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

David J. Clayton, David J. Stensel, Phillip Watson & Lewis J. James*

School of Sport, Exercise and Health Sciences, Loughborough University, Loughborough, Leicestershire, LE11 3TU, UK

*Corresponding author: Dr Lewis J. James

Email: L.James@lboro.ac.uk

Tel: +44 (0) 1509 226305

Fax: +44 (0) 1509 226301

Co-author email addresses

Mr David J. Clayton – D.Clayton@lboro.ac..uk

Dr. David J. Stensel – D.Stensel@lboro.ac.uk

Dr. Phillip Watson – P.Watson2@lboro.ac.uk

Abstract

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

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

Introduction

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

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

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

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

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

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

Methods

Subjects

After ethical approval subjects completed a medical screening questionnaire, a three-factor eating questionnaire (Stunkard & Messick, 1985) and provided written consent. Subjects were twelve healthy, weight stable, recreationally active males (mean ± SD) (age: 24 ± 2 y, 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^{-2}). Subjects were not restrained, disinhibited or hungry eaters.

Preliminary trials

Subjects completed two preliminary trials. During the first, they completed a discontinuous incremental exercise test on an electrically braked cycle ergometer (Lode Corival, Groningen, Holland) to determine peak oxygen consumption (VO$_2$peak). Increments lasted 4 min, were separated by ~5 min rest and work load increased until volitional exhaustion. Expired air was collected into a Douglas Bag during the last min of each increment, whilst heart rate (Polar Beat, Kempele, Finland) and rating of perceived exertion (RPE) (Borg, 1973) were measured at the end of each increment. Expired air was analysed for O_2 and CO_2 concentration (Servomex 1440 Gas Analyser, Sussex, UK), volume (Harvard Dry Gas meter, Harvard Apparatus Ltd, Kent, UK) and temperature (Edale, Cambridge, UK).

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

Pre-trial standardisation

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

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

Experimental design

Participants arrived at the laboratory between 9.30-10.30am and voided their bladder and bowels, before nude body mass was measured. Subjects then completed 30 min steady state cycling exercise at ~60% VO_2peak followed by five min rest and then five 3 min intervals at ~85% VO_2peak, each separated by 2 min rest. Total exercise time was therefore 60 min. Expired air was collected between 14-15 min and 29-30 min steady state exercise and during the final minute of the third and fifth interval. Heart rate and RPE were measured at 15 min and 30 min during steady state exercise and at the end of each interval. Subjects consumed 100 ml of water at 15 min, and prior to intervals one, three and five.

Upon completion of exercise, nude body mass was measured and subjects assumed a seated position. Ten minutes post-exercise, subjects were provided with a recovery drink (Table 1) to consume within five minutes and an *ad-libitum* lunch was provided 75 minutes post-exercise whilst subjects rested in a comfortable environment (23.5 ± 1.8°C).

The lunch meal was designed to closely match UK dietary guidelines for macronutrient proportions, and consisted of pasta, cheese, tomato sauce and olive oil (Tesco, Cheshunt, UK). The meal was homogenous in nature and provided 7.87 ± 0.1 $kJ \cdot g^{-1}$ (14% protein, 53%

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

Post-exercise drinks

Subjects completed three experimental trials with a different post-exercise recovery drink consumed during each trial (Table 1). Drinks investigated were; a whey protein isolate solution (Volactive Hydrapro, Volac International Ltd., Orwell, UK) providing 30g of whey protein (PRO), an energy matched sucrose (Tate and Lyle, London, UK) solution (CHO) or a placebo solution (PLA). The composition of the protein powder per 100 g powder was: 91.7 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 prepared the evening before experimental trials and were refrigerated overnight (4°C). Each drink contained 425 ml of water mixed with 75 ml of lemon squash (Tesco, Cheshunt, UK), was served in an opaque container and was ingested through a straw to minimise any visual or olfactory differences between the drinks. Trials were separated by at least one week and administered in a double-blind, randomised, counterbalanced manner. Subjects were aware that the study was assessing different post-exercise recovery drink compositions, but were not informed what the drinks contained. At the end of the study, subjects were informed about the contents of the experimental drinks, and asked whether they could tell any differences between the drinks and on which visit they thought they consumed each drink. Four out of twelve subjects stated they could taste a difference between the drinks, but only one subject correctly identified the drinks.

