The Effects of Carbohydrate and Protein Hydrolysate co-ingestion upon Exercise Metabolism and Cycling Performance

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by

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Abstract

The ergogenic effects of acute carbohydrate (CHO) supplementation during prolonged exercise are well established. Recently, a number of studies have examined the potential for the inclusion of protein to further augment these efficacious effects. However, observations at present remain equivocal. Furthermore, there is currently a dearth of knowledge regarding the potential physiologic influences of specific protein sources. Therefore, the purpose of the current study was to determine the efficacy of adding whey protein (CHO-PRO) and hydrolysed marine peptides (CHO-PRO-PEP) to a CHO solution when compared to an equally energetic CHO only beverage. Following an initial familiarisation, 12 recreationally active male volunteers performed three randomised, double blind trials. The trials consisted of a 90-minute steady-state cycle preload corresponding to 50% of predetermined maximal power output, followed by a 5 km time-trial. From the onset of exercise and at 15-minute intervals during the initial preload, participants ingested 180 ml of either: CHO (67 g hr\(^{-1}\) of maltodextrin); CHO-PRO (53.1 g hr\(^{-1}\) of maltodextrin, 13.6 g hr\(^{-1}\) of whey protein concentrate) or CHO-PRO-PEP (53.1 g hr\(^{-1}\) of maltodextrin, 11.0 g hr\(^{-1}\) of whey protein concentrate and 2.4 g hr\(^{-1}\) of protein hydrolysate extracted from salmon). Physiological measures including heart rate, blood glucose and blood lactate were also acquired at 15-minute intervals concomitant to expired gas analysis. No significant differences were observed in time-to-complete the 5 km time-trial regardless of the solution ingested (455.49 ± 16.10; 455.57 ± 18.09 and 454.83 ± 20.75 seconds for CHO, CHO-PRO and CHO-PRO-PEP respectively, p = 0.97). Average and peak power output also demonstrated no statistical significance between treatments (p = 0.71 and p = 0.44, respectively). Conversely, significant interaction effects (condition × time) were apparent for both blood lactate (p = 0.02) and the respiratory exchange ratio (p = 0.007). Heart rate
also demonstrated a significant main effect for condition (p = 0.047). No other physiological parameters were significantly different between conditions. This data therefore suggests that CHO-PRO and CHO-PRO-PEP supplementation provide no additional performance enhancing effects in comparison to a typical CHO only solution. However, the inclusion of protein from marine sources within a CHO solution may influence substrate utilisation during prolonged steady-state exercise.

**Key Words:** Protein, cycle ergometry, exercise performance, ergogenic aids.
Chapter 1 - Introduction

The efficacy of carbohydrate (CHO) feedings during exercise is well established and has been discussed at length (Jeukendrup, 2004; 2008; Jeukendrup and Jentjens, 2000). A series of studies conducted during the 1980s were the first to consistently demonstrate that exogenous CHO supplementation enhanced exercise capacity during prolonged (approximately 2 hours), moderate to high intensity activity (Coyle et al., 1983; Hargreaves, Costill, Coggan, Fink and Nishibata, 1984; Ivy et al., 1983). Moreover, numerous studies over the previous three decades have served to reaffirm these observations, resulting in the general consensus that CHO ingestion can improve endurance performance (Jeukendrup, 2004; 2008; Jeukendrup and Jentjens, 2000). Although the specific mechanism(s) responsible for these effects remain to be fully elucidated (Jeukendrup, 2010; Karelis, Smith, Passe and Peronnet, 2010), the current viewpoint is that CHO supplementation attenuates perturbations in CHO metabolism via the maintenance of euglycaemia, higher rates of CHO oxidation and sparing of hepatic glycogen reserves during prolonged exercise that is dependent upon high rates of glycolytic flux (Jeukendrup, 2004; 2008; Karelis et al., 2010).

Similar to endurance exercise, others have also observed performance enhancing benefits during relatively short duration (≤ 1 hour), high intensity exercise (Anantaraman Carmines, Gaesser and Weltman, 1995; Below, Mora-Rodriguez, Gonzalez-Alonso and Coyle, 1995; El-Sayed, Balmer and Rattu, 1997; Jeukendrup, Brouns, Wagenmakers and Saris, 1997). However, such ergogenic effects have not been documented by others (Clark, Hopkins, Hawley and Burke, 2000; McConell, Canny, Daddo, Nance and Snow, 2000). Given that endogenous stores of CHO are unlikely to be a limiting factor during exercise of such duration and any possible ergogenic effects are unrelated to the availability of
supplementary CHO (Carter, Jeukendrup, Mann and Jones, 2004a; McConell et al., 2000), it would appear that a metabolic explanation is not plausible (Jeukendrup and Chambers, 2010). More recently, several investigators have proposed the existence of a CHO-specific central mediated effect as a consequence of the novel method of rinsing CHO solutions in the mouth, as opposed to ingestion, which has been shown to increase the excitability of corticomotor pathways (Chambers, Bridge and Jones, 2009; Gant, Stinear and Byblow, 2010) and enhance performance (Carter, Jeukendrup and Jones, 2004b; Pottier, Bouckaert, Gilis, Roels and Derave, 2010; Rollo, Williams, Gant and Nute, 2008). Collectively, the aforementioned evidence highlights several different mechanisms, which would appear to be duration and intensity dependant, via which ingesting CHO solutions may augment performance.

Concomitant to the premise that CHO administration is ergogenic, several groups have also investigated whether the addition of a small amount of protein to a CHO solution (CHO-PRO) may offer further benefits to that of CHO only beverages. Results to date however are, at best, equivocal (Jeukendrup and Tipton, 2009; Stearns, Emmanual, Volek and Casa, 2010). From a performance perspective studies can be categorised into those employing assessments of exercise capacity (i.e. time-to-exhaustion protocols) and those assessing exercise performance (time-trial), of which the latter are more ecologically valid (Currell and Jeukendrup, 2008a). However, this clarity is lost when the CHO-PRO beverages themselves are considered, given both the intra- and inter-study differences in nutritional and calorific content (Betts and Williams, 2010; Stearns et al., 2010). For example, the consumption of CHO-PRO beverages has been shown to significantly improve time-to-exhaustion by 13-40% (Ivy, Res, Sprague and Widzer, 2003; Saunders, Kane and Todd, 2004; Saunders, Luden and Herrick, 2007). However, despite solutions containing the same
amount of CHO, the feeding strategies employed by those reporting ergogenic effects provided CHO at levels below that considered optimal to attain peak exogenous CHO oxidation from a single CHO source (Jeukendrup, 2004; 2008). Consequently, it is unclear whether the observed benefits were mediated via a protein specific mechanism, or simply the additional energy content in the CHO-PRO solutions (Saunders, Moore, Kies, Luden and Pratt, 2009). Conversely, not all those utilising time-to-exhaustion protocols have reported performance enhancing effects (Ferguson-Stegall et al., 2010; Martinez-Lagunas, Ding, Bernard, Wang and Ivy, 2010; Romano-Ely, Todd, Saunders and St. Laurent, 2006; Valentine, Saunders, Todd and St. Laurent, 2008). Interestingly, in studies matching treatments for total calories (Romano-Ely et al., 2006; Valentine et al., 2008), in which the addition of protein ultimately reduced the CHO content, or in instances where sub-optimal rates of CHO were administered and solutions not matched for energy provision (Ferguson-Stegall et al., 2010; Martinez-Lagunas et al., 2010), no significant differences in performance have been reported. Thus, although not strictly ergogenic, the addition of protein maintained the efficacy of the beverages as performance was not reduced. This may suggest that the inclusion of protein had an independent metabolic effect.

Regarding supplement composition, CHO-PRO solutions have typically contained approximately 2% protein, providing roughly 20 g of the macronutrient per hour (Jeukendrup and Tipton, 2009; Stearns et al., 2010); although some have utilised considerably less (Ferguson-Stegall et al., 2010; Martinez-Lagunas et al., 2010). However at present little attention has been paid to the type of protein ingested, in which whey protein has almost universally been employed (Ferguson-Stegall et al., 2010; Romano-Ely et al., 2006; Saunders et al., 2004; Toone and Betts, 2010).
A limited number of contemporary studies have, however, sought to determine the potential benefits of protein hydrolysates when concurrently ingested with CHO during prolonged aerobic exercise (Breen, Tipton and Jeukendrup, 2010; Saunders et al., 2009; Vegge, Ronnestad and Ellefsen, 2012). Protein hydrolysates consisting of small chain amino acids may offer additional benefits in comparison to their intact proteins. These include increased digestion and absorption kinetics (Calbet and Holst, 2004; Koopman et al., 2009) and a greater insulinaemic response when ingested alone (Koopman et al., 2009), or with CHO (van Loon, Kruijshoop, Verhagen, Saris and Wagenmakers, 2000a; van Loon, Saris, Kruijshoop and Wagenmakers, 2000b; van Loon, Saris, Verhagen and Wagenmakers, 2000c). Protein hydrolysates also differ from one another nutritionally, and may therefore elicit different and very specific physiological effects (Kim and Mendis, 2006; Moughan, Fuller, Han, Kies and Miner-Williams, 2007). Indeed, chronic consumption of hydrolysates produced from fish protein has been shown increase fatty acid oxidation and reduce adipose tissue mass in rats, when compared to an equal-energetic amount of soy protein (Liaset et al., 2009; Vegge et al., 2012). Furthermore, protein hydolysates produced from marine sources may also attenuate vasoconstriction and promote vasodilation by inhibition of the angiotensin converting enzyme within the renin-angiotensin system (Ewart et al., 2009; Hong et al., 2008). The addition of hydrolysates derived from fish protein may therefore offer additional ergogenic effects; potentially reducing the utilisation of finite glycogen stores by increasing fatty acid oxidation (Vegge et al., 2012). Such an effect would be advantageous during prolonged moderate to high-intensity exercise within which glycogen depletion is associated with fatigue (Bergstrom, Hermansen, Hultman and Saltin, 1967; Bergstrom and Hultman, 1967). The inclusion of hydrolysates from marine sources may also reduce cardiovascular strain and better maintain thermoregulatory function during
prolonged exercise. Evidence from a recent conference proceeding (Vegge et al., 2010) suggests that the addition of fish protein hydrolysate to a CHO and whey protein solution may significantly improve exercise performance; in comparison to an iso-energetic CHO-PRO solution and CHO only beverage matched for CHO as opposed to energetic content. However, it was observed that the ergogenic effects of concurrent protein hydrolysate ingestion may be dependent upon individual variation in performance level, with no overall differences in exercise performance when the data was analysed as a collective (Vegge et al., 2012).

To the best of the author’s knowledge, no published study to date has examined the efficacy of energetically-equivalent CHO and CHO-PRO solutions containing protein hydrolysates from marine sources during exercise. Therefore the purpose of the current study was to assess the efficacy of protein hydrolysates derived from salmon meat, when concurrently ingested with CHO and whey protein, upon exercise metabolism during an initial cycle preload and subsequent 5 km cycling time-trial performance.
Chapter 2 - Literature Review

2.1 Introduction

Elite athletes possess several common attributes of which talent is undoubtedly the most influential (Maughan, King and Lea, 2004a). From a physiological perspective, elite performance is the product of favourable genetics, robust training and the sound application of nutrition (Applegate, 1999; Maughan et al., 2004a; Pearce, 2005; Williams and Folland, 2009). The latter is critical, as the quantity and quality of nutritional intake play a central role in the adaptive response to training; which subsequently enables athletes to perform at the limits of their physiological capacity (Breen et al., 2010; van Loon, 2007). Moreover, a commitment to correct nutritional practice can make the difference between success and failure (Maughan et al., 2004a). The application of sports nutrition is far from a novel concept and has existed in various guises dating back to the first Olympia (Grivetti and Applegate, 1997). However, it is only during the last four decades in which an ever advancing knowledge of exercise metabolism has produced a transition from an anecdotal to an increasingly evidenced based approach (Grandjean, 1997; Jeukendrup, 2008; Kreider et al., 2010; Rodriguez, DiMarco and Langley, 2009).

In addition to habitual energy intake, the majority of athletes also consume other exogenous compounds with the desired outcome of further enhancing performance beyond that of making educated food choices alone (Maughan et al., 2004a). Nutritional ergogenic aids represent a diverse and constantly expanding number of food stuffs which appeal to athletes and support staff alike. However, the evidence supporting the efficacy of some supplements is tentative; often due to the poor methodological design of studies (Pearce, 2005). Furthermore, the purported benefits of many nutritional ergogenic aids are exaggerated as
data are extrapolated from studies of best fit as opposed to definitive evidence (Burke, Castell and Stear, 2009; Lopez and Casa, 2009; Maughan et al., 2004a; Pearce, 2005). The spectrum of available supplements is vast, ranging from essential macronutrients, including CHO and protein, to those less familiar such as creatine and sodium bicarbonate (Applegate, 1999; Burke et al., 2009; Pearce, 2005). Although many supplements are targeted towards anaerobic based activity, as evidenced by the latter, there are several others that may benefit exercise that is prolonged in nature (Juhn, 2002). Long-duration aerobic activity is unique as athletes are required to address their nutritional needs not simply pre and post exercise, but most critically, during exercise (Burke, Millet and Tarnopolsky, 2007; Saunders, 2007).

It is well documented that CHO ingestion during endurance exercise, predominately cycling, can improve performance (Jeukendrup, 2004; 2008; 2010; Pfeiffer, Stellingwerff, Zaltas, Hodgson and Jeukendrup, 2011). However, despite its apparent efficacy, several issues remain unclear including the mechanism(s) via which CHO ingestion manifests its ergogenic effects and the optimal type and dose of CHO to confer such benefits (Jeukendrup, 2008; Karelis et al., 2010). Similarly the recent practice of adding a small quantity of protein to further enhance the efficacy of CHO solutions also remains unclear (Stearns et al., 2010). Therefore, it is the purpose of the following discussion to review the current literature implicit to the use of CHO during exercise and the efficacy of simultaneous CHO and protein co-ingestion.
2.2 Carbohydrate and Prolonged Exercise: Performance and Mechanisms

It is generally accepted that the consumption of CHO during prolonged exercise has an ergogenic effect (Jeukendrup, 2004; 2008; 2010). This consensus has been reflected within both past and present position statements published by the American College of Sports Medicine (ACSM) and other authoritative bodies regarding nutritional intake and exercise performance (Jeukendrup, 2004; Rodriguez et al., 2009). However, although CHO ingestion during exercise is widely advocated, the mechanism(s) underlying such benefits remain to be fully elucidated (Jeukendrup, 2008; 2010; Karelis et al., 2010). At present, evidence from empirical data has identified three possible physiologic mechanisms: glycogen sparing, maintenance of euglycaemia and high rates of CHO oxidation (Jeukendrup, 2008; 2010; Tsintzas and Williams, 1998), and more recently, a central mechanism (see Jeukendrup and Chambers, 2010; Rollo and Williams, 2011 for review).

