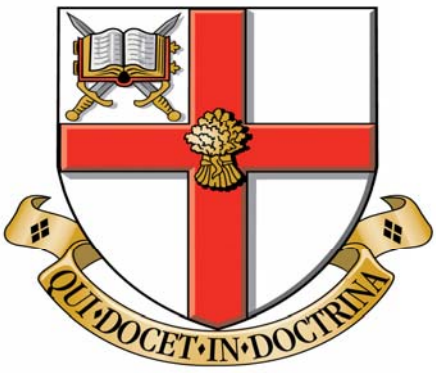

MULTIPLE-SPRINT SPORT EXERCISE AND CARBOHYDRATE-PROTEIN INGESTION IN HUMANS

Thesis submitted in accordance with the requirements of the University of Chester for the degree of Doctor of Philosophy

By Jamie Highton

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Abstract

The aim of the present thesis was to examine the potential for acute carbohydrate-protein (CHO-P) ingestion to enhance performance and recovery from exercise designed to simulate the demands of multiple-sprint sports (MSSs). Chapter 3 of the thesis explored the inter- and intra-day reliability and concurrent validity of non-motorised treadmill ergometry (NMT) for the assessment of short-distance sprint performance (i.e. 10-30 m). There were no significant mean differences between NMT variables recorded on the same day or between days. Ratio limits of agreement indicated that the best agreement was in 20 (1.02 */÷ 1.09) and 30 m (1.02 */÷ 1.07) sprint times, peak (1.00 */÷ 1.06) and mean (0.99 */÷ 1.07) running speed and step length (0.99 */÷ 1.09) and frequency (1.01 */÷ 1.06). The poorest agreement was observed for time to peak running speed (1.10 */÷ 1.47). Significant differences were observed between NMT and over-ground sprint times across all distances, with times being lower (faster) by approximately 25-30% over-ground. The correlations between NMT and over-ground variables were generally modest (r_s = 0.44 – 0.67), and optimal for time to cover 30 m on Day 2 (r_s = 0.8). Chapter 4 sought to examine the efficacy of CHO-P ingestion during 4 h of recovery from the Loughborough Intermittent Shuttle Test (LIST) when compared to CHO matched for energy (ISOEN) or CHO (ISOCHO) in a typical CHO beverage. There were significant increases over time in muscle soreness, and reductions in extensor and flexor peak torque (by approximately 9%, 9% and 8%, and 13 %, 13% and 11% at 60 deg·s^{-1}) and jump performance (10%, 7% and 5%) with the ingestion of CHO-P, ISOEN and ISOCHO, respectively. Beverage type x time interactions were not significant for any of these variables, indicating that changes in each variable were similar for all groups. Decrements in sprint performance assessed

on the NMT were typically small and not different between beverage types (<4%), although sprint times over 20 and 30 m remained elevated for 48 h post-exercise. Accordingly, Chapter 4 provided no clear evidence for a benefit of ingesting CHO-P in the hours after exercise to enhance recovery of muscle function and selected performance variables following MSS activity. Chapters 5 and 6 of the thesis aimed to examine the effect of CHO-P ingestion during simulated MSS exercise. In Chapter 5, it was observed that sprint times, HR and gut fullness increased over the course of the LIST, with no influence of consuming each of the different beverages. In contrast, there was a main effect of time ($P < 0.001$), and drink ($P = 0.042$) observed for RPE, which was lower ($P < 0.001$) during the LIST in the CHO-P condition (16.9 ± 1.4) than in either the ISOCHO (17.8 ± 1.1) or ISOEN (17.7 ± 1.3). However, time to exhaustion was not different ($P = 0.29$) between CHO-P (468.3 ± 268.5 s), ISOCHO (443.4 ± 286.3 s) and ISOEN (446.2 ± 282.08 s), although these times did equate to a non-significant mean improvement of 4% in the CHO-P trial. Chapter 6 demonstrated that during a modified version of the LIST with two self-regulated blocks of exercise intensity, participants had a higher average speed (8.1 ± 0.3 cf. 7.9 ± 0.5 $km \cdot h^{-1}$) during the final (self-regulated) 15 min block of the LIST in the CHO-P condition compared to CHO. Whilst the mechanisms for such an improvement are not certain, the attenuated rise in RPE observed in Chapter 5, and increased blood urea concentration observed in Chapter 6, with CHO-P ingestion may suggest altered central fatigue and/or increased protein oxidation enhances performance during MSSs.

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And so the difficult part. This work is dedicated to the person who deserves it most, to who would have been most proud of me, and to who I can no longer say just how thankful I am. My Mum. My Mum was so proud that I had started a PhD, she carried a piece of card with the title of my work in her handbag so that she could tell anyone who would listen what her son was doing. I think it speaks volumes that anyone who knew her would know just how proud she would be now, and I feel hollow at the thought of not being able to share this moment with her. She was the most kind-hearted, selfless and loving person I've ever known, and it is because of her that I am who I am and have done what I've done. I miss her more than I can express. So Mum, I love you, thank you, and this is for you.

Publications

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Conference proceedings

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Chapter 1

Introduction

1.1 Continuous, intermittent and multiple-sprint sport exercise

The 1960s and 1970s saw a rapid rise in academic interest in the physiology of intermittent human work. Researchers at this time asserted that "In daily life the most common situation is not continuous exercise but work performed as intermittent exercise with bursts of more intense activity interspersed with repeated periods of lighter activity or rest" (Essen et al., 1977, p. 490). Whilst interest was in the potential health benefits and training adaptation with such exercise (Fox et al., 1969), this definition was remarkably similar to that often used to define multiple-sprint sports (MSSs) such as soccer, rugby, hockey, netball and basketball; that is, consisting of "periods of support running and recovery interspersed with brief periods of sprinting" (Nicholas et al., 2000, p. 97) and "fluctuating randomly from brief periods of maximal or near maximal work to longer periods of moderate- and low-intensity activity" (Glaister, 2005, p. 758). Thus, research in this area paved the way for an understanding of the physiology of intermittent compared to continuous exercise, and the need to develop and utilise an intermittent exercise protocol in the study of MSSs.

Investigations at this time employed two distinct methodologies in their comparison of intermittent and continuous exercise, with some matching the absolute exercise intensity performed intermittently (during periods of work) with that performed at a continuous intensity (Essen & Kjaiser, 1978; Fox et al., 1969; Margaria et al., 1969; Christensen et al., 1960b), and others matching the average work output or oxygen

consumption of intermittent and continuous exercise over a set amount of time (Essen, 1978; Essen et al., 1978; Edwards et al., 1973; Astrand et al., 1960a; Christensen et al., 1960a). Thus, in the latter studies, the exercise intensity (power output or running speed) during work periods was approximately twice that of work performed continuously.

When absolute exercise intensity is matched in continuous and intermittent exercise, individuals are able to tolerate much greater workloads when exercising intermittently (Essen & Kaijser, 1978; Margaria et al., 1969). Indeed, recent research which has attempted to optimize work intensities during high-intensity intermittent training, demonstrated that when highly trained runners exercise for 30 s at the running speed associated with VO_{2max} interspersed with 30 s recovery, they are able to maintain VO_{2max} for approximately 10 min, a time nearly three times longer than when the running speed was carried out continuously (Billat et al., 2000). Unsurprisingly, during this type of comparison, intermittent work is associated with a much lower 'physiological strain' and altered metabolism. For example, intermittent work is associated with a lower 'oxygen debt' (calculated as the oxygen uptake during recovery periods minus resting oxygen consumption) (Fox et al., 1969), blood (Fox et al., 1969; Margaria et al., 1969; Christensen et al., 1960b) and muscle lactate (Essen & Kaijser, 1978), phosphocreatine (PCr) and adenosine tri-phosphate (ATP) concentration (Essen & Kjaiser, 1978). In these studies, the ATP:AMP ratio, PCr and citrate accumulation in the muscle acted to inhibit glycolysis during intermittent exercise, potentially at the phosphofructokinase junction, which in turn resulted in greater lipid metabolism during intermittent exercise (Christensen et al., 1960b).

In contrast to studies that compared the physiological responses to continuous and intermittent exercise matched for absolute exercise intensity, studies that compared different types of exercise at the same average intensity provided evidence that the physiological strain (measured via oxygen consumption, heart rate and blood lactate) of intermittent exercise was greater than (Edwards et al., 1973; Astrand et al., 1960a) or equal (Christensen et al., 1960a) to that of continuous exercise. Indeed, higher ratings of perceived exertion during intermittent exercise of the same average intensity have been observed (Drust et al., 2000; Edwards et al., 1972). In these studies, it was thought that a higher proportion of energy is produced via aerobic metabolism due to the replenishment of myohemoglobin oxygen stores, oxidation of accumulated lactate and resynthesis of PCr during rest periods (Astrand et al., 1960b).

Intramuscular and blood-borne substrate utilisation during intermittent and continuous exercise matched for average work intensity also differs, despite inducing similar energy expenditure (Christmass et al., 1999). More specifically, differences are present in fat and CHO utilisation, with fat oxidation approximately three times lower and CHO utilisation 1.2 times higher in intermittent compared to continuous exercise (Christmass et al., 1999). Whilst muscle glycogen utilisation does not appear to be different in intermittent compared to continuous exercise (Essen et al., 1977), the pattern of muscle glycogen utilisation in individual fibre types is altered. Essen (1978) reported that whilst muscle glycogen depletion was similar in type I fibres in intermittent (213 $mmol \cdot kg^{-1}$ dry weight) and continuous exercise (277 $mmol \cdot kg^{-1}$ dry weight), glycogen depletion was greater in type II fibres in intermittent (203 $mmol \cdot kg^{-1}$ dry weight) compared to continuous exercise (113 $mmol \cdot kg^{-1}$ dry weight). Such a

discrepancy between fibres may have important implications for MSS athletes who are required to utilise type II fibres for movements requiring explosive power and speed (e.g. sprinting, jumping and tackling).

Though the use of different methods to compare intermittent and continuous exercise has provided somewhat contrasting results, the physiological demands of these forms of exercise are clearly different. Indeed, the rest periods associated with intermittent exercise having a marked effect on physiological processes, such as substrate utilisation, oxygen consumption and exercise tolerance, occurring during exercise. Thus, interventions aimed at improving intermittent exercise performance and recovery, such as that during MSSs, should be tested using an intermittent protocol. In recent decades, this has been achieved, in some part, through time-motion analysis and physiological measurements taken during competition (detailed in Chapter 2). However, perhaps the most important development in this area has been the creation of so called 'simulation protocols' designed to be equivalent to the movement and physiological demands of MSSs.

By far the most utilised of these so-called simulation protocols has been the Loughborough Intermittent Shuttle Test (LIST; Nicholas et al., 2000). Based on movement demands originally documented by Reilly and Thomas (1976), this protocol involves repeated 20 m shuttle runs at varying intensities dictated via an audio signal from a CD. Patterns of exercise can be completed for 90 min, whilst the inclusion of a 'Part B' at the end of the protocol allows the measurement of time to exhaustion and

fatigue (Nicholas et al., 1995). This protocol has been validated against the movement and physiological demands of soccer (Nicholas et al., 2000) and elicits similar physiological responses to several other MSSs (see Chapter 2). Furthermore, the pattern of recovery post-LIST is similar to that observed after a soccer match (Magalhaes et al., 2009). A brief overview of the similarities between the LIST and soccer is presented in Table 1.1.

Table 1.1. A comparison of physiological and perceptual measures taken during and after the LIST and a soccer match

Variable	LIST	Soccer*
During		
Energy expenditure (kJ)	5550 ± 318	5672
VO_2 ($l \cdot min^{-1}$)	2.86 ± 0.16	2.5-3.5
VE ($l \cdot min^{-1}$)	76.5 ± 3.3	76.0
Average HR ($b \cdot min^{-1}$)	155	170
RER	0.92 ± 0.01	0.88
$\%VO_{2max}$	70.0 ± 2.0	70-75
Total CHO oxidation (g)	215 ± 13	205
Total fat oxidation (g)	49 ± 11	56
After (24 h)		
Leg soreness (1-10)	≈ 4.8	≈ 6
Knee extensor strength (Nm)	≈ -10	≈ -20
Knee flexor strength (Nm)	≈ -20	≈ -20
Plasma CK ($U.l^{-1}$)	900	1200
Jump height (cm)	≈ -7	≈ -5
Sprint time increase (s)	≈ 0.3	≈ 0.2
Muscle glycogen depletion (%)*	40-60%	40-90%

VE = ventilation, HR = heart rate, RER = respiratory exchange ratio, CHO = carbohydrate, CK = creatine kinase. *Data collated from various sources: Magalhaes et al. (2009); Ascensao et al., (2008); Bailey et al. (2007); Krustrup et al. (2006); Stolen et al. (2005); Thompson et al. (2001a); Nicholas et al. (2000); Nicholas et al. (1999); Thompson et al. (1999); Bangsbo et al. (1992); Leatt & Jacobs (1989); Bangsbo (1984); Jacobs et al. (1982); Saltin (1973).*Immediately after exercise.

Given the similar physiological response of the LIST and MSSs such as soccer, the LIST has become a powerful tool to study fatigue and recovery. Indeed, one of the initial studies utilising the LIST reported a 33% improvement in run time to exhaustion with the ingestion of a CHO-electrolyte beverage compared to placebo (Nicholas et al., 1995). Others have subsequently demonstrated the ergogenic potential of carbohydrate (CHO) ingestion during the LIST (Foskett et al., 2008; Patterson & Gray, 2007; Davis et al., 1999), as well examining the influence of water ingestion (McGregor et al., 1999), a hot environment (Sunderland & Nevill, 2005), a honey sweetened beverage (Abbey & Rankin, 2009), caffeine (Gant et al., 2010) and compression garments (Houghton et al., 2009). Additionally, several studies have utilised the LIST to monitor the effects of potential recovery aids such as CHO (Nicholas et al., 1999), cold-water immersion (Bailey et al., 2007) and vitamin C ingestion (Thompson et al., 2001a; Thompson et al., 2001b). Such studies have provided valuable information on the efficacy of interventions in a MSS context, and informed the practice of MSS athletes. It should be noted, however, that the LIST has received criticism, as it fails to consider skill performance and has fairly restricted movement patterns that are not necessarily representative of MSSs. Consequently, several authors have developed the LIST (Welsh et al., 2002), created a protocol to match their respective sport of interest (e.g. Twist & Sykes, 2011; Roberts et al., 2010a; Williams et al., 2010; Currell et al., 2009; Sirotic & Coutts, 2008; Edwards et al., 2003; Rahnama et al., 2003), or included tasks such as the Loughborough Soccer Passing Test and Loughborough Soccer Shooting Test (Ali et al., 2007a). Nevertheless, the LIST remains a popular and convenient method for assessing MSSs in a research setting.

1.2 MSS concerns and carbohydrate-protein supplementation

Dehydration and muscle glycogen depletion have been implicated on the aetiology of fatigue during MSSs (Bangsbo et al., 2006), typically evidenced via a decrease in high-intensity running and sprinting in the latter stages of competition (Mohr et al., 2003). Consequently, authors have sought to develop strategies to offset this fatigue for a competitive advantage. In addition, congested competitive and training schedules in MSSs require athletes and coaches to consider optimal recovery strategies, which may comprise replenishment of endogenous energy stores, repair of damaged skeletal muscle tissue and replacement of lost fluids for MSS players (Reilly & Ekblom, 2005).

Over recent years, the effects of a typical CHO supplement, with the addition of a small amount of protein (CHO-P), have received significant research attention both during endurance cycling and running and in recovery from resistance training. Whilst results in the investigation of CHO compared to CHO-P have been equivocal, the purported benefits of acute ingestion of such a supplement include improved endurance capacity (Saunders et al., 2007; Saunders et al., 2004; Ivy et al., 2003), time trial performance (Saunders et al., 2009), rehydration (Watson et al., 2008; Shirreffs et al., 2007), muscle glycogen restoration rate (Beradi et al., 2006; Williams et al., 2003; Ivy et al., 2002; van Loon et al., 2000, Zawadzki et al., 1992) and recovery from exercise-induced muscle damage (EIMD; Ferguson-Stegall et al., 2010; Saunders et al., 2009; Cockburn et al., 2008; Valentine et al., 2008; Baty et al., 2007; Luden et al., 2007; Romano-Ely et al., 2007; Rowlands et al., 2007; Saunders et al., 2007; Bird et al., 2006; Saunders et al., 2004). Whether such benefits occur during and/or following MSS exercise is currently unknown. However, a basic schematic of MSS concerns, where the potential for CHO-P

ingestion to be beneficial exists, and areas of investigation in the current thesis, are shown in Figure 1.1.

1.3 Thesis aims

Given the benefits of CHO-P ingestion and current performance and recovery concerns for MSS athletes, the investigation of acute CHO-P supplementation in a MSS context appears warranted, particularly given the dearth of research that exists in the area. Accordingly, the aim of the present thesis was to examine the effects of acute CHO-P ingestion on recovery of muscle function and selected performance measures (Chapters 3 and 4), with particular emphasis on EIMD, and to assess the efficacy of a CHO-P supplement on delaying fatigue during simulated MSS exercise (Chapters 5 and 6).

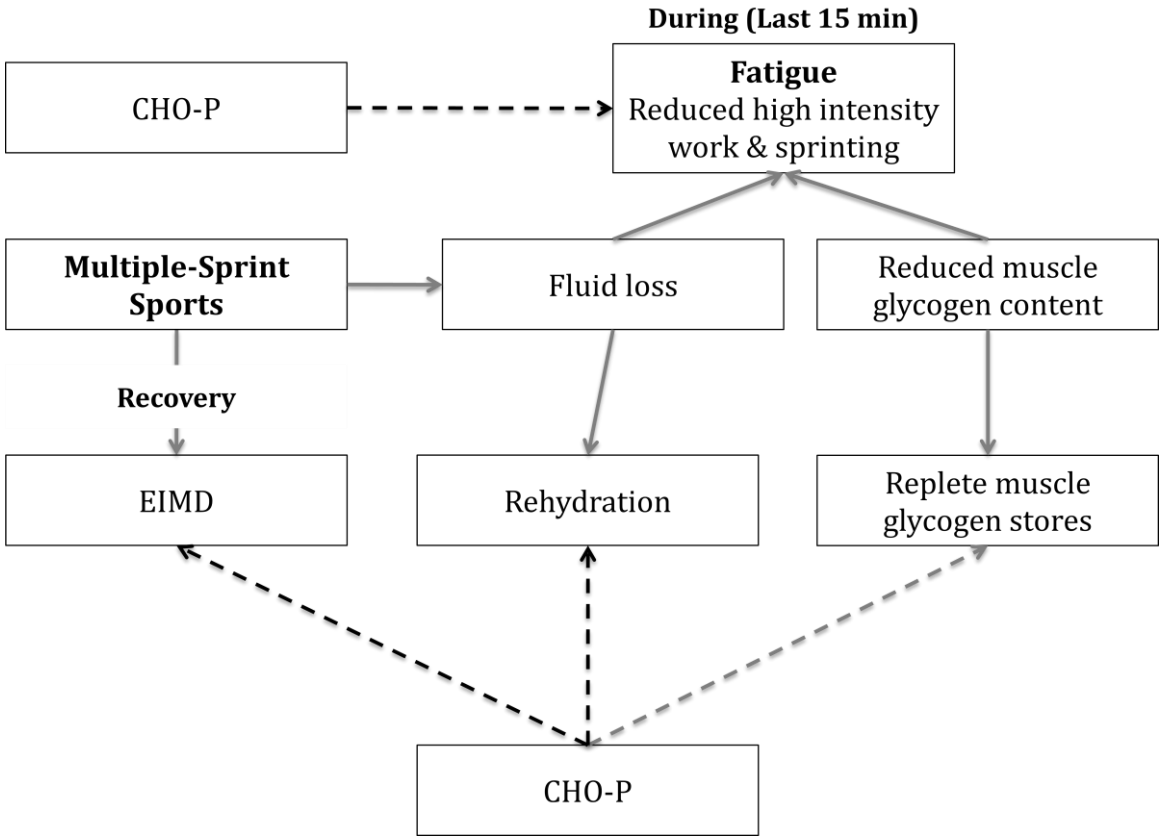

Figure 1.1 Key considerations for MSSs both during the event and in recovery, and potential sites at which acute CHO-P beverage ingestion may be beneficial (dashed lines). Lines in black indicate areas of investigation for the present thesis.

1.3 Organisation of the thesis

Chapter 2 of the thesis is a review of the pertinent literature in the area of MSSs, CHO and CHO-P ingestion. Thereafter, four discrete data chapters present empirical research on the reliability and validity of non-motorised treadmill (NMT) ergometry (Chapter 3), recovery of muscle function and performance with CHO-P ingestion following simulated MSS exercise (Chapter 4), the effects of CHO-P ingestion during MSS exercise (Chapter 5) and the effects of CHO-P on self-regulated MSS exercise (Chapter 6). Finally, Chapter 7 of the thesis presents conclusions on the efficacy of acute CHO-P ingestion for MSS exercise, and potential directions for future research.

Chapter 2

Review of Literature

2.1 Introduction

The aim of this review is to critique the pertinent research on: a) the movement and physiological demands of MSSs and associated recovery considerations and b) the potential for CHO, and CHO-P, to enhance performance during, and recovery following, MSSs.

2.2 Intermittent exercise and MSSs

MSSs such as hockey, soccer and rugby are characterised by periods of high-intensity muscular work interspersed with periods of low-intensity movement and/or rest, and are thus intermittent in nature. This distinguishes MSSs from more continuous modes of exercise, such as endurance running and cycling, as their physiological demands are more complex. Strategies for offsetting fatigue and enhancing recovery from this form of exercise should, therefore, be based on studies which have used intermittent, and not continuous, exercise protocols. The following section provides an overview of the intermittent characteristics of MSSs, the physiological responses to continuous and intermittent exercise, and more specifically the previously reported physiological responses and demands of different MSSs.

2.2.1 MSS Characteristics

2.2.1.1 Time-motion methodologies

Since the early 1970s many studies have documented the number, type, intensity and duration of movement patterns associated with MSSs, commonly termed time-motion analysis. However, time-motion analysis of these sports has incorporated several different and distinct methodologies (for a full review see Barris & Button, 2008; Carling et al., 2008), and thus the potential discrepancies between different methodologies, and the subsequent influence on measured variables, should be considered when evaluating the movement patterns associated with MSSs.

Popular time-motion methodologies in MSS initially included manual real-time notation (Docherty et al., 1988; Lyons, 1988; Andrews, 1985; Withers et al., 1982; Reilly & Thomas, 1976; Brooke & Knowles, 1974) and manual video based analysis (Andersson et al., 2007; Deutsch et al., 2007; Randers et al., 2007; Impellizeri et al., 2006; Duthie et al., 2005; Krustrup et al., 2005; Spencer et al., 2004; Mohr et al., 2003; Meir et al., 2001a; Meir et al., 1993). Both of these methods have been shown to be reliable (albeit through the use of reliability coefficients, which provide measurements of relative, and not absolute, agreement between measurements; Krustrup & Bangsbo, 2001; Reilly & Thomas, 1976), however their time-consuming nature and potential observer error have resulted in them being neglected by many researchers

More contemporary methods of time-motion analysis within MSSs include the use of semi-automated and automated multiple-camera systems (Barros et al., 2007; Di Salvo et al., 2007; Rampinini et al., 2007) and global positioning systems (GPS: Waldron et al., 2011a; Cunniffe et al., 2009). These methods have the advantage of allowing the movements of multiple players per game to be captured, with subsequent analysis of movements not as heavily reliant on observer skill and objectivity (Carling et al., 2008). GPS utilises 27 orbiting satellites, of which at least three must be in contact with a GPS receiver at one time, to determine position on the earth. This is achieved by measuring the time taken for a signal from each satellite to reach the GPS receiver allowing the trigonometric determination of position (Larsson, 2003). Any change in this position can be used to calculate distance covered , and the time taken to cover that distance can be used to calculate movement speed (although many commercial available GPS systems calculate speed utilising measured changes in satellite signal frequency owing to movement of the receiver, termed the Doppler shift).

The reliability and validity of measurements attained via GPS has received considerable interest over recent years. This research has demonstrated that reliability and validity appear to be dependent on the nature of the movement being measured and the sampling frequency of the GPS receiver. More specifically, both the reliability and validity of distance covered and speed is greater during linear movements without changes of direction (Petersen et al., 2009; Townsend et al., 2008), movements over longer distances (Petersen et al., 2009; Waldron et al., 2011b) and at lower speed (Gray et al., 2010; Duffield et al., 2009; Coutts & Duffield, 2008), and utilising a higher sampling frequency (5 Hz as opposed to 1 Hz) (Portas et al., 2010; Duffield et al., 2009).

Interestingly, measurements taken in Europe appear to demonstrate less variability than those collected in Australasia (Portas et al., 2010). Nevertheless, several studies have demonstrated that the measurement error associated with measuring team sport activity is generally small. For example, MacLeod et al. (2008) compared distances measured via trundle wheel and speeds measured via infrared timing gates to those measured via GPS during 14 laps of a circuit involving movements representative of those experienced during field hockey (including walking, jogging cruising, sprinting and changes of direction). Mean distance (6,821 m) and speed (7 $km \cdot h^{-1}$) from the GPS was not significantly different from actual distance (6,818 m) or timing gate measured speed (7 $km \cdot h^{-1}$). Likewise, the mean difference and 95% limits of agreement were no greater than 0.1 ± 0.08 $m \cdot s^{-1}$ and 0.2 ± 1.09 m for speed and distance respectively for individual movements in the circuit (zig-zags, sprints etc.). Similarly, Portas et al. (2010) reported validity was typically between 2.2 to 4.4% for various soccer specific movements (linear sprints, turns and high-intensity soccer drills), whilst the reliability for each measurement ranged from 2 to 5.3%. This is in agreement which has typically reported a coefficient of variation or standard error of the estimate of < 10% for speed and distance measurements associated with MSSs (Waldron et al., 2011; Barbero-Alvarez et al., 2010; Gray et al., 2010).

Whilst the reliability and validity of each of the aforementioned time-motion methodologies appears to be well established, the absolute agreement between measurements produced from each of them is not. Randers et al. (2010) reported that, when comparing a video-based time-motion analysis system, a semi-automatic multiple-camera system and two commercially available GPS systems during a soccer

match, there were similar performance decrements (i.e. changes on distance covered at high intensity from the first to second half and in the final 15 min of the game); however, there were large between-system differences in both total distance covered and distances covered in different locomotive categories. The semi-automatic multiple-camera system measured a total distance covered that was approximately 1 km longer than each of the other methods, whilst both the video analysis systems measured approximately 0.5 km more distance covered during low-intensity running (7-13 km·h^{-1}) than both GPS systems. In addition, distance covered in the high intensity running locomotive category (> 13 km·h^{-1}) was 0.6-1.0 km longer when measured by the semi-automatic multiple camera system compared to all other methods. These results are to some extent supported by Edgecomb and Norton (2006), who reported that both a computer-based tracking and GPS system over-reported total distance covered in an Australian Rules football match by 4.8 and 5.4% respectively. Thus, differences exist in the measurements produced by different methods of time-motion analysis, and as such should be taken into account when making comparisons between studies investigating the movement demands of MSSs. Furthermore, comparisons between different studies are made difficult due to the use of different 'motion descriptors' (e.g. standing, walking, jogging, striding and sprinting) and their definitions. Nevertheless, for the purpose of this review, studies incorporating all of the aforementioned methods of time-motion analysis will be included to provide an overview of the movement characteristics of various MSSs. Furthermore, to enable comparisons between studies, where speed categories are not provided this review will consider standing and walking as a low-intensity movement, jogging as a medium-intensity movement, and striding/cruising and sprinting as high-intensity movements.

2.2.2 Time-Motion analysis of MSSs

Investigations into the movement characteristics of MSSs have primarily been conducted in soccer, however, research has begun to evaluate the movement profiles and physiological and match demands associated with a variety of MSSs, including rugby union, rugby league, hockey, Australian rules football, netball and basketball.

2.2.2.1 Soccer

Both male and female elite standard outfield players appear to cover approximately 9-13 km during a match (see Table 2.1 below), although this has been shown to vary by position, with midfielders typically covering a greater distance than other outfield players (Barros et al., 2007; Di Salvo et al., 2007; Rampinini et al., 2007; Mohr et al., 2003; Reilly & Thomas, 1976). As can be seen in Table 2.1, the distance covered during a soccer match is made up of several different intensities of movement. Indeed, it has been estimated that between 1000 and 1500 discrete movements occur approximately every 4-6 s during a match, with a pause of approximately 3 s every 2 minutes (Krustrup et al., 2005; Strudwick et al., 2002). It should be noted that Table 2.1 is not a comprehensive overview of the types of movement undertaken in a soccer game, as many others such as sideways and backward movement, kicking and jumping are not included, yet are likely to contribute to the physiological demands of a game. Nevertheless, it is clear that soccer is a sport that is highly intermittent in nature.

The majority of the distance covered during a soccer match is in low-intensity locomotive categories such as walking and jogging (see Table 2.1), which is likely to

require little energy turnover. Mohr et al. (2003) reported that elite soccer players spent approximately 20, 42 and 30% of the time during a game standing, walking or running at a low intensity respectively. Conversely, elite players will typically spend approximately 4.5, 2.8, and 1.4 % of the total time during a match in high-intensity locomotive categories such as moderate speed running, high speed running (i.e. striding or cruising) and sprinting respectively, with approximately 69 ± 5 high-intensity runs and 39.2 ± 2 sprints taking place in a game (Rampinini et al., 2007; Mohr et al., 2003). Perhaps unsurprisingly considering the potentially high energy turnover required for such activity, it appears that the time spent in high-intensity movement differentiates between top and moderate-level athletes. Mohr et al. (2003) reported that international soccer players covered a greater distance during high-intensity running (28%) and sprinting (58%) than players from the premier Danish league. Consequently, time spent or distance covered in high-intensity running during a soccer match has become a widely used performance measure in the assessment of professional soccer players (Zubillaga et al., 2007). Other factors that have been shown to affect high-intensity running and sprinting during a game include the quality of opposition (Lago et al., 2010) and, as with distance covered, playing position (Mohr et al., 2003; Reilly & Thomas, 1976).

Table 2. 1. Distance covered (m) in different locomotive categories in soccer

Study	Players	Total	Walking/Jogging	LIR	HIR	Sprinting	Method
Andersson et al. (2007)	Professional (n = 11, female, Scandinavia)	10,000 ± 500			1,600 ± 400		Manual video analysis
Bangsbo et al. (1991)	Professional/Semi-Professional (n = 14, Denmark)	10, 800 (range 9,490-12,930)					
Barros et al. (2007)	Professional (n = 112, Brazil)	10,012 ± 1024	5,537 ± 263	1,615 ± 351	1,731 ± 399	437 ± 171	Automated video tracking
Bradley et al. (2009)	Professional (n = 370, England)	10,714 ± 991	8042	1,706	662	255	Multiple-camera
Di Salvo et al., (2007)	Professional (n = 20, Spain)	11, 393 ± 1016	7061 ± 272	1,965 ± 288	2,743 ± 369	248 ± 116	Multiple-camera
Hewitt et al. (2007)	Professional (n = 6, female, Australia)	9,140	4,500	4,740	620	280	GPS
Krustrup et al., (2005)	Professional (n = 14, female, Denmark)	10,300	9,000		1,310	160	Manual video analysis
Lago et al., (2010)	Professional (n = 27, Spain)	10,491-11,425	8,199-8,656		1,869-2525		Multiple-camera
Lago-Penas et al. (2009)	Professional (n =127, Spain)	10,943-935	6996 ± 308		540 ± 145	219 ± 122	Multiple-Camera
Mohr et al.,	Professional (n =	10,860			2,430	650 ± 60	Manual video

(2003)	18, Italy)	± 180			± 140		analysis
Randers et al. (2007)	Professional (n = 23, Scandinavia)	10,800 ± 170			2,130 ± 100	470 ± 30	Manual video analysis
Rampinini et al., (2007)	Professional (n = 20, Europe)	11,019 ± 331			2,738 ± 220	903 ± 115	Semi-automatic video
Reilly & Thomas (1976)	Professional (n = 40, England)	8,680 ± 1011	2,150 ± 471	3,187 ± 746	1,810 ± 411	974 ± 246	Audio & hand notation
Scott & Drust (2007)	Professional (n = 30, Female, England)	11,979 ± 1,325	3,114	5,390	1,557	359	Manual video analysis

Values are presented as mean ± standard deviation where possible. LIR = Low-intensity running (incorporating jogging or running < 15 km·h^{-1} depending on definition), HIR = High-intensity running (incorporating striding, cruising or running ≥ 15 km·h^{-1} depending on definition). Participants were male unless otherwise stated.

Fatigue, defined as an inability to maintain a given exercise intensity in the presence of sensations of tiredness and an increased sense of effort to maintain said force (Bart & Romain, 2010; Abbiss & Laursen, 2005), has been reported during a soccer match as a reduction in exercise intensity in the both the last half and last 15 min of a match (Bangsbo et al., 1991; Reilly & Thomas, 1976). Some of the physiological mechanisms through which fatigue may occur during MSSs are presented in section 2.2.3.2. Mohr et al. (2003) reported that elite soccer players performed more running at both a low and high intensity during the first compared with the second half (31.1% c.f. 28.4% and 9 % c.f. 7.7% respectively), whilst players sprinted more in the first compared to the second half (1.6% c.f. 1.2%) of a game. In addition, when the game was analysed in 15

segments, players covered 14-45% less distance in high-intensity running compared to the first four blocks of 15 minutes (the first 60 min of a game), whilst sprint distance was reduced by 70 m. Krustrup et al. (2006) examined repeated sprint ability via a test consisting of 5 repeated sprints separated by 25 s recovery, at the start, during half-time, and at the end of a competitive soccer game in 31 Danish soccer players. The mean time to complete the sprints was 2.8 ± 0.7% longer than at the start of the game, with each individual sprint slower after compared with before the game. No difference was found between sprint performance during half-time and before the game, thus impairments in high-intensity exercise capacity are likely to manifest during the latter part of a soccer match. It is worth noting that sprint performance, and the decline thereof associated with fatigue, is considered to be fundamental to success in MSSs. As such, sprint performance is a common marker to monitor recovery and fatigue in MSS players and has typically been measured via infrared timing gates (Magalhaes et al., 2009; Ascensao et al., 2008; Krustrup et al., 2006). However, other methods, such as NMT ergometry and GPS, may provide a much more comprehensive analysis of sprint performance owing to their capacity to provide real-time multiple split times, peak and mean speeds, rates of acceleration, force production and stride length and frequency.

2.2.2.2 Rugby Union

The total distances covered by players during a rugby union match is less than that reported during elite soccer matches. With the use of a multiple-camera system, Roberts et al.s' (2008) examination of the motion characteristics of 31 elite English rugby union players during a match revealed that forwards covered a distance of 5,581 ± 692 m, whilst backs covered a significantly higher distance of 6,127 ± 724 m. This is

in agreement with previous studies that reported distances of approximately 4,500 to 6,200 m during elite rugby union competition (Deutsch et al., 1998; Docherty et al., 1988). More recently, Cunniffe et al. (2009) reported that the distance covered in a game was 6,953 m in a sample of 2 elite international rugby players. The reason for this value being higher than that reported in previous studies is unclear, however the use of GPS, the level of competition and small sample size utilised are likely to be contributing factors.

As with soccer, rugby union is a sport that is highly intermittent in nature, involving movements at several different intensities. For example, in their study of the game movements of 47 elite rugby union players over two competitive seasons, Duthie et al. (2005) reported that forwards spent 41, 27, 20, 1.7 and 0.5% of total match time standing walking, jogging, striding and sprinting, respectively, whilst backs spent 41, 38, 16, 1.6 and 2.1% of percentage total time in each respective locomotive category. Similarly, Cunniffe et al. (2009) reported that 37, 27, 10, 14, 5 and 6% of total distance covered in a match was completed walking, jogging, striding, high-intensity running and sprinting, respectively. These findings are comparable to soccer, in that the majority of time spent on the pitch is in lower intensity movements, although time in high intensity activity appears to be marginally higher in soccer (see section 2.2.2). As with soccer, Duthie et al. (2005) reported that a large number of individual movements take place in a game, with forwards completing 651 discrete movements (not including rugby-specific activities such as static exertions, tackling, jumping and lifting), and backs completing approximately 658. These movements (or efforts) would generally be of a short duration (< 4 s), with rest periods typically less than 20 s. In contrast to

soccer, studies to date have reported that there is no change in movement patterns between the first and second halves of a game (Cunniffe et al., 2009; Duthie et al., 2005), suggesting that fatigue is not as evident in these sports. However, it may be that analysis needs to be to be completed over shorter time-spans (~15 min) as with soccer (e.g. Mohr et al., 2003), to elucidate the role of fatigue during the latter stages of a rugby union match.

2.2.2.3. Rugby league

The average distance covered in a game of rugby league appears to be between 4,000-9,000 m (Waldron et al., 2011a; King, Jenkins & Gabbett, 2009; Sykes et al., 2009; Meir, et al., 2001a), with backs suggested to cover an overall greater distance than forwards (Waldron et al., 2011a). However, when expressed relative to game time, forwards cover a greater relative distance than backs (95 ± 7 c.f. 89 ± 4 $m \cdot min^{-1}$). As with soccer and rugby union, this distance is covered intermittently with a variety of different movement intensities. Sykes et al. (2009) reported that with the ball in play, match-play was comprised of 53.9 ± 7.3% walking, 30.5 ± 5.4% jogging, 6.7 ± 1.4% medium intensity running, 1.8 ± 0.5% high-intensity running and 0.4 ± 0.3% sprinting. As with soccer, these values were shown to vary by playing position, with outside backs performing more high-intensity running than other positions. However, in agreement with other MSSs, for all players the majority of time spent in a rugby match is in low intensity locomotive categories (King et al., 2009; Meir et al., 2001a; Sirotic et al., 2009). Furthermore, time spent in high-intensity activity differentiates between elite and sub-elite level rugby league players, with elite players spending significantly more time (a mean difference of 97.7 s) in movement at high-intensity than their sub-elite

counterparts (Sirotic et al., 2009). Few studies have reported on fatigue during a game, however Sirotic et al. (2009) reported that mean running speed decreased by approximately 0.4 km·h^{-1} in the second half of a match in elite Australian rugby league players, suggesting that fatigue is apparent during the latter part of this form of activity. Indeed, Sykes et al. (2011) subsequently reported 4.9%, 30.5% and 46.8% reductions in the final quarter of a match in overall m·min^{-1}, high-intensity m·min^{-1} and very high intensity m·min^{-1}, respectively.

2.2.2.4 Other MSSs

Several other MSSs have been the subject of time-motion analysis, albeit with limited research. A recent study by Gabbett (2010) utilising GPS has reported that the average distance covered by female hockey players is 6,600 m during a match, with high-intensity running representing approximately 4% of total time during a game. Spencer et al. (2004; 2005b), conducting manual video-based time-motion analysis of field hockey in 14 elite international hockey players, showed that total game time was comprised of 46.5 ± 8.1% walking, 40.5 ± 7% jogging, 4.1% ± 1.1 striding and 1.5 ± 0.6% sprinting, with approximately 780 discrete movements taking place every 5.5 s during the game. As with other sports, these movement patterns were shown to vary by playing position, with more sprints performed by inside-forwards and strikers. Changes in performance during the game due to fatigue were not reported in either of the aforementioned studies.

In a 60 min basketball match, approximately 756-1,220 discrete movements will take place, with a change in movement occurring every 2 s (McInnes et al., 1995). As with other MSSs, the majority of playing time is spent in low-intensity movement (approximately 55-75%), with the remainder spent in high intensity locomotive categories (Bishop & Wright, 2006; McInnes et al., 1995). A reduction in time spent in high-intensity activity (approximately 3%) in the final quarter of a basketball match has also been observed (Abdelkrim et al., 2006).

Much of the work examining netball movement patterns was conducted in the 1980s and early 1990s (Steele & Chad, 1992; Woolford & Angrove, 1991; Otago, 1983), but recently, Davidson and Trewartha (2008) used computerised video analysis of six elite female netball players to establish the movement activities associated with netball competition. Total distance covered in a game was 7,984 ± 767 m, 4,283 ± 261 m and 4,210 ± 477 m for centres, goal keepers and goal shooters, respectively, and the percentage of total time spent standing, walking, jogging, running and sprinting being 12.3, 31.8, 17.2, 14.7 and 2.4%, respectively. Approximately 1,266 ± 62 discrete movements took place over the course of a game, whilst a change in movement occurred every 2.8 s.

In Australian Rules football, players have been reported to cover approximately 10,100 ± 1,400 m (Burgess et al., 2006), spending on average 33.4 ± 5.5% walking, 37.8 ± 5.1% jogging, 18.3 ± 3.2% striding and 7 ± 2.3 % sprinting. Burgess et al. (2006) also provided evidence of fatigue during this form of competition, with elite players shown

to cover a 5% greater distance in the first versus the second half, whilst mean speed was 14% higher in the first half of the game.

2.2.3 Physiology of high intensity intermittent exercise

2.2.3.1. High-intensity and maximal intermittent exercise

High-intensity or maximal intermittent exercise refers to that which involves repeated work periods at or above 100% VO_{2max}. This exercise pattern is closely linked to that which occurs during MSSs, albeit periods of high-intensity work during MSSs are likely to occur in a much less regimented fashion than in laboratory protocols. Nevertheless, an understanding of skeletal muscle metabolism during high-intensity or maximal intermittent work is likely to provide information on the aetiology of fatigue during MSSs.

In an early investigation into repeated maximal efforts, McCartney et al. (1986) examined muscle metabolism and power output during four 30 s maximal efforts on an isokinetic cycle ergometer interspersed with 4 min recovery periods in a sample of eight university students. It was reported that the highest power output occurred during the first exercise period (1,626 ± 102 W), with a significant reduction of 20% (1,321 ± 775 W) and a further 21% in the second and third work periods, respectively. No further change in power output was observed in the final exercise period. Similarly, when maximal cycling work bouts were performed for 10 shorter periods (6 s) with 30 s recovery, Gaitanos et al. (1993) observed that, whilst mean power output was 26.6% lower in the final sprint compared to the first, 47.5% of the reduction in power output

occurred in the first 5 sprints, with a smaller reduction in power output over the last six sprints when compared to the first four. Thus, it appears that decrements in power output, presumably due to fatigue, predominantly occur from the first to early work bouts, with later work bouts in maximal intensity intermittent exercise affected to a lesser degree. The recovery time between repeated high intensity work bouts has a marked impact on fatigue. Balsom et al. (1992) demonstrated that sprint times over 40 m could be maintained for 15 sprints when interspersed with 120 s recovery. However, when recovery time was reduced to 60 and 30 s, reductions were observed in sprint performance from the 11^{th} and 5^{th} sprints respectively.

Muscle biopsies and blood samples taken from studies examining high intensity intermittent work have provided a great deal of information on the potential mechanisms for the altered power output typically observed during this form of exercise. McCartney et al. (1986) discovered that during four maximal work bouts, muscle ATP concentrations decreased by 40% after the first exercise bout and continued to drop throughout the exercise protocol. Similarly, PCr concentrations in the muscle declined by 70, 88 and 96% immediately following the first, third and fourth exercise bouts, respectively. However, both muscle ATP and PCr concentrations were recovered to 90 and 78% of resting levels, respectively, after the end of the third recovery period. Accordingly, whilst PCr appears to provide a large proportion of energy for ATP resynthesis during repeated maximal efforts, the finding that its stores were almost completely restored during rest periods would suggest that reductions in muscle PCr stores were not solely responsible for the reductions in power output during repeated sprints in the McCartney et al. (1986) study. In contrast, a significant

body of research exists showing that creatine supplementation, and associated increased muscle PCr stores, improves repeated sprint performance (Yquel et al., 2002; Mujika et al., 2000; Jones et al., 1999; Aaserud et al., 1998; Balsom et al., 1993). Thus, the role of PCr depletion in the aetiology of fatigue in intermittent work is unclear.

The contribution of glycolysis to energy provision, as evidenced via the accumulation of blood and muscle lactate, during repeated maximal exercise decreases as intermittent exercise continues. Indeed, Gaitanos et al. (1993) estimated that whilst glycolysis accounted for 44% of anaerobic energy provision during the first of 10 sprints, the corresponding value for the 10th sprint was only 16%. McCardle et al. (1986) reported that plasma lactate concentrations progressively increased throughout exercise until the third (out of four) maximal efforts, where a plateau of 21-23 mmol·l^{-1} was reached. Similarly, a large increase in muscle lactate was observed after the first effort (~ 29.0 mmol·kg^{-1}) with no further change after the third and fourth exercise bouts. Interestingly, the progressive decline in work rate and attenuation of lactate production in this study would suggest that lactate accumulation and subsequent acidosis is not an important factor in the development of fatigue during multiple-sprint work. In a similar pattern to muscle lactate production, muscle glycogen was reduced following the first (18 mmol·kg^{-1}) and second (15 mmol·kg^{-1}) exercise periods, with no significant reduction observed thereafter. In contrast, plasma glycerol concentration increased progressively throughout the exercise protocol, reaching almost 500% of resting levels during recovery after the final exercise bout. Such increases led the authors to suggest that intramuscular triglycerides provided a significant proportion of energy during multiple maximal efforts, particularly during rest periods. Similarly,

Spriet et al. (1989) reported that muscle lactate increased by 33.3 ± 9.1 mmol·kg^{-1} dry weight in the second of three exercise bouts, whereas in the final exercise bout it only increased by 22.9 ± 6.8 mmol·kg^{-1} dry weight. Furthermore, it was also reported that muscle glycogen utilisation during the final work bout was only 32% of that observed during the second work bout. Whilst, the exact cause of a reduced glycogenolysis and glycolysis during the latter stages of intermittent exercise are unclear, it has been suggested that citrate, H^+, and potassium accumulation observed during intermittent work (Bangsbo et al., 1996; Spriet et al., 1989; Essen, 1978) may act to inhibit the activity of glycogen phosphorylase and phosphofructokinase, the rate limiting enzymes for glycogenolysis and glycolysis, respectively.

2.2.3.2 Physiological responses to MSSs

As with time-motion analysis, much of the work that has documented the physiological responses to MSSs has been conducted in soccer, with a relative paucity of data existing on the physiological responses to other sports with similar movement patterns. Accordingly, this section of the review will focus on the studies which have documented physiological responses to soccer, and, where possible, reference will be made to other MSSs. It is suggested that due to the similar movement patterns that occur in each MSS (see section 2.2.2), that much of the work conducted in soccer is likely to be relevant to other MSSs.

2.2.3.2.1 Aerobic metabolism

The aerobic contribution to energy provision in MSSs has primarily been investigated via the measurement of heart rate during competition, with values subsequently 'converted' to oxygen consumption based on the known relationship between oxygen uptake and heart rate during sub-maximal running (Krustrup et al., 2005; Bangsbo et al., 1994). However, it should be noted that heart rate measured during a game is likely to over-estimate oxygen consumption, as factors such as dehydration and subsequent hyperthermia (Montain & Coyle, 1992), and mental stress (Rusch et al., 1981) are likely to elevate heart rate without a corresponding increase in oxygen uptake. Nevertheless, it has been shown that the heart rate-oxygen consumption relationship is similar during treadmill running and exercise designed to mimic the demands of soccer (Esposito et al., 2004), and as such heart rate is likely to provide useful information on aerobic metabolism and physiological strain during MSSs.

The predominantly sub-maximal nature of soccer (see section 2.2.2.1), means that this form of activity primarily stresses the aerobic energy systems. Indeed, an inverse relationship between VO_{2max} and decrements in work rate over a game was shown in soccer players over three decades ago (Reilly & Thomas, 1976). The average and maximal heart rate during a soccer game is approximately 85% and 98% of maximal values, respectively, for both males and females (Krustrup et al., 2005; Bangsbo, 1994; Bangsbo et al., 1994; Ali & Farrally, 1991; Reilly & Thomas, 1979). Using the relationship established between heart rate and oxygen consumption, these studies have estimated that oxygen consumption during a soccer match is, on average, approximately 70-75% of VO_{2max} (Bangsbo et al., 2006). Thus, the oxygen consumption during a game for a player with a VO_{2max} of 60, 65 or 70 $ml \cdot kg^{-1} \cdot min^{-1}$ would be 45.0,

48.8 or 52.5 ml·kg^{-1}·min^{-1} respectively, translating into an estimated energy expenditure of between 1,500 and 1,800 kcal for a 75 kg player (Stolen et al., 2005).

Whilst no studies have directly measured oxygen consumption during a soccer match, some have attempted to measure it during soccer-like activities with the use of portable gas analysers. Esposito et al. (2004) demonstrated that the average VO_2 during simulated soccer match play was 3.49 ± 0.29 l·min^{-1} or 48.1 ml·kg^{-1}·min^{-1}, whilst VO_2 was 70, 84 and 94% of maximal values during moderate, high and very high intensity activity, respectively. Accordingly, the estimations for oxygen uptake derived from heart rate during a soccer game appear to be accurate, though, it should be noted that these results were obtained on amateur soccer players, and thus the applicability of these findings to elite players can be questioned. Nevertheless, estimations for VO_2 during soccer are further supported via measurements of core temperature, as a linear relationship between core temperature and relative work-intensity has been established (Saltin & Hermansen, 1966). Core temperatures of between 39 and 40°C have been observed during a game (Mohr, Krustrup et al., 2004; Ekblom, 1986), which is similar to the core temperature (~38.7°C) observed during continuous exercise at 70% VO_{2max} (Saltin & Hermansen, 1966).

Heart rates observed during other MSSs are similar to those observed during elite soccer matches (see Table 2.2 below), with values indicating that many of these sports are also conducted at approximately 70% of VO_{2max}.

Table 2.2 Heart rate responses to different MSSs

Study	Sport	Average HR (b.min^{-1})		Max HR
		First half	Second Half	
Morton (1978)	Rugby Union	161		
Roberts et al. (2010)	Simulated Rugby Union	160 ± 5		
Coutts et al. (2003)	Rugby League	167 ± 9 (86.7 ± 4.4% HRmax)	165 ± 11	
Waldron et al. (2011)	Rugby League	83.5 ± 1.9% HRmax		
MacLeod et al. (2007)	Hockey	174 ± 12	169 ± 11	191 ± 9
Boyle et al. (1994)	Hockey	159 ± 8		
Matther & Delextrat (2009)	Basketball	165 ± 9 (89.1% HRmax)		
McInnes et al. (1995)	Basketball	168 ± 9 (87 ± 2% HRmax)		188 ± 7 (99 ± 1% HRmax)
Rodriguez-Alonso et al. (2003)	Basketball	186 ± 6		94.6% HRmax
Hahn et al. (1979)	Australian Football	164 (range 140-180)		

HRmax = maximum heart rate.