Subjective feelings questionnaires

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

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

Statistical analysis

Data was analysed using SPSS 20.0 (SPSS Inc., Somers, NY, USA). All data were checked for normality of distribution using a Shapiro- Wilk test. Normally distributed data containing one factor was analysed using one-way repeated measures ANOVA and non-normally distributed data was analysed using Friedman’s ANOVA. Data containing two factors was analysed using a two-way repeated measures ANOVA. Post-Hoc analysis were Bonferroni-adjusted paired t-tests or Bonferroni-adjusted Wilcoxon signed-rank tests for normally and non-normally distributed data, respectively. Data sets were determined to be significantly different when $P<0.05$. Data are presented as mean ± standard deviation (normally distributed), or median ± range (non-normally distributed).

Results

Exercise measurements

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

Energy intake, appetite ratings and drink perception

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

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

Subjects perceived no difference between drinks for aftertaste (P=0.934), bitterness (P=0.105), creaminess (P=0.958), refreshment (P=0.226), thickness (P=0.913), stickiness (P=0.088), or fruitiness (P=0.196). CHO was perceived as more pleasant than PRO (P<0.05) and tended to be perceived as more pleasant than PLA (P=0.053). CHO was perceived as sweeter than PRO (*P*<0.05), whilst PRO was perceived as saltier than PLA (*P*<0.05) (Figure 3).

Discussion

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

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

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

Despite several studies reporting a decrease in energy intake following ingestion of protein containing drinks, this is not a universal finding. Poppitt *et al.* (2011) reported that low energy (<350 kJ) 500 ml whey protein enriched water drinks (5-20 g) did not decrease energy intake compared to an energy free placebo, although subjects reported increased fullness, satisfaction and decreased hunger after consumption of the protein drinks compared to the placebo drink. Much of the disparity within the liquid pre-load literature could be attributed to methodological differences, such as pre-load to meal time interval (Poppitt *et al*., 2011), volume of pre-load provided (Almiron-Roig & Drewnowski, 2003), sensory characteristics of the drinks (Bertenshaw *et al*., 2013), or protein source (Anderson & Moore, 2004). In the study of Poppitt et al. (2011), the time between ingesting the pre-load and the ad-libitum meal was 120 min which may be too long to observe a difference between drinks of such low energy density (<0.7 kJ·ml^{-1}). Based on recent findings, the average time interval for voluntary meal requests occurs ~80 min after the cessation of exercise (King, Wasse, & Stensel, 2012). Therefore, in the current study, a 500 ml pre-load with a pre-load to meal time interval of 60 min was utilised (75 min after exercise), along with a more energy dense drink (1.06 kJ·ml^{-1}) formulated to supply 30 g of protein (6%) to ensure maximal stimulation of muscle protein synthesis (Moore *et al*., 2009; Witard *et al*. 2014). Findings from the current study were that energy intake was reduced after protein ingestion at the subsequent meal by approximately 575 kJ representing a mean decrease of 9% compared to the placebo trial intake. However, there was no difference in energy intake after ingestion of the 6% protein compared to the isoenergetic carbohydrate drink, and was not different after ingestion of the carbohydrate and placebo drinks in the current study. When energy consumed in the post exercise drink was considered, total mean energy intake over each of the trials was reduced during PRO (6359 ± 960 kJ) compared to PLA (6431 ± 492 kJ) and CHO (6640 ± 901 kJ) although there were no significant differences between any of the trials (P=0.383). The exercise protocol of this study was conducted in the post-prandial state and it is unclear whether the same effect would be observed if exercise was performed in the fasted state. However, based on these results, the addition of protein to post exercise drinks might not increase energy intake at the next feeding opportunity and the consumption of protein after exercise may incur other benefits such as stimulating myofibrillar and mitochondrial protein synthesis (Wilkinson *et al*., 2008) or enhancing the recovery of muscular force production (Cockburn, Hayes, French, Stevenson, & St Claire Gibson, 2008).

