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The Influence of Hypotonic Carbohydrate Electrolyte Solutions on Muscle Metabolism and Exercise Capacity with regards to Intermittent High Intensity Shuttle Running

by

Andrew Foskett

Submitted in partial fulfilment of the requirements for the award of Doctor of Philosophy of Loughborough University

November 2003

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Performance and exercise capacity in prolonged continuous exercise has been shown to be enhanced by the ingestion of dilute (~6%) carbohydrate-electrolyte (CHO-E) solutions during exercise. There is a wealth of literature on the effects of CHO-E solutions on prolonged, continuous exercise however this is not the case for high-intensity intermittent exercise. One reason for this is the difficulty of replicating the demands of multiple-sprint sports within the laboratory. The development of the Loughborough Intermittent Shuttle Running Test (LIST), a protocol that simulates the physiological demand of multiple-sprint sports, has allowed for further study in this area.

Sports drinks contain a variety of different types of carbohydrate, including synthetic polymers of maltodextrins. The rationale for these is two-fold; firstly there is some evidence to suggest that these glucose polymers of low osmolality may empty faster than isoenergetic glucose solutions especially at dilute (~6%) concentrations. Secondly CHO-E solutions formulated from maltodextrins with low dextrose equivalents are less acidogenic and have lower cariogenic potential than simple glucose solutions, thus making them preferable for tooth health. Therefore the purpose of this thesis was to examine the effects of a 6.4% hypotonic synthetic polymer maltodextrin CHO-E solution on muscle metabolism and physiological performance during prolonged high-intensity intermittent running (LIST).

This thesis contains four experimental studies. From the results of study 1 it was concluded that the ingestion of a 6.4% hypotonic CHO-E solution provided no ergogenic benefit to running capacity during the LIST despite blood and hormonal data suggesting that the solution was effective at delivering glucose. As isotonic CHO-E solutions have shown to have ergogenic properties during LIST running the purpose of study 2 was to compare isoenergetic hypotonic and isotonic CHO-E solutions during LIST running. The results from this study suggested that performance, as measured by exercise capacity and sprint speed, was not affected by either treatment.
In study 3 the protocol was modified to extend the exercise duration so that a greater demand was placed on the subjects' glycogen reserves. The results from this study further confirmed that exercise capacity in LIST running was not influenced by CHO provision during exercise however the habitual CHO intake of the subjects may have masked any ergogenic properties of the CHO-E solution and led to increased performance in the control trials.

In the final study the subjects' pre-trial exercise and dietary CHO intake was manipulated to increase endogenous glycogen concentrations. The results showed that in these subjects the ingestion of a 6.4% hypotonic CHO-E solution increased exercise capacity during the LIST by 21%. Muscle biopsy analyses revealed a lower net muscle glycogen utilisation in the CHO trial post-90 min (p=0.07).

There is evidence in the literature to suggest that there is an ergogenic effect of CHO ingestion during the LIST. However the results presented in this thesis suggest that when endogenous glycogen stores are moderate the ingestion of a CHO-E solution may suppress lipid oxidation without a concomitant increase in CHO oxidation and thus LIST capacity is not enhanced. In contrast, when endogenous glycogen concentrations are elevated through dietary manipulation there is an ergogenic benefit from the ingestion of hypotonic CHO-E solutions during LIST exercise.

**Keywords:** sports drinks, multiple-sprint sports, muscle glycogen, fatigue, endogenous carbohydrate, exogenous carbohydrate

This thesis is dedicated to the memory of Maarten van den Braak.
"Nobody said it was easy,
No-one ever said it would be this hard..."

The Scientist - Coldplay
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CHAPTER 1

INTRODUCTION

1.1 Introduction

It is generally accepted that performance and capacity in prolonged continuous exercise can be enhanced by the provision of carbohydrate, in the form of a dilute carbohydrate-electrolyte solution, during exercise (see Coggan and Coyle, 1991; Coyle and Montain, 1992; Tsintzas and Williams, 1998 for reviews). The mechanisms responsible for the improvements in capacity may be dependent on the modality of exercise but it is thought to be due to either maintenance of blood glucose or decreased utilisation of glycogen in the exercising muscles. Although there is a wealth of literature concerning prolonged, continuous exercise there is far less information on the effects of carbohydrate ingestion on physiological performance or muscle metabolism during high-intensity intermittent exercise. This is paradoxical when one considers that there is far greater participation globally in multiple-sprint sports compared to endurance events. The main reason for the lack of extensive study on intermittent sports is the difficulty in simulating game situations in a valid and reliable manner. Some studies have extrapolated data from intermittent protocols on cycle ergometers however this is erroneous due to the aforementioned differences in exercise mode. Furthermore the mechanisms underpinning the ergogenic properties of the carbohydrate-electrolyte solutions may be specific to the type of exercise undertaken. Similarly investigators have attempted to replicate the physiological demands of intermittent exercise on treadmills but this again would confound results because of the absence of the accelerating and decelerating actions integral to turning during the free running common in most games.

The development of a protocol that elicits physiological demands similar to those experienced in multiple sprint sports – The Loughborough Intermittent Shuttle Run Test [LIST] (Nicholas et al., 2000) has allowed for further study in this area. The LIST has been utilised within our laboratory to investigate the effects on intermittent running on soccer skill performance (McGregor et al., 1999; Ali et al., 2002); as well as exercise in the heat (Morris et al., 1998; 2000; 2003). It has also been adopted by
other laboratories for studies on the influences of branch-chain amino acids and chromium on fatigue (Davis et al., 1999; 2000) as well as carbohydrate ingestion and mental performance (Welsh et al., 2002).

The LIST has also been utilised to examine the effects of exogenous carbohydrate ingestion on physiological performance and muscle metabolism during high-intensity intermittent exercise (Nicholas et al., 1995; 1999). Nicholas et al., (1995) reported an increased high-intensity running capacity following 75min of the LIST. They attributed this improvement in performance to a sparing of glycogen in the working muscle fibres following ingestion of a carbohydrate-electrolyte solution. As with the majority of the studies on prolonged exercise the carbohydrate used in the above studies was provided in the form of an isotonic carbohydrate-electrolyte beverage. The optimal formulation of a fluid and fuel replacement beverage depends on several factors although it is generally agreed that such solutions should have a carbohydrate content in the range of 4-8% carbohydrate. They should not be hypertonic but should contain small amounts of electrolytes (see Murray et al., 1999; Coombes and Hamilton, 2000 for an overview of CHO-E solution properties).

More recently the use of synthetic polymers of maltodextrins, as the main source of carbohydrate in carbohydrate-electrolyte beverages, has increased because they provide carbohydrate without a concomitant increase in osmolality. There is general agreement that osmolality has a negligible effect on the gastric emptying rates of carbohydrate solutions when compared to energy density. Nevertheless there is evidence to suggest that glucose polymers may empty faster than isoenergetic glucose solutions especially at dilute (~5%) concentrations (Foster et al., 1980). Information on the rates of intestinal absorption of hypotonic and isotonic isoenergetic carbohydrate solutions is less clear, with some studies suggesting that hypotonic solutions may be absorbed more readily than isotonic solutions (Wapnir and Lifshitz, 1985, Leiper et al., 1994a). The evidence has been provided by perfusion studies in which the stomach has been by-passed and the solution delivered directly to segments of the small intestine. However when solutions are ingested it appears that differences in osmolality are equilibrated by the time the solution has progressed to the proximal duodenum. Therefore it seems that there may be no practical benefits of hypotonic
solutions (Gisolfi et al., 2001), nevertheless these solutions have not been shown to impair fluid or carbohydrate delivery when compared with isotonic solutions.

The high sugar content and low acidity of carbohydrate-electrolyte beverages coupled with their use during exercise, when dehydration may lead to a reduced salivary flow, may contribute to both dental erosion and caries production (Milosevic, 1997). Although others refute these claims (Murray, 1997; Brouns and Muntjewerf, 1997) and cite a lack of evidence proffering that many beverages consumed are acidic and cariogenic in nature (tea, coffee, fruit juice, soft drinks and alcoholic beverages) there has still been a move by manufacturers to address this dental health issue. Carbohydrate-electrolyte solutions formulated purely from maltodextrins with low dextrose equivalents are less acidogenic and so of a lower cariogenic potential than simple glucose solutions (Duggal et al., 2002).

The relative merits of potentially ‘toothkind’ maltodextrin solutions versus standard glucose solutions on dental health are beyond the scope of this thesis. However the search for a ‘toothkind’ carbohydrate-electrolyte solution is, on balance, a worthwhile pursuit and has had an effect on the current formulation of carbohydrate-electrolyte solutions for distribution to sports performers. As there is a burgeoning need for science to have practical implications it is important therefore to investigate exercise modalities common to the practitioner and also carbohydrate solutions that are manufactured for general consumption. In a reasonably comprehensive review on carbohydrate ‘sports drinks’ Coombes and Hamilton (2000) state that of nearly 70 studies examined only 16 of them investigated commercially available carbohydrate-electrolyte solutions.

Therefore the purpose of this thesis is to examine the effects of a hypotonic synthetic polymer maltodextrin carbohydrate-electrolyte solution on muscle metabolism and physiological performance during prolonged high-intensity intermittent running.
1.2 Organisation of Thesis

This thesis is presented in eight main chapters. The review of literature deals with the most relevant literature on intermittent exercise, metabolism and performance with particular reference to carbohydrate-electrolyte ingestion and factors associated with fatigue.

The general methods chapter (Chapter 3) describes the equipment and the testing procedures used during the administration of the experimental tests and the procedures associated with the collection and analysis of blood samples, expired air samples and muscle extraction and metabolite assays.

The main aim of the first study (Chapter 4) was to investigate the exercise capacity and physiological responses during the latter stages of high intensity intermittent exercise following the ingestion of a hypotonic carbohydrate solution.

The second study (Chapter 5) was similar to the first but offered slight modifications to the exhaustive capacity test in an attempt to better elucidate the point of fatigue. It also offered a direct comparison between isotonic and hypotonic carbohydrate solutions and the ergogenic potential of both. In this study the volume of ingesta was increased to maximise gastric volume and also CHO delivery.

The principal aim of the third study (Chapter 6) was to extend the LIST protocol in an attempt to more realistically simulate the demands of a soccer match (including extra-time) and to examine the effects that exogenous carbohydrate provision, in the form of a hypotonic solution, may have on this.

The purpose of the final study (Chapter 7) was to expand on the previous investigation in order to evaluate the effects that high endogenous carbohydrate stores may have on endurance capacity and whether there was an accumulative effect of exogenous carbohydrate provision. To further elucidate on the mechanisms underpinning fatigue this study also investigated the metabolic changes occurring within the muscle cell during the LIST and at fatigue.
The final chapter of the thesis (Chapter 8) summarises all of the studies and discusses the potential mechanisms that may explain the findings. It also addresses some of the issues on which future research could focus.
CHAPTER 2

LITERATURE REVIEW

2.1 Introduction
The purpose of this chapter is to highlight and critically examine the pertinent literature which has examined the physiological and metabolic responses to intermittent high intensity exercise and the influence of nutritional intervention, namely in the form of exogenous CHO provision, during exercise.

The studies included have examined the efficacy of exogenous CHO provision during intermittent high intensity exercise and the factors affecting the delivery and oxidation of CHO-E solutions during such activity. The review of literature consists of 5 main sections. The first section following the introduction describes the physiological demands of intermittent running exercise. The following section details the aetiology of fatigue during such activity. This is followed by a section detailing the effect of endogenous liver and muscle glycogen concentrations and also the effects of the provision of exogenous carbohydrate during intermittent activity both in field studies and in the laboratory. The final sections concern the factors affecting the delivery of CHO to the systemic circulation namely the rate of gastric emptying and intestinal absorption as well as the uptake of glucose into the muscle cell and its subsequent oxidation rate.

2.2 Physiological Demands of Intermittent Running Exercise
Sports that involve intermittent running activity, such as basketball, hockey, rugby and soccer, are both prolonged in duration and involve periods of high-intensity exercise. It has been speculated that an individual player may exhibit in excess of one thousand changes in playing activity each lasting approximately five seconds during a soccer match (Reilly and Thomas, 1976; Bangsbo et al., 1991) and during women's hockey (Lothian and Farrally, 1994). Basketball players also display on average a thousand discrete activities during a match, changing movement every two seconds (McInnes et al., 1995). Whilst between 500-700 separate rugby specific activities have been reported for players during competitive rugby union matches (Deutsch et al., 1998). As such there is a large variability in the physiological demands imposed on players in a range of intermittent sports.
A number of techniques have been adopted by researchers to collate data on the physiological demands of match play in intermittent sports including time-motion studies (Mayhew and Wenger, 1985) and video analysis (Bangsbo et al., 1991). However due to the different methods adopted by the authors and their individual classifications of movement patterns it is difficult to draw comparisons between these studies. Furthermore the sport specific activities, such as heading in soccer or scrummaging, rucking and mauling in rugby make it difficult to generalise on the physiological demands of intermittent sports. As such the following is a review of the literature concerning the physiological demands of a range of intermittent activities. There is an inherent bias towards the physiological demands of soccer due to the wealth of research into this sport and the paucity of information reported on other intermittent sports. For example, in a review of science and its application to football (Reilly and Gilbourne, 2003) the authors report that soccer accounted for 65% of all scientific studies presented on the various codes of football. Furthermore some of the research on these other intermittent sports may not appear in peer-reviewed journals (Cibich, 1991; Woolford and Angove, 1991); or may have methodologies that are questionable due to small subject pools; limited number of matches analysed (Ramsey et al., 1970; Woolford and Angove, 1991; Coutts et al, 2003) and training status of the players (McArdle et al., 1971).

2.2.1 Distance Covered

Many authors report match analysis data on the total distance covered during a soccer match. Distances range from as low as seven kilometres (Reilly and Thomas, 1976) to thirteen and a half kilometres (Whitehead, 1975). However most of the authors recount distances of between 8-12 km for male players (Withers, 1982; Ekblom, 1986; Van Gool et al., 1988; Bangsbo et al., 1991; Strudwick and Reilly, 2001); with similar distances reported for female players (~10.5km) (Mohr et al., 2003). Shorter distances (3.8-9.6km) have been reported during rugby union matches (Reid and Williams, 1974; Williams 1976; Deutsch et al., 1998); rugby league matches (5-8km) (Brewer and Davis, 1995; Meir et al, 1993) and World Cup hockey matches (5.6km) (Wein, 1981). There appear to be no data on total distance covered during basketball although an early study (Blake, 1941) reported that players averaged two kilometres during
defensive play, thus suggesting that in the modern era and incorporating both
defensive and offensive play the distances would be much greater.

Individual players have exhibited differences of up to 0.92 km in distance covered
between soccer matches (Bangsbo et al., 1991) and differences have also been noted
between playing positions in both soccer (Bangsbo et al., 1991) and rugby (Deutsch et
al., 1998; Docherty et al., 1988). These disparities could serve to explain the ranges
expressed in the literature within sports. Such variance could be attributable to
estimation methods, specificity of positional demands, match tactics or systems of
play and the importance of the match.

2.2.2 Speed and Intensity of Player Movement
Although players may cover distances from as little as a few kilometres up to about
fourteen kilometres during intermittent activity, this value alone tells us little of the
physiological demands of these matches. Of more importance is the intensity at which
these distances are covered. Again the match analysis data on this parameter differ
with some authors stating the proportion of distance covered at various intensities
(Reilly and Thomas, 1976) while others report the percentage of time spent at various
intensities (Mayhew and Wenger, 1985; Bangsbo et al., 1991; McInnes et al., 1995;
Deutsch et al., 1998). Mayhew and Wenger (1985) found that soccer players spent
the total match time (90min) at the following intensities; standing 2.3%, walking
46.4%, jogging 38%, running 11.3% and utility (side and backward movements) 2%. Deutscht et al., (1998) differentiated between certain playing positions (front five,
back row, inside backs and outside backs) but reported that rugby players spent the
total match time (70min) at the following range of intensities; inactive 40-47%,
walking 14-28%, jogging 5-6%, cruising 2-3%, sprinting 2-3%, rucking/mauling 4-
5%, scrummaging 6% and 4-6% in static high-intensity actions. McInnes et al., (1995)
report that basketball players spent the total 'live' time (ie time when ball is in play)
(36min) at the following intensities; standing/walking 35%; jogging 12%; running
13%; striding/sprinting 8%, shuffling 29% and jumping 3%.

