

1 **The effect of post-exercise drink macronutrient content on appetite and energy intake**

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21 **Abstract**

22 Carbohydrate and protein ingestion post-exercise are known to facilitate muscle glycogen
23 resynthesis and protein synthesis, respectively, but the effects of post-exercise nutrient intake
24 on subsequent appetite are unknown. This study aimed to investigate whether protein induced
25 satiety that has been reported at rest was still evident when pre-loads were consumed in a
26 post-exercise context. Using a randomized, double blind, crossover design, 12 unrestrained
27 healthy males completed 30 min of continuous cycling exercise at ~60% VO_2peak , followed
28 by five, 3 min intervals at ~85% VO_2peak . Ten min post-exercise, subjects consumed 500 ml
29 of either a low energy placebo (15 kJ) (PLA); a 6% whey protein isolate drink (528 kJ)
30 (PRO); or a 6% sucrose drink (528 kJ) (CHO). Sixty min after drink ingestion, a homogenous
31 *ad-libitum* pasta lunch was provided and energy intake at this lunch was quantified.
32 Subjective appetite ratings were measured at various stages of the protocol. Energy consumed
33 at the *ad-libitum* lunch was lower after PRO (5831 ± 960 kJ) than PLA (6406 ± 492 kJ)
34 ($P < 0.05$), but not different between CHO (6111 ± 901 kJ) and the other trials ($P > 0.315$).
35 Considering the post-exercise drink, total energy intake was not different between trials
36 ($P = 0.383$). There were no differences between trials for any of the subjective appetite ratings.
37 The results demonstrate that where post-exercise liquid protein ingestion may enhance the
38 adaptive response of skeletal muscle, and this may be possible without affecting gross energy
39 intake relative to consuming a low energy drink.

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44 Key words: Satiety, Protein, Pre-load, Intermittent exercise, Energy balance

45 **Introduction**

46 The maintenance of a stable body weight is achieved through careful balance between energy
47 intake and energy expenditure. However, mismanagement of this balance on a global scale
48 has led to an increase in the prevalence of obesity and obesity related comorbidities (Malik,
49 Willett, & Hu, 2013; Finucane *et al.*, 2011). Exercise and energy restriction are commonly
50 used to create energy deficits during weight loss programs, but these methods appear to have
51 disparate effects on appetite and subsequent energy intake (King *et al.*, 2011). Energy intake
52 appears to be unaffected by an acute bout of exercise, although chronic exercise programs
53 appear to induce some level of compensation (Blundell *et al.* 2003). By contrast, acute energy
54 restriction has been shown to markedly increase feelings of hunger and energy intake (Hubert,
55 King, & Blundell, 1998). Increased feelings of hunger are cited as a key factor culminating in
56 poor dietary adherence (Dansinger, Gleason, Griffith, Selker, & Schaefer, 2005), and as such,
57 developing methods to suppress hunger and energy intake, whilst inducing a negative energy
58 balance, should be the primary goal of modern weight management programmes.

59 Following exercise, the consumption of fluid helps restore any plasma volume losses (Nose,
60 Mack, Shi, & Nadal, 1988; Shirreffs, Taylor, & Leiper, 1996), and the addition of protein to
61 post-exercise drinks might aid post-exercise rehydration (James, 2012), as well as being
62 critically important for myofibrillar and mitochondrial protein synthesis (Wilkinson *et al.*,
63 2008). From a weight management perspective, it is also important to consider whether
64 consuming energy in a post-exercise recovery drink will weaken the energy deficit induced
65 by the exercise session, and how accurately the energy contained in the drink will be
66 compensated for during subsequent feeding.

67 High protein diets have been shown to promote greater feelings of satiety than normal protein
68 diets, whilst promoting losses in body fat and preservation of lean body mass (Leidy *et al.*

69 2007). Significant evidence also exists that acute protein feeding at rest enhances satiety (Hill
70 & Blundell, 1986; Stubbs, van Wyk, Johnstone, & Harbron, 1996) and reduces subsequent
71 energy intake (Poppitt, McCormack, & Buffenstein, 1998; Porrini *et al.*, 1997; Araya, Hills,
72 Alvina, & Vera 2000) compared to carbohydrate and fat. Additionally, protein has an
73 increased thermogenic effect compared to carbohydrate and fat (Feinman and Fine, 2004)
74 which may further decrease energy balance by increasing energy expenditure. Whilst there
75 may be differences in food rheology between providing energy in liquid or solid form,
76 several studies have demonstrated that a liquid protein meal also suppresses appetite and
77 reduces acute energy intake compared to an isoenergetic carbohydrate or water control
78 (Anderson & Moore, 2004; Bowen, Noakes, Trenerry, & Clifton, 2006a; Bertenshaw, Lluich,
79 & Yeomans, 2008; Astbury, Stevenson, Morris, Taylor, & McDonald, 2010). Conversely,
80 other studies have reported no difference in energy intake between protein and carbohydrate
81 pre-loads (Bowen, Noakes, & Clifton, 2007), as well as between low dose whey protein
82 drinks and water (Poppitt *et al.* 2011). Whilst several studies have failed to observe any
83 attenuation in energy intake, the majority of studies have reported an increase in subjective
84 perceptions of satiety after consuming protein containing drinks (Harper, James, Flint, &
85 Astrup, 2007; Bowen *et al.*, 2007; Poppitt *et al.* 2011). This suggests that the consumption of
86 protein containing drinks leads to enhanced satiety which may affect food intake or food
87 choices (i.e. reduced snacking) under free-living conditions (Poppitt *et al.*, 2011).