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

Recent research has highlighted the impact of sensory characteristics of drinks on subsequent energy intake. Bertenshaw *et al.* (2013) observed that when a high carbohydrate drink is artificially thickened, *ad-libitum* energy intake was reduced compared to a high protein drink. The authors suggested that energy intake was primarily governed through the hedonic qualities of the pre-load, with drinks that are described by subjects as being particularly thick or creamy, typically inducing higher feelings of satiety and reducing *ad-libitum* energy intake at a subsequent meal. When reviewing the literature, several studies that have observed differences in energy intake between protein and carbohydrate drinks have also provided drinks that would be expected to differ hedonically (skimmed milk vs. fruit juice) (Dove *et al*., 2009), or subjects have identified differences in the sensory characteristics of the drinks (i.e. thickness and/or creaminess) (Bertenshaw *et al*., 2008; Bertenshaw *et al*., 2009; Astbury *et al.*, 2010). Oreosensory cues have been shown to elicit hormonal changes related to appetite control (Teff, 2006, 2010), as well as enhance fullness and expected satiety of a drink (McCrickerd *et al.*, 2012). Therefore, insufficient blinding of experimental drinks may result in sensory differences that confound any potential effects of macronutrient composition on appetite and subsequent energy intake. In the current study, an acidified whey protein isolate was utilised, which assimilates well in solution, and resulted in no differences in thickness or creaminess reported by participants between any of the experimental drinks (Figure 3). In turn, this may have attenuated the subjective perception of satiety which has been commonly observed after protein ingestion (Bertenshaw *et al.*, 2008; Bertenshaw *et al.*, 2009; Astbury *et al.*, 2010; Poppitt *et al.*, 2011; Dove *et al.*, 2009), as there were no differences in hunger, fullness, prospective food consumption or desire to eat between trials in the current study. This may also help to explain why no difference was observed in *ad-libitum* energy intake after ingestion of the protein or carbohydrate drinks in the present study, despite several studies observing greater energy intake after carbohydrate ingestion compared to protein (Bertenshaw *et al.*, 2008; Bertenshaw *et al.*, 2009; Astbury *et al.*, 2010; Dove *et al.*, 2009).

The consumption of protein and carbohydrate drinks is particularly common after exercise but the interaction between exercise and post-exercise macronutrient intake on appetite has not been well studied. Liquid protein feeding at rest has often been reported to suppress appetite and energy intake relative to carbohydrate (Bertenshaw *et al.*, 2008; Bertenshaw *et al.*, 2009; Astbury *et al.*, 2010; Dove *et al.*, 2009), although this was not observed during the current investigation. The mechanisms behind these findings are not entirely clear, but could conceivably be due to the exercise protocol of the current study having a greater effect on appetite and energy intake than the macronutrient content of the post-exercise drinks. Forty minutes of high intensity interval cycling has been shown to reduce muscle glycogen concentration by approximately 50% (Stepto, Martin, Fallon, & Hawley, 2001). Although the degree of glycogen depletion would have been expected to be less severe after exercise in the current study, the perturbation in glycogen homeostasis may have influenced energy intake (and therefore carbohydrate intake) in order to promote glycogen resynthesis and restore glycogen balance (Hopkins, Jeukendrup, King, & Blundell, 2011). This may have counteracted some of the satiating properties of the post-exercise protein drink culminating in no difference in energy intake between the carbohydrate and protein trials. However, other investigations have found no differences in energy intake between steady state exercise, intermittent exercise and resting conditions, where disparate states of glycogen homeostasis might be expected to influence energy intake significantly (Deighton, Karra, Batterham, & Stensel, 2013).

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

Conclusions

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

Acknowledgements

The authors would like to thank Volac International Ltd. for supplying the protein powder for use in this study and Georgina Mynott for her assistance with data collection.

References

Almiron-Roig, E., & Drewnowski, A. (2003) Hunger, thirst, and energy intakes following consumption of caloric beverages. *Physiology & Behavior, 79(4)*, 767-773.

Anderson, G.H., & Moore, S.E. (2004) Dietary proteins in the regulation of food intake and body weight in humans. *The Journal of Nutrition, 134(4),* 974S-979S.

Araya, H., Hills, J., Alvina, M., & Vera, G. (2000) Short-term satiety in preschool children: a comparison between high protein meal and a high complex carbohydrate meal. *International Journal of Food Science & Nutrition, 51(2),* 119-124.

Astbury, N.M., Stevenson, E.J., Morris, P., Taylor, M.A., & Macdonald, I.A. (2010) Dose–response effect of a whey protein preload on within-day energy intake in lean subjects. *British Journal of Nutrition, 104(12)*, 1858-1867.

Bartlett, J.D., Close, G.L., MacLaren, D.P., Gregson, W., Drust, B., & Morton, J.P. (2011) High-intensity interval running is perceived to be more enjoyable than moderate-intensity continuous exercise: implications for exercise adherence. *Journal of Sports Science, 29,* 547-553.

Bertenshaw, E.J., Lluch, A., & Yeomans, M.R. (2008) Satiating effects of protein but not carbohydrate consumed in a between-meal beverage context. *Physiology & Behavior, 93(3)*, 427-436.

Bertenshaw, E.J., Lluch, A., & Yeomans, M.R. (2009) Dose-dependent effects of beverage protein content upon short-term intake. *Appetite, 52(3)*, 580-587.