Reilly and Thomas (1976) found that soccer players covered the total distance (8.7
km) at the following intensities; 24% walking, 36% jogging, 20% cruising, 11%
sprinting, 7% backing off and 2% moving in possession of the ball. What is pertinent
to note is that authors generally concur that the main difference between high and low ability players is not the total distance covered but the proportion of distance covered at the highest intensity (Bangsbo et al., 1992; Mohr et al., 2003).

The studies that report exercise intensities as percentages of total time would appear to show that intermittent sports players spend relatively large periods of the match at low intensities. These low intensity periods would appear to be to allow players to recover between successive work bouts. McLean (1992) and Deutsch et al., (1998) report work: rest ratios averaging 1:1.9 in competitive rugby; whilst in hockey, ratios of 2:5 for men (Wein, 1981); and 1:5.7 for women (Lothian and Farrally, 1994) have been reported which are similar to that of soccer (Reilly and Thomas, 1976).

2.2.3 Energy Requirements
The intermittent nature of sports, such as soccer, makes them more physiologically demanding than a continuous run of similar distance (Tumilty, 1993). In addition to this, players also utilise energy to overcome inertia (stopping, starting, turning, falling and standing) which would elevate the energetic cost. Furthermore the energetic cost of dribbling a ball was 5.2 kJ·min⁻¹ greater than running at the same speed irrespective of the intensity in soccer (Reilly and Ball, 1984) and 15-16 kJ·min⁻¹ greater in hockey (Reilly and Seaton, 1990). Similar elevated energy demands would be expected for running with the ball in hand in rugby, as this has been reported to negatively affect sprinting ability (Grant et al., 2003); and also dribbling in basketball, although at present there are no energetic data reported on such actions.

Zelenka and colleagues (1967) estimate the energetic cost of a ninety-minute soccer match to be 55 kJ·min⁻¹, a value in accordance with those expressed by Reilly (1990) of between 21-73 kJ·min⁻¹ and similar to the range (30-74 kJ·min⁻¹) reported for hockey players (Reilly, 1981; Boyle et al., 1994) and female college basketball players (30-50 kJ·min⁻¹) (McArdle et al., 1971). However Coutts et al., (2003) report higher values (85 kJ·min⁻¹) for rugby league players. This may be as a consequence of their larger physiques and also the greater energetic demand from the upper body during the contact phases of play.
2.2.4 Aerobic Contribution

Attempts to measure the aerobic contribution during intermittent match play through indirect calorimetry lead to interference in the game with such measurements being impractical in the field (Durnin and Passmore, 1967). Ekblom (1986) monitored the rectal temperature (T_r) of high ability Swedish soccer players and from laboratory comparisons estimated exercise intensities of ~80% VO_2max. Generally heart rate (HR) responses are monitored with the HR-VO_2 relationship being pre-determined in the laboratory (Åstrand and Rodahl, 1986). Values of 157-175 beats·min⁻¹ have been recorded in soccer with estimates of oxygen uptake at 75% VO_2max (Ekblom, 1986; Van Gool et al., 1988; Bangsbo, 1994; Reilly, 1997). Ogushi and colleagues (1993) believe these values to be an overestimation and through expired air collection, in modified Douglas bags, during an actual soccer match report values of oxygen uptake as low as 47-49% VO_2max. However Ogushi only analysed minor parts of the match and therefore these data are not fully representative. Rodríguez and Iglesias (1998) reported that oxygen uptake measured using a portable analyser was 15% lower than that estimated from heart rate data during a friendly soccer match. Using portable expired air analysis, Miyagi and Ohashi (2003) determined oxygen uptake during an elite youth soccer match and report values of ~70% VO_2max. Subjects' heart rates averaged 176 beats·min⁻¹ which is in accordance with earlier reported data.

Boyle (1994) estimated an oxygen uptake of 78% VO_2max for hockey play although this appears to be high when one considers that their subjects displayed average heart rates of approximately 159 beats·min⁻¹. Similar estimations were reported for female soccer players (80% VO_2max) but again this seems an overestimate as average heart rates were 164 beats·min⁻¹. Heart rates reported for rugby union are toward the lower range of soccer players (155 beats·min⁻¹) (Atkinson, 1999), this is probably due to the technicality of the re-starts in the game and the time spent at rest by the backs whilst these set plays occur. Whilst basketball, netball and ice hockey players display heart rates that are toward the higher end (170-180 beats·min⁻¹) of those exhibited by soccer players (Higgs et al., 1982; McArdle et al., 1971; Ramsay et al., 1970; Woolford and Angove, 1991; Green et al., 1976 and 1978b; Paterson, 1979). This is possibly a consequence of the laws of these games that allow multiple ‘rolling’ substitutions whereby players can compete on court/rink at a higher intensity knowing that they
will shortly be 'benched' where they can recover. This may explain why ice hockey players can maintain intensities of approximately 81%VO$_{2\text{max}}$ during their 'on-ice' time, (Green et al., 1976) and basketball players have estimates of oxygen uptake of ~86%VO$_{2\text{max}}$ (McArdle et al., 1971). Similar data have been reported for rugby league players (~80%VO$_{2\text{max}}$) correlating with heart rates of approximately 166 beats·min$^{-1}$.

Bangsbo (1994) offered a more conservative estimate for oxygen uptake of 70%VO$_{2\text{max}}$ for soccer stating that heart rate values overestimate oxygen uptake due to other stresses of soccer match play such as emotional conditions and static muscle contractions causing heart rate to become disproportionate with oxygen uptake (Balsom et al., 1992). Similarly (Deutsch et al., 1998) believe that heart rates in rugby may be elevated due to increased release of catecholamines owing to the contact nature of tackling, rucking and mauling. The data from Ramsay et al., (1970) offer a limited insight into emotional stresses on heart rates. They reported that a basketball player's heart rate remained elevated during foul shots, above levels that reflected the physical demand of the shot, if the subject was making the shot but recovered if another player was making the attempt. This data should be interpreted with caution, however, due to the small sample size. A further factor in using heart rates to estimate aerobic contribution is that sports that involve a lot of upper-body activity, such as rugby, ice hockey and basketball confound estimates of oxygen uptake (Paterson et al., 1979).

Some authors report the percentage of match play spent in relative heart rate zones. During basketball approximately 15% of match play was spent at intensities higher than 95%HR$_{\text{peak}}$ (McInnes et al., 1995) whilst in rugby approximately 20% of activity was at 95% of competitive maximal heart rate (Deutsch et al., 1998) and in hockey approximately 30% of the match was spent at aerobic intensities greater than 92%HR$_{\text{max}}$.

2.2.5 Anaerobic Contribution

Although match analysis data on soccer suggests that only 12% of total activity may be classed as anaerobic (Mayhew and Wenger, 1985), many coaches, players and spectators would argue that it is these high-intensity movements that are the most
crucial to successful performance. Bangsbo et al (1991) report 19 sprint occurrences each lasting approximately 2s from a player during a 90min football match, whilst Reilly et al (Reilly and Thomas, 1976; Reilly, 1994) report a far higher occurrence of sprints (55-60). Studies in soccer report moderate exercise blood lactate concentrations of 4-6 mmol.l⁻¹ (Rohde and Espersen, 1988; Gerisch et al., 1988, Miyagi and Ohashi, 2003), both during and post match, suggesting that energy production was derived primarily from aerobic sources. A further soccer study (Bangsbo et al., 1991) elicited blood lactate concentrations of 10 mmol.l⁻¹ suggesting a larger anaerobic glycolytic contribution, these data concur with those of Ekblom (1986) who reported peak blood lactate values in excess of 12 mmol.l⁻¹ during soccer. McInnes et al., (1995) report mean blood lactate concentrations of 7 mmol.l⁻¹ during a basketball match with the highest peak value for an individual player reaching 13 mmol.l⁻¹. Average blood lactate responses to rugby union were in the range of 5-7 mmol.l⁻¹ (Deutsch et al., 1998) with McLean (1992) reporting a peak concentration of 10 mmol.l⁻¹. A wider range of blood lactate concentrations (3.8-15 mmol.l⁻¹) were reported in rugby league match play (O’Connor, 2003) with an average of ~9 mmol.l⁻¹. Blood lactate concentrations in ice hockey declined with match duration and averaged 8.7 mmol.l⁻¹, 7.3 mmol.l⁻¹ and 5.0 mmol.l⁻¹ for the first, second and final periods respectively (Green et al., 1976).

However since blood lactate concentration reflects a balance between lactate entry into and removal from the blood it therefore cannot really be used to quantify the extent of glycolysis. Furthermore lactate serves as a gluconeogenic precursor and will likely be metabolised during the low intensity bouts that follow the intense exercise periods in a match (Bangsbo et al., 1993). Bangsbo (1997) states that the values reported from intermittent activity are consistent with the activity in the five minutes prior to measurement. Furthermore the differences in blood lactate values found in the literature can be attributed to the importance and standard of the game or the tactics adopted by the teams, with Gerisch and colleagues (1988) reporting higher values in soccer teams employing man-to-man marking than in zonal defence.
2.2.6 Substrate Utilisation

High intensity, intermittent exercise places a high demand on the body's liver and muscle glycogen reserves (Hargreaves, 1994). It is well recognised that energy from carbohydrate oxidation is required to perform exercise greater than 60%VO$_{2\text{max}}$. Performing prolonged intermittent, high intensity exercise of approximately 70%VO$_{2\text{max}}$ for prolonged periods (60-90min), as is common in competitive intermittent sports, causes the body to utilise much of the carbohydrate stores as an energy source. Coggan and Coyle (1991) postulate that for the first hour of cycling exercise at 70-75%VO$_{2\text{max}}$ most of the carbohydrate energy is derived from muscle glycogen and after this blood glucose uptake and oxidation increase progressively. The contribution of protein as a substrate during intermittent running activity is unclear, however during continuous exercise of similar duration and intensity the oxidation of protein contributed to less than 10% of total energy production (Wagenmakers et al., 1991). Although both protein and lipid are utilised as fuel during prolonged intermittent activity it is well recognised that neither source would deplete, unlike carbohydrate, to the extent that the capacity to transduce energy in the working muscles would be impaired (Jacobs, 1988; Shephard, 1992).

Due to the overall intensity of these activities and the highly intensive bouts of exercise it is generally accepted that prolonged intermittent exercise places large demands on the body's limited endogenous CHO stores. This appears substantiated by data from actual soccer games that report an increase in FFA mobilisation towards the latter stages of match play suggesting a shift in substrate utilisation brought about by a compromised availability of CHO (Bangsbo, 1993). Furthermore match analysis data exhibit decreased distances covered in the second half of soccer matches, especially the higher intensity movements (Reilly and Thomas, 1976; Van Gool et al., 1988; Bangsbo et al., 1991) thus suggesting fatigue. Some may argue that the reduction in performance in the latter stages of intermittent sports play may be as a consequence of the outcome of the game being already decided. However a more recent study (Mohr et al., 2003) reported similar findings of fatigue yet stipulated that in 21 of the 27 games analysed the goal difference between the two teams was only one goal or less. Furthermore the work rate of substitute players was higher in the final 15min of match play than regular players suggesting that fatigue may have contributed to the decrements in high-intensity running (Mohr et al., 2003). Finally, Rebelo et al., (1998)
had subjects perform sprint tests and intermittent running tests pre-, half-time and after a friendly match. They reported that both sprint performance and intermittent running performance were negatively affected by the soccer match and that players were showing evidence of fatigue.

2.2.7 Models for Simulating High-Intensity Exercise

Although there are inter-positional and inter-sport differences in the physiological demands of intermittent exercise, from the data reported in the literature it is possible to make some generalisations. Match analysis data informs us that players within intermittent sport are making numerous discrete activities throughout the duration of the match with each of these activities lasting seconds. These discrete movements accumulatively amount to a distance covered in the match that can be measured in kilometres whilst encompassing a broad range of exercise intensities. These intensities range from standing rest to maximal sprints but also include sport specific motions such as jumping, shuffling and dribbling as well as isometric muscular contractions during activities like scrummaging in rugby or 'boxing out' in basketball. Physiological measures from actual match play inform us that players are placing large demands on their aerobic system to provide energy with estimates of oxygen uptake averaging in excess of 70% of the individual’s maximum. Furthermore measures of blood lactate intimate that there is a large anaerobic contribution to these activities and the data reported on work: rest ratios may suggest that there is insufficient recovery between bouts of high intensity activity to maintain maximal performance throughout the duration of the match.

There are obvious limitations with utilising actual match conditions for research processes due to the lack of a controlled environment. For this reason researchers have attempted to simulate the physiological demands of intermittent exercise in the laboratory. Researchers have used data from sporting activities to design cycling (Hargreaves et al., 1984; Fielding et al., 1985; Yaspelkis et al., 1993) and treadmill (Bangsbo et al, 1992; Walton and Rhodes, 1997; Nassis et al., 1998; Quanz, 1999) protocols to simulate intermittent activity although these are limited by the exercise modality and the lack of free running and turning. More recently researchers have devised free running protocols utilising shuttle runs to incorporate turning, accelerating and decelerating which more closely simulate the physiological demands
of intermittent match play (see Nicholas et al., 2000 for a detailed methodology). By utilising such controlled simulations it is possible for researchers to more accurately determine the aetiology of fatigue in intermittent running exercise and also to examine possible interventions to maximise performance, such as nutritional intervention.

2.3 Mechanisms of Fatigue in Intermittent Exercise.

2.3.1 Repeated Sprints

Intermittent sports such as soccer, hockey and rugby have periods of brief maximal intensity effort punctuated by rest or lower intensity effort (see 2.2 Physiological Demands of Intermittent Running Exercise). The ability to reproduce power output during these maximal intensity bouts is of great importance to successful performance in intermittent sports. Power output (PO) is impaired when recovery between sprints is of insufficient duration (Holmyard et al., 1987; Gaitanos et al., 1993). Harris and colleagues (1976) demonstrated that the recovery of maximal muscle performance followed a similar pattern to that of the chemical recovery of the muscle. This is likely a reflection of the regulation of the rate of ATP production during maximal effort or the existence of a common regulatory factor such as the recovery of intramuscular pH.

It has been well documented that PCr concentrations decline with contractile activity (Boobis et al., 1982; Nevill et al., 1996) and that a decrease in maximal performance could be as a consequence of an insufficient restoration of PCr (Balsom, 1992). Sahlin and co-workers (1998) reported that maximum PO declined after 10s of exercise and corresponded with the depletion of PCr. This data is supported by the study of Bogdanis and colleagues (1995) which reported a co-incidental occurrence of peak power output (PPO) and PCr resynthesis. Further support for this are the dietary creatine supplementation trials that demonstrated that elevated muscle [PCr] led to increased sprint performance in cycling, swimming and soccer (Greenhaff et al., 1994; Peyrebrune et al., 1998; Mujika et al., 2000). Harris and associates (1976) found PCr resynthesis to be a biphasic process; following an exponential recovery with the fast portion having a half-time of 21-22s and the slow portion ~170s. Other authors report higher values for the rapid component of 30-40s (DiPrampero and Margaria, 1969) and ~57s (Bogdanis et al., 1995).
Decreases in muscle glycogen during maximal sprinting show that glycogenolytic processes are initiated within six seconds of exercise (Boobis et al., 1982) and subsequent decreases in pH brought about by glycogenolysis may help to contribute to fatigue (Cheetham et al., 1986; Bogdanis et al., 1995). This may account for the findings that fatigue during repeated sprinting occurs before total glycogen depletion (Hermanssen and Vaage, 1977; Cheetham et al., 1986). There are alternative theories on the mechanisms of fatigue due to decreases in intramuscular pH. Nakamura and Schwartz (1971) promulgated that H⁺ inhibits the sarcoplasmic reticulum ATPase thus reducing cellular uptake of Ca²⁺ and indirectly leads to an impairment of the excitation-contraction coupling mechanism. Hermanssen (1981) reported that the increased [H⁺] may directly affect the contractile mechanism of the muscle. This theory is supported by studies on rabbit skeletal myofilaments by Blanchard and colleagues (1984) who demonstrated that H⁺ ions compete with Ca²⁺ ions and reduce their affinity for binding with troponin. For a more detailed overview on metabolic factors that contribute to altered Ca²⁺ regulation in skeletal muscle fatigue the author recommends a review paper by Steele and Duke (2003).