2.2.3.2.2 Anaerobic metabolism

Whilst aerobic metabolism is the predominant energy system engaged during a soccer game, anaerobic metabolism is likely to be taxed considerably during the many brief, high-intensity periods that occur during this form of exercise.

The role of PCr in muscle metabolism during a soccer game has received little attention, however, Krustrup et al. (2006) demonstrated that muscle PCr levels were lower immediately after a game when compared to resting levels (88 ± 2 c.f. 79 ± 3 mmol·kg·$^{-1}$ dry weight) in 31 sub-elite soccer players. Furthermore, muscle PCr levels were lower

still following intense exercise periods (defined as periods involving fast running and sprinting over 18 and 25 km·h^{-1}, respectively) in the first (76 ± 3 mmol·kg·$^{-1}$ dry weight) and second half (67 ± 3 mmol·kg^{-1} dry weight) of a game. Hence, it would appear that PCr is a significant contributor to energy provision during a soccer match, particularly during intense periods of play. However, in agreement with work conducted on repeated high or maximal intensity exercise, it seems unlikely that PCr is the predominant cause of fatigue associated with MSSs, as Krustrup et al. (2003) demonstrated that performance during intermittent running to exhaustion could be maintained with muscle PCr levels much lower (40 ± 5 mmol·kg^{-1} dry weight) than those reported at the end of a soccer game (Krustrup et al., 2006).

Several studies have measured blood lactate levels during a soccer game in attempt to establish the levels of anaerobic metabolism due to lactate-producing glycolysis and the potential causes of fatigue. Mean blood lactate concentrations after a game of 2-10 mmol·l^{-1} have been observed, with individual values as high as 12-15 mmol·l^{-1} (Krustrup et al., 2006; Bangsbo, 1994; Ekblom, 1986), similar to those seen during intermittent exercise to exhaustion (Krustrup et al., 2003), and those reported in other MSSs (see Table 2.3). Interestingly, blood lactate concentrations are lower in the second half of a soccer game than the first. For instance, Bangsbo (1994) demonstrated that mean blood lactate concentrations were 4.9 (range 2.1-10.3) and 3.7 (range 1.8-5.2) mmol·l^{-1} for the first and second halves of a game, respectively, in Danish first and second division soccer players. The finding that blood lactate concentration is lower in the second half of a game is likely to be due to both the shorter distance covered and reduced high-intensity running performed in the second half (see section 2.2.2).

Table 2.3 Blood lactate responses to different MSSs

Study	Sport	Blood Lactate (mmol·L^{-1})	
		Average	Maximum
Deutsch et al. (1998)	Rugby Union	6.6	8.5
Docherty et al. (1988)	Rugby Union	2.8 ± 1.6	-
McLean (1992)	Rugby Union	5.4 ± 0.6	5.9
Coutts et al. (2003)	Rugby League	5.9 ± 2.5	8.4
Ghosh et al. (1991)	Hockey	5.6	-
Rodriguez-Alonso et al. (2003)	Basketball	5.0 ± 2.3	-
Matthew & Delaxtrat (2009)	Basketball	5.2 ± 2.7	10

To date, only one study has directly measured muscle lactate concentrations during soccer matches. Krustrup et al. (2006) reported that mean muscle lactate concentrations were higher after soccer matches (13.0 ± 0.8 mmol·kg^{-1} dry weight) compared with pre-match values (4.2 ± 0.5 mmol·kg^{-1} dry weight), whilst muscle lactate values were higher again following intense periods during a game (15.9 ± 1.9 and 16.9 ± 2.3 mmol·kg^{-1} dry weight in the first and second halves respectively). In contrast to blood lactate concentrations, muscle lactate concentrations in the study of Krustrup et al. (2006) were considerably lower than those observed at exhaustion in intermittent exercise (48.9 ± 6.1 mmol·kg^{-1} dry weight; Krustrup et al., 2003). It is noteworthy that whereas during continuous exercise blood lactate suitably reflects lactate concentrations in the muscle (Bangsbo et al., 1993), Krustrup et al. (2006) observed no significant correlation between muscle and blood lactate concentrations sampled during a soccer game. The authors suggest that this difference is because of the lower clearance rates of lactate in the blood when compared to muscle (Bangsbo et al., 1995), and thus an accumulation of lactate may take place in the blood after several

high-intensity efforts. Consequently, it is possible that during a soccer game, blood lactate concentration may be high without a corresponding high lactate concentration in the muscle. Nevertheless, their findings of high blood and moderate muscle lactate concentrations in a game indicate that the rate of glycolysis is high, in particular during intense periods of movement.

It is unlikely that high concentration of lactate, and associated H^+ accumulation and reduced muscle pH, are the predominant cause of fatigue in MSSs. Krustrup et al. (2006) observed no significant correlation between muscle lactate, blood lactate or muscle pH and sprint performance at any point in a soccer game. Indeed muscle pH was only marginally decreased during the course of the match (from 7.2 to 6.8). Furthermore, a previous investigation has failed to demonstrate that an accumulation of lactate causes fatigue during MSS like exercise (Krustrup et al., 2003), and relatively little evidence exists to show that reduced muscle pH associated with increased H^+ ion concentrations and muscle lactate causes fatigue in working muscle (Stackhouse et al., 2001).

2.2.3.2.3 Substrate utilisation

During intermittent exercise designed to simulate the demands of MSSs, respiratory exchange ratios (RER) of 0.85, 0.87 and 0.91 have been observed at intensities corresponding to 55, 71 and 81% of VO_{2max} respectively, (Bangsbo, 1994). Thus, based on the estimated average VO_2 during a soccer match, Bangsbo (1994) estimated that the mean RER value during soccer is approximately 0.88, corresponding to a

contribution of 60% CHO and 40% fat to energy production. The exact contribution, if any, of protein to metabolism during a soccer match is not clear. However, based upon studies utilising continuous exercise protocols of a similar intensity and duration to soccer, it has been estimated that protein oxidation contributes up to 10% of total energy production (Tarnopolsky, 2004; Wagenmakers et al., 1990; Wagenmakers et al., 1989). However, protein oxidation can increase with lowered energy (Chiang & Huang, 1988) and CHO availability (Howarth et al., 2010; Riddell et al., 2003; Elwyn et al., 1979), and dehydration (Berneis et al., 1999). Thus, amino acid oxidation may make a significant, albeit relatively small, contribution to energy provision during a soccer match.

2.2.3.2.4 CHO metabolism

CHO oxidation during a soccer match has been estimated to total approximately 205 g, equating to a CHO oxidation rate of approximately ≥ 2 g of CHO per minute (Bangsbo, 1994b). Blood glucose concentration is typically marginally higher after (4.9 – 5.5 $mmol \cdot l^{-1}$), when compared to before, a soccer match (approximately 4.5 $mmol \cdot l^{-1}$ Bangsbo, 1994; Ekblom, 1986; Krustrup et al., 2006). Peak blood glucose values typically occur in the first half of a match (6.1 ± 0.3 c.f. 5.3 ± 0.3 $mmol \cdot l^{-1}$), and at 30 min (approximately 7.5 $mmol \cdot l^{-1}$) during simulated soccer performance (Nicholas et al., 2000). Given that these values are not indicative of a hypoglycemic state, (~3.5 $mmol \cdot l^{-1}$), it appears that liver glycogen stores are sufficient to maintain normal blood glucose concentrations during the course of a soccer match. Thus, though previously implicated in the aetiology of fatigue (Coggan & Coyle, 1987; Coyle et al., 1986), hypoglycaemia is unlikely to be the predominant cause of fatigue in MSSs.

After a soccer match, whole muscle glycogen has been reported to be reduced to a concentration below which a maximal glycolytic rate can ensue (~ 200 mmol.kg^{-1} dry weight; Bangsbo et al., 1992), although this is not always the case (Jacobs et al., 1982). The importance of muscle glycogen as a substrate during soccer was originally demonstrated by Saltin (1973), who reported that the concentrations taken from the thigh muscle of five players were 96, 32 and 9 mmol·kg^{-1} wet weight before, at half-time and at the end of a non-competitive match, respectively. In the same study, four other players entered the game with lowered (45 mmol·kg^{-1} wet weight) muscle glycogen levels due to intense physical exercise leading up to the game. For these players, muscle glycogen stores were almost completely depleted by half-time of the match. Other studies have reported reductions in muscle glycogen of between 20-90% following a soccer match (Krustrup et al., 2006; Leatt & Jacobs, 1989; Jacobs et al., 1982), whilst exercise designed to simulate the demands of soccer results in an approximate 60% loss of muscle glycogen, with a greater rate of glycogen utilisation in type II versus type I fibres (Nicholas et al., 1999). Indeed, Krustrup et al. (2006) demonstrated that muscle glycogen depletion was not uniform across all muscle fibres following a non-competitive match. Whilst whole muscle glycogen stores were significantly depleted by 42 ± 6% after the match, approximately 47% of all individual muscle fibres were almost or completely depleted, with type I fibres having the highest proportion of completely depleted fibres, despite a higher rate of glycogen utilisation in type II fibres. Consequently, it would appear that a significant proportion of muscle fibres are depleted of glycogen to a level that would result in impaired energy provision from glycolysis during a soccer match (Bangsbo et al., 1992).

Reviews into the aetiology of fatigue in MSSs have suggested that muscle glycogen depletion is likely to be the predominant cause of reduced work rates observed during the latter stages of competition (Bangsbo et al., 2006; Mohr et al., 2005; Reilly, 1997). The implications of such a depletion become clear when considering that studies utilising the stable isotope method have established that muscle glycogen utilisation increases with increasing exercise intensity, and that during continuous exercise at an exercise intensity similar to that of MSSs (~70% VO_{2max}), it is the predominant substrate utilised for energy production (Van Loon et al., 2001; Romijn et al., 1993).

Nearly 40 years ago, Saltin (1973) demonstrated the importance of muscle glycogen as a substrate for performance during MSSs, in reporting that when soccer players entered a match with low muscle glycogen concentration (~200 mmol·kg^{-1} dry weight) induced by prior exercise, they covered 25% less distance than players entering the game with normal (~400 mmol·kg^{-1} dry weight) concentrations. Those players with normal muscle glycogen concentrations also covered 27 and 24% of total distance walking and sprinting, respectively, whereas the corresponding values for individuals with lower concentrations were 50 and 15%. Importantly, there is strong recent evidence that increased CHO intake before and during exercise is associated with enhanced muscle glycogen concentrations and performance. This is discussed in later sections of this review.

2.2.3.2.5 Fat metabolism

Fat oxidation over the course of a soccer match is estimated to equal approximately 49 g (Bangsbo, 1994b). In contrast to blood glucose, free fatty acid (FFA) concentrations in the blood progressively increase over the course of a soccer game (Krustrup et al., 2006; Bangsbo, 1994b), thus the rate of lipolysis during a game appears to be relatively high. This is supported by the observation of an increase in glycerol concentration of approximately 100-200 $\mu mol \cdot l^{-1}$ in the blood towards the end of a game (Krustrup et al., 2006). Whilst, the exact contribution of intramuscular triglycerides to energy provision during a soccer match has yet to be clearly established. Rico-Sanz et al. (1998) have reported that intramuscular triglyceride content of the soleus, medial gastrocnemius and tibialis muscles measured via magnetic resonance spectroscopy remained unaltered following 90 minutes of variable intensity running (45.3 ± 12.7, 34.8 ± 10.1 and 25.2 ± 7.3 $mmol \cdot l^{-1}$, respectively) when compared to baseline measures (46.4 ± 13.6, 35.0 ± 12.1 and 23.1 ± 4.8 $mmol \cdot l^{-1}$, respectively). However, few rest periods were included in the intermittent protocol of Rico-Sanz et al. (1998), and intramuscular triglycerides have been suggested as a major source of the fat oxidised in periods of rest during intermittent exercise (McCartney et al., 1986; Essen et al., 1977).

2.2.3.2.6 Endocrine response

Changes in several circulating hormone concentrations have been monitored over the course of a soccer match. For instance, insulin, a peptide hormone secreted from the β cells of the islets of Langerhans in the pancreas, has been shown to inhibit lipolysis (Knight & Iliffe, 1973), reduce hepatic glucose output (Matsuuraet al., 1975), increase

glycogen synthesis (Mandarino, 1989), reduce blood glucose (Cowell & Lein, 1963) and increase muscle amino acid uptake (Liu & Barrett, 2002). Insulin concentrations in the blood have been shown to decrease over the course of a soccer match (Bangsbo, 1994b), and more specifically from baseline levels (15.2 ± 2.0 $\mu mol \cdot l^{-1}$) to 7.4 ± 0.5 $\mu mol \cdot l^{-1}$ and 6.0 ± 0.9 $\mu mol \cdot l^{-1}$ in the first and second halves, respectively (Krustrup et al., 2006).

In contrast, catecholamine (both epinephrine and norepinephrine) concentrations tend to increase over the course of a game (Bangsbo, 1994b). It is likely that the increased catecholamine levels and decreased insulin concentrations toward the end of MSSs can explain increases in FFA mobilisation, as lowered insulin concentrations have been shown to increase lipolysis during exercise (Wasserman et al., 1989), whilst whole body lipolytic rate is increased with increasing circulating catecholamines (Arner et al., 1990). Both salivary and blood cortisol concentrations have also been shown to significantly increase at the end of a soccer game (Haneishi et al., 2007; Edwards et al., 2006; Carli et al., 1986), which results in stimulated gluconeogenesis using amino acids as precursors. Indeed, increases in plasma ammonia (NH_3), which is a bi-product of protein deamination required for protein metabolism, have been observed during a game (Krustrup et al., 2006). Consequently, as alluded to earlier, protein may be an important substrate during MSSs.

2.2.3.2.7 Fluid Loss

Fluid loss via sweating during a soccer match or training is reported to be in the region of 0.7 – 2.1 $l \cdot h^{-1}$ (Maughan et al., 2005; Shirreffs et al., 2005), although this is likely to be influenced by factors such as individual sweating rate, environmental temperature and exercise intensity. Nevertheless, losses equivalent to 4-5 l or 3.1% of body mass have been reported in high ambient temperatures (Bangsbo, 1994b; Mustafa & Mahmoud, 1979), and between 1 to 3% in temperate conditions (Krustrup et al., 2006; Bangsbo, 1994b). Such fluid loss may contribute to the fatigue observed during MSSs, especially as dehydration equivalent to an approximate 2% body mass loss can impair prolonged continuous exercise performance (Armstrong et al., 1985; Maughan, 1985) and aerobic capacity (Pinchan et al., 1988), and is associated with an increased core temperature and cardiovascular strain (Sawka & Montain, 2000). Furthermore, dehydration has been shown to impair sprint performance (McGregor et al., 1999) and skill performance (McGregor et al., 1999; Edwards et al., 2007) during simulated MSS exercise. However, reduced sprint performance (Krustrup et al., 2006) has also been observed during a soccer match with only a relatively small amount of fluid loss (~1%), whilst Mohr et al., (2004) observed no change in core or muscle temperature throughout a soccer match. Thus, dehydration is unlikely to be the sole cause of fatigue in MSSs.

2.3 CHO intake and fatigue

In light of the well-documented link between CHO (from muscle and liver glycogen) and endurance performance, many studies have examined the potential for altered

CHO ingestion, whether it be through diet or acute supplementation, to preserve or increase glycogen stores and subsequently have an ergogenic effect. This may be of particular importance to MSSs, where muscle glycogen depletion is thought to be the main cause of fatigue.

2.3.1 CHO loading in the days before exercise

Bergstrom et al. (1967), in their classic 'CHO loading' study, demonstrated that a high muscle glycogen concentration can be achieved via a manipulated dietary CHO intake. In this study, participants initially performed an exhaustive work bout at 70% VO_{2max} followed by 3 days of a high fat (1,300 kcal) high protein (1,300 kcal) diet. Subsequently, participants performed another exercise bout to exhaustion (in which muscle glycogen concentrations became significantly depleted) followed by 3 days of a high CHO diet (2,300 kcal). This regimen resulted in a 'supercompensation' of muscle glycogen stores, whereby muscle glycogen concentration was significantly higher than baseline, reaching values over 900 $mmol \cdot kg^{-1}$ dry weight.

Sherman et al. (1981) later demonstrated that muscle glycogen concentration could be increased to very high values through the use of a more moderate CHO loading regimen. In this study, participants gradually reduced their training load over a 6 day period (beginning with continuous running at 75% VO_{2max} for 90 min and finishing with complete rest on the final day), and ingested either a mixed diet consisting of 50% CHO, 3 days of low CHO intake (25%) followed by 3 days of high CHO intake (70% - classic supercompensation regimen), or a mixed diet with 50% CHO for 3 days followed

by a high CHO intake (70%) for the following 3 days (moderate supercompensation protocol). Sherman et al. (1981) reported significant increases in muscle glycogen concentration from baseline in both the classic and moderate supercompensation protocols, with very little difference between regimens (950 c.f. 918 mmol·kg^{-1} dry weight). Thus, it was concluded that a normal training taper in conjunction with a slightly increased CHO intake is sufficient to raise muscle glycogen levels. Indeed, subsequent studies have demonstrated that muscle glycogen levels (in type I, IIa and IIb) in trained athletes can be increased to maximal values (approximately 810 mmol·kg^{-1} dry weight) within 24 h when 10-12.5 g of CHO per kg body mass per day is provided with little or no physical training (Bussau et al., 2002; Coyle et al., 2001).

In general, CHO loading (causing increased muscle glycogen stores) leads to an improvement in endurance exercise performance (Walker et al., 2000; Pitsiladis & Maughan, 1999; Bergstrom et al., 1997; Pizza et al., 1995; Rauch et al., 1995; Fallowfield & Williams, 1993; Williams et al., 1992; Brewer et al., 1988; Galbo et al., 1979), although this is not always the case (Andrews et al., 2003; Burke et al., 2000). In all instances, CHO loading results in an increased reliance on muscle glycogen during subsequent exercise (Andrews et al., 2003; Coyle et al., 2001; Bosch et al., 1993; Galbo et al., 1979). Discrepancies in performance effects between studies have previously been attributed to the use of capacity versus time trial tests of performance (elciting an approximately 20% c.f. 2-3% improvement in performance; Hawley et al., 1997), and the duration of the exercise test, such that longer protocols (> 90 min) have been associated with larger performance gains (Hargreaves et al., 2004; Sherman et al., 1981).

The benefits of a high CHO diet (9 g·kg^{-1} body mass) on endurance performance appear to be negated when CHO is provided in sufficient quantities in the hours before and during exercise (Burke et al., 2000). In this study, muscle glycogen levels were significantly higher after CHO loading (572 ± 107 c.f. 485 ± 128 mmol·kg^{-1} dry weight during placebo), however, neither time taken to complete a 100 km cycling time trial, nor the time taken to complete a series of 1 or 4 km sprints during the trial, were improved. The authors suggested that the ingestion of both 2 g·kg^{-1} body mass before exercise and 1 g·kg^{-1} body mass per hour CHO during exercise was sufficient to maintain blood glucose levels even in the absence of CHO loading, and as such CHO availability was sufficient to sustain a required exercise intensity during prolonged exercise when CHO is consumed in the hours before, and during, prolonged exercise.

Relatively few studies have examined the effects of increased CHO intake on prolonged intermittent exercise performance similar to that observed during MSSs. As discussed previously, Saltin (1973) originally observed a higher total distance and percentage distance covered sprinting during a soccer match in participants who consumed a high CHO diet than those who consumed a normal diet following a previous soccer match three days earlier. Similarly, when participants consumed 10 g of CHO per kg body mass in the 22 h after prolonged intermittent shuttle running (i.e. the LIST), they were able to run 1.1 km farther in a subsequent exercise bout than when consuming an isocaloric diet providing only 5.4 g of CHO per kg body mass (Nicholas et al., 1997). Bangsbo (1992) investigated the effects of providing a high CHO (15 g·kg^{-1} body mass) versus a control diet (9.2 g·kg^{-1} body mass, a value nearly twice as high as that habitually consumed in the sample) on prolonged intermittent exercise performance

consisting of 46 min of intermittent field running followed by intermittent treadmill running to exhaustion in seven full time professional soccer players. When consuming the high CHO diet, participants were able to cover 17. 1 km, which was 0.9 km longer than that covered when the control diet was consumed. However, it should be noted that the total distance covered in this study is greater than that typically observed during a soccer game, and as such the applicability of these findings to MSSs may be questioned. Interestingly, it appears that CHO ingestion during exercise in addition to a high CHO diet induces greater performance increases in MSS activity than a high CHO diet alone. Foskett et al. (2008) provided participants with 10 g CHO per kg body mass in the two days before performing the LIST to exhaustion, followed by either consumption of a CHO-electrolyte solution (~ 90 g of CHO per hour) or a sweetened placebo consumed during exercise. Participants were able to exercise for approximately 21% longer when they consumed the CHO-electrolyte beverage, with no difference observed in muscle glycogen utilisation between trials. Instead, it was suggested that CHO intake during exercise maintained plasma glucose concentrations towards the end of the LIST, and thus provided a greater amount of CHO for muscle metabolism and central nervous function, therefore delaying fatigue.

2.3.2. CHO in the hours before exercise

A CHO rich meal (approximately 100-300 g) consumed 3-4 h before exercise has been shown to increase markedly muscle glycogen levels (by up to 45%) and increase CHO oxidation during exercise compared to the fasted state (Chryssanthopoulos et al., 2004; Wright et al., 1991; Neufer et al., 1987; Coyle et al., 1985), and in most cases improve endurance performance, particularly when performance is measured via a capacity test

(see Table 2.4). Only one published study to date has reported the effects of a pre-exercise CHO feeding in the hours before prolonged intermittent running performance. Little et al. (2010) provided participants with either a low or high glycemic index meal two hours before performing a 75 min high-intensity running protocol designed to simulate the demands of competitive soccer (Drust et al., 2000). Participants were then required to complete 15 min of repeated 1 min sprints in which they were instructed to go as fast as possible. It was reported that distance covered was higher during the repeated sprints when consuming either a low or high GI meal when compared to fasting for > 12 h (1,450 m cf. 1,500 m). CHO ingestion was also accompanied by higher muscle glycogen concentrations immediately before the repeated sprint test (350 cf. 200 mmol·kg^{-1} dry weight), with a tendency for reduced fat and increased CHO oxidation during exercise. The authors suggested that a CHO meal before prolonged MSS exercise provides greater exogenous CHO for energy provision during exercise, and thus spares muscle glycogen for use towards the end of performance.

Table 2.4. Effects of CHO feeding 3-4 h before endurance exercise

Study	Participants	Feeding	Effect
Chyssanthopoulos et al. (2002)	10 club level runners (male n = 10)	2.5 g CHO per kg body weight 3 h before exercise	9% increase in TTE at 70% VO_{2max}
Little et al. (2010)	16 soccer players (male n = 16)	1.5 g CHO per kg body weight 2 h before exercise	Approximate 3% increase in distance covered during repeated 1 min sprints
Neufer et al. (1987)	10 Cyclists (male n = 10)	200 g CHO 4 h before exercise	22% increase in work output for 15 min after 45 min at 77% VO_{2max}
Schabort et al. (1999)	7 moderately trained endurance cyclists (male n = 7)	100 g CHO 3 h before exercise	19% increase in cycle TTE at 70% VO_{2max}
Sherman et al. (1984)	10 recreational cyclists (male, n = 9, female n = 1)	315 g of CHO 4 h before exercise	15% increase in cycling TT after 95 min at 70% VO_{2max}
Whitley et al. (1998)	8 well-trained cyclists (male n = 8)	215 g CHO per 70 kg body weight	No change in 10 km TT
Williams & Chyssanthopoulos (1996)	Trained runners	2 g CHO 4 h before exercise	No difference in 30 km run TT
Wright et al. (1991)	9 cyclists (male, n = 8, female, n = 1)	5 g CHO per kg body weight 3 h before exercise	44% increase in TTE at 70% VO_{2max}

TT = Time Trial, TTE = Time to Exhaustion

Whilst CHO ingestion 3-4 h before exercise appears to improve endurance performance, the addition of CHO ingestion *during* exercise seems have an additive effect. Chryssanthopoulos et al. (2002) reported that, whilst a CHO meal consisting of 2 g of CHO per kg body mass consumed 3 h before exercise enhanced run time to exhaustion at 70% VO_{2max} by 9% compared to exercising in the fasted state, the additional ingestion of a 6.9% CHO solution during exercise (providing approximately

71 g of CHO) enhanced performance by 22%. Similarly, Wright et al. (1991) reported a 44% increase in endurance capacity when CHO was consumed before and during exercise, whilst CHO before or during exercise resulted in improvements of only 18 and 32%, respectively. Furthermore, whilst the optimal amount of pre-exercise CHO ingestion is yet to be adequately established, the amount of CHO ingested in this period is likely to have important implications for performance. When participants were fed either 45, 156 or 312 g of CHO 4 h before exercise, performance in a 45 min cycling time trial was enhanced by 9% in the 312 g CHO feeding only (Sherman et al., 1989). This improvement in performance was accompanied by a significantly greater amount of CHO oxidation (51%), and a better maintenance of blood glucose.

2.3.2.1 Glycemic index of the pre-exercise meal

The glycemic index (GI) provides a system whereby foods can be ranked by the magnitude and rate of increase in blood glucose and insulin that they elicit over a 2 h period (Jenkins et al., 1981). The GI score is calculated by dividing the incremental area under the blood glucose concentration-time curve by the area under the curve of a reference food (usually glucose or white bread). A higher GI indicates rapid absorption and delivery of CHO into the circulation, and is usually accompanied by a rapid and short-lived increase in circulating insulin levels (Wolever et al., 1991).

The effects of the glycemic index of meals consumed before exercise on subsequent performance are unclear, with some studies reporting improved endurance performance following the consumption of a low GI meal (Wong et al., 2008; Wu &

Williams, 2006), and others reporting no significant difference in performance following high or low GI CHO ingestion (Wong et al., 2009; Stevenson et al., 2005; Earnest et al., 2004). However, it does appear that the consumption of a low GI CHO meal results in increased fat oxidation compared to the ingestion of high GI meals (Wong et al., 2008; Stevenson et al., 2006; Wee et al., 2005), likely as a consequence of reduced plasma insulin concentrations (Stevenson et al., 2009). Still, whether this leads to altered substrate oxidation during subsequent exercise, particularly at a high intensity (> 70% VO_{2max}), is unclear (Wong et al., 2009; Stevenson et al., 2006; Stevenson et al., 2005). Furthermore, when CHO meals of different GI have been provided before prolonged intermittent exercise simulating MSSs, there appears to be no effect on either performance or substrate oxidation (Little et al., 2010; Erith et al., 2006). Nevertheless, there is some evidence that fat oxidation is increased during exercise following low GI CHO ingestion, with a subsequent sparing of muscle glycogen (Wong et al., 2008; Wee et al., 2005). Interestingly, the ingestion of CHO *during* exercise (~ 170 g) would appear to negate any metabolic or ergogenic effects of different glycemic index foods before performance (Wong et al., 2009; Burke et al., 1998).

2.3.3 CHO immediately before exercise

CHO ingested in the hour before exercise will often result in rapid hyperglycemia and hyperinsuliemia (Marmy-Conus et al., 1996), which is typically followed by a subsequent rapid decline in blood glucose concentration at the onset of exercise (Coyle et al., 1997; Marmy-Conus et al., 1996; Horowitz & Coyle, 1993). This 'rebound hypoglycemia' typically proceeds for 15-30 min (Febbraio et al., 2000b), and is independent of the quantity of CHO consumed (Jentjens et al., 2003). The fall in blood

glucose concentration in susceptible individuals is most likely to be due to a concomitant reduced liver glucose output in response to CHO ingestion-induced hyperglycaemia and hyperinsulinemia, and an increased muscle glucose uptake due to muscular contraction at the onset of exercise (Marmy-Conus et al., 1996). In addition, CHO feeding close to exercise has been shown to increase CHO oxidation (Febbraio et al., 2000b) and in some cases muscle glycogenolysis (Febbraio et al., 2000b), whilst lowering long chain fatty acid oxidation due to insulin-mediated reduced lipolysis and lipid oxidation during exercise (Horowitz et al., 2000; Coyle et al., 1997). However, the ingestion of CHO during exercise (~ 2g CHO per kg body mass) in conjunction with pre-exercise CHO feeding appears to offset the transient hypoglycaemia often observed with CHO feeding before exercise alone, whilst maintaining higher CHO oxidation rates in the latter stages of exercise (Febbraio et al., 2000a). As such, it seems prudent to consume CHO during exercise if CHO is consumed immediately before exercise to prevent symptoms of hypoglycaemia.

Despite the CHO-induced hypoglycaemia commonly observed following pre-exercise CHO ingestion, the majority of studies have reported either no (Jentjens & Jeukendrup, 2003b; Jentjens et al., 2003; Febbraio et al., 2000a; Sparks et al., 1998; Febbraio & Stewart, 1996; Hargreaves et al., 1987) or an enhanced effect (Sherman et al., 1991; Gleeson et al., 1986) of pre-exercise CHO ingestion on endurance performance. Furthermore, as with CHO ingestion several hours before exercise, there appears to be an additive effect of consuming CHO during, as well as immediately before, exercise. For instance, when participants were fed both 150 g of CHO before and during 120 min of steady state cycling (at 63% of peak power output), subsequent time trial

performance was significantly improved when compared to CHO intake before exercise or no CHO ingestion at all (Febbraio et al., 2000a). Moreover, there was no difference in time trial performance between the ingestion of CHO during and before exercise compared to ingestion of CHO during exercise only, suggesting that CHO ingestion during exercise is likely to have an important impact on endurance performance.

As with CHO ingestion 3-4 h before exercise, it appears that the glycemic index of CHOs ingested immediately before exercise has little or no influence on performance. Jentjens and Jeukendrup (2003b) provided participants with a 500 ml beverage containing 75 g of either glucose (high GI), trehalose (moderate GI) or galactose (low GI) 45 min before 20 min of steady-state exercise at 65% maximal power output followed by a 702 kJ time trial. Whilst both plasma glucose and insulin were significantly higher 15 min into the glucose trial, with four out of eight participants developing rebound hypoglycaemia, there was no significant difference in time trial performance between any of the three trials.

2.3.4 CHO during exercise

The early work of researchers in the late 1970s to 1980s initially demonstrated that CHO feedings during continuous exercise at 60-80% VO_{2max} and lasting 1-3 h could delay fatigue by up to approximately 60 min (Mitchell et al., 1988; Coggan & Coyle, 1987; Neufer et al., 1987; Coyle et al., 1986; Hargreaves et al., 1984; Coyle et al., 1983; Ivy et al., 1979). Over subsequent decades, the majority of studies have demonstrated that CHO ingestion improves both endurance capacity (Maughan et al., 1996; Tsintzas

et al., 1995; Wilber & Moffat, 1992; Wright, et al., 1991; Maughan et al., 1989) and time trial performance (Rollo & Williams, 2009; Angus et al., 2000; Tsintzas et al., 1993; Zachwieja et al., 1992; Mitchell et al., 1989) over a variety of exercise intensities lasting for > 45 min, such that the ergogenic effect of CHO ingested during exercise has become largely unquestioned (for a review, see Jeukendrup, 2004). However, whilst the continuous exercise intensity of these studies was often similar to that observed during MSSs (70 to 80% VO_{2max}), their protocols do not replicate the more intermittent nature and movement patterns of such sports.

2.3.4.1 CHO and prolonged intermittent exercise performance

There is a significant body of research demonstrating that the ingestion of CHO during prolonged intermittent exercise has an ergogenic effect when compared to a placebo control. Coggan and Coyle (1988) were amongst the first to demonstrate such an effect. During an approximate 210 min intermittent cycle alternating between 60 and 85% VO_{2max}, they provided seven participants with either 1 $g \cdot kg^{-1}$ body weight CHO (85% glucose polymer and 15% sucrose) after 10 min with 0.6 $g \cdot kg^{-1}$ body weight every 20 min thereafter, or equal volumes of a sweetened placebo. Coggan and Coyle (1988) observed that participants were able to maintain a significantly higher exercise intensity during the third hour of exercise during the CHO trial (2.74 ± 0.13 c.f. 2.29 ± 0.09 MJ), and participants were able to cycle for approximately 18% longer until fatigue and thus perform 19% more work in the trial. CHO feeding also resulted in an increased plasma glucose concentration (up to 6 $mmol.l^{-1}$) and a maintained CHO oxidation rate of up to 2 $g \cdot min^{-1}$. An improvement in late intermittent exercise performance with CHO ingestion was also confirmed in studies by Murray et al. (1987)

and Murray et al. (1989), who utilised intermittent cycling protocols with blocks of 15-20 min cycling at 55 to 65% VO_{2max} whilst ingesting CHO at 0.75 g·kg^{-1} body weight every hour followed by a 400 and 1200 revolution time trial. It was observed that participants were able to complete the time trial 12 and 5% quicker, respectively, when compared to placebo. Yaspelkis et al. (1993) were also able to show that the ingestion of 18 g·h^{-1} of CHO during 3.3 h of intermittent cycling at 45-80% VO_{2max} enabled participants to cycle for approximately 10% longer during a subsequent 80% VO_{2max} cycle to fatigue. As with previous observations, this study showed that CHO consumption produced a higher blood glucose concentration than a placebo condition (6.7 ± 0.7 c.f. 4.4 ± 0.1 mmol·l^{-1}), but additionally was the first to show that CHO provided in solid form (25 g·h^{-1}) resulted in a similar improvement in performance as the liquid supplement. CHO supplementation also resulted in a significantly higher muscle glycogen concentration after 3 h of exercise (79 ± 3.5 μmol·g^{-1} wet wt.) when compared to a placebo (58.5 ± 7.2 μmol·g^{-1} wet weight). The implications of the latter finding are clear, as muscle glycogen sparing and/or resynthesis has been implicated as a possible mechanism for enhanced performance with CHO supplementation during exercise.

In contrast, Nassis et al. (1998) demonstrated that exercise capacity at 90% VO_{2max} after 100 min running at speeds of 80, 85 and 90% VO_{2max} interspersed with slow running at 45% VO_{2max} was not improved with the ingestion of a 6.9% CHO-electrolyte solution. Whilst this finding was not explained, it is possible that the rate of ingestion of CHO in the study (35 g·h^{-1}) was too low to improve performance. Indeed, CHO ingestion rates of 60-70 g·h^{-1} are recommended to elicit maximal exogenous CHO oxidation

(Jeukendrup & Jentjens, 2000). Furthermore, Nassis et al. (1998) failed to observe an increase blood glucose concentration or CHO oxidation rate during exercise, suggesting sub-optimal delivery of CHO following ingestion. However, as previously mentioned, an ingestion rate as low as 18 $g \cdot h^{-1}$ has been shown to improve performance during intermittent exercise (Yaspelkis et al., 1993). Arguably, the use of a running protocol and high exercise intensities by Nassis et al. (1998) may have caused a slower rate of gastic emptying when compared to the cycling protocol of Yaspelkis et al. (1993), thus limiting CHO delivery (Brouns et al., 1987).

Thus, laboratory-based studies on intermittent exercise appear to show that CHO can improve late-exercise performance and capacity, regardless of whether CHO is consumed in solid or liquid form. However, such studies have not fully resembled the less rigid structure and range of intensities (including sprints) common to MSSs and have often assessed performance via subsequent constant load exercise, which again does not replicate the movements associated with MSSs.

2.3.4.2 CHO and MSS performance

Studies examining the influence of CHO intake during exercise on performance during actual MSSs are scarce. Indeed, to this author's knowledge, only one study has examined the influence of ingesting a CHO supplement during soccer match play. Leatt and Jacobs (1989) provided CHO in the form of a 7.5% glucose polymer solution at a rate of 35 $g \cdot h^{-1}$ during an outdoor friendly soccer match, and subsequently assessed blood glucose and muscle biopsy samples for glycogen content. It was demonstrated

that the ingestion of CHO resulted in a significantly lower change in muscle glycogen (111 ± 24 mmol·kg^{-1} dry muscle) compared to a placebo (181 ± 24 mmol·kg^{-1}), whilst blood glucose was not different between conditions. However, this study did not include any measures of performance, and as such did not provide any indication of whether CHO ingestion was ergogenic. The study was also subject to several methodological flaws. An independent groups design was adopted, such that five participants consumed CHO whilst five consumed placebo. As such, there was potential for muscle glycogen content to be affected by the two groups working at different rates. To this end, it would have been useful to incorporate time-motion analysis of individual players during the game. The authors also state that muscle biopsy samples were obtained 25-40 min following the game. Such a delay and large time window of sampling may have provided an opportunity for rapid post-exercise resynthesis of muscle glycogen with CHO ingestion (Ivy et al., 1988).

In a landmark study, Nicholas et al. (1995) examined the effects of consuming a 6.9% CHO-electrolyte solution on intermittent run time to fatigue (involving alternating 20 m runs at 55 and 95% VO_{2max}) following 75 min of running the LIST in 9 male MSS players. Consumption of the CHO-electrolyte solution resulted in participants being able to run for approximately 30% longer than when a sweetened placebo was consumed, although there was no effect on 15 m sprint performance throughout the LIST. There was a tendency for blood glucose values to be higher when CHO was consumed, thus potentially providing the working muscles with a greater supply of exogenous CHO for oxidation, whilst preserving muscle glycogen stores. Indeed, Nicholas et al. (2000) later demonstrated that the same CHO-electrolyte beverage

consumed during the LIST reduced muscle glycogen utilisation (in both type I and II fibres) by approximately 22%. Patterson and Gray (2007) have also observed that the ingestion of a CHO gel (providing 43 $g \cdot h^{-1}$) extended run time to fatigue at the end of the LIST from 4.2 ± 1.2 to 6.1 ± 1.3 min, and was accompanied by a significantly higher blood glucose concentration throughout and at the end of the test compared to a placebo condition (6.1 ± 1.1 c.f. 5.2 ± 0.6 $mmol.l^{-1}$). Two further studies, in which the primary aim was to examine the effects of CHO consumed with branched-chain amino acids (Davis et al., 1999) and chromium (Davis et al., 2000), have reported that time to exhaustion is enhanced by 52 and 32% respectively in part B of the LIST when CHO is consumed compared to placebo. Similarly, Foskett et al. (2008) reported that CHO-electrolyte ingestion at a rate of 1 $g \cdot kg^{-1}$ body weight every hour improved run time to fatigue at the end of the LIST by 17% compared to placebo, even when participants consumed a high CHO diet in the days before each condition. This improvement occurred in the presence of similar levels of muscle glycogen utilisation between trials, and thus enhanced performance was attributed to the higher plasma glucose concentrations observed when CHO was consumed during exercise. As with the Nicholas et al. (1995) study, sprint times were not different between trials.

Simulations of sports other than soccer have also reported favourable effects of CHO ingestion. Welsh et al. (2002) examined the influence of a CHO-electrolyte or placebo beverage on 60 min of intermittent exercise designed to replicate the competitive movement patterns of basketball (consisting of four 15 min quarters including walking, sprinting and running at 120% VO_{2max}) and a subsequent shuttle run to fatigue. Here, the ingestion of approximately 128 g of CHO resulted in a significant 37% increase in

run time to fatigue and improved 20 m sprint performance in the final 15 min of exercise along with blood glucose levels that were significantly higher with than the placebo condition. In a similarly study, Winnick et al. (2005) utilised a modified LIST (designed to more closely replicate basketball) consisting of 4 x 15 min blocks of variable exercise, including running at speeds corresponding to 55% and 120% VO_{2max} as well as sprinting, whilst consuming 41 $g \cdot h^{-1}$ of a CHO-electrolyte solution. Though endurance performance was not measured, the authors did report that 20 m sprint time (~2%) and average jump height (~4%) were significantly better in the fourth exercise quarter of the CHO trial.

In contrast, there has been a minority of studies which have reported that CHO ingestion during MSS exercise confers no benefit over a placebo. Morris et al. (2003) reported that ingestion of 0.26 $g \cdot kg^{-1}$ body weight of CHO every 15 min of the LIST (lasting 60 min) failed to improve either sprint performance or subsequent run time to exhaustion (running at 100% VO_{2max} for 60 s interspersed with 60 s rest). The lack of effect may be due to several reasons, not least that the exercise was performed in 30°C, and Morris et al. (2003) reported a significant trial order effect such that the distance covered after the LIST was 8,441 m in the final trial and only 6,839 m in the first trial. Thus, participants may have become acclimatised to performing in the heat (Sunderland et al., 2007), and as such any potential influence of CHO was masked. This may have been confounded further by a learning effect occurring with the time to exhaustion test, whose reliability was unknown. It is also possible that fatigue during the repeated high intensity (~100%VO_{2max}) may have been influenced by

phosphocreatine depletion (Glaister, 2005; Bogdanis et al., 1995), and as such the effect of CHO ingestion would have been less pronounced.

Abbey and Rankin (2009) also reported that CHO-electrolyte ingestion of 1 $g \cdot kg^{-1}$ did not improve sprint performance or prolonged shuttle running capacity following 75 min of intermittent running consisting of jogging at 55 and 120% VO_{2max} and sprinting. However, Abbey and Rankin (2009) did not observe a decline in performance during the placebo condition, suggesting that the protocol was not sufficient to induce fatigue typically observed in MSSs. Furthermore, blood glucose concentrations in the study were not significantly different between trials, suggesting there was an inadequate CHO delivery with the CHO supplement. The authors attributed this to the feeding being provided at the start and 45 min of exercise, as opposed to the ingestion every 15 min typically used in other studies. This may have some merit, as repeated feeding accelerates the rate of gastric emptying and the subsequent delivery of CHO to the intestine (Noakes et al., 1991).

In a study aiming to examine the effects of CHO and caffeine consumption on rugby union performance, Roberts et al. (2010a) also reported that the ingestion of 1.2 $g \cdot kg^{-1}$ body weight of CHO per hour did not result in improved performance in sustained high-intensity running or in 15 m sprint time recorded at 20 min intervals during 102 min of exercise. It is unclear why CHO exhibited no ergogenic effect, but the study was distinct in that it was the first of its kind to incorporate elements such as high intensity movements including contacts with tackle pads and simulated rucks. As such, fatigue in

this form of exercise may be due to factors which are unlikely to be influenced by CHO ingestion, including contact-induced damage and trauma to skeletal muscle (Smart et al., 2007).

In addition to enhanced endurance capacity, several studies have documented that CHO intake during exercise can enhance motor skills and mental function during MSS activity. In the study of Welsh et al. (2002), improvements in physical performance with CHO-electrolyte consumption were accompanied by significant improvements in the time taken to complete a whole body motor skill test (involving a pseudo hopscotch course in which participants were instructed to land on coloured/non-coloured squares with alternate feet) and the Stroop colour and word test (Golden, 1978) at the end of a simulated basketball match. More recently, Ali et al. (2007b) examined the influence of ingesting a 6.4% CHO-electrolyte solution on sprint, soccer shooting and passing performance (Ali et al., 2007a) following completion of the LIST. Participants were depleted of muscle glycogen stores in the evening before the LIST by completing a 75 min cycle at 70% VO_{2max} interspersed with five 50 s maximal sprints. Having consumed the CHO-electrolyte solution, they were better able to maintain shooting performance, whilst there was a non-significant (P = 0.13) trend for better maintenance of passing performance at the end of the LIST. Additionally, participants improved their 15 m sprint performance throughout the LIST when compared to the placebo condition. Currell et al. (2009) later reported that ingestion of a 7.5% maltodextrin solution enhanced dribbling, agility and shooting performance throughout a simulated soccer match relative to consuming a sweetened placebo. In contrast, Zeederberg et al. (1996) observed no benefit of ingesting a 6.9% CHO solution

on skill proficiency assessed via video analysis during actual soccer match play. However, it is likely that the extraneous variables associated with playing soccer in an open environment was a factor, as was the authors use of subjectivity in their assessment of the success of the skill performance indicators (unlike the validated skill tests developed by Ali et al. (2007a) whereby successful passes and shots were quantified according to reliable and valid criteria).

2.3.5 Mechanisms for the ergogenic effect of CHO

The precise mechanism(s) through which CHO ingestion improves exercise performance are not clear. However, theories have been posited, including the better maintenance of endogenous energy stores, enhanced blood glucose concentration and exogenous CHO oxidation and thus energy provision, and most recently, reductions in 'central' fatigue.

As previously alluded to, the ingestion of CHO during MSS exercise and the accompanying enhancement of performance have often coincided with a higher blood glucose concentration in the exercising participant than when water or placebo is consumed (Foskett et al., 2008; Patterson & Gray, 2007; Nicholas et al., 1995). Coyle et al. (1986) originally observed that CHO feeding during exercise at 70% VO_{2max} prevented the drop in blood glucose that was observed when water was consumed. In their study, blood glucose concentrations dropped to levels well beyond hypoglycemia ($\sim$ 2.5 $mmol \cdot l^{-1}$) at exhaustion following 3 h of exercise. Concurrently, CHO oxidation rates were maintained at approximately 2.2 $g \cdot min^{-1}$ in the CHO trial whereas they

dropped to 1.2 g in the placebo trial. These findings led to the proposal that CHO feeding resulted in greater CHO availability (via increased blood glucose) and improved CHO oxidation rates that would improve performance. This assertion was supported in a follow-up study, whereby participants exercised to fatigue at approximately 70% VO_{2max} causing hypoglycemia (~3.1mmol·l^{-1}) (Coggan & Coyle, 1987). Participants were then required to resume exercise 20 min later following either glucose infusion or ingestion of a placebo or glucose drink. Both glucose infusion and ingestion raised blood glucose concentrations (11 and 5.1 mmol·l^{-1} respectively) and improved time to fatigue when compared to placebo (43 and 26 min longer respectively). These early studies supported the idea that blood glucose was an important energy substrate during prolonged exercise. However, it is interesting to note that in the study of Coyle et al. (1988), participants who consumed CHO were still unable to continue exercise when blood glucose levels equalled 4.4 mmol·l^{-1}, and CHO oxidation rates (2 g·min^{-1}) were still relatively high, thus potentially challenging the hypothesis that CHO feeding improved performance via enhanced blood glucose concentrations. Indeed, subsequent studies have demonstrated that improved endurance performance is not associated with increased rates of CHO oxidation (Currell & Jeukendrup, 2008; Febbraio et al., 2000a). As such, the traditional view that improved performance with CHO ingestion is predominantly due to enhanced exogenous CHO oxidation may now be challenged.

Whilst blood glucose may be utilised for contracting skeletal muscle, it is also the predominant fuel source for the central nervous system (Dalsgaard & Secher, 2007). Accordingly, blood glucose concentrations may have important implications for the continued activation of skeletal muscle and perception of effort during exercise, and

indeed may explain the better maintenance of sprint performance and endurance capacity during MSS activity. Nybo (2003) demonstrated that the ingestion of a 6% glucose polymer solution providing approximately 70 $g \cdot h^{-1}$ of CHO enabled participants to maintain a significantly higher force (222 ± 20 N) during a 2 min MVC compared to the placebo condition (197 ± 21 N) following a 3 h cycle at 60% VO_{2max}. Blood glucose was reduced to hypoglycemic concentrations in the placebo condition (~ 3 $mmol \cdot l^{-1}$), whereas it was maintained when CHO was consumed during exercise. Interestingly, Nybo (2003) was able to ascertain the degree to which the observed decline in MVC could be attributed to reduced CNS activation by utilising the twitch interpolation technique, whereby an electrical stimulation was superimposed on to the participants' MVC. In the placebo trial, reduced force output was associated with a significantly lower voluntary activation of skeletal muscle of approximately 15% compared to the CHO condition. Voluntary activation was not significantly different from baseline compared to 3 h after exercise when CHO was consumed, supporting the supposition that euglycemia is important to maintain CNS function. Nybo (2003) also observed a significantly lower rating of perceived exertion (RPE) in the last hour of cycling with CHO ingestion (13 ± 1) compared to the placebo trial (16 ± 1).

In support of the Nybo's (2003) findings, and more specific to MSSs, Winnick et al. (2005) demonstrated that physical and CNS function were maintained when participants were fed a 6% CHO solution at regular intervals during four 15 min quarters of shuttle running at variable intensities ranging from 30-120% VO_{2max}. CNS function was assessed via the Stroop word test, Profile of Mood States, the measurement of force output during which participants were instructed to maintain

the sensation associated with 40% MVC, and performance in a motor-skills test requiring sprinting and changes of direction with specific foot placements throughout. CHO ingestion resulted in significantly reduced force sensations, enhanced motor skills and improved mood late in exercise compared to placebo, suggesting that CNS function was better maintained during MSS exercise with CHO supplementation. However, unlike Nybo (2003), Winnick et al. (2005) did not assess this directly, nor were measurements of blood glucose taken. Nevertheless, the potential for CHO to affect CNS function and perception of effort and subsequent MSS performance appears to be feasible. However, it should be noted that improvements both in sprint performance (Ali et al., 2007; Welsh et al., 2002) and endurance capacity (Patterson & Gray, 2007; Davis et al., 1999; Nicholas et al., 1995) during MSSs have often been reported when participants did not reach hypoglycaemia in the placebo trial. Furthermore, other studies have failed to find a relationship between blood glucose concentration and exercise performance (Felig et al., 1982; Horowitz & Coyle, 1993). Consequently, the potential contribution of hypoglycaemia to fatigue is not clear, and CHO during MSSs may exert its effect via other mechanisms.