The discussion to follow will specifically address the physiological mechanisms related the efficacy of CHO supplementation.

2.2.1 Reduced Glycogen Utilisation

The initial observation of a possible reduction in muscle glycogenolysis following CHO supplementation, commonly referred to as a glycogen “sparing effect”, was reported by Bergstrom and Hultman (1967). Later, Coyle et al. (1983) observed that when participants consumed a glucose polymer (1.0 g·kg\(^{-1}\) after 20 minutes and 0.25 g·kg\(^{-1}\) at 60, 90 and 120 minutes, respectively), that they were able to cycle significantly longer than when prescribed a taste matched placebo (157 ± 5 versus 134 ± 6 minutes at 74 ± 2% VO\(_{2\text{max}}\)). This was accompanied by a significant increase in blood glucose concentration and the maintenance of plasma insulin concentration following ingestion of the CHO beverage.
Intriguingly, three of the participants displayed no significant differences in blood glucose or exercise capacity when prescribed the placebo in comparison to CHO. However, regardless of condition, no differences were observed in the respiratory exchange ratio (RER) or CHO oxidation in which the authors concluded that CHO consumption during exercise slowed the utilisation of muscle glycogen by maintaining euglycaemia (Coyle et al., 1983; Coyle, Coggan, Hemmert and Ivy, 1986).

Subsequently, Coyle and co-workers (1986) measured muscle glycogen content directly. Similar to the authors’ previous study participants again performed cycle ergometry to exhaustion at approximately 74% VO$_{2\text{max}}$ (70-74% VO$_{2\text{max}}$). However, although again consuming a glucose polymer, the initial bolus dose of CHO was 2.0 g·kg$^{-1}$ after 20 minutes of exercise with subsequent feedings of 0.4 g·kg$^{-1}$ at 20 minute intervals for the duration of the trials. Therefore participants consumed significantly more CHO during both the early stages and throughout the cycling trial than the authors’ previous study (Coyle et al., 1983). Concurringly, time-to-exhaustion was significantly longer following the consumption of CHO in comparison to the placebo (4.02 ± 0.33 hours versus 3.02 ± 0.19 hours, respectively). However in contrast to their previously presented data, both the RER and total CHO oxidation significantly deviated between treatments during the latter stages of exercise. The RER (0.84-0.87) and total CHO oxidation (2.2-1.8 g·min$^{-1}$) were maintained throughout the entirety of the protocol with CHO supplementation. Conversely, when consuming the placebo, both the RER and total CHO oxidation declined significantly during the third hour of exercise to values of 0.80 ± 0.1 and approximately 1.4 g·min$^{-1}$ respectively, at the termination of exercise.

Concerning a potential reduction in glycogen utilisation with CHO feedings, the authors observed no significant differences in muscle glycogen content after 2 or 3 hours of steady-
state exercise regardless of condition (Coyle et al., 1986). Upon initial evaluation, CHO supplementation failed to mediate the hypothesised glycogen sparing effect. However, when samples were acquired at exhaustion following CHO ingestion (4 hours), it was observed that no further muscle glycogen had been utilised. Given a modest but non-significant rise in plasma glucose and insulin concentrations and an apparent delay in increased CHO oxidation, it has been suggested by others that the ingested CHO may not have been available to the contracting musculature until after 2 hours of exercise (Tsintzas and Williams, 1998). This data, coupled with the previously stated observation that no further muscle glycogen was utilised during the additional hour of cycling performed following CHO ingestion, suggests that a slowing of muscle glycogen usage may have occurred during the latter stages of exercise as a consequence of CHO feedings (Tsintzas and Williams, 1998).

Similarly, others reported no significant differences in muscle glycogen consumption when either CHO or a placebo was ingested (Flynn et al., 1987; Hargreaves and Briggs, 1988; Mitchell et al., 1989). As with that of Coyle et al. (1986), the latterly cited investigators observed only a modest increase in blood glucose (0.5-1.5 mmolL\(^{-1}\)) and a non-physiologically meaningful increase in plasma insulin levels when participants exercised continuously at a comparable exercise intensity (approximately 70% \(\text{VO}_{2\text{max}}\)); however, again, exercise capacity or performance was improved by maintenance of euglycaemia and higher rates of CHO oxidation compared with a placebo (Flynn et al., 1987; Hargreaves and Briggs, 1988; Mitchell et al., 1989; Tsintzas and Williams, 1998).

The ingestion strategies employed by these preliminary studies, in which CHO was not typically consumed until 10-20 minutes after the onset of exercise (Coyle et al., 1986; Hargreaves and Briggs, 1988; Mitchell et al., 1989), may have impaire
opposed to when CHO is consumed prior to or at the onset of exercise (De Bock, Derave, Ramaekers, Richter and Hespel, 2007; Tsintzas, Williams, Boobis and Greenhaff, 1995; 1996). Therefore it has been suggested that the absence of significant increases in blood glucose, and perhaps more critically, increased insulin secretion during the initial stages of cycle ergometry, may explain why the aforementioned works failed to observe a glycogen sparing effect (Tsintzas and Williams, 1998). Indeed, when continuously infusing glucose, beginning 8 minutes after the onset of exercise to produce sustained hyperglycaemia for the duration of a prolonged steady-state ride (2 hours at 73 ± 2% VO$_{2\text{max}}$), Coyle, Hamilton, Gonzalez-Alonso, Montain and Ivy (1991) reported no significant rise in plasma insulin levels compared to a control condition until the second hour of exercise, peaking at approximately 80 minutes. Accordingly, the authors reported no glycogen sparing effect, regardless of muscle fibre type. Therefore, it appears that hyperinsulinaemia during the early stages of exercise is essential to reduce muscle glycogen utilisation (Tsintzas et al., 1995; 1996; Tsintzas and Williams, 1998).

In addition, two recent investigations have reported reduced glycogenolysis in type I, II (Stellingwerff et al., 2007) and type IIa muscle fibres (De Bock et al., 2007), in which CHO feedings at the onset of exercise produced significant elevations in blood glucose and plasma insulin compared to a placebo and control conditions. Moreover, Stellingwerff and co-workers (2007) found that exogenous CHO supplementation spared muscle glycogen usage in a time-dependent manner during a prolonged bout (3 hours) of cycle ergometry at 63 ± 4% VO$_{2\text{max}}$. The authors concluded that increased plasma glucose uptake and CHO oxidation during the initial stages of exercise reduced muscle glycogen flux during the first hour of activity and in turn reduced net (type I and II) muscle glycogen usage. Not all investigations in which CHO was ingested prior to or upon the onset of exercise (or in
combination) have observed such benefits (Arkinstall, Bruce, Nikolopoulos, Garnham and Hawley, 2001; Jeukendrup et al., 1999a; 1999b). Methodological differences may however explain the equivocal nature of the respective findings. For example, despite inducing a two-to-three fold increase in plasma insulin and an approximate 1.5 mmol L\(^{-1}\) rise in plasma glucose, individuals participating in both studies by Jeukendrup and colleagues (1999a; 1999b) cycled for 120 minutes at 50% VO\(_{2\text{max}}\), a notably lower intensity than that of those reporting reduced glycogen degradation (De Bock et al., 2007; Stellingwerff et al., 2007). However, based on previous observations that suggest cycling at lower intensities (45-48% VO\(_{2\text{max}}\)), either continuously or intermittently, reduces muscle glycogen breakdown in type I fibres, it is perhaps surprising that Jeukendrup et al. (1999a; 1999b) did not report similar findings. This point also highlights a further methodological inconsistency between the studies in which muscle glycogen content was not determined directly via biopsy samples in the instance of Jeukendrup and co-workers (1999a, 1999b). Instead, glycogen utilisation was calculated from total CHO and plasma CHO oxidation. Use of this method may therefore have negated the potential reduction in glycogen usage within specific muscle fibres.

Conversely, in the case of Arkinstall et al. (2001) both exercise intensity (70% VO\(_{2\text{max}}\)) and the direct determination of muscle glycogen content make the methodologies more comparable to those reporting reduced glycogenolysis following CHO ingestion (De Bock et al., 2007; Stellingwerff et al., 2007). Similarly, plasma glucose increased in a similar fashion between the studies (approximately 2 mmol L\(^{-1}\)), with plasma insulin levels also mirroring this trend (Arkinstall et al., 2001). Interestingly, Stellingwerff et al. (2007) reported that reduced glycogen usage during the initial 60 minutes of exercise resulted in a net reduction in glycogen degradation in both type I and II muscle fibres over the entirety
of the 3 hour protocol. In contrast, despite the protocol of Arkinstall et al. (2001) being 60 minutes in total, they reported no significant differences in muscle glycogen reserves. However this may be explained by the direct determination of mixed muscle glycogen content prior to and upon completion of the 60-minute exercise bout in the case of Arkinstall et al. (2001) and indirect calculation of muscle glycogen stores at the 60 minute point, representative of a third of the total trial, by Stellingwerff et al. (2007).

Collectively, these studies highlight more than the methodological differences regarding exercise intensity, CHO ingestion and subsequent effects on metabolism, as previously discussed. The contrasting analytical techniques regarding direct and indirect determination of muscle glycogen content also merit discussion. As eluded to previously, Jeukendrup and colleagues (1999a; 1999b) calculated muscle glycogen oxidation indirectly on a whole body level via indirect calorimetry and stable isotopic methods (total CHO oxidation - plasma glucose oxidation). However, this method removes the ability to discriminate between glycogenolysis in individual muscle fibres or specific muscle site as reported by others (De Bock et al., 2007; Stellingwerff et al., 2007). It is also based on the assumptions that muscle glycogen and blood glucose are the original sources of CHO oxidation during exercise and the disappearance of CHO from the blood is reflective of blood glucose oxidation (Coyle, 1996). On the other hand, this indirect measure overcomes the confounds of the traditional method of assessing pre and post exercise changes in muscle glycogen content via muscle biopsy specimens, due to the inherent variability in samples acquired from the same, or different, incision sights and at separate times (De Bock et al., 2007). This is particularly pertinent when comparing studies examining mixed muscle fibre glycogen usage and others evaluating glycogen depletion in specific fibres (Tsintzas and Williams, 1998). More contemporary works have combined several methods such as
indirect calorimetry, stable isotope infusion and biopsy sampling, to determine overall CHO usage during exercise (Arkinstall et al., 2001; Stellingwerff et al., 2007). It is therefore evident that not simply the exercise protocol, but also the methods of muscle glycogen assessment, are essential as to whether or not a “sparing” effect is observed.

Exercise modality may also be an important methodological consideration. Indeed, it has been suggested by some that glycogen sparing may have greater relevance to unsupported modes of exercise such as running, as opposed to supported modes such as cycling, given the apparent physiological responses evoked by the two (Tsintzas and Williams, 1998). Based on evidence from their laboratory (Tsintzas et al., 1995; 1996), Tsintzas and Williams (1998) proposed that glycogen sparing may be related to the higher insulin response apparent during the initial stages of treadmill running compared with cycle ergometry. The authors reported a reduction in glycogen degradation after 60 minutes of running in type I muscle fibres only (Tsintzas et al., 1995). Later, in a test of exercise capacity, Tsintzas and colleagues (1996) employed the same exercise intensity and 5.5% CHO solution to that of their previous study. In contrast, the actual CHO feeding strategy and subject demographics (VO2max: 54.5 ± 2.0 ml·kg⁻¹·min⁻¹ versus 61.8 ± 2.3 ml·kg⁻¹·min⁻¹) were different. Regardless of these discrepancies, a glycogen sparing effect was again observed in type I muscle fibres, accompanied by similar increases in blood glucose and serum insulin concentrations during the initial 20 minutes of exercise (Tsintzas et al., 1996).

However, these authors did not directly compare running and cycling within the same study. The most recent evidence, published by Pfeiffer et al. (2011), illustrates contrary to the suggestions of Tsintzas and Williams (1998), that there appears to be no difference in the blood-borne substrate or hormone responses implicit to CHO metabolism during
running compared with cycling; when volunteers were fed identical CHO solutions and exercised at the same relative intensity (~60% VO2max). Moreover, significant differences were only apparent within conditions when water and CHO were ingested as opposed to between exercise modes. Such changes may be mediated via a dose-response relationship with exogenous CHO supplementation as opposed to a specific exercise modality effect as inferred previously (Tsintzas and Williams, 1998). However, although demonstrating similar metabolic responses between the two modalities, muscle glycogen content was not specifically determined (Pfeiffer et al. 2011). These authors did, however, observe a trend towards reduced endogenous CHO oxidation during the final 60 minutes of running compared with cycling when fed CHO, which was allocated to reduced hepatic glycogenolysis, although this did not reach statistical significance (p = 0.09).

To the best of the author’s knowledge, the novel work of Arkinstall et al. (2001) is the only investigation to-date to directly compare the physiological responses between cycle ergometry and treadmill running within the same population. Firstly, concerning mixed muscle glycogen content, Arkinstall et al. (2001) reported no statistically significant differences in glycogen utilisation in the vastus lateralis when water or CHO (64 ± 3 g hr⁻¹) were ingested during either cycling or running. Despite this, CHO supplementation resulted in approximately a 20% reduction in muscle glycogen degradation in the latter exercise modality. This finding further highlighting the pitfalls of assessing muscle glycogen using mixed as opposed to single fibres. Moreover, by the authors own admissions, the low subject number and lack of statistical power may have also impaired their ability to report a statistically meaningful effect (Arkinstall et al., 2001). Secondly, with reference to metabolic factors, plasma glucose values were significantly elevated (peaking at approximately 6.5 mmol L⁻¹) following CHO ingestion during both exercise modalities,
similar to those the values recently reported by Pfeiffer and associates (2011). However, despite these values being similar during the initial 30 minutes of exercise, they gradually declined close to baseline levels in the study of Arkinstall et al. (2001), whilst in contrast, remaining elevated for the duration of the protocol in the instance Pfeiffer et al. (2011). However, plasma insulin concentrations were not comparable between the studies. Moreover, given the methodological differences between the studies relating to exercise intensity and CHO consumption, it is perhaps not surprising that differences in the metabolic response were evident. Indeed, as proposed by Pfeiffer et al. (2011), the insulin response may have been maximised during both exercise modalities in their study due a greater consumption of CHO.