88 A recent meta-analysis stated that studies utilising interventions that combine exercise with
89 dietary restriction are the most successful for long term, sustainable weight loss and
90 maintenance (Franz *et al.*, 2007). High intensity intermittent exercise is characterised by brief
91 vigorous exercise bouts interspersed with periods of rest, and has been shown to be a time-
92 efficient and enjoyable training method for cardiovascular and skeletal muscle adaptations,
93 linked to improved health outcomes (Gibala, Little, McDonald & Hawley, 2012; Bartlett *et al.*

94 2011). Both dietary restriction and exercise have an influence on appetite, and whilst the
95 acute appetite response to a protein pre-load provided at rest has been well researched, no
96 studies have attempted to investigate this in combination with exercise. Due to the popularity
97 of consuming commercial protein and carbohydrate drinks after exercise, the aim of this
98 study was to assess whether the macronutrient content of a drink has any effect on subsequent
99 appetite and energy intake following 60 minute exercise session consisting of endurance and
100 high-intensity intermittent exercise. As protein consumption at rest has been shown to
101 attenuate subsequent energy intake, it was hypothesised that consuming protein in a post-
102 exercise recovery drink may lead to a reduction in energy intake at a subsequent meal. These
103 is some evidence to suggest that chronic exercise may increase energy intake in some
104 individuals (Blundell *et al.* 2003), and as such the consumption of a protein containing drink
105 after exercise may have the potential to offset this effect, therefore becoming an effective aid
106 for weight loss and management. A 30 g dose of protein has been shown to maximally
107 stimulate muscle protein synthesis after exercise (Moore *et al.* 2009; Witard *et al.* 2014) and
108 whey protein has been shown to attenuate appetite to a greater extent than other forms of
109 protein (Hall, Millward, Long, & Morgan, 2003) Therefore, in this study a 6% (500 ml) whey
110 protein isolate drink was compared to an isoenergetic carbohydrate drink and low energy
111 placebo.

112 **Methods**

113 *Subjects*

114 After ethical approval subjects completed a medical screening questionnaire, a three-factor
115 eating questionnaire (Stunkard & Messick, 1985) and provided written consent. Subjects
116 were twelve healthy, weight stable, recreationally active males (mean \pm SD) (age: 24 ± 2 y,
117 weight: 71.2 ± 5.7 kg, height: 1.75 ± 0.05 m, BMI: 23.2 ± 1.4 kg·m⁻², VO_{2peak}: 52 ± 8 ml·kg⁻¹·
118 min⁻¹). Subjects were not restrained, disinhibited or hungry eaters.

119 *Preliminary trials*

120 Subjects completed two preliminary trials. During the first, they completed a discontinuous
121 incremental exercise test on an electrically braked cycle ergometer (Lode Corival, Groningen,
122 Holland) to determine peak oxygen consumption (VO_{2peak}). Increments lasted 4 min, were
123 separated by ~5 min rest and work load increased until volitional exhaustion. Expired air was
124 collected into a Douglas Bag during the last min of each increment, whilst heart rate (Polar
125 Beat, Kempele, Finland) and rating of perceived exertion (RPE) (Borg, 1973) were measured
126 at the end of each increment. Expired air was analysed for O₂ and CO₂ concentration
127 (Servomex 1440 Gas Analyser, Sussex, UK), volume (Harvard Dry Gas meter, Harvard
128 Apparatus Ltd, Kent, UK) and temperature (Edale, Cambridge, UK).

129 During the second preliminary trial, subjects completed a full replication of an experimental
130 trial including the *ad-libitum* pasta meal, with water ingested as the post-exercise drink.

131 *Pre-trial standardisation*

132 Subjects completed a weighed food diary in the 24 h preceding the first experimental trial and
133 replicated this in the 24 h before each subsequent trial. Strenuous exercise and alcohol
134 ingestion were not permitted during this period.

135 On the day of each experimental trial subjects consumed a standard breakfast providing 15%
136 of estimated energy requirements (RMR (Mifflin *et al.*, 1990) multiplied by 1.7) 2 h before
137 exercise commenced. This amounted to 1810 ± 80 kJ and is consistent with the absolute
138 amount of energy provided at breakfast in studies of this nature (Bertenshaw *et al.*, 2008;
139 Poppitt *et al.*, 2011; Bertenshaw *et al.*, 2013). The breakfast consisted of cereal (Rice Snaps,
140 Tesco, Cheshunt, UK) and semi-skimmed milk (Tesco, Cheshunt, UK) in a ratio of 30 g
141 cereal: 125 ml milk. Water was permitted *ad-libitum* and recorded on the morning of the first
142 trial until subjects arrived at the lab, and was then repeated prior to subsequent trials.

143 *Experimental design*

144 Participants arrived at the laboratory between 9.30-10.30am and voided their bladder and
145 bowels, before nude body mass was measured. Subjects then completed 30 min steady state
146 cycling exercise at $\sim 60\%$ VO_{2peak} followed by five min rest and then five 3 min intervals at
147 $\sim 85\%$ VO_{2peak} , each separated by 2 min rest. Total exercise time was therefore 60 min.
148 Expired air was collected between 14-15 min and 29-30 min steady state exercise and during
149 the final minute of the third and fifth interval. Heart rate and RPE were measured at 15 min
150 and 30 min during steady state exercise and at the end of each interval. Subjects consumed
151 100 ml of water at 15 min, and prior to intervals one, three and five.

152 Upon completion of exercise, nude body mass was measured and subjects assumed a seated
153 position. Ten minutes post-exercise, subjects were provided with a recovery drink (Table 1)
154 to consume within five minutes and an *ad-libitum* lunch was provided 75 minutes post-
155 exercise whilst subjects rested in a comfortable environment ($23.5 \pm 1.8^{\circ}C$).

156 The lunch meal was designed to closely match UK dietary guidelines for macronutrient
157 proportions, and consisted of pasta, cheese, tomato sauce and olive oil (Tesco, Cheshunt, UK).