Whichever mechanism is responsible, it is necessary for pH to return towards baseline values in order to reproduce maximal performance. For this to occur H⁺ ions must translocate from the muscle cell. The half-time recovery for pH is reported to be between 3-9.5 min (Sahlin et al., 1975); however data are equivocal as other authors report unchanged muscle pH following 3-10 min of recovery (Allsop et al., 1990; Bogdanis et al. 1996). Furthermore data from actual intermittent match play suggest that although muscle pH is significantly lower during intense periods of soccer play than it is at rest (Krustup et al., 2003) it does not fall below 6.9 due to the extended low intensity recovery periods between high-intensity activities. Therefore sprint performance may not be compromised due to the effects of acidity on excitation contraction coupling during such activity.

The literature regarding repeated multiple sprint exercise generally follow similar generic protocols, that is brief maximal ergometric effort (6-30s) separated by a fixed recovery duration (>20s). During competitive intermittent sports play the timing of sprint activity and the length of recovery duration are often dictated by the pattern of play. As such players may often have fractional recovery periods between successive
sprints. Short lasting maximal intensity exercise causes muscle cells to gain Na\(^+\) and lose K\(^+\) continuously (Sjøgaard et al., 1985; Sejersted and Hallen, 1987) creating an ionic imbalance dependent on the pattern of muscle activation. Cherry and colleagues (1998) had subjects sprint on a cycle ergometer for 30s and then with a fractional recovery period (2-3s) sprint again for 6s. They reported that the rapid initial recovery phase of PO was not associated with restoration of PCr and speculated that the ability to generate maximal PO may be related to ionic factors.

It would appear that for maximal intensity effort to be repeated indefinitely and for fatigue to be delayed would necessitate sufficient recovery duration. The duration of the recovery period would depend on the rate of resynthesis of PCr and of the restoration of intramuscular pH. The majority of the studies examining repeated sprint performance utilise passive recovery between successive bouts. This would lead to a pooling of blood and an overestimation of recovery time for metabolic resynthesis and restoration of ionic imbalances. An active recovery, as evidenced in intermittent sports performance, would lead to an elevated blood flow and a concomitant increase in PCr resynthesis (Bogdanis et al., 1996); an increased removal of lactate (Signorile et al., 1993); and a greater restoration of anaerobic power in successive sprints (Ahmaida et al., 1996).

Holmyard et al., (1987) investigated the influence of recovery duration on repeated sprinting on a non-motorised treadmill. On two occasions subjects completed ten 6s sprints separated by either a 30s recovery or a 60s recovery between successive sprints. With the shorter recovery duration subjects’ mean power output (MPO) and peak power output (PPO) fell by 21.4% and 13.2% respectively between the first to the last sprint. Conversely with the 60s recovery protocol there were negligible decrements in power output of 4.2% and 3% for MPO and PPO respectively.

The mean duration of maximal intensity sprinting in intermittent sports is approximately 2s. This is of shorter duration than the sprint bouts in most studies and therefore subsequent recovery duration required between bouts will be less. This is demonstrated by Balsom et al., (1992) who investigated the effect of 30s recovery on maximal repeated sprint performance over 15m, 30m and 45m. Subjects could not replicate maximal performance over 30m and 45m however the authors report no
decline in sprint performance over 15m (~2.5s) through sprints 1-40. As this latter
distance is that most commonly covered at maximal intensity during actual
intermittent game play it would suggest that 30s is sufficient recovery duration.

It may be that 30s recovery is sufficient to restore [PCr] following a brief sprint and
this may explain the data from Balsom et al., (1992). Whether sprint performance can
be maintained over a longer duration of intermittent activity is equivocal. Some data
from our laboratory demonstrate a slow but significant decline in 15m sprint
performance throughout 90min of intermittent shuttle running (LIST) (Ali et al.,
2002). Whereas earlier data from our laboratory report similar 15m sprint times
throughout 75min (Nicholas et al., 1995) and 90min of LIST (Nicholas et al, 1999)
although the same author reports a tendency for faster sprint times in the first 20min
of a 70min LIST protocol (Nicholas et al., 1997). The decrements in sprint
performance over a sustained duration are likely to be as a consequence of a
continuous accumulation of H+ ions and the subsequent decrease in intramuscular pH.
The nature of the LIST protocol is such that subjects experience a 15m maximal sprint
approximately every 82s, although probably sufficient to restore [PCr] this may be
insufficient, despite the active recovery, to fully translocate H+ ions from the muscle
cell. Although blood lactate concentrations from this protocol (Ali et al., 2002) do not
reflect the decrements in sprint performance these do not reflect the true muscle
lactate concentration. Some of this lactate will be metabolised within the active
muscle or taken up by other organs and inactive muscle during the recovery period
following the sprint but prior to blood sampling (Bangsbo, 1993).

2.3.2 Fatigue Due to Substrate Availability
There is a well established relationship between both muscle glycogen stores and
blood glucose values and endurance performance at relatively high exercise intensities
(Coyle and Montain, 1992). As previously mentioned intermittent activity,
characteristic of games play, places a large demand on the anaerobic provision of
energy and therefore utilises carbohydrate as a fuel source. This imposes demands on
hepatic glucose release, blood-borne substrate and muscle glycogen stores. Fatigue
during these types of activity could be as a consequence of insufficient energy
provision and therefore studies have investigated the effects of both muscle glycogen
concentrations and blood glucose concentrations on performance in actual and simulated intermittent game situations.

2.3.2.1 Muscle Glycogen
During exercise of 60-85%\(\text{VO}_2\text{max}\) fatigue has been reported to occur co-incidentally with depletion of muscle glycogen to low levels (Essen et al., 1978; Sherman et al., 1995; Sahlin et al., 1998). In an early study Saltin (1973) investigated the influence of low and normal muscle glycogen concentration on performance during soccer match play. He found that players with low pre-match muscle (vastus lateralis) glycogen concentrations (~200 mmol glucosyl units·kg\(^{-1}\)dm) covered 24% less distance and spent longer walking compared to those with normal pre-match glycogen concentrations (~400 mmol glucosyl units·kg\(^{-1}\)dm). Muscle biopsies taken during the half-time intermission revealed muscle glycogen to be almost depleted in the players who started with low concentrations compared to about 30% of pre-game values in the players who began the match with normal muscle glycogen concentrations. Similar glycogen depletion patterns are reported from competitive ice-hockey play (Green et al., 1978b). Players pre-match muscle (vastus lateralis) glycogen concentrations were reported as being normal (~375 mmol glucosyl units·kg\(^{-1}\)dm) but declined by 60% to post-match concentrations of ~150 mmol glucosyl units·kg\(^{-1}\)dm. Although these values are more consistent with the values reported by Saltin (1973) for soccer players at half-time, one must consider the duration of matches for ice hockey (60 min) and soccer (90 min). More recently Krustrup and colleagues (2003) report muscle glycogen utilisation rates from a soccer match that concur with those cited. They found that subjects utilised ~200 mmol glucosyl units·kg\(^{-1}\)dm during a match and that even with normal pre-match glycogen concentrations (450 mmol glucosyl units·kg\(^{-1}\)dm) approximately 36% of muscle fibres measured were almost depleted whilst 12% were completely depleted at the end of the match.

A study by Nicholas et al., (1997) confirms the need for subsequent high muscle glycogen concentrations for players that are participating in intermittent activity frequently. In their study subjects performed intermittent shuttle running (LIST) for 70 min followed by an exhaustive capacity test. They then had a 22h recovery period where they consumed either a high CHO diet (10g·kg\(^{-1}\) BM) or an isocaloric mixed
diet. Although concentrations of muscle glycogen were not measured the protocol was designed to follow recommendations on glycogen loading (Keizer et al., 1986) and so it may be assumed that muscle glycogen was resynthesised to a greater extent in the high CHO diet trial. Following the dietary intervention subjects repeated the initial running protocol and during the high CHO trial they were able to not only reproduce the pre-diet performance but actually improve their running capacity. In contrast following the mixed diet the subjects were unable to replicate their baseline running performance. Similarly Bangsbo et al., (1992) increased subjects' dietary CHO intake from 39% to 65% for two days and found a resultant improvement (0.9km) in intermittent treadmill running capacity following a field-based simulated soccer activity (6856m).

It has long been established that pre-exercise muscle glycogen concentrations positively correlate with cycle ergometry exercise capacity (Bergstrom et al., 1967). More specifically, Rico-Sanz et al., (1999) investigated the relationship between pre-exercise muscle (gastrocnemius) glycogen concentrations and time to exhaustion in a shuttle running protocol designed to simulate a soccer match. From determination of muscle glycogen concentrations by natural abundance of $^{13}$C-MRS the authors report a positive correlation between high resting muscle glycogen concentrations and muscle glycogen utilisation. This relationship has long been established in cycling studies that have manipulated glycogen concentrations through exercise and diet (Gollnick et al., 1972), but more importantly Rico-Sanz and colleagues report some evidence of a relationship between resting muscle glycogen concentrations and run time to exhaustion in soccer related activity. Although the correlation was only modest ($r=0.62; p<0.01$) this may be as a consequence of the study design. Despite utilising elite youth soccer players, the inter-individual differences in exercise capacity raise questions about the validity of the protocol. The mean time to exhaustion was 42 min with only 2 (of 17) subjects able to complete 90 min of activity, with some fatiguing as early as 15 min.

In a related study the same authors reported the dietary intake of a similar cohort of elite youth players (Rico-Sanz et al., 1998b). Interestingly the total energy intake ($\approx$2500kcal·day$^{-1}$), the carbohydrate intake ($\approx$4.5g·kg$^{-1}$·day$^{-1}$) and the relative
contribution of carbohydrate (~50%) to the daily intake of these players were lower than those recommended for soccer players (Clark, 1994). Similar poor dietary practices have been reported in youth (LeBlanc et al., 2002) and adult (Jacobs et al., 1982; Maughan, 1997; Riach et al., 2003) soccer players. Jacobs et al., (1982) found that, in players adopting poor nutritional strategies, muscle glycogen concentrations had returned to only ~300mmol glucosyl units·kg⁻¹·dm seventy-two hours after a soccer match thus suggesting that low pre-match muscle glycogen stores may be a common occurrence within the sport of soccer. This may be especially true when players are training or playing regularly and not adopting sound nutritional practices. These data show the need for adequate pre-exercise nutritional strategies for games players and they also suggest that players could have significant benefits from exogenous CHO provision during match-play.

2.3.2.2 Blood glucose

Although liver glycogenolysis can help maintain euglycaemia during exercise, if the duration and intensity of exercise is sustained and elevated then reductions in blood glucose concentration can occur. Prolonged cycling studies suggest that fatigue occurs due to a reduction in blood glucose concentrations late in exercise. The ingestion of exogenous CHO maintains normal blood glucose late in exercise and appears to delay fatigue in prolonged continuous cycling studies (Coyle et al., 1983; 1986). However this has not been posited as the reason for improvements in continuous running performance or capacity. This would also appear to be the case with intermittent running protocols as illustrated by data from Walton and Rhodes (1997) on female soccer players. Their subjects ingested either solid CHO (50g) and water (400ml); a 12.5% CHO solution (50g in 400ml H₂O) or an isovolumetric placebo prior to a treadmill based simulated soccer type activity. They ran at variable exercise intensities for two 19 min halves separated by a 10 min half-time break before alternating between 10s at 120%VO₂max and 10s rest to fatigue. They found that regardless of CHO composition subjects ran for significantly longer, and fatigue was delayed, when consuming CHO compared to the placebo trial. Although blood glucose concentration was reported to be higher in the CHO trials for the early stages of the protocol these differences were eliminated before the run to exhaustion. This suggests that low blood glucose concentration in the placebo trial did not account for
the decrements in exhaustive capacity and that the improvements in the CHO trial were attributable to other mechanisms - either a sparing of muscle glycogen or a resynthesis of muscle glycogen during the periods of low intensity and rest.

Similar results have been reported in several laboratory studies on intermittent shuttle running (LIST) (Nicholas et al., 1995; 1999, Davis et al., 1999; 2000, Welsh et al., 2002) which report that ingestion of exogenous CHO leads to an initial increase in blood glucose concentrations followed by a gradual return to baseline values, compared to maintenance of blood glucose concentrations in the control trials. None of these authors report evidence of hypoglycaemia in their subjects in the control trial nor posit this as a mechanism responsible for the reduced exercise capacity in these trials.

In contrast to these results Ingle et al., (2000) compared four different solutions: an 8% CHO-E solution; a 2.5% CHO-E solution; an electrolyte solution and water during intermittent shuttle running (LIST). They reported higher blood glucose concentrations from the 8% solution compared to the three other solutions and posited this as an explanation for the increased endurance capacity (~5 min or ~1km) in this trial. Moreover Quanz (1999), in a study where players were able to run for ~30% longer (~84s) during a simulated soccer treadmill protocol following the ingestion of a 10% CHO solution, stated that fatigue was not attributable to glycogen depletion but rather to a lack of available blood-borne substrate late in exercise. The author's interpretation of the data must be viewed with caution however as it should be noted that blood glucose concentrations never fell below baseline values during the study even in the placebo trial. Furthermore blood lactate concentrations did not peak above 3mmol·l⁻¹ during the protocol which would suggest that the simulation was not as intense as a competitive soccer match. Interestingly, the author did appear to contradict his initial point and concluded that there may have been a decrease in muscle glycogen concentrations specific to the type II fibres and that the provision of exogenous CHO allowed for resynthesis of glycogen in these fibres during the low intensity bouts. It appears far more likely that increases in intermittent exercise capacity are as a consequence of either glycogen sparing in the working muscle fibres or resynthesis of glycogen during the low intensity periods of activity.
2.3.2.3 Intra-Myocellular Triacylglycerol

Intra-myocellular triacylglycerol (IMTG) are small stores of triacylglycerol present as droplets within the cytoplasm of skeletal muscle cells near to the mitochondria and as such are an important potential energy source for exercising muscle. There is growing evidence to suggest that during prolonged moderate intensity exercise IMTG provide a significant contribution to oxidative energy production. However there is not universal agreement about the role of IMTG, opinions appear to be associated with the methods used to quantify IMTG degradation. Watt et al., (2002) present an interesting review on this area. Briefly, it would appear that studies utilising isotopic tracers and $^1$H-magnetic resonance spectroscopy (MRS) report utilisation of IMTG during 90-120min of exercise at intensities of 55-70%VO$_{2\text{max}}$. However data from studies utilising muscle biopsy techniques do not concur. The main reason for this appears to be that the between-biopsy variation in IMTG content is larger than any potential decrements in IMTG concentration during exercise and so differences may be attributable to sample variation or net IMTG degradation. These data are further complicated by the large inter-individual variations in IMTG content and also the between muscle variations.