Regarding the observed differences in exercise modality, it is apparent that during cycle ergometry glycogen is predominantly utilised in the vastus lateralis, the almost universal site of muscle biopsy sampling regardless of exercise mode. In comparison, during treadmill running, the gastrocnemius and soleus demonstrate the greatest amount of glycogen degradation (Costill, Gollnick, Jansson, Saltin and Stein, 1973 and Costill, Jansson and Gollnick, 1974 as cited by Arkinstall et al., 2001). Therefore, although selection of the same muscle biopsy sampling site, regardless of mode, allows for inter-study comparison, given the mode specific differences as outlined above, it is understandable why some studies have reported reduced glycogen usage during running (Tsintzas et al., 1995; 1996). Conversely, had the musculature of the lower leg been the predominate site of acquiring muscle biopsy specimens in previous studies, it may be that the preceding discussion would have focused upon the glycogen sparing effect of CHO ingestion during cycle ergometry as opposed to treadmill running.
It is evident that given the plethora of methodological discrepancies and subsequent equivocal findings, that at present there is limited evidence to suggest CHO consumption during prolonged continuous exercise may mediate a glycogen sparing effect. Although it has been suggested that reduced glycogenolysis may have greater significance to treadmill running in contrast to cycle ergometry, given the current lack of studies providing direct comparisons between the two, no firm conclusions can be drawn. Therefore the prevailing view is that CHO ingestion most likely mediates its ergogenic effects by mechanisms other than reducing muscle glycogen degradation during exercise.

2.2.2 Maintenance of Euglycaemia and Carbohydrate Oxidation

Two further metabolic mechanisms - maintenance of euglycaemia and high rates of CHO oxidation - appear to be implicit pathways via which CHO ingestion mediates its ergogenic effects during prolonged exercise (see reviews: Jeukendrup, 2004; 2008; 2010; Jeukendrup and Jentjens, 2000; Jeukendrup and Tipton, 2009). A series of studies during the 1980s were the first to establish the importance of maintaining blood glucose, and subsequently CHO oxidation, upon exercise capacity, and later exercise performance.

Coyle and colleagues (1986) observed that when ingesting a taste matched placebo, participants’ plasma glucose concentrations began to gradually decline after 60 minutes of cycle ergometry at approximately 70% VO\textsubscript{2max}, in which subjects became hypoglycaemic at the point of exhaustion (~2.5 mmol\textsuperscript{L\textsuperscript{-1}}). Carbohydrate oxidation mirrored this trend and similarly declined in an almost linear fashion to the point of fatigue. In contrast, when consuming CHO at regular intervals, plasma glucose was maintained within a homeostatic range throughout exercise (4.2-5.2 mmol\textsuperscript{L\textsuperscript{-1}}), becoming significantly different to the placebo after 80 minutes. Subsequently, CHO oxidation was maintained throughout the
entirety of the protocol when CHO was ingested, in which time-to-exhaustion was also significantly greater in comparison to the placebo. However, given that such favourable metabolic conditions were maintained throughout exercise, and that muscle glycogen concentration did not decline significantly further during the final hour of the CHO trial, it remains to be established why subjects experienced fatigue. As noted by Jeukendrup (2004), it is interesting that plasma glucose did not decline, even with CHO ingestion during the latter stages of exercise, in accordance with other early empirical works, including those from the same laboratory (Coyle et al., 1983; Flynn et al., 1987; Neufer et al., 1987).

Regarding performance, such ergogenic effects were not unique, as the same, and several other laboratories reported similar benefits, although not all (see Jeukendrup, 2004 for review). Furthermore, Coggan and Coyle (1987) later demonstrated that when glucose was administered either intravenously or orally at the point of exhaustion, and so restoring euglycaemia, participants were able to continue for significantly longer during a second successive fatiguing bout of cycle ergometry, compared to the placebo. The authors also reported similar observations when CHO was ingested late in exercise as opposed to at exhaustion (Coggan and Coyle, 1989). It was therefore evident that maintenance of blood glucose, and so CHO oxidation, were essential to prolonged exercise capacity.

However, the plethora of inter and intra-study differences regarding CHO feeding strategies made it challenging to provide recommendations for CHO intake during exercise, with no optimal ingestion strategy identified to maintain blood glucose and maximise CHO oxidation. These methodological discrepancies were also responsible, at least in part, for some of the conflicting metabolic and performance data as highlighted above.
Subsequently, a number of studies were designed with the aim of identifying the optimum amount of CHO required to mediate performance enhancing effects.

2.2.3 Search for the Optimum Dose and Performance

Fielding and co-workers (1985) were among the first of a limited number of studies to assess the efficacy of various CHO doses upon exercise performance. In what was a novel study, participants performed 4 hours of intermittent cycle ergometry (8 × 30 minute intervals consisting of 20 minutes of continuous cycling at 50% VO$_{2\text{max}}$, followed by 10 minutes of intermittent cycling [100% VO$_{2\text{max}}$ for 30 seconds followed by 2 minutes recovery], with the final 100% VO$_{2\text{max}}$ bout at the end of the 4 hour ride performed to exhaustion and taken as the performance measure). During the three trials participants ingested either water (control) or solid CHO (sucrose). The CHO feedings provided identical amounts of CHO each hour (21.5 g hr$^{-1}$). This dose was either administered on the hour commencing at the onset of exercise, or halved and provided at 30 minute intervals, in which the authors referred the former trial as dosage and latter as frequency. Blood glucose concentration remained relatively constant throughout the entirety of the protocol when CHO was ingested frequently at 30 minute intervals, in contrast to the large but predictable fluctuations (peaks and troughs) during the dosage condition. Performance was significantly improved when CHO was consumed frequently compared to control, however no differences were observed between either of the CHO feeding strategies or when the dosage treatment was compared to control.

However, it is interesting to note that the feeding strategy within the aforementioned study has been incorrectly interpreted by recent reviews (Jeukendrup, 2004; 2008), in which it was stated that Fielding and colleagues (1985) compared different hourly CHO dosages (11
and 22 g·hr⁻¹ respectively). In fact, the hourly rate of CHO delivery was identical, as alluded to above. It was only the frequency at which subjects received feedings that was manipulated. Similarly the performance results were also misconceived. Despite no difference in performance between CHO conditions (Fielding et al., 1985), Jeukendrup (2004; 2008) concluded that performance was significantly improved following the ingestion of 22 g·hr⁻¹ in contrast to 11 g·hr⁻¹ (in reality 11 g every 30 minutes), and therefore that the minimum amount of CHO required to confer an ergogenic effect was 22 g·hr⁻¹.

More recent evidence suggests that as little as 16 g·hr⁻¹ of CHO may be sufficient to confer a performance enhancing effect (Maughan, Bethell and Leiper, 1996). However this data should be interpreted with caution given that the hypotonic, low CHO solution, despite improving median time-to-exhaustion by 14%, was not statistically different when compared to water when assessed by non-parametric methods.

Flynn and colleagues (1987) assessed the efficacy of four different solutions including an artificially sweetened and flavoured placebo and three experimental beverages containing 5% or 10% CHO consisting of various amounts of glucose, fructose and maltodextrin. However, in contrast to that of both of the previous cited studies (Fielding et al., 1985; Maughan et al., 1996), Flynn and associates (1987) reported no significant differences in exercise performance regardless of condition despite using a similar feeding strategy that proved a comparable dosage of CHO (16-22 g·hr⁻¹) and, more notably, when also employing greater dosages (45 g·hr⁻¹). The numerous differences between the studies, such as the contrasting methods of evaluating performance (e.g. time-to-exhaustion and time-trial), is of one a number of methodological disparities that may elucidate the contrasting findings. Similarly, the prolonged intermittent nature of the protocol by Fielding et al.
(1985) was very different to the continuous protocols of both Flynn et al. (1987) and Maughan et al. (1996).

Both the investigations of Fielding et al. (1985) and Flynn et al. (1987) utilised a taste-matched placebo which one would assume made it challenging for the subjects to distinguish between the latter and the actual CHO containing beverages. Given the similar metabolic differences between the studies when the placebo was ingested (gradual decline in blood glucose over the duration of the trials), it is clear that within the study of Flynn et al. (1987) that a placebo effect may have been present given that there were no differences in the amount of work completed or average power output between trials.

Subsequently in a series of two studies, Mitchell and colleagues (1988; 1989) investigated the effects of various CHO beverages upon a similar isokinetic time-trial (“all-out” 12 and 15 minute bout respectively). Within the initial study, this performance measure was preceded by a preload of 105 minutes of intermittent cycling (7 × 12 minute blocks at 70% \( \text{VO}_{2\text{max}} \)). Each interval was followed by 3 minutes of recovery in which subjects consumed one of three CHO solutions (5%, 6% and 7.5%) that provided 33g, 40g and 50 g hr\(^{-1}\) of CHO correspondingly or a taste matched placebo. In comparison to the latter, subjects performed significantly more work during the maximal effort time-trial following CHO ingestion. However, there were no differences between CHO solutions, regardless of intake.

Later when utilising a continuous cycle preload (105 minutes at 70% \( \text{VO}_{2\text{max}} \)), the same research group investigated the efficacy of both similar and much larger amounts of CHO upon exercise performance (37 g hr\(^{-1}\), 74 g hr\(^{-1}\) and 111 g hr\(^{-1}\) in 6%, 12% and 18% solutions, respectively). The rationale for the use of these solutions was that they were
representative of commercially available CHO beverages at the time (Mitchell et al., 1989). In comparison to the authors previous observations, it was only when subjects consumed the 12% CHO beverage (74 g hr\(^{-1}\)) that an ergogenic effect was observed when compared to the placebo. Equivocal findings have also been presented by Murray and associates (1987; 1989), when utilising an intermittent preload preceding a cycle sprint. However, a subsequent investigation by this group demonstrated enhanced cycle time-trial performance (4.8 km) when preceded by a similar varying intensity, intermittent preload with regular CHO feedings totalling 26 g hr\(^{-1}\) and 78 g hr\(^{-1}\) (Murray, Paul, Seifert and Eddy, 1991).

More recently, Galloway and co-workers (Galloway, Wootton, Murphy and Maughan, 2001) further addressed this issue, concomitant to the influence of ambient temperature. In this instance the authors investigated the effects of 2%, 6% and 12% CHO solutions fed relative to the participants’ body mass in which volunteers cycled to exhaustion in an atypical environment (10°C). As the authors hypothesised, based on previous observations from their laboratory (Galloway and Maughan, 1998), CHO ingestion failed to elicit an ergogenic influence upon time-to-exhaustion. However, as CHO oxidation rates were comparable to other empirical data in which cycle ergometry was performed at typical ambient temperatures, the authors advocated the use of 6-12% CHO solutions.

Given the number of intra and inter-study differences, and the resulting equivocal findings, it is difficult to draw firm conclusions regarding the amount of CHO required to mediate a performance enhancing effect. One may conclude, perhaps, that approximately 20 g hr\(^{-1}\) of CHO is the minimum amount required to elicit performance benefits based on the aforementioned literature (Fielding et al., 1985; Jeukendrup, 2008; Maughan et al., 1996). In the main, the majority of studies have provided between 40-75 g hr\(^{-1}\) of CHO and subsequently observed a performance enhancing effect, in which results have greater
consistency as CHO supplementation approaches the higher end of this spectrum (Jeukendrup, 2004; 2008). This has been reflected in the most recent position statement by the ACSM in which it was recommend that athletes should consume 30-60 g·hr⁻¹ of CHO during prolonged exercise (Rodriguez et al., 2009).

2.2.4 Exogenous Carbohydrate Oxidation

To this end discussion has centred on the amount of CHO required to produce favourable improvements in performance. However such benefits may not be implicitly dependent upon the absolute amount of CHO consumed. Alternatively, the desired ergogenic effects may primarily be reliant upon the oxidation of CHO supplied from external sources, referred to as exogenous CHO oxidation (Jeukendrup, 2008; 2010). The latter is dependent upon several factors including the form, amount and type of CHO, feeding strategy, exercise intensity and the training and pre-exercise nutritional status of the participant. It has been established that some factors are more influential than others (Jeukendrup 2004; 2008; 2010; Jeukendrup and Jentjens, 2000). For example, regarding the form of CHO, it has recently been observed that mean and peak exogenous CHO oxidation rates were comparable regardless of whether CHO was obtained from a nutritionally matched liquid and gel or liquid and solid source (Pfeiffer, Stellingwerff, Zaltas and Jeukendrup, 2010a; 2010b). In contrast, the amount and type of CHO consumed may have profound limitations upon its subsequent oxidation (Jeukendrup, 2010; Jeukendrup and Jentjens 2000). The optimum amount of ingested CHO should result in the maximum rate of exogenous CHO oxidation with minimal malabsorption and gastrointestinal discomfort (Jeukendrup, 2008).

The following discussion will consider the most pertinent factors to exogenous CHO oxidation: the type and amount of CHO consumed; both of which have been the subject of
intense investigation over the previous two decades (Jeukendrup, 2010; Jeukendrup and Jentjens 2000). Given the abundance of factors that limit the oxidation of supplementary CHO, it is beyond the scope of the current review to consider each caveat independently. For a detailed review of other factors see Jeukendrup and Jentjens (2000).

2.2.5 Factors Effecting Exogenous Carbohydrate Oxidation

2.2.5.1 Type and Amount of Carbohydrate

Several studies have investigated the oxidation rates of various types of CHO (see Jeukendrup, 2008; Jeukendrup and Jentjens, 2000 for review). Concerning the monosaccharides (glucose, fructose and galactose), it is evident that glucose is the most readily oxidised form of supplementary CHO, given that it is rapidly absorbed and is the universal form of CHO oxidised within the sarcoplasm (Jeukendrup, 2008). In contrast fructose and galactose must be initially converted into glucose in the liver prior to being catabolised within the muscle (Jeukendrup, 2010); therefore, both of these types of CHO are oxidised at slower rates. In addition, fructose is transported from the intestinal lumen into the cytosol of the intestinal epithelial cells via a specific facilitated transporter (GLUT 5); whereas glucose and galactose are transport by a sodium-dependant glucose co-transporter (SGLT 1) (Ferraris, 2001; Ferraris and Diamond, 1997). From the aqueous milieu of the epithelial cells all of the monosaccharides are transported by a universal facilitative transporter (GLUT 2) across the basolateral membrane into the systemic blood (Ferraris, 2001; Ferraris and Diamond, 1997).

Regarding fructose, it has recently been demonstrated that during exercise that this hexose is oxidised at a slightly lower rate to that of glucose, however various rates of fructose oxidation have been reported within the literature (Burelle, Lamoureux, Peronnet,
Massicotte and Lavoie, 2006; Jentjens, Moseley, Waring, Harding and Jeukendrup, 2004; Jeukendrup, 2010). In contrast, galactose is oxidised at much lower rates (50-60% less) to that of the both glucose and fructose (Burelle et al., 2006; Jeukendrup, 2010; Leijssen, Saris, Jeukendrup and Wagenmakers, 1995).

Concerning other types of CHO, the disaccharides maltose (glucose-glucose) and sucrose (glucose-fructose) and glucose polymers (maltodextrins) can also be oxidised at high rates (~1.0 g min⁻¹), comparable to that of glucose, during prolonged, moderate intensity cycling exercise (Jeukendrup, 2008; Rehrer et al., 1992; Venables, Brouns and Jeukendrup, 2008; Wagenmakers, Brouns, Saris and Halliday, 1993). Following enzymatic hydrolysis at the brush border membrane via sucrase and maltase, these various forms of CHO are subsequently transported across the epithelial cells and into the portal circulation as their respective monosaccharides (Jeukendrup and Gleeson, 2009).