In a follow-up study to their initial work demonstrating the ergogenic effect of CHO ingestion during the LIST (Nicholas et al., 1995), Nicholas et al. (1999) demonstrated that, compared to a placebo, consumption of a 6.9 % CHO-electrolyte reduced muscle glycogen utilisation by 22% when compared to placebo. The authors hypothesised that this was due to increased oxidation of exogenous CHO that spared muscle glycogen, hyperinsulinemia-induced increase of the pyruvate dehydrogenase complex and resynthesis of muscle glycogen during periods of low intensity exercise. Whilst

increased exogenous CHO oxidation may have contributed to the reduced muscle glycogen utilisation observed by Nicholas et al. (1999), it should be noted that only a small amount of ingested CHO (~ 15 g) is oxidised in the first hour of exercise (Jeukendrup et al., 1997), and thus endogenous CHO sparing is likely to be minimal until the latter stages of exercise. There is currently little evidence for CHO ingestion resulting in increased entry of pyruvate into the mitochondria, and the indirect evidence of a decreased lactate concentration observed during the LIST by Nicholas et al. (1999) has not been reported in similar studies (Patterson & Gray, 2007; Davis et al., 1999). As such, it is plausible that the resynthesis of muscle glycogen due to elevated plasma glucose and insulin concentrations (Kuipers et al., 1989) may well have contributed to the observed preservation of glycogen stores, and may be of particular importance in MSSs involving large periods of low-intensity activity.

The sparing and resynthesis of muscle glycogen with CHO ingestion may partially explain the observed increases in endurance capacity and sprint performance during MSS exercise (Philips et al., 2011). There is a well-established link between muscle glycogen availability at the onset of exercise and endurance capacity (Saltin, 1973), whilst muscle glycogen is the predominant fuel source during exercise > 70% VO_{2max} (Romijn et al., 1993). Thus, it is reasonable to assume that increased muscle glycogen availability would enhance intermittent exercise capacity of a similar intensity to MSS exercise. It has been shown that short-duration maximal-intensity exercise performance can be impaired when muscle glycogen levels reach a critically low level of < 200 $mmol \cdot kg^{-1}$ dry weight (Bangsbo et al., 1992a; Bangsbo et al., 1992b). Such values have been observed during simulated MSS exercise (Nicholas et al., 1999), and

in individual muscle fibres after a soccer match (Krustrup et al., 2006), and thus CHO supplementation and muscle glycogen sparing may offset reductions in performance owing to the level of glycogen depletion associated with this form of exercise.

A further potential mechanism for an ergogenic effect of CHO is via a central effect. Several studies have observed improvements in performance during exercise of high intensity (> 70% VO_{2max}) and lasting ~ 60 min when CHO is ingested (for a review, see Jeukendrup, 2004). This is surprising, as CHO availability was not thought to be a limiting factor for such exercise. In addition, there is a growing body of evidence that a CHO mouth-rinse can improve performance compared to a placebo (Jeukendrup & Chambers, 2010), suggesting that CHO does not only provide a metabolic advantage, but also a central affect which can modulate motor output. Whilst not yet fully understood, evidence is emerging that there are taste-independent oral CHO receptors that activate areas of the brain, such as the anterior cingulate cortex and ventral striatum, which may alter CNS function and improve exercise performance (Jeukendrup et al., 2008). To date, no study has examined the effects of a CHO mouth rinse on MSS performance, and the potential for a central effect of CHO on such exercise are unknown.

2.3.6 Optimal CHO consumption

Several factors have been suggested to influence exogenous CHO oxidation rates during exercise, including the type and amount of CHO consumed, feeding schedule and exercise intensity. These factors have previously been comprehensively reviewed

elsewhere (Jeukendrup & Jentjens, 2000), and more recently from a team sport perspective (Phillips et al., 2011). Accordingly, the key regulating factors for maximal CHO oxidation during exercise will briefly be highlighted here.

2.3.6.1 Timing of CHO ingestion

During continuous exercise, the timing of CHO ingestion appears to have little influence on rates of CHO oxidation. Indeed, when glucose is provided in a single dose of 100 g at the onset of exercise lasting approximately 90 min (Pirnay et al., 1977a; Pirnay et al., 1977b), CHO oxidation rates (0.4-0.6 $g.min^{-1}$) are similar to those reported when the same amount of CHO is provided with regular feedings (Moodley et al., 1992; Massicotte et al., 1989). This is in spite of evidence that regular CHO feedings accelerate gastric emptying and thus CHO delivery to the intestine (Noakes et al., 1991). Research demonstrating an ergogenic effect of CHO during MSS exercise has typically utilised regular CHO feedings at approximate 15 min intervals (Patterson & Gray, 2007; Nicholas et al., 1995), whilst Abbey and Rankin (2009) failed to show an effect on performance in the LIST when CHO was provided as a bolus at 45 min. In contrast, Clarke et al. (2008) reported that the metabolic responses to CHO provision at 15 min or 45 min intervals during the LIST were not significantly different, as evidenced by blood glucose and insulin concentrations and CHO and fat oxidation. Though, no performance measures were taken in this study, the authors did report a reduced sensation of gut fullness with regular CHO feedings. Therefore, frequent CHO intake during MSSs where possible may be considered prudent.

2.3.6.2 Amounts and types of CHO

Jeukendrup et al. (1999) showed that CHO oxidation during exercise does not increase in a linear fashion with CHO intake. During cycling at 50% VO_{2max}, participants consumed either a 4% or 22% glucose solution, providing 36 and 180 g·min^{-1} respectively. The CHO oxidation rate was approximately 0.6 g·min^{-1} in the 4% trial, but did not increase further than 0.9 g·min^{-1} in the 22% trial. Jeukendrup & Jentjens (2000) later composed a graph showing the relationship between CHO ingestion and CHO oxidation rate from numerous studies. Remarkably, it would seem that above an ingestion rate of approximately 1.2 g·min^{-1}, exogenous CHO oxidation does not increase beyond 1 g·min^{-1}. The authors suggested that CHO oxidation during exercise is likely to be limited by the rate of digestion, absorption and transport of glucose into the systemic circulation. Accordingly, recommendations for prolonged exercise are that 60-70 g·h^{-1} of CHO should be consumed. The optimal CHO ingestion rate during MSSs is yet to be established, however, studies reporting an ergogenic effect have typically used ingestion rates of 30-60 g·h^{-1} (Ali et al., 2007; Patterson & Gray, 2007; Welsh et al., 2007; Davis et al., 1999; Nicholas et al., 1995).

Several types of CHO have been provided during exercise in an attempt to determine their ergogenic properties in comparison to glucose, including fructose, sucrose and galactose, the disaccharides maltose and lactose and glucose polymers or maltodextrin. Of these, glucose, maltose, sucrose and maltodextrin can be oxidised at the highest rates (~ 1 g·min^{-1}; Hawley et al., 1992), with sucrose and fructose are oxidised at a lower rate (~ 0.6 g·min^{-1}; Leijssen et al., 1995; Massicotte et al., 1994). Interestingly,

the ingestion of multiple types of CHO may enhance exogenous CHO oxidation above that observed with single CHO ingestion (Jentjens et al., 2004a; Jentjens et al., 2004b), with rates of 1.7 $g \cdot min^{-1}$ reported for the combined ingestion of glucose and fructose (Jentjens & Jeukendrup, 2005). This has been attributed to the separate transport mechanisms across the intestinal luminal membrane for glucose and fructose (Shi et al., 1995), and thus a greater amount of exogenous CHO is made available for oxidation following ingestion. To the author's knowledge, there are no published studies examining whether such an increase in CHO oxidation can occur with the ingestion of multiple-CHOs during MSSs, or whether such supplementation is ergogenic.

2.3.6.3 Solution composition

Dehydration has been shown to impair sprint performance (McGregor et al., 1999) and skill performance (Edwards et al., 2007; McGregor et al., 1999) during MSS exercise, though this effect can be countered by fluid ingestion designed to limit or match the amount of fluid lost during exercise (McGregor et al., 1999). Consequently, it seems prudent that CHO feedings be provided in the form of a beverage during exercise, and that the composition of such beverages favours high fluid uptake and CHO oxidation. Euhydration can also be aided by the inclusion of electrolytes, and in particular sodium, in the beverage, which act to increase water absorption from the small intestine as well as the retention of fluid (Maughan & Murray, 2000; Nielsen et al., 1986). More specific recommendations on fluid intake are difficult, as factors such as body mass and composition, individual sweating rate and composition, environmental conditions and exercise intensity are likely to influence fluid requirements during exercise.

Earlier studies proposed that the osmolality of a CHO-electrolyte beverage was an important determinant of gastric emptying and subsequent fluid and CHO absorption (Coyle et al., 1978; Hunt & Pathak, 1960). However, it was subsequently demonstrated that when isocaloric CHO solutions with different osmolality are consumed, osmolality accounts for only a small proportion (~5%) of the variance in gastric emptying observed (Murray et al., 1994). As osmolality typically increases with increased CHO content, it is likely that CHO concentration is a more important mediator of gastric emptying. Indeed, it has been demonstrated that gastric emptying rates are similar when beverages of equal CHO content but different osmolality are consumed (Brouns et al., 1995). Indeed, there is an apparent negative correlation between CHO concentration and the rate of gastric emptying (Brouns et al., 1995; Calbet & MacLean, 1997).

Whilst not necessarily important for gastric emptying, osmolality and CHO type are thought to influence the rate of intestinal absorption of water and glucose, particularly when CHO concentration reaches > 8% (Gisolfi et al., 1992). Yet, the consumption of relatively high concentrations of glucose via glucose polymers such as maltodextrin, can increase glucose delivery as their osmotic load is typical low (Sole & Noakes, 1989). Nevertheless, the amount of CHO appears to be the most important mediator of exogenous CHO oxidation rate, as the consumption of equal CHO solutions with different osmolality and concentration does not influence exogenous CHO oxidation during steady-state exercise (Jandrain et al., 1989).

2.3.6.4 Exercise intensity

Exogenous CHO oxidation has been shown to increase in a linear fashion with increased exercise intensity. However, this relationship levels off at approximately 50% VO_{2max}, with no further increase in exogenous CHO oxidation at intensities > 60% VO_{2max} (Pirnay et al., 1982; Pimay et al., 1995). In addition, exercise above 80% VO_{2max} appears to reduce gastric emptying and absorption of glucose and water due to a reduced blood flow to the gut (Brouns & Beckers, 1993). Whilst continuous exercise cannot be maintained for a long enough period to be limited by CHO and fluid delivery, it is relevant for MSSs as such exercise requires intermittent periods of work that will be above 80% VO_{2max}. Indeed, Leiper et al. (2005) demonstrated that gastric emptying of a 6.4% CHO-electrolyte sports drink was significantly lower during 30 min of the LIST than during rest or steady state walking. This study raises the question of whether recommendations for maximal exogenous CHO oxidation are suitable for MSSs, and studies directly comparing different CHO-electrolyte fluid compositions for maximal CHO oxidation are warranted.

2.4. CHO-P ingestion during endurance exercise

Over the last decade, there has been much interest into the potential for a relatively small amount of protein (approximately 7-20 $g \cdot h^{-1}$), added to a classic CHO supplement, to further enhance performance beyond that typically seen with CHO ingestion alone. In one of the first of these studies, Ivy et al. (2003) compared the effects of ingesting a 200 ml bolus of 7.75% liquid CHO plus 1.94% protein supplement, providing approximately 47 g of CHO and 12 g of whey protein per hour, against a 7.75% CHO or

placebo solution during exercise. The nine cycle trained participants were required to cycle at varying exercise intensities of between 45 and 75% VO_{2max} for 3 h, followed by a cycle at 85% VO_{2max} until volitional fatigue. Ivy et al. (2003) reported that, whilst CHO ingestion significantly increased cycle time to fatigue when compared to placebo (19.7 ± 4.6 c.f. 12.7 ± 3.1 min), endurance capacity was a further 36% more with the addition of whey protein to the CHO supplement (26.9 ± 4.5 min). These findings were later supported by Saunders et al. (2004), who reported that the ingestion of a 7.3% CHO and 1.8% whey protein (providing 37 and 9 g of CHO and protein per hour, respectively) resulted in a significant 29% increase in cycle time to fatigue at 75% VO_{2max} (106.3 ± 45.2 min) when compared to ingestion of a 7.3% CHO solution (82.3 ± 32.6 min).

Whilst the above studies appeared to demonstrate that protein consumed during exercise provided a clear and relatively large ergogenic effect above CHO alone, subsequent studies have failed to confirm such an advantage, and thus the evidence is currently equivocal (see Tables 2.5 and 2.6 below). It is likely that several methodological differences in these studies can explain the majority of differing effects observed in the CHO-P literature. Perhaps most importantly, it should be noted that the large and significant effects observed in the research of Ivy et al. (2003) and Saunders et al. (2004) occurred when drinks of different *energy* content were provided during exercise, and thus supplements were only matched for CHO content. This, in conjunction with supplements providing CHO at a rate below the 60-70 $g \cdot h^{-1}$ considered optimal for maximal exogenous CHO oxidation (Jeukendrup & Jentjens, 2000), may have led to amino acids derived from the protein being directly oxidised to

provide energy for muscular contraction during exercise. Thus, there would be greater exogenous energy availability for performance when participants exercised with the CHO-P versus CHO beverage, and performance enhancement may not have been due to a protein specific mechanism. Indeed, when studies have matched beverages for *energy* and not *CHO* content, or provided optimal CHO ingestion rates, either a much smaller (2.4-12%) or no performance change has often been reported. However, such a finding is still of relevance, as it would seem that when exogenous CHO availability is sub-optimal, protein ingestion can provide a suitable replacement whilst potentially providing several recovery benefits discussed later in the thesis (section 2.5.3).

Table 2.5 Studies examining the effects of CHO-P versus CHO matched supplement

Reference	Sample	Protocol	Ingestion Rates*	%Improvement in Performance	Significant Difference
Breen et al. (2010)	12 M cyclists	120 min @ 55% VO_{2max}followed by 1 h TT	65 g·h^{-1} CHO + 19 g·h^{-1} P	0% **CHO-P** = 247 ± 13 W, **CHO** = 247 ±11 W	N
Lee et al. (2008)	8 M	TTE @ 70% VO_{2max}	60 g·h^{-1} CHO + 33 g·h^{-1} P	-7.5% **CHO-P** = 102.8 min, **CHO** = 110.6 min	N
Osterberg et al. (2008)	13 M cyclists	120 min @ 5% below OBLA followed by 7 Kj·kg^{-1} TT	75 g·h^{-1} CHO + 10 g·h^{-1} P	-4.4% **CHO-P** = 38.8 ± 5.5 min, **CHO** = 37.1 ± 3.8 min	N
Saunders et al. (2007)	13 cyclists (8 M, 5 F)	TTE @ 75% VO2peak	41 g·h^{-1} CHO + 10 g·h^{-1} P	13% **CHO-P** = 116.6 ± 28.6 min, **CHO** = 102.8 ±25 min	Y
Saunders et al. (2009)	13 M cyclists	60 km TT (performance measured in the last 5 km)	60 g·h^{-1} CHO + 16 g·h^{-1} P	3% **CHO-P** = 16.5 ± 0.6 min, **CHO** = 16.9 ± 0.6 min	Y
Valentine et al. (2008)	11 M	TTE @ 75% VO_{2max}	77.5 g·h^{-1} CHO + 19.4 g·h^{-1} P	7.4% **CHO-P** = 124 ± 8 s, **CHO** = 117 ± 6 s	N
Van Essen & Gibala (2006)	10 M cyclists	80 km TT	60 g·h^{-1} CHO + 20 g·h^{-1} P	0% **CHO-P** = 135 ± 9 min, **CHO** = 135 ± 9 min	N

M = male, F = female. TT = time trial, TTE = time to exhaustion. CHO = CHO. P = protein. *CHO beverages in these studies contained the amount of CHO indicated without protein

Table 2.6 Studies examining the effects of CHO-P versus a calorie matched CHO supplement

Reference	Sample	Protocol	Ingestion Rates	%Improvement in Performance	Significant
Martinez-Lagunas et al. (2010)	12 cyclists (7 M, 5 F)	150 min @ 55-75% VO_{2max} followed by TTE @ 80% VO2max	34.5 g·h^{-1} CHO + 8.8 g·h^{-1} P c.f. 46 g CHO	12% **CHO-P** = 30.5 ± .9 min, **CHO** = 26.9 ± 6.1min	N
Romano-Ely et al. (2006)	14 M cyclists	TTE @ 70% VO_{2max}	45 g·h^{-1} CHO + 11.3 g·h^{-1} P c.f. 60 g CHO	2.4% **CHO-P** = 98.1 ± 28.7 min, **CHO** = 95.8 ± 29.7 min	N
Toone & Betts (2010)	12 M cyclists/triathletes	45 min @ 60-90% VO_{2max} followed by 6 km TT	66 g·h^{-1} CHO + 24 g·h^{-1} P c.f. 90 g CHO	- 1.2% **CHO-P** = 438 ± 22 s, **CHO** = 433 ± 21 s	N
Valentine et al. (2008)	11 M	TTE @ 75% VO_{2max}	77.5 g·h^{-1} CHO + 19.4 g·h^{-1} P c.f. 86.9 g CHO	4% **CHO-P** = 124 ± 8 s, **CHO** = 120 ± 11 s	N

M = male, F = female. TT = time trial, TTE = time to exhaustion. CHO = CHO. P = protein.

A further criticism of the early studies by Ivy et al. (2003) and Saunders et al. (2004) is the use of exercise capacity tests to assess the effects of CHO-P ingestion. That is, such tests are considered to possess poor ecological validity as participants do not regulate their own exercise intensity, whilst their reliability is relatively poor when compared to that of time trials (CV ~ 26.6 and 3.49% respectively, Jeukendrup et al., 1996). It is also worthy of note that to-date no studies have examined the potential for CHO-P ingestion

during exercise to enhance MSS-like activity. Considering the lack of clarity on the effects of CHO-P and endurance, and some evidence that CHO-P may improve performance late on during exercise (Saunders et al., 2009), such an investigation would appear warranted, particularly when it seems clear that performance declines in the latter stages of MSSs.

2.4.1 Types and amounts of protein

Unlike the current understanding of optimal types and amounts of CHO to be consumed during exercise, little is known on what the most ergogenic form and amount of protein is, or indeed whether the discrepancies in findings between different studies are due to the types of protein being used (see Tables 2.5, 2.6 and 2.7). Previous studies have utilised whole proteins, such as whey and casein, as well branched chain amino acids (BCAAs) or hydrolysed whole proteins. Whilst not the focus of this review, early work which provided BCAAs during exercise has typically reported that there is no ergogenic effect on performance (Watson et al., 2004; Madsen et al., 1996; Van Hall et al., 1995; Varnier et al., 1994). Furthermore, some studies have reported increased plasma ammonia (NH_3) levels with BCAA supplementation (Watson et al., 2004; Madsen et al., 1996; Van Hall et al., 1995), which has been implicated in the aetiology of fatigue during prolonged aerobic exercise (Banister & Cameron, 1990). This increase in NH_3 is thought to be due to an increase in BCAA deamination during exercise for energy provision (MacLean et al., 1996). However, Colombani et al. (1999) reported that providing whole protein (milk protein hydrolysate) during a marathon run did not yield a significant rise in NH_3, despite observing a significant rise in plasma BCAAs with the ingestion of a CHO-P beverage. The authors suggested that the ingestion of whole

proteins would be likely to be advantageous, as such a supplement would not only provide NH_3 producing amino acids, but NH_3 binding amino acids, such as glutamate, alanine and glutamine. Accordingly, improved performance has only been reported in studies utilising whole proteins (whey isolate and casein hydrolysate) when combined with CHO (Table 2.7).

The precise type of protein most likely to elicit an ergogenic effect is unclear. Whey and casein, whilst both derived from milk, have been shown to have markedly different digestive properties. Whey is a soluble protein, whereas casein clots in the stomach, resulting in a slower rate of gastric emptying (Mahe et al., 1996). Consequently, it has been shown that whey ingestion elicits amino acid concentrations in the blood that are higher, but less prolonged, than when casein is ingested (Boirie et al., 1997). Due to this more rapid absorption of amino acids, whey has been the most popular form of protein used in endurance studies (see Table 2.7). However, studies have not reported a consistent benefit. More recently, Saunders et al. (2009) provided CHO with casein hydrolysate during endurance exercise, which is split into tri- and di-peptide bonds and thus results in a faster absorption of amino acids than intact protein and free amino acid ingestion (Grimble & Silk, 1989; Silk et al., 1985). Plasma amino acid concentrations were not reported in this study, although Saunders et al. (2009) did observe a significant 3% improvement in 60 km cycling time trial performance when the protein hydrolysate was consumed compared to CHO alone. However, recently Breen et al. (2010) reported no difference in 1 h time trial performance when hydrolysed whey was consumed with CHO, thus the effects of different forms of protein

ingestion are unclear. As such, studies which directly compare the use of different proteins during endurance exercise are warranted.

The ingestion rate of protein during endurance exercise has varied from approximately 8 – 33 g·h^{-1}, although this has been provided with varying amounts of CHO (see Tables 2.5 and 2.6). To date, no study has directly compared varying amounts of protein during endurance exercise, although significant effects have been typically observed with a protein ingestion rate of approximately 15 g·h^{-1} (see Tables 2.5 and 2.6). As CHO is the predominant energy source during endurance exercise, and CHO oxidation seems to be limited up to an intake of approximately 60-70 g·h^{-1}, it would seem prudent to provide CHO and protein at rates of approximately 40-60 and 10-20 g·h^{-1} respectively. However, the optimal rate of protein ingestion during endurance exercise is yet to be elucidated.

Table 2.7. Drink characteristics in CHO-P studies

Reference	Protein Source	Significant Difference
Breen et al. (2010)	Whey Hydrolysate	N
Greer et al. (2011)	BCAAs	N
Ivy et al. (2003)	Whole Protein (not specified)	Y
Lee et al. (2008)	Milk	N
Martinez-Lagunas et al. (2010)	Whey	N
Osterberg et al. (2008)	Whey	N
Romano-Ely et al. (2006)	Whey	N
Saunders et al. (2004)	Whey	Y
Saunders et al. (2007)	Not specified	Y
Saunders et al. (2009)	Casein Hydrolysate	Y
Toone & Betts (2010)	Whey Isolate	N
Valentine et al. (2008)	Whey Concentrate	N
Van Essen & Gibala (2006)	Whey Isolate	N

N = no, Y = yes.

2.4.2 Potential mechanisms for enhanced performance with CHO-P

The limited understanding of a mechanism through which protein ingested during exercise exerts its effect exacerbates the lack of consensus on the effects of CHO-P ingestion. As previously alluded to, one potential mechanism is through the provision of energy via protein oxidation. Whilst protein oxidation during exercise is relatively small (approximately 5-10% of total substrate oxidation) when compared to CHO and fats, its oxidation rate is increased with increasing exercise intensity (Babij et al., 1983)

and in the glycogen depleted state (Wagenmakers et al., 1991), and thus it may provide an important energy substrate. Indeed, several studies have reported that CHO-P ingestion enhances protein oxidation rates during exercise. Koopman et al. (2004) examined protein balance during 6 h of running and cycling at 50% VO_{2max} with either a CHO (0.7 g CHO·kg^{-1}·h^{-1}) or CHO-P solution (0.7 g CHO·kg^{-1}·h^{-1} and 0.25 g P·kg^{-1}·h^{-1}). Whole body protein turnover was assessed via the infusion, and subsequent collection via breath and blood, of leucine, phenylalanine and urea tracers. It was reported that protein oxidation was 32% higher when CHO-P was consumed (25 ± 2 mg protein·kg^{-1}·h^{-1}) when compared to CHO alone (17 ± 1 mg protein·kg^{-1}·h^{-1}). In agreement, Colombani et al. (1999) reported greater levels of plasma and urine metabolic by-products of protein metabolism such as urea, nitrogen and 3-Methylhistidine when a CHO-P beverage was consumed during a marathon, compared to the ingestion of CHO. Similarly, plasma amino acid availability has been reported to increase and decrease during a run at 75% VO_{2max} with CHO-P and CHO ingestion respectively (Miller et al., 2002). Thus, it could be hypothesised that ingested protein during exercise provides a fuel that potentially acts to spare the body's endogenous CHO stores (i.e. blood glucose and muscle glycogen), and that can be used to maintain a higher intensity during the latter stages of exercise. This being said, current evidence suggests that despite a potentially enhanced rate of protein oxidation, CHO-P ingestion does not alter the rate of either muscle glycogen utilisation (Cermak et al., 2009) or the maintenance of euglycemia (Toone & Betts, 2010; Cermak et al., 2009; Saunders et al., 2009; Valentine et al., 2008; Saunders et al., 2007; Van Essen & Gibala, 2006; Saunders et al., 2004).

A further possible explanation for improved performance with CHO-P ingestion is its elicitation of a greater insulin response during exercise, such as that sometimes observed with CHO-P intake during recovery (see section 2.5.3). If this were the case, it is possible that the increased circulating insulin would potentially act to improve CHO delivery across the muscle cell membrane via enhanced GLUT-4 translocation (Jentjens & Jeukendrup, 2003), which may subsequently provide more exogenous CHO for muscular contraction. Such an increased insulin release may be of particular importance during intermittent exercise such as MSSs, as it has been hypothesised that muscle glycogen resynthesis can occur during periods of low-intensity exercise and rest (Jeukendrup, 2004), thus providing extra substrate for muscular contraction. However, whilst amino acids are a potent stimulant for insulin release from the pancreatic beta-cells (Nuttall et al., 1984), and higher insulin values have been observed with CHO-P ingestion versus placebo (Saunders, 2007), most studies have failed to report a significant increase in serum insulin with CHO-P ingestion versus CHO alone during exercise (van Essen & Gibala, 2006; Ivy et al., 2003). That being said, van Loon et al., (2000) demonstrated that the composition of amino acids provided in a supplement can have a marked impact on the subsequent insulin response, and as such future studies investigating different types of protein supplement on exercise performance are justified.

CHO-P ingestion may also enhance performance through an attenuation of central fatigue in accordance with the 'central fatigue hypothesis', as originally proposed by Newsholme et al. (1987). This hypothesis is built on the common observation that during exercise there is an increased free fatty acid mobilisation from adipose tissue

and subsequent appearance in the blood for use as fuel for exercising muscle (Havel et al., 1967). As the mobilisation of FFA in the blood is greater than its rate of utilisation, blood FFA concentration steadily increases during exercise (Spriet, 2002). As both FFA and the amino acid free tryptophan (f-TRP) compete to bind to albumin for transportation in the blood, the increased FFA concentration results in a concomitant increase in circulating f-TRP (Curzon & Knott, 1974). This in turn causes an alteration in the f-TRP:BCAA ratio (i.e. a greater concentration of tryptophan relative to BCAAs) and an increase in the transportation of f-TRP across the blood brain barrier. This is thought to occur as both f-TRP and the BCAAs compete for entry into the central nervous system via the large neutral amino acid transporter (Hargreaves & Pardridge, 1988). In turn, it is predicted that f-TRP is converted to serotonin in the brain, a neurotransmitter that has been associated with increased feelings of tiredness and sleep and is an important determinant of mood and aggression (Meeusen et al., 2006; Cooper et al., 2003). Consequently, alterations in mood and perceived effort or fatigue may lead an athlete to lower their exercise intensity or stop exercise completely.

A CHO-P supplement has the potential to alter the chain events described above in different ways. Firstly, as with the ingestion of CHO alone during exercise, CHO and protein will lower the rate of FFA mobilisation through insulin-mediated reductions in lipolysis (Horowitz et al., 2000), and thus a lowered blood concentration of FFAs ensues. In addition, ingested protein has been shown to increase the concentration of BCAAs in the blood (Miller et al., 2002), thus further reducing the f-TRP:BCAA ratio. However, whether this in turn leads to lowered serotonin concentrations in the central nervous system and lowered feelings of fatigue is unknown and such a supposition is

purely speculative. Nevertheless, there have been several studies which have reported a significantly lower rating of perceived exertion with the ingestion of CHO-P versus CHO alone, in particular in the latter stages of exercise. In the study of Valentine et al. (2008), a significantly lower mean RPE (6-20) of 15.0 ± 1.8 versus 16 ± 1.4 was reported during the last 30 min of a cycle test to exhaustion at 75% VO_{2max} when CHO-P was consumed compared to CHO alone. Similarly, Martinez-Lagunas et al. (2010) reported that CHO-P ingestion resulted in a significantly lower average RPE (12.5 ± 0.4) when compared to CHO (12.8 ± 0.4) alone. Greer et al. (2011) also reported a significantly attenuated rise in RPE during a 90 min cycle at 55% VO_{2max} when CHO with added BCAAs was consumed compared to a placebo, whilst there was no reported significant difference between an isocaloric CHO beverage and a placebo.

Whether lowered RPE with CHO-P ingestion provides an indication of attenuated central fatigue is unclear, as a multitude of afferent inputs have been suggested to inform an individual's perception of effort, including heart rate, ventilation, substrate depletion and the anticipation of the remaining exercise intensity (for reviews, see Marcora, 2009; Tucker, 2009; Hampson et al., 2006). Moreover, lowered RPE with CHO-P ingestion is not a universal finding, with Toone and Betts (2010) reporting a significantly higher RPE, attributed to greater sensations of gastrointestinal discomfort, when protein was added to a CHO supplement. At present there is little scientific support for the central fatigue-hypothesis, with only a few studies reporting a lowered perception of effort with BCAA ingestion (Greer et al., 2010; Crowe et al., 2006), and others reporting no effect (Watson et al., 2004). Furthermore, whilst van Hall et al. (1995) demonstrated that BCAA ingestion lowered brain tryptophan uptake by

approximately 10%, they failed to find any negative effect of tryptophan ingestion on time to exhaustion at 70% of maximal power output, despite a concomitant 7-fold increase in brain tryptophan uptake. However, no perceptual measures of fatigue were recorded in this study.

Several authors have suggested that protein provided in a CHO beverage may provide amino acid precursors for anaplerotic reactions taking place in the Krebs cycle (Saunders, 2007; Ivy et al., 2004). Krebs cycle flux can increase more than 80-fold in the transition from rest to exercise, whilst a concomitant 5- to 10-fold increase in the concentration of Krebs cycle intermediates in skeletal muscle in humans has been observed (Wagenmakers, 1998). Thus, it is thought that an increase in Krebs cycle intermediates, and their synthesis via the conversion of carbon from amino acids such as glutamate (Wagenmakers, 1998), is necessary to maintain a high level of ATP resynthesis via mitochondrial respiration (Gibala et al., 1997). However, Cermak et al. (2009) recently reported that Krebs cycle intermediate (citrate and malate) expansion was not significantly different when CHO-P (223 ± 31 mmol·kg^{-1} dry weight) or CHO (185 ± 38 mmol·kg^{-1} dry weight) was consumed during a 90 min cycle at 70% VO_{2max}. Thus, as of now there appears to be little evidence of such a mechanism being responsible for any observed improvement in performance with CHO-P ingestion.

2.5 Recovery from MSSs

The high rates of energy utilisation and muscular fatigue observed during MSSs not only have implications for acute performance, but the subsequent recovery of

performance, as many consequences of participating in MSSs will have an effect for 48 - 72 h post-exercise. Considering the congested competitive and training schedules of many MSS athletes, who are often required to compete on consecutive days, the recovery of performance has become a key concern for applied sports scientists. Accordingly, the following section will discuss some of the key considerations for recovery following MSS competition.

2.5.1 Exercise-induced muscle damage

It is well documented that strenuous, unaccustomed exercise or exercise with an increased duration or intensity often results in ultrastructural damage to skeletal muscle (Byrne et al., 2004; Gleeson et al., 2003; Allen, 2001; Clarkson et al., 1992). The principle cause of this exercise-induced muscle damage (EIMD) is the eccentric muscle action (Clarkson et al., 1986) whereby the muscle is forcibly lengthened under tension with lower muscle unit activation than concentric contractions (Enoka, 1996). Indeed, EIMD has been shown to occur following activities with a high eccentric component or repeated stretch-shortening cycles such as plyometrics (Twist & Eston, 2005), resistance training (Byrne & Eston, 2002a; Paul et al., 1989) and distance running (Sherman et al., 1984). EIMD also occurs frequently in athletic populations, in particular during periods of overtraining or overreaching (Palazzetti et al., 2003; Eichner, 1995). However, of particular relevance to the current research is the now well-documented occurrence of symptoms of EIMD that occur following MSSs or exercise designed to simulate its physiological and movement demands (discussed in greater detail in section 2.5.1.3 below).

The symptoms of EIMD include the disruption of the intracellular muscle structure, sarcolemma and extracellular matrix, (Friden & Lieber, 2001a; Friden & Lieber, 1992; Hortobagyi et al., 1998), delayed-onset muscle soreness (DOMS; characterised by muscle pain and tenderness upon palpation which generally peaks at 24-48 hours and subsides within 96 hours; Friden, 2002; Cleak & Eston, 1992a; Cleak & Eston, 1992b), muscle swelling and a decreased range of motion, (Clarkson et al., 1992; Cleak & Eston, 1992a), and a leakage of intramuscular proteins such as creatine kinase (CK), myoglobin (Mb) and lactate dehydrogenase (LDH) into the blood (van der Meulen, Kuipers & Drukker, 1991; Clarkson et al., 1986). However, perhaps of greatest importance to sports performers is the commonly observed long-lasting impairment of muscle function (i.e. reductions in strength and power) and the resulting changes in performance associated with EIMD. Furthermore, a review of the literature on markers of EIMD has identified that measures of muscle function provide the most effective means for evaluating the magnitude and time-course of damage (Warren, Lowe & Armstrong, 1999).

2.5.1.1 Mechanisms and time course of EIMD

The mechanisms by which EIMD impairs muscular function are not yet fully understood, and a full review is beyond the scope of this thesis (for reviews, see Proske & Morgan, 2001; Armstrong et al., 1991). As such, a brief overview of the key phases of EIMD will be presented.

EIMD is thought to be caused predominantly by mechanical stress on the muscle sarcomere and connective tissue, though metabolic stress has also been implicated in the aetiology of EIMD. From a mechanical perspective, it is proposed that repeated eccentric actions results in non-uniform lengthening of sarcomeres when active muscle is stretched beyond its optimum length (i.e. during an eccentric contraction), with some sarcomeres rapidly over-extending beyond myofilament overlap and failing to re-interdigitate upon relaxation (Proske & Morgan, 2001). This has been termed the 'popping-sarcomere hypothesis'. Biopsy samples from human muscle have indeed shown non-uniform registration of z-lines, termed 'z-line' streaming, following EIMD (Lieber & Friden, 2002; Friden & Lieber, 2001a). Thus, it appears that repeated eccentric actions result in permanently weakened or overstretched sarcomeres which alter the contractile properties of the muscle. Additionally, EIMD is associated with the early loss of cytoskeletal proteins that are responsible for sarcomere stability and the transmission of force both across a single fibre and from fibre to fibre. Indeed, researchers have reported losses in desmin (Lieber et al., 1996) and distrophin (Lovering & De Deyne, 2004) immediately after eccentric exercise associated with significant reductions in force generating capacity.

Whilst the precise time-course is unclear, the initiation of EIMD is also associated with impairments in E-C coupling. E-C coupling is the physiological mechanism whereby an electrical discharge at the muscle initiates chemical events that ultimately cause muscle action, starting with the action potential along the plasmalemma and ending with the release of calcium from the sarcoplasmic reticulum (Ingalls et al., 1999). Support for a reduction in efficiency of the E-C coupling system in humans has been shown through

the observation of greater force loss when muscle is stimulated at low-frequencies (Allen, 2001; Jones, 1996), and maximally activated tetanic force when compared to caffeine activated (which induces Ca^{2+} release from the sarcoplasmic reticulum) force production (Ingalls et al., 1999).

EIMD is also thought to be initiated by metabolic events which cause either metabolic deficiencies or by-products that directly damage the skeletal muscle tissue or make individual muscle fibres susceptible to damage. More specifically, prolonged endurance exercise may result in insufficient mitochondrial respiration (Tee et al., 2007), and ultimately lowered ATP in muscle fibres which may decrease the action of Ca^{2+}-ATPase in the sarcoplasmic reticulum and compromises the removal of cytosolic Ca^{2+} (Duchen et al., 1990). This in turn would result in proteolysis and muscle fibre degeneration (Duncan, 1987). Such an effect may be exacerbated in the presence of depleted muscle glycogen stores which may limit ATP resynthesis. Indeed, Warhol et al. (1985) reported that damage was confined to fibres that were almost completely depleted of glycogen following a marathon. This has implications for MSSs, where it has been observed that competition results in 50% of individual muscle fibres being either completely or nearly depleted of glycogen (Krustrup et al., 2005). Free radical, or reactive oxygen species (ROS), production has been implicated in metabolically-induced EIMD. Elevated oxygen consumption for a prolonged period produces ROS (Knez, et al., 2007), which are molecules with an unpaired electron in their outer valence shell. These molecules are highly reactive, and may cause lipid peroxidation in the muscle cell membrane and subsequent impairments in Ca^{2+} handling in the sarcoplasmic reticulum and damage to many cellular components (Best et al., 1999).

Following the initial damaging event, skeletal muscle is thought to undergo an autogenic phase whereby calcium homeostasis is disturbed (Armstrong, 1990). Ultimately this is thought to result in calpain (a non-lysosomal protease) activation (Belcastro et al., 1998) and ensuing proteolysis of key contractile and structural proteins, such as titin and desmin, and thus sarcolemmal stability is further reduced (Verbeurg et al., 2005; Goll et al., 1991). This cycle in turn damages the muscle such that large muscular proteins leak into the blood. Subsequently, it is thought that apoptosis of the muscle fibre ensues and an inflammatory response and muscle tissue repair is initiated (Pyne, 1994). This stage involves the selective breakdown of muscular proteins and their subsequent resynthesis via inflammatory cells such as neutrophils and macrophages, ROS and finally satellite cells (Tidball, 2002). The process is thought to take approximately seven days, but is dependent on the severity of the damaging event (Clarkson & Hubal, 2000).

2.5.1.2 Applied implications

Studies utilising eccentric exercise such as plyometrics, downhill running and resistance exercise have shown that EIMD causes significant immediate and prolonged impairments in isometric (Larsen et al., 2005; Sayers & Clarkson, 2001; Child et al., 1998; Clarkson et al., 1992; Cleak & Eston, 1992a) and isokinetic (Sherman et al., 2004; Michaut et al., 2002; Deschenes et al., 2000; Byrne et al., 2000; Eston et al., 1996; Gibala et al., 1995; Golden & Dudley, 1992; Friden et al., 1983) strength by approximately 20%. Such impairments typically peak at 24-48 h, and last for up to one week. In turn, this alteration in muscle function has been shown to impair several performance measures in the days after exercise which have implications for MSS athletes, including

reduced vertical jump performance (Marginson et al., 2005; Byrne & Eston, 2002a; Komi, 2000; Avela et al., 1999; Horita et al., 1999; Chambers et al., 1998), power generating ability (Twist & Eston, 2005; Byrne & Eston 2002b), single and repeated sprint performance (Highton et al., 2009; Twist & Eston, 2005), agility (Highton et al., 2009) and endurance capacity (Twist & Eston, 2009; Marcora & Bosio, 2007). However, EIMD response can be affected by the mode of damaging exercise (Tee et al., 2007; Vickers, 2001), and therefore it is important to examine the specific muscle damage response associated with MSS exercise.

2.5.1.3 EIMD and MSSs

Table 2.8 below provides a brief summary of the documented responses to MSS competition, including a variety of sports. These studies appear to demonstrate that such exercise consistently leads to EIMD which may persist for up to 72 h. This is not surprising considering the high aerobic demand of MSSs, and the repeated eccentric contractions that are likely to occur during sprints, changes of direction, jumping and accelerating/decelerating (Osgnach et al., 2010). However, it is interesting to note that the majority of studies have utilised CK as a marker of EIMD, despite its poor temporal relationship with skeletal muscle function (and therefore muscular damage) following eccentric exercise (Friden & Lieber, 2001b). This apart, several studies have reported alterations in muscle function, jump height and sprint performance of between 2 and 18% following MSSs (McLellan et al., 2011; Fatouros et al., 2009; Rowsell et al., 2009; Magalhaes et al., 2009; Ascensao, et al., 2008), which are similar to those observed following eccentrically biased exercise detailed previously.

Table 2.8 Muscle damage response to MSS competition

Study	Sample	Sport	EIMD	Notes
Ascensao et al. (2008)	16 M Semi-elite	Soccer match	↑ Mb, CK, DOMS, ↓ Isok strength (10%), 30 m sprint speed (7%)	EIMD markers present until 72 h post-exercise
Cunniffe et al. (2002)	10 M Elite	Rugby Union Match	↑ CK	EIMD markers present until 38 h. T/C sig. lowered 14 h after exercise
McLellan et al. (2011)	17 M Elite	Rugby League	↑ CK, ↓ PP during CMJ (18%)	Up to 48 h
Montgomery et al. (2008)	29 M Elite	3 d basketball tournament	↑ Mb, CK, DOMS	
Rowsell et al. (2009)	20 M Junior	Soccer	↑ CK, LDH, Mb, ↓ 12 x 20 m sprint speed (2%) & CMJ (7%)	No effect of cold water immersion
Smart et al. (2008)	23 M Elite	Rugby Union	↑ CK	CK sig correlated to game time and number of contacts
Fatouros et al. (2009)	30 M Semi-elite	Soccer	↑ CK, DOMS, ↓ 20 m sprint speed (2%) & CMJ (10%)	
Takarada (2003)	15 M Amateur	Rugby Union	↑ CK, LDH, Mb	Sig related to contacts
Magalhaes et al. (2009)	16 M Semi-elite	Soccer	↑ CK, Mb, ↓ Isok strength (7%), 20 m Sprint speed (10%) & CMJ (11%)	
McLellan et al. (2010)	17 M Elite	Rugby League	↑ CK	

M = Male, d = day, CK = Creatine Kinase, Mb = Myoglobin, LDH = Lactate dehydrogenase, Isok = Isokinetic, Isom = Isometric, DOMS = delayed onset muscle soreness, CMJ = counter-movement jump, T/C = testosterone to cortisol ratio, PP = peak power.

Several studies have also demonstrated that participation in the LIST results in EIMD similar to that observed after MSS competition. Thompson et al. (1999) reported a significant increase in the intensity of general muscle soreness among 16 habitually active males in the 72 hours following the LIST, peaking at 24-48 h. Peak increases in CK were also observed at 24 h post exercise. Interestingly, muscle soreness was most

frequently reported in the hamstring muscles. Thompson et al. (2001b) later observed significant decreases post-LIST in muscular function of the knee flexors (but not extensors) up to 48 h post-exercise in nine habitually active males. This was the case for both isometric and isokinetic contractions at 60 deg·s^{-1}. As with their earlier study (Thompson et al., 1999), both CK and muscle soreness were elevated for up to 72 hours following the LIST. Finally, Bailey et al. (2007) reported that muscle soreness, plasma CK activity and myoglobin concentration were significantly elevated up to 48 h following completion of the LIST in 20 healthy males. Significant reductions in isometric peak torque of the knee flexors (but not the knee extensors) and vertical jump performance of 18% and 6%, respectively, were also observed.

To conclude, it would appear that participation in a MSS, or a protocol designed to simulate the demands of MSSs, produces the same symptoms of EIMD commonly observed following eccentric exercise. These symptoms last for up to 72 hours post exercise, with impairments of muscle function peaking at 48 h and being primarily located on the knee flexors. As such, a key consideration in the recovery of performance for coaches and athletes needs to incorporate the prevention and/or treatment of exercise-induced muscle damage.

2.5.2 Muscle glycogen depletion and fluid loss

Section 2.2.3.2 has highlighted the importance of muscle glycogen depletion and dehydration to fatigue *during* MSS exercise, and how CHO-electrolyte solutions may exert their ergogenic effect via an attenuation of such factors, respectively. However,

considering that muscle glycogen levels can be depleted by 40-90%, and fluid losses can total 4-5 l following MSS competition, the rapid restoration of each is considered an important *recovery* consideration to ensure optimal performance in a subsequent exercise bout. Indeed, the restoration of muscle glycogen may require particular attention as part of post-MSS recovery strategy, since EIMD has been shown to slow this process (Asp et al., 1997; Asp et al., 1995; Costill et al., 1990; Sherman et al., 1983). To this end, Bangsbo et al. (2006) demonstrated that muscle glycogen levels did not reach pre-exercise levels 42 h after a soccer match, even when participants were fed a high CHO (~600 g) diet. Similarly, Jacobs et al. (1982) reported that muscle glycogen concentrations were only 50% of baseline values 48 h following a competitive match.

2.5.3 CHO and protein ingestion for recovery

Acute CHO-P ingestion both during and after exercise has been proposed to attenuate EIMD, increase rates of muscle glycogen resynthesis and improve fluid balance. Consequently, such a supplement may be particularly valuable as a recovery aid following MSS competition. Accordingly, the following section will discuss the effects of CHO-P on post-exercise recovery.

2.5.3.1 EIMD

A multitude of different recovery strategies has been employed to try and attenuate EIMD, each with limited success (for a review, see Howatson & van Someren, 2008). These strategies, including antioxidants, cryotherapy, b-hydroxy-b-methylbutyrate supplementation, massage, compression garments, and non-steroidal ant-inflammatory

drugs, have sought predominantly to dampen the inflammatory response associated with EIMD, and have typically been utilised following resistance or eccentrically biased exercise. Indeed, a relative paucity of data on EIMD recovery strategies exists following MSS competition, often with conflicting results. Thompson et al. (2001b) reported that acute ingestion of the antioxidant vitamin C, proposed to counter the effects of ROS, had no effect on CK or muscle soreness in the days after completing the LIST. As with eccentrically biased exercise, Montgomery et al. (2008) demonstrated that neither compression garments nor cryotherapy via cold water immersion affected plasma CK or Mb in the days following a 3 day basketball tournament. In contrast, Gill, Beaven and Cook (2005) observed that CK activity was significantly higher in a group of players who performed passive recovery than groups who underwent contrast-water therapy or wore a compression garment in the days following a rugby union match. However, the usefulness of such findings are questionable considering the large variations observed in CK response to EIMD (Clarkson & Ebbeling, 1988), and lack of functional measures to assess recovery. Rowsell et al. (2009) demonstrated that cryotherapy had no effect on CK, counter-movement jump height and sprint performance 22 h after a soccer match, although perceived soreness was significantly lower when participants underwent cold-water immersion. Thus, cryotherapy may counter some of the negative perceptual responses associated with EIMD following MSSs. Indeed, Bailey et al. (2007) observed muscle soreness was significantly lower at 24 and 48 h, whilst plasma CK was not different, following the LIST when participants underwent cold water immersion.

2.5.3.1.1 CHO-P and EIMD

Over recent years, the potential for the ingestion of a small amount of protein in combination with CHO to attenuate EIMD and accelerate recovery has received considerable interest. Table 2.9 provides a summary of the findings in this area.

Table 2.9. Summary of studies examining the effects of CHO-P on markers of exercise-induced muscle damage.

Study	Sample	Exercise	Protein amount	Protein type	Ingestion Time	Effects
Baty et al. (2007)	34 M	Whole body RE (3 x 8 8-RM)	13.5 g	Milk-based	Before, during & after	↔ Strength ↓ CK, Mb
Betts et al. (2009)	17 M	90 min intermittent run	0.4 g·kg·h^{-1}	Whey isolate	During & 4 h after	↔ Isometric strength, CK, LDH, soreness
Bird et al. (2006)	32 M	3 x 10 75%-RM	6 g	EAAs	During	↓ 3-MH
Breen et al. (2010)	12 M Cyclists	120 min @ 55% VO_{2max} + 1 h TT	0.27 g·kg·h^{-1}	Whey Hydrolysate	During	↔ MVC, soreness, CK
Cockburn et al. (2008)	24 M	6 x 10 eccentric-concentric contractions	0.2 g·kg·h^{-1}	Milk-based	2 h after	↔ Soreness ↓ CK, Mb ↑ MVC
Ferguson-Stegall et al. (2010)	8 M & 7 F	3 h cycle @ 45-70 VO_{2max}	Not specified	Whey isolate	During	↓ Mb
Gilson et al. (2010)	13 M	4 day intensified training	0.3 g·kg·h^{-1}	Milk-based	1 h after	↔ MVC ↓ Mb, CK
Green et al. (2008)	18 F	30 min downhill running	0.3 g·kg·h^{-1}	Whey	1 h after	↔ MVC, soreness, CK
Luden et al. (2007)	23 M	6-day cross-country training	0.365 g·kg·h^{-1}	Whey	During	↓ Soreness, CK

Millard-Stafford et al. (2005)	8 M Runners	21 km run	0.2 g·kg·h^{-1}	Whey isolate	During	↓ Soreness ↔ CK
Pritchett et al. (2009)	10 M Cyclists	6 x 3 x 30 s WAnT	0.25 g·kg·h^{-1}	Milk-based protein	2 h after	↔ Soreness, CK
Romano-Ely et al. (2006)	14 M Cyclists	TTE @ 70% VO_{2peak}	0.39 g·kg·h^{-1}	Whey	During and 15 min after	↓ Soreness, CK, LDH
Rowlands et al. (2007)	10 M Cyclists	150 min intermittent cycling	0.3 g·kg·h^{-1}	Whey isolate, casein & soy	4 h after	↓ Soreness, CK
Saunders et al. (2004)	15 M Cyclists	TTE @ 75% VO_{2peak}	0.18 g·kg·h^{-1}	Whey	During and 30 min after	↓ CK
Saunders et al. (2007)	8 M & 5 F	TTE @ 75%VO_{2peak}	0.18 g·kg·h^{-1}	Not specified	During and 1 h after	↓ CK
Saunders et al. (2009)	13 M Cyclists	60 km TT	0.25 g·kg·h^{-1}	Casein hydrolysate	During and 1 h after	↓ Soreness, CK
Seifert et al. (2005)	9 M & 9 F	Skiing	0.17 g·kg·h^{-1}	Not specified	During	↓ Mb, CK
Skillen et al. (2008)	12 M Cyclists	TTE @ 85%VO_{2peak}	0.07 g·kg·h^{-1}	EAAs	Before, during and 15 min after	↓ Soreness, CK ↑ VJ
Stock et al. (2010)	17 M	6 x 6 squats @ 75% 1-RM	0.025 g·kg·h^{-1}	Leucine	15 min after	↔ LDH, CK, soreness
Valentine et al. (2008)	11 M Cyclists	TTE @ 70%VO_{2peak}	0.26 g·kg·h^{-1}	Whey concentrate	During	↓ Mb, CK ↑ Reps @ 70% 1-RM
White et al. (2008)	27 M	50 Eccentric contractions	0.28 g·kg·h^{-1}	Whey concentrate	1 h after	↔ MVC, CK, soreness
Wojcik et al. (2001)	27 M	100 x 120% 1-RM eccentric contractions	0.17 g·kg·h^{-1}	Not specified	2 h after	↔ Isokinetic strength, 3-MH

M = males, F = females. TTE = time to exhaustion, TT = time trial, RE = resistance exercise, RM = repetition maximum. EAAs = essential amino acids. MVC = maximal voluntary contraction, PT = peak torque, CK = creatine kinase, LDH = lactate dehydrogenase, Mb = myoglobin, VJ = vertical jump height.