The use of isotopic tracer methodology allows for a non-invasive measure of IMTG degradation during exercise however the accuracy of such data is dependent on a number of assumptions. These include the assumption that all of the plasma FFA taken up by the skeletal muscle is being oxidised and that the difference between this and total lipid oxidised is the contribution from IMTG. Furthermore it must be assumed that values of RER are determined accurately, which probably is the case during steady-state submaximal exercise. However RER values may be less meaningful when subjects perform intermittently and supra-maximally, as is the case during high-intensity intermittent running during sports like football. By utilising MRS techniques it is also possible to measure IMTG non-invasively. However problems arise with this methodology due to both the technical difficulty of accurately re-measuring at a specific site and also the difficulty in quantifying IMTG content when changes to muscle water content have occurred, such as during exercise. There is evidence to suggest that IMTG may contribute to energy provision during sustained exercise. However due to the limitations in accurately quantifying net IMTG degradation, especially during high-intensity intermittent exercise there are as yet no
data in the literature regarding the contribution of IMTG during multiple-sprint exercise and whether IMTG availability contributes to fatigue in this type of exercise.

2.3.3 Central Fatigue

Central fatigue is a subset of fatigue associated with alterations in CNS function that cannot be explained by dysfunction in the muscle itself. However due to the scarcity of objective measures for examining CNS function there is much debate as to the exact mechanisms underlying central fatigue. Much of the current interest in central fatigue focuses on possible exercise induced alterations in neurotransmitter function in the brain.

Newsholme and colleagues (1987) first proposed serotonin (5-hydroxytryptamine; 5-HT) to be a potential mediator of CNS fatigue. Increases in brain 5-HT have important effects on arousal, lethargy, sleepiness and mood that could be linked to altered perceptions of effort and muscular fatigue (Young, 1986). Increases in brain 5-HT synthesis occur in response to an increased delivery to the brain of blood borne tryptophan (TRP), an amino acid precursor to 5-HT. The majority of TRP circulates bound to albumin but free tryptophan (f-TRP) is able to permeate the blood-brain barrier. Brain 5-HT synthesis will occur when the f-TRP: branch chain amino acid (BCAA) ratio rises. Curzon and colleagues (1973) propose that this occurs during prolonged exercise since BCAAs are taken up by the blood and oxidised by skeletal muscles and plasma free fatty acids (FFA) increase in the blood causing a rise in f-TRP as the FFAs displace TRP from albumin binding sites. Blomstrand and associates (1988) report a 45% increase in f-TRP and a 29% decrease in BCAA following an intermittent army training programme, of similar duration to a soccer match, thus adding support to the promulgation that this central fatigue hypothesis helps explain deterioration in sport and exercise performance during intermittent running activity (Newsholme et al., 1987).

Blomstrand et al., (1991) examined whether BCAA supplementation could improve marathon performance or 30km cross-country performance. They reported an increase in mental performance in subjects in the BCAA trial following the 30km race, although they did not report any improved running performance. Furthermore although marathon performance time was improved in the BCAA trial for the less
elite runners (>3h run time), they reported no improvements in performance in the faster runners. Davis and colleagues (1999) supplemented subjects with BCAA to investigate whether the potential alterations to the f-TRP:BCAA ratio would reduce the synthesis of brain 5-HT and subsequently delay central fatigue during high-intensity intermittent exercise (LIST). Subjects ingested either a 6% CHO-E solution plus 7g BCAA; a 6% CHO-E solution or a placebo on three separate occasions in a cross-over design. Subjects ran 75 min of LIST before an exhaustive capacity test. Although subjects ran for significantly longer on the two CHO-E trials there were no additional benefits of the BCAA supplementation.

During exercise ammonia (NH₃) is released through the deamination of adenosine monophosphate (AMP) to inosine monophosphate (IMP). Bannister and Cameron (1990) propose that NH₃ is toxic to the brain and that it alters brain membrane permeability to selected amino acids that are precursors to various neurotransmitters. There is increasing evidence that substantial increases in NH₃ can result from BCAA catabolism and this is most evident during the latter stages of prolonged exercise when carbohydrate stores are depleted (Wagenmakers et al., 1991). This increase in NH₃ by deamination of BCAAs would be via the glutamate dehydrogenase (GDH) reaction (Wagenmakers et al., 1991), however Bangsbo (1993) reports that GDH activity in skeletal muscle is low and that deamination of AMP is likely to be the primary source of NH₃ during soccer which has been reported to be elevated after a match (Bangsbo, 1993). Furthermore Nicholas et al., (1995) and Ali (2002) reported elevated plasma NH₃ concentrations during 75-90 min of laboratory based simulated soccer activity (LIST).

A further mechanism postulated for central factors associated with fatigue are those concerning substrate delivery to the brain. It is suggested that when cerebral glucose uptake becomes restricted it may limit cerebral energy turnover (Wahren et al., 1999). During hypoglycaemia there is a reduced cerebral oxygen uptake. Although lactate and ketone bodies can be metabolised as a fuel for the brain during high-intensity exercise it is not clear whether these can fully compensate during neuroglucopenia. Nybo et al., (2003a and 2003b) had subjects cycle for 3h at 60% VO₂max on either a 6% CHO solution or on a placebo. Arterial blood glucose was maintained in the CHO trial but fell to hypoglycaemic values (<3 mmol·l⁻¹) in the placebo trial. Consequently
subjects reported higher perceived ratings of exertion, greater subjective feelings of dizziness and one subject even failed to complete the protocol in the placebo trial. The authors reported that the ingestion of glucose stabilised the cerebral glucose uptake and prevented central fatigue. It should be noted, however, that the exercise intensity, duration and modality utilised in the above study differed from intermittent running exercise and therefore the findings may not be applicable to such activity. It is generally agreed that liver glycogenolysis can maintain euglycaemia during exercise at \(\sim 75\%\) \(\text{VO}_2\text{max}\) for about 2h and this is probably why hypoglycaemia is not reported in studies on intermittent running even in the absence of exogenous CHO provision (Nicholas et al., 1995; 1999, Davis et al., 1999; 2000, Welsh et al., 2002).

2.4 Carbohydrate and Intermittent Exercise

Numerous studies conclude that CHO feedings during prolonged continuous exercise improve exercise capacity or performance regardless of the mode of exercise (See Tzintzas and Williams, 1998 for a review). The majority of sports participated in globally are intermittent in nature, characterised by free running and turning, these include team games such as football, hockey and rugby as well as individual activities, for example the racket sports. As these sports are generally played with an overall exercise intensity that may be classified as high (70-80\%\(\text{VO}_2\text{max}\)) and are prolonged in duration (>60 min) it is relevant to investigate whether similar ergogenic benefits may apply during these activities. Less research has been conducted in the area of intermittent exercise, and that which has been reported often utilised cycling as the exercise modality. Although cycling studies are easily replicable in the laboratory and readily lend themselves to physiological measurements, they are not representative of the type of activity characteristic in intermittent sports.

Furthermore differences have been suggested for the mechanisms responsible for improvements in performance in continuous running and cycling exercise following the ingestion of CHO (see Tsintzas and Williams, 1998 for a review). The following is a review of the relevant literature concerning CHO ingestion and intermittent running studies both in the laboratory and field setting, however at times data are reported from intermittent cycling studies as these either serve to strengthen the data
from intermittent running or fill gaps in the literature where similar experiments have yet to be conducted using running.

2.4.1 Field Studies
In an early study on the effects of exogenous CHO ingestion and actual soccer match play Muckle (1973) found that when players ingested a glucose syrup prior to performing they scored more and conceded less goals in the second halves of these matches compared to the games when they did not. Although this study would appear to advocate the benefits of exogenous CHO ingestion there are numerous methodological failings to consider. The study was lacking in experimental control and although the ratio of goals scored to conceded is an objective measure there is no way of knowing whether this was as a consequence of the experimental variable. The author does not report any work rates during these matches only the number of ball contacts for one random player per match. Furthermore the data are from 40 matches over the course of a season (20 with CHO, 20 without) there are no controls over the opposition, the importance of the matches, the ambient conditions or many other extraneous variables including the carbohydrate intake in the days preceding the matches. Nor does it report whether a placebo or any other form of fluid was ingested in the other matches.

To expand on this latter point, McGregor et al., (1999) found that the ingestion of fluid alone significantly reduced the exercise induced changes to heart rate, ratings of perceived exertion, plasma concentrations of cortisol, aldosterone and osmolality during 90 min of intermittent shuttle running (LIST) compared to a no-fluid trial. This would appear to be more a consequence of an increased thermal load in the no-fluid trial rather than a substrate issue as there were no trial differences between glucose, free fatty acids, insulin or glycerol concentrations. However, other authors have suggested that there is an increased CHO oxidation during prolonged exercise in the absence of fluid (Fallowfield et al., 1996; Hargreaves et al., 1996). Therefore the effects of CHO solutions on muscle metabolism and exercise performance may not solely be a consequence of energy provision per se and may be due to differences in fluid delivery between test solutions. It is therefore difficult to ascertain whether the exogenous CHO had an effect on the match outcomes in Muckle’s study.
In a slightly more controlled study Kirkendall et al., (1988) investigated the effects of a 23% CHO solution on soccer match play during both outdoor 9-aside play (90 min) and indoor 5-aside play (60 min). They reported an increase in distance covered in the outdoor match and a greater incidence of high-intensity movements in the indoor match following CHO ingestion (80-90g·h⁻¹) compared to a placebo. Similar findings were reported recently (Reilly and Keane, 2002) for work rates of players during actual Gaelic Football match play, where the ingestion of CHO allowed players to perform more discrete movements and to walk for less duration compared to a placebo. Kirkendall et al., (1988) posited that CHO ingestion either slowed muscle glycogen depletion or the exogenous CHO was used as an alternative fuel source late in exercise once muscle glycogen depletion had occurred. However since no measurements of blood glucose or muscle glycogen were taken the authors were unable to confirm their hypotheses.

Another field study that concurs with these theories is that of Leatt and Jacobs (1989). They matched five players from opposing teams during an 8 aside soccer match and fed 500ml of a 7% glucose polymer to one team, and an isovolumetric amount of placebo to the other, prior to the match and at half-time. The authors reported a higher rate of muscle glycogen utilisation in the control group (39%) compared to the CHO group. The reasons cited by the authors were that the ingested CHO allowed for a sparing of muscle glycogen or that there was an increased rate of resynthesis of muscle glycogen during the low intensity periods of play.

To the best of the author’s knowledge there do not appear to be any studies on intermittent activity that have attempted to conclusively show an occurrence of glycogen resynthesis during the low intensity bouts due to inherent methodological difficulties. However during 3h of cycling at 40%VO₂max, an exercise intensity similar to the low intensity bouts of many intermittent exercise protocols, Kuipers et al., (1989) report glycogen resynthesis rates of ~21mmol glucosyl units·kg⁻¹·dm⁻¹·h⁻¹. This is towards the lower end of what one may expect immediately post-exercise following CHO supplementation (20-50mmol glucosyl units·kg⁻¹·dm⁻¹·h⁻¹) (Jentjens and Jeukendrup, 2003) although it could make a significant contribution to delaying glycogen depletion and increasing exercise capacity.
A reduction in muscle glycogen utilisation was also reported in a field study on ice hockey players. Simard et al., (1988) investigated the performance and metabolic effects of exogenous CHO during actual ice hockey match play. Players were fed either a CHO solution or a placebo before and during two competitive matches comprised of three 20 min periods with 15 min intervals. The lack of control over the competitiveness of the opposition must be noted, however the exogenous CHO allowed players to skate for 5.6% longer duration and for 10.2% greater distance than the placebo. In addition, muscle biopsies (vastus lateralis) revealed that players utilised 10.3% less muscle glycogen, despite the increased work rate, in the CHO trial. The authors attribute this to glycogen sparing in the working muscle fibres.

2.4.2 Laboratory Simulations
Although it would appear that exogenous CHO provision may lead to a decreased utilisation of muscle glycogen in field studies, thus explaining the ergogenic properties of these solutions, it is difficult to quantify whether subjects are performing at the same relative workloads. Therefore it is erroneous to conclude that the reduced muscle glycogen utilisation is a consequence of the exogenous CHO per se. In order to reduce any extraneous variables apparent in field studies investigators have tried to replicate the physiological demands of intermittent sports in the laboratory setting. Although these studies cannot completely reflect the demands of a sports match they should at least offer the minimal physiological demands that competitors may face.

Nicholas et al., (1995) reported an increase in endurance shuttle running capacity following 75 min of intermittent exercise (LIST) with the ingestion of exogenous CHO. They found that the provision of a 6.9% CHO-E solution at a rate of 47g·h⁻¹ throughout exercise allowed subjects to run for 33% longer than when ingesting a similar volume of a taste matched placebo. This increase in capacity is similar to studies on intermittent cycling (Fielding et al., 1985; Yaspelkis et al., 1993; Hargreaves et al., 1984). Furthermore, following the same exercise protocol, Davis and colleagues (1999) reported a 52% increase in shuttle run capacity following CHO (6%) ingestion and a 42% increase following the ingestion of CHO (6%) + BCAA (7g). Additionally the same authors found that subjects ran for approximately 32% longer duration following the ingestion of either a 6% CHO solution or a 6% CHO + Cr³⁺ (400µg) solution compared to a placebo (Davis et al., 2000).
Similarly Welsh et al., (2002) found that CHO ingestion allowed subjects to run for longer following a modified LIST protocol. Subjects ran four 15 min LIST blocks separated by a 20 min half time break before a high-intensity run to fatigue. Subjects ingested a bolus of a 6% CHO solution pre-game with serial feedings every 15 min, they also received a bolus of an 18% CHO solution during the half time break. This led to CHO ingestion of approximately 90g·h\(^{-1}\) and allowed subjects to run for 37% longer in the run to fatigue compared to the placebo trial. These data are supported by Shirreffs and Merson (2003) during a similar exercise protocol.

Finally, MacLaren and Close (2000) examined the effects of a 6% maltodextrin solution on exercise capacity during a modified LIST protocol designed to mimic the physiological demands of Rugby League referees. Subjects ran in 8 min blocks interspersed with 2 min rests, they ran 4 blocks before a 10 min half-time break and then a further 3 blocks before an intermittent shuttle run to exhaustion. Test solutions were consumed in 200ml boli during the rest breaks. The ingestion of the CHO resulted in a 25% improvement in endurance capacity.

It would appear therefore that CHO ingestion leads to an increased intermittent shuttle running capacity in well controlled laboratory studies. Nicholas et al., (1995) attributed the improvements in capacity to glycogen sparing in the working muscles. This was based on findings from within their laboratory on CHO provision during continuous running (Tsintzas et al., 1995). To examine this hypothesis the authors had subjects perform 90 min of LIST activity and obtained muscle (vastus lateralis) glycogen concentrations pre and post exercise (Nicholas et al., 1999). They found that exogenous carbohydrate provision led to glycogen sparing in the active muscle fibres. They reported a 22% decrease in muscle glycogen utilised following the ingestion of a 6.9% CHO-E solution compared to a taste matched placebo. The sparing occurred in all fibre types but predominantly in the Type II muscle fibres.

Unfortunately it is not possible to definitely ascertain whether the higher post exercise muscle glycogen concentrations in the CHO trial from Nicholas and colleagues (1999) is as a consequence of glycogen sparing or increased muscle glycogen resynthesis. It is interesting to note, however, that muscle glycogen resynthesis during low intensity cycling (Kuipers et al., 1987) also occurs predominately in the fast-twitch muscle
fibres. Further confirmation of the mechanism for improved exercise capacity in intermittent exercise is yielded from a study on cycling (Yaspelkis et al., 1993). Although the findings should be viewed with caution due to the exercise modality, the protocol combined high intensity exercise, low intensity periods and an exhaustive test making similar demands on the subjects as the intermittent running studies; furthermore the enhancements to exercise capacity were of a similar magnitude. Yaspelkis et al., (1993) found that exogenous CHO provision increased intermittent cycling capacity compared to a placebo. Subjects completed a cycling protocol designed to simulate a stage of the Tour de France which comprised of time spent cycling at intensities of 45%\( VO_{2\text{max}} \) and 75%\( VO_{2\text{max}} \) for 190 min, culminating in a ride to exhaustion at 80%\( VO_{2\text{max}} \). From muscle (vastus lateralis) biopsy samples they found that glycogen concentration was significantly higher in the two CHO trials compared to the placebo trial prior to the exhaustive test. They reported a \( \sim 33\% \) increase in cycling capacity following CHO ingestion and attributed this to glycogen sparing. Although blood glucose was significantly higher in the CHO trials the authors did not feel that this substrate was responsible for the improvements in capacity as blood glucose oxidation has been reported to be unable to support exercise at intensities greater than 75%\( VO_{2\text{max}} \) late in exercise (Coggan and Coyle 1988).