Bertenshaw, E.J., Lluch, A., & Yeomans, M.R. (2013) Perceived thickness and creaminess modulates the short-term satiating effects of high-protein drinks. *British Journal of Nutrition*, *110(3)*, 578-586.

Blundell, J.E., Stubbs, R.J., Hughes, D.A., Whybrow, S., & King, N.A. (2003) Cross talk between physical activity and appetite control: does physical activity stimulate appetite? *Proceedings of the Nutrition Society, 62*, 651-661.

Boirie, Y., Dangin, M., Gachon, P., Vasson, M., Maubois, J., & Beaufrère, B. (1997) Slow and fast dietary proteins differently modulate postprandial protein accretion. *Procedings of the National Academy of Sciences*, *94(26)*, 14930-14935.

Borg, G. (1973) Perceived exertion: a note on" history" and methods. *Medicine & Science in Sports & Exercise*, *5(2)*, 90-93.

Bowen, J., Noakes, M., Trenerry, C., & Clifton, P.M. (2006) Energy intake, ghrelin, and cholecystokinin after different carbohydrate and protein preloads in overweight men. *Journal of Clinical Endocrinology & Metabolism, 91(4)*, 1477-1483.

Bowen, J., Noakes, M., & Clifton, P.M. (2006) Appetite regulatory hormone responses to various dietary proteins differ by body mass index status despite similar reductions in ad libitum energy intake. *Journal of Clinical Endocrinology & Metabolism*, *91(8)*, 2913-2919.

Bowen, J., Noakes, M., & Clifton, P.M. (2007) Appetite hormones and energy intake in obese men after consumption of fructose, glucose and whey protein beverages. *International Journal of Obesity*, *31(11)*, 1696-1703.

Cockburn, E., Hayes, P.R., French, D.N., Stevenson, E., & St Clair Gibson, A. (2008) Acute milk-based protein-CHO supplementation attenuates exercise-induced muscle damage. *Applied Physiology, Nutrition, & Metabolism, 33(4)*, 775-783.

Dansinger, M.L., Gleason, J.A., Griffith, J.L., Selker, H.P., & Schaefer, E.J. (2005) Comparison of the Atkins, Ornish, Weight Watchers, and Zone diets for weight loss and heart disease risk reduction. *JAMA: The Journal of the American Medical Association, 293(1)*, 43-53.

Deighton, K., Karra, E., Batterham, R.L., & Stensel, D.J. (2013) Appetite, energy intake and PYY3-36 responses to energy-matched continuous exercise and submaximal high intensity exercise. *Applied Physiology, Nutrition, & Metabolism, 38(9)*, 947-952.

Dove, E.R., Hodgson, J.M., Puddey, I.B., Beilin, L.J., Lee, Y.P., & Mori, T.A. (2009) Skim milk compared with a fruit drink acutely reduces appetite and energy intake in overweight men and women. *American Journal of Clinical Nutrition, 90(1)*, 70-75.

Feinman, R.D., & Fine, E.J. (2004) “A calorie is a calorie” violates the second law of thermodynamics. *Nutrition Journal,*3(9).

Finucane, M.M., Stevens, G.A., Cowan, M.J., Danaei, G., Lin, J.K., Paciorek, C.J., et al. (2011) National, regional, and global trends in body-mass index since 1980: systematic analysis of health examination surveys and epidemiological studies with 960 country-years and 9.1 million participants. *The Lancet, 378(9785)*, 31-40.

Franz, M.J., VanWormer, J.J., Crain, A.L., Boucher, J.L., Histon, T., Caplan, W., et al. (2007) Weight-loss outcomes: a systematic review and meta-analysis of weight-loss clinical trials with a minimum 1-year follow-up. *Journal of American Dietary Association*, *107(10),* 1755-1767.

Gibala, M.J., Little, J.P., MacDonald, M.J., & Hawley, J.A. (2012) Physiological adaptations to low-volume, high-intensity interval training in health and disease. *Journal of Physiology, 590,* 1077-1084.

Hall, W., Millward, D., Long, S., & Morgan, L. (2003) Casein and whey exert different effects on plasma amino acid profiles, gastrointestinal hormone secretion and appetite. *British Journal of Nutrition, 89(2),* 239-248.

Harper, A., James, A., Flint, A., & Astrup, A. (2007) Increased satiety after intake of a chocolate milk drink compared with a carbonated beverage, but no difference in subsequent ad libitum lunch intake. *British Journal of Nutrition, 97(3),* 579-583.

