

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

3

4 **Running Title:** Carbohydrate, Protein and Rehydration

## 5 **Abstract**

6 The addition of whey protein to a carbohydrate-electrolyte drink has been  
7 shown to enhance post-exercise rehydration when a volume below that  
8 recommended for full fluid balance restoration is provided. We  
9 investigated if this held true when volumes sufficient to restore fluid  
10 balance were consumed, and if differences might be explained by changes  
11 in plasma albumin content. Sixteen participants lost ~1.9% of their pre-  
12 exercise body mass by cycling in the heat and rehydrated with 150% of  
13 body mass lost with either a 60 g·L<sup>-1</sup> carbohydrate drink (CHO) or a 60  
14 g·L<sup>-1</sup> carbohydrate, 20 g·L<sup>-1</sup> whey protein isolate drink (CHO-P). Urine and  
15 blood samples were collected pre-exercise, post-exercise, post-  
16 rehydration and every hour for 4 h post-rehydration. There was no  
17 difference between trials for total urine production (CHO 1057±319 mL;  
18 CHO-P 970±334 mL; *P*=0.209), drink retention (CHO 51±12%; CHO-P  
19 55±15%; *P*=0.195) or net fluid balance (CHO -393±272 mL; CHO-P -  
20 307±331 mL; *P*=0.284). Plasma albumin content relative to pre-exercise  
21 was increased from 2-4 h during CHO-P only. These results demonstrate  
22 that the addition of whey protein isolate to a carbohydrate-electrolyte  
23 drink neither enhances nor inhibits rehydration. Therefore, where post-  
24 exercise protein ingestion might benefit recovery, this can be consumed  
25 without effecting rehydration.

26

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

## 29 **Introduction**

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

36 Rehydration can be separated into three main physiological phases:  
37 gastric emptying, intestinal absorption, and fluid retention. Several  
38 factors have been shown to influence the rate of gastric emptying for a  
39 drink including volume, osmolality and energy density (Vist & Maughan  
40 1994; Vist & Maughan 1995). Intestinal absorption is also influenced by a  
41 number of interrelated factors such as availability/ efficiency of  
42 transporters and osmotic gradients between the intestine and the blood  
43 (Leiper & Maughan 1986; Shi et al., 1994). Finally, fluid retention is  
44 influenced by hormonal secretion, serum osmolality and osmotic/ oncotic  
45 pressures (Nose, Mack, Shi & Nadel, 1988).

46 The addition of protein to a rehydration drink has the potential to  
47 influence each physiological phase of the rehydration process. The rate of  
48 gastric emptying and thus delivery of fluid to the intestine is similar for  
49 isoenergetic protein and carbohydrate solutions (Maughan, Leiper & Vist,  
50 2004). Protein is co-transported out of the intestine with sodium (Stevens,  
51 Kaunitz & Wright, 1984) and since protein and carbohydrate use non-

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

59 While studies have shown that the addition of whey protein (Seifert et al.,  
60 2006) or milk protein (James, Clayton & Evans, 2011) to a carbohydrate  
61 rehydration drink might decrease urine production, the possible  
62 mechanisms of action remain unclear. Proposed potential mechanisms  
63 include proteins assisting in water and sodium absorption from the  
64 intestine (Wapnir, Wintertzahn & Teichberg, 1997), increased plasma  
65 protein synthesis resulting in higher oncotic pressure (Okazaki et al.,  
66 2009) or a slowing of gastric emptying. Increased water and sodium  
67 absorption will assist in the restoration of plasma volume and osmolality,  
68 while increased synthesis of plasma albumin, which is the main plasma  
69 protein, draws fluid into the vascular space. Both these effects will  
70 increase plasma volume, which might enhance the restoration of fluid  
71 balance after exercise. If the rate of gastric emptying is slowed, then the  
72 rate of water delivery to the circulation might be reduced and the diuresis  
73 attenuated (Clayton, Evans & James, In press).

