scale; Adam Equipment Co Ltd., Milton Keynes, UK). Following this,

participants 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). Participants exercised in blocks of 10 min, separated by 5 min rest, during which they were re-weighed. Initial exercise intensity was ~2 W·kg body mass^{-1} and participants continued until they had lost 1.7% of their pre-exercise body mass. Participants then showered and dried, before being re-weighed to determine their total body mass loss. A 20g plastic cannula was then inserted into an antecubital vein and after 15 min seated rest a second 7.5 mL blood sample was drawn, after which participants provided another urine sample (-1 h).

Over a 1 h period participants were then rehydrated with a 60 g·L^{-1} carbohydrate drink (CHO) or a 60 g·L^{-1} carbohydrate, 20 g·L^{-1} whey protein isolate drink (CHO-P) (Volactive Hydrapro; Volac International Ltd., Orwell, UK) (Table 1.). 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). Drinks were made up using bottled mineral water (Volvic; Danone UK Ltd., London, UK). The 60 g·L^{-1} carbohydrate in both drinks was made up of 30 g·L^{-1} glucose (Myprotein.co.uk, Manchester, UK) and 30 g·L^{-1} maltodextrin (Myprotein.co.uk, Manchester, UK). Sodium chloride was also added to drinks to give a final sodium concentration of ~25 mmol·L^{-1}. Drinks had similar sodium concentration (CHO 26±2 mmol·L^{-1}; CHO-P 26±2 mmol·L^{-}

^{1}) and potassium concentration (CHO 1.3±0.3 mmol·L^{-1}; CHO-P 1.3±0.3 mmol·L^{-1}), but osmolality was greater for CHO-P (329±4 mosmol·kg^{-1}) than CHO (312±4 mosmol·kg^{-1}) (P<0.001). 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 (0, 15, 30 and 45 min). At the end of the 1 h rehydration period, participants rated the taste characteristics of the drinks. Questions asked were how 'sweet', 'salty', 'bitter' and 'pleasant' does the drink taste? And were assessed using a 100 mm visual analogue scale, with the verbal anchors 'not at all' at 0 mm and 'extremely' at 100 mm. Participants then remained in the laboratory for a 4 h monitoring period during which further blood (7.5 mL) and urine samples were collected at the end of the rehydration period (0 h) and every hour thereafter (1 h, 2 h, 3 h and 4 h). Finally, participants body mass was measured at the end of the trial. All blood samples were drawn after 15 min in an upright seated position.

Sample collection and analysis

Blood samples were drawn into dry syringes and 1.3 mL of blood was mixed with EDTA (1.75 mg·L^{-1}) and used for the analysis of haemoglobin by the cyanmethaemoglobin method (Sigma-Aldrich Company Ltd., Gilliangham, UK) and haematocrit by microcentrifugation. Haemoglobin and haematocrit values were used to estimate changes in plasma volume relative to the pre-exercise sample (Dill & Costill, 1974). A further 1.3 mL was dispensed into a pre-chilled tube containing 1.75 mg·L^{-1} EDTA and

was placed in ice, before plasma was separated by centrifugation (3000 g, 10 min, 4°C) and stored at -80°C. The remainder of each blood sample was dispensed into a plain tube and allowed to clot, before serum was separated by centrifugation (3000 g, 10 min, 4°C). Plasma was analysed for glucose concentration using the glucose oxidase peroxidase amino antipyrine phenol method (ABX Pentra 400; Horiba Medical, Northampton, UK) and albumin concentration using the bromocresol green method (ABX Pentra 400; Horiba Medical, Northampton, UK). Serum was analysed for osmolality by freezing point depression (Gonotec Osmomat 030 Cryoscopic Osmometer; Gonotec, Berlin, Germany).

For each urine sample, participants were instructed to completely empty their bladder and collect the entire volume produced. Sample volume was measured, with a sample retained and analysed for osmolality by freezing point depression. Drink samples were also analysed for osmolality by freezing point depression, as well as for sodium and potassium concentration by flame photometry (Corning Clinical Flame Photometer 410C; Corning Ltd., Essex, UK).

Statistical analysis and calculations

Data were analysed using IBM SPSS Statistics v20 (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 (*e.g.* 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 mean±1SD.