Hill, A.J., & Blundell, J.E. (1986) Macronutrients and satiety: the effects of a high-protein or high-carbohydrate meal on subjective motivation to eat and food preferences. *Nutrition & Behavior*, *3*, 133-144.

Hopkins, M., Jeukendrup, A., King, N.A., & Blundell, J.E. (2011) The relationship between substrate metabolism, exercise and appetite control. *Sports Medicine*, *41(6)*, 507-521.

Hubert, P., King, N., & Blundell, J. (1998) Uncoupling the effects of energy expenditure and energy intake: appetite response to short-term energy deficit induced by meal omission and physical activity. *Appetite*, *31(1),* 9-19.

James, L. (2012) Milk protein and the restoration of fluid balance after exercise. *Medicine Sport Science*, *59*, 120-126.

King, J.A., Wasse, L.K., Ewens, J., Crystallis, K., Emmanuel, J., & Batterham, R.L.(2011) Differential acylated ghrelin, Peptide YY3–36, appetite, and food intake responses to equivalent energy deficits created by exercise and food restriction. *Journal of Clinical Endocrinology & Metabolism, 96(4)*, 1114-1121.

King, J.A., Wasse, L.K., & Stensel, D.J. (2012) Acute exercise increases feeding latency in healthy normal weight young males but does not alter energy intake. *Appetite, 61(1)*, 45-51.

Leidy, H.J., Carnell, N.S., Mattes, R.D., & Campbell, W.W. (2007) Higher protein intake preserves lean mass and satiety with weight loss in pre-obese and obese women. *Obesity, 15(2),* 421-429.

Long, S., Jeffcoat, A., & Millward, D. (2000) Effect of habitual dietary-protein intake on appetite and satiety. *Appetite, 35(1),* 79-88.

Malik, V.S., Willett, W.C., & Hu, F.B. (2013) Global obesity: trends, risk factors and policy implications. *Nature Reviews Endocrinology, 9(1)*, 13-27

McCrickerd, K., Chambers, L., Brunstrom, J.M., & Yeomans, M.R. (2012) Subtle changes in the flavour and texture of a drink enhance expectations of satiety. *Flavour, 1(1),* 20-30.

Mifflin, M.D., St Jeor, S., Hill, L.A., Scott, B.J., Daugherty, S.A., & Koh, Y. (1990) A new predictive equation for resting energy expenditure in healthy individuals. *American Journal of Clinical Nutrition, 51(2),* 241-247.

Moore, D.R., Robinson, M.J., Fry, J.L., Tang, J.E., Glover, E.I., Wilkinson, S.B., et al. (2009) Ingested protein dose response of muscle and albumin protein synthesis after resistance exercise in young men. *American Journal of Clinical Nutrition, 89(1),* 161-168.

Nose, H., Mack, G.W., Shi, X., & Nadel, E.R. (1988) Role of osmolality and plasma volume during rehydration in humans. *Journal of Applied Physiology, 65(1),* 325-331.

Poppitt, S.D., McCormack, D., & Buffenstein, R. (1998) Short-term effects of macronutrient preloads on appetite and energy intake in lean women. *Physiology & Behavior, 64(3),* 279-285.

Poppitt, S.D., Proctor, J., McGill, A., Wiessing, K.R., Falk, S., Xin, L., et al. (2011) Low-dose whey protein-enriched water beverages alter satiety in a study of overweight women. *Appetite, 56(2),* 456-464.

Porrini, M., Santangelo, A., Crovetti, R., Riso, P., Testolin, G., & Blundell, J. (1997) Weight, protein, fat, and timing of preloads affect food intake. *Physiology & Behavior, 62(3),* 563-570.

Shirreffs, S.M., Taylor, A.J., Leiper, J.B., & Maughan, R.J. (1996) Post-exercise rehydration in man: effects of volume consumed and drink sodium content. *Medicine & Science in Sports & Exercise, 28(10),* 1260-1271.

Stepto, N.K., Martin, D.T., Fallon, K.E., & Hawley, J.A. (2001) Metabolic demands of intense aerobic interval training in competitive cyclists. *Medicine & Science in Sports* & Exercise, 33(2), 303-310.

Stubbs, R.J., van Wyk, M.C., Johnstone, A.M., & Harbron, C.G. (1996) Breakfasts high in protein, fat or carbohydrate: effect on within-day appetite and energy balance. *European Journal of Clinical Nutrition, 50(7),* 409-417.

Stunkard, A.J., & Messick, S. (1985) The three-factor eating questionnaire to measure dietary restraint, disinhibition and hunger. *Journal of Psychosomatic Research, 29(1),* 71-83.