158 The meal was homogenous in nature and provided 7.87 ± 0.1 $kJ \cdot g^{-1}$ (14% protein, 53%

159 carbohydrate, 33% fat). Subjects ate in a custom built isolated feeding booth to prevent any
160 distractions and to allow food to be provided by an experimenter with minimal interaction.
161 Subjects were instructed to ‘eat until comfortably full and satisfied’ and they had 30 min in
162 which to eat. Food was made up in excess of expected consumption, distributed into five
163 bowls and warmed before being provided to subjects. Fresh warm food was provided to
164 subjects before they had finished each bowl to ensure that finishing a bowl did not serve as a
165 satiety cue. *Ad-libitum* water intake was permitted during lunch. Food and water intake was
166 quantified by weighing bowls and glasses before and after consumption. Subjects remained in
167 the feeding area for the entire 30 min and then rested in the laboratory for 60 min before
168 being allowed to leave.

169 *Post-exercise drinks*

170 Subjects completed three experimental trials with a different post-exercise recovery drink
171 consumed during each trial (Table 1). Drinks investigated were; a whey protein isolate
172 solution (Volactive Hydrapro, Volac International Ltd., Orwell, UK) providing 30g of whey
173 protein (PRO), an energy matched sucrose (Tate and Lyle, London, UK) solution (CHO) or a
174 placebo solution (PLA). The composition of the protein powder per 100 g powder was: 91.7
175 g protein, 0.1 g carbohydrate, 0.2 g fat, 20 mg sodium, 10 mg potassium, 10 mg chloride
176 (data supplied by the manufacturer). Drinks were prepared the evening before experimental
177 trials and were refrigerated overnight (4°C). Each drink contained 425 ml of water mixed
178 with 75 ml of lemon squash (Tesco, Cheshunt, UK), was served in an opaque container and
179 was ingested through a straw to minimise any visual or olfactory differences between the
180 drinks. Trials were separated by at least one week and administered in a double-blind,
181 randomised, counterbalanced manner. Subjects were aware that the study was assessing
182 different post-exercise recovery drink compositions, but were not informed what the drinks
183 contained. At the end of the study, subjects were informed about the contents of the

184 experimental drinks, and asked whether they could tell any differences between the drinks
185 and on which visit they thought they consumed each drink. Four out of twelve subjects stated
186 they could taste a difference between the drinks, but only one subject correctly identified the
187 drinks.

188 *Subjective feelings questionnaires*

189 Subjects rated their feelings of hunger, stomach fullness, desire to eat and prospective food
190 consumption (PFC) on a 100mm visual analogue scale with 0 mm representing ‘not at all’
191 and 100mm representing ‘extremely’. Ratings of muscle soreness, mouth taste, satisfaction
192 and nausea were also included to distract subjects from the main outcomes. Questionnaires
193 were provided pre-exercise (0 min), post-exercise (60 min), post-recovery drink (75 min),
194 pre-meal (135 min), post-meal (165 min), 30 minutes post-meal (195 min) and 60 minutes
195 post meal (225 min).

196 Additional questions related to drink perception (pleasantness, aftertaste, saltiness, bitterness,
197 sweetness, creaminess, thickness, stickiness, fruitiness, and how refreshing) were asked
198 immediately after drink ingestion.

199 *Statistical analysis*

200 Data was analysed using SPSS 20.0 (SPSS Inc., Somers, NY, USA). All data were checked
201 for normality of distribution using a Shapiro- Wilk test. Normally distributed data containing
202 one factor was analysed using one-way repeated measures ANOVA and non-normally
203 distributed data was analysed using Friedman’s ANOVA. Data containing two factors was
204 analysed using a two-way repeated measures ANOVA. Post-Hoc analysis were Bonferroni-
205 adjusted paired t-tests or Bonferroni-adjusted Wilcoxon signed-rank tests for normally and
206 non-normally distributed data, respectively. Data sets were determined to be significantly

207 different when $P < 0.05$. Data are presented as mean \pm standard deviation (normally
208 distributed), or median \pm range (non-normally distributed).

209

210 **Results**

211 *Exercise measurements*

212 Subjects pre-exercise body mass ($P=0.828$) and subjective appetite ratings ($P>0.219$) were
213 not different between trials. There was no difference between trials for VO_2 , heart rate or
214 RPE response during exercise (Table 2). Gross energy expenditure during the exercise
215 session was 2880 ± 295 kJ (PLA), 2851 ± 321 kJ (PRO) and 2823 ± 310 kJ (CHO) and was
216 not different between trials ($P=0.629$). Additionally there was no difference in RER
217 ($P=0.364$), fat oxidation ($P=0.303$) and carbohydrate oxidation ($P=0.723$) between trials.

218 *Energy intake, appetite ratings and drink perception*

219 Energy intake at the *ad-libitum* test meal (Figure 1) was reduced during PRO compared to
220 PLA ($P<0.05$), with no other differences between trials ($P>0.315$). When energy consumed
221 in the post-exercise drink was included, total energy intake was 6431 ± 492 kJ (PLA), $6359 \pm$
222 960 kJ (PRO) and 6640 ± 901 kJ (CHO) and there was no difference between trials
223 ($P=0.383$). Water intake during the test meal was not different between trials ($P=0.751$) and
224 amounted to 568 ± 366 ml, 479 ± 210 ml and 472 ± 151 ml during PLA, PRO and CHO,
225 respectively.

226 There was a main effect of time ($P<0.01$) for all subjective appetite measures (hunger, desire
227 to eat, prospective food consumption and fullness), but no main effects of trial ($P>0.219$) or
228 interaction effects ($P>0.164$) (Figure 2a-d).