Net fluid balance (NFB) was calculated relative to pre-exercise, at which time participants were assumed to be in NFB. NFB at each time point was determined using fluid lost through sweat 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. Pre-exercise, participants 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 variables

Pre-exercise body mass (CHO 75.78±13.19 kg; CHO-P 75.91±13.45 kg; $P=0.471$), urine osmolality (CHO 356±225 mosmol·kg^{-1}; CHO-P 387±248 mosmol·kg^{-1}; $P=0.378$) and serum osmolality (CHO 288±3 mosmol·kg^{-1};

CHO-P 288±4 mosmol·kg^{-1}; P=0.862) were not different between trials, indicating that participants started both trials in a similar state of hydration.

Exercise variables

Body mass loss during exercise was similar in both trials (CHO 1.45±0.23 kg; CHO-P 1.44±0.21 kg), equating to an overall reduction of 1.92±0.1% and 1.91±0.13% body mass loss for CHO and CHO-P (P=0.660). As such, drink volume consumed during the rehydration phase, calculated based on body mass loss, was also not different between trials (CHO 2166±354 mL; CHO-P 2166±314 mL; P=1.000).

Urine variables and fluid balance

The total volume of urine produced after drinking was not different between trials (CHO 1057±319 mL; CHO-P 970±334 mL; P=0.209), meaning that 51±12% (CHO) and 55±15% (CHO-P) of the ingested drinks were retained (P=0.195). The volume of urine produced each hour during the study showed a main effect of time (P<0.001), but no main effect of trial (P=0.419) or interaction effect (P=0.217). Compared to -1 h, urine volume was increased at 1-3 h during both trials (P<0.05; Figure 1).

There was a main effect of time (P<0.001), but no main effect of trial (P=0.284) or interaction effect (P=0.213) for NFB (Figure 2). Compared to pre-exercise, NFB was decreased at -1 h and increased at 0 h during both trials (P<0.001) and was then negative at 3 h and 4 h during CHO

($P<0.01$) and 4 h during CHO-P ($P<0.05$). There was a main effect of time ($P<0.001$) and an interaction effect ($P<0.05$), but no main effect of trial ($P=0.285$) for urine osmolality (Table 2).

Blood variables

There was a main effect of time ($P<0.001$) and an interaction effect ($P<0.001$), but no main effect of trial ($P<0.785$) for plasma glucose concentration (Table 2). For serum osmolality there was no main effect of trial ($P=0.723$) or interaction effect ($P=0.258$), but there was a main effect of time ($P<0.001$; Table 2). For change in plasma volume (Figure 3), there was a main effect of time ($P<0.001$), a tendency for a main effect of trial ($P=0.087$) and no interaction effect ($P=0.218$). Compared to pre-exercise, plasma volume was decreased at -1 h during both trials ($P<0.001$) and increased at 1-4 h during CHO-P ($P<0.05$) and 2-4 h during CHO ($P<0.01$).

There was a main effect of time ($P<0.001$), but no main effect of trial ($P=0.458$) or interaction effect ($P=0.944$) for plasma albumin concentration (data not shown). Plasma albumin concentration compared to pre-exercise was increased at -1 h during both trials ($P<0.05$). For plasma albumin content (Figure 4) there was a main effect of time ($P<0.001$), and a tendency for a main effect of trial ($P=0.086$), but no interaction effect ($P=0.448$). Compared to pre-exercise, plasma albumin content was increased at 2 h, 3 h and 4 h during CHO-P ($P<0.05$), but did not change significantly during CHO.

Drink perception

Participants perceived the CHO drink to be more pleasant than the CHO-P drink (71±14 vs. 51±21 mm; $P<0.01$) and they perceived the CHO-P drink to be more bitter than the CHO drink (27±18 vs. 20±13 mm; $P<0.01$), but there was no perceived difference for sweetness ($P=0.771$) or saltiness ($P=0.689$).

Discussion

The main aim of this study was to investigate if the addition of whey protein isolate to a carbohydrate electrolyte drink influenced rehydration. We hypothesised that drink retention would be greater on the CHO-P trial compared to the CHO trial, and that this would be due to the role of plasma albumin in plasma volume expansion. While there was a tendency for an increased plasma albumin content and an increased plasma volume as hypothesised, the extent of this increase would appear to have been insufficient to elicit changes in net fluid balance after the consumption of a rehydration drink containing 60 $g \cdot L^{-1}$ carbohydrate and 20 $g \cdot L^{-1}$ whey protein isolate in a volume equivalent to 150% of sweat losses when consumed over 1 h compared to a drink containing only 60 $g \cdot L^{-1}$ carbohydrate.