Other data from intermittent cycling studies are equivocal in their results. Davis et al., (1997) reported an increase in intermittent cycling capacity when subjects ingested CHO. Subjects cycled at 120-130%\( VO_{2\text{max}} \) for 1 min and then rested for 3 min repeatedly until fatigue. Following the ingestion of an 18% CHO bolus and serial feedings of a 6% CHO solution every 20 min subjects were able to delay fatigue by approximately 27 min (7 cycle bouts) compared to a placebo. The authors reported similar findings irrespective of gender and attributed the increased exercise capacity to an increased resynthesis of muscle glycogen during the rest periods.

However in an earlier study Fielding et al., (1985) investigated the effects of frequency and dosage of CHO ingestion during prolonged continuous cycling (4h) interspersed with intermittent high-intensity bouts. They provided subjects with 86g of solid CHO either in small amounts (10.75g) every half an hour (frequency trial) or in larger amounts (21.5g) every hour (dosage trial) versus a placebo. The protocol
consisted of thirty minute bouts; twenty minutes at 50%VO$_{2\text{max}}$ and then ten minute high-intensity bouts alternating between 30s at 100%VO$_{2\text{max}}$ and 3 min rest. The final high intensity bout following 4h of exercise was at 100%VO$_{2\text{max}}$ until exhaustion. In this final exhaustive bout subjects cycled significantly longer in the frequency feeding trial than in the placebo trial and this would appear to be due to the maintenance of elevated blood glucose concentrations throughout the exercise period. There were no differences between trials for muscle glycogen utilisation therefore there was no evidence of glycogen sparing occurring in the CHO trials. The authors concluded that large infrequent doses of CHO causes fluctuations in blood glucose, whereas regular administration of smaller quantities allows for maintenance of blood glucose which is able to provide substrate to the working muscles late in exercise. These findings appear to be more in line with studies on continuous cycling (see Tsintzas and Williams, 1998 for a review).

Although the majority of the literature suggests that exogenous CHO ingestion results in greater intermittent exercise performance (see Table 2.1 for a summary of studies) not all studies concur with these findings. Nassis et al., (1998) had subjects run intermittently on a treadmill to exhaustion and compared the ergogenic properties of a 6.9% CHO solution (30g·h$^{-1}$) against a placebo. They alternated between 15s of high intensity running and 10s at a lower intensity (45%VO$_{2\text{max}}$). The high intensity running began at 80%VO$_{2\text{max}}$ for the first hour, increased to 85%VO$_{2\text{max}}$ for the next 40 min and then increased once more to 90%VO$_{2\text{max}}$ until fatigue. The authors reported neither differences in endurance capacity between trials nor any differences in the rate of CHO oxidation. It should be noted that the nature of the protocol, although intermittent, was fairly unique and was not a simulation of any form of recognised intermittent activity. There were also tendencies for heart rates to be higher in the CHO trial suggesting an increased work rate or elevated thermal strain.
## Intermittent Running Studies

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<td>Foster et al (1986)</td>
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<td></td>
</tr>
<tr>
<td>Leatt and Jacobs (1989)</td>
<td>90min 8-aside football match</td>
<td>7% glucose polymer</td>
<td>40g.h⁻¹</td>
<td>39% less net muscle glycogen utilisation in CHO trial</td>
<td>Glycogen sparing or increased rate of glycogen resynthesis</td>
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<tr>
<td>Kirkendall et al (1988)</td>
<td>90min 9-aside football match</td>
<td>23% CHO solution</td>
<td>83g.h⁻¹</td>
<td>Increase in distance covered in CHO trial</td>
<td>Slowing of glycogen depletion or use of substrate late in exercise</td>
</tr>
<tr>
<td>Kirkendall et al (1988)</td>
<td>60min 5-aside football match</td>
<td>23% CHO solution</td>
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<td>Simard et al (1988)</td>
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<td></td>
<td></td>
<td>Skated longer (5.6%) and further (10.2%) in CHO trial</td>
<td>Glycogen sparing in the working muscle fibres</td>
</tr>
<tr>
<td>Nicholas et al (1995)</td>
<td>75min LIST then capacity test</td>
<td>6.9% CHO-E solution</td>
<td>47g.h⁻¹</td>
<td>Ran 33% longer in capacity test</td>
<td>Glycogen sparing or increased rate of glycogen resynthesis</td>
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Table 2.1 Summary of studies on CHO ingestion during intermittent exercise
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<th>Author</th>
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<th>CHO Ingestion</th>
<th>Key Findings</th>
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<tr>
<td>Northcott et al</td>
<td>90min simulated soccer circuit</td>
<td>8% CHO solution</td>
<td>48g.h⁻¹</td>
<td>Ran 6% further first half and 11% further second half</td>
<td></td>
</tr>
<tr>
<td>(1995)</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Walton and</td>
<td>Treadmill soccer simulation then capacity test</td>
<td>12.5% CHO</td>
<td>50g.h⁻¹</td>
<td>Ran for longer (~11min) on both CHO solution and solid CHO</td>
<td>Blood glucose higher early in both CHO trials but not prior to capacity test. Glycogen sparing or increased rate of glycogen resynthesis</td>
</tr>
<tr>
<td>Rhodes (1997)</td>
<td>(females)</td>
<td>solution v 50g</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wilkinson et al</td>
<td>90min treadmill run</td>
<td>8% glucose</td>
<td>55g.h⁻¹</td>
<td>No difference in sprint speed</td>
<td>Blood glucose higher in CHO but had no effect on sprint performance</td>
</tr>
<tr>
<td>(1997)</td>
<td></td>
<td>polymer</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Nassis et al</td>
<td>Treadmill intermittent running</td>
<td>6.9% CHO-E</td>
<td>30g.h⁻¹</td>
<td>No differences in running capacity</td>
<td>Fatigue due to inadequate PCr resynthesis and reduction in muscle glycogen</td>
</tr>
<tr>
<td>(1998)</td>
<td></td>
<td>solution</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Davis et al</td>
<td>75min LIST then capacity test</td>
<td>6% CHO solution</td>
<td>52g.h⁻¹</td>
<td>Ran for 52% longer on CHO and 42% longer on CHO + BCAA</td>
<td>Glycogen sparing or increased rate of glycogen resynthesis</td>
</tr>
<tr>
<td>(1999)</td>
<td>v 6% CHO + BCAA solution</td>
<td>(including 18% CHO)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nicholas et al</td>
<td>90min LIST</td>
<td>6.9% CHO-E</td>
<td>44g.h⁻¹</td>
<td>22% less net muscle glycogen utilisation in CHO trial</td>
<td>Glycogen sparing or increased rate of glycogen resynthesis (especially in Type II fibres)</td>
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<tr>
<td>(1999)</td>
<td></td>
<td>solution</td>
<td></td>
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Table 2.1 Summary of studies on CHO ingestion during intermittent exercise (cont)
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<td>75min test</td>
<td>ChO2 + 6% CH2O</td>
<td>10% CH2O, treadmill soccer simulation, then ChO2 test</td>
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<tr>
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<td>75min test</td>
<td>Energy test</td>
<td>10% CH2O, Test CH2O, then CH2O treadmill soccer simulation, test CH2O</td>
</tr>
<tr>
<td>Taylor et al.</td>
<td>(2000)</td>
<td>75min test</td>
<td>6% CH2O test</td>
<td>Modified test, then CH2O treadmill soccer simulation, test CH2O</td>
</tr>
<tr>
<td>Taylor et al.</td>
<td>(2000)</td>
<td>75min test</td>
<td>6% CH2O test</td>
<td>Modified test, then CH2O treadmill soccer simulation, test CH2O</td>
</tr>
<tr>
<td>Taylor et al.</td>
<td>(2002)</td>
<td>75min test</td>
<td>8% CH2O test</td>
<td>Modified test, then CH2O treadmill soccer simulation, test CH2O</td>
</tr>
<tr>
<td>MacLaren</td>
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<td>CH2O test, then treadmill soccer simulation, test CH2O</td>
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<tr>
<td>Welsh et al.</td>
<td>(2002)</td>
<td>75min test</td>
<td>6% CH2O test</td>
<td>Modified test, then CH2O treadmill soccer simulation, test CH2O</td>
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<td>CH2O test, then treadmill soccer simulation, test CH2O</td>
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<td>Table 2.1</td>
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<td>CH2O test, then treadmill soccer simulation, test CH2O</td>
</tr>
</tbody>
</table>

Summary of studies on CH2O Ingestion during Intermittent Exercise (cont)
<table>
<thead>
<tr>
<th>Author</th>
<th>Protocol</th>
<th>Energy Density</th>
<th>CHO Ingestion</th>
<th>Key Findings</th>
<th>Proposed Mechanisms</th>
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</thead>
<tbody>
<tr>
<td>Hargreaves et al (1984)</td>
<td>8x30min bouts 20min at 50% VO_{2max} and 10min of 4x30s with 2min rest</td>
<td>43g of solid CHO</td>
<td>43g.h^{-1}</td>
<td>39% less net muscle glycogen utilisation in CHO trial</td>
<td>Glycogen sparing or increased rate of glycogen resynthesis</td>
</tr>
<tr>
<td>Fielding et al (1985)</td>
<td>8x30min bouts 20min at 50% VO_{2max} and 10min of 4x30s with 2min rest</td>
<td>86g of solid CHO</td>
<td>21.5g.h^{-1}</td>
<td>Cycled longer in frequency trial v dose trial and placebo</td>
<td>No differences in net muscle glycogen utilisation prior to capacity test therefore no sparing. Elevated blood glucose late in exercise.</td>
</tr>
<tr>
<td>Yaspelkis et al (1993)</td>
<td>Intervals of 45% and 75%VO_{2max} for 190min then capacity test at 80%</td>
<td>10% CHO solution v 50g solid CHO</td>
<td>50g.h^{-1}</td>
<td>Cycled 30% longer in both CHO trials</td>
<td>Blood glucose and insulin both higher. Glycogen sparing or increased rate of glycogen resynthesis</td>
</tr>
<tr>
<td>Davis et al (1997)</td>
<td>1min at 120-130%VO_{2max} then 3min rest repeated to fatigue</td>
<td>18% CHO pre 6% CHO during</td>
<td></td>
<td></td>
<td>Blood glucose and insulin both higher. Increased rate of glycogen resynthesis</td>
</tr>
</tbody>
</table>

Table 2.1 Summary of studies on CHO ingestion during intermittent exercise (cont)
On this latter note Morris et al., (2003) found that intermittent exercise (LIST) in the heat (30°C) was compromised by the ingestion of a 6.5% CHO-E solution compared to either a taste matched placebo or flavoured water. They found that the rate of rise in rectal temperature was greater in the CHO trial and 6 of their 9 subjects were forced to withdraw during this trial due to elevated core temperatures, thus finding that there was a tendency for increased running capacity following the ingestion of the other two solutions. This latter finding is probably more a consequence of a reduction in splanchnic blood flow in the heat affecting gastric emptying and as such is beyond the scope of this review however it does suggest that ambient environmental conditions in addition to the protocol utilised can confound interpretation of the results.

2.4.3 Carbohydrate and Sprint Performance in Intermittent Exercise

Thus far the literature reviewed has concentrated on the efficacy of exogenous CHO to increase exercise capacity during intermittent activity or to elevate the proportion of time spent at the higher exercise intensities. Obviously there are important ramifications for participants in all intermittent activities if exogenous CHO can be shown to have such an ergogenic effect. In addition it is also important to examine whether the provision of exogenous CHO can increase the performance or occurrence of maximal intensity efforts (ie sprints) during intermittent activity. As mentioned earlier, glycogenolytic processes are initiated within seconds of maximal exercise (Boobis et al., 1982) and lead to decreases in muscle glycogen concentrations. Therefore CHO supplementation has been proposed as an intervention to delay fatigue in repeated sprint activity. The data on this appear to be equivocal.

Symons and Jacobs (1989) investigated the effects of low muscle glycogen on very high-intensity intermittent exercise. They reported no differences in force production during electrically evoked, isometric or isokinetic maximal leg extension between subjects with low (153 mmol glucosyl units·kg⁻¹·dm) and normal (426 mmol glucosyl units·kg⁻¹·dm) muscle glycogen concentrations. It should be noted that this exercise protocol was of higher intensity and shorter duration than would be experienced in a game type activity and as such force production may not have been limited by glycogen but more likely high energy phosphate availability.
Wilkinson et al., (1997) investigated the effect of exogenous CHO on sprint performance during a treadmill simulation of the physiological demands of soccer match play. Despite higher blood glucose concentrations in the CHO trial the authors reported no differences in sprint performance between trials. Similar findings have been reported by Nicholas et al., (1995; 1997; 1999) during intermittent shuttle running (LIST). However in contrast to this MacLaren and Close (2000) and Shirreffs and Merson (2003) reported faster sprint times throughout 80 min and 60 min, respectively, of LIST activity following ingestion of a 6% CHO solution. Similarly Welsh et al., (2000) reported faster sprint times in the final quarter of a modified LIST protocol in their CHO trial and Ali et al., (2002) demonstrated that CHO ingestion attenuated the decrements in sprint performance observed during 90 min of LIST activity in subjects who had previously performed glycogen lowering activity. Finally Sugiura and Kobayashi (1998) found that the ingestion of a 20% glucose polymer during the 15 min half-time break of 90 min of intermittent cycling exercise, at an average exercise intensity of 75%VO$_{2\text{max}}$, led to improved sprint performance in a 40s Wingate test following the exercise protocol.

2.5 Gastrointestinal Function

In order for carbohydrate-electrolyte solutions to provide any ergogenic benefits then they must be effectively delivered to the site of oxidation. The transport of carbohydrate-electrolyte solutions through the gastrointestinal system is a complex process and is influenced by many factors including fluid volume, solute osmolality, caloric content, carbohydrate type and exercise intensity and modality. The efficacy of such solutions for replenishment of fluid and carbohydrate is both a function of their rates of gastric emptying and intestinal absorption. Many of the studies investigating the rates of gastric emptying and intestinal absorption of carbohydrate-electrolyte solutions state equivocal findings. Costill and Gisolfi et al. have written comprehensive review chapters on gastric emptying and intestinal absorption respectively (Gisolfi and Lamb, 1990) and the influence of exercise. Also a review of sports drinks by Coombes and Hamilton (2000) details the effects of exercise on gastric emptying and intestinal absorption. What follows is a brief overview of the literature on these areas paying specific attention to intermittent high-intensity running and also solute osmolality.
2.5.1 Gastric Emptying

There appears to be a general consensus of opinion that gastric volume plays a large role in the rate of emptying of the gastric contents (Costill and Saltin, 1974; Duchman et al., 1997; Rehrer et al., 1990; Ryan et al., 1989). Large gastric volumes stimulate stretch receptors in the gastric mucosa and promote faster rates of emptying. However, there does appear to be an upper limit for gastric volume above which the rates of emptying may slow. Costill and Saltin (1974) investigated the rates of emptying of differing volumes of a glucose solution and the rate of gastric emptying increased with ingestate volume up to 600mL, beyond this volume the rate was impaired. Hunt and Spurrel (1951) report similar findings but stipulate an upper volume of 750mL. Mitchell and Voss (1990) found that gastric emptying increased up to volumes of 1600mL. Despite these differences which could be attributable to methodological differences or inter-individual variations the authors generally concur that there is an initial fast phase of emptying of large volumes that progressively slows as gastric volume decreases. It is therefore beneficial to keep the volume of fluid in the stomach high so that this initial exponential rate of emptying can be maintained. This finding has led many investigators to offer subjects a large bolus of solution prior to exercise (~500-600mL) followed by serial feedings of smaller volumes (100-200mL) at frequent intervals (15-20min). Such feeding strategies are designed to maximise gastric emptying (see Figure 2.1) but to avoid any gastrointestinal distress that may be caused by too large a gastric volume.