229 Subjects perceived no difference between drinks for aftertaste ($P=0.934$), bitterness
230 ($P=0.105$), creaminess ($P=0.958$), refreshment ($P=0.226$), thickness ($P=0.913$), stickiness
231 ($P=0.088$), or fruitiness ($P=0.196$). CHO was perceived as more pleasant than PRO ($P<0.05$)
232 and tended to be perceived as more pleasant than PLA ($P=0.053$). CHO was perceived as

233 sweeter than PRO ($P<0.05$), whilst PRO was perceived as saltier than PLA ($P<0.05$) (Figure
234 3).

235

236

237 Discussion

238 The aim of this investigation was to examine whether post-exercise drink composition would
239 affect energy intake at an *ad-libitum* lunch served 60 minutes after drink ingestion (i.e. 75
240 min post-exercise). The primary finding from this study was that energy intake was
241 suppressed by approximately 9% (575 kJ) after consumption of a 6% whey protein isolate
242 drink compared to a low energy placebo. These results suggest that consuming a protein
243 containing drink after exercise might be an effective method of reducing energy intake at a
244 subsequent meal compared to a low energy placebo drink.

245 Protein intake immediately after exercise potentiates the exercise-induced stimulation of
246 myofibrillar and mitochondrial protein synthesis (Wilkinson *et al.*, 2008). Furthermore, whey
247 protein seems to induce a greater muscle protein synthetic response compared to casein or
248 soy (Tang, Moore, Kujbida, Tarnopolsky, & Phillips, 2009), which is likely due to
249 differences in postprandial absorption kinetics (Boirie *et al.*, 1997). In the present study, 30 g
250 of whey protein was provided, which has been shown to be within the optimal range to
251 maximise the protein synthetic response (Moore *et al.*, 2009; Witard *et al.* 2014). However,
252 from a weight management perspective, the additional energy ingested in a post-exercise
253 drink may compromise the energy deficit induced by the exercise session if the energy
254 consumed is not compensated for at the next feeding opportunity. Results of the present study
255 suggest that protein can be added to a post-exercise recovery drink without affecting gross
256 energy intake. In addition to the effects of protein on satiety, protein also has an increased
257 thermogenic effect compared to carbohydrate or fat (Feinman and Fine, 2004), and
258 consequently post-exercise protein ingestion might further decrease energy balance by
259 increasing energy expenditure, although this was not measured in the current investigation.

260 There is increasing evidence that acute protein feeding at rest may enhance satiety (Hill &
261 Blundell, 1986; Stubbs *et al.*, 1996) and reduce energy intake at a subsequent meal (Poppitt *et*
262 *al.*, 1998; Porrini *et al.*, 1997; Araya *et al.*, 2000) compared to isoenergetic carbohydrate and
263 fat meals. Although this effect is less conclusive when energy is provided in liquid form,
264 several studies have demonstrated a suppression of appetite and energy intake when high
265 protein drinks are provided at rest, compared to water and carbohydrate drinks (Bertenshaw
266 *et al.*, 2008; Bertenshaw *et al.*, 2009; Astbury *et al.*, 2010; Dove *et al.*, 2009). Bertenshaw *et*
267 *al.* (2008) found that a 300 ml drink enriched with 37.7 g of protein (50% of total energy)
268 reduced energy intake after an interval of both 30 and 120 min compared to an isoenergetic
269 high carbohydrate drink containing 1.7 g of protein (2% of total energy) or a low energy
270 placebo. Similarly, Astbury *et al.* (2010) found that the addition of protein to mixed
271 macronutrient 400 ml pre-load drinks reduced subsequent energy intake after 90 min
272 compared to an energy free placebo although systematically increasing pre-load protein
273 intake did not further reduce energy intake until a very high protein content of 50.4 g (50% of
274 total energy) was achieved. Blinding subjects to drinks with such disparate macronutrient
275 contents can prove difficult, and in both of these investigations, subjects reported protein
276 containing drinks to be thicker and/or creamier than low protein or placebo control drinks
277 which may have influenced energy intake (Bertenshaw, Lluch, & Yeomans, 2013), as well as
278 the expected satiety of the drink (McCrickerd, Chambers, Brunstrom, & Yeomans, 2012).

279 Despite several studies reporting a decrease in energy intake following ingestion of protein
280 containing drinks, this is not a universal finding. Poppitt *et al.* (2011) reported that low
281 energy (<350 kJ) 500 ml whey protein enriched water drinks (5-20 g) did not decrease energy
282 intake compared to an energy free placebo, although subjects reported increased fullness,
283 satisfaction and decreased hunger after consumption of the protein drinks compared to the
284 placebo drink. Much of the disparity within the liquid pre-load literature could be attributed