Participants were in negative net fluid balance on both trials by the end of the study, despite consuming fluid volumes equivalent to 150% of losses,

in line with the current recommendations (Sawka et al., 2007). This is similar to the level of negative fluid balance shown by James et al. (2012), where energy matched carbohydrate and carbohydrate protein drinks were ingested. The addition of macronutrients such as carbohydrate or protein to rehydration drinks may not be sufficient to prevent net fluid balance becoming negative in the hours post rehydration with volumes equivalent to 150% body mass loss. Conversely, the addition of sodium to rehydration drinks has been shown to influence drink retention in a dose dependent manner (Maughan & Leiper 1995; Shirreffs & Maughan 1998). Drinking large volumes of fluids with no/ low sodium content can cause a diuresis (Shirreffs, Taylor, Leiper & Maughan, 1996) and so it is possible that the 25 $mmol \cdot L^{-1}$ sodium concentration used in the CHO and CHO-P trials was not sufficient to restore/ maintain net fluid balance, although this is a similar sodium concentration to that used by Seifert et al. (2006), who did not show a large drink induced diuresis.

An apparent drink induced diuresis, such as seen here and by James et al. (2012), could mask any potential benefits of adding whey protein to rehydration drinks. Blunting the extent of the drink induced diuresis, possibly through drinking a reduced volume or drinking at a slower rate over a longer time period (Jones, Bishop, Green & Richardson, 2010), may result in a detectable effect of added whey protein. The present study aimed to replicate the findings of Seifert et al. (2006), but using a drink volume equivalent to 150% of body mass loss, in line with current recommendations (Sawka et al., 2007). Due to the larger volume to be

consumed, participants were given an increased time limit in which to consume the drink, in an attempt to avoid a diuresis, which was not seen by Seifert et al. (2006). Interestingly however, the volume and rate of consumption of the drinks in the study by Seifert and colleagues could be expected to cause a substantial drink induced diuresis. Although using a volume equivalent to 100% of mass loss, this was only 400-500 mL less (1662±519 mL CHO and 1726±662 mL CP) than was consumed by participants in the present study where 150% of mass loss was used (2166±354 mL CHO and 2166±314 mL CHO-P). Furthermore, the entire volume was consumed over a 20 minute period in the study by Seifert et al. (2006), equating to a drinking rate of ~84 $mL \cdot min^{-1}$, rather than the 60 minute rehydration period employed in the present study which equated to an average drinking rate of ~36 $mL \cdot min^{-1}$. That said, it is likely to be the rate of delivery of fluid to the circulation rather than the rate of drinking that influences drink retention. Clearly the interplay between volume, composition and rate of consumption of rehydration drinks is complex and warrants further investigation in order to prevent or minimise any diuresis which occurs as a result of a flawed rehydration strategy.

In the present study we did not match the energy density of the two drinks, as has been done in previous studies (James et al., 2011; James et al., 2012; James et al., 2013), in an attempt to replicate the findings of Seifert et al. (2006). Potential mechanisms for the purported actions of protein enhancing fluid retention during rehydration are likely to be

329 related to alterations in gastric emptying, intestinal absorption and/ or
330 fluid retention.

331 With regard to the gastric emptying phase, the CHO drink would be
332 expected to empty from the stomach faster than the CHO-P drink, since
333 the rate of gastric emptying has a linear relationship with energy density
334 (Calbet & MacLean 1997). A slower rate of gastric emptying, and
335 therefore intestinal absorption, might delay alterations in plasma
336 osmolality, thereby minimising diuresis and allowing greater drink
337 retention when a carbohydrate protein drink is consumed. Indeed, Seifert
338 et al. (2006) showed a significantly greater serum osmolality during the
339 carbohydrate-protein trial compared to either the carbohydrate or water
340 trials, suggesting that energy density, and its influence on gastric
341 emptying, may be the determining factor for the beneficial influence of
342 protein on rehydration drinks. However, the data presented here do not
343 support the theory that energy density is a main factor determining a
344 difference in fluid retention between carbohydrate and carbohydrate
345 protein rehydration drinks as there was no difference between the two
346 trials for serum osmolality, nor for net fluid balance.