Based on this evidence the various types of CHO have been categorised according to the rate at which they are oxidised (Achten, Jentjens, Brouns and Jeukendrup, 2007; Jeukendrup, 2008). Those oxidised at a rate of ~1.0-1.1 g min⁻¹ (~60-70 g hr⁻¹) including glucose, sucrose, maltose and maltodextrins are considered to be rapidly oxidised CHO. The second distinguishable group are those oxidised at lower rates (~0.6 g min⁻¹, ~30-50 g hr⁻¹) and include fructose, galactose, isomaltulose and trehalose (Achten et al., 2007; Jeukendrup, 2008).

From a practical perspective, it is important that recommendations regarding the amount of CHO required to maximise exogenous CHO oxidation can be made. The oxidation of supplementary CHO has been assessed with the use of isotopic tracers which involves labelling the ingested CHO solution with either a radioactive (i.e.¹⁴C) or, most commonly, a
stable isotope ($^{13}$C), given the potential hazards of increased radiation exposure (Jeukendrup and Jentjens, 2000; Moseley et al., 2005). Typically, when labelled CHO is consumed at the onset of exercise and at frequent intervals thereafter, exogenous CHO oxidation will increase and continue to do so until reaching a plateau between 60-90 minutes, at which point it will not increase further (Jeukendrup, 2010; Jeukendrup and Jentjens, 2000). Until recently it was thought that CHO consumed during exercise could not be oxidised at a rate greater than 1.1 g min$^{-1}$ when CHO intake exceeded 1.0-1.2 g min$^{-1}$ (Jentjens et al., 2004; Jeukendrup, 2008; 2010; Jeukendrup et al., 1999a; Jeukendrup and Jentjens, 2000). It was therefore evident that the relationship between CHO intake and CHO oxidation was not linear, to which an apparent ceiling existed (Jeukendrup, 2010). Based upon contemporary evidence it would appear that the limiting step was located within the intestine, and specifically the saturation of the sodium-dependent glucose transporter, SGLT 1 (Jeukendrup, 2008; 2010). Several studies have now demonstrated that exogenous CHO oxidation may be increased well above 1.1 g min$^{-1}$ when CHO sources that utilise different intestinal transporters, for example glucose and fructose, referred to as multiple transportable CHOs, are simultaneously ingested (see Jeukendrup, 2010 for review). Indeed, exogenous CHO oxidation has been shown to peak at 1.75 g min$^{-1}$ with high levels of glucose and fructose ingestion (Jentjens and Jeukendrup, 2005; Jeukendrup, 2010). Providing that energy requirements are high enough, a preferred CHO intake from multiple transportable sources may be 90 g hr$^{-1}$ in order to maximise supplementary CHO oxidation (Jeukendrup, 2008; 2010). Furthermore, multiple transportable CHOs have also been shown to significantly improve exercise performance (Currell and Jeukendrup, 2008b; Jeukendrup, 2010). Therefore, current guidelines from leading authoritative bodies, such as
the ACSM, will have to be amended to take these findings into account (Burke, Hawley, Wong and Jeukendrup, 2011; Jeukendrup, 2010).

2.3 The Efficacy of Carbohydrate and Protein Co-ingestion

A topical issue and one pertinent to the current study is the recent practice of adding a small amount of protein (20 g L\(^{-1}\), approximately 2%) to a typical CHO beverage (Jeukendrup and Tipton, 2009; Stearns et al., 2010; Vandenbogaerde and Hopkins, 2011). However, in contrast to the established ergogenic effects of CHO supplementation, the equivocal nature of the literature regarding CHO-PRO co-ingestion suggests no additional benefits over that of a CHO only beverage (Betts and Stevenson, 2011; Jeukendrup and Tipton, 2009). Similarly, in comparison to the plausible metabolic mechanisms of CHO ingestion, there would appear to be only a tentative mechanistic rationale for the addition of protein to a CHO solution (Betts and Williams, 2010; Jeukendrup and Tipton, 2009). Notwithstanding this data, a recent meta-analysis by Vandenbogaerde and Hopkins (2011), the first meta-analytical review of CHO supplementation, suggested that the most effective CHO supplement was one that contained a combination of multiple transportable CHOs and protein. Therefore the addition of protein to a CHO solution may improve its efficacy (Betts and Stevenson, 2011).

A number of research groups have explored the possibility that CHO-PRO co-ingestion may further augment the beneficial effects of CHO supplementation alone (see Stearns et al., 2010 for review). However, given the plethora of intra- and inter-study differences in methodological design it is perhaps not surprising that results to date are equivocal (Jeukendrup and Tipton, 2009; Stearns et al., 2010). Issues surrounding beverage composition, variability in exercise preload and the subsequent methods of assessing
exercise capacity or exercise performance are common caveats (Stearns et al., 2010). The only customary feature is exercise modality, in which all studies have been performed using cycle ergometers. All data at present advocating the efficacy of CHO-PRO solutions have come from those utilising methods of exercise capacity as opposed to exercise performance (Breen et al., 2010; Rowlands and Wadsworth, 2011; Stearns et al., 2010). For the purpose of the proceeding discussion studies will be categorised according to their method of performance assessment: blind end-point tests of exercise capacity (time-to-exhaustion) or non-blind, known end-point tests of exercise performance (time-trial) (Rowlands and Wadsworth, 2011).

2.3.1 Time-to-Exhaustion Protocols

In a novel study Ivy and colleagues (2003) examined the efficacy of a CHO-PRO beverage (7.75 g 100 ml⁻¹ of CHO, 1.94 g 100 ml⁻¹ of whey protein) to that of an iso-CHO only (7.75 g 100 ml⁻¹) and an artificially sweetened placebo solution. Participants ingested 200 ml of each respective solution 30 minutes prior to and at 20 minute intervals during a 3 hour intermittent cycle preload. As hypothesised by the authors, exercise capacity was significantly improved when subjects consumed the CHO-PRO solution (26.9 ± 4.5 minutes) in contrast to both the CHO only (19.7 ± 4.6 minutes) and the placebo beverage (12.7 ± 3.1 minutes). However, the mechanistic hypothesis of the study, that the addition of protein would increase plasma insulin concentration, was not proven. Therefore the mechanisms underlying the observed improvement in exercise capacity were unclear (Ivy et al., 2003).

Saunders and co-workers (2004) also examined the efficacy of the addition of protein to a CHO solution. Similarly the authors assessed exercise capacity, however without a cycle
preload. Instead, participants were required to cycle to volitional fatigue at initially 75% \( \text{VO}_{2\text{peak}} \) and again 12-15 hours later at 85% \( \text{VO}_{2\text{peak}} \), in which they consumed 1.8 ml\( \text{kg}^{-1} \) of either a CHO or CHO-PRO solution (approximately 9.64 g of CHO and 2.38 g of protein) at 15-minute intervals throughout each bout. Time-to-exhaustion was significantly greater during both the first (106.3 ± 45.2 vs. 82.3 ± 32.6 minutes) and second (43.6 ± 12.5 vs. 31.2 ± 8.7 minutes) exhaustive effort following ingestion of the CHO-PRO solution in comparison to the CHO only beverage. Similar observations were also later presented by the same laboratory when comparing the effectiveness of CHO and CHO-PRO supplementation in gel form (Saunders et al., 2007).

Collectively the aforementioned data suggest that CHO-PRO co-ingestion may prolong exhaustive exercise capacity within the region of 13-40%, when compared to solutions matched for CHO content or a placebo (Ivy et al., 2003; Saunders et al., 2004; 2007). A caveat of the latter works, however, are that the feeding strategies employed provided inadequate amounts of CHO (37-47 g\( \text{hr}^{-1} \)) to produce maximal exogenous oxidation rates from a single CHO source (Jeukendrup, 2004; Jeukendrup and Jenjtens, 2000; Saunders et al., 2009). Furthermore, given that the experimental beverages were matched for CHO as opposed to energy content, in which the addition of protein also provided additional calories, it was not possible to determine if the ergogenic effects of CHO-PRO supplementation were the consequence of a specific protein mediated effect (Saunders et al., 2009; Stearns et al., 2010). Also of note is the possibility of additional placebo effects with CHO-PRO supplementation (Vandenbogaerde and Hopkins, 2011). Previously, placebo effects with CHO feedings have been observed (Beedie and Foad, 2009; Clark et al., 2000). Therefore, although speculative, the addition of subsequent trials within studies examining the efficacy of CHO-PRO supplementation may result in subjects second
guessing that they have been prescribed a potentially beneficial treatment, which may further augment performance (Vandenbogaerde and Hopkins, 2011). Similarly it is also challenging to blind beverages containing protein (Breen et al., 2010; Vandenbogaerde and Hopkins, 2011).

The majority of existing data employing tests of exercise capacity have failed to report ergogenic effects following concurrent CHO-PRO ingestion when compared to energy matched CHO solutions (Romano-Ely et al., 2006; Valentine et al., 2008). Valentine et al. (2008) utilised identical CHO, CHO-PRO and placebo beverages to that of Ivy and colleagues (2003), with the addition of an iso-energetic CHO solution (9.69 g 100 ml$^{-1}$) relative to the CHO-PRO beverage. It was observed that when matched for energy content, CHO-PRO ingestion (126.2 ± 25.4 minutes) provided no further benefits to that of an iso-energetic CHO solution (121.3 ± 36.8 minutes). Although significantly improving performance in comparison to a placebo, neither the CHO-PRO nor iso-energetic CHO solution proved more effective than that of the iso-CHO beverage (corresponding to CHO-PRO). Thus, this study demonstrates that when CHO is fed at rates considered optimal for peak exogenous CHO oxidation, that protein provides no further metabolic benefit. It also suggests that the performance enhancing benefits observed by others may have been related to the additional calorific content provided by the addition of protein, when consuming inadequate amounts of CHO (Ivy et al., 2003; Saunders et al., 2004; 2007). Comparable observations were made by Romano-Ely et al. (2006) when also comparing energy matched CHO and CHO-PRO solutions.

Recent data from investigations by John Ivy and colleagues at the University of Texas laboratory, in which the inclusion of sub-optimal levels of single or multiple transportable CHO$_i$s in the CHO-PRO beverages and the energy content not matched to a CHO control,
have also failed to show performance enhancing effects (Ferguson-Stegall et al., 2010; Martinez-Lagunas et al., 2010). The rationale for the use of such solutions was the possibility that the addition of protein may allow for a reduction in CHO and calorie content, whilst maintaining or even improving performance. Such beverages would offer an alternative to those athletes wanting to maintain or reduce body mass when competing in weight category events. However, although exercise capacity in the latterly cited studies was not improved, time-to-exhaustion was not significantly different between treatments despite a reduced CHO and energy content in the CHO-PRO solutions, when compared to the CHO only beverages. As a result it would appear that the addition of protein maintained the efficacy of these solutions in comparison to a CHO beverage containing up to double the CHO content, and 40-60% more energy (Betts and Stevenson, 2011; Ferguson-Stegall et al., 2010; Martinez-Lagunas et al., 2010).

The most recent publication from this research group suggests it may be possible to reduce both the energy and CHO content whilst accommodating for the addition of protein and, surprisingly, improve as opposed to simply maintaining, exercise capacity (McCleave et al., 2011). Similar to previous investigations from the same laboratory (Ferguson-Stegall et al., 2010; Ivy et al., 2003; Martinez-Lagunas et al., 2010), subjects again performed a prolonged intermittent cycle preload prior to cycling to volitional exhaustion at individually determined ventilatory threshold (approximately 75% VO$$_{2\text{max}}$$). Participants were required to consume 275 ml of either a CHO or CHO-PRO solution immediately prior to, and at 20 minute intervals throughout both the cycle preload and the blind-ended test of exercise capacity. As the study was a continuation of the previously cited work (Ferguson-Stegall et al., 2010; Martinez-Lagunas et al., 2010), the CHO-PRO solution was again atypical and contained 3 g 100 ml$$^{-1}$$ of multiple transportable CHOs (1 g of glucose, maltodextrin and
fructose respectively) and 1.2 g 100 ml\(^{-1}\) of whey protein isolate. Conversely, the CHO beverage was a conventional CHO solution containing 6 g 100 ml\(^{-1}\) of glucose only and therefore provided double the CHO content and one third more energy to that of the CHO-PRO solution (McCleave et al., 2011); in which both beverages had been employed in a previous study by the same research group (Ferguson-Stegall et al., 2010).

Exercise capacity was improved by 15.2\% following ingestion of the CHO-PRO solution (49.94 ± 7.01 minutes) in comparison to the CHO only beverage (42.36 ± 6.21 minutes) despite the evident differences in CHO content. This study therefore suggests that the addition of protein may potentiate independent metabolic effects. However this outcome may also be explained, at least in part, by the inclusion of multiple transportable CHOs within the CHO-PRO solution; although total CHO oxidation and the RER were comparable. Therefore, it is not possible at present to unequivocally conclude that the inclusion of protein, in this instance, mediated a specific metabolic effect. Preferably, the CHO only beverage should have included multiple transportable CHOs similar to the CHO-PRO solution, as well as a placebo condition to assess the true performance benefit.

Notwithstanding the methodological differences surrounding beverage content, the use of time-to-exhaustion as an assessment tool also merits discussion. Although tests of exercise capacity are common, as evidenced by the aforementioned articles, the ecological validity of such protocols is limited given that competitive events do not require an athlete to maintain an externally paced, predetermined percentage of VO\(_{2\text{max}}\) or power output for an indefinite period until exhaustion (Breen et al., 2010; Currell and Jeukendrup, 2008a). Therefore, the examination of exercise capacity fails to simulate not only the performance requirements, but also the physiological responses to that of an actual sporting event (Currell and Jeukendrup, 2008a).
In contrast, the more recent practice of utilising time-trials, in which individuals either complete a set distance or amount work as quickly as possible or are required to cover the greatest distance or complete as much work as possible in a predetermined period (Laursen, Francis, Abbiss, Newton and Nosaka, 2007; Paton and Hopkins, 2001), appear to have both greater ecological validity and reliability to that of time-to-exhaustion protocols (Currell and Jeukendrup, 2008a; Jeukendrup, Saris, Brouns and Kester, 1996). When comparing the reproducibility of time-to-exhaustion and time-trial protocols, Jeukendrup and colleagues (1996) reported that the coefficient of variation (CV) for time-trial cycling (3.35%) was significantly less than that of time-to-exhaustion (26.6%) of a similar duration. Generally, tests of exercise capacity have a CV of > 10% in contrast to < 5% for that of methods assessing exercise performance which are therefore more reliable (Currell and Jeukendrup, 2008a).