285 to methodological differences, such as pre-load to meal time interval (Poppitt *et al.*, 2011),
286 volume of pre-load provided (Almiron-Roig & Drewnowski, 2003), sensory characteristics of
287 the drinks (Bertenshaw *et al.*, 2013), or protein source (Anderson & Moore, 2004). In the
288 study of Poppitt *et al.* (2011), the time between ingesting the pre-load and the ad-libitum meal
289 was 120 min which may be too long to observe a difference between drinks of such low
290 energy density ($<0.7 \text{ kJ}\cdot\text{ml}^{-1}$). Based on recent findings, the average time interval for
291 voluntary meal requests occurs ~ 80 min after the cessation of exercise (King, Wasse, &
292 Stensel, 2012). Therefore, in the current study, a 500 ml pre-load with a pre-load to meal time
293 interval of 60 min was utilised (75 min after exercise), along with a more energy dense drink
294 ($1.06 \text{ kJ}\cdot\text{ml}^{-1}$) formulated to supply 30 g of protein (6%) to ensure maximal stimulation of
295 muscle protein synthesis (Moore *et al.*, 2009; Witard *et al.* 2014). Findings from the current
296 study were that energy intake was reduced after protein ingestion at the subsequent meal by
297 approximately 575 kJ representing a mean decrease of 9% compared to the placebo trial
298 intake. However, there was no difference in energy intake after ingestion of the 6% protein
299 compared to the isoenergetic carbohydrate drink, and was not different after ingestion of the
300 carbohydrate and placebo drinks in the current study. When energy consumed in the post
301 exercise drink was considered, total mean energy intake over each of the trials was reduced
302 during PRO ($6359 \pm 960 \text{ kJ}$) compared to PLA ($6431 \pm 492 \text{ kJ}$) and CHO ($6640 \pm 901 \text{ kJ}$)
303 although there were no significant differences between any of the trials ($P=0.383$). The
304 exercise protocol of this study was conducted in the post-prandial state and it is unclear
305 whether the same effect would be observed if exercise was performed in the fasted state.
306 However, based on these results, the addition of protein to post exercise drinks might not
307 increase energy intake at the next feeding opportunity and the consumption of protein after
308 exercise may incur other benefits such as stimulating myofibrillar and mitochondrial protein

309 synthesis (Wilkinson *et al.*, 2008) or enhancing the recovery of muscular force production
310 (Cockburn, Hayes, French, Stevenson, & St Claire Gibson, 2008).

311 No blood parameters were measured in the present investigation making the mechanisms
312 behind the observed appetite suppression after protein administration difficult to elucidate.
313 Bowen and colleagues (Bowen *et al.*, 2006a; Bowen, Noakes, & Clifton, 2006b) have studied
314 the effects of protein intake on appetite regulatory hormone profiles and have shown that
315 lower post-prandial plasma concentrations of ghrelin as well as higher concentrations of
316 satiety hormones glucagon-like peptide-1 (GLP-1) and cholecystokinin (CCK) are present up
317 to 3 h after protein ingestion compared to glucose ingestion. It is possible that the reduction
318 in energy intake observed after protein ingestion during the current study was caused by
319 alterations in gut peptide profiles, with protein stimulating an increase in satiety hormones
320 (e.g. GLP-1 and CCK) and a reduction in appetite stimulatory hormones (e.g. ghrelin)
321 compared to ingestion of a low energy placebo control. However, alterations in appetite
322 hormone profiles do not always accurately predict energy intake (Bowen *et al.*, 2007).

323 Recent research has highlighted the impact of sensory characteristics of drinks on subsequent
324 energy intake. Bertenshaw *et al.* (2013) observed that when a high carbohydrate drink is
325 artificially thickened, *ad-libitum* energy intake was reduced compared to a high protein drink.
326 The authors suggested that energy intake was primarily governed through the hedonic
327 qualities of the pre-load, with drinks that are described by subjects as being particularly thick
328 or creamy, typically inducing higher feelings of satiety and reducing *ad-libitum* energy intake
329 at a subsequent meal. When reviewing the literature, several studies that have observed
330 differences in energy intake between protein and carbohydrate drinks have also provided
331 drinks that would be expected to differ hedonically (skimmed milk vs. fruit juice) (Dove *et*
332 *al.*, 2009), or subjects have identified differences in the sensory characteristics of the drinks
333 (i.e. thickness and/or creaminess) (Bertenshaw *et al.*, 2008; Bertenshaw *et al.*, 2009; Astbury

334 *et al.*, 2010). Oreosensory cues have been shown to elicit hormonal changes related to
335 appetite control (Teff, 2006, 2010), as well as enhance fullness and expected satiety of a
336 drink (McCrickerd *et al.*, 2012). Therefore, insufficient blinding of experimental drinks may
337 result in sensory differences that confound any potential effects of macronutrient composition
338 on appetite and subsequent energy intake. In the current study, an acidified whey protein
339 isolate was utilised, which assimilates well in solution, and resulted in no differences in
340 thickness or creaminess reported by participants between any of the experimental drinks
341 (Figure 3). In turn, this may have attenuated the subjective perception of satiety which has
342 been commonly observed after protein ingestion (Bertenshaw *et al.*, 2008; Bertenshaw *et al.*,
343 2009; Astbury *et al.*, 2010; Poppitt *et al.*, 2011; Dove *et al.*, 2009), as there were no
344 differences in hunger, fullness, prospective food consumption or desire to eat between trials
345 in the current study. This may also help to explain why no difference was observed in *ad-*
346 *libitum* energy intake after ingestion of the protein or carbohydrate drinks in the present study,
347 despite several studies observing greater energy intake after carbohydrate ingestion compared
348 to protein (Bertenshaw *et al.*, 2008; Bertenshaw *et al.*, 2009; Astbury *et al.*, 2010; Dove *et al.*,
349 2009).

350 The consumption of protein and carbohydrate drinks is particularly common after exercise
351 but the interaction between exercise and post-exercise macronutrient intake on appetite has
352 not been well studied. Liquid protein feeding at rest has often been reported to suppress
353 appetite and energy intake relative to carbohydrate (Bertenshaw *et al.*, 2008; Bertenshaw *et*
354 *al.*, 2009; Astbury *et al.*, 2010; Dove *et al.*, 2009), although this was not observed during the
355 current investigation. The mechanisms behind these findings are not entirely clear, but could
356 conceivably be due to the exercise protocol of the current study having a greater effect on
357 appetite and energy intake than the macronutrient content of the post-exercise drinks. Forty
358 minutes of high intensity interval cycling has been shown to reduce muscle glycogen

359 concentration by approximately 50% (Steputo, Martin, Fallon, & Hawley, 2001). Although the
360 degree of glycogen depletion would have been expected to be less severe after exercise in the
361 current study, the perturbation in glycogen homeostasis may have influenced energy intake
362 (and therefore carbohydrate intake) in order to promote glycogen resynthesis and restore
363 glycogen balance (Hopkins, Jeukendrup, King, & Blundell, 2011). This may have
364 counteracted some of the satiating properties of the post-exercise protein drink culminating in
365 no difference in energy intake between the carbohydrate and protein trials. However, other
366 investigations have found no differences in energy intake between steady state exercise,
367 intermittent exercise and resting conditions, where disparate states of glycogen homeostasis
368 might be expected to influence energy intake significantly (Deighton, Karra, Batterham, &
369 Stensel, 2013).