Table 2.9 shows that that the effects of CHO-P ingestion on EIMD are currently equivocal. Fifteen studies have reported attenuation in at least one marker of EIMD, however it is notable that only 10 studies have measured strength. Of these, only three have reported lower strength loss or enhanced recovery following the ingestion of CHO-P. The reasons for different findings between studies are difficult to elucidate, however it is likely that variations in the quantity, type and timing of ingested protein, matching of CHO-P supplements with an equal CHO or energy content for comparison, mode of damaging exercise and measurement method of EIMD have confounded findings in the area. These factors are discussed in greater detail in Chapter 4 of this thesis.

Only one study has examined the effects of acute CHO-P ingestion following MSS exercise. Betts et al. (2009) reported that the ingestion of either 1.2 g·kg·h^{-1} of CHO or the same solution plus 0.4 g·kg·h^{-1} whey protein isolate in the 4 h after the LIST resulted in similar losses of peak isometric torque of the knee extensors (8%) and flexors (30%), and increases in serum CK, Mb and LDH. However, this study failed to match the drinks provided for energy content, and as such it is yet to be seen whether and iso-caloric volume of protein can replace CHO to attenuate markers of EIMD after MSS exercise. Furthermore, it would have been useful to see if measures of muscle function more closely related to athletic performance than isometric strength, such as sprint and jump performance, were affected by CHO-P ingestion compared to CHO alone.

The precise mechanism through which CHO-P ingestion may affect EIMD is currently not known, however it is hypothesised higher rates of muscle protein degradation than synthesis limit the functional restoration rate of skeletal muscle tissue following EIMD (Ingalls et al., 1998). CHO-P has the potential to increase muscle protein synthesis and/or reduce protein degradation after injury (Lowe et al., 1995). This may occur via two mechanisms. Firstly, both CHO and protein ingestion will increase circulating insulin as previously discussed. Indeed, several studies have reported a greater plasma insulin concentration with CHO-P ingestion compared to CHO alone after exercise (Betts et al., 2008; Betts et al., 2007; Betts et al., 2005; Jentjens et al., 2001; Zawadzki et al., 1992), although others have failed to report such an effect (Millard-Stafford et al., 2005; Ivy et al., 2003; Ivy et al., 2002; Tarnopolsky et al., 1997). This discrepancy is thought to be due to the lower protein ingestion rates in the latter studies (~0.1 g·kg·h^{-1}), and as such an ingestion rate closer to that used in the former studies (~0.3 g·kg·h^{-1}) protein has been recommended (Betts & Williams, 2010). Insulin has been shown to stimulate protein synthesis *in vitro* (Lawrence, 2001), although *in vivo* insulin concentrations within the normal physiological range reduce net muscle protein breakdown only (Wolfe, 2001). Nevertheless, hyperinsulinemia induced by CHO-P ingestion may enhance protein balance by reducing protein breakdown after exercise.

Secondly, it is thought that CHO-P ingestion will provide amino acids, which serve as building blocks for muscle protein resynthesis, as well as activating several signalling pathways responsible for the up-regulation of protein synthesis (Koopman et al., 2007; Hawley et al., 2006). In fact, whilst CHO ingestion elicits an insulin response that reduces protein breakdown after exercise, net positive protein balance (greater rates of

protein synthesis than degradation) only ensues in the presence of an adequate supply of amino acids (Levenhagen et al., 2002). Furthermore, several studies have reported better protein balance when protein is co-ingested with CHO compared to CHO alone (Bird et al., 2006; Borsheim et al., 2004; Koopman et al., 2004; Miller et al., 2003). Interestingly, ingestion of protein immediately after exercise yields protein synthesis rates three-fold greater than those observed when it is ingested 3 h after exercise (Levenhagen et al., 2001), suggesting that it is important to commence feeding immediately after exercise to maintain protein balance.

2.5.3.2 CHO-P and muscle glycogen

2.5.3.2.1 CHO and glycogen resynthesis

A high daily CHO intake can significantly increase muscle glycogen stores (see section 2.3.1), and as such this strategy can be adopted to resynthesise muscle glycogen in the days after MSSs (Nicholas et al., 1997). However, the immediate hours after exercise appear to provide an opportunity for enhanced rates of muscle glycogen resynthesis due to increased insulin sensitivity to glucose uptake (Cartee et al., 1989). This is thought to be mediated by an increased GLUT-4 translocation to the muscle cell surface that can last for approximately 4 h (Hansen et al., 1998). Accordingly, muscle glycogen resynthesis rates during short term recovery are typically much higher with CHO ingestion (approximately 20-50 $mmol \cdot kg^{-1}$ dry $weight \cdot h^{-1}$) compared to when no CHO is consumed (7-12 $mmol \cdot kg^{-1}$ dry $weight \cdot h^{-1}$) (Jentjens & Jeukendrup, 2003). Indeed, Jeuekendrup and Jentjens (2003), and later Betts and Williams (2010), collated data from studies examining CHO ingestion and muscle glycogen resynthesis rates after

exercise, and demonstrated a linear relationship between the amount of CHO ingested and glycogen resynthesis, with maximal synthetic rates occurring at an ingestion rate of approximately 1.2 g·kg·h^{-1}.

As with protein ingestion, delaying CHO feeding by 2 h post-exercise can result in a 50% decrease in muscle glycogen resynthesis rates compared to ingestion immediately after exercise (Ivy et al., 1988). Furthermore, as high GI CHOs elicit a larger insulin response and make glucose available in the blood quicker than low GI CHOs, their ingestion facilitate greater muscle glycogen storage in the hours after exercise (Kiens et al., 1990; Blom et al., 1987). Finally, the ingestion of CHO at regular intervals rather than as a large bolus has been suggested to enable higher glycogen synthesis rates in order to maintain a constantly elevated plasma insulin and glucose concentration (van Loon et al., 2000). However, to the author's knowledge no study has directly tested this hypothesis.

2.5.3.2.2 CHO-P and glycogen resynthesis

In light of the relationship between insulin and muscle glycogen resynthesis, several researchers have sought to examine whether CHO-P ingestion can enhance further rates of muscle glycogen resynthesis, and subsequent endurance performance, in the hours after exercise. In the first study to examine this link, Williams et al. (2003) examined the effects of ingesting a CHO-P versus a 6% CHO-electrolyte sports beverage on glycogen restoration and time to exhaustion at 85% VO_{2max} 4 h following glycogen depleting exercise in eight trained cyclists. They reported that the CHO-P beverage, in

comparison to the CHO-electrolyte beverage, increased time to exhaustion during the submaximal run by 55%, storage of muscle glycogen by 128%, insulin concentration by 92%. This study corroborated the findings of others who showed that muscle glycogen synthesis rates are indeed greater when protein is ingested with CHO (Berardi et al., 2006; Ivy et al., 2002; van Loon et al., 2000; Zawadzki et al., 1992), and subsequent performance is enhanced (Karp et al., 2006; Saunders et al., 2004), in the hours after exercise. However, a common aspect of these studies has been that they have either provided CHO in sub-optimal quantities (< 1 $g \cdot kg \cdot h^{-1}$), or compared the supplement to another which has not been matched for energy content. Indeed, in the case of Williams et al. (2003), an approximate 368 extra calories was provided in the CHO-P supplement than the CHO drink over the four hour recovery period.

van Loon et al. (2000) utilised a research design that compared the effects of a CHO-P drink providing 0.8 $g \cdot kg \cdot h^{-1}$ CHO plus 0.4 $g \cdot kg \cdot h^{-1}$ protein, and drinks providing 0.8 $g \cdot kg \cdot h^{-1}$ and 1.2 $g \cdot kg \cdot h^{-1}$ CHO-only, and as such were matched for CHO and energy content respectively. Five hours following a glycogen depleting intermittent cycle, it was shown that muscle glycogen rates were in fact highest in the CHO condition providing 1.2 $g \cdot kg \cdot h^{-1}$, despite a much higher insulin concentration (approximately 40%) throughout the recovery period in the CHO-P trial. Thus, although insulin is likely to be important for muscle glycogen resynthesis after exercise, the availability of CHO is likely to limit synthetic rates at the upper end of ingestion rates. Indeed, studies which have compared CHO-P to an energy matched equivalent CHO drink, or CHO provided at an optimal rate (> 1 $g \cdot kg \cdot h^{-1}$) have failed to show an improvement in the rate of muscle glycogen resynthesis (Howarth et al., 2009; Jentjens et al. 2001;

Carrithers et al., 2000; van Hall et al., 2000; van Loon et al., 2000), or subsequent endurance performance (Betts et al., 2007; Millard-Satfford et al., 2005). In agreement, data collated by Betts & Williams (2010) have demonstrated that glycogen synthesis rates are not enhanced when protein is consumed with adequate amounts of CHO (> 1 $g \cdot kg \cdot h^{-1}$).

2.5.3.3 CHO-P and post-exercise fluid balance

Current recommendations for the post-exercise restoration of fluid losses are that 1.5 l of fluid should be consumed for every 1 kg of body mass lost during exercise (Shirreffs et al., 1996). Plain water is considered a poor choice for post-exercise rehydration as large volumes are likely to reduce plasma sodium concentration and plasma osmolality, the results of which are increased urine output and decreased desire to drink (Nose et al., 1988). As such, electrolytes, and particularly sodium, included in a drink enable euhydration after exercise much more quickly than plain water alone (Shireffs & Maughan, 2000). Indeed, Shirreffs et al. (1996) demonstrated that, with adequate volumes of ingested fluid, fluid balance six hours after exercise was only achieved when sodium intake was greater than sodium losses during exercise. For MSSs, this is likely to necessitate a sodium intake of > 4 g, as Maughan et al. (2005) have demonstrated sweat sodium concentrations of 42.5 ± 13 $mmol \cdot l^{-1}$ and salt losses of 4.3 ± 1.8 g during soccer training lasting for 90 min. CHO intake in a recovery beverage is also encouraged to replenish muscle glycogen stores after exercise, but small amounts may also aid fluid absorption in the gut (Rehrer et al., 1992). Over recent years some studies have demonstrated how CHO-P, in the form of milk ingestion, can result in greater fluid balance than CHO-electrolyte beverages. Shirreffs et al. (2007) reported that drinking a

volume ~150% of sweat losses of milk and milk with added sodium produced a lower urine output (611 and 550 ml respectively) than either water (1184 ml) or a CHO-electrolyte drink (1,205 ml) in the 5 h after completing intermittent exercise inducing a 1.8% body mass loss. This meant that a net positive fluid balance was maintained throughout the post-exercise recovery period with milk ingestion, with a negative fluid balance 1 h after drinking the other fluids. Watson et al. (2008) later confirmed these findings, showing that milk resulted in a markedly lower urine output and thus fluid balance was maintained in the 3 h after exercise. The mechanisms for greater fluid retention with CHO-P ingestion resulting in improved fluid balance are currently not known, however it has been proposed that increased sodium concentrations in milk, and slower gastric emptying of fluid with the ingestion of protein, lowers plasma osmolality, which prevents diuresis (Watson et al., 2008). This area of research is relatively new, and further studies examining the use of CHO-P as a post-exercise recovery aid are warranted.

2.6 Conclusions

This review has discussed several pertinent aspects of MSSs and acute CHO-P supplementation. Significant developments in time-motion analysis have allowed the accurate measurement of external load and movement demands associated with MSSs. Accordingly, it is now clear that athletes cover distances of between 6,000 to 11,000 m, predominantly at low movement speeds. Importantly, several studies have demonstrated that in the latter stages of competition there are reductions of up to 45 and 31% in high-intensity running and sprinting, respectively. Consequently, MSSs seem to induce fatigue in their participants. The physiological responses to MSSs

suggest that these sports predominantly tax the aerobic energy systems and are performed at an average of 70% VO_{2max}. However, rates of glycolysis are likely to be high as evidenced via increased muscle lactate concentrations. In addition, it is likely that causes of fatigue during MSSs are depletion of muscle glycogen stores and fluid loss.

CHO supplementation both in the days before, 3-4 h before and during endurance exercise is likely to be ergogenic. There is also a large body of research demonstrating that CHO ingestion during simulated MSS exercise can improve endurance capacity and skill performance. The mechanisms for this improvement are not fully understood, but centre on increased exogenous energy availability, better maintenance of endogenous energy stores and potential central effects. However, there is a wealth of evidence on the optimal composition of a CHO supplement for endurance exercise. Maximal CHO oxidation can be achieved via CHO delivery of 60-70g·h^{-1}, whilst CHO oxidation may be further increased with consumption of multiple-transportable CHOs. CHO concentration appears to mediate gastric emptying, and as such concentrations > 8% during exercise should be avoided.

The addition of protein to CHO has, in some instances, been shown to further enhance endurance performance. However, research on the effects of CHO-P and its mechanisms of action have thus far been equivocal, and little consensus exists among researchers. The optimal composition of a CHO-P supplement is unknown, but the current review suggests that the delivery of CHO and protein at rates of approximately

40-60 and 10-20 $g \cdot h^{-1}$, respectively, is appropriate. However, to date, there is a dearth of knowledge on the effects of CHO-P during MSS activity. With regard to MSS recovery, it is likely that recovery from EIMD (and associated reductions in muscle strength and performance), muscle glycogen repletion and rehydration are significant concerns for athletes. Whilst it has been speculated that CHO-P may have additive effects to CHO for each of these aspects of recovery, again, much of the research in this area is unclear, and little information exists on the efficacy of CHO-P following MSSs. Accordingly, the aims of the current body of research are to document the effects of CHO-P on MSS recovery and performance.

Chapter 3

The Reliability and Concurrent Validity of Non-Motorised Treadmill Sprint Performance

3.1 Introduction

Sprint running performance is considered to be a fundamental component of success in a variety of competitive MSSs (e.g. soccer, rugby, hockey). Time-motion analysis has shown that on average approximately 20-60 sprints, operationally defined as "maximal effort, a rapid motion" (Bloomfield et al., 2004), per player per game will take place (Spencer et al., 2005a), with many of these bouts occurring during 'crucial' patterns of play (i.e. chasing attacking players, counter-attacking, scoring tries, and chasing through-balls). Mean sprint times and distances in such sports are between 2-3 seconds and 10-20 m respectively (Spencer et al., 2005a), with sprints rarely exceeding distances of 40 m (Bangsbo & Mohr, 2005; Meir et al., 2001a). Consequently, the physiological assessment of team sport players is often confined to their sprinting performances over distances of 5 to 40 m (Ellis et al., 2000), whether following training interventions (Spinks et al., 2007) or different periods of recovery (Highton et al., 2009; Magalhaes et al., 2009; Ascensao et al., 2008).

Non-motorised treadmill (NMT) ergometry, as originally described by Lakomy (1987), provides a potentially useful tool for the assessment of maximal sprint running performance. Its use allows the benefits associated with testing in controlled laboratory conditions and the calculation of many performance measures (for example

time to peak running speed, step length and step frequency) which are potentially of interest to athletes, coaches and researchers. By utilising NMT ergometry, these sprint parameters can be measured continually, providing real-time information for coaching or research purposes.

The importance of ensuring that measurements made as part of research or athlete support are both reliable and valid is widely recognised (Atkinson & Nevill, 1998). Such an approach allows researchers and sports scientists to detect 'real' changes in performance with confidence, and ensures that a test or instrument is measuring what it purports to. In recent years, several studies adopting appropriate statistical tests have documented the reliability of certain performance (Hopker et al., 2009; Sirotic & Coutts 2008; Lim & Chia 2007; Hughes et al., 2006; Tong et al., 2001) and physiological (Sirotic & Coutts 2008) variables obtained via NMT ergometry. Such variables include maximal force, maximal power, maximal running speed, heart rate and oxygen consumption; the reliability of which has generally been interpreted as favourable. However, there are several potentially beneficial performance measurements that can be obtained via NMT ergometry for which reliability has yet to be assessed, including mean speed, split times over multiple distances and step rate and length. In addition, the statistical agreement between measures obtained on the NMT over a variety of distances and those obtained over-ground remains to be elucidated, with only the early work of Lakomy (1987), and more recently Hopker et al. (2009), offering direct comparisons between performances in each. As such, the potential utility of the NMT for the assessment of sprint performance in team sport athletes, whether for research or in the applied setting, is yet to be fully explored. Accordingly, the purpose of this

study was to report on both the inter- and intra-day reliability and concurrent validity of a variety of sprint performance measures obtained on a commercially available NMT over distances commonly associated with MSS competition.

3.2 Methods

Participants and Experimental Design

Twelve healthy male university level team sport (soccer, rugby union and rugby league) players (mean age: 22.3 ± 3.6 years; body mass: 80.3 ± 8.4 kg; stature: 1.80 ± 0.10 m) volunteered to participate in the study. Prior to data collection all participants provided written informed consent and completed a pre-test health questionnaire to reveal if there were any contraindications to exercise. Ethical approval was granted by the Ethics Committee of the Department of Sport and Exercise Sciences, University of Chester.

This study utilised a repeated measures design in which participants, following a period of familiarisation, were required to complete two laboratory trials on separate days involving the assessment of NMT sprint performance (to assess the inter-day reliability of measured variables) and one trial on an outdoor all-weather surface for the assessment of over-ground sprinting performance (see Figure 3.1). The order of sprint assessment was randomised for each participant, with each of the three trials separated by 24 h. On each testing occasion participants performed three maximal sprints, which in the case of the NMT were used to assess the intra-day reliability of values obtained. Participants were instructed to refrain from any strenuous physical

activity and maintain their normal diet during the period of testing to avoid any possible interference that this might have on their sprinting abilities.

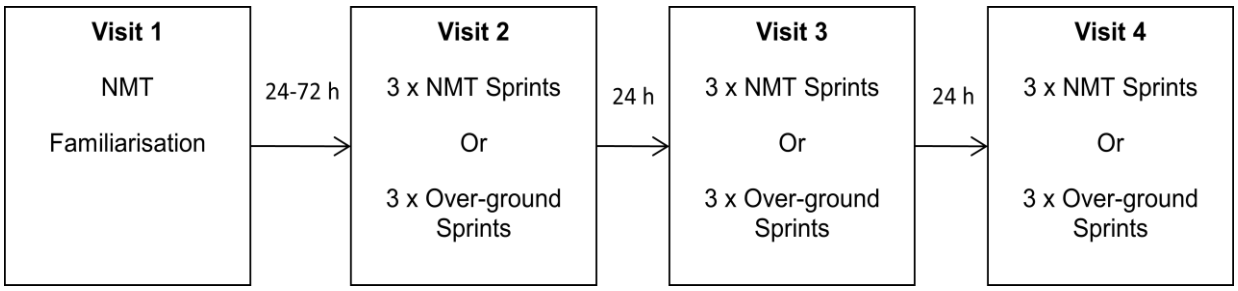

Figure 3.1. Schematic of the study design

Procedures

NMT Familiarisation

The NMT (Woodway, Force 3.0, USA) utilised in this study was an updated version of the system originally presented by Lakomy (1984; 1987), and is similar to systems that have been described in detail in previous studies (e.g. Hopker et al., 2009; Sirotic & Coutts 2008; Lim & Chia 2007; Hughes et al., 2006; Tong et al., 2001). Approximately 48-72 h prior to data collection, participants were familiarised with the NMT over three sessions within a 24 h period. These sessions provided detailed verbal instructions on the technique required to run on the NMT (which were then reinforced before each sprint performance session) and allowed participants to walk and then jog at a low intensity before completing 3 sprints for 30 m at gradually increasing speeds (i.e. from approximately 60 to 100% of the their perceived maximum speeds). The familiarisation session was terminated when participants indicated that they were

comfortable to sprint maximally and were achieving a consistent running speed (within 1 $m \cdot s^{-1}$) at each maximal sprint.

Assessment of NMT Sprint Performance

NMT sprint performance variables were measured following a warm-up on the NMT consisting of three minutes continuous jogging interspersed with one maximal sprint for 6 s (Tong et al., 2001). This also served to 'warm-up' the treadmill rollers and thus minimise the resistance of the treadmill belt (Lakomy, 1987). During the sprints that followed, participants were connected to a mounted strain gauge via a non-elastic tether and harness which was attached around their waists (Figure 3.2). The height of the strain gauge was adjusted so that the tether was at an angle of 8° (measured via a goniometer) above horizontal for each participant (whilst standing) so as to maintain the horizontal position of the tether during the forward lean adopted when sprinting on the NMT (Lakomy, 1987). Participants were instructed to sprint maximally from a standing start on the researcher's instruction and to maintain the effort until they had reached a distance of 30 m. Split times were also recorded at 10 and 20 m, with speed sampled at a rate of 100 Hz. For measurements of peak running speed, data were also averaged over 1 s to provide peak measurements, and are referred to as 'peak averaged' hereafter. This procedure was repeated twice more interspersed with 2 minutes passive recovery.

Figure 3.2. A participant performing a maximal sprint on the NMT.

Assessment of over-ground sprint running performance

To assess the concurrent validity of sprint performance variables obtained on the NMT, participants performed three maximal 30 m sprints from a standing start on an outdoor all-weather surface, interspersed with two minutes passive recovery. Sprints were conducted in dry conditions, with the direction of the sprints being perpendicular to any prevailing wind direction (Gabbett 2006; Meir et al., 2001b). Sprint times were recorded using 6 electronic photo cells (Speedtrap II, Brower Timing Systems, Utah, USA) positioned at 10, 20 and 30 m from the start line. The initial gate at the start of the sprint was replaced by a switch activated by the participant's foot leaving the ground, which served to reduce the degree of momentum developed before the start of the sprint and prevent extraneous movement influencing the recorded sprint times (Duthie et al., 2006). Sprint times to each of these distances were recorded to the nearest 0.01 s via telemetry to a hand-held system, with the lowest value (i.e. best performance) from the three sprints used for data analysis.

Statistical Analyses

The measures obtained from the NMT included 10, 20 and 30 m sprint times (s), mean and peak (averaged and instantaneous) running speeds ($m \cdot s^{-1}$), time to peak running speed (s), step length ($m \cdot step^{-1}$) and step frequency ($steps \cdot s^{-1}$). Optimum values (best performances) from the three sprints were used in the analysis of concurrent validity against over-ground sprint performance and inter-day reliability. Data from trials 2-3 on the same day (since these were typically where the fastest sprints occurred) were used to assess the intra-day reliability of the sprint variables. Following an examination of the distributions of the variables via the Shapiro-Wilk test of normality, descriptive statistics (mean ± standard deviation) were generated. Reliability was assessed via the coefficient of variation (CV) and the 95% limits of agreement (LoA; bias ± 1.96 x SDdiff) as originally described by Bland and Altman (1986). The ratio LoA (see Atkinson & Nevill (1998) for a review) were also calculated owing to the presence of heteroscedastic errors among certain residuals (Atkinson & Nevill, 1998), which were reduced (and in some cases removed) by applying logarithmic (natural) transformations. The validity of the NMT measures against over-ground sprinting was examined via correlation analysis (Spearman's rho; r_s as the variables were not always normally distributed), and the 95% and ratio LoA statistics. Alpha was set at 0.05 and all statistical analyses were conducted using SPSS for Windows (Version 14.0, 2006).

3.3 Results

The inter-day reliability statistics of 11 variables measured on the NMT are presented in Table 3.1. Whilst there were no significant biases ($P > 0.05$) between the

measurements taken on separate days for any of these, the best levels of agreement (within 7%) between days were observed for the measurements of time to 30 m (1.02 */÷ 1.07), peak instantaneous (0.99 */÷ 1.07), peak averaged (1 */÷ 1.06), mean speeds (1 */÷ 1.06) and step frequency (1.01 */÷ 1.06). Time to peak speed exhibited the poorest agreement, with a difference of up to 47%.

The intra-day reliability (Day 1) statistics on the NMT (Table 3.2) show no significant biases ($P > 0.05$) for any of the measures, and the best agreement (within 7%) occurred for the time to 20 (0.99 */÷ 1.07) and 30 m (0.99 */÷ 1.06), peak averaged (0.95 */÷ 1.05), mean speed (1.01 */÷ 1.07) and step length (0.99 */÷ 1.07) and frequency (1.01 */÷ 1.05). As with measurements taken between days, it was time to peak speed which demonstrated the poorest agreement between trials (up to 33% difference). Similar results were observed for intra-day reliability on day 2 (Table 3.3).

Table 3.1 Inter-day NMT descriptive (mean ± SD) and reliability statistics

Performance Variable	Day 1	Day 2	Limits of Agreement	Ratio Limits of Agreement	Coefficient of Variation
Time to 10 m (s)	2.39 ± 0.17	2.30 ± 0.22	0.09 ± 0.34	1.04 */÷ 1.16	4.2%
Time to 20 m (s)	4.23 ± 0.26	4.13 ± 0.25	0.10 ± 0.37	1.02 */÷ 1.09	2.8%
Time to 30 m (s)	6.10 ± 0.36	6.00 ± 0.3	0.11 ± 0.42	1.02 */÷ 1.07	2.2%
10-20 m (s)	1.80 ± 0.11	1.82 ± 0.11	0.02 ± 0.14	1.01 */÷ 1.08	2.3%
20-30 m (s)	1.83 ± 0.11	1.83 ± 0.14	-0.01 ± 0.19	0.99 */÷ 1.12	2.9%
Peak Instantaneous Speed ($m \cdot s^{-1}$)	5.62 ± 0.28	5.60 ± 0.26	-0.02 ± 0.35	1.00 */÷ 1.06	1.8%
Peak Averaged Speed ($m \cdot s^{-1}$)	5.56 ± 0.28	5.54 ± 0.26	-0.02 ± 0.33	1.00 */÷ 1.06	1.8%
Mean Speed ($m \cdot s^{-1}$)	4.94 ± 0.27	5.02 ± 0.25	-0.08 ± 0.33	0.99 */÷ 1.07	2.1%
Time to Peak Speed (s)	3.41 ± 0.73	3.09 ± 0.65	0.32 ± 1.16	1.10 */÷ 1.47	10.8%
Step Length ($m \cdot step^{-1}$)	1.16 ± 0.09	1.16 ± 0.99	-0.01 ± 0.09	0.99 */÷ 1.09	2.3%
Step Frequency ($steps \cdot s^{-1}$)	4.43 ± 0.33	4.48 ± 0.33	0.06 ± 0.25	1.01 */÷ 1.06	1.6%

Table 3.2 Intra-day NMT descriptive (mean ± SD) and reliability statistics (Day 1)

Performance Variable	Trial 2	Trial 3	Limits of Agreement	Ratio Limits of Agreement	Coefficient of Variation
Time to 10 m (s)	2.48 ± 0.24	2.50 ± 0.24	-0.02 ± 0.33	0.99 */÷ 1.14	2.8%
Time to 20 m (s)	4.28 ± 0.26	4.34 ± 0.34	-0.06 ± 0.31	0.99 */÷ 1.07	1.7%
Time to 30 m (s)	6.16 ± 0.36	6.23 ± 0.42	-0.07 ± 0.39	0.99 */÷ 1.06	1.8%
10-20 m (s)	1.80 ± 0.11	1.85 ± 0.14	-0.04 ± 0.16	1.02 */÷ 1.08	1.9%
20-30 m (s)	1.88 ± 0.11	1.89 ± 0.13	-0.01 ± 0.22	0.99 */÷ 1.13	3%
Peak Instantaneous Speed ($m \cdot s^{-1}$)	5.58 ± 0.26	5.55 ± 0.33	0.03 ± 0.27	1.01 */÷ 1.07	1.2%
Peak Averaged Speed ($m \cdot s^{-1}$)	5.52 ± 0.26	5.50 ± 0.33	-0.02 ± 0.26	0.95 */÷ 1.05	1.1%
Mean Speed ($m \cdot s^{-1}$)	4.89 ± 0.26	4.81 ± 0.37	0.08 ± 0.52	1.02 */÷ 1.12	2.4%
Time to Peak Speed (s)	3.78 ± 0.75	3.76 ± 0.98	0.02 ± 1.11	1.02 */÷ 1.33	8.1%
Step Length ($m \cdot step^{-1}$)	1.14 ± 0.08	1.13 ± 0.07	-0.01 ± 0.08	0.99 */÷ 1.07	1.7%
Step Frequency ($steps \cdot s^{-1}$)	4.37 ± 0.37	4.43 ± 0.33	0.04 ± 0.23	1.01 */÷ 1.05	1.4%

Table 3.3 Intra-day NMT descriptive (mean ± SD) and reliability statistics (Day 2)

Performance Variable	Trial 2	Trial 3	Limits of Agreement	Ratio Limits of Agreement	Coefficient of Variation
Time to 10 m (s)	2.51 ± 0.34	2.41 ± 0.16	0.10 ± 0.50	1.03 */÷ 1.20	5.1%
Time to 20 m (s)	4.34 ± 0.39	4.24 ± 0.19	0.10 ± 0.52	1.02 */÷ 1.12	3.1%
Time to 30 m (s)	6.20 ± 0.44	6.11 ± 0.26	0.09 ± 0.47	1.01 */÷ 1.07	2.1%
10-20 m (s)	1.83 ± 0.09	1.83 ± 0.09	0.00 ± 0.05	1.00 */÷ 1.03	0.7%
20-30 m (s)	1.85 ± 0.16	1.87 ± 0.11	-0.02 ± 0.16	0.99 */÷ 1.09	1.9%
Peak Instantaneous Speed ($m \cdot s^{-1}$)	5.53 ± 0.27	5.55 ± 0.26	-0.02 ± 0.24	1.00 */÷ 1.04	1.2%
Peak Averaged Speed ($m \cdot s^{-1}$)	5.48 ± 0.27	5.50 ± 0.26	0.02 ± 0.21	1.00 */÷ 1.04	1.1%
Mean Speed ($m \cdot s^{-1}$)	4.85 ± 0.35	4.92 ± 0.21	-0.08 ± 0.40	0.98 */÷ 1.08	2.3%
Time to Peak Speed (s)	3.65 ± 0.98	3.52 ± 0.88	0.13 ± 1.58	1.03 */÷ 1.49	10.8%
Step Length ($m \cdot step^{-1}$)	1.11 ± 0.08	1.14 ± 0.10	0.04 ± 0.1	1.03 */÷ 1.09	2.6%
Step Frequency ($steps \cdot s^{-1}$)	4.41 ± 0.37	4.37 ± 0.38	-0.05 ± 0.25	0.99 */÷ 1.06	1.5%

In terms of concurrent validity, all of the performance measures on the NMT were found to be significantly inferior ($P < 0.05$) to those obtained from over-ground sprinting on both days (Tables 3.4 and 3.5). For example, the 30 m split times were, on average, 1.88 (Day 1) and 1.77 s (Day 2) faster over-ground than on the NMT, and the mean speeds were 2.09 (Day 1) and 2.02 $m \cdot s^{-1}$ (Day 2) lower. The correlations between NMT and over-ground variables were typically modest (0.44 – 0.67), though as high as 0.80 (time to 30 m, Day 2) and as low as 0.30 (10-20 m time, Day 2).

Table 3.4 Concurrent validity statistics of the NMT variables (Day 1)

Performance Variable	NMT	Over-ground	Limits of Agreement	Ratio Limits of Agreement	Coefficient of Variation	r_s
Time to 10m (s)	2.39 ± 0.17	1.70 ± 0.20	-0.68†† ± 0.45	0.71 */÷ 1.26	23.9%	0.43
Time to 20m (s)	4.23 ± 0.26	3.01 ± 0.22	-1.22†† ± 0.51	0.71 */÷ 1.14	23.8%	0.54
Time to 30m (s)	6.10± 0.36	4.23 ± 0.25	-1.88†† ± 0.60	0.69 */÷ 1.11	25.7%	0.58†
10-20 m (s)	1.80 ± 0.11	1.30 ± 0.05	-0.50†† ± 0.19	0.72 */÷ 1.11	22.8%	0.50
20-30 m (s)	1.83 ± 0.11	1.20 ± 0.06	-0.64†† ± 0.15	0.65 */÷ 1.08	29.7%	0.67†
Mean Speed ($m \cdot s^{-1}$)	4.94 ± 0.27	7.02 ± 0.42	-2.09†† ± 0.71	0.70 */÷ 1.12	25.5%	0.58†

† $P < 0.05$; †† $P < 0.05$, reflecting the presence of a systematic bias between measurements.

Table 3.5 Concurrent validity statistics of the NMT variables (Day 2)

Performance Variable	NMT	Over-ground	Limits of Agreement	Ratio Limits of Agreement	Coefficient of Variation	r_s
Time to 10m (s)	2.30 ± 0.22	1.70 ± 0.20	-0.60†† ± 0.48	0.74 */÷ 1.26	21.2%	0.44
Time to 20m (s)	4.13 ± 0.25	3.01 ± 0.22	-1.12†† ± 0.45	0.73 */÷ 1.12	22.1%	0.66†
Time to 30m (s)	6.00 ± 0.30	4.23 ± 0.25	-1.77†† ± 0.47	0.70 */÷ 1.09	24.5%	0.80†
10-20 m (s)	1.82 ± 0.09	1.30 ± 0.05	-0.52†† ± 0.16	0.71 */÷ 1.11	23.7%	0.30
20-30 m (s)	1.83 ± 0.14	1.20 ± 0.06	-0.63†† ± 0.15	0.65 */÷ 1.08	29.4%	0.48
Mean Speed ($m \cdot s^{-1}$)	5.02 ± 0.25	7.02 ± 0.42	-2.01†† ± 0.71	0.71 */÷ 1.12	24.5%	0.60†

† $P < 0.05$, †† $P < 0.05$ reflecting the presence of a systematic bias between measurements.

3.4 Discussion

The present study has demonstrated that particular measures of sprint performance can be generated reliably on the NMT. Specifically, for measurements obtained on the same day, times to 20 and 30 m, peak averaged and mean speed, and step length and frequency demonstrated the best levels of agreement. Similarly, for inter-day measurements, agreement was best for times to 30 m, peak instantaneous, peak averaged and mean speeds and step frequency. Whilst these findings are consistent with previous studies which have generally reported that NMT measurements of running speed and distance covered in a set time interval are reliable (Hopker et al., 2009; Sirotic & Coutts 2008; Lim & Chia 2007; Hughes et al. 2006; Tong et al. 2001), they are unique in that no previous studies have identified step length and frequency measures to be the amongst the most reliable. The implication of this discovery is not trivial considering that the goal of many sprint training programmes is to improve either one or both of these kinematic parameters (Moir et al. 2007; Myer et al. 2007; Spinks et al. 2007; Zafeiridis et al. 2005). Step length and frequency are typically measured via video analysis, which is often time-consuming and will generally preclude the provision of real-time feedback. Based on these findings, it appears that NMT may offer a reliable and time-effective method of assessing sprint kinematics over 30 m.

The acceptability of the measurement error (reliability) observed for the majority of the measures of sprint performance is endorsed when one considers it in the context of whether a future study involving a 'practical' sample size could detect a genuine change in sprint performance (due to an intervention, for instance). The employment of such

an ‘analytical goal’ (Atkinson & Nevill, 1998) has seldom been done in studies of this kind, though it adds quality to the interpretation of the reliability data. Previous research has demonstrated that changes in sprint performance, either following training programmes or during periods of fatigue or recovery, are usually small, typically falling between 2 and 7% (Highton et al., 2009; Magalhaes et al., 2009; Harris et al., 2008; Markovich et al., 2007; Moir et al., 2007; Myer et al., 2007; Krustrup et al., 2006; Zafereidis et al., 2005). Therefore, given the calculated ratio LoA for these measures, the nomogram of Atkinson et al. (1999) reveals that a sample size of approximately 10-30 participants would be required to detect a 5% change in each of these measures. Accordingly, we can posit that the measurement error associated with these is acceptable given that the practical sample size used in the current study would be sufficient to detect such a change.

To the author’s knowledge, this study is one of the first of its type to present information on sprint performance over several different distances. Importantly, the data indicate that the reliability of sprint times recorded on the NMT improves as the distance increases, with 10 m sprint times, whilst still considered reliable based upon the sample size required to detect a meaningful change in this variable (approximately 25 participants), demonstrating the poorest levels of agreement (both between trials on the same day and between days), in comparison to times for 20 and 30 m, and the 10-20 and 20-30 m split times. A possible explanation for this finding is the relatively high degree of force required to overcome the inertia of the treadmill belt at the start of the sprint compared to running over-ground. This, combined with the high retarding forces which act on the participant throughout NMT running (Lakomy, 1987), provides

the scope for higher degrees of performance variability in general, and particularly at the outset. Moreover, the participant is likely to be more vulnerable to such effects when not habituated to NMT running, fatigued or lacking in motivation.

Of all the variables reported in this study it was only time to peak running speed which demonstrated unacceptable levels of intra- and inter-day levels of agreement. For this variable, a sample size of up to 100 participants would be required to detect a genuine 5% change in performance, whilst the intra- and inter-day variation was as high as 47%, a value way beyond any expected change in sprint performance associated with either training or fatigue. In addition, the coefficient of variation was above 10% (a common, if arbitrary, cut-off point for the assessment of reliability, (Atkinson & Nevill, 1998).

Despite the potential utility of time to peak running speed as a marker of an individual's acceleration, no study to date has examined its reliability. However, that the data demonstrated poor levels of both intra- and inter-day reliability, it would seem to be of little value to coaches and researchers, and likely reflects issues with NMT reliability during the early stages of a sprint. In an attempt to counteract this, and to provide a meaningful measure of acceleration, measurements of running speed at fixed time points which corresponded to average sprint durations during team sport activity (i.e. 1, 2 and 3 s) were analysed for reliability (Table 3.6). Measurements of running speed at both 2 and 3 seconds demonstrated high levels of intra- (1.02 */÷ 1.12 and 1.00 */÷ 1.04, respectively) and inter-day (1.01 */÷ 1.06 and 1.01 */÷ 1.05,

respectively) agreement. However, as with time to 10 m, values obtained earlier on in the sprint (1 s) demonstrated poor levels of intra- and inter-day reliability (1.09 */÷ 1.49 and 1.05 */÷ 1.22, respectively). As such, it is suggested that measurements of running speed at 2 and 3 seconds, and not time to peak running speed, may provide more valuable feedback on acceleration during maximal sprint running on the NMT.

Table 3.6 Reliability of running speeds at 1, 2 and 3 seconds

Variable ($m \cdot s^{-1}$)	Mean ± SD	Mean ± SD	Limits of agreement	Ratio limits of agreement
Between Trials: Day 1	**Trial 1**	**Trial 2**		
Speed at 1 s	4.06 ± 0.6	4.03 ± 0.6	-0.03 ± 0.93	0.99 */÷ 1.28
Speed at 2 s	5.14 ± 0.4	5.2 ± 0.41	0.05 ± 0.41	1.01 */÷ 1.08
Speed at 3 s	5.46 ± 0.29	5.42 ± 0.4	-0.03 ± 0.29	0.99 */÷ 1.06
Between Trials: Day 2	**Trial 1**	**Trial 2**		
Speed at 1 s	3.93 ± 0.86	4.21 ± 0.44	0.28 ± 1.3	1.09 */÷ 1.49
Speed at 2 s	5.16 ± 0.46	5.25 ± 0.28	0.09 ± 0.56	1.02 */÷ 1.12
Speed at 3 s	5.47 ± 0.28	5.48 ± 0.28	0.01 ± 0.23	1.00 */÷ 1.04
Between Days	**Day 1**	**Day 2**		
Speed at 1 s	4.24 ± 0.39	4.45 ± 0.53	0.21 ± 0.85	1.05 */÷ 1.22
Speed at 2 s	5.28 ± 0.29	5.23 ± 0.31	0.05 ± 0.34	1.01 */÷ 1.06
Speed at 3 s	5.49 ± 0.3	5.52 ± 0.27	0.03 ± 0.29	1.01 */÷ 1.05

With respect to the concurrent validity of the NMT measures against over-ground running, the observation of the presence of systematic bias for times to 10, 20 and 30 m and mean running speed concur with the original study of Lakomy (1987). That is, the participants in this study were significantly slower on the NMT (25-30% c.f. 20%) than when sprinting over-ground. Similarly, in the only other study making a direct comparison between non-motorised treadmill and over-ground sprint performance, Hopker et al. (2009) reported that 20 m sprint time was higher by approximately 2.59 s on the NMT compared to running over-ground. As alluded to above, this attenuation of performance is probably a consequence of the participants having to overcome the high intrinsic resistance of the NMT belt. In addition, the participant does not reach the inertia characteristic of over-ground sprint running when maximal speed is reached on the NMT, but instead must constantly accelerate the treadmill belt between steps.

The association between performance on the NMT and over-ground running was also generally moderate in this study, with only the correlations for time to 20 and 30 m and mean speed being significant and in excess of 0.58 (r_s = 0.66, 0.8 and 0.6, respectively). Nonetheless, the trend for individuals who were faster on the NMT over distances of 20 and 30 m to be faster over-ground could provide a useful measure of relative sprint performance. That is, based on the findings herein it is likely that individuals who are faster over-ground will, in most cases, be faster on the NMT, particularly over distances > 20 m. Thus, it may be possible to identify faster athletes utilising NMT ergometry. However, this may not always be the case, with factors such as body mass likely to influence NMT sprint performance irrespective of over-ground sprint ability. Indeed, Lakomy (1987) originally reported that individuals with larger body masses were at an

advantage on the NMT due to the force required to overcome the resistance of the NMT belt being relatively higher (per kilogram body mass) for lighter individuals.

In conclusion, the present data offer support for NMT ergometry as a reliable tool for measuring particular sprint performance variables, namely time to 20 and 30 m, peak and mean running speeds and step length and frequency. This information can be used by sport and exercise practitioners not only to identify which measurements of sprint performance are reliable enough to be considered worthwhile, but also to detect what might be considered 'real' changes in performance. For example, for somebody who attained a 30 m sprint time of 6 seconds, when tested on another day they could attain anywhere between 5.69 and 6.53 seconds according to the 95% LoA (as a worst case scenario). Thus, in theory changes in sprint performance would have to be outside of these limits to be considered to be due solely to factors other than reliability. A further finding of the current study is that the NMT is likely to be able to detect changes of 5% in many of the sprint performance variables measured with a sample size of approximately 10. Such information is useful for the design of future studies concerned with the NMT.

In relation to over-ground sprinting, it would appear that, in absolute terms, individuals were consistently slower on the NMT by approximately 25-30%. In relative terms, however, superior performances over-ground over 30 m were reflected in superior NMT performances, and as such, faster sprinters over-ground are likely to be identifiable via NMT ergometry over this distance. It is concluded that the NMT is a useful tool for researchers and sports science practitioners wishing to examine changes in sprint

performance, in particular over longer distances associated with MSS activity. However, exercise practitioners should be cognisant that sprint times on the NMT might not always be representative of speed over-ground during competition and therefore, should not disregard over-ground running as being an appropriate method of assessing sprint ability, particularly as it does not require familiarisation (Glaister et al., 2007). Nonetheless, there is the potential for a future, larger-scale study to examine whether over-ground sprint performance can be predicted with an acceptable degree of accuracy from the NMT measurements. More broadly, there is scope to embark on a more comprehensive evaluation of the efficacy of NMT sprint performance in relation to over-ground running by using force platform and video analysis systems.

Chapter 4

The Effects of a CHO-P Beverage on Muscular Function and Maximal-Intensity Exercise Performance following Simulated Multiple-Sprint Sport Exercise

4.1 Introduction

Strenuous unaccustomed exercise often leads to a disruption of the ultrastructure of skeletal muscle. The primary cause of this exercise-induced muscle damage (EIMD) is eccentric muscle action, whereby the muscle is forcibly lengthened under tension by a mechanical stress per motor unit that is higher than encountered during a concentric muscle action (Enoka, 1996). In addition, the contribution of metabolic or 'oxidative' stress during prolonged aerobic exercise, including high muscle temperature, lowered pH, insufficient mitochondrial respiration and oxygen free radical production, has also been implicated in the initial aetiology of EIMD (Cleak & Eston, 1992b; Armstrong, 1990). Thereafter, it is thought that skeletal muscle is subjected to autogenic processes and subsequent phagocytic and regenerative phases (Kendall & Eston, 2002; Armstrong, 1990), which serve ultimately to repair and regenerate skeletal muscle proteins and tissue as part of an exercise-induced adaptive response.

The period of EIMD is characterised by z-line streaming, the appearance of muscular proteins in the blood (possibly indicating a compromised muscle cell membrane stability), delayed onset muscle soreness, stiffness, swelling, and a decreased range of motion; the symptoms of which can last for up to one week, but which will typically peak 24-48 h after exercise (Byrne et al., 2004; Friden & Lieber, 2001a; Clarkson et al., 1992;

Cleak & Eston, 1992a). However, perhaps of greatest importance to the exercising athlete is the immediate and prolonged impairment in neuromuscular function (i.e. reductions in strength and power) associated with EIMD (for a review, see Byrne et al., 2004). Indeed, there is now a wealth of evidence demonstrating the negative consequences of EIMD on measures of athletic performance, several of which are likely to have implications for MSS performance and training, such as single and repeated sprint ability (Highton et al., 2009; Twist & Eston, 2005), vertical jump height (Twist & Eston, 2007; Marginson et al., 2005; Byrne & Eston, 2002a; Chambers et al., 1998), and agility (Highton et al., 2009).

Considering the many actions involving repeated eccentric muscular contraction during MSS (e.g. acceleration, deceleration, jumping, jogging/sprinting, rapid changes of direction and kicking), and the high aerobic nature of these sports (Bangsbo et al., 2006), it has been well documented that participation in this form of activity, and indeed protocols designed to simulate their demands, results in symptoms associated with EIMD in the days after exercise (Cunniffe et al., 2010; Mclellan et al., 2010; Magalhaes et al., 2009; Ascensao et al., 2008; Bailey et al., 2007; Takarada, 2003; Thompson et al., 2001b; Thompson et al., 1999). In the main, these studies have reported only blood biochemical and endocrine (typically plasma CK) responses to exercise, despite the poor correlation these markers share, in terms of magnitude and time-course, with both muscular tissue injury (Komulainen et al., 1995), and impairment in muscular function (Friden & Lieber, 2001b; Margaritis et al., 1999). Whilst relatively few studies have investigated decrements in muscle function in the days after MSS exercise, those that have report reductions of approximately 10-15% in isometric or isokinetic peak torque,

generally peaking at 24 h (Magalhaes et al., 2009; Bailey et al., 2007; Thompson et al., 2001a; Thompson et al., 2001b). As such, the congested competitive schedules of elite MSS athletes, combined with the potential influence of EIMD on muscular function, performance, and even injury risk (Brockett et al., 2004), would suggest that the consideration of EIMD as part of a recovery strategy is highly appropriate for MSS athletes.

A strategy that has accumulated interest in recent years is that of the acute post-exercise ingestion of relatively small amounts of protein (≤ 100 g) added to a CHO beverage. Such a recovery strategy is appealing as it is likely to aid rehydration (Watson et al., 2008), and may accelerate muscle glycogen resynthesis following exercise (Berardi et al., 2006; Ivy et al., 2005; van Loon et al., 2000). Studies examining acute CHO-P ingestion have provided equivocal findings, with some authors reporting an attenuated response to EIMD markers (Cockburn et al., 2008; Green et al., 2008; Valentine et al., 2008; Baty et al., 2007; Miles et al., 2007; Saunders et al., 2007; Millard-Stafford et al., 2005; Seifert et al., 2005; Saunders et al., 2004), and others reporting no benefits (Breen et al., 2010; Gilson et al., 2010; Betts et al., 2009; Green et al., 2008; White et al., 2008; Wojcik et al., 2001). Such discrepancies probably reflect methodological differences relating to amounts, type and timing the CHO-P, and the muscle damaging protocol and measures of EIMD utilised. For example, several studies have compared CHO-P supplements with other supplements *not* matched for energy or CHO content (Cockburn et al., 2008; Baty et al., 2007; Millard-Stafford et al., 2005; Seifert et al., 2005), thus making it difficult to establish whether attenuated markers of EIMD are due to a protein specific mechanism or the extra kilocalories available. Furthermore, relatively few

studies (Breen et al., 2010; Betts et al., 2009; Cockburn et al., 2008; Green et al., 2008; Valentine et al., 2008; White et al., 2008; Wojcik et al., 2001) have utilised measurements of muscle function to assess EIMD, despite this measure being thought to offer the most reliable and valid indication of the time-course and magnitude of EIMD (Warren et al., 1999). That being said, measurements of muscle function often do not closely resemble the more dynamic, multi-joint movements associated with athletic activity. Indeed, the velocity of leg flexion during sprinting can be up to 975 deg·s^{-1} (Baltzopoulos & Gleeson, 2009), a value much higher than can be measured with isokinetic dynamometry.

To date, the only study that has examined the influence of ingesting a CHO-P beverage on markers of EIMD (including muscle function) after MSS exercise (Betts et al., 2009), reported no significant effects. However, no 'performance' markers relevant to MSS players, such as sprinting and jumping, were examined in that study, and only isometric muscle function was reported, which bears little resemblance to such sporting movements. Thus, further research on this theme is warranted, especially with respect to the potential effects of CHO-P ingestion on markers of performance. It is also interesting to note that relatively few studies have provided CHO-P before and during exercise, as well as during post-exercise recovery. Such an approach may be beneficial due to enhanced protein synthesis rates and increased amino acid delivery (Tipton et al., 2001) and a protective effect of amino acids on muscular disruption during exercise (White et al., 2008). Accordingly, the ingestion of a CHO-P solution before, during and after MSS exercise may be more likely to attenuate EIMD than ingestion after exercise alone. Therefore the aim of this study was to examine the effects of ingesting a CHO-P

beverage immediately before, during and after exercise, in comparison to CHO alone, on the recovery of muscle function and maximal-intensity exercise performance following simulated MSS activity.