Tang, J.E., Moore, D.R., Kujbida, G.W., Tarnopolsky, M.A., & Phillips, S.M. (2009) Ingestion of whey hydrolysate, casein, or soy protein isolate: effects on mixed muscle protein synthesis at rest and following resistance exercise in young men. *Journal of Applied Physiology*, *107(3)*, 987-992.

Teff K.L. (2006) Learning hunger: conditioned anticipatory ghrelin responses in energy homeostasis. *Endocrinology*, *147(1)*, 20-22.

Teff, K.L. (2010) Cephalic phase pancreatic polypeptide responses to liquid and solid stimuli in humans. *Physiology & Behavior, 99(3),* 317-323.

Wilkinson, S.B., Phillips, S.M., Atherton, P.J., Patel, R., Yarasheski, K.E., Tarnopolski, M.A., et al. (2008) Differential effects of resistance and endurance exercise in the fed state on signaling molecule phosphorylation and protein synthesis in human muscle. *Journal of Physiology*, *586(15)*, 3701-3717.

Witard, O.C., Jackman, S.R., Bleen, L., Smith, K., Selby, A., & Tipton, K.D. (2014) Myofibrillar muscle protein synthesis rates subsequent to a meal in response to increasing doses of whey protein at rest and after resistance exercise. *American Journal of Clinical Nutrition*, 99(1), 86-95.

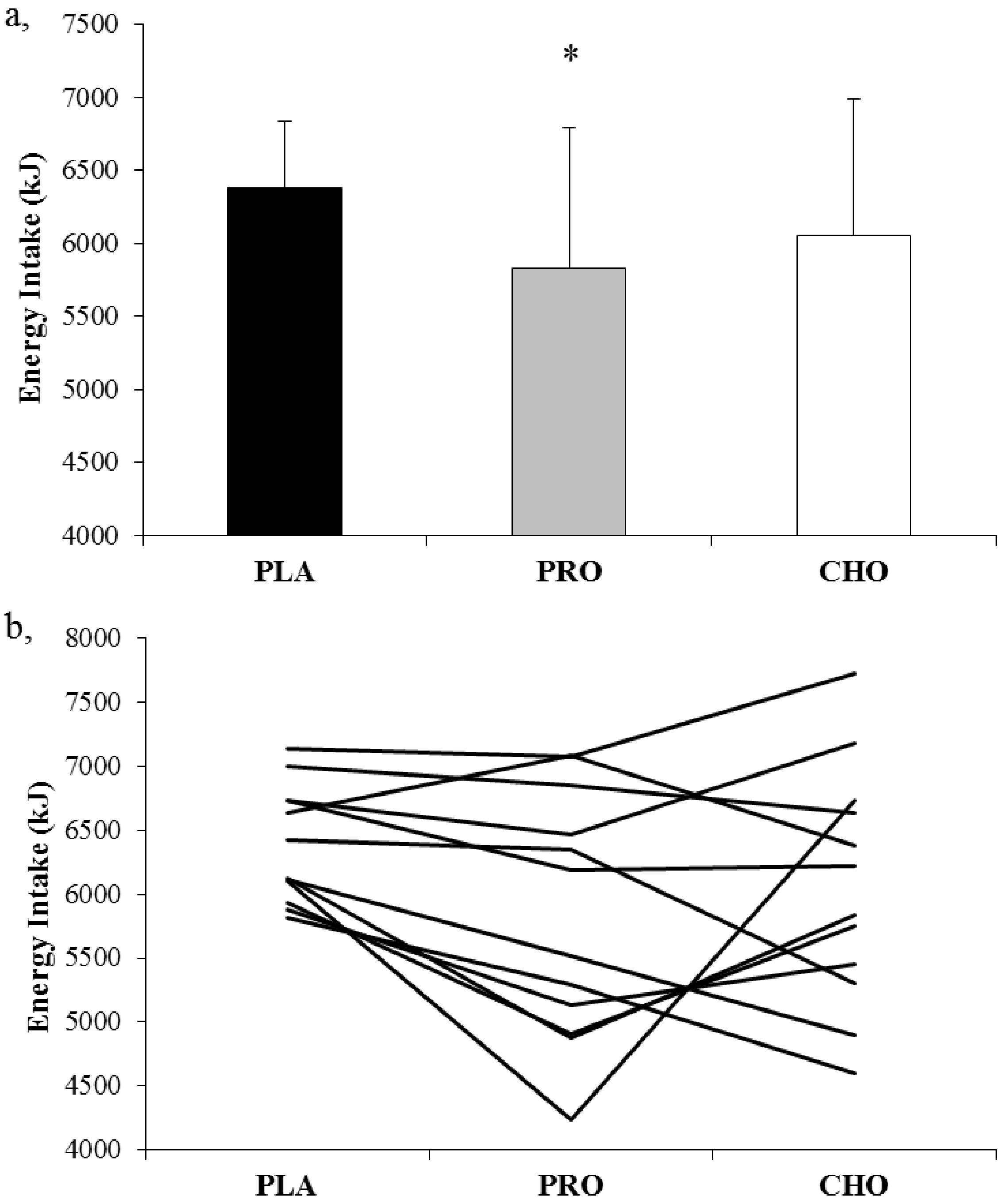
Captions (for figures 1-3)