370 Inter subject variability for energy intake appeared to be greater during the carbohydrate and
371 protein trials compared to the placebo trial (Figure 1b) . The reason for this is not clear, but
372 might be due to differences in participant's habitual intakes of these nutrients. Indeed, a study
373 by Long, Jeffcoat, and Millward (2000) found that individuals who consumed a high protein
374 diet habitually were less sensitive to the satiating properties of a high protein meal compared
375 to habitual low protein consumers. Likewise, we could speculate that a similar response may
376 exist in subjects who consume a high carbohydrate diet habitually or perhaps regularly ingest
377 high carbohydrate drinks in particular. Habitual dietary intakes were not collected as part of
378 the current study and therefore these hypotheses remains speculative based on these results.

379

380 **Conclusions**

381 The present study investigated the effects of altering the composition of a post-exercise drink
382 on subjective appetite and voluntary energy intake. When a whey protein isolate drink was
383 consumed 10 minutes after exercise, energy intake was reduced at a subsequent meal
384 provided 75 minutes post-exercise compared to a low energy placebo drink. This suppression
385 of food intake was not observed after ingestion of a carbohydrate drink. Matching the drinks
386 for sensory characteristics such as thickness and creaminess may explain why no difference
387 in subjective satiety and food intake was observed after ingestion of the carbohydrate and
388 protein drinks. Previous studies have shown that protein ingestion immediately post-exercise
389 may enhance the adaptive response of skeletal muscle by increasing myofibrillar and
390 mitochondrial protein synthesis and the present findings suggest that this adaptation might be
391 possible without affecting gross energy intake relative to consuming a low energy/ energy
392 free drink.

393

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541 **Captions (for figures 1-3)**

542

543 **Figure 1.** (a) Mean energy intake at the *ad-libitum* test meal (kJ) and (b) subjects individual
544 energy intakes (kJ) during each trial Values are means, with vertical error bars representing
545 standard deviation.* Significantly different from PLA ($P<0.05$)

546

547 **Figure 2.** Subjective feelings of hunger (a), desire to eat (b), prospective food consumption
548 (c), and fullness (d) after consuming the placebo (■), protein (▲) and carbohydrate (○) drinks.
549 Hatched shaded rectangle represents exercise, grey rectangle represents ingestion of the post-
550 exercise recovery drink, and black rectangle represents the ad-libitum buffet meal. Data
551 points are medians. All subjective measures of appetite showed a main effect of time ($P<0.01$)

552

553 **Figure 3.** Subjective perceptions of test drinks (mm): PLA (■), PRO (■) and CHO (□).
554 Subjective perceptions of salty, sweet, creamy, refreshing and thick were non-normally
555 distributed, however all values presented are means, with vertical error bars representing
556 standard deviation for consistency. * significantly different from PLA ($P<0.05$). †
557 significantly different from CHO ($P<0.05$).