4.2 Methods

Participants and Experimental Design

Twenty-seven recreationally active university level team-sport athletes (males, *n* = 15; females, *n* = 12) volunteered to participate in the study (age = 22.4 ± 1.8 y, stature = 1.74 ± 8.4 m, body mass = 68.1 ± 11.3 kg, predicted VO_{2max} = 48.9 ± 5.4 ml·kg^{-1}·min^{-1}). Prior to data collection, participants provided written informed consent and completed a pre-test health questionnaire to check for any contraindications to exercise. Ethical approval for the study was granted by the University of Chester's Faculty of Applied and Health Sciences Research Ethics Committee.

The study incorporated an independent groups design in which the participants were randomly allocated to one of three supplement (beverage) groups (see Table 4.1). An independent groups design was adopted to avoid the potential influence of the repeated bout effect on any measured markers of EIMD and performance (McHugh et al., 1999). The beverages were prepared by an independent third party and administered in a double-blind manner. Participants visited the laboratory on five separate occasions over the course of 10 days. The dependent variables were measurements of muscle soreness, muscle function, vertical jump height and sprint speed.

Table 4.1. Participant characteristics.

	CHO-P (*n* = 9)	**ISOCHO** (*n* = 9)	**ISOEN** (*n* = 9)
Age (years)	**23.2 ± 2.2**	**22.1 ± 1.5**	**22.0 ± 1.6**
Males	23.6 ± 3.0	22.2 ± 1.8	22.6 ±1.7
Females	22.8 ± 0.9	22.0 ± 1.2	21.3 ± 1.3
Stature (m)	**1.73 ± 0.1**	**1.76 ± 0.1**	**1.71 ± 0.1**
Males	1.76 ± 0.1	1.84 ± 0.1	1.76 ± 0.1
Females	1.69 ± 0.1	1.67 ± 0.1	1.67 ± 0.1
Body Mass (kg)	**70.0 ± 7.6**	**68.8 ± 14.6**	**65.6 ± 11.7**
Males	75.6 ± 4.5	78.7 ± 9.1	73.6 ± 9.3
Females	63.0 ± 3.1	56.3 ± 9.1	55.5 ± 2.7
MSSRT (stage)	**10.4 ± 1.7**	**10.9 ± 1.6**	**10.5 ± 1.5**
Males	11.4 ± 1.5	12.0 ± 0.9	11.4 ± 1.2
Females	9.2 ± 1.1	9.5 ± 1.2	9.4 ± 1.1
$\dot{V}O_{2max}$ (ml·kg^{-1}·min^{-1})	**48.1 ± 5.7**	**49.9 ± 5.5**	**48.7 ± 5.4**
Males	51.5 ± 4.9	53.6 ± 3.1	51.8 ± 4.3
Females	43.9 ± 3.5	45.3 ± 3.9	44.8 ± 4.0

CHO + P = Carbohydrate and Protein, ISOCHO = Carbohydrate-only matched for carbohydrate with the CHO + P group, ISOEN = Carbohydrate only matched for energy content with the CHO + P group, MSSRT = Multi-Stage Shuttle Run Test

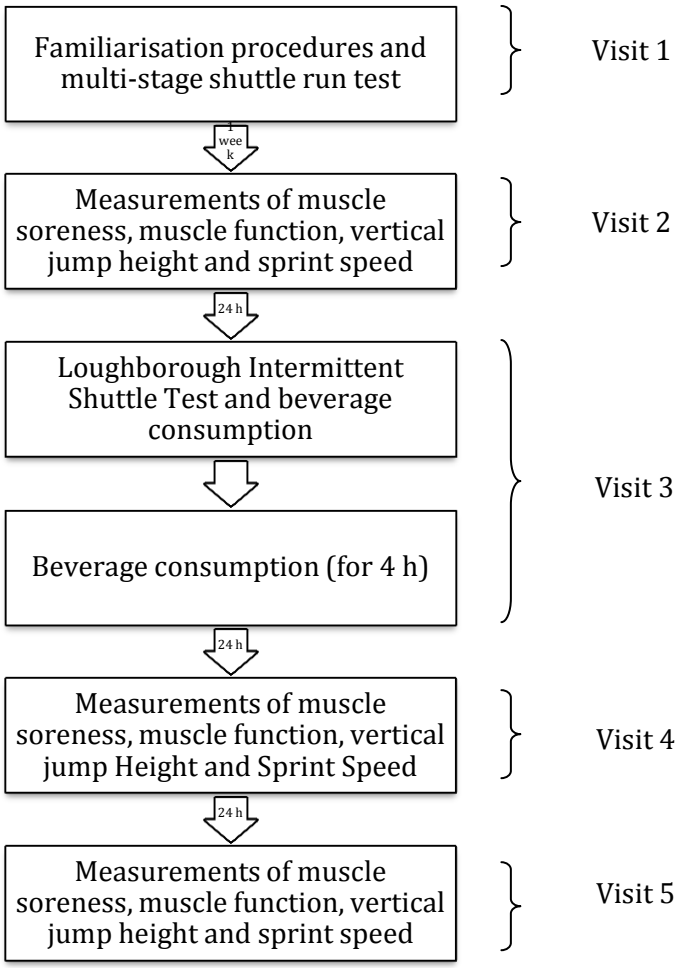

Figure 4.1. Schematic of the study design

Procedures

Participants completed an initial laboratory visit where they were familiarised with running on the NMT and then performed the multi-stage shuttle run test to exhaustion. On the second visit, baseline measurements of perceived muscle soreness and peak isokinetic torque of the knee extensors and flexors, squat-, countermovement-, and drop-jump height and sprint performance over 10, 20 and 30 m on a NMT were recorded. Participants were then randomly allocated to one of three groups which in visit three and consumed either a 7.5% carbohydrate (ISOCHO), or a 10% CHO (ISOEN) or a 7.5% CHO plus 2.5% protein (CHO-P) beverage, both during and in the 4 h after

completing the Loughborough Intermittent Shuttle Test (LIST). The two remaining visits at 24 and 48 h post-LIST required participants to repeat the baseline measurements. For two days prior to, and during the days of testing, participants completed a food diary to enable the investigator to check for any foods that might confound the potential impact of the beverages. Dietary intake for each group was; CHO-P ~ 2014 kcal·d^{-1}, 53% CHO, 25 % fat, 18 % protein; ISOEN ~ 1960 kcal·d^{-1}, 55% CHO, 23 % fat, 21 % protein; ISOCHO ~ 1965 kcal·d^{-1}, 57% CHO, 26 % fat, 15 % protein. Participants were also instructed to refrain from any physical activity in the day before and during the period of data collection.

The multi-stage shuttle run test

Following a standardised warm up, participants completed the multi-stage shuttle run test (MSSRT) on an indoor wooden surface. Briefly, the test consisted of shuttle running between two markers placed 20 m apart at increasing running speeds (0.14 m·s^{-1}) until exhaustion (Leger & Gadoury, 1989). Maximal heart rate was recorded at the end of the test via a heart rate monitor (Polar Electro, Oy, Finland). Maximal oxygen uptake (VO_{2max}) was estimated from the level and stage reached using the table of Ramsbottom et al. (1988). From this**,** running speeds corresponding to 55 and 95% of VO_{2max} were calculated for use with the LIST as speeds corresponding to 'jog' and 'cruise' respectively.

Assessment of perceived muscle soreness

Perceived muscle soreness of the knee extensors and flexors was assessed using a visual analogue scale (VAS). The VAS is numbered from 1 to 10 (on the reverse side of a sliding scale unseen by the participant) with 0 indicating no muscle soreness and 10 indicating the muscles are too sore to move. Participants were instructed to squat down and adopt a knee angle of approximately 90° and then indicate the level of perceived soreness in each muscle group based upon the rating scale. This method has been used successfully in previous studies to indicate muscle soreness (Marginson et al., 2005; Twist & Eston, 2005), and has been shown to correlate extremely well (r = 1.0, P < 0.05) with multi-dimensional methods of assessing muscular pain (Cleather & Guthrie, 2007).

Assessment of muscle function

Measurements of peak isokinetic torque in the knee extensors and flexors of the dominant leg were measured at 60 deg·s^{-1} and 240 deg·s^{-1} using an isokinetic dynamometer (Biodex, Biodex Medical, New York, USA). Participants were tested in the seated position with the lateral femoral epicondyle aligned to the dynamometer's axis of rotation. The upper body and active limb were secured with restraining straps to prevent extraneous movement (Baltzopolous & Gleeson, 2009), and the dynamometer lever arm length and the vertical, horizontal and seat positions were recorded for each participant to replicate testing positions at each time interval. The range of motion for each contraction was set prior to testing for each participant, and limb mass was assessed to enable a gravity correction of values (Gleeson & Mercer, 1996). Participants performed a familiarisation trial consisting of three submaximal and one maximal

practice repetitions, which also served as a warm-up at each angular velocity (Byrne et al., 2001). During testing, participants performed five maximal repetitions with a 60 s interval between sets of repetitions, with the highest peak torque achieved during the repetitions recorded. Participants performed the slower angular velocity first to enhance the reproducibility of results (Wilhite et al., 1992). Visual feedback displaying real time force was used to encourage maximal efforts (Gleeson & Mercer, 1996).

Assessment of vertical jump height

Vertical jump height was measured via an infra-red timing system (Optojump, Microgate S.r.l., Bolzano, Italy), which measures flight time and is triggered by participants removing their feet from the infra-red beam on takeoff and then breaking the beam again on landing. This flight time (t_{air}) was then used to calculate vertical take-off velocity (v) as follows:

$$v = 0.5(t_{air} \times g)$$

where g is acceleration due to gravity (9.81 $m \cdot s^{-2}$) .Subsequently, jump height was calculated as:

$$\text{Height} = \frac{v^2}{2g}$$

according to procedures previously documented by Byrne & Eston (2002).

Participants performed three different types of vertical jump; the squat- (SJ), countermovement- (CMJ) and drop-jump (DJ). The SJ involved participants bending the

knees to approximately 90 degrees for 3 s, and then on the instruction of the researcher jumping maximally for vertical height without any 'dipping' movement at the start of the jump. The CMJ required participants to adopt an upright position, and then on the instruction of the researcher participants flexed their knees to 90 degrees and then jumped vertically for maximal height. The DJ was performed from a 50 cm high platform (Horita et al., 1999) and required participants to step off the platform on the researcher's instruction and then jump vertically for maximal height with minimal ground contact. Each jump was performed three times, with participants instructed to keep their hands on their hips at all times and maintain a fully extended leg position during the jump and on landing. The highest of the three jumps for each method was used for data analysis.

Assessment of sprint performance on the non-motorised treadmill

After familiarisation (for details, see Chapter 3), sprint performance variables (time to 10, 20 and 30 m (s), and peak speed ($m \cdot s^{-1}$) were measured on a NMT after a warm-up consisting of three minutes continuous jogging interspersed with one maximal sprint for six seconds (Tong et al., 2001). This also served to 'warm-up' the treadmill rollers and thus minimise the resistance of the treadmill belt (Lakomy, 1987). During the sprints that followed, participants were connected to a mounted strain gauge via a non-elastic tether and harness which was attached around the participant's waist. The height of the strain gauge was adjusted so that the tether was at an angle of 8° above horizontal for each participant (whilst standing) so as to maintain the horizontal position of the tether during the forward lean adopted when sprinting on the NMT (Lakomy, 1987). Participants were instructed to sprint maximally from a standing start on the

researcher's instruction and to maintain the effort until they had reached a distance of 30 m. Split times were also recorded at 10 and 20 m, with speed sampled at a rate of 100 Hz. This procedure was repeated a further six times interspersed with 2 min passive recovery. The fastest of the six sprints were used for data analysis. The dependent variables of 10, 20 and 30 m sprint time (s), peak sprint speed ($m \cdot s^{-1}$) and step length ($m \cdot step^{-1}$) and frequency ($steps \cdot s^{-1}$) were used to assess sprint performance as they have been shown to be amongst the most reliable for NMT ergometry (Chapter 3).

MSS simulation

MSS activity was simulated using the LIST (Figure 4.2), as previously described by Nicholas et al. (2000). This test has been shown to elicit a similar physiological response to that observed during a soccer match (Nicholas et al., 2000), and more importantly has been shown to yield changes in systemic and functional markers of EIMD similar to those observed in the days after a soccer match (Magalhaes et al., 2009). The test was completed on a wooden floor over 20 m, identified by cones and floor markings. A set pattern of exercise was performed for 90 min, with running and walking speeds dictated by an audio CD throughout the test. Trials were conducted in temperatures of between 18 and 22˚C.

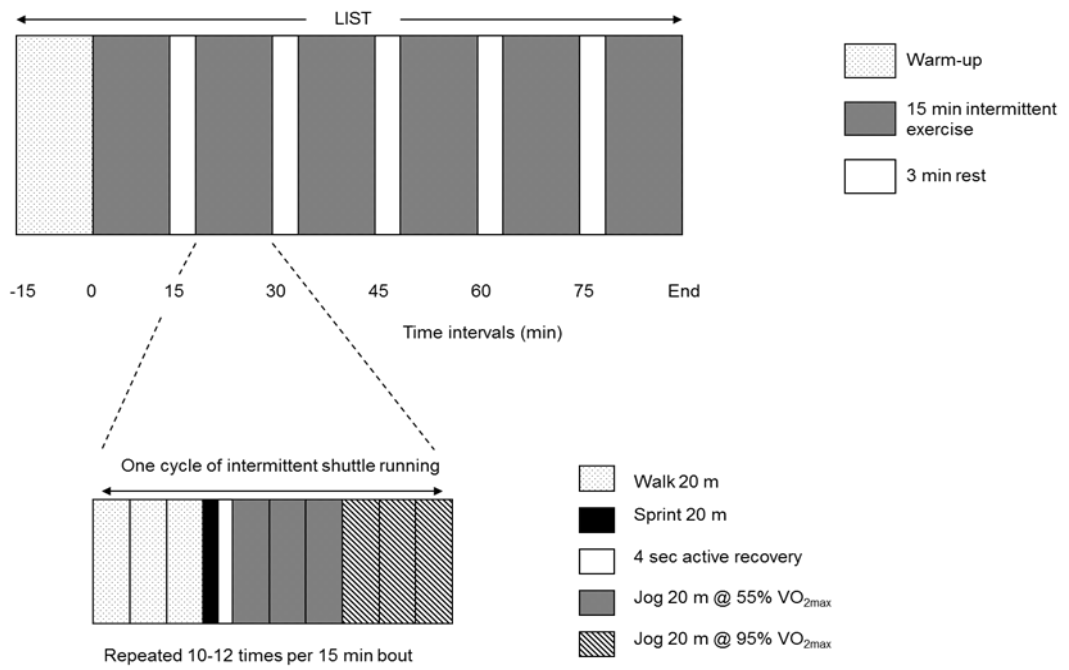

Figure 4.2. Schematic of the Loughborough Intermittent Shuttle Test

Beverage composition and ingestion schedule

Participants arrived for the completion of the LIST protocol following a 12 h overnight fast, during which time only water was allowed. Thereafter, participants ingested one of three beverages during and in the four hours after completing the LIST. The make-up of each drink is presented in Table 4.2. The CHO-P beverage contained 7.5% CHO (in the form of dextrose and maltodextrin) via a commercially available CHO-electrolyte powder (Lucozade Sport, GlaxoSmithKline) and 2.5% whey protein isolate (Volactive UltraWhey 90 XP Instant), the amino acid profile of which is shown in Table 4.3. During the LIST, participants consumed approximately 5.6 ml·kg^{-1} bw $^{-1}$·h^{-1} of their allocated test beverage, whilst a 200 ml bolus was provided one hour before exercise. In the 4 h of recovery, participants consumed this beverage at 15 min intervals at a rate of 0.9 g·kg^{-1} body mass for CHO and 0.3 g·kg^{-1} body mass for protein. This rate of ingestion (i.e. 1.2 g·kg^{-1} body mass·h^{-1}) was selected in accordance with the guidelines for CHO

consumption for maximal post-exercise glycogen resynthesis (Betts & Williams, 2010; Jentjens & Jeukendrup, 2003b). The ISOCHO beverage was matched for CHO content with the CHO-P beverage and thus contained 7.5% CHO. Again, this was ingested at 15 min intervals over the 4 h after the LIST, with a CHO ingestion rate of 0.9 $g \cdot kg^{-1}$ bw^{-1}. The ISOEN beverage was matched for energy content with the CHO-P beverage, with a concentration of 10% CHO, and yielded a CHO intake of 1.2 $g \cdot kg^{-1}$ bw^{-1}. Thus, drink concentrations were within the limits associated with enhanced performance without undue gastrointestinal distress (Coggan & Coyle, 1991). Accordingly, a hypothetical 70 kg participant consumed either 252 g CHO, 336 g CHO, or 252 g CHO + 84 g protein in the ISOCHO, ISOEN and CHO-P groups respectively, during the post-LIST recovery period, whilst total fluid intake was ~ 3.4 l.

Table 4.2 Selected nutrient content of supplement drinks per 100 ml

Ingredient	**CHO-P**	**ISOEN**	**ISOCHO**
CHO (g)	7.5	10	7.5
Protein (g)	2.5	0	0
Fat (g)	0.025	0	0
Sodium (g)	0.05	0.05	0.04

Table 4.3 Amino acid profile of the whey protein supplement

Amino Acid	%
Alanine	5.0
Arginine	2.1
Aspartic acid	11.0
Cystine	2.2
Glutamic Acid	18.1
Glycine	1.4
Histidine	1.7
Isoleucine*	6.4
Leucine*	10.6
Lysine	9.6
Methionine	2.2
Phenylalanine	.03
Proline	5.5
Serine	4.6
Threonine	6.7
Tryptophan	1.4
Tyrosine	2.6
Valine*	5.9

* Branched Chain Amino Acid

Statistical Analysis

All descriptive statistics are presented as means ± standard deviation (SD). To assess changes in each of the dependent variables between groups, all variables (with the exception of muscle soreness and step length and frequency) are expressed as a percentage of the values attained at baseline. However, raw values (mean ± SD) are presented in Table 4.4 for reference. To assess changes in perceived muscle soreness, jump height and sprint performance, a two-way (group [3] x time [3]) mixed factorial analyses of variance (ANOVA) was performed for each variable. For muscle function (peak torque), a three-way ANOVA (group x angular velocity [2] x time) was applied. Homogeneity of variance was assessed via Levene's test, whilst sphericity was assessed via Mauchly's test of sphericity, with any violations accounted for via the Greenhouse-Geisser adjustment to the degrees of freedom. Where any statistically significant *F* ratios

were found, post-hoc analysis consisting of Bonferroni adjusted (P = 0.025) paired sample t-tests were utilised to isolate where the differences lay. The alpha level for the ANOVAs was set at P = 0.05.

4.3 Results

The mean baseline values for all dependent variables were not significantly different (P > 0.05) between groups. Likewise, the mean energy, CHO, protein and fat intakes were not significantly (P < 0.05) different between groups over the period of testing.

Perceived muscle soreness

There was a significant main effect for time on perceived muscle soreness in both the knee extensors ($F_{2, 48}$ = 31.93, P < 0.05) and flexors ($F_{2, 46}$ = 86.38, P < 0.05) after completion of the LIST. There was no main effect for group or a group x time interaction (P > 0.05). Values for the knee extensors were significantly elevated (P < 0.025) at 24 (t_{26} = -8.304) and 48 h (t_{26} = -3.84) in all groups (Figure 4.3), as were values for knee flexors (t_{26} = -11.834 and t_{26} = -7.986).

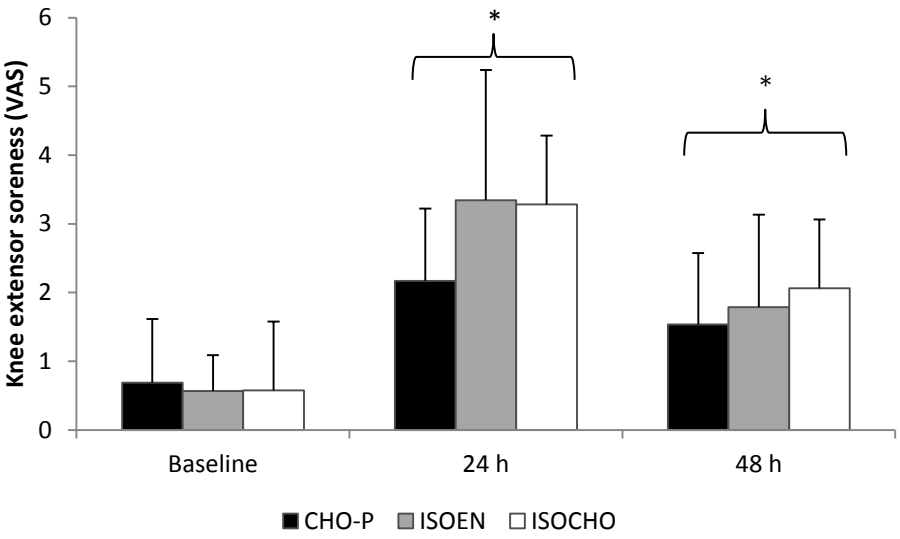

Figure 4.3 Changes in muscle soreness of the knee extensors following completion of the LIST. * Significantly different to baseline ($P < 0.025$).

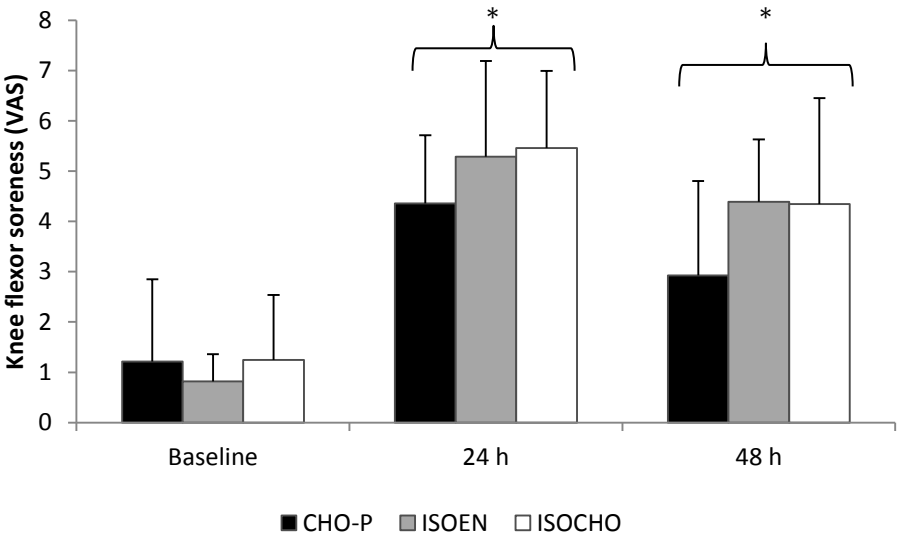

Figure 4.4 Changes in perceived muscle soreness of the knee flexors following completion of the LIST. * Significantly different to baseline ($P < 0.025$).

Isokinetic muscle function

Whilst the effect of time on peak torque of the knee extensors was significant ($F_{2, 48}$ = 39.61, $P < 0.05$), the effects of group, group x time and group x time x angular velocity were not ($P > 0.05$). There was, however, a significant effect for both angular velocity ($F_{1, 2}$ = 38.43, $P < 0.05$) and angular velocity x time interaction ($F_{2, 4}$ = 11.18, $P < 0.05$). Post-hoc analysis showed that peak torque decrements overall were significantly ($P < 0.025$) higher at 60 than 240 deg·s^{-1}, and that reductions at 60 deg·s^{-1} were evident at both 24 (t_{26} = 8.29) and 48 h (t_{26} = 6.89, $P < 0.025$; Figure 4.5), but only at 24 h for the 240 deg·s^{-1} velocity (t_8 = 3.2, $P < 0.025$).

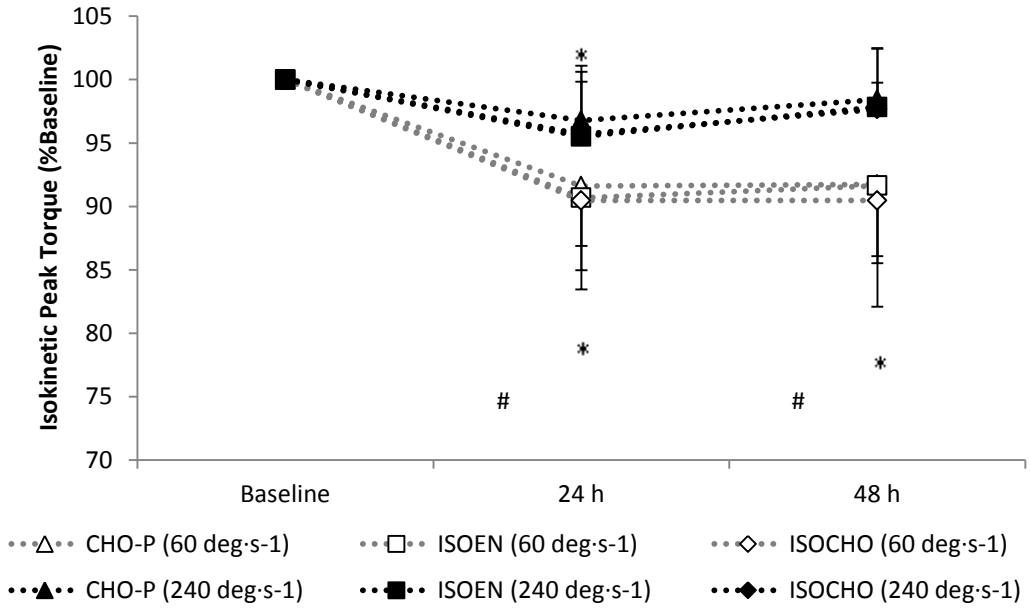

Figure 4.5. Changes in peak isokinetic torque of the knee extensors after completion of the LIST. * Significantly different from baseline ($P < 0.025$). #Significantly different between speeds ($P < 0.025$).

Similarly, peak torque values for the knee flexors decreased over time ($F_{2, 48}$ = 35.94, P < 0.05), but did not differ between groups (P = 0.51) or due to the group x time interaction (P = 0.76). There was an effect of angular velocity (F_2 = 37.65, P < 0.05), and an angular velocity x time interaction ($F_{2, 4}$ = 14.2, P < 0.05). As for knee extension, post-hoc analysis showed that the peak torque decrements during knee flexion overall were significantly (P < 0.025) higher at 60 than 240 deg·s^{-1}, and peak torque values were significantly (P < 0.025) lower than baseline at both 24 (t_{26} = 11.25) and 48 h (t_{26} = 5.85) at 60 deg·s^{-1}, but only at 24 h (t_{26} = 4.44) for the higher angular velocity (Figure 4.6).

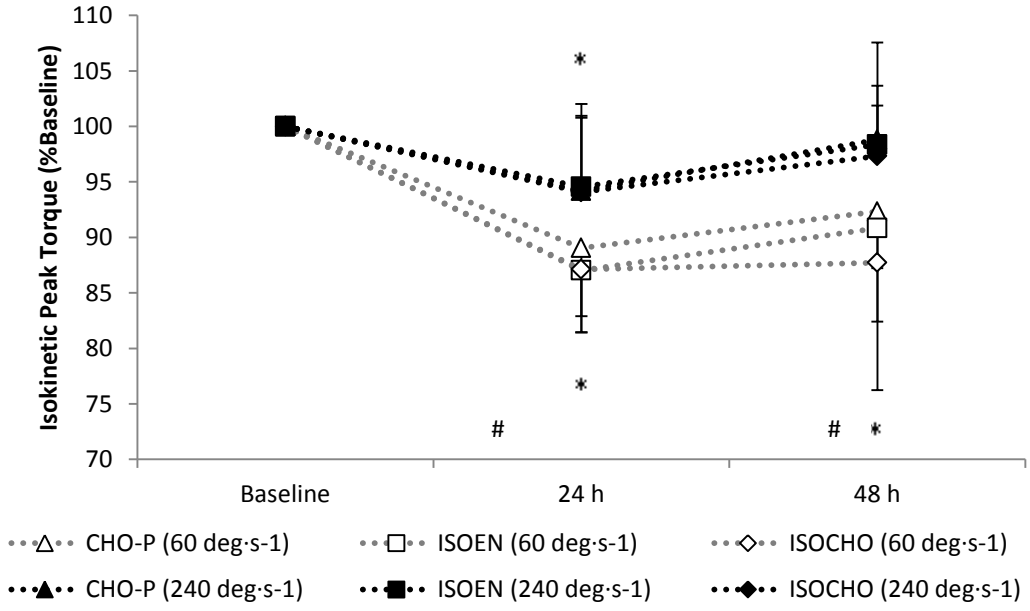

Figure 4.6. Changes in peak isokinetic torque of the knee flexors following completion of the LIST. * Significantly different from baseline (P < 0.025). #Significantly different between speeds (P < 0.025).

Vertical jump performance

Significant main effects of time were observed on SJ ($F_{2, 48}$ = 31.65, P < 0.05), CMJ ($F_{(GG)\ 2,\ 23.367}$ = 27.63, P < 0.05) and DJ ($F_{2,\ 48}$ = 15.497, P < 0.05) heights. The main effects of group and the group x time interactions were not significant for any of the three jumps (P > 0.05). Post-hoc analysis revealed that the overall heights for each type of jump were significantly (P < 0.025) lower than baseline at both 24 and 48 h (Figures 4.7-4.9).

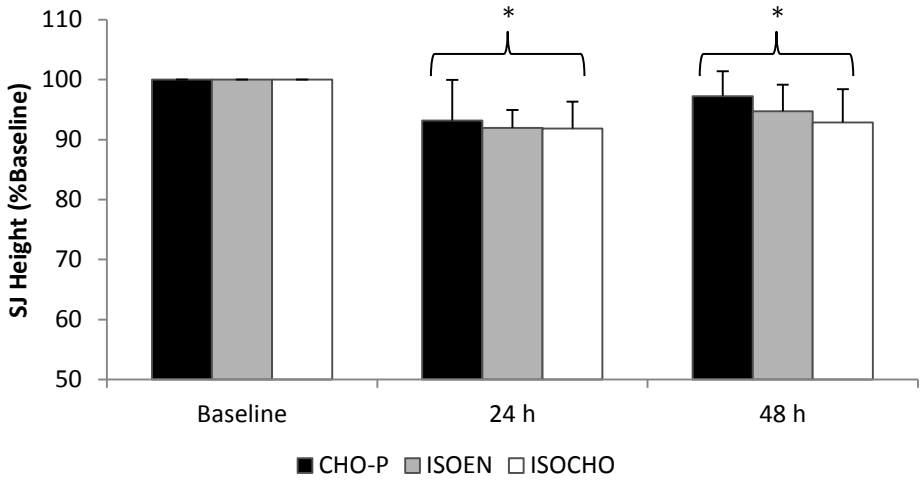

Figure 4.7. Changes in SJ height after completion of the LIST. * Significantly different to baseline (P < 0.025)

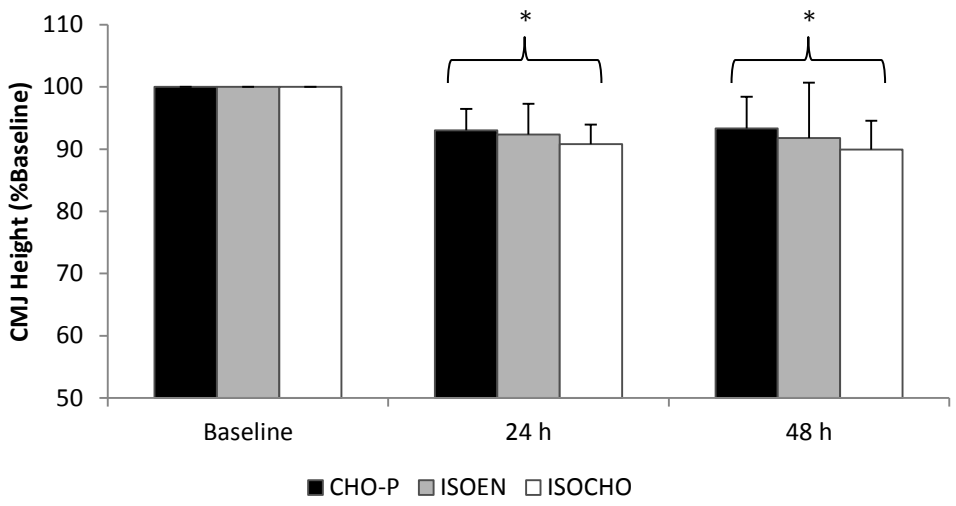

Figure 4.8. Changes in CMJ height after completion of the LIST. * Significantly different to baseline ($P < 0.025$)

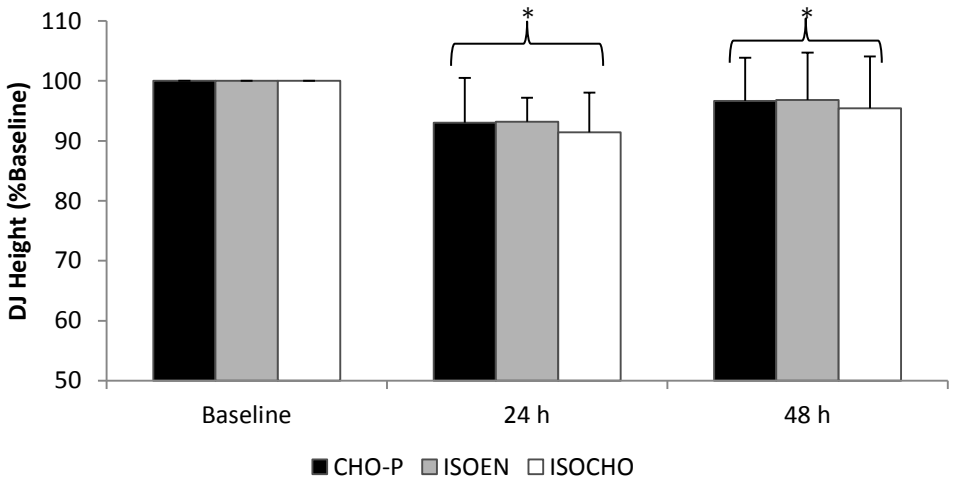

Figure 4.9. Changes in DJ height after completion of the LIST. * Significantly different to baseline ($P = 0.025$)

Sprint performance

Sprint times varied significantly over time at 10 ($F_{2, 48} = 13.81$, $P < 0.05$), 20 ($F_{2, 48} = 20.10$, $P < 0.05$) and 30 m ($F_{2, 48} = 21.45$, $P < 0.05$), but not there was no effect of group

and no time x group interaction for any of the distances ($P > 0.05$). Overall, 10 m sprint times were increased from baseline at 24 h ($t_{26} = 5.85$, $P < 0.05$), but not significantly at 48 h (Figure 4.10). Sprint times over 20 and 30 m were significantly ($P < 0.025$) elevated at both 24 and 48 h. Similarly, peak NMT speed over 30 m was affected by time ($F_{2, 48} = 11.33$, $P < 0.05$), but not by group, or by the interaction of group and time ($P > 0.05$) Post-hoc analysis identified that peak NMT speed was significantly slower than baseline at 24 h ($P < 0.025$), but not at 48 h.

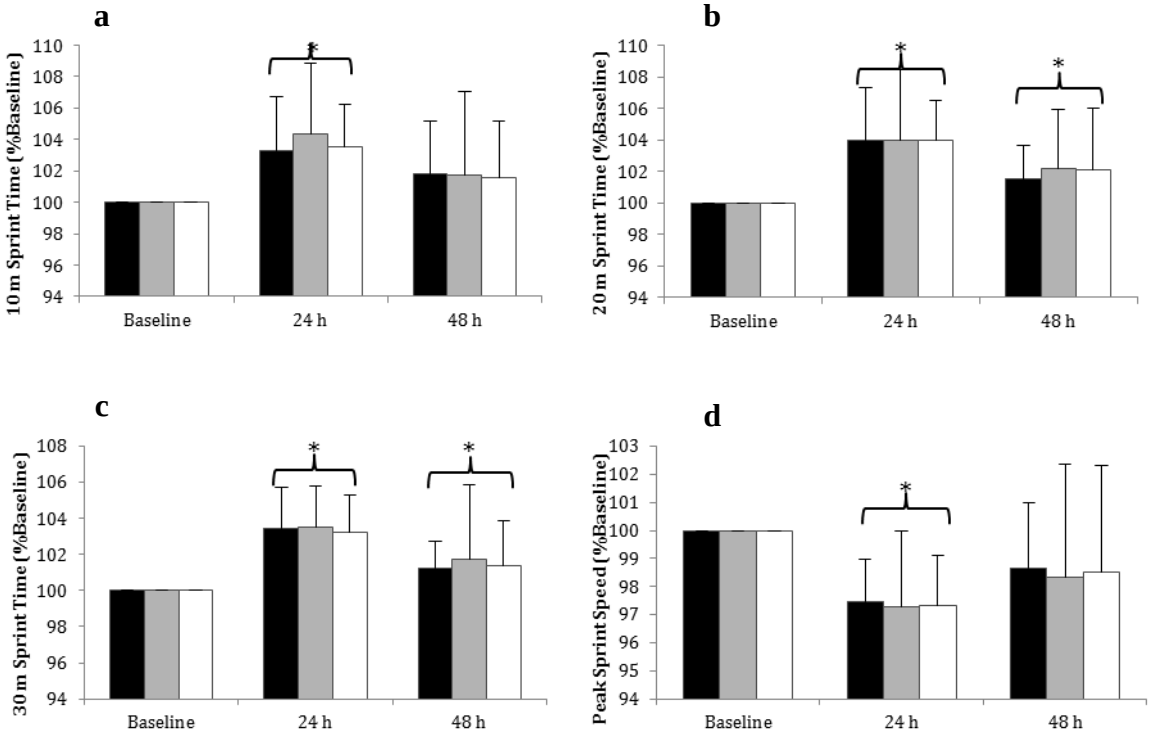

Figure 4.10. Changes in a) 10 b) 20 and c) 30 m sprint time and d) 30 m peak speed for CHO-P (■), ISOEN (■) or ISOCHO(□).

Whilst mean values were lower at 24 h in all groups, step length showed no main effect for time ($F_{2, 48} = 0.25$, $P > 0.05$), group ($F_{2, 24} = 0.04$, $P > 0.05$) or group x time interaction ($F4, 48 = 0.07$, $P > 0.05$). Similarly, despite a mean decrease in step frequency, no effect

of time ($F_{2, 48}$ = 1.7, P > 0.05), group ($F_{2, 24}$ = 0.4, P > 0.05), or group x time interaction ($F_{4, 48}$ = 0.1, P > 0.05) was evident (Figure 4.11).

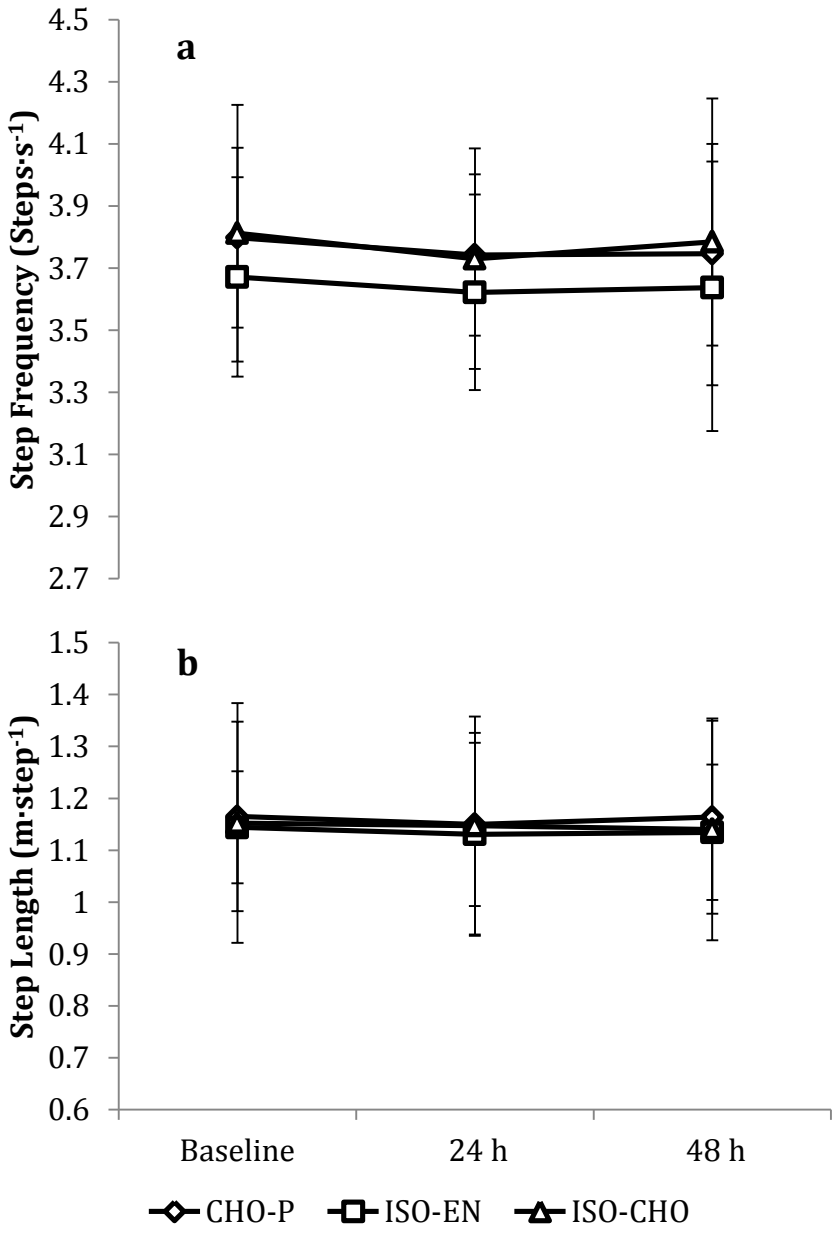

Figure 4.11. Changes in a) step frequency and b) step length after the LIST.

Table 4.4. Means ± standard deviation of isokinetic peak torque values (N·m-1) before and after completing the LIST

	Baseline			24 h			48 h		
	CHO-P	**ISOEN**	**ISOCHO**	**CHO-P**	**ISOEN**	**ISOCHO**	**CHO-P**	**ISOEN**	**ISOCHO**
Extension 60 deg·s^{-1}	190 ± 47.5	212.5 ± 64.7	208.5 ± 75.5	174.5 ± 47.2	194.5 ± 64.9	186 ± 60	175.5 ± 48.8	196.1 ± 63.4	190.9 ± 77.2
Flexion 60 deg·s^{-1}	112 ± 29.6	110.5 ± 37.4	116.2 ± 40.8	100.4 ± 29.6	96.4 ± 34.9	100.6 ± 35.2	104.2 ± 31.4	100.9 ± 37.3	102.7 ± 40.8
Extension 240 deg·s^{-1}	121 ± 29.7	129 ± 37.2	131.6 ± 50.7	117.3 ± 32.1	123.2 ± 36.1	125.4 ± 48.1	119.1 ± 32.1	126 ± 36.4	128.2 ± 48.3
Flexion 240 deg·s^{-1}	81.5 ± 19	75.3 ± 26.4	79.1 ± 31.6	76.7 ± 19.4	70.6 ± 23.8	73.1 ± 26.4	80.6 ± 20.5	74.2 ± 26.4	76.4 ± 28.9

Table 4.5. Means ± standard deviation of jump heights and sprint speeds before and after completing the LIST

	Baseline			24 h			48 h		
	CHO-P	**ISOEN**	**ISOCHO**	**CHO-P**	**ISOEN**	**ISOCHO**	**CHO-P**	**ISOEN**	**ISOCHO**
Jumps									
SJ (cm)	27.6 ± 5	32 ± 5.6	29.2 ± 8.1	25.8 ± 5.5	29.4 ± 5.5	26.7 ± 7	26.8 ± 4.4	30.2 ± 5.3	26.9 ± 7
CMJ (cm)	30.1 ± 6.7	34.4 ± 6	33.2 ± 9.8	28.0 ± 6.3	31.8 ± 6.3	30 ± 8.3	28.2 ± 7.1	31.6 ± 6.9	29.7 ± 8.7
DJ (cm)	30.4 ± 6.9	33.7 ± 5.7	31.9 ± 10.4	28.2 ± 6.1	31.4 ± 5.5	28.9 ± 8.4	29.4 ± 7	32.8 ± 6.8	29.9 ± 8.1
Sprints									
10 m (s)	2.62 ± 0.4	2.75 ± 0.3	2.62 ± 0.4	2.70 ± 0.4	2.87 ± 0.3	2.72 ± 0.4	2.66 ± 0.4	2.81 ± 0.4	2.67 ± 0.39
20 m (s)	4.70 ± 0.7	4.90 ± 0.6	4.70 ± 0.7	4.89 ± 0.87	5.12 ± 0.8	4.89 ± 0.8	4.77 ± 0.7	5.03 ± 0.7	4.79 ± 0.7
30 m (s)	6.82 ± 1.1	7.10 ± 1	6.89 ± 1.1	7.07 ± 1.3	7.40 ± 1.1	7.11 ± 1.5	6.91 ± 1.1	7.28 ± 1.1	6.97 ± 1.1
Peak Speed ($m \cdot s^{-1}$)	5.08 ± 0.8	4.84 ± 0.7	5.12 ± 0.9	4.96 ± 0.8	4.71 ± 0.8	4.98 ± 0.9	5.01 ± 0.8	4.77 ± 0.8	5.02 ± 0.8

4.4 Discussion

The observed changes in perceived muscle soreness, isokinetic peak torque, jump height and sprint times in the current study provide indirect evidence that EIMD was present in the days after completion of the LIST. Perceived muscle soreness in the knee extensors and flexors was increased, peaking at 24 h, and remaining elevated at 48 h. However, it is noteworthy that perceptions were not influenced by the type of beverage consumed, though they were consistently lower in the knee extensors and flexors in the CHO-P group (by approximately 17%) than the ISOEN and ISOCHO groups. Similarly, vertical jump performances and peak torque values were diminished 24 h after the LIST, and generally remained lower than baseline values at 48 h, as did NMT peak speed and sprint performances over 10, 20 and 30 m. However, unlike perceived muscle soreness, there were no discernable trends with respect to the type of beverage consumed.

Previously, Millard-Stafford et al. (2005) reported that muscle soreness of the legs 24 h after a 21 km run was approximately 50% lower when participants were provided with a CHO-P supplement 2 h post-exercise than when they consumed a CHO beverage matched for energy content. Similar reductions in muscle soreness have been reported elsewhere (Saunders et al., 2009; Skillen et al., 2008; Luden et al., 2007; Romano-Ely et al., 2006). However in the studies of Luden et al., (2007) and Romano-Ely et al. (2006), antioxidants were also provided in the CHO-P supplement, which have been shown to attenuate muscle soreness in some cases (Thompson et al., 2001a). Whilst muscle soreness is thought to relate poorly with the magnitude and time-course of EIMD (Nosaka et al., 2002), there is evidence that increases in muscle soreness can alter an individual's perception of effort during subsequent exercise (Proske et al., 2004), and potentially cause neuromuscular

inhibition of maximal muscle activation and subsequently reduced force output (Horita et al., 1999). Whilst effort perceptions were not recorded in the current study, such implications are important as it is thought they inform pacing strategies during endurance exercise (St Clair Gibson et al., 2006), whilst many sporting movements require high degrees of muscle force, including actions common in MSS such as sprinting and jumping (Rampinini et al., 2011; Mohr et al., 2003). Thus, a supplement which can reduce perceived muscle soreness may have benefits for subsequent athletic performance in the days following MSS exercise.

Soreness associated with EIMD is thought to originate from activation of unmyelinated group IV afferent fibres in response to an accumulation of chemical and noxious stimuli associated with inflammation and degradation of muscle proteins (Byrnes & Clarkson, 1986; Armstrong, 1984). Therefore, if acute protein ingestion could prevent muscle protein degradation and subsequent inflammation, it is reasonable to assume that there would be a concomitant reduction in perceived muscle soreness. However, the current data showing no effect of CHO-P ingestion on muscle function, or indeed jump and sprint performance, suggests that muscle soreness was not reduced via an attenuation in EIMD. As such, the differences in soreness in the present study may have been due to inter-subject variations in perception of pain (Ebbeling & Clarkson, 1989). To this end, and in agreement with the current findings, several other studies have reported that acute CHO-P ingestion does not significantly alter perceived muscle soreness when compared to either CHO (Breen et al., 2010; Betts et al., 2009; Cockburn et al., 2008; Green et al., 2008) or water/placebo (Cockburn et al., 2008; Green et al., 2008).

The present study indicates that participation in the LIST results in a significant reduction in muscle function 24 and 48 h post-exercise. This was evidenced by reductions in isokinetic peak torque of the knee extensors and flexors, regardless of CHO-P, ISOEN, or ISOCHO ingestion. Such decrements (CHO-P ~ 8.4 and 10.9%, ISOEN ~ 9.4 and 13%, ISOCHO ~ 9.5 and 12.9%, respectively) are within the range (10-20%) previously reported following a soccer match (Magalhaes et al., 2009) and the LIST (Magalhaes et al., 2009; Bailey et al., 2007; Thompson et al. 2001a), but lower than values of ~40% after high-load eccentric isokinetic contractions (Byrne et al., 2004). Moreover, the greater change in force, and indeed muscle soreness, in the knee flexors compared to the extensors following the LIST is consistent with previous studies (Bailey et al., 2007), and likely reflects their susceptibility to EIMD following MSS activity due to their smaller muscle mass (Byrne et al., 2004). Interestingly, it was notable that the decrements in muscle function were more pronounced at a slower angular velocity of movement. These findings support previous observations that strength loss following eccentric exercise is greater at lower angular velocities of movement (Michaut et al., 2002; Deschenes et al., 2000; Gibala et al., 1995), and may be due to a possible force-related mechanism leading to an inverse relationship between activation failure and the velocity of movement (Michaut et al., 2002). Furthermore, slower movement velocities, which demonstrate a greater capacity to generate force, might be regulated by the intensity of volitional activation against resistance and not ultimately by the velocity of movement (Deschenes et al., 2000), whilst neural inhibition might also be more pronounced in such actions in an attempt to prevent further damage (Westing et al., 1991).