Similarly given that time-trials are internally, as opposed to externally paced, they better mimic both the performance and physiological demands of a true to life sporting event (Currell and Jeukendrup, 2008a). The typical profile of a pacing strategy adopted during a cycling time-trial of approximately 1 hour in duration, entails a gradual decline in power output during the first three-quarters of the time-trial, before power output increases dramatically as the participant approaches the known end-point (Carter et al., 2004b; Jeukendrup et al., 1997; Rollo and Williams, 2011). This ability to independently self pace allows for either an increase, or indeed a decrease, in power output as a consequence of perhaps a nutrition intervention, which also make time-trials a more sensitive performance measure. Evidently, such a strategy cannot be adopted during time-to-exhaustion protocols, in which power output is externally fixed and the end-point blind. Such factors may therefore, at least in part, explain why a number of studies have reported beneficial
outcomes following CHO-PRO co-ingestion when utilising assessments of exercise capacity as opposed to exercise performance.

2.3.2 Time-Trial Protocols

In contrast to the eight studies to date examining the efficacy of CHO-PRO co-ingestion utilising time-to-exhaustion protocols, an additional five studies have also examined the potential benefits of such solutions when employing time-trial methodologies (Breen et al., 2010; Osterberg, Zachwieja and Smith, 2008; Saunders et al., 2009; Toone and Betts, 2010; Van Essen and Gibala, 2006). Of interest is the study of Saunders and colleagues (2009) in which participants performed a simulated 60 km time-trial. Male cyclists consumed 200 ml of either a CHO only (3% glucose, 3% maltodextrin mixture) or CHO-PRO solution (identical CHO blend plus 1.8% casein hydrolysate) upon completion of each 5 km of the time-trial, leading to a total CHO and protein intake of approximately 60 g hr$^{-1}$ and 14 g hr$^{-1}$, correspondingly. Subsequently, the authors reported that time-trial performance was not significantly different between either of the treatments. These results are consistent with previous observations from the same laboratory when feeding volunteers iso-CHO (Valentine et al., 2008) and iso-energetic solutions (Romano-Ely et al., 2006), at a rate considered optimal to produce maximal exogenous CHO oxidation, however when utilising time-to-exhaustion protocols. Such observations also directly contradict findings from the same authors, who reported efficacious effects when providing subjects with sub-optimal amounts of CHO within both the CHO and CHO-PRO beverages when comparably assessing exercise capacity (Saunders et al., 2004; 2007).

Despite finding no improvement in time-to-complete the time-trial, Saunders and co-workers (2009) postulated that CHO-PRO co-ingestion had significantly improved “late
exercise time-trial performance”. However, their protocol was not designed to specifically determine the impact of additional protein during the latter stages of exercise (Breen et al., 2010). Similarly, this finding may have been an artefact of the methodological design, in which participants were informed of their performance time from the initial trial and competed against this time during their second visit. Consequently a significant trial order effect was observed. Therefore, it was not possible to independently determine the effect of the additional protein (Betts and Williams, 2010; Saunders et al., 2009). This may have also been influenced by the absence of a familiarisation and a placebo trial.

In a robust study, controlling for confounding variables not documented by earlier empirical works, Breen et al. (2010) comparably observed no performance enhancing effects when also prescribing iso-CHO solutions, in which the additive protein again provided additional calorific content in the CHO-PRO beverage. During an initial 120-minute cycle preload at 50% $W_{max}$, participants were provided with either a 6% maltodextrin solution (65 g hr$^{-1}$ of CHO) or an identical beverage containing 1.8% of an unspecified protein hydrolysate (19 g hr$^{-1}$) from the onset of exercise, and at 15-minute intervals thereafter in 275 ml boluses. During the proceeding time-trial of approximately 60 minutes in duration, volunteers ingested the same fluid volumes upon completion of each 25% of the trial. Consistent with that of Saunders et al. (2009), time-to-complete the time-trial was not significantly different regardless of treatment consumed (60:13 ± 1:33 and 60:51 ± 2:40 [minutes:seconds] for CHO and CHO-PRO, respectively). In contrast however, Breen and associates (2010) reported no improvement in late exercise time-trial performance, in which their protocol which was specifically designed to determine such a possibility, measuring power output at each 25% of the time-trial completed.
When also utilising iso-CHO solutions delivering CHO at optimal rates, Van Essen and Gibala (2006) observed no beneficial performance effects with the addition of 1.8% whey protein within their CHO-PRO beverage during a simulated 80 km time-trial. At present, it therefore appears that when CHO is consumed at rates to produce maximal exogenous CHO oxidation, that the addition of protein serves no further metabolic benefit during ecologically valid time-trial protocols (Jeukendrup and Tipton, 2009). Therefore the proposal of an independent, protein specific mechanism, would not appear plausible at this stage.

Collectively, these articles also demonstrate that the inherent differences in feeding strategy as a consequence of time-trial and time-to-exhaustion methodologies, may explain the apparent contrasting results between the two methods of quantifying endurance performance, and that it may not simply be a caveat relating to the ecological validity and reliability of the measures. For example, regardless of the time taken to complete a given amount of work or cover a set distance, all participants will consume the same amount of CHO or CHO-PRO when utilising time-trials. Conversely, when the end-point is not known, each individual subject will consume various absolute volumes of a respective beverage. This may be further compounded when solutions provide undesirable amounts of CHO, in which the additional energetic stimulus provided by the additive protein content may allow subjects to continue for longer periods when compared to CHO alone. The longer an individual is able to sustain the externally fixed power output, the greater amounts of the respective solution they will receive. This may therefore explain why a small cohort of investigations utilising time-to-exhaustion protocols and providing sub-optimal amounts of CHO have reported performance enhancing benefits (Ivy et al., 2003; Saunders et al., 2004; 2007). Similarly, pertinent factors such as the conditioning status,
pre-exercise nutritional state and motivation of an individual may have greater significance during exhaustive exercise tests (Bergstrom et al., 1967; Currell and Jeukendrup, 2008a; Stearns et al., 2010).

It is also possible that CHO-PRO solutions may confer ergolytic effects during tests of exercise performance (Toone and Betts, 2010). Briefly, subjects performed a 45-minute intermittent cycle preload (60-90% VO$_{2\text{max}}$) preceding a 6 km time-trial. Participants ingested 2.5 ml kg$^{-1}$ of either a CHO (9% sucrose) or CHO-PRO solution (6.8% sucrose, 2.2% whey protein isolate), from the onset of exercise and at 15-minute intervals during the preload period. Subsequently, mean time-to-complete the time-trial was significantly longer post CHO-PRO consumption (438 ± 22 seconds, p = 0.048), in contrast to the CHO only beverage (433 ± 21 seconds). These results may, however, be explained by the composition of the respective solutions. The authors justification for both the CHO content and feeding strategy was that both the CHO and CHO-PRO beverages would not fail to meet the recommendations of Jeukendrup (2004) of providing ≥ 1 g min$^{-1}$ of CHO. However, given that sucrose is metabolised to glucose and fructose and therefore provides a multiple transportable, as opposed to a single CHO source which can be oxidised at much higher rates (Jeukendrup, 2010), it is not surprising that the reduced CHO content in the CHO-PRO beverage to accommodate for the additional protein, also compromised the efficacy of the solution. Ingestion of the CHO solution resulted in a total CHO intake of 95 ± 7 g and therefore coinciding with the 90 g hr$^{-1}$ of CHO deemed optimal to produced maximal exogenous oxidation rates from a glucose and fructose mixture (Jeukendrup, 2008; 2010; Jeukendrup and Tipton, 2009). Conversely, the CHO-PRO beverage provided a total of 72 ± 5 g of CHO which is considered the desirable intake from only a single, as opposed to a
multiple transportable, CHO source (Jeukendrup, 2004; 2008; 2010; Jeukendrup and Jentjens, 2000).

Therefore, despite the equivocal nature of the literature at present, it is evident that the contemporary practice of adding protein to a typical CHO solution provides no synergistic effects when CHO is delivered at recommended rates during ecologically valid exercise performance tests (Betts and Stevenson, 2011; Breen et al., 2010; Jeukendrup and Tipton, 2009).

2.4 Potential Mechanisms of Carbohydrate and Protein Co-ingestion

It would appear that when CHO is prescribed at optimal rates to produce maximal exogenous CHO oxidation, that the addition of protein provides no independent ergogenic effects as performance is not further augmented (Breen et al., 2010; Saunders et al., 2009; Valentine et al., 2008; Van Essen and Gibala, 2006). However, other than the empirical work of Valentine and collaborators (2008), no other study has provided CHO at recommended rates within both a CHO and CHO-PRO solution, with both also matched for energetic content, in an attempt to specially isolate the potential protein mediated effects. Therefore, more studies utilising iso-caloric solutions in which CHO is consumed at optimal rates are required to isolate the independent effects of the addition of protein in the absence of calorific variability between beverages (Stearns et al., 2010). Although recent performance data suggests no further favourable effects with CHO-PRO co-ingestion, it is not until more data specifically isolating protein are available that it can be concluded with certainty that protein confers no independent influence (Stearns et al., 2010).

A number of potential avenues have been identified in which protein when added to CHO may further increase the efficacy of a typical CHO solution (Betts and Williams, 2010;
Rowlands and Wadsworth, 2011; Saunders, 2007; Saunders et al., 2007; Valentine et al., 2008). One such avenue is the possibility that CHO-PRO co-ingestion may increase plasma insulin levels and so increase glucose uptake by the sarcoplasm and consequently increase CHO oxidation (Rowlands and Wadsworth, 2011; Saunders, 2007). However, no significant differences in neither plasma insulin nor glucose levels, nor RER and total CHO oxidation (measured via indirect calorimetry), have been reported collectively within the previously cited studies. However, only the initial study of Ivy et al. (2003) assessed all four of these metabolic measures within the same study.

It has also been postulated that the increased insulinaemic response observed with post exercise CHO-PRO ingestion, and the subsequent increase in glycogen resynthesis, may also be a potential mechanism during exercise (Betts and Williams, 2010; Cermak, Solheim, Gardner, Tarnopolsky and Gibala, 2009; Ivy et al., 2003). However, recent evidence in which muscle glycogen content was directly determined via biopsy samples contradicts such a construct (Cermak et al., 2009). Moreover, any potentially advantageous insulinaemic response is likely to be inhibited via the catecholamine-mediated suppression of insulin release during exercise (Hunt and Ivy, 2002; Toone and Betts, 2010). Similarly, it has been suggested that although protein oxidation contributes minimally to energy transfer (5-10%), that this fraction may increase substantially during the latter stages of endurance exercise when individuals are in a glycogen depleted state (Jeukendrup and Tipton, 2009; Saunders, 2007). It has been reported that protein oxidation (leucine) may increase two-to-three fold during ultra-endurance exercise when a CHO-PRO in contrast to a CHO solution is consumed (Koopman et al., 2004). Therefore, it may be that simultaneous CHO-PRO ingestion may modify substrate usage and reduce endogenous and exogenous CHO utilisation late in exercise (Saunders, 2007; Stearns et al., 2010). However, the authors of
the latter study observed that the widely used isotopic tracing model of leucine overestimates whole body protein oxidation and underestimates protein synthesis during prolonged exercise, in comparison to other tracing methods (Gibala, 2007; Koopman et al., 2004).

It is evident from studies utilising multiple transportable CHO's that the intestinal lumen is a critical limiting step to subsequent exogenous CHO oxidation (Jeukendrup, 2008; 2010). It has subsequently been suggested that the addition of protein to a CHO beverage may, similar to different forms of CHO, up-regulate additional facilitative intestinal transporters resulting in enhanced fluid, electrolyte and glucose absorption (Rowlands and Wadsworth, 2011; Saunders, 2007; Stearns et al., 2010). Again however, there would appear to be little support for such a premise given that CHO oxidation is not further augmented when optimal or suboptimal amounts of CHO are concurrently ingested with protein (Breen et al., 2010; Rowlands and Wadsworth, 2011; Saunders et al., 2004; 2007; 2009). Conversely, it is also possible that the inclusion of protein within a typical CHO beverage may reduce the efficacy of such a solution via an increase in solution osmolality and reduced gastric emptying (Rowlands and Wadsworth, 2011; Vandenbogaerde and Hopkins, 2011). However, this is unlikely to occur with the inclusion of small quantities of protein employed in contemporary studies (Vandenbogaerde and Hopkins, 2011). Another potential protein specific mechanism is the possibility of anaplerotic reactions resulting in the retention or replenishment of tricarboxylic acid cycle (TCA) intermediates, and subsequently maintaining oxidative phosphorylation (Betts and Williams, 2010; Cermak et al., 2009; Ivy et al., 2003; Saunders, 2007). However, direct evidence suggests that such a mechanism is not plausible in which the total concentration of malate and citrate, which account for approximately 70% of the TCA intermediates in humans (Gibala, Gonzalez-
Alonso and Saltin, 2002), were not significantly different following CHO or CHO-Pro co-ingestion during 90 minutes of cycle ergometry (Cermak et al., 2009).

Aside from the potential metabolic mechanisms, is it also possible that protein ingested with CHO may have centrally mediated effects upon fatigue (Rowlands and Wadsworth, 2011; Saunders, 2007; Stearns et al., 2010). It has been postulated that plasma branch-chain amino acid (BCAA) content either remains unchanged or decreases during prolonged exercise, in contrast to an increase in free tryptophan (f-TRP) (Meeusen, Watson, Hasegawa, Roelands and Piacentini, 2006; Rowlands and Wadsworth, 2011). Both the BCAAs and f-TRP share the same facilitative transporter across the blood-brain barrier, therefore an increase in the f-TRP to BCAA ratio augments the flux of f-TRP into the cerebrum and thereby increasing the presence of this prerequisite to serotonergic neurons and increasing serotonin production (Meeusen et al., 2006; Meeusen and Watson, 2007). Subsequently, serotonin is believed to inhibit central nervous system function, resulting in diminished drive for exercise and reduced motor output (Davis and Bailey, 1997; Meeusen et al., 2006; Meeusen and Watson, 2007). It is therefore enticing to believe that the ingestion of protein and the provision of BCAAs may readdress this balance and consequently attenuate central fatigue (Meeusen et al., 2006). However, again there is a lack of evidence to support such a premise in which studies feeding both intact proteins and BCAAs have failed to report any ergogenic effects (Meeusen et al., 2006; Stearns et al., 2010). Moreover, given that fatigue is a multifaceted phenomenon, it is challenging to comprehend that simply f-TRP delivery to the brain is the universal mechanism of serotonin release, and similarly, that such a simple hypothesis may explain the intricate central fatigue process (Jeukendrup and Gleeson, 2009; Meeusen et al., 2006).
Therefore in the absence of an empirically supported physiological mechanism, it would appear that the additive effect of protein to a typical CHO beverage is simply additional energetic content (Betts and Stevenson, 2011; Breen et al., 2010; Saunders et al., 2009; Valentine et al., 2008). However, it must be highlighted that more robust studies are required to isolate the potential ergogenic effects and mechanisms of protein when simultaneously ingested with recommended amounts of CHO (Stearns et al., 2010).