Early studies by Hunt and Pathak (1960) suggested that an osmoreceptor mechanism in the intestine inhibited gastric emptying and therefore beverage osmolality would control the rate of gastric emptying. Similarly, from the same laboratory Barker et al., (1974) found that the rate of gastric emptying of water was progressively slowed as osmolality was increased by adding a range of concentrations of glucose. However, Hunt (1960) found no differences in the rate of emptying of glucose and starch meals despite an initial hypothesis that the higher osmotic pressure of the glucose meal would impair its rate of emptying. In support of this, Elias et al., (1968) reported similar emptying rates for isocaloric amounts of monosaccharides and disaccharides in test meals and more recently Brouns et al., (1995) found no differences in the emptying rate of six 6% CHO solutions with a range of osmolalities (243-374
mOsmol·kg⁻¹). However, the authors did report differences in rates of gastric emptying between 6 solutions of matched osmolalities (330 mOsmol·kg⁻¹) with a range of concentrations (4.5%-9%) with the rate of emptying being inversely related to energy density. This is further substantiated by Shi et al., (2000) who found there to be no differences in the gastric emptying rates of four isoenergetic 6% carbohydrate solutions despite the type of carbohydrate or the number of transportable carbohydrates within the solution. The authors concluded that neither carbohydrate type nor osmolality (in a range of 1-424 mOsm·kg⁻¹) influences the rate of gastric emptying of a dilute (<6%) carbohydrate solution (Shi et al., 2000). More recently Simpson et al., (2002) reported no differences in rates of gastric emptying between 6.4% solutions with osmolalities of 25 mOsm·kg⁻¹, 47 mOsm·kg⁻¹, 229 mOsm·kg⁻¹ and 390 mOsm·kg⁻¹.

Figure 2.1 A diagram to show the effect on gastric emptying of maintaining a high gastric volume through serial feedings versus a single bolus. Modified from Rehrer et al., 1990
Other recent studies concur, reporting similar emptying rates for isoenergetic solutions with differing osmolalities (Vist and Maughan, 1994, Murray et al., 1994, Leiper et al., 1999) thus upholding the concept that caloric content is a stronger regulator of gastric emptying than osmolality. Vist and Maughan (1995) utilised a double sampling gastric aspiration technique and compared the rate of gastric emptying of 600ml of 2%, 4% and 6% glucose solutions against a water control every 10 min for an hour. They found that the 6% solution emptied the slowest, followed by the 4% solution suggesting a pattern between energy density and gastric emptying rate. They reported no difference between the 2% solution and water suggesting that at low concentrations of CHO the effect on gastric emptying was negligible. Coyle (1978) found that a 4.6% carbohydrate solution resulted in an approximately 35-40% slower rate of gastric emptying than 2.5% or 1.1% solutions and water. This is in accordance with other authors (Costill and Saltin., 1974, Neufer et al., 1986, Sole and Noakes, 1989, Houmard et al., 1991) who report delayed gastric emptying when carbohydrate concentration exceeds 2.5%. More specifically Simpson et al., (2001) report an improved fluid delivery from a hypotonic 2% solution compared to both hypotonic (118 mOsmol·kg⁻¹) and isotonic (283 mOsmol·kg⁻¹) 6.4% solutions. They reported no differences between the 6.4% solutions despite the large variation in solution osmolality. Although an increasing CHO concentration leads to decreases in the volume of fluid emptied from the stomach it has been reported that the emptying rate of CHO, within the range 5% to 25%, is constant (8.91 kJ·min⁻¹) regardless of the concentration (Brener et al., 1983) this would suggest that there is a feedback mechanism in place that determines a fixed caloric flow through the pylorus, although these data await confirmation from other investigations.

Conversely, although Sole and Noakes (1989) found a constant delivery rate of CHO from glucose solutions regardless of energy content, further confirming that CHO content may regulate gastric emptying, they did not report the same findings for glucose polymers. Ingestion of glucose polymers caused the rate of energy delivery to increase in parallel with the energy density of the solution, thus suggesting that energy density was not the only regulatory factor for gastric emptying. Moreover, Foster and colleagues (1980), when comparing the emptying rates of glucose and glucose polymers at various concentrations (5%, 10%, 20% and 40%), found that although energy density did affect gastric emptying of the more concentrated solutions (>10%)
with there being similar emptying rates regardless of osmolality there was a difference at the lower CHO concentration. The 5% glucose polymer did in fact induce a faster rate of gastric emptying than the 5% glucose solution and attributed this to an effect of osmolality (glucose polymer 75 mOsm·kg\(^{-1}\) and glucose 266 mOsm·kg\(^{-1}\)).

Furthermore, both Vist and Maughan (1995) and Sole and Noakes (1989) report that a concentrated (15-19%) glucose polymer solution emptied faster than an isocaloric glucose solution. However Vist and Maughan (1995) found that a 4% glucose monomer emptied faster than a 19% glucose polymer despite having a higher osmolality thus suggesting that although osmolality may play a small role in the rate of gastric emptying the energy density of the solution had a more marked effect. Sole and Noakes (1989) conclude that neither carbohydrate content nor osmolality fully explain the differences observed in gastric emptying of CHO solutions at rest. Following an investigation where they compared the resting emptying rates of thirteen CHO drinks of differing type, concentration and osmolality they report an inverse correlation between the osmolality of the gastric aspirate and the rate of emptying of all solutions. They also report a similar correlation between the osmolality of the ingested solution and its emptying rate. Thus suggesting that osmolality does affect emptying rates. However the glucose polymers did not empty as fast as their osmolalities would suggest, therefore implying that emptying rates may be more regulated by the rate of hydrolysis of these polymers prior to their arrival at the duodenal osmoreceptors. This theory is supported by Neufer et al., (1986) who found that although glucose polymers had a faster initial rate of gastric emptying, once the polymer was hydrolysed within the small intestine stimulation of glucose-specific osmoreceptors probably inhibited further gastric emptying.

It would therefore appear that gastric emptying may be affected by both osmolality and energy density and that the latter may play more of a regulatory role the more concentrated the solution is. However, rates of gastric emptying of both carbohydrate and water from a 6% glucose solution were reported as 1.8g·min\(^{-1}\) and 1.8 l·h\(^{-1}\) respectively (Duchman et al., 1997). As this is higher than the reported maximal rate (1-1.5g·min\(^{-1}\)) necessary to optimise exogenous carbohydrate oxidation (Jeukendrup and Jentjens, 2000), gastric emptying does not appear to be a limiting factor for CHO delivery and should also be adequate to maintain hydration in temperate conditions.
Finally, although it would appear that there is little intra-individual variation between the rates of emptying of various solutions (Beckers et al., 1991) the same cannot be said for the differences in gastric emptying rate between individuals. It is suggested that hyperphagia can lead to adaptations of the gastrointestinal system and that endurance training and the accompanying high energy intakes of athletes may lead to increases in the rates of gastric emptying (Carrio, 1989). Moreover, there are data to suggest that the gastrointestinal tract may undergo training adaptations with regards to fluid and solute ingestion, and that individuals who regularly consume CHO solutions will have faster rates of gastric emptying than those who do not (Horowitz et al., 1996). Both of these factors may have an influence on the results garnered from studies dependent on the training and nutritional status of the subjects recruited.

2.5.2 Gastric Emptying During Exercise

A number of authors have investigated the gastric emptying rates of water and carbohydrate solutions during running and cycling exercise. Many of these investigators (Costill and Saltin, 1974, Neufer et al., 1986, Mitchell et al., 1989, Houmard et al., 1990, Coyle et al., 1993, Murray et al., 1997) report similar gastric emptying rates between water and dilute carbohydrate solutions (<7% carbohydrate), whilst some (Vist and Maughan 1995, Murray et al., 1997) have reported delayed gastric emptying rates, especially with concentrated carbohydrate solutions (>8% carbohydrate). Rehrer et al., (1989) investigated the cumulative effects of exercise, training status and energy density on gastric emptying and reported that the drink composition had a far greater impact on the rate of emptying than either the training status of the individual or the intensity of exercise (<70%Work\text{max}).

Early studies from Campbell et al., (1928, op cit Costill and Saltin, 1974) report enhanced gastric emptying, above resting rates, during light activities such as walking. These findings were subsequently confirmed by Costill and colleagues who found that 120 min of running (70%VO\text{2max}) induced a faster rate of gastric emptying of a 6% carbohydrate solution than either a similar time spent cycling (70%VO\text{2max}) or at rest (Costill, 1990). Similarly Neufer et al., (1986) found that running for 15 min at intensities of approximately 50-70%VO\text{2max} led to faster rates of gastric emptying for some carbohydrate solutions than an equal time spent at rest. The exact mechanism for the differences attributable to exercise mode have not been defined although it is
suggested that the bodily motion involved during the activity may facilitate the delivery of chime to the duodenum (Neufer et al., 1986). Rehrer and Meijer (1991) examined the biomechanical vibration of the abdominal region of 6 subjects during both running and cycling activity at 60-70% of maximal performance and confirmed that running caused a two-fold increase in abdominal vibration compared to cycling. However data from the same laboratory (Rehrer et al., 1990) reported no differences attributable to exercise mode between gastric emptying rates of various solutions during both running and cycling protocols at 80%VO₂max. This is in agreement with the data by Houmard and colleagues (1990) who conducted a similar experiment but at a slightly lower exercise intensity (75%VO₂max).

Fordtran and Saltin (1967) compared the relative rates of emptying of water and a 13.3% carbohydrate solution at rest and during treadmill running at 70% VO₂max. Although the authors report a slowing of gastric emptying in the glucose solution at rest they found that exercise had no real effect on the emptying rates. Conversely, Sole and Noakes (1989) found that during treadmill running for 90 min at 75%VO₂max the rate of gastric emptying of water was slower than at rest. Interestingly they report that the same was not true for a 10% glucose polymer solution and exercise appeared to have no detrimental effect. Coyle et al., (1993) found there to be no difference in the rate of gastric emptying of water and three carbohydrate solutions of different osmolality, type and energy density during cycling at 70%VO₂max. Similarly no differences in gastric emptying were reported during cycling at 70% of maximal workload compared to resting (van Niewenhoven et al., 1999). Murray et al., (1997) confirm these findings reporting no differences between emptying rates of water and a 6% carbohydrate solution during 70 min of cycling at 60% VO₂max. These authors did report a significant slowing of a 20% carbohydrate solution which is in agreement with the findings of Vist and Maughan (1995) who found no impairment of gastric emptying of a 4% solution at rest but slower rates of emptying of an 18% solution.

Bennett and Dobson (1990) performed a meta-analysis of 15 studies incorporating a total of 188 subjects and concluded that exercise caused increased gastric emptying when compared to rest. They also reported that a glucose/fructose solution caused less inhibition of gastric emptying in both conditions compared to other types of carbohydrate. Unfortunately they report no data on solution osmolality.
Gisolfi et al., (1998) found that both hypotonic and isotonic solutions emptied at 17ml·min⁻¹ during cycling exercise at 64%VO₂max which was almost as fast as the rate of fluid ingestion. Lambert et al., (1997) concurred with these findings reporting rates of 18-19ml·min⁻¹ for both a water placebo and a CHO solution during similar exercise. In a graphical depiction of 11 studies on gastric emptying Coyle and Montain (1992) summarise that most people can empty 1000ml·h⁻¹ (~17ml·min⁻¹) during exercise. It is posited that exercise has no effect on gastric emptying (~13ml·min⁻¹) until the exercise intensity exceeds 65-80% of the subject's maximal oxygen uptake (Costill and Saltin, 1974, Gisolfi et al., 2000). Above these intensities it is suggested that gastric emptying may be delayed by potential inhibitory effects of increased catecholamine and hormonal levels on splanchnic blood flow and gastric motility (Murray 1987; Neufer et al., 1989; Leiper et al 2001c)

Moodley at al., (1992) found that there was a greater rate of gastric emptying from a glucose polymer solution than from a glucose solution regardless of energy density of the solutions during treadmill running for 90 min at 70%VO₂max. They also reported that intestinal CHO delivery increased as a linear function of CHO content thus suggesting that earlier thoughts of gastric emptying being wholly regulated by energy density may be unfounded. Furthermore Owen et al., (1986) found a trend for osmolality to affect gastric emptying during constant paced treadmill running in the heat. A 10% glucose polymer appeared to empty faster than an isocalorific glucose solution and this was attributed to the different osmolalities of the solutions. The glucose polymer delivered 51.3g of CHO whilst the glucose solution only delivered 41.3g, equivalent to ~14% and ~11% of the total calorific cost of the run respectively. However Hawley et al., (1991) report no differences in gastric emptying between a 15% glucose polymer and a 15% starch solution during 90 min cycling at 70%VO₂max, which is in agreement with the findings of Gisolfi et al., (1998). Since moderately intense exercise has been postulated to have negligible effects on gastric emptying compared to rest it is no surprise that data are equivocal as to whether hypotonic solutions offer faster rates of emptying during exercise compared to other solutions, much as they are in resting individuals.
Campbell et al., (1928) also suggested that gastric emptying may be enhanced when exercise was performed in intermittent bouts. It is assumed that during intermittent exercise, despite overall exercise intensities being high, the time spent at relatively low activity levels allows for sufficient gastric emptying to occur, compensating for any inhibitory effects during the periods of high intensity activity and sprinting. However these assumptions were not confirmed in an investigation by Mitchell and colleagues (1989) or by Leiper et al., (2001a) who reported slower gastric emptying of a 6% carbohydrate solution from intermittent cycling at an average intensity of 66%VO₂max compared to continuous cycling at the same intensity and also rest. Furthermore, the same laboratory (Leiper et al., 2001b) investigated the effects of intermittent running in the form of a five aside soccer match and found that gastric emptying of a 6% solution was slower during the first 15 min compared to walking and although no statistical differences were found for the second 15 min there was a tendency (p=0.055) for the soccer activity to significantly slow the rate of emptying despite a lower overall exercise intensity (~60%VO₂max) than one would expect for soccer match play (~75%VO₂max). In a more recent study (Leiper et al., 2003), controlled simulated soccer activity of a higher intensity (~75%VO₂max) did slow the rate of emptying of both a 6.4% carbohydrate solution and water compared to low intensity walking, however no differences were reported in emptying rates during the soccer activity between water and the carbohydrate solution. These data would suggest therefore that although intermittent exercise may decrease the rate of emptying of solutions compared to rest there do not appear to be any additional gastric disturbances caused by including substrate and electrolytes to water.

### 2.5.3 Intestinal Absorption

There are a number of mechanisms in the small intestine for the absorption of water, electrolytes and substrate. Both water and electrolytes can pass through the mucosal barrier across osmotic and hydrostatic gradients and, via diffusion, along electrochemical gradients (Leiper, 2001c). This polar route is not accessible to substrate as the monosaccharides are too large to pass through the mucosal barrier. As water absorption is largely a passive process caused by local osmotic gradients, hypotonicity of the luminal contents could establish osmotic gradients that promote water absorption (Maughan, 1991). It has therefore been postulated that hypotonic
solutions are more efficacious for the treatment of diarrhoeal disease than isotonic or hypertonic solutions (Thillaninayagam et al., 1998). This should therefore make such solutions more effective as fluid replacement drinks during exercise, although other authors disagree (Hunt et al., 1992, Shi et al., 1994, Gisolfi et al., 1998). The findings of Thillaninayagam and colleagues are supported by Wapnir and Lifshitz (1985) who suggested that there is a negative correlation between osmolality of the luminal contents and the rate of water absorption and that fluids that are hypotonic (<280 mOsm·kg⁻¹) promote water absorption. However the data regarding this view are equivocal: a study by Leiper et al. (1994b) found that there were no differences between the rates of absorption of hypotonic (200 mOsm·kg⁻¹) and isotonic (300 mOsm·kg⁻¹) solutions, therefore concluding that osmolality was not the major influence on intestinal absorption.