Given that measurements of muscle function are thought to provide the most suitable gauge of the time-course and magnitude of EIMD (Warren et al., 1999), this study indicates that the ingestion of a CHO-P supplement before, during and after the LIST does not attenuate EIMD. This concurs with studies that have reported no change in systemic markers of EIMD following CHO-P ingestion (Stock et al., 2010; Betts et al., 2009; Breen et al., 2009; Pritchett et al., 2009; Saunders et al., 2009; Green et al., 2008; White et al., 2008; Millard-Stafford et al., 2005; Wojcik et al., 2001), but is in contrast to several studies that have reported lowered systemic markers of EIMD following CHO-P ingestion, including CK (Pritchett et al., 2009; Romano-Ely et al., 2008; Cockburn et al., 2008; Skillen et al., 2008; Valentine et al., 2008; Baty et al., 2007; Luden et al., 2007; Rowlands et al., 2007; Saunders et al., 2007; Seifert et al., 2005; Saunders et al., 2004), lactate dehydrogenase (Romano-Ely et al., 2006), and myoglobin (Ferguson-Stegall et al., 2010; Cockburn et al., 2008; Valentine et al., 2008; Baty et al., 2007; Seifert et al., 2005).

To the author's knowledge, there are only two published studies that have reported alterations in post-exercise muscle function with CHO-P ingestion versus CHO alone. Cockburn et al. (2008) reported that isokinetic peak torque of the knee flexors was significantly higher at 48 h following 6 x 10 eccentric contractions in a group that consumed a CHO-P milkshake (approximately 125 $N \cdot m^{-1}$) versus CHO-only (approximately 80 N·m-1) or water (approximately 85 $N \cdot m^{-1}$). Similarly, Valentine et al. (2008) reported that the consumption of a CHO (7.8%) and protein (1.94%) solution resulted in an improvement in the maximal amount of repetitions participants were able to perform at 70% of their 1-repetition maximum 24 h following a cycle to exhaustion at 70% VO_{2max}. However, the precise mechanism through which ingestion of CHO and protein can reduce

EIMD is unclear and not well understood. It is known though that muscle protein synthesis is depressed for several hours after injury (Lowe et al., 1995), whilst protein degradation is elevated for several days post-injury (Ingalls et al., 1998; Lowe et al., 1995), both of which are likely to impair the functional restoration rate of the muscle. It is also thought that the combination of an increased availability of amino acids, in conjunction with an increase in pancreatic insulin release associated with CHO-P ingestion (van Loon et al., 2000), results in an anabolic environment favouring increased rates of protein synthesis and decreased rates of protein degradation (Bird et al., 2006; Borsheim et al., 2004; Miller et al., 2003; Levenhagen et al., 2002; Roy et al., 2000). This positive protein balance, in turn, may attenuate the contractile protein loss and myofibrillar disruption following exercise (Bird et al., 2006). Whilst this chain of events provides a plausible explanation for the observed lessening of markers of EIMD in previous studies, it should be noted that a further five studies have reported no effect of CHO-P ingestion on muscle function following EIMD (Breen et al., 2010; Betts et al., 2009; Green et al., 2008; White et al., 2008; Wojcik et al., 2001). Of particular note are the findings of Betts et al. (2009), who, as with the current study, observed no differences in muscle function, albeit assessed isometrically, in the two days following the LIST between the ingestion of CHO-P and an CHO-only beverage. The current study has shown this to be the case even when compared to what is considered to be a sub-optimal, in terms of the recommendations for maximal muscle-glycogen resynthesis (Betts & Williams, 2010; Jentjens & Jeukendrup, 2003), amount of CHO (i.e. 0.8 $g \cdot kg^{-1}$ bw^{-1}) is consumed post-exercise.

The discrepancies in the findings of the above studies are, in part, likely due to methodological inconsistencies between studies reporting a positive or no effect. For

example, several different types of protein have been administered for post-exercise recovery, including whole proteins such as whey isolate (Ferguson-Stegall et al., 2010; Betts et al., 2009; Green et al., 2008; Valentine et al., 2008; Luden et al., 2007; Millard-Stafford et al., 2005), whey hydrolysate (Breen et al., 2010) and casein (Gilson et al., 2010; Saunders et al., 2009; Cockburn et al., 2008; Pritchett et al., 2008; Baty et al., 2007). Additionally, some studies have provided individual branched chain or essential amino acids (BCAAs and EAAs respectively; Stock et al., 2010; Skillen et al., 2008; Bird et al., 2006). It is well-established that different proteins will elicit a markedly different insulin response (van Loon et al., 2000), and will release amino acids into the blood at different rates (Dangin et al., 2001; Boirie et al., 1997), whilst long term casein supplementation can induce greater protein synthesis and muscle hypertrophy than whey (Boirie et al., 1997). However, on the basis that several studies have reported attenuations in EIMD with whey (Gilson et al., 2010; Saunders et al., 2009; Valentine et al., 2008; Luden et al., 2007; Saunders et al., 2007; Romano-Ely et al., 2006; Millard-Stafford et al., 2005; Saunders et al., 2004), casein (Cockburn et al., 2008; Baty et al., 2007) and EAAs (Skillen et al., 2008; Bird et al., 2006) whilst others have not (Breen et al., 2010; Gilson et al., 2010; Stock et al., 2010; Betts et al., 2009; Green et al., 2008; Pritchett et al., 2008; White et al., 2008), there appears to be no pattern based on protein type. Furthermore, the amounts of protein provided in studies have varied, ranging from 0.17 to 0.4 $g \cdot kg \cdot h^{-1}$. Although the optimal amount of protein in a post-exercise supplement is currently unknown, effects have been observed with as little as 0.18 $g \cdot kg \cdot h^{-1}$ (Saunders et al., 2004), whilst no effect has been observed with 0.4 $g \cdot kg \cdot h^{-1}$ (Betts et al., 2009). As such, different protein amounts do not appear to account for the differences in EIMD response in CHO-P studies.

As previously alluded to, the timing of a protein supplement has the potential to affect EIMD response. A supplement provided before and during exercise may elicit increased amino acid delivery (Tipton et al., 2001) and a protective effect of amino acids on muscle disruption during exercise (White et al., 2008), whilst CHO-P intake immediately after exercise will increase amino acid uptake compared to 3 h later (Levenhagen et al., 2001). Furthermore, CHO-P supplementation during long-duration endurance exercise has been shown to enhance protein balance via both reduced protein degradation and increased protein synthesis (Koopman et al., 2004). However, White et al. (2008) have reported that the time-course and magnitude of EIMD is not different when CHO-P is consumed either during or after exercise, albeit after resistance exercise. As the current study has revealed no benefit of CHO-P consumed before, during and after exercise, the potential for timing of the beverage to affect EIMD response is unclear.

One further potential cause of the equivocal findings is the magnitude of the decrement in muscle function associated with EIMD. Cockburn et al. (2008) reported decrement of ~40% (compared to the 8 – 13% in the current study), implies that a greater disruption to myofibrillar proteins is required to elicit a benefit of CHO-P ingestion. Yet, other studies have reported similarly large decrements in muscle function with little effect of CHO-P supplementation (White et al., 2008; Wojcik et al., 2001). Nonetheless, this study would suggest that acute CHO-P ingestion confers little benefit with regard to muscle function or EIMD post-MSS exercise.

A further extension on the work of Betts et al. (2009), and indeed previous studies examining the effects of CHO-P and EIMD, is the observation that decrements in selected measures of performance following the LIST are not affected by CHO-P ingestion, compared to CHO alone. Whilst measurements of muscle function may be considered appropriate for the assessment of EIMD, they bear little resemblance to the more dynamic, multi-joint movements associated with athletic performance. In the current study SJ and CMJ height were significantly reduced at both 24 (SJ = 6.9, 8 and 8.2%; CMJ = 7, 7.7 and 9.2%) and 48 h (SJ = 2.8, 3.2 and 7.1%; CMJ = 6.7, 8.3, 10.1%) in the CHO-P, ISOEN, and ISOCHO groups respectively. Similarly, DJ height was significantly reduced at 24 h and 48 h (7-9%). These decrements are similar to those previously reported after the LIST and a soccer match (Magalhaes et al., 2009). Ostensibly, the present data suggest that the LIST induces similar decrements in jump performance that regardless of whether the stretch shortening cycle (SSC) is used or not, which is surprising as Byrne & Eston (2002) have previously reported that the SJ (which does not utilize the SSC) is more susceptible to reductions due to EIMD. However, a factor might be the mode of muscle damaging exercise, as Byrne & Eston (2002) employed 100 leg squats that induced a 20% reduction in leg strength, much greater than that observed in the current study (10%).

Finally, the current study demonstrated reductions in sprint performance from 10-30 m, as well as peak speed attained over 30 m, which did not vary due to the ingestion of CHO-P, ISOEN and ISOCHO. These reductions (2-4%) are consistent with the 5% reduction in short-distance over-ground sprint performance associated with EIMD following soccer (Magalhaes et al., 2009; Ascensao et al., 2008; Krustrup et al., 2006) and the LIST (Magalhaes et al., 2009) previously reported. It is interesting that the observed

decrements are similar to those reported during over ground running considering the apparent lack of validity of the NMT, whereby participants were observed to attain a speed that, on average, was 30% lower than they did over ground (Chapter 3). However, this data may support the previous assertion that the NMT possesses 'relative' validity, whereby those who are faster over ground are typically faster on the NMT (Chapter 3). As such, the participants' sprint times in the current study were probably lower than they would have attained over ground, but the effect of EIMD would have been similar. In addition, the observed decrements in the current 20 and 30 m sprint performances only at 48 h is likely due to the 10 m distance being the least reliable of the three performed (Chapter 3).

The reduction in sprint performance associated with EIMD is thought to be due to several potential mechanisms, including reduced muscle force output and reduced reflex sensitivity, due to muscle soreness and thereby a decline in the ability to utilise the SSC, and fluctuations in stride length and frequency that are associated with muscle stiffness and a reduced range of motion (Highton et al., 2009). In the present study, no significant difference was observed between step length and step frequency over time, although mean values were reduced at each visit. These differences amounted to an approximate 1% change in performance, which according to data from Chapter 4, would have required a sample size of approximately 80 (Atkinson et al., 1999) to detect a significant difference. Thus, the lack of significant change in step length and frequency in the present study was probably due to insufficient sample size. The absence of a performance effect between supplement groups in the current study is likely to be a direct consequence of the lack of an effect of CHO-P on EIMD and thus muscle function. Given the importance of short-

distance sprint performance to MSS competition, nutritional supplements which can aid the recovery of muscle function and sprint performance are likely to be worthwhile. However, it would appear that acute CHO-P ingestion has no effect on sprint recovery following MSS exercise.

In conclusion, this study indicates that participation in simulated MSS causes EIMD and significant impairments in muscle function and maximal intensity exercise performance, such as jumping and sprinting. However, it would appear that acute CHO-P ingestion before, during and after this form of exercise confers little benefit in selected markers of EIMD and performance in the following days when compared to CHO consumption alone, even when CHO consumption is considered sub-optimal. These findings may endorse previous views on nutrition and muscle glycogen repletion, whereby immediate post-exercise nutrient intake is considered relatively unimportant when recovery time longer than 24 h between exercise bouts is available (Burke et al., 2004). The current findings reaffirm that either CHO *or* CHO with protein can be used as a post-exercise nutritional aid for MSS athletes. Nevertheless, athletes may still consider immediate post-exercise consumption of protein prudent, as delayed protein feeding by as little as 2 hours can reduce whole body and leg protein accretion (Levenhagen et al., 2001). Furthermore, such a beverage is likely to aid rehydration, and may promote muscle glycogen repletion (Berardi et al., 2006).

Chapter 5

The Effects of a CHO-P Beverage on Simulated Multiple-Sprint Sport Exercise

5.1 Introduction

MSSs are prolonged in nature (60-90 min), and are characterised by periods of high-intensity movement (i.e. sprinting and fast-running or 'cruising') interspersed with periods of low-intensity movement and rest. The average intensity of such sports is approximately 70% VO_{2max} (Bangsbo et al., 2007; Bangsbo et al., 2006; Esposito et al., 2004), with players typically covering distances between 6 and 12 km (Waldron et al., 2011; Bradley et al., 2009; Gabbett et al., 2008; Roberts et al., 2008; Rampinini et al., 2007; Randers et al., 2007; Scott & Drust, 2004), and as such they place a high demand on the aerobic capacity of their players. In addition to this high aerobic activity, players will perform approximately 20-40 sprints (Spencer et al., 2005a) and (in soccer) approximately 150-200 brief intense actions, such as jumping, tackling and kicking (Mohr et al., 2003), all of which will place a high demand on the anaerobic energy systems at certain times in a game.

A consequence of the above physiological/movement profile is that fatigue typically develops during MSSs. Several studies have reported that the total distance covered, and the time spent performing high-intensity work (>15 $km \cdot h^{-1}$), is lower in the second half of a MSS than in the first (Sykes et al., 2011; Waldron et al., 2011; Sirotic et al., 2009; Bangsbo et al., 2007; Burgess et al., 2006; Reilly & Thomas, 1976). More specifically, Mohr et al. (2003) reported that the distance covered both at high-intensity and sprinting is

significantly reduced in the last 15 min of a soccer game, similar to rugby league (Sykes et al., 2011; Waldron et al., 2011), Australian Rules football (Burgess et al., 2006) and basketball (Abdelkrim, et al., 2006).

Whilst the exact cause of such fatigue is unclear, it is known that the predominant fuel source during exercise at >70% VO_{2max}, muscle glycogen, can be depleted by 40-90% during the course of a soccer match (Krustrup et al., 2006; Leatt & Jacobs, 1989; Jacobs et al., 1982), and that significant proportions of type I (55%) and II (45%) fibres are almost completely or fully depleted of glycogen at the end of a game. Furthermore, Saltin (1973) reported that players covered a greater distance and spent more time sprinting when they entered a game with high-levels of muscle glycogen compared to a glycogen depleted state, and Krustrup et al. (2006) established that reduced muscle glycogen was associated with impaired 30 m sprint performance after a soccer match. Thus, muscle glycogen depletion is likely to be implicated in the aetiology of fatigue during MSSs.

The development of fatigue in MSSs could also be explained by exercise-induced fluid losses and subsequent dehydration. Soccer matches induce fluid losses up to 3-5 l depending on environmental conditions (Bangsbo, 1994), whilst a fluid loss of as little as 2% body mass has been shown to impair performance in the YO-YO intermittent recovery test immediately after 45 min of soccer match play (Edwards et al., 2007). Indeed, McGregor et al. (1999) have reported that 15 m sprint time in the last 15 min of a soccer simulation protocol was slower by approximately 4% when participants consumed no fluid compared to when they consumed 8 $ml \cdot kg \cdot h^{-1}$ of a sugar-free cordial. Other potential,

if speculative, mechanisms of fatigue during prolonged aerobic exercise and MSSs include central fatigue and reduced central drive, hypoglycemia, excitation-contraction coupling impairment, damage to skeletal muscle contractile proteins and ATP and PCr degradation (for reviews, see Abbiss & Laursen, 2005 and Bangsbo, 2006).

Importantly, it is now well established that the consumption of CHO during exercise of moderate to high-intensity (60-80% VO_{2max}) lasting > 1 h has the potential to delay fatigue and improve performance (Jeukendrup, 2004). Although the mechanism for such an effect is poorly understood, maintenance of euglycemia, muscle glycogen sparing and/or re-synthesis during low intensity activity, reduced central fatigue and increased central drive have all been implicated (Karelis et al., 2010). It should also be noted that, when consumed in beverage form in conjunction with electrolytes (such as sodium and potassium), a CHO supplement may serve to further aid hydration status (Brouns et al., 1992). Clear evidence demonstrates that consumption of a CHO-electrolyte solution enhances performance during exercise designed to simulate the demands of MSSs. Previously reported benefits include up to a 50% increase in prolonged intermittent shuttle running capacity (Foskett et al., 2008; Patterson & Gray, 2007; Welsh et al., 2002; Nicholas et al., 1995) as well as better maintenance of 15 m sprint (Ali et al., 2007), agility (Currell et al., 2009) and soccer skill performance (Currell et al., 2009; Ali et al., 2007). Over recent years, there has been a growing interest in the ergogenic potential of adding a relatively small amount of protein (<20 $g \cdot h^{-1}$) to a CHO solution, on the basis that it might enhance the stimulation of insulin release and protein oxidation, causing CHO sparing, reduced central fatigue and better maintenance of tricarboxylic acid intermediates (Saunders, 2007). However, empirical findings in this area have been equivocal, with studies reporting either enhanced exercise

capacity with the addition of protein (Saunders et al., 2009; Saunders et al., 2007; Ivy et al., 2004; Saunders et al., 2004) or no difference (Breen et al., 2010; Toone & Betts, 2010; Lee et al., 2008; van Essen & Gibala, 2006) when compared to CHO alone. These inconsistencies can probably be explained by methodological differences, not least with respect to the provision of optimal, or calorie-matched, amounts of CHO in beverages used for comparison. Nevertheless, to-date no study has examined the potential for CHO-P ingestion to enhance performance in MSS. Consequently, the aim of the current study was to examine the effects of a CHO-P beverage, in comparison to CHO alone, matched for both energy content and CHO content, on selected performance, physiological and perceptual measures during exercise simulating the typical physiological demands of MSSs.

5.2 Methods

Participants and Experimental Design

After the attainment of written informed-consent and the completion of a pre-test health questionnaire to check for any contraindications to exercise, 10 (males, n = 6, females n = 4) healthy university-level team-sport athletes (soccer, rugby and netball) participated in the study. It should be noted that endurance performance effects with CHO-P ingestion do not differ between males and females (Saunders et al., 2007). Participant characteristics are shown in Table 5.1. Ethical approval for the study was granted by the University of Chester Department of Sport and Exercise Sciences Ethics Committee.

Table 5.1 Participant characteristics.

Variable	Overall	Males (n = 6)	Females (n = 4)
Age (years)	21.7 ± 2.1	22.7 ± 1.8	20.3 ± 1.9
Stature (m)	1.77 ± 0.1	1.80 ± 0.1	1.73 ± 0.1
Body mass (kg)	72.0 ± 13	78.2 ± 12	62.7 ± 9.9
MSSRT score	11.3 ± 2.2	12.7 ± 1.4	9.2 ± 1.1
VO_2max ($ml \cdot kg^{-1} \cdot min^{-1}$)*	51 .0 ± 7.6	55.9 ± 4.9	43.7 ± 3.8

MSSRT = Multi-Stage Shuttle Run Test. * Estimated from the Tables of Ramsbottom et al. (1988).

The current study adopted a repeated-measures, double-blind, randomised cross-over design, where participants were required to attend testing sessions on four separate occasions. A full schematic of the study design is presented in Figure 5.1. In brief, participants performed a multi-stage shuttle run test (MSSRT; Leger & Gadoury, 1989; Leger & Lambert, 1982) followed by a 15 min familiarisation with the Loughborough Intermittent Shuttle Test (LIST; Nicholas et al., 2000) on the initial visit. They then completed the LIST on three separate days one week apart, where they consumed either a 6% CHO plus 2% whey protein (CHO-P), an 8% (ISOEN) or a 6% (ISOCHO) CHO-only beverage during each trial in a random order. Participants were instructed to refrain from any strenuous physical activity in the 48 h before any testing session, and to complete a 48 h food diary in the 48 h before the first LIST, which they were informed should be replicated in the 48 h preceding each subsequent trial. Daily dietary intake was 2127 ± 390 kcal, 311.3 ± 79 g CHO, 53.5 ± 12.2 g fat and 100.3 ± 23 g protein.

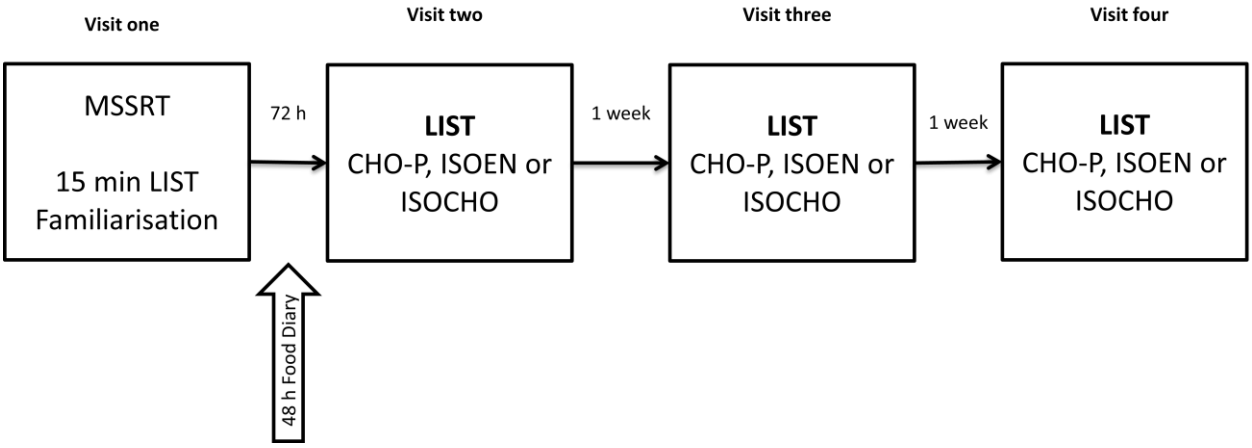

Figure 5.1. Schematic of the study design

Procedures

Multi-Stage Shuttle Run Test

The MSSRT for the estimation of aerobic capacity and running speeds for the LIST was completed in accordance with the procedures previously described in Chapter 4.2. Each participant's VO_2max was estimated using the Table of Ramsbottom et al. (1988) based on the end-stage achieved on the test.

The Loughborough Intermittent Shuttle Test

After a 12 h overnight fast, participants completed the LIST as previously described by Nicholas et al. (2000), and similar to that described in Chapter 4 of the thesis (Figure 5.2). However, a key difference to that used in Chapter 4 was the inclusion of Part B of the LIST, during which participants were required to run at alternating running speeds of 55% and 95% VO_2max until volitional exhaustion to provide a measure of prolonged, intermittent shuttle running capacity. Part A of the LIST consisted of 5 x 15 min periods of exercise

interspersed with 3 min passive recovery. The tests were conducted on an indoor wooden surface, in ambient temperatures of between 19 and 23°C.

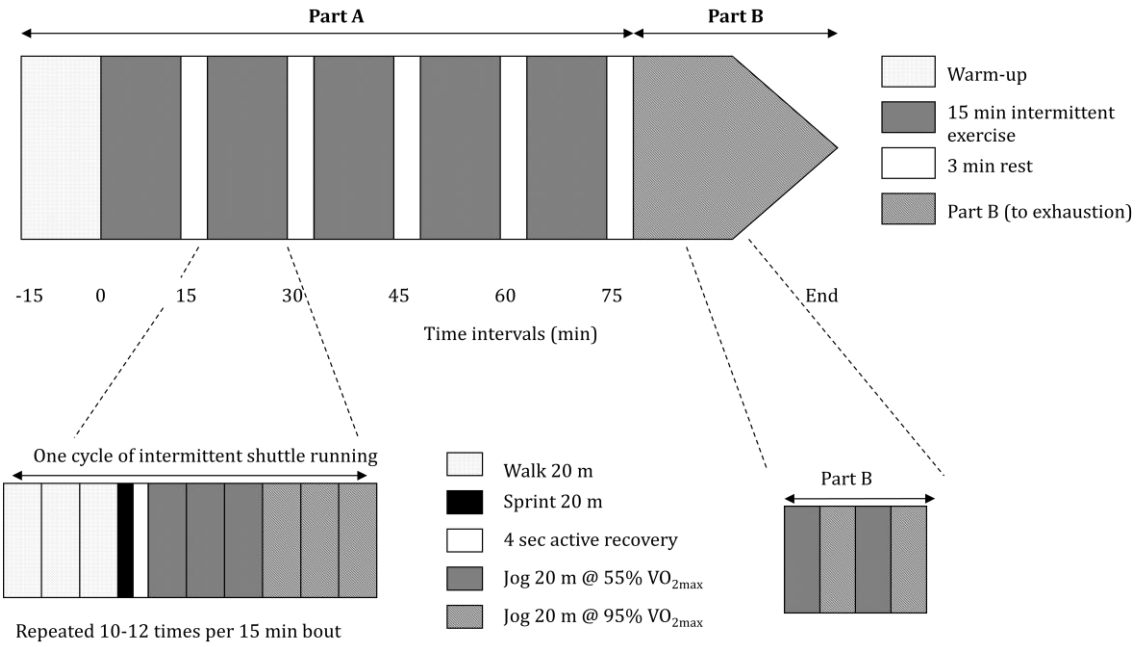

Figure 5.2 The Loughborough Intermittent Shuttle Test including a time to exhaustion test (Part B).

Nude body mass was recorded immediately before and after the LIST via balance beam scales (Seca, 712, Hamburg, Germany) to estimate fluid loss induced via exercise. Heart rate was recorded throughout the protocol via heart rate telemetry (Polar Electro, Oy, Finland), whilst measurements of gastrointestinal discomfort due to gut fullness (measured on a scale of 1-10 ranging from completely empty [1] to completely full [10]) and rating of perceived exertion (RPE) on a scale of 6-20 (Borg, 1970) were recorded during the 3 min intervals following each 15 min period of running. Sprint times over 15 m during each cycle of the LIST were also recorded using infra-red timing gates positioned at the start line and 15 m from the start line (Speedtrap II, Brower Timing Systems, Utah, USA).

Drink Composition and Ingestion Schedule

During each LIST participants consumed a 6% CHO plus 2% protein (CHO-P), or an 8% CHO (ISOEN), or a 6% CHO solution (ISOCHO). Participants consumed 5 ml·kg^{-1} of the solution 30 min before exercise, and then consumed 2.5 ml·kg^{-1} every 15 min during exercise. This resulted in a mean ingestion rate of 0.83 ±0.02 g·min^{-1} CHO plus 0.27 ± 0.06 g·min^{-1} protein in the CHO-P condition, 1.10 ± 0.19 g·min^{-1} CHO in the ISOEN condition and 0.87 ± 0.14 g·min^{-1} CHO in the ISOCHO condition, whilst mean fluid intake was 1,260.5 ± 230.2 ml over the course of the trial. A hypothetical 70 kg participant would have consumed 65.3 g·h^{-1} CHO during exercise in the ISOEN trial, and as such would have met the upper limit of CHO ingestion for maximal exogenous CHO oxidation (60-70 g·h-^{1}, Jeukendrup & Jentjens, 2000), whilst the CHO-P (49 g·h^{-1} CHO and 16.5 g·h^{-1} protein) and ISOCHO (49 g·h^{-1} CHO) trials were designed to deliver CHO at a rate considered sub-optimal. As such, the potential for any observed effects to be due to extra energy availability could be determined. Protein was provided in the form of whey protein isolate (Volactive UltraWhey 90 XP Instant), the amino acid profile of which has already been presented in Chapter 4. CHO, in the form of maltodextrin and dextrose, was provided via a commercially available sports drink (Lucozade Sport, GlaxoSmithKline). The pertinent details of each drink composition are presented in Table 5.2.

Table 5.2. The macronutrient and electrolyte concentration of beverages consumed during each trial (expressed per 100 ml of fluid).

Ingredient	CHO-P	ISOEN	ISOCHO
CHO (g)	6	8	6
Protein (g)	2	0	0
Fat (g)	0.025	0	0
Sodium (g)	0.05	0.05	0.04

Statistical Analysis

Descriptive statistics (mean ± standard deviation) were calculated for all dependent variables. The mean values over the 15 min blocks of the LIST for both heart rate and sprint performance were used for all analyses. To assess for differences in heart rate, 15 m sprint time, gut fullness and RPE between trials, two-way (beverage condition [3] x time [5]) separate repeated measures analysis of variance (ANOVA) were performed. Further two-way ANOVAs were performed to assess changes in body mass from pre- to post-LIST (beverage condition [3] x time [2]) and one-way separate repeated measures ANOVAs were utilised to examine any potential differences between trials for time to exhaustion, heart rate and RPE at exhaustion. Sphericity was assessed via Mauchly's test, with any violations accounted for via the Greenhouse-Geisser statistic ($_{GG}$). In the presence of a statistically significant *F* ratio, a series of post-hoc Bonferroni adjusted paired *t*-tests were conducted to see where the differences lay. The alpha level was set at $P < 0.05$ for all ANOVAs, whilst the Bonferroni adjusted *P* value was set at $P = 0.017$ for the time to exhaustion and RPE and heart rate at exhaustion. The alpha level was set at $P = 0.005$ for all other dependent variables. In addition, the mean effect size and accompanying 95% confidence interval of the difference was calculated for RPE (as there was a significant difference between beverage conditions observed here) and time to exhaustion, as

suggested by Batterham and Hopkins (2006). Effect sizes were calculated as the difference between the means divided by the pooled standard deviation, with the following quantitative criteria for effect sizes used to explain the practical significance of the findings: trivial <0.2, small 0.2-0.6, moderate 0.6-1.2, large 1.2-2.0, and very large >2.0 (Hopkins, 2006).

5.3 Results

No significant (P > 0.05) trial order effects were observed for any of the measured variables.

Body mass and fluid loss

ANOVA revealed that there was a significant ($F_{1, 9}$= 82, P < 0.0001) reduction in body mass over time (corrected for ingested fluid) from pre- to post-LIST in the CHO-P (Pre = 72.03 ±13.35 kg, Post = 70.18 ± 12.98, %Body mass loss = 2.56 ± 0.67%), ISOEN (Pre = 72.09 ± 13.35 kg, Post = 70.24 ± 12.83 kg, %Body mass loss = 2.53 ± 0.62%) and ISOCHO (Pre = 72.07 ± 13.36 kg, Post = 70.17 ± 12.28 kg, %Body mass loss = 2.6 ± 0.82%) conditions, with no main effect for beverage condition ($F_{2, 18}$ = 0.112, P = 0.895) or beverage by time interaction ($F_{2, 18}$ = 0.16, P = 0.85).

Heart Rate, Gut Fullness and RPE

ANOVA showed a significant ($F_{4, 36}$ = 17.1, P < 0.0001) effect for time on HR, but a non-significant effect for beverage condition ($F_{2, 18}$ = 0.53, P = 0.476) or beverage condition x

time interaction ($F_{8, 72}$ = 0.11, P = 0.43). Post-hoc analysis revealed that heart rate increased from 15 to 30 min (t_9= -3.825, P = 0.004) (Figure 5.3).

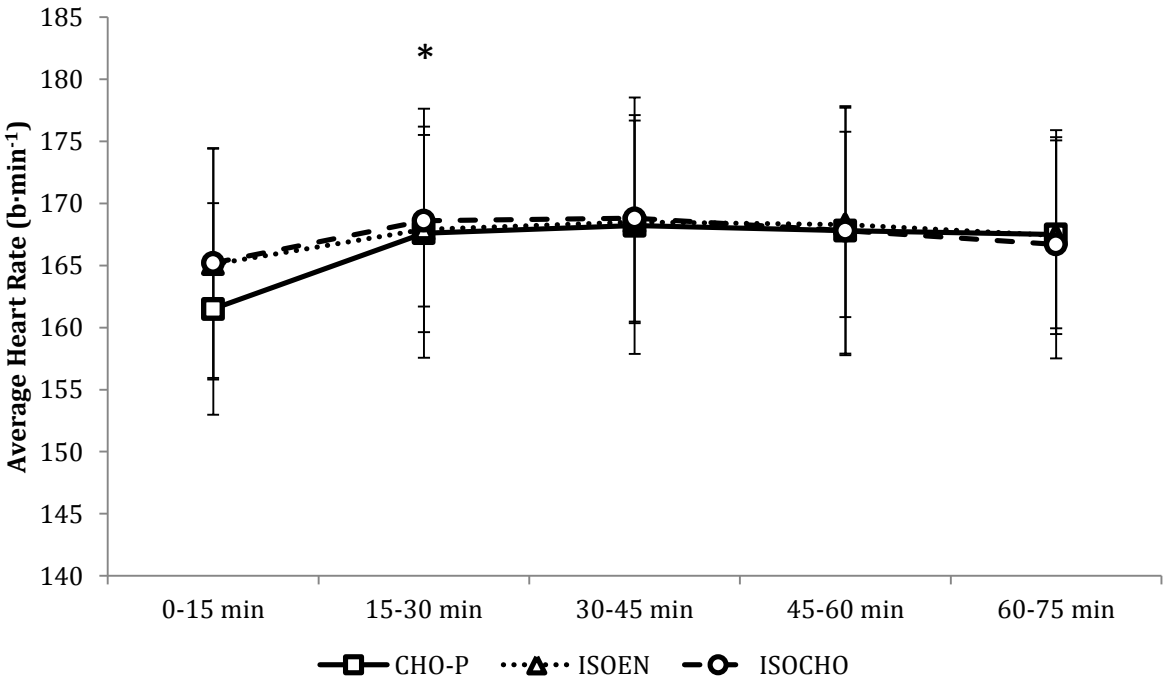

Figure 5.3 Average heart rate over each 15 min block of the LIST. *Significantly higher than 15 min.

ANOVA on gut fullness demonstrated a main effect for time ($F_{(GG)2.1, 19.3}$ = 46.9, P < 0.0001), but no effect for beverage condition ($F_{(GG)1.3, 13.7}$ = 1.29, P = 0.3) or beverage x time interaction ($F_{8, 72}$ = 0.51, P = 0.84). Post-hoc analysis revealed that gut fullness increased at 30 min from the measurement taken at 15 min in all conditions (P < 0.005), whilst increasing further from 30 min onward (Figure 5.4).

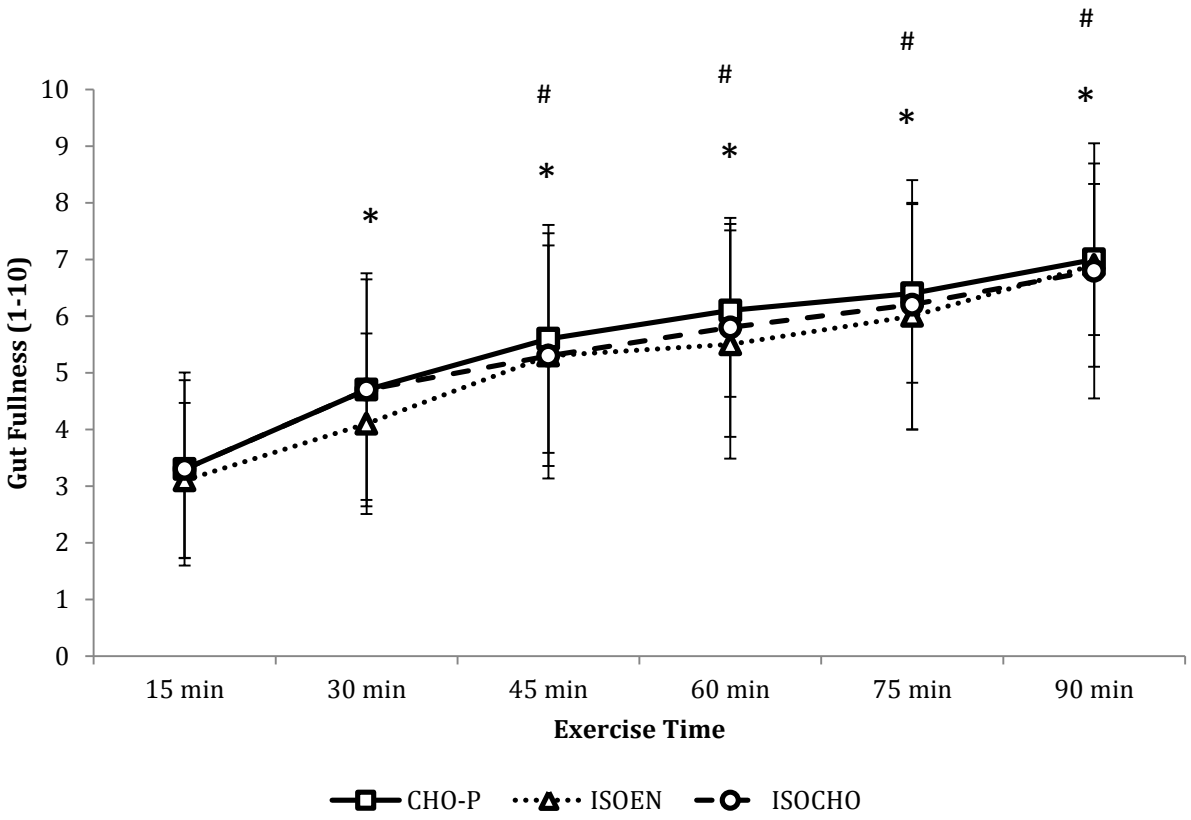

Figure 5.4. Changes in sensations of gut fullness during the LIST.* Significantly (P < 0.005) higher than 15 min. #Significantly higher than 30 min.

There was a main effect for time with RPE ($F_{4, 36}$ = 43.9, P < 0.0001), increasing significantly from the measurement taken at 15 min at all time points in all conditions (P < 0.0001, Figure 5.5). RPE was also significantly higher than 30 min at 60 and 75 min in all beverage conditions (P < 0.0001). There was a significant main effect for beverage condition ($F_{2, 18}$ = 4.3, P = 0.024), with post-hoc analysis showing that RPE was lower in the CHO-P trial when compared to the ISOEN (t_9 = 6, P = 0.004) and ISOCHO (t_9 = 3.857, P < 0.0001) trials. However, no significant beverage x time interaction ($F_{8, 72}$ = 1.679, P = 0.118) was observed, although differences between mean values appeared to be largest in the final 15 min of exercise (Figure 5.5). Effect sizes and qualitative interpretations for RPE are presented in Table 5.4.

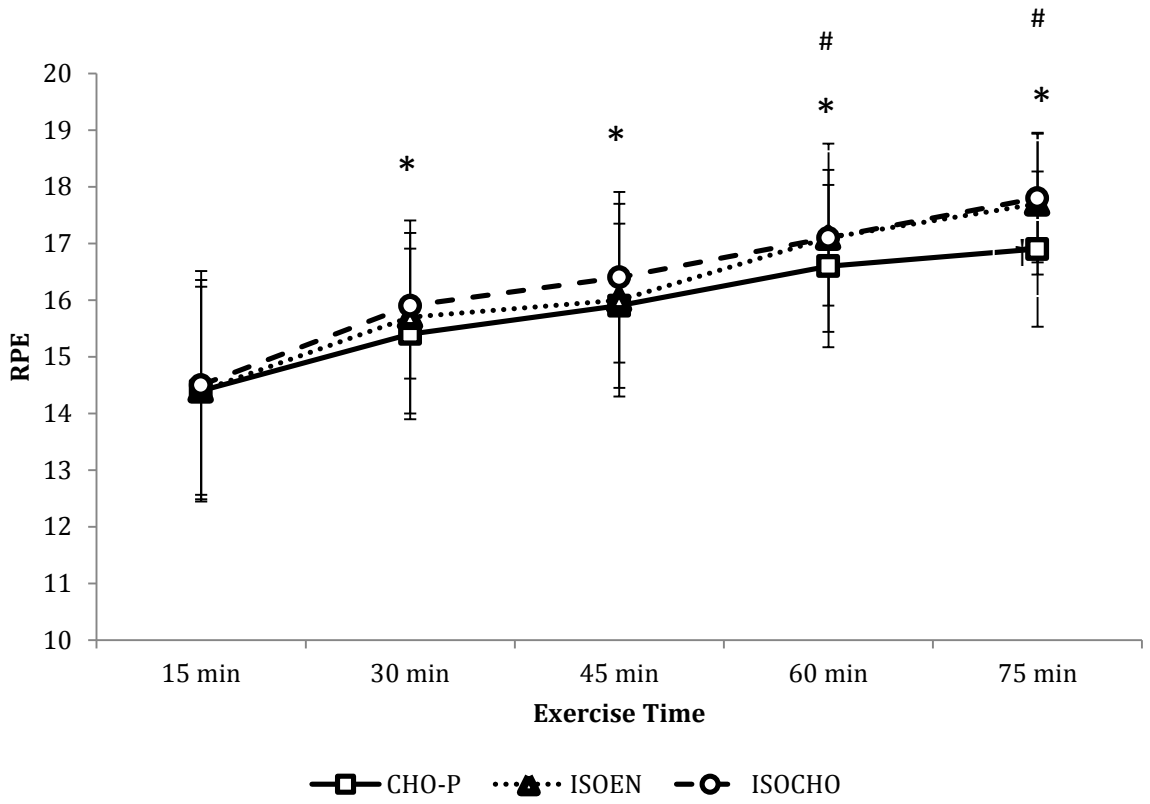

Figure 5.5 Changes in RPE measure at the end of each 15 min block of part A of the LIST. *Significantly higher than 15 min. #Significantly higher than 30 min. †Significantly lower than ISOEN and ISOCHO.

Sprint Performance

There was a significant increase in average time to sprint 15 m over the course of the LIST ($F_{(GG)1.6,\ 14.7}$ = 21.93, P < 0.0001). Average sprint time was higher at 45-60 min (CHO-P = 2.79 ± 0.37 s, ISOEN = 2.78 ± 0.38 s, ISOCHO = 2.79 ± 0.37 s, P < 0.005), and 60-75 min (CHO-P = 2.82 ± 0.37 s, ISOEN = 2.82 ± 0.37 s, ISOCHO = 2.83 ± 0.37 s, P < 0.005) when compared to 0-15 min (CHO-P = 2.65 ± 0.33 s, ISOEN = 2.66 ± 0.32 s, ISOCHO = 2.66 ± 0.35 s), and also higher at 60-75 min than 15-30 min, (CHO-P = 2.69 ± 0.35 s, ISOEN = 2.7 ± 0.34 s, ISOCHO = 2.7 ± 0.32 s, P < 0.005). There was no main effect for beverage condition ($F_{2,\ 18}$ = 0.45, P = 0.644) or group x beverage interaction ($F_{8,\ 72}$ = 0.2, P = 0.99), and as such no

difference in sprint time between different beverage conditions was observed at any time point (Figure 5.6).

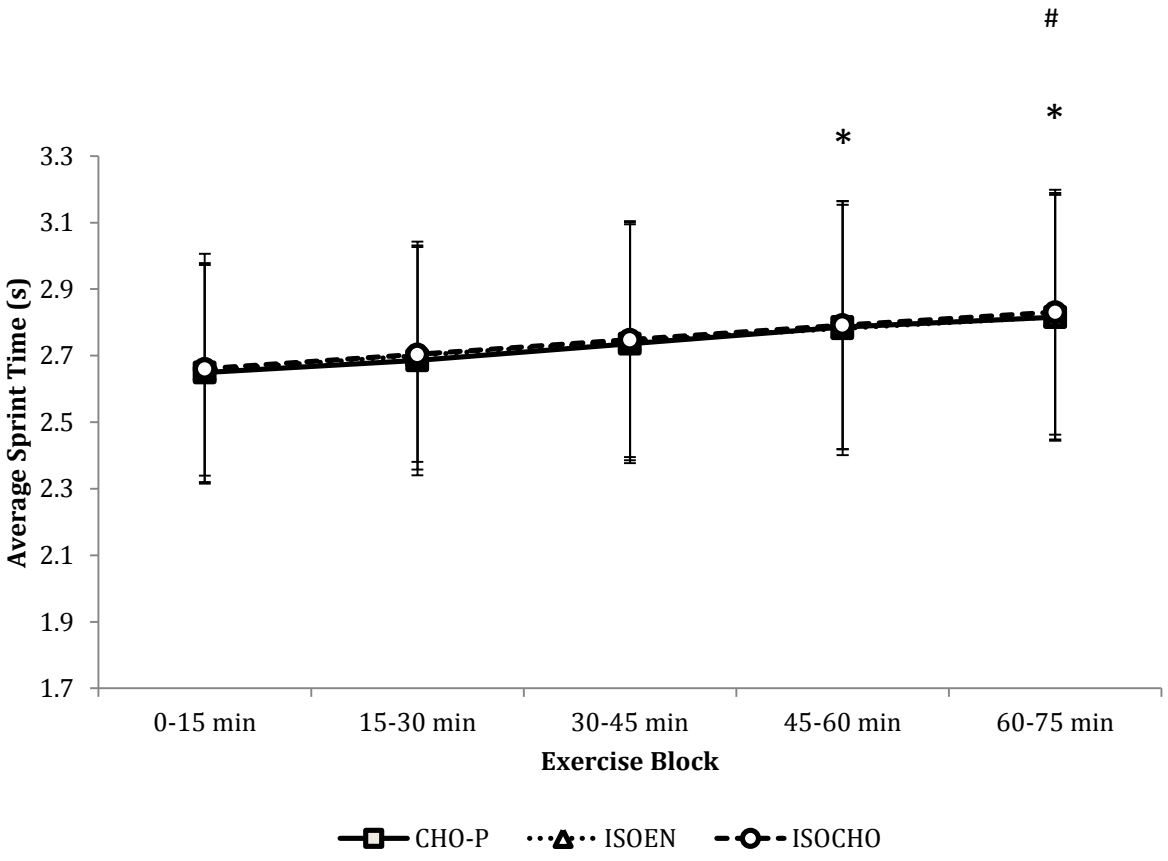

Figure 5.6 Average 15 m sprint time during each 15 min block of the LIST. * Significantly ($P < 0.005$) different to 0-15 min, #Significantly ($P < 0.005$) different to15-30 min.

Time to Exhaustion

Time to exhaustion for each trial, along with measurements taken upon volitional exhaustion, is presented in Table 5.3, whilst participants' individual time to exhaustion scores are shown in Figure 5.7. ANOVA revealed no significant difference in the time to exhaustion between different beverages ($F_{2, 18}$ = 1.3, P = 0.29), nor were there any differences in HR ($F_{2, 18}$ = 0.61, P = 0.553), RPE ($F_{2, 18}$ = 0.73, P = 0.496) or gut fullness ($F_{2, 18}$ = 0.153, P = 0.86) at exhaustion. Effect sizes for time to exhaustion are shown in Table 5.4.

Table 5.3 Time to exhaustion and heart rate, RPE and gut fullness (mean ± standard deviation) at the end of part B of the LIST.

	CHO-P	**ISOEN**	**ISOCHO**
TTE (s)	468.3 ± 268.5	446.2 ± 282.1	443.4 ± 286.3
Heart Rate ($b \cdot min^{-1}$)	186 ± 8	186 ± 7	185 ± 7
RPE (6-20)	19.5 ± 0.5	19.6 ± 0.5	19.4 ± 0.7
Gut Fullness (1-10)	7 ± 1.3	6.9 ± 1.8	6.8 ± 2.3

TTE = Time to Exhaustion.

Table 5.4 Mean differences (95% confidence intervals) for TTE and RPE in the CHO-P and ISOEN and ISOCHO conditions in part B of the LIST. Values are accompanied by effect sizes and qualitative interpretations, which are presented directly below in italics.

	Mean difference and Effect Size		
	CHO-P	**ISOEN**	**ISOCHO**
TTE (s)	-	-22.1 (-166 – 175) *-0.08* *Trivial* ↓	-24.9 (-166 - 178) *-0.09* *Trivial* ↓
RPE	-	0.8 (0.49 – 1.1) *0.63* *Moderate* ↑	0.9 (0.37 – 1.43) *0.7* *Moderate*↑

Effect sizes are calculated as the difference between the means divided by the pooled standard deviation. Qualitative interpretations of effect sizes are based on those of Hopkins (2006): trivial <0.2, small 0.2-0.6, moderate 0.6-1.2, large 1.2-2.0, and very large >2.0. ↑ = increase, ↓ = decrease. TTE = Time to Exhaustion.

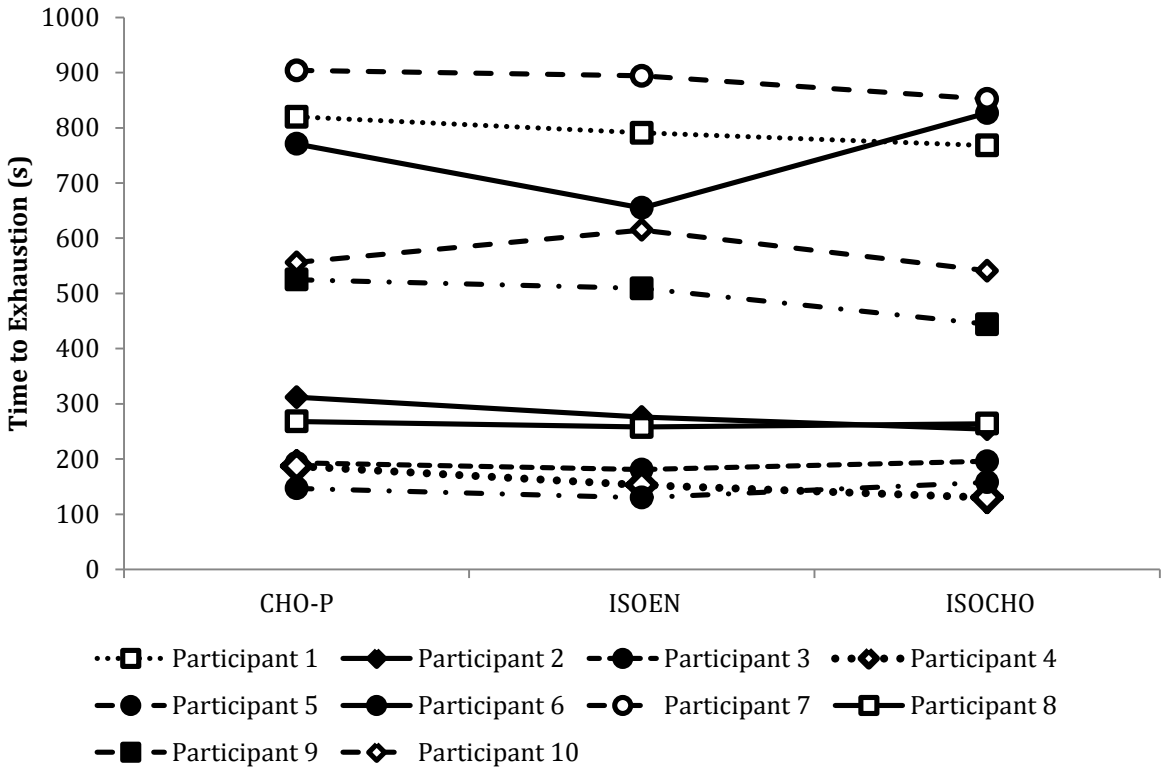

Figure 5.7. Individual time to exhaustion scores for each trial in part B of the LIST

5.4 Discussion

To the author's knowledge, this is the first study to document the effects of a CHO-P supplement, in comparison to CHO alone, consumed during exercise designed to simulate the demands of MSS performance. The key findings were that: a) CHO-P appears to confer little advantage over CHO alone with regard to the maintenance of sprint performance over the course of such exercise; b) CHO-P may lower participants' perception of effort during simulated MSS exercise; and c) alterations in perception of effort with CHO-P did not translate into a significant improvement in individuals' endurance capacity measured via time to exhaustion.