2.5 Additional Benefits of Protein Hydrolysates

At present, the majority of studies investigating the efficacy of CHO-PRO solutions have utilised whey protein as their protein source, including those in which performance was improved as a consequence of CHO-PRO co-ingestion (Ivy et al., 2003; Saunders et al., 2004; 2007; Stearns et al., 2010). However, to date, limited attention has been directed towards the types of protein utilised. To the best of the author’s knowledge, no study has specifically compared the effects of various types of protein when simultaneously ingested with CHO during endurance exercise performance (Stearns et al., 2010). Two contemporary investigations have recently examined the potential of including protein hydrolysates, as opposed to commonly utilised whey protein, within their CHO-PRO solutions. The novel study of Saunders and associates (2009) was the first published study to assess the efficacy of the inclusion of protein hydrolysates within a CHO beverage. The protein itself was derived from a specific casein protein hydrolysate. Subsequently, although finding no significant difference in the time-to-complete the time-trial, the authors reported improvement in late exercise time-trial performance, as highlighted in previous sections. However, the methodology of the study has since been criticised by others for not being appropriately designed to specifically assess performance during the latter stages of the time-trial (Breen et al., 2010). Also, by the authors own admission, a significant trial
order effect may have confounded the outcomes of the study (Betts and Williams, 2010; Saunders et al., 2009). Conversely, Breen and colleagues (2010) when utilising an unspecified protein hydrolysate observed no improvement in time-trial performance in what was a well controlled study.

Concerning the possible benefits of protein hydrolysate inclusion, it has been observed that protein hydrolysates containing mostly di- and tri-peptides are absorbed at much higher rates in comparison to whole protein sources and individual amino acids (Koopman et al., 2009; Manninen, 2004; 2006; 2009), making them more suitable for consumption during exercise. Indeed, the industrial hydrolytic process mimics that of the human digestive system in which, following intestinal and subsequently brush border membrane peptidase digestion, only di- and tri-peptides are absorbed intact (Manninen, 2006; 2009). Additional potential benefits include an increased insulinaemic response when protein hydrolysates are co-ingested with CHO over CHO alone, which has been reported during both post exercise recovery (Manninen, 2006; van Loon et al., 2000a; 2000b), and similarly during a resting fasted state (Claessens, Calame, Siemensma, van Baak and Saris, 2009; van Loon et al., 2000c). However, it remains to be determined if such effects are apparent during exercise given the very different metabolic conditions.

A novel application is the use of protein hydrolysates as an alternative to antihypertensive medications in the treatment of hypertension (Ewart et al., 2009; Hong et al., 2008; Moughan et al., 2007). Angiotensin converting enzyme (ACE) operates within the rennin-angiotensin system to catalyze the biologically inactive angiotensin I to angiotensin II, of which the latter is a potent vasoconstrictor and also inhibits the vasodilator bradykinin (Ewart et al., 2009; Hong et al., 2008). Protein hydrolysates operate to inhibit ACE by occupying its active binding sites to prevent its interaction with angiotensin I and thus,
conversion to angiotensin II (Ewart et al., 2009; Hong et al., 2008). Although such a mechanism has an evident clinical application, it is also possible that these benefits may be desirable during endurance exercise to perhaps increase thermoregulatory responses and maintain nutrient delivery to the target tissues (i.e. muscle) (Cathcart, Murgatroyd, McNab, Whyte and Easton, 2011). Indeed, it was recently observed that CHO-PRO ingestion improved endurance cycling performance in the heat; however the authors proposed different mechanisms to those postulated here to explain the ergogenic benefits (Cathcart et al., 2011). Furthermore, given that protein hydrolysates differ from one another nutritionally, it may be that they confer independent and very specific metabolic and physiological effects that have not yet been identified (Moughan et al., 2007).

2.6 Summary

In summary, it is evident that CHO ingestion during prolonged exercise may improve exercise capacity and performance, in which contemporary data suggests that the maintenance of euglycaemia and high rates of exogenous CHO oxidation are the most pertinent mechanisms to confer such benefits (Jeukendrup, 2004; 2008; 2010; Jeukendrup and Jentjens, 2000). Such outcomes may be achieved when individuals consume 60-70 g·hr⁻¹ of CHO from a rapidly oxidised, single CHO source, or 90 g·hr⁻¹ from multiple transportable CHO sources, if energy expenditure is high enough (Jeukendrup, 2004; 2008; 2010; Jeuknedrup and Jentjens, 2000). However, other mechanisms may also play a role to which not all have been elucidated (Jeukendrup, 2010; Karelis et al., 2010).

Regarding the potential synergistic effects of CHO-PRO co-ingestion, the equivocal nature of the literature at present highlights no additional ergogenic effects over a typical CHO only solution (Stearns et al., 2010). Indeed, negating the three studies that have observed
beneficial outcomes when employing methods of exercise capacity (Ivy et al., 2003; Saunders et al., 2004; 2007), no subsequent investigations when supplying CHO at recommended rates have reported performance enhancing effects (Breen et al., 2010; Stearns et al., 2010). Concerning the possible mechanisms of CHO-PRO ingestion it would appear that at present, other than increasing the energetic content of a typical CHO solution, there are no other plausible protein specific mediated effects (Breen et al., 2010; Jeukendrup and Tipton, 2009; Valentine et al., 2008). Indeed, the proposed mechanisms are tentative at best with little supporting empirical evidence (Betts and Williams, 2010; Rowlands and Wadsworth, 2011; Saunders, 2007). However, it must be noted that additional studies are required with a remit to isolate the effects of additive protein aside from calorific variability (Stearns et al., 2010). Similarly, there is also a current dearth in knowledge concerning the efficacy of different protein types when co-ingested with CHO (Stearns et al., 2010), in which protein hydrolysates may potentially offer additional benefits over conventional proteins.
Chapter 3 - Methodology

3.1 Participants

Twelve apparently healthy men volunteered to participate in the study (characteristics [M ± SD] Age: 24.6 ± 5 years; height: 176.5 ± 5.7 cm; body mass: 76.0 ± 8.3 kg; VO\textsubscript{2max}: 52.5 ± 5.2 ml kg\textsuperscript{-1} min\textsuperscript{-1}; W\textsubscript{max}: 294 ± 19 W). All were engaged in physical training (3-5 d wk\textsuperscript{-1}) prior to and throughout the data collection period. The experimental protocol was approved by the departmental research ethics committee and each participant provided written informed consent.

3.2 Experimental Design and General Procedures

The study followed a randomized, double-blind, cross-over design. Initial testing consisted of an assessment of maximal oxygen uptake (VO\textsubscript{2max}) and maximal power output (W\textsubscript{max}) utilising an incremental cycle test to exhaustion. Participants then returned to the laboratory on a further four occasions (7-10 days apart) to complete firstly a familiarisation and the subsequent blinded trials. All trials consisted of a 90-minute steady-state cycle at 50% W\textsubscript{max} followed by a 5 km time-trial. All testing was conducted between the hours of 08:30 and 11:30 to minimise any influence of circadian variation, and subjects arrived at the laboratory in a fasted state (approximately 12-hours postprandial). All procedures were conducted at sea level in a thermo-neutral environment (laboratory environment [M ± SD] temperature: 21.0 ± 1.2°C; humidity: 40 ± 6%; barometric pressure: 1014 ± 11 millibars).
3.3 Initial Testing

3.3.1 Maximal Oxygen Uptake and Maximal Power Output

During their initial visit to the laboratory each individual’s VO$_{2\text{max}}$ and $W_{\text{max}}$ were determined utilising a step-incremented protocol to exhaustion on an electromagnetically braked cycle ergometer (Lode Sport Excalibur, Lode B.V. Medical Technology, Groningen, Netherlands). Body mass (SECA digital weighing scales, SECA, Birmingham, UK) and height (Holtain stadiometer, Holtain, Crymych, Dyfed) were recorded prior to testing along with each subjects’ desired ergometer orientation which was replicated during subsequent visits. The protocol consisted of a 3-minute warm-up at 95 W proceeded by an increase of 35 W every 3-minutes until fatigue, with the ergometer set in cadence independent (hyperbolic) mode (Breen et al., 2010; Currell and Jeukendrup, 2008b). Fatigue was defined as the point when participants could not maintain a cadence of at least 60 rotations per minute on two occasions despite strong verbal encouragement after the first failure to do so. This was the only point at which verbal encouragement was given.

Pulmonary oxygen uptake (VO$_{2}$), carbon dioxide production (VCO$_{2}$) and RER were measured continuously during exercise via an automated metabolic gas analyser (Cortex Metalyzer 3B-R2, Cortex Biophysic, Leipzig, Germany). The modular gas analysers were calibrated with gases of known concentrations (17.05% O$_{2}$, 4.98% CO$_{2}$, Cranlea, Birmingham, UK) and ambient air. The volume sensor was calibrated with a 3-L calibration syringe (Hans Rudolph model 5530, Hans Rudolph, Kansas, USA) immediately prior to data acquisition. Heart rate was recorded continuously using a heart rate monitor (Polar, Polar Electro, OY, Finland). During the third minute of each stage and again upon termination of the test the subjects’ rating of perceived exertion (RPE) was obtained using
the 6-20 Borg scale (Borg, 1982). The highest 11-breath rolling average (centred to the middle breath) was considered to be VO\textsubscript{2}\text{max} (Robergs, Dwyer and Astorino, 2010). This value was considered maximal with a plateau in VO\textsubscript{2} (< 2 ml\,kg\,min\textsuperscript{-1}) with increasing test duration/workload. In the absence of a discernible plateau, secondary criteria which included 1) heart rate within 10 b\,min\textsuperscript{-1} of age predicted maximum heart rate (220 - age), 2) RER > 1.10 and 3) RPE > 17 were utilised. Maximum power output was calculated from the power output during the last completed stage, plus the fraction of time spent in the final non-completed stage multiplied by the work rate increment (Currell and Jeukendrup, 2008b) (i.e. \( W_{\text{max}} = W_{\text{com}} + \frac{t}{180} \times 35 \), where \( W_{\text{com}} \) is the power output during the last completed stage, \( t \) is the time in seconds spent in the final non-completed stage and 35 is the work rate increment in watts). These values were then used to determine the power output for the preloaded steady-state cycle bout corresponding to 50\% \( W_{\text{max}} \).

3.4 Familiarisation and Blinded Trials

3.4.1 90-minute Steady-State Cycle Preload

During their second visit to the laboratory subjects performed a familiarisation trial consuming water only following the identical feeding strategy to that of the actual treatment beverages. Participants arrived at the laboratory in a fasted state (approximately 12-hours postprandial) and had been instructed to consume 500 ml of water before bed and the same volume again on waking to ensure they were adequately hydrated. A urine sample was initially obtained and assessed for osmolality (Osmometer, Advanced Instruments Model 3320, Advanced Instruments Inc., Massachusetts, USA) and pH (pH book [pH 2-12], Scientific Laboratory Supplies, UK). Participants with a urine osmolality of \( \leq 700 \) mOsmol\,kg\textsuperscript{-1} were considered euhydrated (Sawka et al., 2007). Each individuals’ body
mass was then recorded with participants wearing shorts only and repeated again post exercise along with urine osmolality and pH. Subjects were then fitted with a heart rate monitor and mounted the electromagnetically braked cycle ergometer. They then began a 90-minute steady-state cycle bout corresponding to 50% of their previously determined $W_{\text{max}}$ ($147 \pm 10$ W), with the cycle ergometer set in cadence independent mode and instructed to maintain a comfortable cadence above 60 rotations per minute. During this period capillary blood samples were obtained every 15 minutes excluding the 90-minute mark; concomitant to heart rate and RPE. VO$_2$, VCO$_2$ and RER were measured during each 10-minute period between feedings (i.e. 5-15, 20-30, 35-45, 50-60, 65-75 and 80-90 minutes) when the oso-nasal mask was removed for a 5-minute interval. Subjects were blinded to all physiological data. A fan was used to cool participants for which the settings and orientation were recorded and replicated during subsequent trials.

3.4.2 5 km Time-Trial

On completion of the 90-minute preloaded cycle subjects were immediately transferred to an air braked cycle ergometer (Wattbike, Wattbike Ltd, Nottingham, UK) to perform a 5 km time-trial. The time-trial began exactly 1 minute after the termination of the steady-state ride. The ergometer display was covered so that participants could only view the distance remaining to completion. No other visual feedback regarding performance was given, however subjects were strongly verbally encouraged to complete the time-trial as quickly as possible. Feedback regarding performance was only provided once all trials had been completed. No physiological data was collected during the time-trial.
3.5 Substrate Oxidation

The rate of carbohydrate oxidation during the steady-state cycling was calculated via indirect calorimetry using the stoichiometric equations of Jeukendrup and Wallis (2005) for moderate to high intensity exercise (50-75% VO\textsubscript{2max}).

\[ \text{CHO oxidation rate} = 4.210 \cdot \text{VCO}_2 \text{ (L min}^{-1}) - 2.962 \cdot \text{VO}_2 \text{ (L min}^{-1}) - 0.40 \]

3.6 Blood Sampling and Analysis

All blood samples were obtained aseptically from the finger tip via lancet (Accu-Chek Safe-T-Pro Plus single-use sterile lancets, Roche Diagnostics, Mannheim, Germany) and collected in 100 µL electrolyte balanced heparin coated capillary tubes (Radiometer, West Sussex, UK). Samples were immediately analysed (95 µL) for whole blood pH, acid-base and metabolite status using a clinical blood gas and electrolyte analyser (ABL 800 basic, blood gas and electrolyte analyser, Radiometer, West Sussex, UK). In instances where sampling and analysis could not occur simultaneously, samples where immediately capped and placed on ice in an insulted container for subsequent analysis (always within 10 minutes following collection). All samples were obtained in duplicate with a range of intraclass correlation coefficients of 0.85-0.97, p < 0.01, respectively for all dependant whole blood measures.