74 Seifert et al. (2006) reported that adding 15 g·L<sup>-1</sup> of whey protein to a 60  
75 g·L<sup>-1</sup> carbohydrate-electrolyte drink consumed in a volume equal to 100%  
76 of body mass loss after dehydrating exercise increased drink retention. In  
77 contrast, James, Gingell and Evans (2012) observed no difference in post-  
78 exercise rehydration between a 65 g·L<sup>-1</sup> carbohydrate drink and a drink  
79 containing 50 g·L<sup>-1</sup> carbohydrate plus 15 g·L<sup>-1</sup> whey protein isolate when  
80 the volume of drink consumed was equivalent to 150% body mass loss.  
81 The difference in findings between these two studies might be related to  
82 differences in the volume of drink ingested or the energy density of the  
83 drinks.

84

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

95

96 **Methods**

## 97 *Participants*

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

109

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

## 114 *Protocol*

115 Experimental trials commenced in the morning after an overnight fast  
116 (~10 h), with the exception of 500 mL water ingested 1.5 h before arrival  
117 at the laboratory. Upon arrival participants assumed a seated position and  
118 after 15 min a 7.5 mL venous blood sample was taken by venepuncture of  
119 an antecubital vein. A urine sample was then provided, before body mass  
120 (in underwear only) was measured to the nearest 0.01 kg (Adam CFW  
121 150 scale; Adam Equipment Co Ltd., Milton Keynes, UK). Following this,

122 participants then exercised on a cycle ergometer (Monark Ergomedic 874E;  
123 Cranlea, Birmingham, UK) in a temperature (35°C) and humidity (60%  
124 relative humidity) controlled environment (Design Environmental Ltd.,  
125 Ebbw Vale, UK). Participants exercised in blocks of 10 min, separated by  
126 5 min rest, during which they were re-weighed. Initial exercise intensity  
127 was  $\sim 2 \text{ W}\cdot\text{kg body mass}^{-1}$  and participants continued until they had lost  
128 1.7% of their pre-exercise body mass. Participants then showered and  
129 dried, before being re-weighed to determine their total body mass loss. A  
130 20g plastic cannula was then inserted into an antecubital vein and after  
131 15 min seated rest a second 7.5 mL blood sample was drawn, after which  
132 participants provided another urine sample (-1 h).

133 Over a 1 h period participants were then rehydrated with a  $60 \text{ g}\cdot\text{L}^{-1}$   
134 carbohydrate drink (CHO) or a  $60 \text{ g}\cdot\text{L}^{-1}$  carbohydrate,  $20 \text{ g}\cdot\text{L}^{-1}$  whey  
135 protein isolate drink (CHO-P) (Volactive Hydrapro; Volac International Ltd.,  
136 Orwell, UK) (Table 1.). The composition of the protein powder per 100 g  
137 powder was: 89 g protein, 0.1 g carbohydrate, 0.2 g fat, 20 mg sodium,  
138 10 mg potassium, 10 mg chloride (data supplied by the manufacturer).  
139 Drinks were made up using bottled mineral water (Volvic; Danone UK Ltd.,  
140 London, UK). The  $60 \text{ g}\cdot\text{L}^{-1}$  carbohydrate in both drinks was made up of  $30$   
141  $\text{g}\cdot\text{L}^{-1}$  glucose (Myprotein.co.uk, Manchester, UK) and  $30 \text{ g}\cdot\text{L}^{-1}$  maltodextrin  
142 (Myprotein.co.uk, Manchester, UK). Sodium chloride was also added to  
143 drinks to give a final sodium concentration of  $\sim 25 \text{ mmol}\cdot\text{L}^{-1}$ . Drinks had  
144 similar sodium concentration (CHO  $26 \pm 2 \text{ mmol}\cdot\text{L}^{-1}$ ; CHO-P  $26 \pm 2 \text{ mmol}\cdot\text{L}^{-1}$ ).