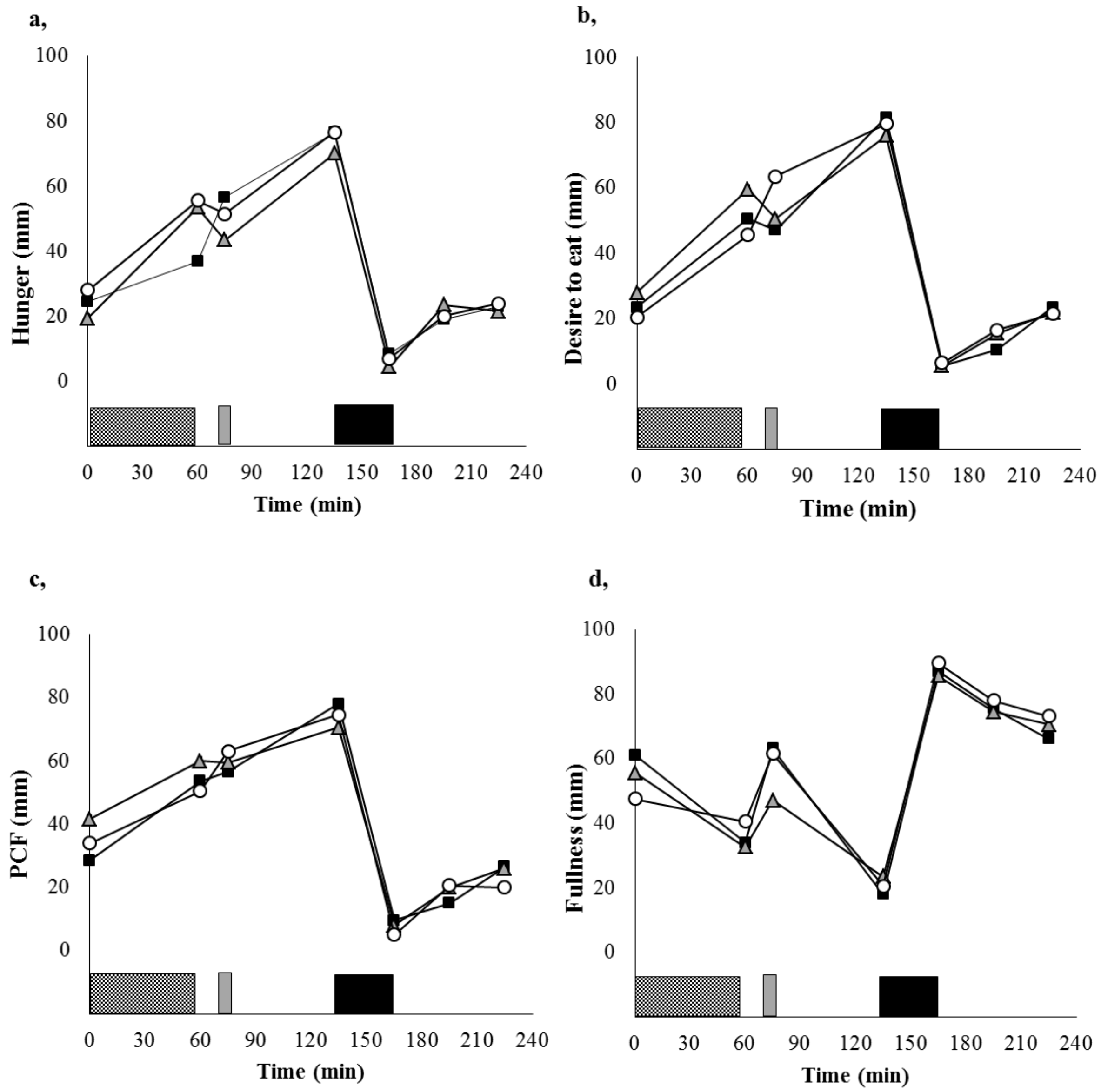
Figure 1. (a) Mean energy intake at the *ad-libitum* test meal (kJ) and (b) subjects individual energy intakes (kJ) during each trial Values are means, with vertical error bars representing standard deviation.* Significantly different from PLA (*P*<0.05)