The reason for these differing findings is that fluid absorption is not merely a passive process. There are a number of different models (see Leiper, 2001c for a more detailed overview) proposing that intestinal absorption of solutes, such as glucose, promote net water uptake. This in turn increases the non-selective transport of additional solute from the intestinal lumen. There are carrier mediated transporters located in the brush-border membrane of the enterocytes of the small intestine. Some carriers are driven by concentration differences that favour the absorption of a specific solute and enhance the rate of absorption compared to diffusion alone. This process is called facilitated diffusion. Although these transporters are energy and sodium independent they are less efficient, especially at low solute concentrations, than active transporters, fructose absorption by GLUT5 is an example of facilitated diffusion.

More effective are the active transporters which are energy dependent and able to move the solute against a chemical gradient. This is achieved through utilisation of an electrochemical potential gradient of a co-transported cation, usually sodium, to supply the required energy. In this manner glucose is mainly absorbed by being actively co-transported with sodium by SGLT1. Furthermore, using a rodent model, Madara and Pappenheimer (1987) demonstrated that Na⁺-coupled solute transport triggers a contraction of perijunctional actomyosin which increases the permeability of the tight junctions between adjacent plasma membranes thereby enhancing the absorption of both solute and fluid via an intracellular route. The flow of water
through these tight junctions can augment solute uptake by a process called solvent drag whereby a heterogenous sample of the intestinal contents can travel across the intestinal mucosa. It is therefore recognised that the absorption of both water and solute are closely related and each can augment the absorption of the other. The addition of CHO or electrolytes to a solution will increase the osmolality of that solution and will therefore affect the passive absorption of fluid, however the active uptake of solute and accompanying fluid may compensate for this osmotic effect. Therefore it is not osmolality *per se* that is the sole mechanism regulating fluid and CHO delivery. It is also a consequence of the CHO type, the addition of electrolytes and the energy density of the CHO.

From measures of doubly labelled water Rehrer and co-workers (1992) reported an increase in net water absorption from a sodium containing dilute CHO solution (4.5%) than from water. In the same study the authors also examined the effect of solution osmolality by comparing the intestinal absorption rates of fluid from a 17% hypotonic maltodextrin solution and a 17% isotonic glucose solution. They concluded that the lower osmolality maltodextrin promoted net water absorption despite its high concentration whilst the 17% glucose solution promoted net water secretion. The authors state that glucose polymers may therefore be a more attractive solution for supplementation during exercise, not due to an increased rate of CHO delivery or utilisation *per se* but more through an enhanced attenuation of dehydration due to a decrease in gastrointestinal secretions and the associated decreased losses of fluid from plasma and tissues to the lumen.

From jejunal perfusion studies at rest Leiper and Maughan (1986) concluded that the optimal osmolality for intestinal absorption was 200-250 mOsm.kg⁻¹. In a later study with a similar methodology (Leiper et al. 1994a) the authors concurred with these earlier findings stating that water absorption was inversely related to osmolality and that carbohydrate absorption was also faster in moderately hypotonic solutions. By substituting glucose polymers for equimolar amounts of monomer it is possible to maintain energy density of solutions but decrease the solution osmolality. The argument to support this is that water uptake is faster from the low osmolality maltodextrin solutions and therefore increased glucose absorption can be attributed to an augmentation of solvent drag rather than to an increase in the active co-transport of
glucose and sodium (Leiper et al 1994a). Furthermore Leiper et al. (1994b) found there to be faster rates of intestinal absorption from glucose solutions at rest compared to fructose solutions. They also report that the addition of small concentrations of NaCl enhanced this glucose uptake preferentially to solute osmolality suggesting that the presence of exogenous sodium was more effective than moderate hypotonicity in enhancing uni-directional water absorption.

In perfusion studies glucose absorption from the intestine increases up to a concentration of 200mmol·l⁻¹ beyond this it appears that the active glucose transporters (SGLT1) become saturated and any additional absorption must be through diffusion along concentration gradients or by solvent drag. A 3.6% glucose solution will offer glucose in a concentration of approximately 200mmol·l⁻¹ (Leiper, 2001c). As CHO solutions up to approximately 7% have been reported to have negligible adverse effects on gastrointestinal function this has led investigators to experiment with multiple substrates in an attempt to maximise the transport of glucose from the intestinal segment. It is important however that multiple substrates do not compete for transporter mechanisms (for example GLUT2 is both a carrier mediated transporter for glucose and galactose).

It has been postulated that sucrose solutions may absorb faster because they would maximise both active and passive glucose transporters, although data appear equivocal. Shi et al., (1995) report that solutions containing two transportable substrates enhance solute and water flux above solutions with just one substrate. This observation is supported by Leiper et al., (1996) who compared five hypotonic solutions of different multiple CHO types (various concentrations of two or more of glucose, sucrose and maltodextrin) with an isotonic glucose monomer. They found similar absorption rates for both fluid and CHO in the five hypotonic solutions which were faster than those of the isotonic glucose monomer but were unable to differentiate between the different combinations as to which was the optimal combination. In their review paper Coombes and Hamilton (2000) state that sucrose/glucose solutions are reported to promote greater fluid and Na⁺ absorption whilst glucose/fructose solutions promote greater carbohydrate absorption however they offer no data to support these claims. Moreover Gisolfi and Duchman (1992), in
a review paper, reported that from dilute carbohydrate solutions (<6%) the rate of intestinal absorption is independent of carbohydrate type.

Much of the conflicting data reported concerning osmolality and intestinal absorption is in part due to the methodology utilised and the intestinal test segment studied. As osmolality changes as solutions progress along the intestinal segments it is important to note whether studies are reporting data from the duodenum, jejunum or both. Similarly data reported from perfusion studies do not truly reflect the actual absorption rates of solutions that are ingested orally because the osmolality of a perfused solution will not be the same as a solution that has progressed along the gastrointestinal tract. Shi et al., (1994) found no differences in intestinal absorption between hypotonic (186 mOsm·kg⁻¹), isotonic (283 mOsm·kg⁻¹) and hypertonic (403 mOsm·kg⁻¹) 6% carbohydrate solutions that were orally ingested. The authors reported progressive changes in the osmolality of the ingested solutions with all three approaching isotonicity by the proximal duodenum. Similar results were found in earlier studies (Hunt and Knox, 1968) and are as a consequence of net movement of water and electrolytes along osmotic and electrochemical gradients (Turnberg, 1973). However in contrast to this Simpson (unpublished data) found that the ingestion of 6.4% CHO solutions with a range of osmolalities (25-390 mOsm·kg⁻¹) reached the intestines with essentially the same composition as when ingested.

The evidence from the literature would therefore suggest that there are a multitude of factors that effect intestinal absorption of carbohydrate solutions at rest. Although data are equivocal as to the effects of solution osmolality and the benefits of hypotonic solutions over isotonic solutions there is little evidence to suggest that hypotonic solutions will empty more slowly. Results in the literature are complicated not only by the number of factors affecting intestinal absorption but also by the differing methodologies used in the published studies. This issue is further complicated when one considers the additional effects that exercise may confer.

2.5.4 Intestinal Absorption and Exercise
Theoretically the release of endorphins and catecholamines during exercise should enhance absorption, but a reduction in splanchnic blood flow could counteract this (Gisolfi et al., 1990). Studies investigating the effects of exercise on the rate of
intestinal absorption found that treadmill running at 64-78% VO$_{2\text{max}}$ (Fordtran and Saltin, 1967) and cycling at 65% VO$_{2\text{max}}$ (Spranger et al., 1989) had no influence on intestinal absorption of water. Fordtran and Saltin (1967) concluded that intestinal blood flow was not reduced enough by exercise to reduce the rate of either active or passive absorption. Costill (1973) supports these findings, he indirectly measured intestinal absorption during exercise by calculating the time to initial appearance of orally ingested labelled glucose in the blood and in breath CO$_2$. In contrast Maughan et al., (1990) report a decreased appearance of deuterium in the blood following ingestion of doubly labelled water during exercise compared to rest. The decrease was proportional to exercise intensity thus suggesting a slowing of gastric emptying or intestinal absorption as intensity increases. It should be noted that the methodology utilised in this study only measures uni-directional water flow and as such does not give a true measure of intestinal fluid absorption and secretion (Davis et al., 1987), such methodological limitations should serve to overestimate net intestinal absorption.

It would appear that similar rates of intestinal absorption occur during exercise as at rest therefore it would be pertinent to assume that the addition of CHO and electrolytes to solutions could enhance intestinal absorption during exercise to similar magnitudes as at rest. This is supported by the study of Spranger et al., (1989) that showed a greater fluid absorption from a carbohydrate-electrolyte solution than from water during cycling at 65%VO$_{2\text{max}}$. However Gisolfi et al., (1998) found that the total fluid absorbed from the duodenojejunum was no different for orally ingested hypotonic, isotonic and hypertonic solutions during cycling at 64% VO$_{2\text{max}}$ compared to a water placebo. The apparent contradictory findings of these studies are probably a consequence of the methodology utilised and the intestinal segment studied. Lambert et al., (1997) utilised techniques where they could simultaneously determine gastric emptying and intestinal absorption of ingested solutions during exercise (see Lambert et al., 1996 for methodology). They found that during continuous cycling at ~64%VO$_{2\text{peak}}$ fluid absorption in the proximal small intestine was dependent on both the segment studied and also the composition of the ingested solution. They reported greater fluid absorption from a water placebo compared to a CHO solution in the duodenum yet this pattern was reversed in the jejunum. The authors report that the duodenum plays a crucial role in fluid absorption therefore it should be included in
segmental perfusion studies and to draw conclusions based purely on jejunal absorption of beverages may be somewhat misleading. To the best of the author’s knowledge there are no studies that report absorption data for the entire duodenojejunum during intermittent running activity.

It would appear that continuous mild to heavy exercise (30-80% VO\textsubscript{2max}) lasting 60-90 min does not serve as a limiting factor for fluid or nutrient uptake via the intestinal system (Gisolfi, 2000). However since these data are from studies that used continuous exercise, as with gastric emptying, the same principles may not apply to intermittent activity. To the best of the author’s knowledge there are no studies that have investigated the intestinal absorption rates of fluids and solutes during intermittent running due to inherent methodological difficulties. Although intermittent exercise as characterised by sports such as soccer are of a similar duration and have an average exercise intensity within the range stated by Gisolfi (2000) they exhibit periods of maximal intensity exercise interspersed with lower intensity recovery. It would seem logical that the bouts of sprinting in these activities would reduce absorption at least to a similar extent as it affects gastric emptying (Leiper, 2001c) although this theory awaits testing.

2.6 Type of CHO and Oxidation Rates
The efficacy of a CHO solution to improve exercise performance or capacity is ultimately dependent on the rate of oxidation of this exogenous CHO. The rate of exogenous CHO oxidation is itself dependent upon the rate of gastric emptying, intestinal absorption and subsequent transport of the ingested CHO into the systemic circulation. What follows is an examination of the pertinent literature regarding exogenous CHO oxidation. For further detail Jeukendrup and Jentjens (2000) offer a comprehensive review of the factors affecting the oxidation of exogenous carbohydrate during prolonged exercise.

Peak values for exogenous CHO oxidation appear not to exceed \(~1\text{g} \cdot \text{min}^{-1}\) even when CHO is ingested at rates in excess of this. Gastric emptying would not appear to be a limiting factor since the rates of gastric emptying (\(~1.8\text{g} \cdot \text{min}^{-1}\)) reported in the literature exceed the maximal rates of CHO oxidation (Moodley et al., 1992; Rehrer et
al., 1992). For example Saris et al., (1993) reported similar rates of gastric emptying between two different CHO solutions, yet only ~48% of the volume of the soluble CHO emptied was oxidised compared to only ~32% of the insoluble CHO. Moodley and colleagues (1992) report similar small fractions (30-38%) of the emptied CHO being oxidised during prolonged cycling exercise. Massicote and colleagues (1989) report far higher fractions (65-72%) of the emptied CHO being oxidised during prolonged cycling at a lower exercise intensity however the subjects were only fed CHO at a rate of 0.8g·min⁻¹ and were therefore not maximising the rate of emptying or oxidation.

It has been shown by infusion studies that intramuscular factors are not the limitation to exogenous glucose uptake (Hawley et al., 1994). Hawley and colleagues bypassed both intestinal absorption and hepatic glucose uptake by infusing glucose into subjects cycling at 70%VO₂max. They report CHO oxidation rates for the infused glucose of 1g·min⁻¹. Therefore limitations to exogenous CHO oxidation appear to occur at the intestinal or hepatic level rather than within skeletal musculature. Maximal absorption rates from the intestine have been reported in the range of 1.3-1.8g·min⁻¹ (Duchman et al., 1997) which although slightly higher than oxidation rates could be a limiting factor, especially if intestinal function is compromised by exercise (see Figure 2.2).

Since intestinal absorption has been posited as a factor that could limit exogenous CHO oxidation, especially during exercise, a number of authors have experimented with using multiple transportable CHO to maximise intestinal absorption. Jeukendrup et al (2003) found that a glucose and sucrose mixture exhibited higher exogenous CHO oxidation rates (~1.25g·min⁻¹) than isoenergetic glucose or glucose/maltose solutions (~1.1g·min⁻¹) during 150 min of cycling at 60%VO₂max. Similarly, data from the same laboratory (Jentjens et al. 2003) found higher rates of exogenous CHO oxidation from a glucose/fructose solution (~1.3g·min⁻¹) during 2h of cycling at 65%VO₂max compared to (~0.8g·min⁻¹) from an isoenergetic and a lower energy density glucose solution.

Although these data suggest that it is possible to elevate exogenous CHO oxidation above 1g·min⁻¹ it is important to note the volume of ingestate necessary to deliver
CHO at those rates. The authors do not state the CHO content of the solutions but if one assumes that the solutions are in the range common to most commercial CHO solutions (6-8%) then it would necessitate the ingestion of 1350-1800m1-h⁻¹ of solution. Although this may be possible during prolonged cycling there would be potential gastrointestinal discomfort during high-intensity intermittent running activity. Although there are no quantitative data to confirm this, there is qualitative information from Rehrer et al., (1989) who reported high incidences of gastrointestinal discomfort during 25km races (25% of subjects) and marathon races (52%). Although the authors report no correlation between gastrointestinal problems and volume of intake, in a further study (Rehrer et al., 1992) they report a far greater incidence of gastrointestinal discomfort in triathletes during the running phase compared to the cycle and the swim. This could be as a consequence of the increased abdominal movement brought about by running as mentioned earlier (Rehrer et al., 1991), this would most likely be exacerbated by intermittent shuttle running and the associated acceleration and deceleration phases.

Since solution osmolality has been reported to potentially increase rates of intestinal absorption some authors have investigated whether replacing glucose with glucose polymers could affect the rates of exogenous CHO oxidation. In one study glucose polymers were found to have higher CHO oxidation rates than glucose during 90 min of cycling at 75%VO₂max (Moodley et al., 1992). The authors reported exogenous CHO oxidation rates increasing to 0.6g·min⁻¹ towards the end of the exercise protocol for the polymers compared to 0.4g·min⁻¹ for glucose solutions. However these rates are low compared to the maximal rates of exogenous CHO oxidation reported. In contrast Massicote et al., (1989) found similar rates of CHO oxidation from 7% glucose polymer and monomer solutions. They report that ~18% and ~16% of the total energy requirement of continuous cycling at 53%VO₂max was provided by the exogenous provision of the glucose polymer or monomer respectively.