These results show that there are very few differences in the physiological, performance and perceptual measures obtained from participants when consuming a 6% CHO plus 2% protein supplement compared to CHO alone, whether it be matched for energy or CHO content. The current protocol induced an approximate fluid loss of 1.8 – 1.9 l, equivalent to ~2.5% loss of body mass and a sweat rate of 1,260 $ml \cdot h^{-1}$, comparable to those seen in elite soccer competition and training (Krustrup et al., 2006; Maughan et al., 2005; Rehrer & Burke, 1996; Bangsbo, 1994). In the present study, changes in body mass, used as an indirect marker of hydration status, were not different in any of the three beverage conditions. There now appears to be some compelling evidence that additional protein serves to increase fluid retention in the hours after exercise (James et al., 2011; Watson et al., 2008; Shireffs et al., 2007), potentially due to a slowed rate of fluid entering the circulation and a concomitant reduction in the rise in vasopressin seen after the ingestion of large volumes of fluid (Mitchell et al., 1994). However, little is known about the hydrating properties of a CHO-P beverage consumed during exercise. Whilst there was no difference in fluid losses in the current study, a more detailed analysis of hydration status during, and fluid retention after, MSS exercise would appear warranted.

An unexpected finding of the present study was that, whilst RPE increased over the course of part A of the LIST, this increase was ameliorated by the ingestion of CHO-P when compared to CHO alone. Whilst there was no interaction effect, it appeared that this was most pronounced in the latter stages (~75 min) of exercise. This is a finding which has been reported elsewhere, but which is not a consistent result in previous CHO-P studies. Valentine et al. (2008) reported RPE to be significantly lower (15.0 ± 1.8 versus 16 ± 1.4) during the last 30 min of a cycle to exhaustion at 70% VO_2max with CHO-P ingestion,

whilst Martinez-Lagunas et al. (2010) reported that CHO-P ingestion resulted in a significantly lower average RPE (12.5 ± 0.4) when compared to CHO (12.8 ± 0.4) alone during intermittent cycling to fatigue. In similar findings to this study, Greer et al. (2011) also reported an attenuated rise in RPE at 75 min during a 90 min cycle at 55% VO_{2max} when CHO with BCAA was consumed. In addition, other studies have shown improved performance with CHO-P ingestion compared to CHO with no difference in RPE, suggesting that participants perceive more intense or longer exercise to require the same effort (Saunders et al., 2009; Saunders et al., 2007; Saunders et al., 2004; Ivy et al., 2003).

In contrast, several studies have failed to show an altered RPE with CHO-P ingestion (Breen et al., 2010; Lee et al., 2008; Osterberg et al., 2008; Romano-Ely et al., 2006; van Essen & Gibala, 2006). Indeed, Toone and Betts (2010) even reported that RPE was marginally higher during 45 min of variable-intensity cycling and a 6 km time trial when CHO-P was consumed compared to an isocaloric CHO beverage. This finding was attributed to a greater sensation of gastrointestinal discomfort in the CHO-P trial, a finding which was not observed in the current study. That being said, measurements of gut fullness were relatively high during the LIST in all trials. This may be due to the slower rates of gastric emptying associated with intermittent activity (Leiper et al., 2005), and also the up and down movements associated with running compared to cycling (Brouns, 1991). Nevertheless, sensations of gut fullness were the same between trials, and so were likely to influence RPE equally in the present study.

The attenuated rise in RPE observed in the current study can potentially be explained by the 'central fatigue hypothesis' (Newsholme et al., 1987). Indeed, this is one mechanism that has previously been put forward to explain CHO-P's ergogenic effect (Saunders et al., 2007), and has been described in section 2.4.2. Put briefly, this hypothesis states that exercise-induced lipolysis initiates a chain of events that ultimately lead to increased serotonin production, a neurotransmitter associated with feelings of tiredness (Cooper et al., 2003), and thus fatigue and/or altered effort perception ensues. It is possible that CHO-P can affect this hypothesis in several ways. Firstly, CHO and protein are known to stimulate insulin release from the pancreatic beta-cells (Nutall et al., 1984), an effect of which is to reduce lipolysis during exercise (Horowitz et al., 2000; Coyle et al., 1997). Thus, consumption of a CHO-P beverage may reduce circulating FFA acid concentrations and thus a greater amount of albumin is available to bind to f-TRP and prevent its conversion to serotonin. Indeed, CHO ingestion alone has been shown to lower RPE during prolonged running (Utter et al., 2004). Secondly, protein increases amino acid availability in the blood following its ingestion (Miller et al., 2002), which has the potential to reduce the BCAA:f-TRP ratio, and prevent the increase of f-TRP uptake in the brain. Whether these events occurred in the current study is unknown. However, we would acknowledge that there is currently little support for the central fatigue hypothesis, as a reduced RPE with BCAA ingestion has not always been observed (Watson et al., 2004). Furthermore, ingestion of tryptophan has been shown to have no negative effect on performance (van Hall et al., 1995).

A number of factors are thought to inform an individual's RPE, including inputs such as knowledge of the task end point and past experience, as well as afferent inputs to the

central nervous system including heart rate, ventilatory rate, muscle pH, temperature, muscle strain perception and energy reserves such as muscle glycogen (for reviews see Marcora, 2009 and Hampson et al., 2001). Interestingly, CHO-P ingestion has been suggested to work via an increase in circulating insulin concentration and therefore the use of exogenous rather than endogenous CHO stores and better maintenance of muscle glycogen stores during exercise (Saunders, 2007). Furthermore, there is evidence that the rate of muscle glycogen restoration after exercise is significantly enhanced with the ingestion of a CHO-P mixture compared to CHO alone (Berardi et al., 2005). This may be of particular importance during intermittent exercise, whereby CHO ingestion may result in muscle glycogen resynthesis during periods of low-intensity activity (Jeukendrup, 2004). Accordingly, better maintenance or replenishment of endogenous energy stores could have been responsible for the lowered RPE with CHO-P ingestion in the current study.

In spite of the observed lowered RPE with CHO-P ingestion during the LIST in the current study, subsequent time to exhaustion was not different between any of the three beverage conditions, with effect sizes calculated as being trivial. Thus, the present findings are in agreement with those that have reported no significant effect of adding protein to a CHO beverage on endurance performance (Breen et al., 2010; Martinez-Lagunas et al. 2010; Toone and Betts, 2010; Lee et al., 2008; Osterberg et al., 2008; Valentine et al., 2008; Romano-Ely et al., 2006; van Essen & Gibala, 2006). However, in this study this is surprising, as RPE is thought to heavily influence an individual's pacing strategy (St Clair Gibson, 2006). Indeed, RPE possesses scalar qualities, such that RPE will increase in a linear fashion proportional to time left until exhaustion (Trent et al., 2008; Noakes et al., 2004). Thus, it would be expected that a lower RPE at the onset of the time to exhaustion

test would lead to a greater exercise time. Indeed, a moderate negative correlation between RPE at the onset of the time to exhaustion test and subsequent exercise capacity was observed in the current study, such that a lower RPE often meant better performance. However, performance was not affected according to beverage condition, which might suggest that fatigue during this form of exercise was due more to peripheral factors such as substrate depletion and dehydration. That being said, it should be noted that the mean score for the time to exhaustion was approximately 4% higher than the CHO conditions, whilst six of the participants had a greater time to exhaustion in the CHO-P trial than either CHO trial. As such, the lack of effect in the current study may be due to the relative heterogeneity of the sample (i.e. the large range of time to exhaustion) and associated large standard deviations. Furthermore, it is acknowledged that the time to exhaustion test is not performed at self-regulated exercise intensity, and as such does not replicate the type of exercise performed during MSSs. Considering RPE may inform pacing strategy, an investigation into the effects of CHO-P on self-regulated exercise intensity during MSS like activity would appear warranted.

Finally, no difference was observed between either of the CHO conditions for any of the measured variables in the current study, despite the ISOCHO condition providing CHO at a rate that is considered sub-optimal for maximal CHO oxidation during steady state exercise. Gastric emptying is slower during intermittent activity (Leiper et al., 2005), and, along with intestinal absorption, may be a regulating factor of exogenous CHO oxidation rates during exercise (Jeukendrup & Jentjens, 2000). As such, CHO ingestion rates of 60 $g \cdot h^{-1}$ could be too high during intermittent MSS activity, as similar benefits may be observed with a lower CHO ingestion rate (Nicholas et al., 1995). Accordingly, the optimal

amount of CHO to consume during this form of exercise without undue gastrointestinal distress is currently unknown, and warrants future investigation.

In conclusion, the present study offers an insight into the effects of a CHO-P beverage on exercise designed to simulate the demands of MSSs. We observed no statistically effect on either sprint performance or time to exhaustion during this form of exercise, although CHO-P did appear to lower RPE in the latter stages of exercise. Future investigations should examine whether this effect translates into performance improvements during self-regulated exercise intensities which more closely replicate the demands of MSSs.

Chapter 6

The Effects of a CHO-P Beverage on Self-Regulated Simulated Multiple-Sprint Sport Exercise

6.1 Introduction

The previous chapter of this thesis demonstrated that the addition of a small amount of protein to a typical CHO sports drink lowered the perception of effort in the latter stages of simulated MSS exercise. However, this reduction in RPE was not associated with a significant improvement in endurance capacity assessed via 'Part B' of the LIST. This component of the LIST, or variations incorporating time to exhaustion, has been used extensively in studies aiming to examine the effects of various interventions on MSS performance (Foskett et al., 2008; Patterson & Gray, 2007; Erith et al., 2006; Kingsley et al., 2005; Sunderland & Nevill, 2005; Morris et al., 2003; Welsh et al., 2002; Nicholas et al., 1997; Nicholas et al., 1995). The justification for such an approach has often been that this time to exhaustion provides an indication of an individual's capacity to perform high-intensity work in the latter stages of this form of exercise (Phillips et al., 2011).

Despite the popularity of measuring time to exhaustion in Part B of the LIST, it is questionable whether performance of an exercise capacity test at a prescribed intensity, where the goal is to run for as long as possible, adequately replicates the requirements of MSSs (Drust et al., 2007). More specifically, as opposed to exercising at a regulated exercise intensity for an indefinite period of time, MSS players are required to self-regulate their exercise intensity for a set period of time (typically 60 – 90 min), much like a time

trial. Thus, when assessing the potential for supplementation to alter the fatigue associated with the latter stages of MSSs, it seems prudent that a simulation protocol should allow participants to self-pace their exercise intensity in that time. In particular, this is relevant for supplements that can influence central fatigue and perception of exertion such as CHO-P (Saunders, 2007). Perception of exertion, whether informed via afferent feedback from a variety of physiological systems (St Clair Gibson et al., 2006), or via efferent signals increasing 'corollary discharge' in response to a progressive reduction in neuromuscular responsiveness (Marcora, 2008), is thought to inform strongly an athlete's pacing strategy during self-paced exercise (Tucker, 2009). Therefore, a lower RPE for a given workload with CHO-P ingestion, as observed in Chapter 6 of this thesis, may have important implications for self-selected exercise intensity. Indeed, supplements such as caffeine (see Doherty & Smith, 2005) and CHO mouth rinsing (Rollo et al., 2010; Rollo et al., 2008) have been associated with an attenuated RPE during improved time trial performance where athletes are required to self-pace their exercise intensity.

Several researchers have recently suggested that decrements in high-intensity running in the latter stages of MSSs (i.e. the last 15 min) are indicative of athletes pacing their efforts in order to be able to complete the exercise task (Aughey, 2010; Edwards & Noakes, 2009). Moreover, and in opposition to, the more traditionally held view that peripheral fatigue ensues owing to muscle glycogen depletion and dehydration (Bangsbo et al., 2006; Krustrup et al., 2006; Nicholas et al., 1999) directly compromising skeletal muscle contractile capacity, Edwards and Noakes (2009) have proposed that MSS athletes down-regulate their exercise intensity based on a variety of factors to reach the exercise end-point without causing severe damage to peripheral physiological systems. Such factors

may well include levels of substrate depletion and thermal stress, but may also include health status, quality of the opposition, crowd support and match score (St Clair Gibson et al., 2006). It is proposed that these variables provide feedback to the athlete, who will attempt to conclude the match having exercised vigorously throughout whilst maintaining an acceptable level of physical discomfort or perceived exertion. How athletes pace in MSSs is unclear, although, Rampinini et al. (2011) recently demonstrated that fatigue observed in soccer was likely to be due, in part, to central factors (i.e. incomplete muscle activation) as evidenced by alterations in muscle function (reduced strength and sprint speed immediately after a match) assessed via twitch interpolation. Furthermore, it is now generally accepted that *sensations* of fatigue and/or exertion, whether as a response to (Psychobiological Model) or in anticipation of (Central Governor Model) alterations in physiological homeostasis, will result in a down-regulation of exercise intensity (Ament & Verkerke, 2009; Marcora, 2008; Noakes et al., 2004). If the model of Edwards & Noakes (2009) is correct, then the importance of perceived exertion to MSS performance cannot be underestimated.

Given the potential significance of pacing to MSSs, a protocol that allows participants to self-regulate or pace their exercise intensity may provide valuable information on the ergogenic potential of certain supplements during such activity. To the author's knowledge, there are relatively few MSS simulation protocols that involve self-regulated periods of exercise intensity. Ali et al. (2009) developed a modification of the original LIST (Nicholas et al., 2000), whereby for the final 30 min of the total 90 min exercise time, participants no longer received an audio signal to regulate their exercise intensity. Instead, the audio signal was removed, and participants are instructed to maintain their exercise

intensity from the previous 60 min as best they could, thus allowing them to self-regulate or pace their exercise intensity. Ali et al. (2009) reported favourable test-retest reliability for both running speed and distance covered (see procedures of this Chapter), making it a potentially useful tool to assess MSS performance. Accordingly, the aim of the current study was to assess the effect of a CHO-P beverage, compared to CHO, to alter self-regulated exercise intensity during simulated MSS exercise. More specifically, it was hypothesised that CHO-P ingestion would result in participants adopting a higher self-selected running speed in the latter stages of a modified LIST.

6.2 Methods

Participants and Experimental Design

After receiving institutional ethical approval, nine male university-level team-sport athletes participated in the study (age 23.4 ± 1.8 years, stature 177.5 ± 9.3 cm, body mass 75.3 ± 12 kg; VO_{2max} of 52.5 ± 3.8 $ml \cdot kg^{-1} \cdot min^{-1}$). All participants provided written informed consent and a pre-test health questionnaire to check for any contraindications to exercise.

The study adopted a double-blind, randomised crossover design, whereby participants completed three testing sessions on separate days (Figure 6.1). On the first occasion, they completed the MSSRT and 15 min familiarisation with a modified LIST detailed in the procedures section. Thereafter, participants completed two modified LISTs separated by approximately one week, during which they either consumed a 6% CHO plus 2% whey protein isolate beverage (CHO-P) or an isoenergetic 8% CHO-only beverage (CHO). Trials

were completed in a random order, with four participants completing the CHO-P trial first. Participants completed a food diary in the 48 h preceding the first modified LIST, and were asked to repeat the same dietary intake in the 48 h before completing the second LIST. Daily dietary intake was 2086 ± 279 kcal, 282.5 ± 67 g CHO, 55.3 ± 17 g fat and 114.8 ± 32 g protein. Participants were also asked to refrain from any strenuous physical activity during the 48 h before each LIST.

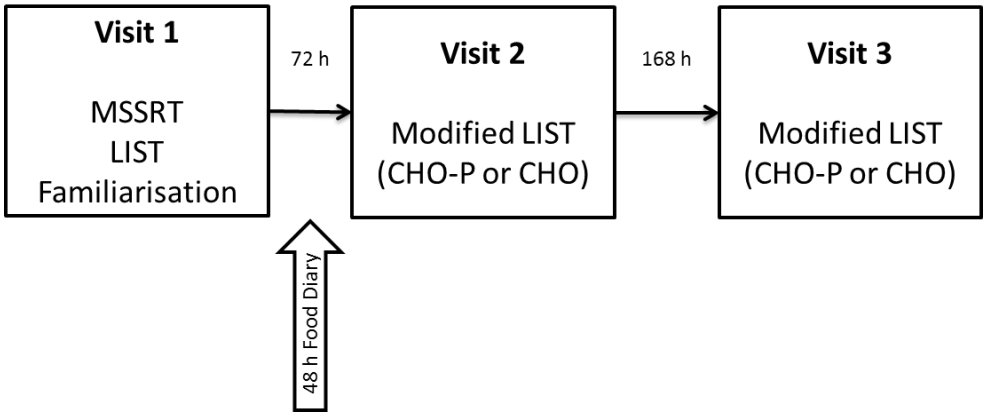

Figure 6.1 Schematic of the study design.

Procedures

Multi-Stage Shuttle Run Test

The MSSRT for the estimation of aerobic capacity and running speeds for the LIST was completed in accordance with the procedures previously described in Chapter 4.2.

The Modified Loughborough Intermittent Shuttle Test

Following a 12 h overnight fast, participants completed a modified LIST (Ali et al., 2009) adapted from that previously described by Nicholas et al. (2000) (see Figure 6.2). The modified LIST involved two distinct segments; part A lasting for 60 min with an exercise intensity regulated via an audio CD, and part B lasting for 30 min during which no audio cue was provided and participants were asked to match the cycle of activity in part A as best they could. Thus, part B of the LIST required participants to self-regulate their exercise intensity to match that which they had previously achieved in part A. This protocol has previously been shown to possess a CV of 1.7 and 5.4% for distance covered and sprint performance respectively (Ali et al., 2009).

During each LIST participants wore the same portable 5 Hz global positioning system (GPS; SPI-Pro, GPSports, Canberra, Australia) to measure distance covered and running speed throughout the protocol. The GPS model utilised in this investigation has previously been shown to have a CV ranging from 1.84 to 2.06% for measurements of distance and speed over 10-30 m (Waldron et al., 2011). Participants were fitted with a custom-made vest positioning the GPS monitor between the scapulae, whilst a compatible heart rate monitor (Polar Electro Oy, Kempele, Finland) was placed around their chests for the measurement of HR during exercise. Satellite availability ranged from 8 to 11 for all testing sessions. All data were downloaded using SPI Ezy v.2.1 (GPSports, Canberra, Australia) and analysed using Team AMS v. 2.1 (GPSports, Canberra, Australia). The variables taken from the GPS included peak and average running speed ($km \cdot h^{-1}$), distance covered (m) over each 15 min block, and peak speed ($km \cdot h^{-1}$) for each 20 m shuttle completed in the last self-regulated exercise block of the modified LIST.

The use of GPS precluded the LIST being completed indoors. Consequently, participants completed the LIST on an outdoor grass pitch whilst wearing studded boots. Testing was not conducted if it was raining or if the grass was wet. Average temperature and humidity were not significantly different between trials (CHO-P ~ 10.9 ± 2°C; CHO ~ 10.9 ± 1.9°C; $P > 0.05$). Nude body mass (Seca, 712, Hamburg, Germany) was measured immediately before and after completion of the LIST to determine fluid losses during exercise, whilst RPE (Borg, 1982) and gut fullness were recorded at 15 min intervals throughout the protocol (see Figure 6.2).

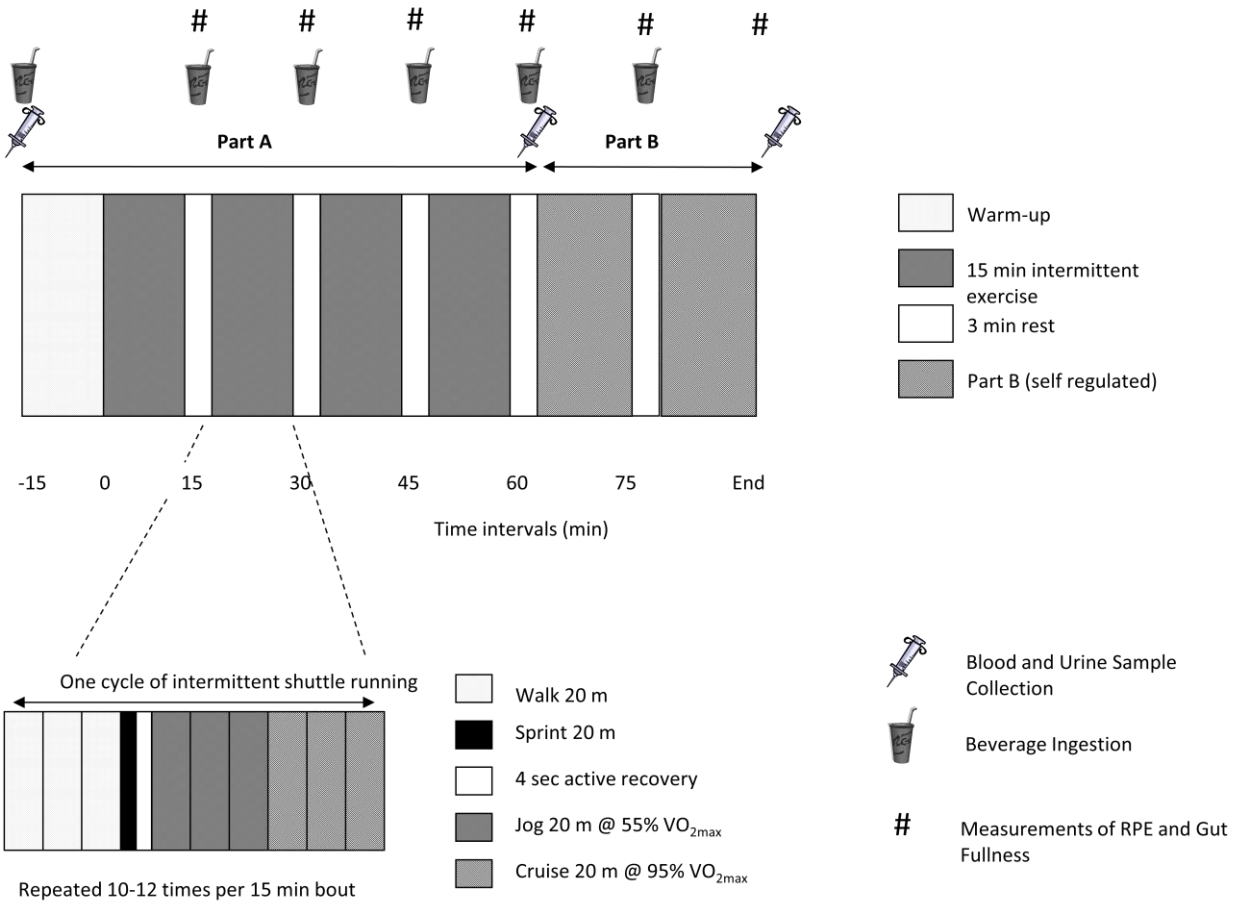

Figure 6.2 Trial schematic showing the modified Loughborough Intermittent Shuttle Test including two x 15 minute self-regulated exercise blocks (Ali et al., 2009).

Urine and blood sampling and analysis

Urine samples were collected in the hour before, at 60 min during (if necessary) and immediately after completion of the modified LIST. Urine volume was measured for the samples taken during and after the LIST, and urine osmolality was assessed for all samples via freeze-point depression (Advanced Instruments, 3300 Micro Osmometer, Massachusetts, USA). Venous blood samples (20 ml) were taken 30 min before, at 60 min during and immediately after the LIST from the veins of the antecubital fossa. Of the whole blood sample, 5 ml was dispensed into an anti-coagulant coated tube (ethylene diamine tetra-acetic acid, EDTA) for the assessment of haematocrit (Haematospin 1400, Hawkley, W Sussex, UK) and haemoglobin (b-haemoglobin photometer, Hemocue AB, Angelholm, Sweden). Subsequent calculations of changes in plasma volume throughout the trials were made in accordance with the equations of Dill and Costill (1974). Blood glucose and urea concentrations were also measured from 30 µl whole-blood samples immediately after collection using reflectance photometry (Reflotron, Type 4, Boehringer Mannheim, Germany). The remaining whole blood sample was stored at room temperature for 1 h in a non-anti-coagulant tube. Samples were then centrifuged at 2000 g for 10 min at 4°C before the serum fraction was collected and stored at -80°C for later analysis of insulin concentration. Insulin analysis took place in duplicate via a commercially available human iso-insulin instant enzyme-linked immunosorbent assay (ELISA, eBioscience, Bender MedSystems, Vienna). CV of the assay ranged from 1.1 to 6.3% for a range of standards, whilst the CV for measured samples was 2.8%.

Drink Composition and Ingestion Schedule

During each LIST trial participants consumed either a 6% CHO plus 2% protein (CHO-P) or an 8% CHO (CHO) solution. Participants consumed 5 ml·kg^{-1} body mass of the solution 30 min before exercise, and then consumed 2.5 ml·kg^{-1} every 15 min during exercise. This resulted in a mean ingestion rate of 52.7 ± 8.35 g·h^{-1} CHO plus 17.6 ± 2.8 g·h^{-1} protein in the CHO-P condition, and 70.24 ± 11.1 g·min^{-1} CHO in the CHO trial, whilst mean fluid intake was 1.3 ± 0.21 l over the course of the protocol. A hypothetical 70 kg participant would have consumed 65.3 g·h^{-1} CHO during exercise in the CHO trial, and as such would have met the upper limit of CHO ingestion for maximal exogenous CHO oxidation (60-70 g·h-^{1}, Jeukendrup & Jentjens, 2000), whilst the CHO-P (49 g·h^{-1} CHO and 16.5 g·h^{-1} protein) trial was designed to deliver CHO at a rate considered sub-optimal (Jeukendrup & Jentjens, 2000). As such, the potential for any observed effects to be due to extra energy availability could be discounted. Protein was provided in the form of whey protein isolate (Volactive UltraWhey 90 XP Instant), the amino acid profile of which has already been presented in Chapter 5. CHO, in the form of maltodextrin and dextrose, was provided via a commercially available sports drink (Lucozade Sport, GlaxoSmithKline). Pertinent details of each drink composition are presented in Table 6.2 in the previous Chapter of this thesis.

Statistical Analysis

All data are presented as mean ± standard deviation. The mean heart rate values over 15 min blocks of the LIST were used for all analyses. Two-way (beverage condition [2] x time [6]) fully repeated measures ANOVAs were performed to assess the variability of mean HR, peak and average running speed, distance covered, gut fullness and RPE. Further two-

way ANOVAs were performed to assess changes in body mass from pre- to post-LIST and changes in plasma volume (beverage condition [2] x time [2]), as well as serum insulin concentration, blood glucose and urea (beverage condition [2] x time [3]). Paired samples *t*-tests were utilised to assess differences in post-exercise urine volume and urine osmolality before starting the LIST. Sphericity was assessed via Mauchly's test of sphericity, with any violations accounted for via the Greenhouse-Geisser statistic ($_{GG}$). In the presence of a statistically significant *F* ratio, a series of post-hoc Bonferroni adjusted paired *t*-tests were conducted to see where the differences lay. Paired *t*-tests were also used to examine differences in the speed of individual movement categories (i.e. walk, jog, cruise and sprint speed) in the final self-regulated block of the LIST. The alpha level was set at $P < 0.05$ for ANOVA, whilst the Bonferoni adjusted *P* value was set at $P = 0.008$ for distance covered, average and maximum speed, gut fullness, RPE and heart rate. The alpha level was set at $P = 0.017$ for serum insulin, blood glucose and urea and $P = 0.025$ for plasma volume and body weight. Mean effect sizes, as suggested by Batterham and Hopkins (2006), were also calculated for GPS variables in the final 15 min of the modified LIST as these were considered key performance variables. These were calculated as previously described in Chapter 5.

6.3 Results

No significant ($P > 0.05$) trial order effects were observed for any of the measured variables.

Modified LIST Performance

There was a significant main effect of time ($F_{5, 40}$ = 7.5, P < 0.0001) for distance covered in each 15 min segment of the LIST, with no main effect for beverage condition ($F_{1, 8}$ = 0.93, P = 0.362) or time x beverage condition interaction ($F_{5, 40}$ = 1.8, P = 0.143). Post-hoc analysis revealed that distance covered was significantly lower in the final 15 min of exercise than in the first 15 min (t_{17} = 3.9, P = 0.001, Figure 6.3). Similarly, the maximal speed attained in a single sprint for each 15 min block changed over time ($F_{5, 40}$ = 18.2, P < 0.0001), but not due to beverage condition ($F_{1, 8}$ = 0.01, P = 0.931) or time x beverage condition interaction ($F_{5, 40}$ = 7.5, 0.86, P = 0.517). Dependent t-tests showed that maximal speed was lower than the first 15 min from 30-45 min onwards (P < 0.008, Figure 6.4). However, ANOVA revealed a significant effect for both time ($F_{5, 40}$ = 11.0, P < 0.0001) and a time x beverage condition interaction ($F_{5, 40}$ = 4.3, P = 0.003) for the average speed attained over each 15 min block of the LIST. Post-hoc analysis showed that average speed at 75-90 min was significantly lower than 0-15 min (t_9 = 0.47, P = 0.002) in the CHO trial only (Figure 6.6).

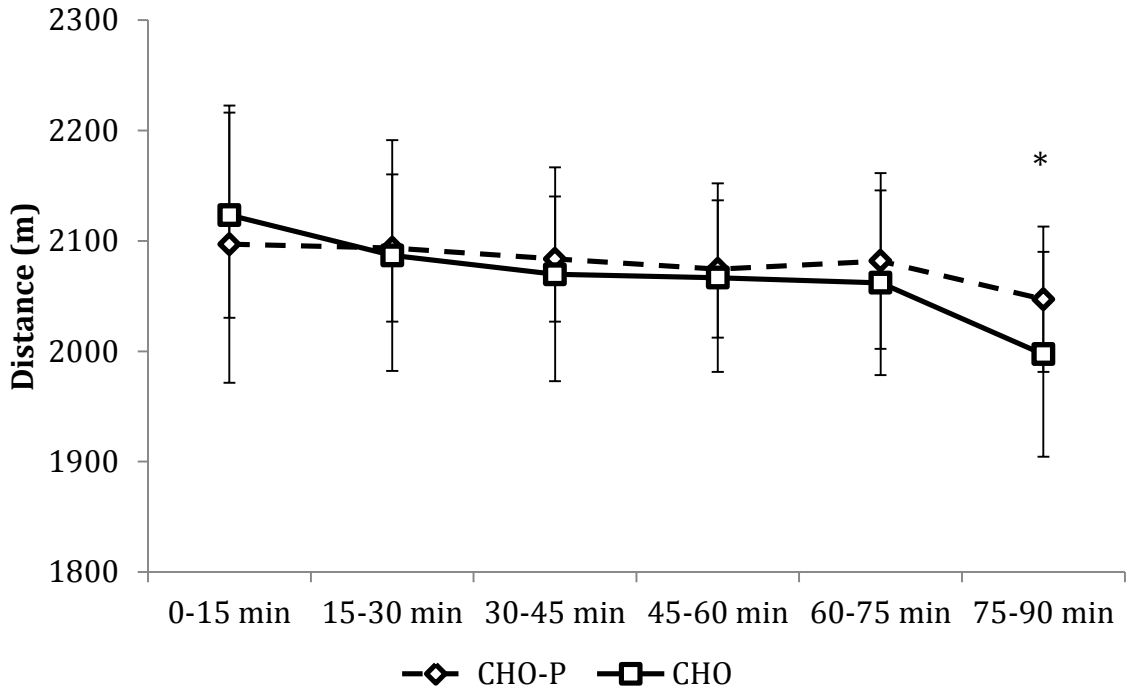

Figure 6.3. Distance covered during each 15 min block of the modified LIST. *Significantly ($P < 0.008$) different to 0-15 min. Values are mean ± SD.

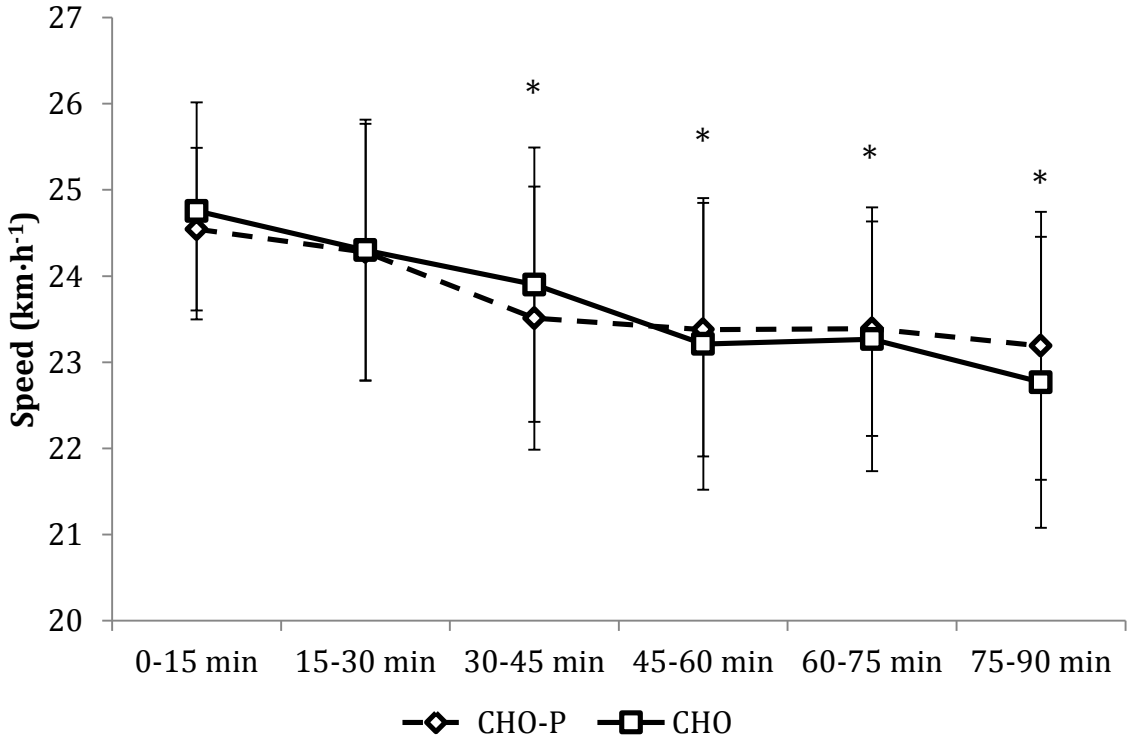

Figure 6.4. Maximum speed during each 15 min block of the modified LIST. *Significantly ($P < 0.008$) different to 0-15 min. Values are mean ± SD.

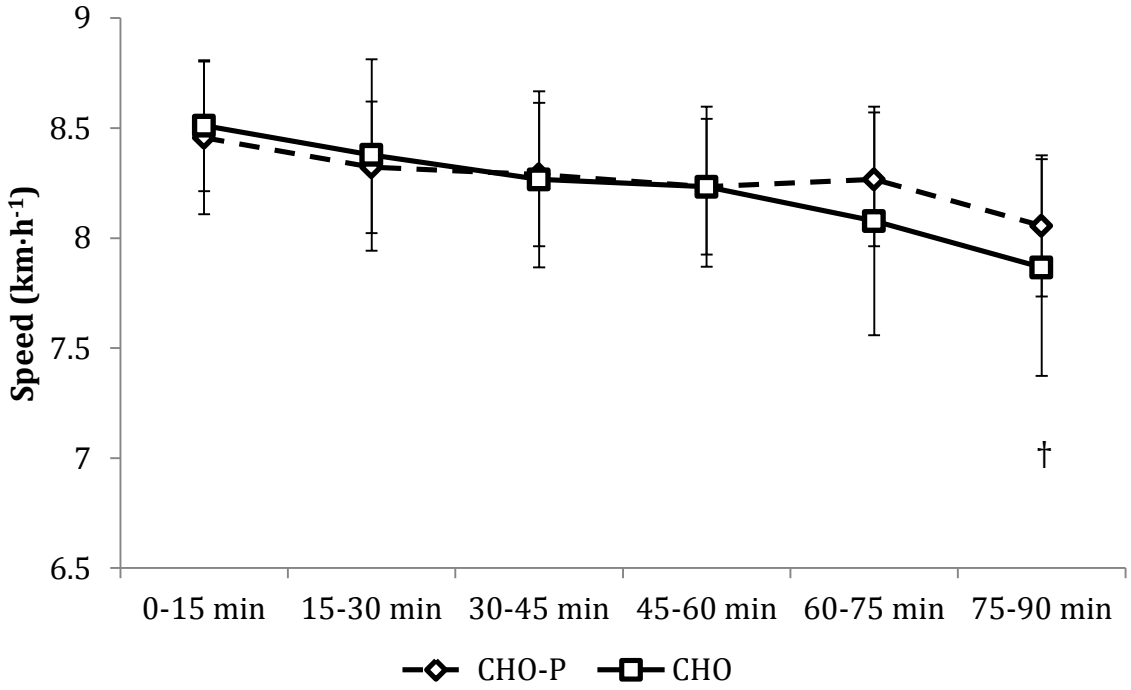

Figure 6.5. Average running speed during each 15 min block of the modified LIST. †Significantly (P < 0.008) different to 0-15 min in CHO trial. Values are mean ± SD.

Further analysis of the final block of the modified LIST (self-regulated block number 2 at 75-90 min) failed to show a significant difference between individual average walking (t_8 = 0.49, P = 0.637), jogging (t_8 = 0.43, P = 0.676), cruising (t_8 = -1.6, P = 0.146) and sprinting speed (t_8 = -2.1, P = 0.073) in the CHO and CHO-P trials (see Figure 6.6).

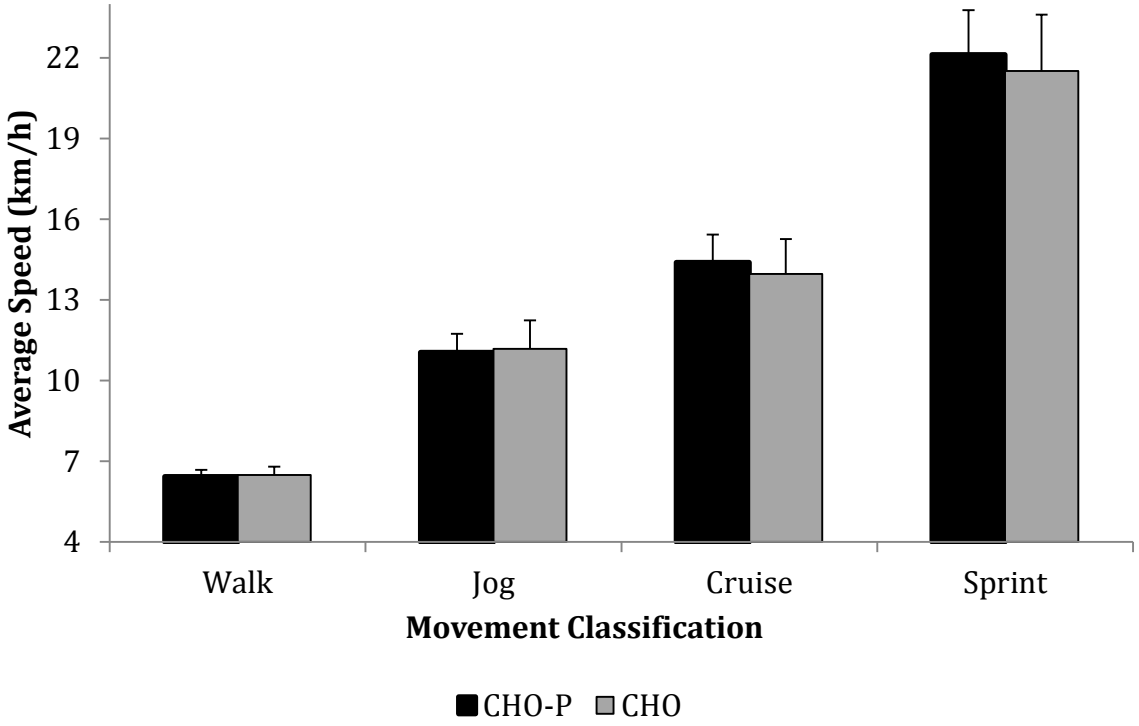

Figure 6.6. Average running speed for different movement intensities in the final (75-90 min) self-regulated block of the modified LIST. Values are mean ± SD.

Effect sizes calculated for performance variables in the final 15 min of exercise are presented in Table 6.1. Whilst not significant, a moderate increase in distance covered, and small increase in average cruise and sprint speed in the CHO-P trial was apparent. In agreement with ANOVA above, there was also a small increase in average running speed in the CHO-P condition.

Table 6.1. Mean differences (95% confidence intervals) between CHO and CHO-P for selected variables in the final block of the modified LIST. Values are accompanied by effect sizes and qualitative interpretations presented in italics

	Mean difference	**Effect size**	**Interpretation**
Distance covered (m)	50.2 (-39.3-61.3)	*0.67*	*Moderate ↑*
Maximum speed (km·h^{-1})	0.30 (-0.85-1.29)	*0.19*	*Trivial ↑*
Average speed (km·h^{-1})	0.18 (0.25-0.78)	*0.46*	*Small ↑*
Walk speed (km·h^{-1})	-0.05 (-0.35-0.00)	*-0.2*	*Trivial ↓*
Jog speed (km·h^{-1})	-0.14 (-0.62-0.56)	*-0.16*	*Trivial ↓*
Cruise speed (km·h^{-1})	0.40 (-0.17-1.26)	*0.48*	*Small ↑*
Sprint speed (km·h^{-1})	0.60 (-0.7-1.7)	*0.34*	*Small ↑*

Effects sizes are calculated as the differences between the means divided by the pooled standard deviation. Qualitative interpretations are based on those of Hopkins (2006): trivial <0.2, small 0.21-0.6, moderate 0.61-1.2, large 1.21-2.0, and very large >2.0. ↑ increase, ↓ decrease.

Hydration Indices

Pre-exercise urine osmolality did not differ (t_7 = 1.1, P = 0.32) in the CHO-P trial (808 ± 194 mOsmol·kg^{-1}) to the CHO trial (839 ± 181 mOsmol·kg^{-1}). Body mass, corrected for fluid intake, decreased ($F_{1, 8}$ = 28.1, P = 0.001) from pre- to post-LIST by 2 ± 0.5 and 2.1 ± 0.5 kg in the CHO-P and CHO trials respectively, with no effect of beverage condition ($F_{1, 8}$ = 0.26, P = 0.63) or time x beverage condition interaction ($F_{1, 8}$ = 0.57, P = 0.47). This amounted to an approximate 2.7% reduction in body mass in the CHO-P trial and a 2.9% reduction the

CHO trial. Plasma volume changes at 60 (t_8 = 0.69, P = 0.51) and 90 min (t_8 = -.01, P = 0.92) were also not different in the CHO-P (-5.6% and 0% respectively) and CHO (-2.7% and -0.7% respectively) trials. Post-exercise urine volume was not different between beverage conditions (t_8 = 0.53, P = 0.61, see Figure 6.7).

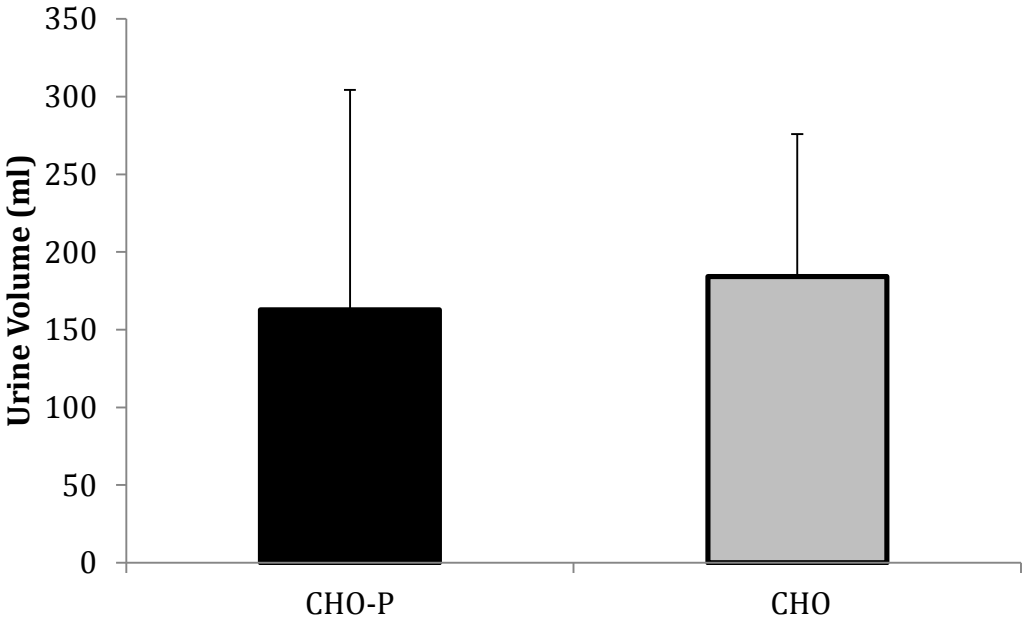

Figure 6.7. Post-exercise urine volume in the CHO-P and CHO trials. Values are mean ± SD.

Heart Rate, Gut Fullness and RPE

ANOVA revealed a significant main effect of time ($F_{(GG)\ 5,\ 40}$ = 4.6, P = 0.031) for HR (see Table 6.2), but no main effect of beverage condition ($F_{1,\ 8}$ = 0.02, P = 0.895) or time x beverage condition interaction ($F_{5,\ 40}$ = 2.7, P = 0.577). Average HR was significantly lower in the last 15 min of exercise (t_{17} = 4.47, P < 0.001) compared to the highest average HR at 15-30 min. Main effects for time were also observed for gut fullness ($F_{\ 5,\ 40}$ = 21.6, P < 0.0001) and RPE ($F_{5,\ 40}$ = 28.4, P = 0.001), with no effect for beverage condition or beverage

condition x time interaction for either (P > 0.05). Post-hoc analysis showed that both gut fullness and RPE increased (P < 0.008) from baseline at 15-30 min onwards.

Table 6.2 Heart rate, gut fullness and rating of perceived exertion during the modified LIST. Values are mean ± SD.

	0-15 min	**15-30 min**	**30-45 min**	**45-60 min**	**60-75 min**	**75-90 min**
			Heart Rate (b·min^{-1})			
Condition						*
CHO	165 ± 8	168 ± 8	167 ± 8	165 ± 9	162 ± 8	162 ± 7
CHO-P	165 ± 8	167 ± 7	167 ± 7	164 ± 8	163 ± 7	163 ± 7
			RPE (6-20)			
		*	*	*	*	*
CHO	14.6 ± 1.2	15.1 ± 1.4	15.6 ± 1.2	16.2 ± 1.0	16.9 ± 0.6	17.8 ± 0.7
CHO-P	14.9 ± 1.4	15.3 ± 1.1	15.9 ± 0.9	16.4 ± 1.0	16.9 ± 0.8	17.6 ± 0.7
			Gut Fullness (1-10)			
		*	*	*	*	*
CHO	2.3 ± 1	3.2 ± 1.2	3.9 ± 1.7	4.1 ± 1.5	4.6 ± 1.4	4.6 ± 1.4
CHO-P	2 ± 0.7	3 ± 1.3	3.6 ± 1.5	4.3 ± 1.3	4.4 ± 1.3	4.7 ± 1.5

* Significantly (P < 0.008) different to 0-15 min independent of beverage condition (i.e. CHO or CHO-P)

Blood Glucose, Urea and Serum Insulin

There was a significant main effect for time for blood glucose ($F_{(GG)2,\ 14}$ = 8.8, P = 0.015) with no effect of beverage condition ($F_{1,\ 7}$ = 1.4, P = 0.275) or time x beverage condition interaction ($F_{2,\ 14}$ =1.48, P = 0.26). Post-hoc analysis showed a significantly higher blood glucose concentration at 60 ($t_{17,}$ = -4.4, P < 0.0001) and 90 min ($t_{16,}$ = -2.7, P = 0.017)

compared to baseline. In contrast, there was no main effect for time for blood urea concentration ($F_{2, 14}$ = 1.58, P = 0.242) but effects for beverage condition ($F_{1, 7}$ = 10.2, P = 0.015) and a time x beverage condition interaction ($F_{2, 14}$ = 5.2, P = 0.021). Dependent t-tests showed that blood urea increased at 60 min in the CHO-P trial only (t_8 = -4.27, P = 0.003). Serum insulin concentrations did not vary due to time ($F_{2, 14}$ = 0.2, P = 0.825), beverage condition ($F_{1,7}$ = 1.25, P = 0.301) or time x beverage condition ($F_{2, 14}$ = 2.3, P = 0.135, see Table 6.3).

Table 6.3 Blood measurements obtained before, during and after the modified LIST. Values are mean ± SD.