3.7 Treatment Beverages

Subjects consumed three different beverages, either a CHO only (67 g hr\textsuperscript{-1} of maltodextrin derived from cornstarch), CHO-PRO (53.1 g hr\textsuperscript{-1} of maltodextrin, 13.6 g hr\textsuperscript{-1} of whey protein concentrate) or CHO-PRO-PEP (53.1 g hr\textsuperscript{-1} of maltodextrin, 11.0 g hr\textsuperscript{-1} of whey protein concentrate, 2.4 g hr\textsuperscript{-1} of peptides [Pep] [fish meat hydrolysate extracted from
salmon]) solution in which all were matched for energy content. Treatment beverages were provided blinded from the manufacturer (Nutrimarine Life Science, Bergen, Norway) in powder form. Prior to each trial the powder was weighed (Kern EW 120-4NM electronic bench-top scales, Kern & Sohn GmBH, Belingen, Germany) and subsequently mixed with water (magnetic stirrer HI-200M, Hanna Instruments, Bedfordshire, UK) in accordance with the manufacturer’s recommendations with the addition of 5 ml of lemon food flavouring (ASDA Stores Ltd., Leeds, UK) added to each total dose (1080 ml) to enhance blinding and palatability. All solutions were administered in a randomised fashion. Participants consumed 180 ml of each respective beverage every 15 minutes of the 90-minute steady-state bout starting at the onset of exercise, however excluding the 90-minute time point (1080 ml in total). Subjects were given one bolus at a time which was provided in an opaque drinks bottle.

3.8 Nutritional and Exercise Control

Participants were instructed to maintain their habitual dietary and fluid intake prior to both the familiarisation and blinded experimental trials, in which they were provided with a food diary to record all food stuffs and fluids consumed 24 hours prior to entering the laboratory. Subjects were briefed and provided with instructions on how to keep an accurate food diary. A copy of the food diary completed prior to the initial familiarisation trial was provided to the subjects before the subsequent blinded treatments with instructions to replicate their food and fluid intake as closely as possible. Volunteers were also instructed to abstain from alcohol and caffeine for 24 hours prior to all visits and none were known to be consuming any prescription medications or other ergogenic substances that may have affected energy transfer (Jeacocke and Burke, 2010). Participants were instructed to maintain the same training frequency, volume and intensity at the initiation of the study for
the duration of the investigation; however to refrain from exercise during the 24 hours prior to entering the laboratory.

3.9 Statistical Analysis

All statistical procedures were performed using SPSS 18 (SPSS Inc., Chicago, USA). Central tendency and dispersion of the data are reported as the mean and standard deviation (M ± SD). Mean differences between conditions are presented with 95% confidence intervals. Linear mixed models were used to determine the effect of the three experimental conditions (CHO, CHO-PRO and CHO-PRO-PEP) on all within-subject variables. Condition was modelled as a factor and time as a covariate. Marginal models were utilised to determine statistical significance between conditions for time-trial performance (condition modelled as a repeated factor) and pre-post exercise measures including body mass, urine osmolality and pH (condition and time modelled as repeated factors). In all cases model fit was assessed by Hurvich and Tsai’s criterion. Where a significant main effect for condition was observed, pairwise comparisons with post hoc Sidak adjustments were made. The effect size for these comparisons was calculated using Cohen’s $d$ and the magnitude of the effect interpreted according to Hopkins, Marshall, Batterham and Hanin (2009). Statistical significance was accepted as $p < 0.05$. All graphs were produced using SigmaPlot 12.0 (Systat Software Inc., USA).
Chapter 4 - Results

4.1 Steady-State Exercise

4.1.1 Blood Glucose and Lactate

Statistical analysis highlighted a significant main effect for time for blood glucose \((F = 9.6, p = 0.01, \text{Figure 1})\) with a mean rate of decrease of 0.05 mmol\(\text{L}^{-1}\) over each 15-minute sampling period. However, no differences were evident between conditions \((F = 0.2, p = 0.84)\) or for the interaction of condition and time \((F = 0.1, p = 0.90)\). Although Figure 1 depicts an apparent quadratic trend for time in blood glucose in both the CHO-PRO and CHO-PRO-PEP conditions, this term proved not to be of statistical significance and so was excluded from the model.

A significant condition \(\times\) time interaction \((F = 4.3, p = 0.02)\) and a main effect for time \((F = 27.6, p = 0.0001)\) was observed for blood lactate (\text{Figure 1}). The slope for lactate in the CHO condition was -0.20 mmol\(\text{L}^{-1}\) \((p < 0.001)\), -0.24 mmol\(\text{L}^{-1}\) \((p < 0.001)\) in CHO-PRO and -0.16 mmol\(\text{L}^{-1}\) in CHO-PRO-PEP \((p = 0.001)\) for each 15-minute sampling period. The rate of decrease of the slope for CHO-PRO-PEP was significantly less to that of CHO-PRO (mean difference [MD]: 0.006, 95% confidence interval [CI]: 0.002 to 0.01, \(p = 0.004\)). In contrast the slopes of CHO-PRO-PEP and CHO (MD 0.002, 95% CI -0.001 to 0.006, \(p = 0.2\)) and those of CHO-PRO and CHO (MD: 0.003, 95% CI: -0.0006 to 0.01, \(p = 0.1\)) were not significantly different. There was no main effect for condition \((F = 0.7, p = 0.53)\).
Figure 1: Mean blood glucose concentration (A), blood lactate concentration (B) and rating of perceived exertion (C) over the 90-minute preload cycling bout for each of the experimental conditions (CHO = carbohydrate; CHO-PRO = carbohydrate and protein; CHO-PRO-PEP = carbohydrate, protein and peptides). Error bars have been omitted for clarity.
4.1.2 Heart rate, VO₂, VCO₂, RER and Total Carbohydrate Oxidation

Main effects for both time ($F = 68.2$, $p < 0.001$) and condition ($F = 3.4$, $p = 0.047$) were observed for heart rate (Figure 2). However there was no significant interaction of the two terms ($F = 0.2$, $p = 0.79$). Although a significant effect for condition was observed, pairwise comparisons with post hoc Sidak corrections failed to identify any significant differences between the individual conditions (CHO and CHO-PRO: MD: -3.7, 95% CI: -7.9 to 0.5, $p = 0.095$, Cohen’s $d = 0.31$ [small effect]; CHO and CHO-PRO-PEP: MD: -2.3, 95% CI: -6.5 to 1.9, $p = 0.42$, Cohen’s $d = 0.20$ [small effect]; CHO-PRO and CHO-PRO-PEP: MD: 1.4, 95% CI: -2.8 to 5.5, $p = 0.80$, Cohen’s $d = 0.11$ [small effect]). Therefore the mean increase in heart rate was 2.5 b.min⁻¹ at each 15-minute interval.

Oxygen consumption significantly increased over the duration of the steady-state ride at a mean rate of 0.03 L.min⁻¹ at each 10-minute gas analysis interval ($F = 14.4$, $p = 0.004$, Figure 2). However, there were no significant effects for condition ($F = 0.5$, $p = 0.62$) or condition × time ($F = 0.9$, $p = 0.40$). Similarly a significant effect for time was observed for VCO₂ ($F = 9.2$, $p = 0.012$) with a mean increase of 0.02 L.min⁻¹ at each collection interval; whereas no such effects were apparent for condition ($F = 1.2$, $p = 0.32$) or the interaction of condition and time ($F = 1.2$, $p = 0.31$).

Conversely, there were significant effects for both condition × time ($F = 5.1$, $p = 0.007$) and condition ($F = 3.8$, $p = 0.039$, Figure 2) for RER. Regarding the interaction effects, the rate of decrease of the slope for the CHO-PRO beverage was significantly greater to that of CHO (MD: 0.00024, 95% CI: 0.00005 to 0.00042, $p = 0.012$) and CHO-PRO-PEP (MD: 0.0003, 95% CI: 0.00008 to 0.0005, $p = 0.004$). There was no statistical significance between the slopes of conditions CHO-PRO-PEP and CHO (MD: 0.00003, 95% CI: -
0.0002 to 0.00022, p = 0.75). As with heart rate, although a significant main effect for condition was observed, pairwise comparisons with post hoc Sidak adjustments failed to highlight any significant differences between the individual treatments (CHO and CHO-PRO: MD: -0.031, 95% CI: -0.076 to 0.015, p = 0.26, Cohen’s d = 0.72 [moderate effect]; CHO and CHO-PRO-PEP: MD: -0.002, 95% CI: -0.049 to 0.045, p = 1.0, Cohen’s d = 0.03 [small effect]; CHO-PRO and CHO-PRO-PEP: MD: 0.028, 95% CI: -0.017 to 0.074, p = 0.32, Cohen’s d = 0.71 [moderate effect]). No main effect for time was observed for RER although this did approach statistical significance (F = 9.9, p = 0.06).

Total CHO oxidation increased at a mean rate 0.02 g min⁻¹ at each 10-minute gas analysis interval (F = 10.9, p = 0.008, Table 1). However, no significant effects were apparent for condition (F = 0.01, p = 0.99) or condition × time (F = 0.04, p = 0.96).

Table 1: Total carbohydrate oxidation during each 10-minute gas analysis interval during the 90-minute preload cycling bout for each of the three experimental conditions.

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>CHO Oxidation (g min⁻¹)</th>
<th>CHO-PRO Oxidation (g min⁻¹)</th>
<th>CHO-PRO-PEP Oxidation (g min⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-15</td>
<td>3.10 ± 0.61</td>
<td>3.38 ± 0.27</td>
<td>3.07 ± 0.53</td>
</tr>
<tr>
<td>20-30</td>
<td>3.00 ± 0.68</td>
<td>3.26 ± 0.36</td>
<td>2.97 ± 0.51</td>
</tr>
<tr>
<td>35-45</td>
<td>3.09 ± 0.72</td>
<td>3.42 ± 0.40</td>
<td>3.01 ± 0.55</td>
</tr>
<tr>
<td>50-60</td>
<td>3.06 ± 0.75</td>
<td>3.36 ± 0.40</td>
<td>2.98 ± 0.55</td>
</tr>
<tr>
<td>65-75</td>
<td>3.15 ± 0.77</td>
<td>3.33 ± 0.45</td>
<td>3.05 ± 0.68</td>
</tr>
<tr>
<td>80-90</td>
<td>3.05 ± 0.88</td>
<td>3.30 ± 0.43</td>
<td>2.99 ± 0.67</td>
</tr>
</tbody>
</table>

CHO = carbohydrate; CHO-PRO = carbohydrate and protein; CHO-PRO-PEP = carbohydrate, protein and peptides; min = minutes. Data presented as M ± SD.
Figure 2: Mean heart rate (A) pulmonary oxygen uptake (B) and respiratory exchange ratio (C) responses over the 90-minute preload cycling bout for each of the experimental conditions (CHO = carbohydrate; CHO-PRO = carbohydrate and protein; CHO-PRO-PEP = carbohydrate, protein and peptides). Error bars have been omitted for clarity.
4.1.3 Rating of Perceived Exertion

Rating of perceived exertion displayed a significant main ($F = 31.03$, $p < 0.001$) and quadratic effect for time ($F = 17.6$, $p = 0.002$); increasing at a mean rate of 1.1 units at each sampling interval with a deceleration of -0.01 units over the course of the steady-state ride (Figure 1). In contrast, no such effects were apparent for condition ($F = 0.1$, $p = 0.94$) or the interaction of condition and time ($F = 0.2$, $p = 0.86$).

4.2 Time-Trial Performance

Table 2 shows the mean (SD) time-trial completion time, mean power output and peak power output for the 5 km cycling time-trial for each of the three experimental conditions. Statistical analysis revealed no significant effect for condition on time-trial performance ($F = 0.02$, $p = 0.98$). Similarly, there was no main effect for condition upon mean power output ($F = 0.43$, $p = 0.67$) or peak power output ($F = 0.96$, $p = 0.39$).

Table 2: Mean time-trial time, mean power output and peak power output for the 5 km cycling time-trial during each of the three experimental conditions.

<table>
<thead>
<tr>
<th></th>
<th>CHO</th>
<th>CHO-PRO</th>
<th>CHO-PRO-PEP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time-trial time (sec)</td>
<td>455.49±16.10</td>
<td>455.57±18.09</td>
<td>454.83±20.75</td>
</tr>
<tr>
<td>Mean power output (W)</td>
<td>241±22</td>
<td>244±28</td>
<td>245±32</td>
</tr>
<tr>
<td>Peak power output (W)</td>
<td>526±105</td>
<td>583±113</td>
<td>559±85</td>
</tr>
</tbody>
</table>

CHO = carbohydrate; CHO-PRO = carbohydrate and protein; CHO-PRO-PEP = carbohydrate, protein and peptides; sec = seconds; W = watts. Data presented as $M$ ± SD.
4.3 Pre-Post Exercise Measurements

4.3.1 Body Mass, Urine pH and Osmolality

Pre and post exercise values for body mass, urine pH and urine osmolality are shown in Table 3. Statistical analysis revealed a significant effect for time ($F = 24.1$, $p < 0.001$) between pre and post body mass measurements. However there were no such effects for condition ($F = 2.3$, $p = 0.11$) or the interaction of condition $\times$ time ($F = 0.85$, $p = 0.42$).

Similarly, there was no significant effect for condition ($F = 3.2$, $p = 0.06$) upon the percentage change in body mass between pre and post exercise values.

A significant main effect for time ($F = 4.5$, $p = 0.040$) was observed between pre and post exercise values for urine pH. In contrast the main effect for condition ($F = 0.1$, $p = 0.91$) and the interaction of condition $\times$ time ($F = 1.42$, $p = 0.25$) failed to reach statistical significance. Similarly, there was a significant main effect for time ($F = 5.15$, $p = 0.041$) between measurements acquired prior to and upon the completion of exercise for urine osmolality. Again however, no such effects were apparent for condition ($F = 0.6$, $p = 0.94$) or condition $\times$ time ($F = 0.6$, $p = 0.94$).
Table 3: Pre and post exercise values for body mass, urine pH and urine osmolality for each of the three experimental conditions.

<table>
<thead>
<tr>
<th>Condition</th>
<th>Pre-exercise</th>
<th>Post-exercise</th>
<th>% change body mass</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Body mass (kg)</td>
<td>Urine pH</td>
<td>Urine osmolality (mOsmol kg⁻¹)</td>
</tr>
<tr>
<td>CHO</td>
<td>76.7 ± 9.0</td>
<td>7 ± 1</td>
<td>392 ± 231</td>
</tr>
<tr>
<td>CHO-PRO</td>
<td>76.8 ± 9.1</td>
<td>7 ± 1</td>
<td>373 ± 285</td>
</tr>
<tr>
<td>CHO-PRO-PEP</td>
<td>76.7 ± 8.8</td>
<td>7 ± 1</td>
<td>383 ± 223</td>
</tr>
</tbody>
</table>

CHO = carbohydrate; CHO-PRO = carbohydrate and protein; CHO-PRO-PEP = carbohydrate, protein and peptides. Data presented as M ± SD.
Chapter 5 - Discussion

The purpose of the present investigation was to determine the efficacy of the addition of hydrolysed marine peptides to a CHO-PRO beverage (CHO-PRO-PEP) in comparison to an equally energetic CHO and CHO-PRO solution. The primary findings of the study are related to substrate utilisation, in which significant interaction effects for both RER and blood lactate were observed. Furthermore, there was a significant main effect for condition for heart rate. However, no such effects were apparent for 5 km time-trial performance. Therefore, although this preliminary data does not demonstrate an ergogenic effect of CHO-PRO-PEP co-ingestion upon time-trial performance, it is evident that the inclusion of protein from marine sources may influence exercise metabolism during prolonged steady-state cycle ergometry.