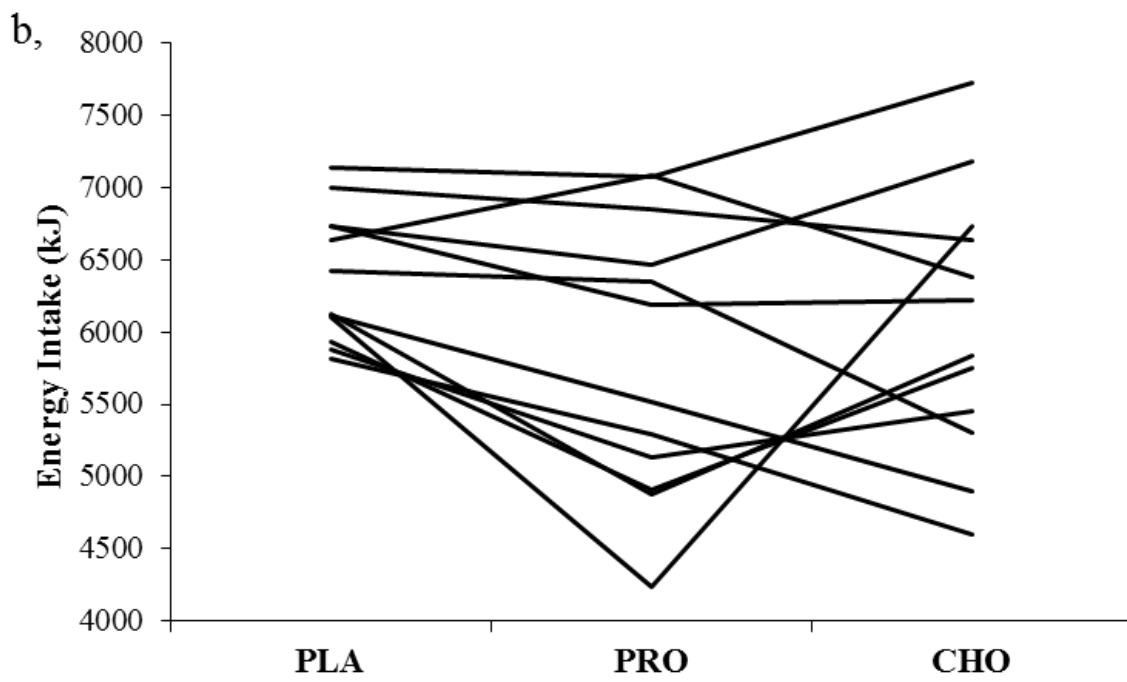
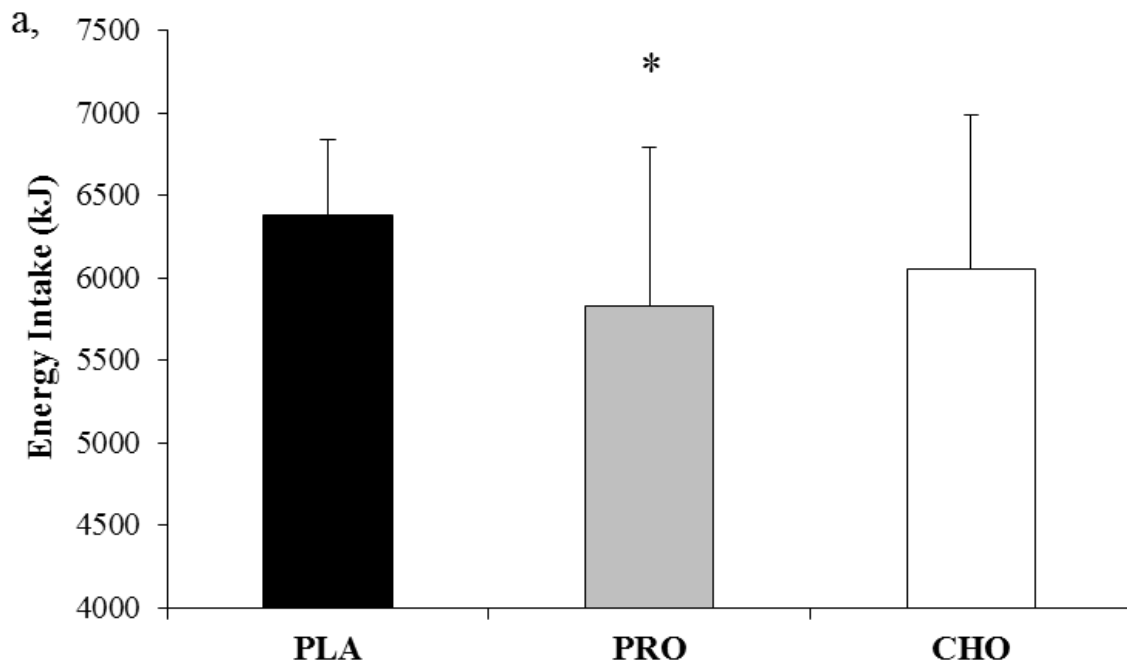
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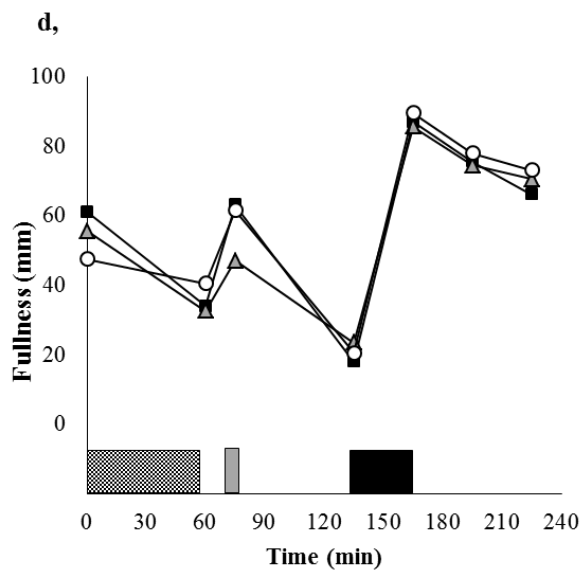
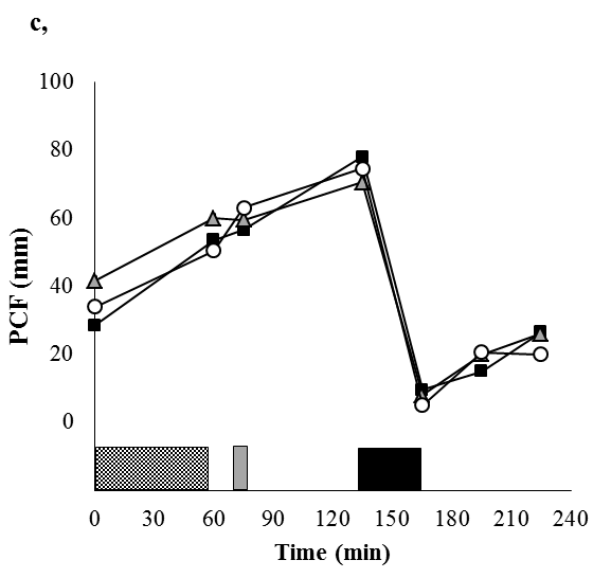
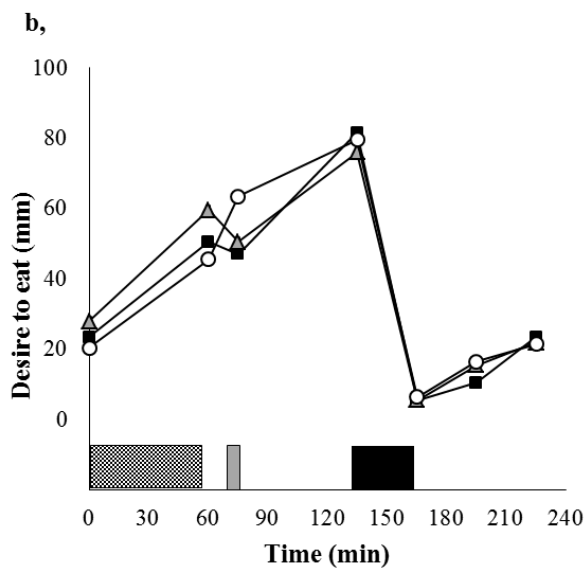
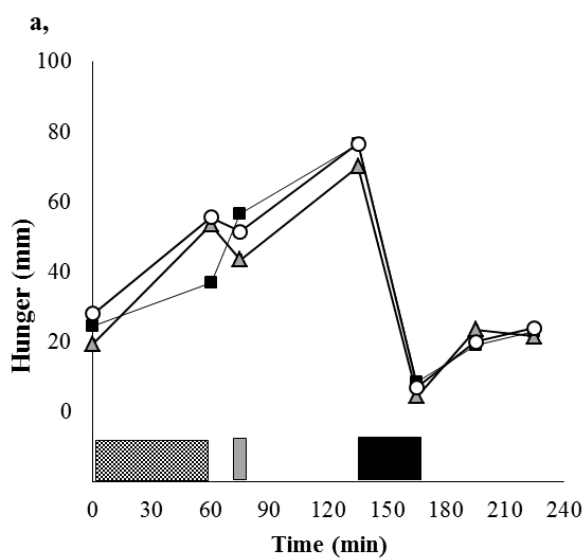
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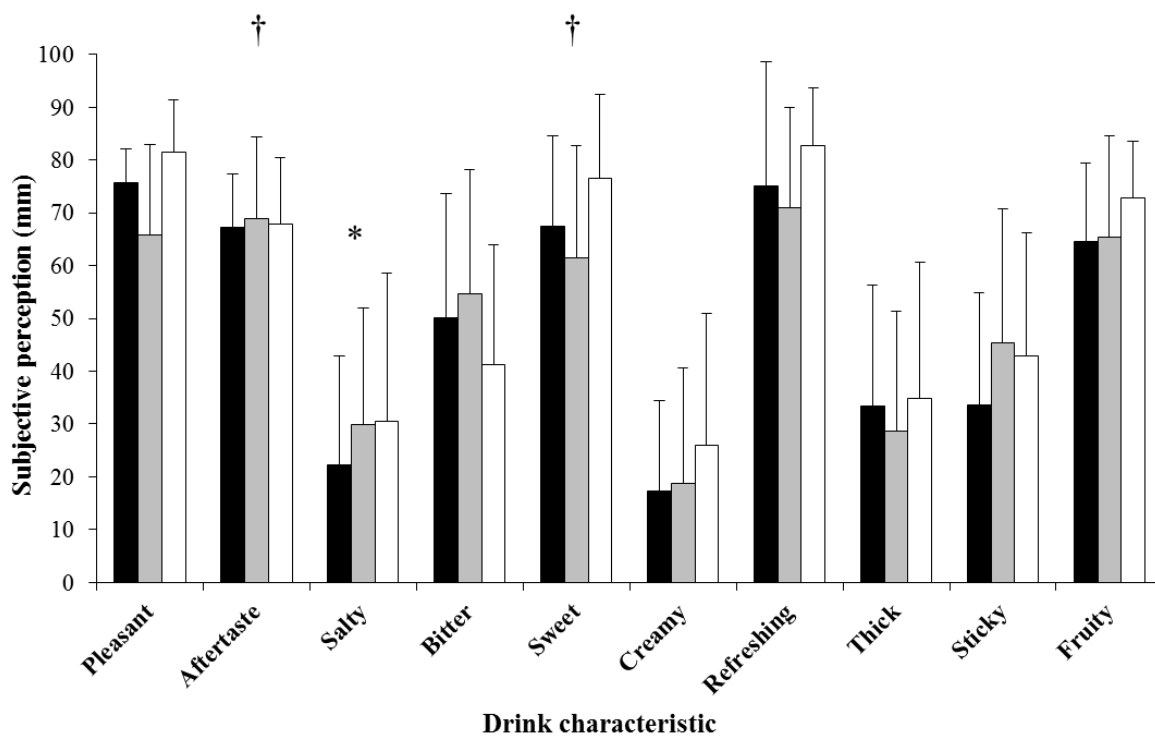
562 Artwork – Figures 1-3