	Baseline	**60 min**	**90 min**
		BGl (mmol·L^{-1})	
Condition		*	*
CHO	3.9 ± 0.8	5.1 ± 0.5	4.8 ± 4.5
CHO-P	4.1 ± 0.8	4.6 ± 0.7	4.5 ± 0.7
		Urea (mmol·L^{-1})	
CHO	6.8 ± 2.1	6.7 ± 1.9	6.4 ± 2.3
CHO-P	7.0 ± 1.4	7.7 ± 1.7*	7.6 ± 1.6
		Insulin (pmol·L^{-1})	
CHO	33.7 ± 24.4	43.8 ± 25.5	33.1 ± 16.3
CHO-P	32.4 ± 19.3	52.3 ± 27.7	32.8 ± 16.3

* Significantly different to baseline. BGl = Blood glucose

6.4 Discussion

In this first study to examine CHO-P ingestion on self-regulated running performance during MSS-like activity, a notable finding was that CHO-P ingestion results in a higher

self-regulated exercise intensity in the latter stages of exercise than CHO ingestion alone. Specifically, a significant decline was observed in the average exercise intensity in the final 15 min of exercise with ingestion of CHO, but not during the trial with CHO-P ingestion. It appeared that differences in high-intensity running speed, although not statistically different between groups, accounted for the differences in late-exercise performance. Indeed, effect sizes showed moderate increases in distance covered and small increases in average speed, average cruise speed and average sprint speed with CHO-P ingestion in the last 15 min of exercise. Average running speeds in the CHO-P and CHO trials were 8.1 ± 0.3 km·h^{-1}and 7.9 ± 0.5 km·h^{-1} respectively, whilst low intensity movement speeds were very similar in the respective trials (walking ~ 6.4 ± 0.2 c.f. 6.5 ± 0.3 km·h^{-1}; jogging ~ 11.1 ± 0.7 c.f. 11.2 ± 1.1 km·h^{-1}). However, small non-significant differences in mean cruise (14.4 ± 1 c.f. 14 ± 1.3 km·h^{-1}) and mean sprint (22.1 ± 1.6 c.f. 21.5 ± 2.1 km·h^{-1}) speed, as well as accompanying small effect sizes, were evident between the CHO-P and CHO trials. An increase in high-intensity running, if present with CHO-P ingestion, may be particularly relevant for MSS athletes as it is this exercise intensity which is typically reduced in the latter stages of performance (Mohr et al., 2003; Bangsbo, 1994; Bangsbo et al., 1991; Reilly & Thomas, 1976). Furthermore, high-intensity running capacity in the latter stages of soccer has been shown to differentiate between sub-elite and elite soccer players (Mohr et al., 2003). Nevertheless, differences in the average running speed observed in the current study were likely due to a protein-specific mechanism, as these results were observed when an equal amount of calories were provided in a CHO supplement. To the author's knowledge, this, in conjunction with data from Chapter 5 of this thesis, is amongst the first to report on the potential for whole-protein ingestion to be ergogenic during MSS-like exercise.

There remains little consensus on the effects of CHO-P ingestion on endurance performance. Interestingly, a recent meta-analysis examining CHO supplementation during exercise concluded that an acutely ingested supplement providing 0.9 $g \cdot kg^{-1} \cdot h^{-1}$ of glucose polymers and fructose with the addition 0.2 $g \cdot kg^{-1} \cdot h^{-1}$ protein was likely to have the largest ergogenic effect on endurance performance (Vandenbogaerde & Hopkins, 2011). The findings of this study would support this assertion; however there is a large variation in the reported performance effects of CHO-P supplements provided during exercise (Stearns et al., 2010). Current discrepancies can in part be explained by differences in both the test and comparison supplement contents, timing of ingestion, and in accordance with findings of this study, the nature of the exercise test. MSS possess several characteristics that make them unique from other endurance sports, including their repeated maximal intensity efforts and periods of low-intensity work such as rest, walking and slow jogging. It is also likely that they exert different physiological responses and patterns of substrate depletion when compared to continuous exercise (see Chapter 1). Consequently, it is important that recommendations for the ingestion of CHO-P during MSS exercise should be based on findings utilising such a form of exercise.

Chapter 5 of this thesis provided evidence that CHO-P ingestion lowered RPE at a regulated exercise intensity in the latter stages of simulated MSS exercise. Whilst this did not translate into a significant improvement in time to exhaustion, this study hypothesised that a lowered RPE, given its potential importance to pacing strategy (Edwards & Noakes, 2009; Tucker, 2009), may translate into changes in self-regulated exercise intensity. Previous authors (Vandenbogaerde & Hopkins, 2011; Saunders, 2007) have suggested that CHO-P may indeed exert its effects via a central mechanism in accordance with the central

fatigue hypothesis of Newshome et al. (1987). In the current study, RPE was not different between the beverage conditions at any time point during exercise. However, rather than indicating a lack of central effect of CHO-P ingestion, participants were exercising at a higher exercise intensity in the CHO-P trial compared to CHO and yet were rating their exertion as the same. Thus, it may be inferred that participants perceived a higher exercise intensity to be no harder with the addition of protein to a CHO beverage. On this basis, future studies might consider measuring plasma BCAA and f-TRP concentrations and brain f-TRP uptake (van Hall et al., 1995) during this form of exercise to elucidate the role of reduced central fatigue on enhanced performance with CHO-P ingestion.

A potential contributor to pacing strategy and performance during MSSs is substrate depletion, and more specifically the utilisation of muscle glycogen (Noakes et al., 2004a; Hampson et al., 2001). Krustrup et al. (2006) have reported that a soccer match can result in the full or partial depletion of 50% of individual muscle fibres, whilst more generally lower muscle glycogen has been associated with reduced high intensity work (Saltin, 1973) and sprint performance (Krustrup et al., 2006) in the latter stages of a soccer match. Interestingly, the unique periods of low intensity work and rest in MSSs may provide the opportunity for the provision of a nutritional supplement to resynthesise muscle glycogen and thus enhance the rate of ATP resynthesis in the latter stages of exercise (Jeukendrup, 2004). As muscle glycogen resynthesis can be insulin dependent (Jentjens & Jeukendrup, 2003), and CHO-P ingestion in the post-exercise recovery period has often been shown to enhance circulating insulin concentrations (Betts et al., 2008; Betts et al., 2007; Betts et al., 2005; Jentjens et al., 2001; van Loon et al., 2000; Zawadzki et al., 1992) it follows that such an intervention accelerates the rate of muscle glycogen restoration (Berardi et al., 2006;

Williams et al., 2003; Ivy et al., 2002; van Loon et al., 2000; Zawadzki et al., 1992). Additionally, CHO-P ingestion in some instances has been associated with a marginally higher insulin concentration *during* exercise (Saunders, 2007; Ivy et al., 2003), suggesting that in the case of MSSs, higher concentrations of muscle glycogen may be present in the latter stages of exercise owing to its intermittent resynthesis (Jeukendrup, 2004). In the current study, however, no significant difference in insulin concentration between beverage conditions during exercise was observed. As such, it seems unlikely that the mechanism described above was responsible for the observed improvements in performance. Nonetheless, future studies involving muscle biopsy samples would provide direct evidence of the effect of CHO-P ingestion on muscle glycogen content during MSS exercise. Furthermore, the potential for different CHO-P mixtures to alter pancreatic insulin release during this form of exercise are worthy of exploration, particularly as van Loon et al. (2000) reported that a mixture of CHO with protein hydrolysate, leucine and phenylalanine was the most insulinoptropic compared to a range of other CHO-P mixtures. Whether such a supplement is likely to exert a different effect to the current CHO-P supplement during MSS exercise is unknown and warrants further investigation.

The ergogenic effect of CHO-P ingestion could also be because the different transport mechanisms of amino acids and glucose across the intestinal wall (Stevens et al., 1982; Hellier et al., 1973) cause an increased fluid and/or fuel uptake in a similar concept to multiple-transportable CHOs (Saunders et al., 2007). In the present study, no difference in any measurements of hydration status between the two beverage conditions was present, including body mass change or plasma volume change. Whilst the former is more likely to reflect sweat rate in the acute exercise period (Maughan et al., 2005), an increase in the

latter might be expected if greater volumes of fluid were taken up into the systemic circulation from the intestine following CHO-P ingestion. Thus, alterations in hydration status were unlikely to account for performance differences in the present study. Interestingly, there was no difference in post-exercise fluid balance (assessed via post-exercise urine volume) between the CHO-P and CHO trials. Previous studies have observed that post-exercise fluid balance is enhanced with the addition of protein to a CHO supplement ingested after exercise (Watson et al., 2008; Shirreffs et al., 2007). This effect is thought to be due to a lower urine production, associated with the slower gastric emptying of slowly digested proteins, such as casein (Ten Have et al., 2007; Boirie et al., 1997) and increased sodium intake (Watson et al., 2008; Shirreffs et al., 2007). However, the present study only provided fluid during exercise whereby exercise-induced increases in circulating catecholamines may influence fluid retention (Wemple et al., 1997). Furthermore, measurements of urine volume may need to be taken for a longer period (~4 h) than in the present study (~1 h) to observe differences in fluid balance (Watson et al., 2008; Shirreffs et al., 2007).

No direct measure of energy expenditure, or indeed specific substrate utilisation, during exercise was recorded in the current study. As such it is unclear whether fuel transport across the intestinal wall, and subsequent increased rates of energy expenditure due to greater exogenous energy availability, were responsible for the increased average speed in the CHO-P trial. However, a higher plasma urea concentration at 60 min in the CHO-P trial compared to CHO was present, which may have been indicative of the deamination of amino acids for oxidation and subsequent energy provision or gluconeogenesis (Koopman et al., 2004; Colombani et al., 1999). In agreement, Koopman et al. (2004) reported

increased protein oxidation during endurance exercise when protein is added to a CHO supplement. Whether this took place in the current study is unknown, but such an effect may have increased energy availability for ATP resynthesis or potentially spared endogenous CHO stores for the latter stages of exercise, and thus improved performance. It is also worthy of note that blood glucose concentrations were not different between trials, as a depletion of this substrate has been implicated in the aetiology of fatigue during MSSs (Patterson & Gray, 2007; Nicholas et al., 1995). In agreement with previous studies (Toone & Betts, 2010; Saunders et al., 2009; Saunders et al., 2007; van Essen & Gibala, 2006; Saunders et al., 2004; Ivy et al., 2003), these data would suggest that replacing some CHO with protein during exercise does not compromise CHO availability for skeletal muscle or the central nervous system (Nybo, 2003).

It cannot be discounted that the observed performance differences in the present study were in some part due to a placebo effect. Indeed, Vandenbogaerde and Hopkins (2011) suggested that the addition of protein was likely to exert a greater effect on endurance performance than CHO alone, due, in some part, to the well-known difficulty of being able to match protein supplements with CHO for taste and texture. In an elegant study design, Clark et al. (2000) performed a study whereby participants performed a 40 km time trial whilst consuming water, and were then separated into six groups who performed a 40 km time trial with either CHO or placebo ingestion. Within each of these groups, participants were either told they were receiving CHO, a placebo, or given no information. It was reported that when participants were told they were receiving CHO, the difference in performance against water was 4.3%, where as it was only 0.3% when they were told they were consuming a placebo. Whilst every attempt was made to match taste and texture of

the two supplements by the supplement provider in the current study, and participants were blinded to which drink they were receiving, unfortunately it can not be discounted that participants perceived themselves to be consuming a supplement which is ergogenic and thus adjusted their exercise intensity. Perhaps a study similar to that of Clark et al. (2000) may be required to determine the placebo effect associated with CHO-P ingestion.

In conclusion, the present study shows that when participants are allowed to self-regulate their exercise intensity, CHO-P ingestion leads to attenuated decrements in average exercise intensity during simulated MSS exercise. More specifically, the addition of 2% whey protein isolate to a 6% CHO solution, delivered at a rate of approximately 49 $g \cdot h^{-1}$ and 0.27 $g \cdot h^{-1}$ respectively, reduced fatigue compared to CHO alone. The mechanisms for such an effect remain unclear, however the current study did not refute previous hypotheses that protein alters perceptions of exertion owing to reduced central fatigue or that protein oxidation is increased resulting in increasing energy availability or sparing of endogenous energy stores. As such, MSS athletes may wish to consider consuming such a CHO-P mixture during exercise to enhance their performance and attenuate fatigue.

Chapter 7

Conclusions

The effects of acute CHO-P ingestion, both in the post-exercise recovery period and during endurance exercise, have been studied extensively. The purported benefits of this supplement include improved endurance capacity and performance (Saunders et al., 2009; Saunders et al., 2007; Saunders et al., 2004; Ivy et al., 2003), rehydration (Watson et al., 2008; Shirreffs et al., 2007), muscle glycogen restoration rate (Beradi et al., 2006; Williams et al., 2003; Ivy et al., 2002; van Loon et al., 2000, Zawadzki et al., 1992) and recovery from EIMD (Ferguson-Stegall et al., 2010; Saunders et al., 2009; Cockburn et al., 2008; Valentine et al., 2008; Baty et al., 2007; Luden et al., 2007; Romano-Ely et al., 2007; Rowlands et al., 2007; Saunders et al., 2007; Bird et al., 2006; Saunders et al., 2004). Such effects make CHO-P supplementation attractive to MSS players given their physiological demands and recovery considerations. However, differences in physiological responses to continuous and intermittent exercise (such as MSSs) require the use of a specific exercise protocol to determine the effects of CHO-P in a MSS context. Accordingly, Chapters 3-6, utilising a simulation of MSSs, have comprised data collected with the intention of advancing knowledge of the potential benefits of the addition of a small amount of protein to a typical CHO supplement.

7.1 CHO-P and EIMD following MSS exercise

CHO-P supplementation has been shown to result in positive protein balance post-exercise (Koopman et al., 2004; Levenhagen et al., 2002), which may accelerate the restoration rate of damaged skeletal muscle proteins and reduce contractile protein loss during EIMD

(Bird et al., 2006). However, when a 7.5% CHO plus 2.5% protein solution was consumed in the 4 h post LIST, no statistical difference in the recovery of soreness or muscle function in the knee extensors and flexors when compared to CHO supplementation matched for either energy of CHO content was observed (Chapter 4). It is also noteworthy that ingestion of CHO-P compared to CHO elicited no significant difference in serum insulin concentrations during MSS exercise (Chapter 6), a peptide hormone which may reduce protein breakdown (Wolfe, 2001) and which has been implicated in altered EIMD (Saunders, 2007). Thus, in agreement with several previous studies (Breen et al., 2010; Green et al., 2008; White et al., 2008; Wojcik et al., 2001), and the only previous study to examine CHO-P and MSS exercise (Betts et al., 2009), indirect evidence from Chapter 4 demonstrates that CHO-P ingestion after MSS exercise does not influence EIMD. The discrepancy between the present findings and those that have reported an attenuated EIMD response with CHO-P ingestion are not clear, but may be explained in part by differences in exercise protocols, methods of EIMD assessment, severity of muscle damage and supplement composition and ingestion schedules.

7.2 CHO-P and recovery of dynamic muscle function

Few studies have reported on recovery of muscle function more closely associated with athletic performance with CHO-P ingestion. As with muscle function and soreness, CHO-P after simulated MSS exercise did not influence the recovery of any vertical jump parameter up to 48 h post-LIST (reduced by approximately 5-10 %). Furthermore, no differences were observed in several NMT derived sprint performance variables (e.g. peak speed, split times and step length and frequency), which had previously been shown to be reliable and sufficient to detect a difference of approximately 5% with the current sample size (Chapter

3). Thus, acute CHO-P ingestion, compared to CHO ingestion alone in the 4 h after MSS exercise, appears to confer little benefit for the recovery of selected performance measures over 48 h.

7.3 CHO-P supplementation and fatigue during MSS exercise

A notable finding of the present body of research was that CHO-P provided during MSS exercise, compared to CHO alone, resulted in participants maintaining a significantly higher (~2.5%) self-regulated average speed in the final 15 min of exercise, whilst moderate-small effect sizes were also observed for maximal speed and distance covered (Chapter 6). In contrast, Chapter 5 showed no significant improvement in run time to exhaustion between CHO-P ingestion and the ingestion of isocaloric or CHO matched supplements at the end of simulated MSS exercise. However, a wide range of time to exhaustion scores, potentially due to the use of too wide a range of fitness levels (assessed via the MSSRT) and the inclusion of males and females, were evident in this chapter, which may have precluded the statistical analyses from detecting differences in performance. Indeed, there was a non-significant mean improvement in performance above CHO with CHO-P of approximately 4%, whilst the majority of the sample (n = 6) demonstrated improvements in time to exhaustion with CHO-P ingestion.

Improvements in self-regulated MSS performance (Chapter 6) can potentially be explained by a reduced RPE during exercise (Chapter 5), in accordance with the 'central fatigue hypothesis' of Newsholme et al. (1987). Accordingly, a lower RPE may have altered the pacing strategy of participants (Tucker, 2009), particularly when they were allowed to

self-regulate their exercise intensity (Chapter 6). More specifically, the lower RPE as a consequence of CHO-P ingestion may have resulted in an up-regulation in exercise intensity, such that RPE was increased to the participants' expected levels at that stage of exercise (Edwards & Noakes, 2009). It is unlikely that differences in hydration status, serum insulin concentrations and blood glucose can explain the observed effects during MSS exercise as no difference in any of these parameters were observed in the present studies (Chapters 5 and 6). However, an increase in plasma urea during exercise with CHO-P ingestion (Chapter 6) may have been indicative of an increased rate of protein utilisation (Koopman et al., 2004; Colombani et al., 1999) and thus endogenous CHO stores may have been spared to maintain exercise intensity in the latter stages of exercise.

7.4 CHO-P supplementation in MSSs

The combination of studies in the present thesis appear to suggest that benefits associated with the acute ingestion of CHO-P, when compared to CHO alone, are confined to an attenuation in fatigue when ingested during exercise, and not acute post-exercise recovery of muscle function. The mechanism for such an effect is not yet clear, however it is likely to be protein specific, as such effects were observed when compared with CHO supplementation matched for energy content. Accordingly, MSS athletes may benefit from ingestion of a 6% CHO plus 2% whey protein isolate beverage during competition, and as such may choose to incorporate it into their nutritional strategy on a match day. However, several limitations to the current thesis should be noted.

7.2 Potential limitations

7.2.1 Participant training status

As noted in previous research reviews (e.g. Burke, 2008), studies in the area of sports nutrition are rarely conducted with elite athletes. Whist the data derived from studies may be relevant to the exercising public, it is questionable whether the same effects with nutritional supplementation would be present in highly trained elite athletes who likely possess a number of neuromuscular and cardiovascular adaptations that separate them from sub-elite MSS players. For example, training at the elite level may elicit the 'repeated bout effect' (McHugh et al., 1999), whereby EIMD is attenuated post-exercise, and thus may alter the efficacy of a recovery supplement such as CHO-P. Moreover, training is likely to result in alterations in substrate metabolism, with a general reduced reliance on CHO utilisation after training (Friedlander et al., 1997). In the current research, participants who regularly engaged in MSSs, predominantly at University level, were utilised. The collated characteristics of each of the samples used in Chapters 4-6 are presented below.

Table 7.1 Participant characteristics throughout the thesis and in elite soccer

	Chapter 3	Chapter 4	Chapter 5	Chapter 6	Elite soccer*
Age (years)	22.3 ± 3.6	22.4 ± 1.8	21.7 ± 2.1	23.4 ± 1.8	26.1 ± 4
Stature (m)	1.8 ± 0.1	1.74 ± 8.4	1.77 ± 0.1	1.78 ± 0.1	1.77 ± 0.1
Body Mass (kg)	80.3 ± 8.4	68.1 ± 11.3	72.0 ± 13	75.3 ± 12	76.4 ± 7
MSSRT	-	10.6 ± 1.6	11.3 ± 2.2	11.6 ± 1.1	13 ± 1.2
$\dot{V}O_{2max}$ ($ml \cdot kg \cdot min^{-1}$)	-	48.9 ± 5.4	51 .0 ± 7.6	52.5 ± 3.8	56 - 69

* As reported by Reilly, Bangsbo & Franks (2000) and Balsom (1994).

Whilst there are many factors that may influence an individual's rise to elite performance, it is evident that there are differences between the participant characteristics in the present thesis and those in elite soccer. Most notably, elite soccer players typically have a higher aerobic capacity, which appears to lower the decrement observed in high intensity work in the latter stages of MSSs compared to sub-elite players (Mohr et al., 2003). Whether this would have affected the efficacy of CHO-P ingestion during or post-exercise is unclear, and a study examining this potential effect would be interesting. However, coaches and athletes are often unwilling to participate in such empirical studies.

7.2.2 Pre-exercise fasting

Chapters 4, 5 and 6 were conducted after an overnight fast (~12 h). This is a common aspect of research in sports nutrition, primarily due to the potential confounding effects of pre-exercise nutritional intake. However, such practice is likely to be rare amongst competing athletes, where a nutritious high CHO meal 3-4 h before competition is often recommended (Williams & Serratosa, 2006). The influence of the pre-exercise fasting on the potential efficacy of acute CHO or indeed CHO-P supplementation during exercise is unclear (Vandenbogaerde & Hopkins, 2011; Jeukendrup, 2004). However, a recent review of the literature into CHO mouth rinsing, the primary effects of which are thought to be central in origin, clearly demonstrated that ingestion of a nutritious meal in the hours before exercise dramatically lowered the supplement's ergogenic potential (Rollo & Williams, 2011). Whether such an effect is evident with CHO-P ingestion is not known, and as such an experimental study in which pre-exercise nutrient ingestion is manipulated would appear warranted.

7.2.3 Lack of placebo

No studies in the present research incorporated a placebo trial, predominantly because of the consistent ergogenic property of CHO ingestion that has been demonstrated during both prolonged endurance exercise (Jeukendrup, 2004) and MSS exercise (Phillips et al., 2011) when compared to a placebo. Thus, it was assumed that any difference between CHO-P ingestion and CHO would, by implication, demonstrate that CHO-P ingestion was more effective than a placebo. However, as previously acknowledged, the potential for a placebo effect with CHO-P ingestion may be strong owing to the well-known difficulties in

matching taste and texture (Vandenbogaerde & Hopkins, 2011). Consequently, it can not be dismissed that any observed effects in the current programme of research were due to a placebo effect, rather than a physiological protein-specific mechanism.

7.3 Directions for future research

7.3.1 Optimal type and rate of ingestion of CHO-P during MSS

The optimal rate of ingestion of CHO-P is currently not known, although based on rates of maximal CHO oxidation (60-70 $g \cdot h^{-1}$, Jeukendrup & Jentjens, 2000), and rates of protein ingestion with observed effects (~ 20 $g \cdot h^{-1}$, see section 2.4), the ingestion rates utilised in the current studies (CHO = 49 $g \cdot h^{-1}$, P = 18 $g \cdot h^{-1}$) seemed appropriate. However, no direct comparison of rates of CHO-P ingestion has been made, and such a study would be useful for informing the practice of athletes. By monitoring changes in exogenous substrate oxidation and intestinal absorption of fluid, glucose and amino acids via ingested tracers, a clearer picture of optimal rates of ingestion should be available. Interestingly, to date no study has examined the optimal rates of ingestion of CHO during MSS exercise, despite the popularity of CHO ingestion during MSSs. As the present research demonstrated no changes in performance, physiological or perceptual measures during MSS exercise when CHO was ingested at a rate approximately 65 or 49 $g \cdot h^{-1}$, it is likely that ingestion of CHO > 49 $g \cdot h^{-1}$ will not benefit MSS performance, even though higher rates of exogenous CHO oxidation are possible with higher ingestion rates (Jeukendrup & Jentjens, 2000).

The effect of the type of protein ingested during exercise is not clear, but different proteins are known to affect the rate of appearance of amino acids in the blood (Boirie, et al., 1997),

and also muscle anabolism (Boirie et al., 1997) and insulin response (van Loon et al., 2000). Saunders et al. (2009) recently demonstrated that late exercise performance was enhanced with a protein hydrolysate, which is known to increase the rate of amino acid absorption and insulin response. Whether hydrolysates would be additive during MSSs is unknown, and a study directly comparing protein types is warranted.

7.3.2. Mechanisms through which CHO-P exerts its effect

The mechanisms through which CHO-P may enhance recovery from EIMD or endurance performance are currently speculative. The present research has not provided any irrefutable evidence for a mechanism of CHO-P in either scenario. With regard to recovery, it has been demonstrated that CHO-P ingestion is more effective than CHO at improving post-exercise protein balance via increased and decreased rates of protein synthesis and degradation respectively (Koopman et al., 2004; Levenhagen et al., 2002). However, whether this leads to an increase in the functional rate of recovery of skeletal muscle is unknown. A measurement of protein balance in Chapter 4 of this thesis may have helped to provide an answer to such a question.

During exercise, several mechanisms of the ergogenic effect of CHO-P have been suggested, none of which have been proven. The current research suggests that increased insulinemic response can be discounted, and that reduced central fatigue and increased protein oxidation may be important. However, this assertion is based on indirect evidence (RPE and blood urea respectively). Additionally, a potential placebo effect could not be discounted. Measurements of brain f-TRP uptake as previously described (see van Hall et

al., 1995), protein oxidation via ingested protein tracers and muscle glycogen utilisation via muscle biopsies or near magnetic resonance spectroscopy, and the use of a design elucidating placebo effects of CHO-P during MSS exercise in future work may help clarify any of these potential mechanisms. Indeed, it is the author's opinion that little consensus will exist on the effects of CHO-P ingestion until physiologically plausible and proven mechanisms for enhanced recovery and/or improved performance have been elucidated.

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Appendices

Ethical approval letter for Chapter 3

DEPARTMENT OF SPORT & EXERCISE SCIENCES

MODULE SS5060

Research Project Ethics Application 2007-08

Title: Reliability and concurrent validity of non-motorised treadmill ergometry

Student(s): Edd Thompson, Ben Massey, Cindi Chaplin and Mike Lee

Supervisor(s): Mr Jamie Highton and Dr Craig Twist

On behalf of the Area Ethics Committee, I am pleased to confirm a favourable ethical opinion for the above research on the basis described in the application form and supporting documentation.

DATE: 10/05/08

Area Leader, Exercise Physiology

Ethical approval letter for Chapter 4

Jamie Highton
Room CFS014
Department of Sport and Exercise Sciences
University of Chester

31 March 2008

Dear Jamie

Study title: **The effect of carbohydrate and protein ingestion on the recovery of maximal intensity exercise performance**

FREC reference:196/08/JH/SES

Version number: **2**

Thank you for sending the above-named application to the Faculty of Applied and Health Sciences' Research Ethics Committee for review.

The application has been considered on behalf of the Committee by Steve Lewis as Lead Reviewer and reported to the Faculty's Research Ethics Committee.

On behalf of the Committee, I am pleased to confirm a favourable ethical opinion for the above research on the basis described in the application form and supporting documentation.

This approval is given provided that you comply with the conditions set out in the attached document. You are advised to study the conditions carefully.

The Committee would also like to suggest the following points; amendments should be provided to the Committee but do not need to be seen before approval is granted:

- Use the phrase 'open wounds' in place of 'blood wounds';
- Note that a written protocol for blood insulin measurement via ELISA should be provided for the applicant's file.

The final list of documents reviewed and approved by the Committee is as follows:

Document	Version	Date
Application form	1	January 2008
Participant information sheet	1	January 2008
Participant consent form	1	January 2008
Participant health questionnaire	2	March 2008
Protocol for DOMS management	1	January 2008
Risk Assessment forms x9	1	January 2008
Recruitment poster	2	March 2008
Schematic representation of data collection process	1	March 2008
Response to FRECs request for additional information	1	March 2008

With the Committee's best wishes for the success of this project.

Yours sincerely,

Dr. Stephen Fallows

Chair, Faculty Research Ethics Committee

Enclosures Standard conditions of approval

c.c. Supervisor

FREC Representative

Ethical approval letter for Chapter 5

DEPARTMENT OF SPORT & EXERCISE SCIENCES

MODULE SS5060

Research Project Ethics Application 2009-10

Title: The effect of carbohydrate plus protein ingestion on exercise capacity during simulated multiple-sprint sport activity

Student(s): Adam Flannigan, Daniel Gruic, Joe Thompson, Abigail Wright, Danielle Wrigley

Supervisor(s): Mr Jamie Highton and Dr Ceri Nicholas

On behalf of the Area Ethics Committee, I am pleased to confirm a favourable ethical opinion for the above research on the basis described in the application form and supporting documentation.

Area Leader, Exercise Physiology DATE: 04/05/10

Ethical approval letter for Chapter 6

Jamie Highton

c/o Department of Sport and Exercise Sciences

University of Chester

Chester campus

20 April 2010

Dear Jamie

Study title: **Effects of carbohydrate and protein on multiple-sprint sport performance**

FREC reference:399/10/JH/SES

Version number: **2**

Thank you for sending the above-named application to the Faculty of Applied and Health Sciences Research Ethics Committee for review.

The application has been considered on behalf of the Committee by Helen Lewis as Lead Reviewer and reported to the Faculty Research Ethics Committee.

On behalf of the Committee, I am pleased to confirm a favourable ethical opinion for the above research on the basis described in the application form and supporting documentation.

The favourable opinion is given provided that you comply with the conditions set out in the attached document. You are advised to study the conditions carefully.

The final list of documents reviewed and approved by the Committee is as follows:

Document	Version	Date
Application Form	1	March 2010
Reference list	1	March 2010
Summary CV of applicant	1	March 2010
Participant information sheet	2	April 2010
Consent form	1	March 2010
Risk assessment forms	1	March 2010
Participant health questionnaire	1	March 2010
Protocol diagram	1	March 2010
DOMS management protocol	1	March 2010

With the Committee's best wishes for the success of this project.

Yours sincerely,

Cynthia VBurek

Prof. Cynthia Burek

Chair, Faculty Research Ethics Committee

Enclosures Standard conditions of approval.

c.c. Supervisor

FREC Representative

Participant information sheet for Chapter 3

Participant Information Sheet

Reliability and concurrent validity of non-motorised treadmill ergometry

You are being invited to take part in a research study. Before you decide, it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with others if you wish. Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part.

Thank you for reading this.

What is the purpose of the study?

This study is being conducted as part of a PhD in Sport and Exercise Sciences which aims to examine the consistency with which a non-motorised treadmill can measure short distance sprint performance.

Why have I been chosen?

The study is seeking to investigate the above response in healthy active adults (18-30 years).

Do I have to take part?

It is up to you to decide whether or not to take part. If you decide to take part you will be given this information sheet to keep and be asked to sign a consent form. If you decide to take part you are still free to withdraw at any time without giving a reason.

What will happen to me if I take part?

Initially you will come to the laboratory to be familiarised with running on the non-motorised treadmill. This session will take up to one hour. You will then be asked to sprint on the non-motorised treadmill on two separate days (1 hour each) and outdoors (1 hour) on another day in a random order. Each day will involve three 30 m sprints. In total, testing will be completed within two weeks.

What are the possible disadvantages and risks of taking part?

It is important that participants attend all laboratory and exercise sessions. We realise that this is a significant commitment for participants and will involve approximately four hours of your time over a period of approximately two weeks.

What are the possible benefits of taking part?

By taking part, you will be enabling researchers to gain a greater understanding of the use of a non-motorised treadmill for the assessment of sprint performance. Subsequently, it is hoped that this will inform the development of appropriate nutritional strategies for performance enhancement and recovery as part of a wider body of research. Additionally, participants will gain useful information about their own physiological capabilities, which may be useful for training purposes.

What if something goes wrong?

If you wish to complain or have any concerns about any aspect of the way you have been approached or treated during the course of this study, please contact Professor Sarah Andrew, Dean of the Faculty of Applied and Health Sciences, University of Chester, Parkgate Road, Chester, CH1 4BJ, 01244 513055.

Will my taking part in the study be kept confidential?

All information that is collected about you during the course of the research will be kept strictly confidential so that only the researcher carrying out the research project will have access to such information. All data will be coded to ensure anonymity.

What will happen to the results of the research study?

Results of this project may be published but any data included will in no way be linked to any specific participant. You are welcome to request a copy of the results of the project should you wish. The data collected will be securely stored in such a way that only those mentioned above will be able to gain access to it.

Who is organising and funding the research?

The Department of Sport and Exercise Sciences at the University of Chester.

Who may I contact for further information?

If you have any questions about the project, either now or in the future, please feel free to contact Jamie Highton via the Department of Sports and Exercise Sciences, Tel: 01244 511189, j.highton@chester.ac.uk.

Thank you for your interest in this research.

Participant information sheet for Chapter 4

Participant Information

The Effect of Carbohydrate and Protein Ingestion on the Recovery of Maximal Intensity Exercise Performance

You are being invited to take part in a research study. Before you decide, it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with others if you wish. Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part.

Thank you for reading this.

What is the purpose of the study?

This study is being conducted as part of a PhD in Sport and Exercise Sciences which aims to examine any potential benefits of ingesting a carbohydrate plus protein sports beverage following exercise designed to simulate the demands of multi-sprint sports (e.g. soccer, hockey and rugby). More specifically, this study will aim to examine whether the ingestion of the carbohydrate plus protein beverage can enhance the recovery of maximal intensity exercise performance (i.e. sprinting and jumping) in the days following completion of intermittent high-intensity exercise and the potential mechanisms behind it.

Why have I been chosen?

The study is seeking to investigate the above response in healthy active adults (18-30 years).

Do I have to take part?

It is up to you to decide whether or not to take part. If you decide to take part you will be given this information sheet to keep and be asked to sign a consent form. If you decide to take part you are still free to withdraw at any time without giving a reason.

What will happen to me if I take part?

One week prior to testing you will be asked to attend the laboratory to familiarise yourself with the procedures we will be using, these are described below.

Initially, we will measure your upper-leg strength using a specific piece of apparatus which will involve you performing maximal contractions of the thigh and hamstring muscles at different speeds in a seated position. Then we will measure your running speed on a non-motorised treadmill (whereby you generate the force to move the treadmill belt) and your jumping ability. These measurements will provide a baseline against which we can compare the effect of the intervention. You will be given time to recover between each test, which in total should take no more than 1 hour. Next you will be asked to carry out a multi stage fitness test called the bleep test. This involves continuous running between two lines 20 metres apart in time to recorded beeps until exhaustion, and will provide a measure of your fitness.

After a weeks rest, you be asked to return to the laboratory to perform 90 min of intermittent shuttle running, involving periods of walking, jogging and all out sprinting. There will be 3 min rest periods between each of the shuttles, which are performed in 15 min blocks. In the 4 h following the completion of the intermittent shuttle run you will be provided with a drink to consume. In total you could expect to drink no more than 2 litres of fluid. Prior to commencing the shuttle running we will ask you to have a capillary (finger prick) sample of blood taken to measure your resting level plasma urea. Once you have completed the exercise test, we will ask you to have a capillary sample of blood taken, to jump for maximal height, to sprint on the non-motorised treadmill and we will measure the strength of your upper-leg muscles again. You will also be asked to provide a perceived rating of muscle soreness, measured by you bending at the knees and moving a pointer along a sliding scale to indicate discomfort. After a day's rest we will ask you to return to the laboratory and have these measurements (not including the intermittent shuttle run and blood sampling) taken again. Forty-eight hours after completing the exercise test, you will return to the laboratory to have the measurements taken for the last time.

From 9 o'clock the night before the 90 min exercise test until competition of testing the next day, you will be asked to fast; water will be allowed. You will also be asked to abstain from exercise the day prior to exercise testing. On the latter 3 days of testing, we will also ask you to complete a food diary detailing the type and amount of food and drink ingested. This information will be used to examine any potential influence of your diet on muscle-damage response.

What are the possible disadvantages and risks of taking part?

It is likely that you may experience a short bout of muscle soreness, a decreased range of motion, swelling and stiffness in the upper leg muscles. This will be most evident approximately two days following the intermittent shuttle run, after which all symptoms will subside and will have disappeared after one week with no lasting effect. It is unlikely, but you may experience some gastrointestinal upset following ingestion of the nutritional supplement. If this occurs, symptoms should subside 24 h after the ingestion of the supplement drink.

It is important that participants attend all laboratory and exercise sessions. We realise that this is a significant commitment for participants and will involve approximately 6 hours of your time.

What are the possible benefits of taking part?

By taking part, you will be enabling researchers to gain a greater understanding of the effects nutritional supplementation on dynamic exercise performance and muscle damage following high intensity, intermittent exercise. Subsequently, this will inform the development of appropriate nutritional strategies for the management of post-exercise muscle soreness. Additionally, participants will gain useful information about their own physiological capabilities, which may be useful for training purposes. A bout of muscle-damaging exercise will also provide a protective effect and reduce the occurrence of symptoms following a repeat of similar exercise in the future.

What if something goes wrong?

If you wish to complain or have any concerns about any aspect of the way you have been approached or treated during the course of this study, please contact Professor Sarah Andrew, Dean of the Faculty of Applied and Health Sciences, University of Chester, Parkgate Road, Chester, CH1 4BJ, 01244 513055.

If you are harmed by taking part in this project, there are no special compensation arrangements. If you are harmed due to someone's negligence (but not otherwise), then you may have grounds for legal action but you may have to pay for this.

Will my taking part in the study be kept confidential?

All information that is collected about you during the course of the research will be kept strictly confidential so that only the researcher carrying out the research project will have access to such information. All data will be coded to ensure anonymity.

What will happen to the results of the research study?

Results of this project may be published but any data included will in no way be linked to any specific participant. You are welcome to request a copy of the results of the project should you wish. The data collected will be securely stored in such a way that only those mentioned above will be able to gain access to it.

Who is organising and funding the research?

The Department of Sport and Exercise Sciences at the University of Chester.

Who may I contact for further information?

If you have any questions about the project, either now or in the future, please feel free to contact Jamie Highton via the Department of Sports and Exercise Sciences, Tel: 01244 511189, j.highton@chester.ac.uk.

Thank you for your interest in this research.

Participant information sheet for Chapter 5

Participant Information Sheet

The effect of carbohydrate plus protein ingestion on exercise capacity during simulated multiple-sprint sport activity.

You are being invited to take part in a research study. Before you decide to participate, it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with others if you wish. Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part.

Thank you for reading this.

What is the purpose of the study?

The potential for carbohydrate to enhance endurance capacity and offset fatigue during multiple-sprint sport activity has already been established. The purpose of this study is to examine the effect of ingesting a small amount of protein added to a carbohydrate beverage on endurance capacity towards the end of simulated soccer match-play.

Why have I been chosen?

You have been chosen because you are a University team sports player aged 18-30 and have volunteered to be available for testing at the required times.

Do I have to take part?

It is up to you to decide whether or not to take part. If you decide to take part you will be given this information sheet to keep and be asked to sign a consent form. If you decide to take part you are still free to withdraw at any time and without giving a reason. A decision to withdraw at any time, or a decision not to take part, will not affect your rights in any way.

What will happen to me if I take part?

If you decide to take part, initial measurements will include the assessment of estimated VO_2 max from the multi-stage fitness test (Bleep test to exhaustion) and familiarisation with the Loughborough Intermittent shuttle test (LIST). You will return to the laboratory between 48 and 72 hours later for baseline measurements of plasma glucose and urea and serum insulin taken via a venous blood sample following an overnight fast. The first trial of the LIST will then be completed including the ingestion of one of the three beverages and subsequent measurements of plasma glucose, urea and serum insulin. Both one and two weeks later participants will complete the LIST consuming each of the different beverages (carbohydrate plus protein, carbohydrate only matched for energy content and carbohydrate only matched for carbohydrate content), You will be asked to maintain a food diary in the 48 hours before the initial completion of the LIST, and then will be asked to replicate this before all other trials. You will also be asked to refrain from strenuous physical exercise in the 24 hours before each main trial. The LIST involves a standardized 15 minute warm-up, followed by a series of 15 minute exercise blocks interspersed with three minute rest periods. The approximate commitment time from you is two hours per session, per week for three weeks taking place on the same day and time of each week. This will also involve 2 hours during the first week for familiarisation and preliminary testing before the trials begin.

What are the possible disadvantages and risks of taking part?

You will be asked to exercise until exhaustion and may experience fatigue which may result in muscle soreness. Venous blood sampling will also need to be carried out immediately pre-and-post-LIST during each trial. If you do not wish to participate in venous blood sampling you can choose not to do so. There is also a possible risk of gastrointestinal discomfort as a result of the beverage ingestion which should subside within a few hours.

What are the possible benefits of taking part?

Participants will receive information about their own fitness capabilities. If results reveal that the addition of protein to a carbohydrate sports drink improves exercise capacity then results can be used in applied settings with elite and non-elite athletes.

What if something goes wrong?

If you wish to complain or have any concerns about any aspect of the way you have been approached or treated during the course of this study, please contact Professor Ken Green, Head of Department, Department of Sport and Exercise Sciences, University of Chester, Parkgate Road, Chester, CH1 4BJ, 01244 513426.

If you are harmed by taking part in this research project, there are no special compensation arrangements. If you are harmed due to someone's negligence (but not otherwise), then you may have grounds for legal action, but you may have to pay for this.

Will my taking part in the study be kept confidential?

All information which is collected about you during the course of the research will be kept strictly confidential so that only the three researchers carrying out the research will have access to such information.

What will happen to the results of the research study?

The results will be written up into a student dissertation and, possibly, a research paper that will be submitted to an academic peer-reviewed journal. Individuals who participate will not be identified in any subsequent report or publication.

Who is organising and funding the research?

The research is organised and conducted by a student of the Department of Sport and Exercise Sciences at the University of Chester.

Who may I contact for further information?

If you would like more information about the research before you decide whether or not you would be willing to take part, please contact:

Name: Mr Jamie Highton

University E-mail: j.highton@chester.ac.uk

Thank you for your interest in this research.

Participant information sheet for Chapter 6

Participant Information Sheet

Effects of Carbohydrate and Protein on Multiple-Sprint Sport Performance

You are being invited to take part in a research study. Before you decide, it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with others if you wish. Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part.

Thank you for reading this.

What is the purpose of the study?

This study is being conducted as part of a PhD in Sport and Exercise Sciences which aims to examine any potential benefits of ingesting a carbohydrate plus protein sports beverage both during and after exercise designed to simulate the demands of multi-sprint sports (e.g. soccer, hockey and rugby). More specifically, this study will aim to examine whether the ingestion of the carbohydrate plus protein beverage can enhance performance during multiple-sprint sports, and the potential mechanisms behind it.

Why have I been chosen?

The study is seeking to investigate the above response in healthy active adults (18-30 years).

Do I have to take part?

It is up to you to decide whether or not to take part. If you decide to take part you will be given this information sheet to keep and be asked to sign a consent form. If you decide to take part you are still free to withdraw at any time without giving a reason.

What will happen to me if I take part?

Initially, we will measure your endurance capacity using the multi-stage shuttle run test (commonly known as 'the bleep test'). This test involves continuous running between two lines 20 metres apart in time to recorded beeps until exhaustion, and will provide a

measure of your fitness. In order to complete the study, it will be a requirement that you reach a pre-determined level (level 8) on this test, so that participants are representative of an athletic population. If you do not reach this level, then you will not be required to take part in the remainder of the study.

After a weeks rest, you be asked to return to the laboratory to perform 90 min of intermittent shuttle running (i.e. 'the LIST') involving periods of walking, jogging and all out sprinting. During the intermittent shuttle run you will be provided with a drink to consume. In total you should expect to drink no more than 2 litres of fluid, and during this time you will not be permitted to consume any food. Prior to commencing the shuttle running we will ask you to have a venous blood sample taken to measure your resting level plasma urea, glucose and insulin. These blood measurements will also be taken during (once) and then at the end of the intermittent shuttle running. Approximately 1 week later you will be asked to repeat all of the above measurements with the exception of the multi-stage shuttle run test.

From 9 o'clock the night before the 90 min exercise test until competition of testing the next day, you will be asked to fast; water will be allowed. You will also be asked to abstain from exercise the day prior to any exercise testing. On the days of testing, we will also ask you to complete a food diary detailing the type and amount of food and drink ingested. You will then be asked to repeat your diet based on the food diary during the second bout of testing (i.e. during the second intermittent shuttle run test).

What are the possible disadvantages and risks of taking part?

It is likely that you may experience a short bout of muscle soreness, a decreased range of motion, swelling and stiffness in the upper leg muscles. This will be most evident approximately two days following the intermittent shuttle run, after which all symptoms will subside and will have disappeared after one week with no lasting effect. It is unlikely, but you may experience some gastrointestinal upset (i.e. feelings of fullness, bloating and/or nausea) following ingestion of the nutritional supplement. If this occurs, symptoms should subside within the 24 h after ingestion of the supplement drink.

It is important that participants attend all laboratory and exercise sessions. We realise that this is a significant commitment for participants and will involve approximately 3 and half hours of your time over a period of approximately 1 week.

What are the possible benefits of taking part?

By taking part, you will be enabling researchers to gain a greater understanding of the effects nutritional supplementation on high intensity, intermittent exercise. Subsequently, this will inform the development of appropriate nutritional strategies for the performance enhancement and recovery. Additionally, participants will gain useful information about their own physiological capabilities, which may be useful for training purposes.

What if something goes wrong?

If you wish to complain or have any concerns about any aspect of the way you have been approached or treated during the course of this study, please contact Professor Sarah Andrew, Dean of the Faculty of Applied and Health Sciences, University of Chester, Parkgate Road, Chester, CH1 4BJ, 01244 513055.

Will my taking part in the study be kept confidential?

All information that is collected about you during the course of the research will be kept strictly confidential so that only the researcher carrying out the research project will have access to such information. All data will be coded to ensure anonymity.

What will happen to the results of the research study?

Results of this project may be published but any data included will in no way be linked to any specific participant. You are welcome to request a copy of the results of the project should you wish. The data collected will be securely stored in such a way that only those mentioned above will be able to gain access to it.

Who is organising and funding the research?

The Department of Sport and Exercise Sciences at the University of Chester.

Who may I contact for further information?

If you have any questions about the project, either now or in the future, please feel free to contact Jamie Highton via the Department of Sports and Exercise Sciences, Tel: 01244 511189, j.highton@chester.ac.uk.

Thank you for your interest in this research.

Participant health questionnaire

Participant Health Questionnaire

Effects of Carbohydrate and Protein on Multiple-Sprint Sport Performance

(PLEASE NOTE THAT THIS INFORMATION WILL BE CONFIDENTIAL)

Individuals will not be permitted to take part in any experimental testing if they:

- Have been instructed to exercise under medical supervision only
- Have a known history of medical disorders
- Have a history of infectious diseases
- Are pregnant

Name:……. DOB:…….. Age:……

Resting Blood Pressure……mmHg

Resting Heart Rate…….bpm

Please answer these questions truthfully and completely. The purpose of this questionnaire is to ensure that you are fit and healthy enough to participate in this research project.

	Yes	**No**
1. Has your doctor ever indicated that you have a heart condition and recommended only medically supervised exercise?	☐	☐

	Yes	**No**
2. Have you ever experienced chest pain brought on by physical exertion?	☐	☐

3. Do you suffer, or have you suffered from:

	Yes	No
Asthma	☐	☐
Diabetes	☐	☐
Bronchitis	☐	☐
Epilepsy	☐	☐
High blood pressure	☐	☐

	Yes	No
4. Are you suffering from any infectious skin diseases, sores, blood wounds (i.e. cuts and grazes), or infections (i.e., Hepatitis B, HIV, etc.)?	☐	☐
5. Are you currently taking any medication	☐	☐

If Yes, please provide details

……
……
……

	Yes	No
6. As far as you are aware, are you pregnant?	☐	☐

	Yes	No
7. Is there anything to your knowledge that may prevent you from participating in the testing that has been outlined to you?	☐	☐

If Yes, please provide details

……
……
……

	Yes	No
8. Have you taken part in any systematic resistance training in the last 6 months?	☐	☐

If Yes, please provide details

……
……
……

9. How many hours of physical activity do you participate in per week? ……

My responses to the above questions are true to the best of my knowledge and I am assured that they will be held in the strictest confidence.

_____	_____	_____
Name of participant	Date	Signature

_____	_____	_____
Researcher	Date	Signature

Informed consent form example

Informed Consent Form

Title of Project: Effects of Carbohydrate and Protein on Multiple-Sprint Sport Performance

Name of Researcher: Jamie Highton

	Yes	No
1. I confirm that I have read and understand the information sheet dated…… for the above study and have had the opportunity to ask any questions.	☐	☐
2. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason and without my legal rights being affected.	☐	☐
3. I understand that the results of this research may be published but that my name or identity will **not** be revealed at any time. In order to keep my records confidential, Jamie Highton will store all information as numbered codes in computer files that will only be available to him or his research assistant.	☐	☐
4. I agree to take part in the above study.	☐	☐

_____	_____	_____
Name of participant	Date	Signature
_____	_____	_____
Name of person taking consent (If different from researcher)	Date	Signature
_____	_____	_____
Researcher	Date	Signature

NMT familiarisation instructions

Instructions for Using the Force Treadmill

Firstly give precise instructions:

1. Give a good description to the running technique required e.g. 'It is like running up a hill or in sand'.
2. Reassure the subject that they need to trust the belt/harness will hold them. Instruct them to lean into it. Spend time on this.
3. Instruct them to keep their head up when sprinting (VERY IMPORTANT!) perhaps get them to focus on the speed column on the display when they sprint, or another object in front of the treadmill/wall if you do not wish them to see their speed.

Secondly perform the familiarisation sessions as follows:

1. Ask the subject to walk, and then jog at their own pace to get used to it and how it feels.

2. Perform a sprinting test which involves 3 sprints separated by 3 minutes of active recovery (This is to prevent sickness and nausea). Ease them into the sprints not going all out until the last sprint. Instruct them that they need to overstride a little at the end of their stride to generate maximal belt speed. The duration of the sprints should be informed by your research protocol.

3. Stand where they can see you, and constantly talk to them to see how they are feeling. Remind them to keep their head up, and constantly reassure and encourage them.

4. Repeat test as above anther 2 times. Most subjects will have picked up the technique confidently by this stage. It may be necessary to repeat the familiarisation test one more time with some subjects.

Daily Food Diary

Name_____

The following food diary should be completed over the **48 hours** prior to, and on **the day of**, performing the Loughborough Intermittent Shuttle Test, and is designed to provide a comprehensive overview of your food and fluid intake which you will be asked to repeat on your second testing visit. NOTE – you should also **not** consume any food or fluid in the morning before you perform the LIST. In completing this food diary, you should aim to adhere to the following guidelines, using the example below as a template:

- Record all food and fluid intake during the 48 hours (even water), including the time of ingestion
- Provide information on how meals are prepared (i.e. fried, poached, grilled etc.)
- Include ingredients which are added to foods during cooking, such as olive oil, salt, butter etc.
- Provide the amount and type of food consumed in the most accurate way possible. Weighing your food is ideal, but if this isn't possible then include rough estimates on portion size (i.e. 1 cup full, teaspoon etc.)

Time	Food/Drink	Amount
9 am	Quaker porridge oats with skimmed milk	50 g, ¼ pint
	Muller fat-free Yogurt	1 average pot
10 am	Bacon sandwich (wholemeal bread) with ketchup	2 rashers, 2 slices, tablespoon

Day 1

Time	Food/Drink	Amount

Day 2

Time	Food/Drink	Amount

Day 3

Time	Food/Drink	Amount