145 <sup>1</sup>) and potassium concentration (CHO  $1.3 \pm 0.3$  mmol·L<sup>-1</sup>; CHO-P  $1.3 \pm 0.3$   
146 mmol·L<sup>-1</sup>), but osmolality was greater for CHO-P ( $329 \pm 4$  mosmol·kg<sup>-1</sup>)  
147 than CHO ( $312 \pm 4$  mosmol·kg<sup>-1</sup>) ( $P < 0.001$ ). The volume of drink ingested  
148 was 150% of the total body mass loss and was ingested in four aliquots of  
149 equal volume at 15 min intervals (0, 15, 30 and 45 min). At the end of  
150 the 1 h rehydration period, participants rated the taste characteristics of  
151 the drinks. Questions asked were how 'sweet', 'salty', 'bitter' and  
152 'pleasant' does the drink taste? And were assessed using a 100 mm visual  
153 analogue scale, with the verbal anchors 'not at all' at 0 mm and  
154 'extremely' at 100 mm. Participants then remained in the laboratory for a  
155 4 h monitoring period during which further blood (7.5 mL) and urine  
156 samples were collected at the end of the rehydration period (0 h) and  
157 every hour thereafter (1 h, 2 h, 3 h and 4 h). Finally, participants body  
158 mass was measured at the end of the trial. All blood samples were drawn  
159 after 15 min in an upright seated position.

#### 160 *Sample collection and analysis*

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

168 was placed in ice, before plasma was separated by centrifugation (3000 g,  
169 10 min, 4°C) and stored at -80°C. The remainder of each blood sample  
170 was dispensed into a plain tube and allowed to clot, before serum was  
171 separated by centrifugation (3000 g, 10 min, 4°C). Plasma was analysed  
172 for glucose concentration using the glucose oxidase peroxidase amino  
173 antipyrine phenol method (ABX Pentra 400; Horiba Medical, Northampton,  
174 UK) and albumin concentration using the bromocresol green method (ABX  
175 Pentra 400; Horiba Medical, Northampton, UK). Serum was analysed for  
176 osmolality by freezing point depression (Gonotec Osmomat 030  
177 Cryoscopic Osmometer; Gonotec, Berlin, Germany).

178 For each urine sample, participants were instructed to completely empty  
179 their bladder and collect the entire volume produced. Sample volume was  
180 measured, with a sample retained and analysed for osmolality by freezing  
181 point depression. Drink samples were also analysed for osmolality by  
182 freezing point depression, as well as for sodium and potassium  
183 concentration by flame photometry (Corning Clinical Flame Photometer  
184 410C; Corning Ltd., Essex, UK).

#### 185 *Statistical analysis and calculations*

186 Data were analysed using IBM SPSS Statistics v20 (Chicago, IL, USA). All  
187 data were checked for normality of distribution using a Shapiro-Wilk test.  
188 Data containing two factors were then analysed using a two-way repeated  
189 measures ANOVA. The Mauchly test was used, and where it indicated that  
190 the assumption of sphericity had been violated, the degrees of freedom

191 for the data set were corrected using the Greenhouse-Geisser estimate.  
192 Significant differences were located using Bonferroni adjusted paired t-  
193 tests for normally distributed data or Bonferroni-adjusted Wilcoxon  
194 signed-ranked tests for non-normally distributed data. Variables  
195 containing one factor (*e.g.* drink perception) were analysed using paired  
196 t-tests or Wilcoxon signed-ranks tests as appropriate.  $P \leq 0.05$  was used to  
197 determine statistical significance. Data are presented as mean  $\pm$  1SD.

198 Net fluid balance (NFB) was calculated relative to pre-exercise, at which  
199 time participants were assumed to be in NFB. NFB at each time point was  
200 determined using fluid lost through sweat during exercise (estimated from  
201 total body mass loss during exercise) and cumulative urine production,  
202 and fluid gained through drink ingestion. Albumin content was determined  
203 using plasma albumin concentration and the change in plasma volume.  
204 Pre-exercise, participants were assumed to have a plasma volume (in L)  
205 equivalent to 5% of body mass (in kg) and plasma volume at each time  
206 point was determined using this pre-exercise plasma volume and the  
207 relative change in plasma volume.

208

## 209 **Results**

### 210 *Pre-trial variables*

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

214 CHO-P  $288 \pm 4$  mosmol $\cdot$ kg $^{-1}$ ;  $P=0.862$ ) were not different between trials,  
215 indicating that participants started both trials in a similar state of  
216 hydration.