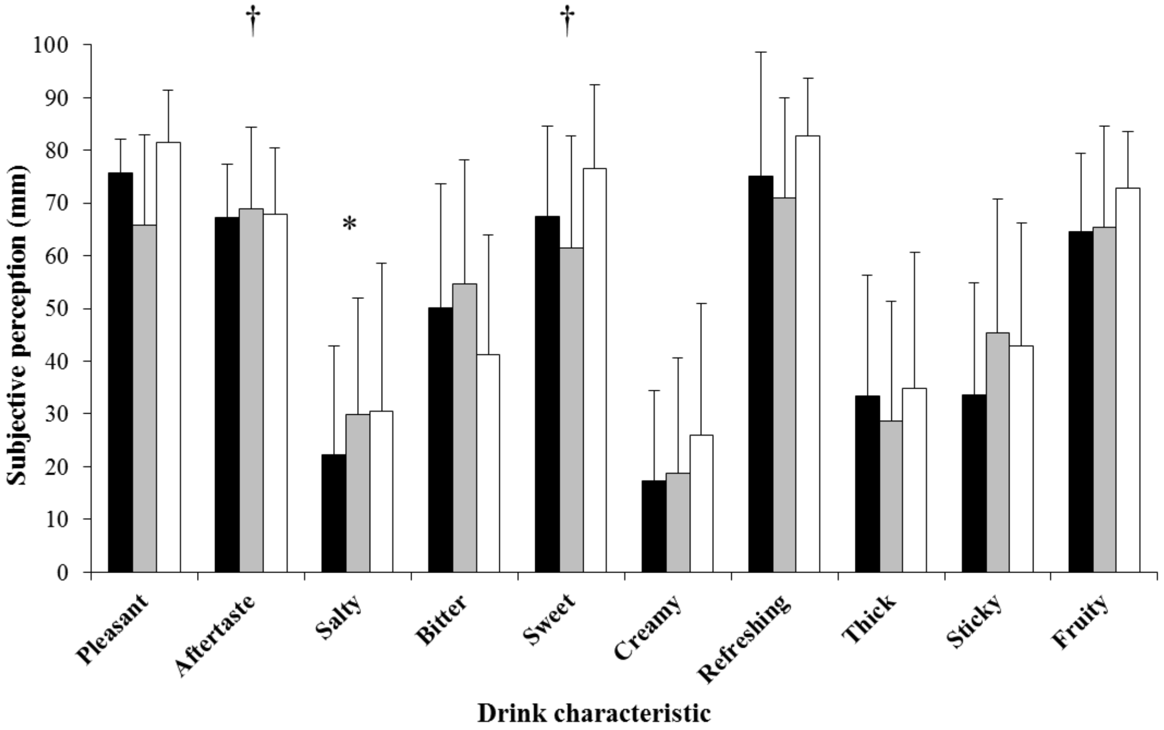
Figure 2. Subjective feelings of hunger (a), desire to eat (b), prospective food consumption (c), and fullness (d) after consuming the placebo (■), protein (▲) and carbohydrate (○) drinks. Hatched shaded rectangle represents exercise, grey rectangle represents ingestion of the post-exercise recovery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

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

Artwork – Figures 1-3




a,
Hunger (mm)
b,
Desire to eat (mm)
c,
PCF (mm)
d,
Fullness (mm)
0
20
40
60
80
100
0 30 60 90 120 150 180 210 240
Time (min)


†
†
*
Subjective perception (mm)
100
90
80
70
60
50
40
30
20
10
0
Pleasant
Aftertaste
Salty
Bitter
Sweet
Creamy
Refreshing
Thick
Sticky
Fruity
Drink characteristic

Tables with captions

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

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

	PLA	PRO	CHO	*P*-value
Initial 30 min				
VO_2 ($L \cdot min^{-1}$)	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 ($b \cdot min^{-1}$)	152 ± 10	153 ± 8	153 ± 9	0.748
RPE	13 ± 1	13 ± 1	13 ± 1	0.395
Intervals				
VO_2 ($L \cdot min^{-1}$)	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 ($b \cdot min^{-1}$)	177 ± 9	176 ± 7	176 ± 8	0.645
RPE	17 ± 1	17 ± 1	17 ± 1	0.925