347 With regard to the intestinal absorption phase of rehydration, glucose and
348 protein are both transported across the intestinal wall by sodium
349 transporters. Therefore, it could be that rehydration drinks containing
350 both macronutrients need to contain a greater concentration of sodium to
351 allow maximal absorption in a similar time frame to a glucose only drink,

and thereby increase fluid retention. However, in the present study both the drinks had a sodium concentration of 25 mmol·L^{-1} which is similar to the concentrations used by Seifert et al. (2006), and more than that used by James et al. (2011), both of whom showed a difference in fluid retention. This suggests that an increase in sodium concentration may not be required when protein is added to carbohydrate rehydration drinks.

A potential increase in the osmotic/ oncotic pressure after drinking a rehydration drink containing carbohydrate and protein rather than just carbohydrate would be expected to decrease urine production and therefore influence the fluid retention phase of rehydration. As the main plasma protein, albumin is the major contributor to oncotic pressure and plasma albumin content is known to influence plasma volume (Francessconi, Sawka, Hubbard & Mager, 1983). Indeed, in the present study there is a trend for a higher plasma albumin content on the CHO-P trial compared to the CHO trial (P=0.086), and a trend for a greater plasma volume on the CHO-P trial compared to the CHO trial (P=0.087). Plasma albumin content was increased compared to pre-exercise during the CHO-P trial only from 2-4 h post-rehydration, a difference not observed during the CHO trial. As the majority of the drinking induced diuresis occurred in the 2 h post-rehydration (Figure 1), it appears that the time course of changes in plasma albumin content might not have been rapid enough to enhance rehydration, in the present study design.

The consumption of protein increases circulating amino acid concentrations. In the present study participants consumed approximately 43 g of whey protein over the 1 hour rehydration period, and although not measured here, this could be expected to increase total amino acid concentration by 1-2 $mmol \cdot L^{-1}$ in the hours after drinking (Hall, Millward, Long & Morgan, 2003). However, it would appear that this level of increase in plasma amino acid concentration might not be enough to alter serum osmolality to a sufficient extent to exert influence over urine production and therefore net fluid balance was unaffected by the addition of whey protein to the carbohydrate drink.

Finally, in the present study the CHO drink was rated as ~20% more pleasant than the CHO-P drink, which may affect *ad libitum* fluid intake in a free living setting. *Ad libitum* intake is vital in determining the efficacy of a rehydration drink and therefore, the palatability of any rehydration drink containing protein should be considered during manufacture.

Conclusion

These results suggest that the addition of whey protein isolate to a carbohydrate-electrolyte rehydration drink does not enhance rehydration when a volume equal to 150% of body mass loss is consumed. Since this study also shows that the addition of whey protein isolate to a carbohydrate-electrolyte rehydration drink does not inhibit rehydration, in situations where the ingestion of protein after exercise might infer some

benefit for post-exercise recovery, whey protein isolate can be added to rehydration drinks without interfering with the rehydration process.

Conflict of interest

This study was funded by Volac International Ltd., Orwell, UK. The authors have no other conflict of interest to declare.

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482 **Tables**

483 **Table 1.** Final composition of the rehydration drinks. Values are mean±
484 SD.

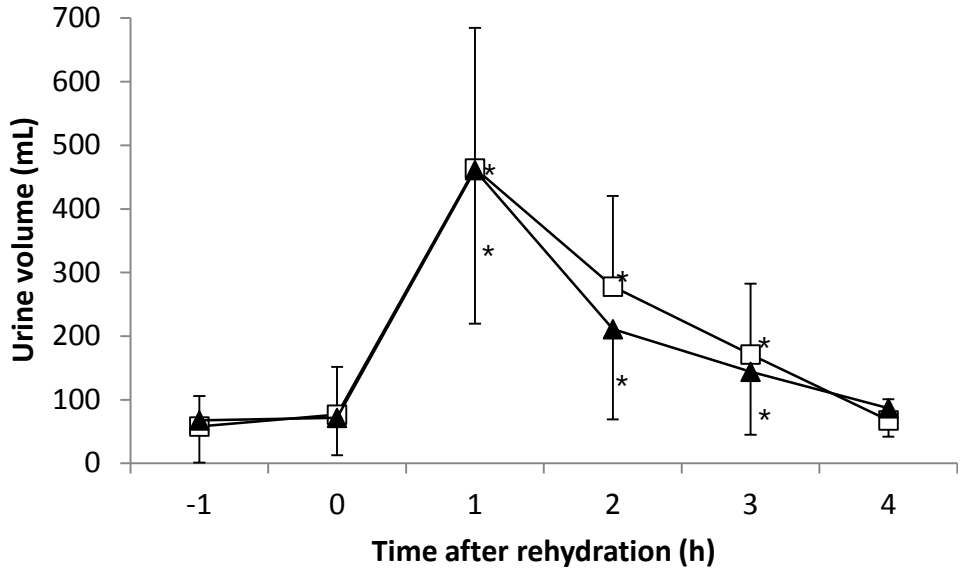
	CHO	CHO-P
Energy ($kJ \cdot L^{-1}$)	1064±0	1406±0
Protein ($g \cdot L^{-1}$)	0.4±0	20.4±0
Carbohydrate ($g \cdot L^{-1}$)	62.2±0	62.2±0
Fat ($g \cdot L^{-1}$)	0±0	0±0
Sodium ($mmol \cdot L^{-1}$)	26±2	26 (2)
Potassium ($mmol \cdot L^{-1}$)	1.3±0.3	1.3±0.3
Osmolality ($mosmol \cdot kg^{-1}$)	312±4	329±4