#### 217 *Exercise variables*

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

#### 224 *Urine variables and fluid balance*

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

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

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

### 239 *Blood variables*

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

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

259 *Drink perception*

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

265

266 **Discussion**

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

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

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

296 An apparent drink induced diuresis, such as seen here and by James et al.  
297 (2012), could mask any potential benefits of adding whey protein to  
298 rehydration drinks. Blunting the extent of the drink induced diuresis,  
299 possibly through drinking a reduced volume or drinking at a slower rate  
300 over a longer time period (Jones, Bishop, Green & Richardson, 2010),  
301 may result in a detectable effect of added whey protein. The present  
302 study aimed to replicate the findings of Seifert et al. (2006), but using a  
303 drink volume equivalent to 150% of body mass loss, in line with current  
304 recommendations (Sawka et al., 2007). Due to the larger volume to be

305 consumed, participants were given an increased time limit in which to  
306 consume the drink, in an attempt to avoid a diuresis, which was not seen  
307 by Seifert et al. (2006). Interestingly however, the volume and rate of  
308 consumption of the drinks in the study by Seifert and colleagues could be  
309 expected to cause a substantial drink induced diuresis. Although using a  
310 volume equivalent to 100% of mass loss, this was only 400-500 mL less  
311 ( $1662 \pm 519$  mL CHO and  $1726 \pm 662$  mL CP) than was consumed by  
312 participants in the present study where 150% of mass loss was used  
313 ( $2166 \pm 354$  mL CHO and  $2166 \pm 314$  mL CHO-P). Furthermore, the entire  
314 volume was consumed over a 20 minute period in the study by Seifert et  
315 al. (2006), equating to a drinking rate of  $\sim 84$  mL $\cdot$ min $^{-1}$ , rather than the  
316 60 minute rehydration period employed in the present study which  
317 equated to an average drinking rate of  $\sim 36$  mL $\cdot$ min $^{-1}$ . That said, it is likely  
318 to be the rate of delivery of fluid to the circulation rather than the rate of  
319 drinking that influences drink retention. Clearly the interplay between  
320 volume, composition and rate of consumption of rehydration drinks is  
321 complex and warrants further investigation in order to prevent or  
322 minimise any diuresis which occurs as a result of a flawed rehydration  
323 strategy.

324 In the present study we did not match the energy density of the two  
325 drinks, as has been done in previous studies (James et al., 2011; James  
326 et al., 2012; James et al., 2013), in an attempt to replicate the findings of  
327 Seifert et al. (2006). Potential mechanisms for the purported actions of  
328 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,

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

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

374 The consumption of protein increases circulating amino acid  
375 concentrations. In the present study participants consumed approximately  
376 43 g of whey protein over the 1 hour rehydration period, and although not  
377 measured here, this could be expected to increase total amino acid  
378 concentration by 1-2 mmol·L<sup>-1</sup> in the hours after drinking (Hall, Millward,  
379 Long & Morgan, 2003). However, it would appear that this level of  
380 increase in plasma amino acid concentration might not be enough to alter  
381 serum osmolality to a sufficient extent to exert influence over urine  
382 production and therefore net fluid balance was unaffected by the addition  
383 of whey protein to the carbohydrate drink.

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

389

## 390 **Conclusion**

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

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

399

400 **Conflict of interest**

401 This study was funded by Volac International Ltd., Orwell, UK. The  
402 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  $\pm$   
484 SD.

|                                       | CHO           | CHO-P         |
|---------------------------------------|---------------|---------------|
| Energy (kJ·L <sup>-1</sup> )          | 1064 $\pm$ 0  | 1406 $\pm$ 0  |
| Protein (g·L <sup>-1</sup> )          | 0.4 $\pm$ 0   | 20.4 $\pm$ 0  |
| Carbohydrate (g·L <sup>-1</sup> )     | 62.2 $\pm$ 0  | 62.2 $\pm$ 0  |
| Fat (g·L <sup>-1</sup> )              | 0 $\pm$ 0     | 0 $\pm$ 0     |
| Sodium (mmol·L <sup>-1</sup> )        | 26 $\pm$ 2    | 26 (2)        |
| Potassium (mmol·L <sup>-1</sup> )     | 1.3 $\pm$ 0.3 | 1.3 $\pm$ 0.3 |
| Osmolality (mosmol·kg <sup>-1</sup> ) | 312 $\pm$ 4   | 329 $\pm$ 4   |