A novel finding of the current study was that RER decreased at a significantly greater rate within the CHO-PRO condition when compared to both CHO and CHO-PRO-PEP throughout the steady-state ride (Figure 2). Moreover, RER demonstrated a trend to be higher with CHO-PRO ingestion ($p = 0.039$). Although these results appear to emphasise the outcomes of specifically CHO-PRO ingestion, it also highlights that the physiologic responses to both CHO and CHO-PRO-PEP supplementation were similar. This is of interest given that the solutions were matched for energy content in which the CHO beverage provided an additional 14 g hr$^{-1}$ of CHO in comparison to both CHO-PRO and CHO-PRO-PEP. Therefore, the inclusion of protein and peptides within these solutions may have potentially facilitated a greater insulinaemic response and subsequently increased glucose uptake and utilisation (Saunders 2007; van Loon et al., 2000a; 2000b; 2000c). However in the absence of directly determining the insulin response and no significant differences in blood glucose concentration or total CHO oxidation, such an explanation is
tentative and requires further investigation. In contrast, such a premise has been dispelled by others when directly assessing the insulinaemic response to CHO and CHO-PRO ingestion during prolonged exercise (Ivy et al., 2003; Van Essen and Gibala, 2006). However, it must also be noted that the solutions within these studies were not matched for energetic content and therefore it was not possible to determine the existence of a specific protein mediated effect, due to the caloric variability between beverages (Ivy et al., 2003; Van Essen and Gibala, 2006).

A further mechanistic explanation for the observed differences in RER with CHO-PRO ingestion maybe related to the potential increase in solution osmolality and the subsequent reduction in gastric emptying with the addition of intact proteins (Hunt and Stubbs, 1975; Rowlands and Wadsworth, 2011; Vandenbogaerde and Hopkins, 2011). Exogenous CHO oxidation may have been reduced as a consequence of supplementary CHO remaining in the gastrointestinal tract, in which finite endogenous CHO stores will have provided the predominant source of CHO flux. This is supported by the trend towards higher RER values with CHO-PRO ingestion and the significant difference in the rate of decline of RER within the CHO-PRO condition. In contrast, it is possible that inclusion of peptides within the CHO-PRO-PEP solution may have enhanced gastric emptying and gastrointestinal uptake (Gabriella et al., 2007; Koopman et al., 2009; Maughan, Leiper and Vist, 2004b; Moughan et al., 2007). This is again reflected in the similar RER values between CHO and CHO-PRO-PEP ingestion despite the disparity in CHO content between the solutions. Similarly, the rate of decrease of blood lactate was significantly less with CHO-PRO-PEP supplementation in comparison to CHO-PRO, which, given that exercise intensity was predetermined, may suggest higher levels of glycolytic flux with the inclusion of marine peptides. However without the direct determination of variables including exogenous and
endogenous CHO utilisation and gastric emptying, and in the absence of differences in blood glucose and total CHO oxidation, these potential mechanisms require clarification. It must also be noted that although statistically significant, the mean differences between the significant effects outlined above would appear small. Therefore the translation of these effects into actual “real-world” physiological and performance benefits is tentative and remains unclear. Our results are also inconsistent with the observations of others, who have not reported significant differences in RER or blood lactate with CHO-PRO supplementation during prolonged exercise (Breen et al., 2010; Saunders et al., 2007; 2009; Toone and Betts, 2010). However the plethora of methodological differences relating to beverage composition and exercise protocols make inter-study comparisons challenging. It is also important to note that no previously published investigation has examined the efficacy of concomitantly ingesting more than one protein type with CHO during exercise.

The issue of solution osmolality is also pertinent when considering cardiovascular and thermoregulatory function. A significant main effect for condition was observed for heart rate with a trend towards higher heart rate values with CHO-PRO and CHO-PRO-PEP supplementation in comparison to CHO alone (Figure 2). As highlighted previously, the inclusion of intact protein within the CHO-PRO condition may have reduced gastric emptying (Hunt and Stubbs, 1975; Rowlands and Wadsworth, 2011; Vandenbogaerde and Hopkins, 2011). Subsequently fluid remaining in the gastrointestinal tract and its delayed delivery to the circulation may have caused disturbances in fluid balance, particularly plasma volume, and thereby increased cardiovascular strain. However, as heart rate also demonstrated a trend to be higher within the CHO-PRO-PEP condition, such a premise would directly contradict the proposal of enhanced gastrointestinal permeability with the inclusion of peptides (Gabriella et al., 2007; Koopman et al., 2009; Maughan et al., 2004b;
Moughan et al., 2007). The apparent higher heart rate values with CHO-PRO-PEP consumption are also contradictory to contemporary evidence suggesting that hydrolysed peptides produced from marine sources may act as angiotensin converting enzyme antagonists, thereby inhibiting the formation of the potent vasoconstrictor angiotensin II (Ewart et al., 2009; Hong et al., 2008). Again however, in the absence of the direct assessment of gastric emptying rates and plasma volume changes, coupled with no significant effect for condition for urine osmolality or body mass measurements (Table 3), these potential mechanisms to explain the differences in heart rate values remain speculative. Similarly, the solutions within the present study were matched for energy content in which the ingestion of equally energetic CHO and protein solutions would appear to not affect gastric emptying or fluid availability (Maughan et al., 2004b). Furthermore, others assessing the efficacy of CHO-PRO co-ingestion have reported no between-condition differences in heart rate (Breen et al., 2010; Osterberg et al., 2008; Saunders et al., 2004; 2007; 2009; Toone and Betts, 2010).

Regarding exercise performance, the findings of the present study are largely consistent with the observations of others who have also reported no additional ergogenic effects with CHO-PRO ingestion over that of CHO alone (Breen et al., 2010; Fergusen-Stegall et al., 2010; Martinez-Lagunas et al., 2010; Romano-Ely et al., 2006; Toone and Betts, 2010; Valentine et al., 2008; Van Essen and Gibala, 2006). In contrast, a limited number of studies have shown significant improvements in exercise performance with simultaneous CHO-PRO supplementation (Ivy et al., 2003; Saunders et al., 2004; 2007). However the methodological disparities between those reporting and not reporting ergogenic effects with CHO-PRO ingestion may account for these conflicting findings. For example, the addition of protein within the CHO-PRO solutions increased the energetic content of these
beverages above that of CHO only in those studies advocating the efficacy of CHO-PRO (Ivy et al., 2003; Saunders et al., 2004; 2007). CHO was also provided in suboptimal quantities to produce maximal exogenous CHO oxidation from a single CHO source (Ivy et al., 2003; Jeukendrup, 2004; 2008; Saunders et al., 2004; 2007). The notion that exercise performance was improved is also contentious as the latterly cited studies actually measured exercise capacity as opposed to exercise performance *per se* (Breen et al., 2010; Currell and Jeukendrup, 2008a; Ivy et al., 2003; Saunders et al., 2004; 2007). The ecological validity of blind-ended exhaustive tests is questionable as athletes are not required to maintain a fixed power output or percentage of maximal aerobic capacity for an indefinite period of time (Breen et al., 2010; Currell and Jeukendrup, 2008a). The reliability of such tests is also limited (Currell and Jeukendrup, 2008a; Jeukendrup et al., 1996). Conversely, assessments of exercise performance which are internally paced and have a predetermined endpoint, as utilised in the current investigation, have greater ecological validity and also reliability (Currell and Jeukendrup, 2008a; Hopkins, Schabort and Hawley, 2001; Jeukendrup et al., 1996). In contrast to the three studies within which the efficacy of CHO-PRO supplementation is founded (Ivy et al., 2003; Saunders et al., 2004; 2007), no other investigation has reported ergogenic effects when providing CHO at optimal rates (Breen et al., 2010; Saunders et al., 2009; Van Essen and Gibala, 2006; Valentine et al., 2008), matching solutions for energetic content (Romano-Ely et al., 2006; Toone and Betts, 2010; Valentine et al., 2008) or neither CHO nor energetic content (Fergusen-Stegall et al., 2010; Martinez-Lagunas et al., 2010; Osterberg et al., 2008). Furthermore, no studies utilising measurements of exercise performance have observed performance enhancing effects with CHO-PRO consumption (Breen et al., 2010; Osterberg
et al., 2008; Saunders et al., 2009; Toone and Betts, 2010; Van Essen and Gibala, 2006), with which our results are consistent.

Only two of the aforementioned studies have utilised protein hydrolysates similar to that of the current investigation (Breen et al., 2010; Saunders et al., 2009). Despite no significant difference in overall 60 km time-trial performance, Saunders and colleagues (2009) reported an improvement in late exercise time-trial performance with CHO-PRO in comparison to CHO only supplementation. However, the methodological design of the study has been scrutinised by others for not being adequately designed to examine exercise performance during the latter stages of the time-trial (Breen et al., 2010). Similarly it was also apparent that the efficacy of the CHO-PRO solution, in which a casein hydrolysate was utilised, may have been trial dependent due to a significant trial order effect being observed (Betts and Williams, 2010; Saunders et al., 2009). In a well controlled study, Breen and associates (2010) observed no additional ergogenic effects with CHO-PRO ingestion in approximately 40 km time-trial performance. Neither did these authors report any significant difference in exercise performance during the latter stages of the time-trial, in which their protocol was designed to specifically examine late exercise time-trial performance. Therefore, the observation that 5 km time-trial performance was not significantly different between treatments in the current study is consistent with others also employing protein hydrolysates (Breen et al., 2010; Saunders et al., 2009).

However, despite similar performance outcomes, perhaps the most striking difference between the current study and those also utilising protein hydrolysates, lies within the treatments provided to participants. The CHO and CHO-PRO treatments within the previous empirical works of Breen et al. (2010) and Saunders et al. (2009) were matched for CHO content, within which CHO was provided at optimal rates from a single CHO
source (Jeukendrup, 2004; 2008; Jeukendrup and Jentjens, 2000). In contrast, all solutions within the present study were matched for energetic content, in which, although CHO was provided in desirable quantities within the CHO solution, the CHO fraction within the CHO-PRO and CHO-PRO-PEP solutions was reduced to accommodate for the addition of protein. Therefore, a potential limitation of the current study, was the use of less than recommended quantities of CHO (maltodextrin) within the CHO-PRO and CHO-PRO-PEP solutions to produce maximal, exogenous CHO oxidation, from a single CHO source (Jeukendrup, 2004; 2008; Jeukendrup and Jentjens, 2000); although it must be noted that this amount was near optimal (53.1 g hr\(^{-1}\)). Preferably, the addition of protein should have not displaced the CHO content within the CHO-PRO and CHO-PRO-PEP solutions, thereby maintaining recommended rates of CHO intake as within the CHO only solution. However, this was an inherent compromise of conducting an iso-caloric study in an attempt to isolate any specific protein mediated effects. Conversely, had the treatments been matched for CHO content, it would have not been possible to distinguish whether any potential metabolic or performance benefits were a consequence of an independent protein mediated effect \textit{per se}, or simply a generic effect of the additional energetic content with the inclusion of protein. However, a potential solution for such a compromise may have been to adopt a feeding strategy in which the CHO content within the CHO solution was increased above recommended levels, to allow for the displacement of CHO within the CHO-PRO and CHO-PRO-PEP beverages, yet still provide CHO at desirable levels whilst ensuring all solutions were still matched for energetic availability (Valentine et al., 2008). In the current study for example, this would have resulted in the respective solutions containing the following: CHO (73.6 g hr\(^{-1}\) of maltodextrin); CHO-PRO (60 g hr\(^{-1}\) of maltodextrin, 13.6 g hr\(^{-1}\) of whey protein concentrate) and CHO-PRO-PEP (60 g hr\(^{-1}\) of
maltodextrin, 11.0 g hr\(^{-1}\) of whey protein concentrate, 2.4 g hr\(^{-1}\) of salmon meat hydrolysate). Subsequent investigations are therefore required to clarify the potential efficacy of CHO-PRO-PEP supplementation when providing CHO at recommend rates, and ensuring that treatments are matched for energetic content in an attempt to isolate any specific protein mediated effects.

5.1 Conclusion

In conclusion, the results of the present study suggest that when matching CHO, CHO-PRO and CHO-PRO-PEP solutions for energetic content, the inclusion of protein hydrolysates produced from marine sources may have significant effects upon exercise metabolism during prolonged, steady-state exercise. However, the translation of these statistically significant metabolic effects into subsequently meaningful performance benefits remains unclear. Indeed, although statistically significant, the magnitude of these differences appears small with regards to having an actual meaningful effect. The latter is highlighted by the fact that there were no significant differences in exercise performance regardless of the treatment received. Moreover, in the absence of an empirically supported mechanism, further investigations with a greater basic science approach are warranted to potentially elucidate mechanisms not yet uncovered, and to further determine the efficacy of CHO-PRO-PEP co-ingestion.

5.2 Directions for Future Research

It is important to note that investigations regarding the efficacy of CHO-PRO solutions are relatively scarce. It is evident that a greater number of iso-caloric studies are required to specifically isolate the potential independent metabolic effects of protein inclusion, in the absence of simply adding energy content (Stearns et al., 2010). Furthermore, it is
recommended that such investigations adopt feeding strategies that provide CHO at recommended rates from either a single (60-70 g hr$^{-1}$), or multiple transportable (~90 g hr$^{-1}$) CHO sources, as opposed to displacing CHO content and thereby potentially compromising the efficacy of the solution when accommodating for protein. To achieve this, as discussed above, CHO content within the CHO only solutions will inevitably have to be increased so that recommended rates of CHO delivery are attained, whilst concurrently ensuring that solutions are energetically equivalent (Valentine et al., 2008). Indeed, a novel avenue yet to be explored is the efficacy of CHO-PRO co-ingestion when optimal quantities of multiple transportable CHOs are employed. In addition, there must also be increased interest in the protein sources utilised and the potential synergistic influences of combined protein ingestion which have been neglected to date.

Concerning other methodological considerations, it is apparent that future investigations should also utilise ecologically valid exercise performance tests (i.e. time-trials), as opposed to the equivocal data at present emanating from the contrasting methods of assessing exercise capacity and exercise performance. Furthermore, regarding realistic, true to life simulation of exercise performance, more prolonged bouts of exercise, for example ultra-endurance exercise, also merit investigation with the results having greater application to road/tour cycling. Regarding the mechanistic approach, it is suggested that subsequent investigations apply a greater number of more informative physiological measures, particularly those pertaining to substrate utilisation and metabolism, including the use of isotopes to calculate supplementary CHO and protein oxidation, as well as insulin, free fatty acids and catecholamine responses which remain to be determined within the same study. Such a comprehensive basic science approach may potentially elucidate mechanisms that are not yet apparent.
References


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