485

Table 2. Urine osmolality (mosmol·kg^{-1}), serum osmolality (mosmol·kg^{-1}) and plasma glucose concentration (mmol·L^{-1}). Values are mean±SD. * denotes a significant difference from pre-exercise. # denotes a significant difference from CHO.

	Pre-ex	-1 h	0 h	1 h	2 h	3 h	4 h
Urine osmolality (mosmol·kg^{-1})							
CHO	356 ±225	593 * ±224	614 * ±221	135 * ±75	212 ±101	331 ±171	583 ±192
CHO-P	387 ±248	589 * ±212	582 * ±199	160 * ±119	317 # ±168	466 # ±201	567 ±158
Serum osmolality (mosmol·kg^{-1})							
CHO	288 ±3	295 * ±3	292 * ±4	288 ±5	289 ±4	287 ±4	287 ±4
CHO-P	288 ±4	293 * ±4	292 * ±5	291 * ±4	289 ±4	288 ±5	287 ±4
Plasma glucose concentration (mmol·L^{-1})							
CHO	4.43 ±0.33	4.57 ±0.86	6.96 * ±2.08	5.63 * ±0.89	4.37 ±1.16	4.06 ±0.71	3.99 ±0.67
CHO-P	4.50 ±0.38	4.69 ±0.47	6.01 # ±1.70	5.26 * ±0.91	4.98 # ±0.70	4.24 ±0.73	4.17 * ±0.42

492 **Figures**

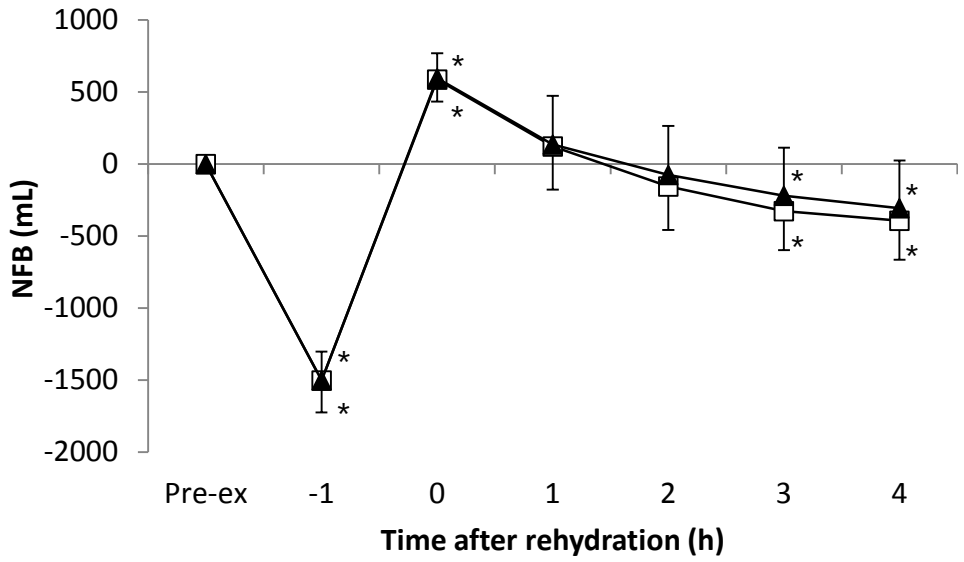


493

494 Figure 1. Mean urine volume (mL) produced each hour after exercise on
495 CHO (□) and CHO-P (▲) trials. Error bars represent SD and * denotes a
496 significant difference from -1 h.