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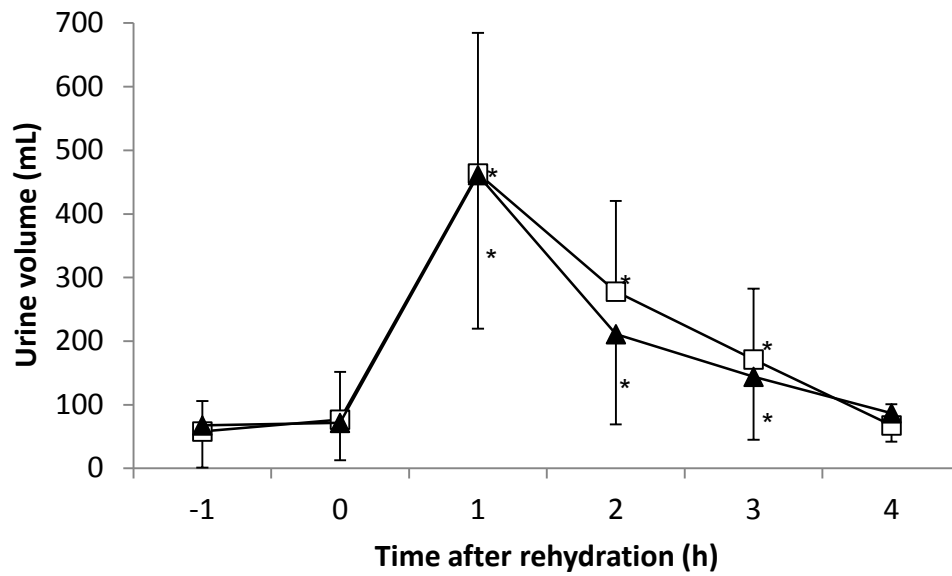


486 **Table 2.** Urine osmolality ( $\text{mosmol}\cdot\text{kg}^{-1}$ ), serum osmolality ( $\text{mosmol}\cdot\text{kg}^{-1}$ )  
 487 and plasma glucose concentration ( $\text{mmol}\cdot\text{L}^{-1}$ ). Values are mean $\pm$ SD. \*  
 488 denotes a significant difference from pre-exercise. # denotes a significant  
 489 difference from CHO.

490

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

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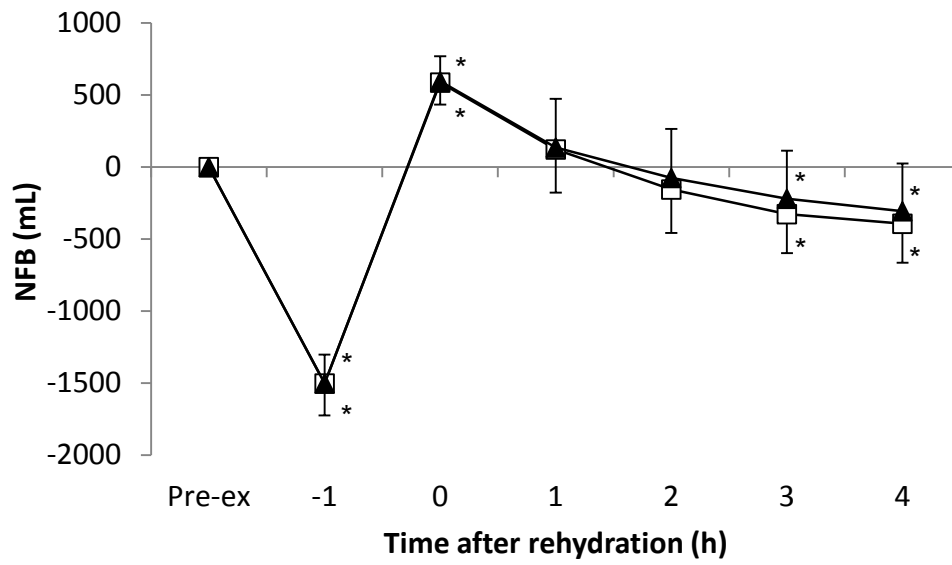
492 **Figures**