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577 **Tables with captions**578 **Table 1.** Composition of test drinks.

	Placebo (PLA)	Protein (PRO)	Sucrose (CHO)
Energy (kJ)	15	528	528
Protein (g)	0.3	30.3	0.6
Carbohydrate (g)	0.6	0.3	30.8
Fat (g)	0	0.1	0

579

580 **Table 2.** Mean variables during initial 30 min exercise and intervals for each trial. *P*-value
581 represents main effect.

	PLA	PRO	CHO	<i>P</i> -value
<i>Initial 30 min</i>				
VO ₂ (L·min ⁻¹)	2.35 ± 0.27	2.34 ± 0.25	2.39 ± 0.33	0.414
VO ₂ (% of peak)	63 ± 3	63 ± 3	63 ± 4	0.565
Heart rate (b·min ⁻¹)	152 ± 10	153 ± 8	153 ± 9	0.748
RPE	13 ± 1	13 ± 1	13 ± 1	0.395
<i>Intervals</i>				
VO ₂ (L·min ⁻¹)	3.20 ± 0.46	3.19 ± 0.41	3.23 ± 0.44	0.737
VO ₂ (% of peak)	85 ± 3	85 ± 4	86 ± 3	0.642
Heart rate (b·min ⁻¹)	177 ± 9	176 ± 7	176 ± 8	0.645
RPE	17 ± 1	17 ± 1	17 ± 1	0.925

582