497

498



499

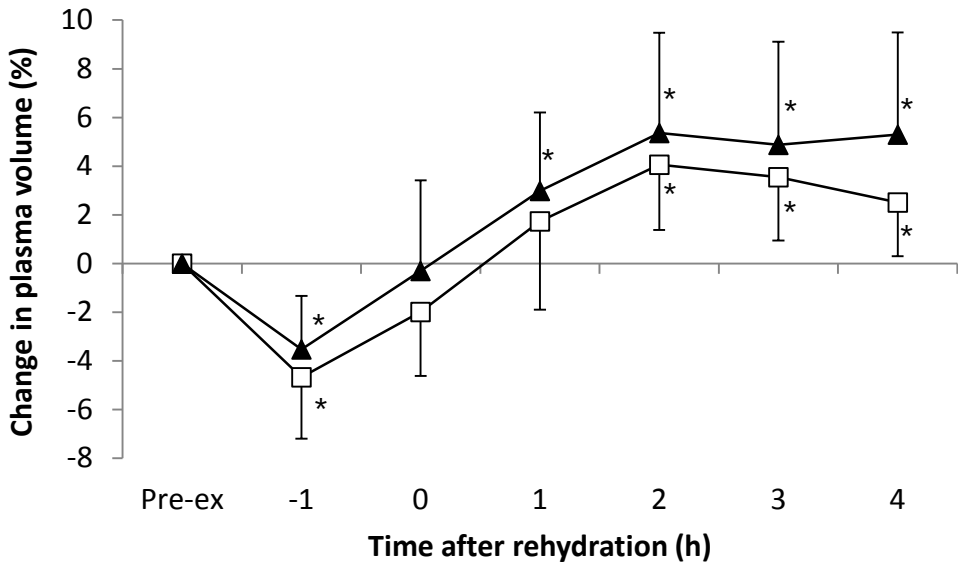
500 Figure 2. Mean whole body net fluid balance (mL) on CHO (□) and CHO-P

501 (▲) trials. Error bars represent SD and * denotes a significant difference

502 from pre-exercise.

503

504

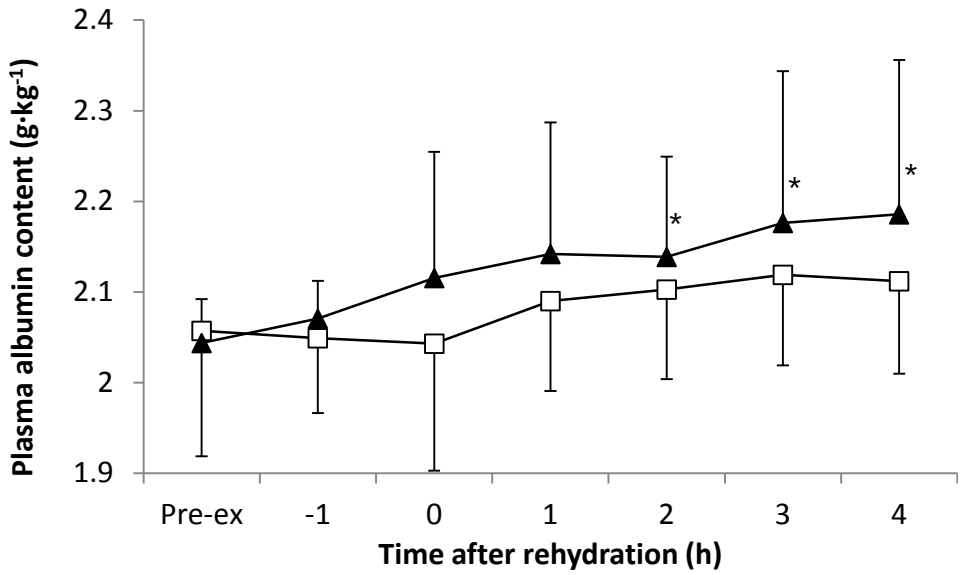


505

506 Figure 3. Mean change in plasma volume relative to pre-exercise (%) on
507 CHO (□) and CHO-P (▲) trials. Error bars represent SD and * denotes a
508 significant difference from pre-exercise.

509

510



511

512 Figure 4. Mean plasma albumin content ($g \cdot kg^{-1}$) on CHO (□) and CHO-P

513 (▲) trials. Error bars represent SD and * denotes a significant difference

514 from pre-exercise.

515