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

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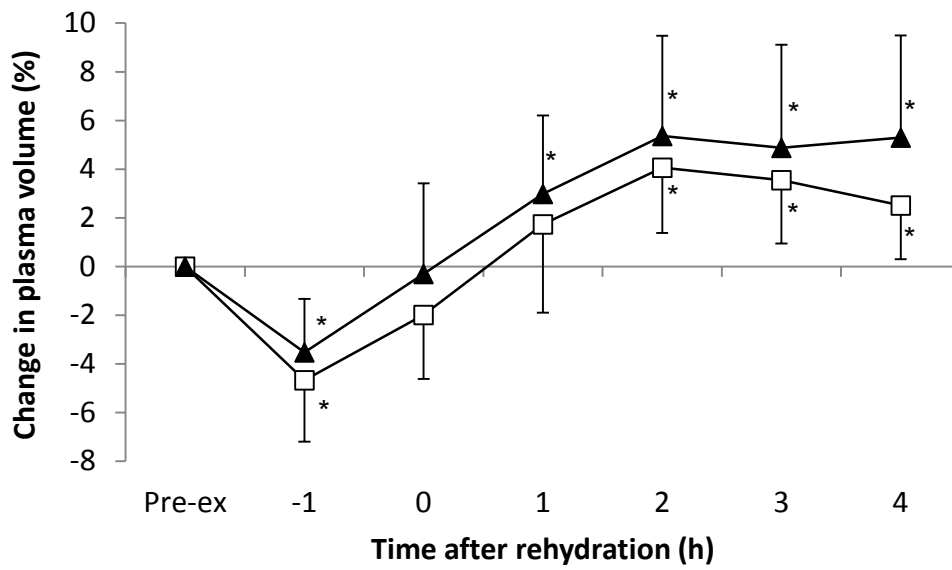


499

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.

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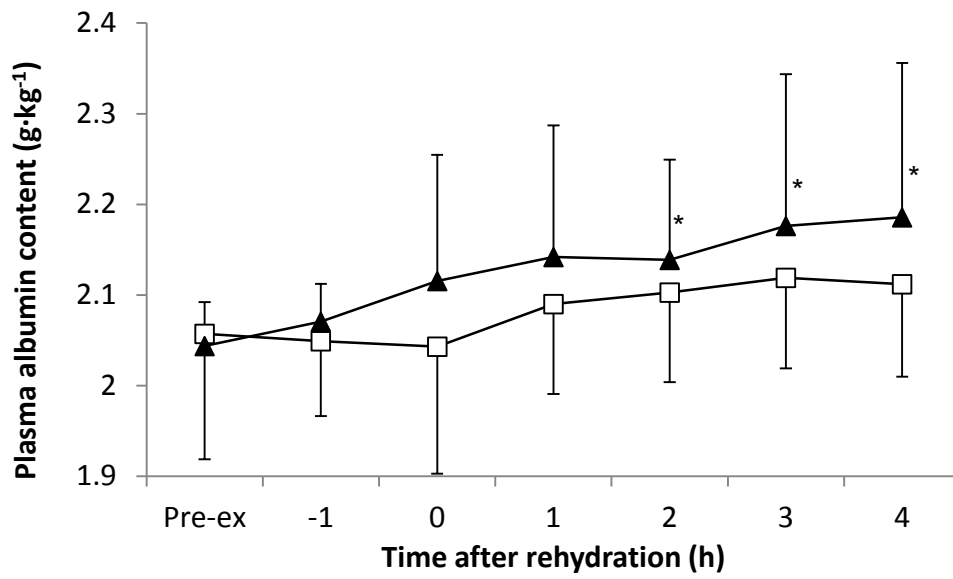


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506 Figure 3. Mean change in plasma volume relative to pre-exercise (%) on  
507 CHO (□) and CHO-P (▲) trials. Error bars represent SD and \* denotes a  
508 significant difference from pre-exercise.

509

510



511

512 Figure 4. Mean plasma albumin content (g·kg<sup>-1</sup>) on CHO (□) and CHO-P

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

514 from pre-exercise.

515