It would appear that the data from the literature concerning the oxidation rates of different CHO types, whether monomers, polymers or multiple transportable CHO, during exercise are equivocal. Wagenmakers et al., (1993) compared the oxidation rates of various ingestible CHO solutions during two hours of cycling at 65%VO₂max.
They examined 4%, 8%, 12% and 16% maltodextrin solutions and an 8% sucrose solution. They report similar CHO oxidation rates for all solutions, except the 4% maltodextrin, reaching a plateau through the later stages of exercise of \(-1\text{g·min}^{-1}\) regardless of energy density or carbohydrate type. A CHO content of 8% appeared to be optimal for maximising CHO oxidation relative to the amount being ingested. In both the 8% sucrose and maltodextrin solutions exogenous CHO oxidation accounted for almost 100% of the ingested solution during the final 30 min of exercise, again confirming that gastric emptying was not a limiting factor. Increasing the amount of CHO ingested did not elevate the rate of oxidation thus suggesting that there may be an accumulation of CHO and fluid in the stomach or intestine that could lead to gastrointestinal discomfort. This is likely to be exacerbated in exercise modalities that are neither body mass supported nor continuous such as intermittent running.

**Figure 2.2** A schematic to illustrate potential regulatory pathways of exogenous CHO oxidation. From Jeukendrup and Jentjens (2000) with permission
In support of this, Hawley et al., (1992) reported peak CHO oxidation rates from exogenous glucose and maltose solutions of ~1 g·min⁻¹ during 90 min of cycling at 70% VO₂max. The same authors also report similar CHO oxidation rates for glucose or glucose polymers (maltodextrins) (Hawley et al., 1992). They conclude that there are no physiologically important differences in the rate of exogenous CHO oxidation from glucose; maltose; sucrose or glucose polymers.

Conversely the same authors found higher rates of CHO oxidation from a 15% soluble starch compared to a glucose polymer with the equivalent energy density during 90 min of cycling at 70% VO₂max (Hawley et al., 1991). They report that exogenous CHO oxidation from the glucose polymer (0.9 g·min⁻¹) accounted for 19% of the total CHO oxidised compared to a 40% contribution (1.8 g·min⁻¹) from the soluble starch. However there are methodological failings to consider with this study.

In a study employing isotopic tracers Saris et al., (1993) firstly found that unless tracer is matched with tracee then artificially high oxidation rates of exogenous CHO can be exhibited. This appears to be the case for Hawley’s high value of 1.8 g·min⁻¹ as they did not account for these potential elevations. Saris and colleagues compared soluble and insoluble CHO with a water placebo during continuous cycling at 60% for 150 min. They report a similar total CHO oxidation rate (~2.7 g·min⁻¹) between trials, however there was a significantly higher exogenous contribution in the soluble CHO trial. This necessitated a larger utilisation of the finite endogenous muscle glycogen in the other trials.

A further limiting factor to CHO oxidation could be the regulatory effect of the liver on blood glucose concentrations. There appears to be an inversely proportional relationship between exogenous glucose concentrations and endogenous glucose production. As exogenous glucose concentration rises endogenous glucose production has been shown to fall (Vella et al., 2003) this would appear to be caused by the associated increased hormonal response. The elevated insulin response to glucose ingestion has an inhibitory effect on hepatic glycogenolysis thus leading to a potential sparing of liver glycogen (McConell et al., 2000). Ingestion of moderate amounts of glucose (~33 g·h⁻¹) during prolonged cycling at 50% VO₂max leads to partial suppression of endogenous glucose production whilst higher amounts of glucose
ingestion (~164g·h⁻¹) completely suppress endogenous glucose production. Despite exogenous CHO provision of 2.7g·min⁻¹ the exogenous CHO oxidation rate still did not increase above 1g·min⁻¹ (Jeukendrup et al., 1999). Moreover McConell et al., (1994) found that ingestion of a 10% CHO solution at rates of 250ml every 15min (~100g·h⁻¹) suppressed hepatic glucose release by about 50% during continuous cycling at 69%VO₂max.

Bosch et al., (1994) found that endogenous glucose production was lowered by the ingestion of a 10% CHO solution (50g·h⁻¹) during cycling at 70%VO₂max in glycogen loaded subjects. The rate of appearance of endogenous CHO in the CHO trial was only 65% of that reported for the placebo trial. The suppression of liver or splanchnic glucose release was due to increased blood glucose oxidation in the CHO trial. Glucose oxidation rate as a contribution of total CHO oxidation was significantly higher (65%) in the CHO trials than in the placebo trial (53%) and was ~0.75g·min⁻¹ this was similar to the rate of CHO ingestion (0.83g·min⁻¹), further supporting the concept that gastric emptying is not a limiting factor. The authors reported that despite the sparing of liver glycogen the provision of exogenous CHO did not appear to have a muscle glycogen sparing effect. It would appear that hepatic glucose output balances the release of CHO from the gut with gluconeogenesis and glycogenolysis to allow blood glucose release to be maintained at approximately 1g·min⁻¹. When large amounts of exogenous CHO are ingested the liver may increase the rate of glycogenesis.

The main finding from the literature is that the maximal rate of exogenous CHO oxidation appears to be about 1g·min⁻¹ irrespective of the type of carbohydrate ingested. It would appear that the factors limiting exogenous CHO oxidation are the rates of intestinal absorption along with a regulatory mechanism within the liver. These maximal rates can be achieved by ingestion of volumes of solutions containing CHO in the range of 1-1.5g·min⁻¹. Although some studies have reported elevated rates of exogenous CHO oxidation these involve volumes of ingestate that probably could not be tolerated during certain intensities and modes of exercise. To the author's knowledge there are no reported data on rates of exogenous CHO oxidation from intermittent exercise, regardless of mode, probably due to methodological
considerations caused by the lack of a 'steady-state' being achieved. As such one cannot speculate on the optimal CHO concentration, type or amount to optimise the rate of oxidation although it would be pertinent to follow similar recommendations as those for continuous running of similar intensity. Furthermore as rates of intestinal absorption could limit the rate of exogenous CHO oxidation it would be prudent to provide a solution that would have a negligible negative effect on intestinal absorption.

2.7 Summary

The weight of evidence from the literature is that sports involving intermittent high-intensity running place large demands on the physiological capacity of participants. Players cover large distances, often at maximal intensities; involving hundreds of discrete actions whilst maintaining an average exercise intensity of about 70% VO2max; as such these types of activities place large demands on the individual’s limited glycogen reserves. Pre-exercise dietary interventions, in the form of high carbohydrate intakes, increase players’ muscle glycogen concentrations and lead to enhanced work capacity as shown in both field studies and laboratory studies (in which aspects of the sports have been simulated). In addition work capacity during intermittent exercise can be further enhanced by ingesting carbohydrate during exercise; again this has been shown both in the field and in the laboratory.

Despite the ergogenic potential of ingesting carbohydrate both before and during exercise, subjects in studies on intermittent running capacity still fatigue and often within the time frame of a match situation (i.e. <90 min). As this fatigue is still attributable to a depletion of muscle glycogen it would suggest that the delivery of the exogenous carbohydrate is unable to meet the demands for carbohydrate oxidation during such activity. To address this issue there is a continual search to discover the optimal properties of a carbohydrate-electrolyte solution for delivery into the systemic circulation and for maximising exogenous carbohydrate oxidation.

The available evidence shows that maximal rates of oxidation of exogenous carbohydrate appear to be about 1g·min⁻¹. However since the intravenous infusion of carbohydrate can lead to even greater oxidation rates it would suggest that the limitations to the effect of carbohydrate-electrolyte solutions could be their transport through the gastrointestinal tract. Therefore if it is possible to enhance the transport of
exogenous carbohydrate through the gastrointestinal tract and into the systemic circulation then this could increase the rates of exogenous carbohydrate oxidation and subsequently prolong exercise capacity. Although the data from the literature is equivocal there is some evidence to suggest that hypotonic carbohydrate-electrolyte solutions may have a faster rate of gastric emptying, and also intestinal absorption, than isotonic solutions. For this reason the investigation of hypotonic carbohydrate-electrolyte solutions during high-intensity intermittent running exercise warrants further examination.
CHAPTER 3

GENERAL METHODS

3.1 Introduction
Methods that are generic and common to all studies throughout this thesis are outlined in this chapter as are methodologies for procedures relating to the collection and analysis of blood and muscle samples. Methods that are specific to a study are described in more detail in the relevant section of that experimental chapter.

All procedures received approval from the Ethical Advisory Committee of Loughborough University and were performed in accordance with the ‘Code of Practice for Workers having Contact with Body Fluids’. All volunteers who expressed a willingness to be included as subjects for these studies were fully informed about the aims, procedures and demands that the study would place upon them. Furthermore they were instructed of any potential risks and discomforts that they may face, only then was written consent obtained (Appendix A). Subjects were required to complete a general health history questionnaire (Appendix B) with subjects displaying any adverse medical conditions being excluded from participation. In addition subjects were required to complete a questionnaire outlining their physical activity status (Appendix C). Subjects were asked to complete an acute health status questionnaire (Appendix D) on the days of the main trials to ensure that they were feeling up to the demands of the experiment and were informed of their right to withdraw from the study, without reason, at any stage.
3.2 Experimental Testing Procedures

3.2.1 Body Mass and Height
Nude body mass was determined to the nearest 0.1 kg using beam balance scales (Avery Ltd. Model 3306ABV). Body mass was recorded pre-exercise (after the subject had voided), and immediately post-exercise following sweat removal from the skin. The indwelling catheter was accounted for in determination of body mass. From these two measurements, after correcting for fluid intake, it was possible to calculate body mass losses and estimate sweat rates. Height was evaluated to the nearest 0.1 cm using a stadiometer (Holtain Ltd). The investigator aimed to compensate for any intervertebral disc shrinkage by applying upward force to the mastoid processes with his fingers whilst ensuring that the subject's feet remained firmly on the floor and the head was maintained in the Frankfort plane.

3.2.2 Testing Facility
All experimental trials were performed in the School of Sport and Exercise Sciences’ Fitness Suite at Loughborough University. The Fitness Suite is a large ventilated sports hall with a flat, non-slip wooden surface. Lines were clearly marked with coloured tape on this surface at 0m, 10m and 20m to designate the start, midway and end lines for both the Multi-Stage Fitness Test (Ramsbottom et al, 1988) and the Loughborough Intermittent Shuttle Test (LIST) (Nicholas et al, 2000).

3.2.3 Preliminary Measurement – Estimation of Maximal Oxygen Uptake
Maximal oxygen uptake (VO2max) was estimated by means of an incremental treadmill test (Study 3, Chapter 6) or the Multi Stage Fitness Test (Studies 1, 2 and 4, Chapters 4, 5 and 7). Subjects were allowed a 10 min warm up and stretching period prior to either test and were informed that the tests were both progressive and maximal and were urged to continue running at the required pace until volitional fatigue.

3.2.3.1 Incremental Treadmill Test
The incremental treadmill test was an adaptation of a previous model (Taylor et al, 1955). Subjects were required to run for 3 min intervals against a specified gradient at a constant speed, determined from heart rate (~155 beats·min⁻¹) and RPE (12-13)
(Borg, 1973) during a four-stage speed-VO₂ trial. The treadmill was set at a 3.5% incline and was elevated by 2.5% every 3 min. Expired air was routinely collected between 1 min 45 s and 2 min 45 s of each 3 min stage. Subjects continued with this incremental protocol until they felt that they could only sustain one further minute of exercise at that intensity, at this point a final one min expired air sample was obtained and exercise ceased. From analysis of the expired air samples maximal oxygen uptake (VO₂max) was determined; this value was accepted as being valid if two or more of the following criteria were met: (1) volitional exhaustion; (2) attainment of age-predicted maximal heart rate, (3) RER equal to or greater than 1.1; (4) a plateau in oxygen consumption (increase less than 2 ml·kg·min⁻¹) with increased work intensity.

3.2.3.2 Multi Stage Fitness Test

The Multi Stage Fitness Test requires subjects to complete 20m shuttle runs at progressively increasing speeds as dictated by audio signals from a pre-recorded compact disc. Level one begins at a shuttle speed of 2.22m·s⁻¹ increasing every minute by 0.14m·s⁻¹ for each new level. Subjects were required to place one foot over the marked line at the end of the 20m shuttle in time with the auditory signal. No less than two subjects performed the test at any one time to encourage competition and increase motivation. Individuals continued with the shuttles until they were unable to maintain the desired pace and withdrew by volition, or were withdrawn by one of the investigators. The level and shuttle number at the point of withdrawal were recorded and maximal oxygen uptake was estimated using a table of predicted values (Ramsbottom et al., 1988)

From the maximal oxygen uptakes obtained from the two tests running speeds equivalent to 55% and 95% of each individual's VO₂max were then calculated for use in the LIST. The actual running speeds utilised in the LIST were not of importance. By using shuttle speeds corresponding to percentages of predicted VO₂max it was possible to ensure that subjects were exercising at intensities relative to their aerobic capacity. The important consideration was that subjects ran at identical speeds for each experimental trial. Subjects were first matched for VO₂max values and then sprint speeds where possible. This was in order to administer the experiments using paired subjects to increase motivation.
On a separate visit subjects ran three 15 min blocks of the LIST to familiarise themselves with the activity order and exercise intensities. This familiarisation trial also enabled them to become accustomed to the Borg Scale of Perceived Exertion, heart rate telemetry and the procedures for collecting expired air.

3.2.4 The Loughborough Intermittent Shuttle Test (LIST)

The LIST (Nicholas et al., 2000) is a free-running exercise protocol designed to simulate the activity patterns of a game of soccer. The experimental protocols utilised throughout this thesis are based on the LIST with the notable alteration that the order of the Cruise (95% VO$_{2\text{max}}$) and Jog (55% VO$_{2\text{max}}$) phases have been switched. This was in response to recommendations from colleagues (McGregor et al., 1999) who had noticed that subjects were running faster during the Jog phase to allow themselves a slower Cruise phase thus making it difficult to differentiate between the speeds of the two phases.

The test required subjects to run between two marked lines, 20m apart, at various speeds related to their individual VO$_{2\text{max}}$ values. The exercise speed for each 20m shuttle was dictated by an audible signal from a microcomputer (BBC Master Series). Unidirectional sprint times were measured over the first 15m using infra-red photoelectric cells (RS Components Ltd, Switzerland) interfaced to the microcomputer. The running intensities followed a cyclical pattern consisting of 10 shuttles as follows:

- 3 x 20m at walking pace (1.54 m·s$^{-1}$)
- 1 x 20m at maximal running speed
- 4s recovery
- 3 x 20m at a running speed corresponding to 95% of individual VO$_{2\text{max}}$
- 3 x 20m at a running speed corresponding to 55% of individual VO$_{2\text{max}}$

Subjects repeated this cyclical activity pattern for approximately 15 min (10 complete cycles plus the three walks and the sprint of the eleventh cycle). This constituted one LIST block; subjects had a 3 min recovery period between exercise blocks. Figure 3.1 depicts the basic LIST protocol; modifications to this basic protocol are illustrated in the methods section of the relevant chapters.
In the first study (Chapter 4) subjects ran 5 blocks of the LIST separated by the 3 min recovery periods making a fixed exercise duration of 75 min plus 12 min of rest, subsequently referred to as 'Part A'. Following a further 3 min recovery subjects then ran 20m shuttles alternating between a Cruise (95% VO_{2max}) and a Jog (55% VO_{2max}) until fatigue ('Part B'). Fatigue was defined as an inability to maintain the desired running speed. The time taken to reach fatigue was recorded as a measure of endurance capacity.

The second study (Chapter 5) replicated the protocol of Study 2 except that for 'Part B' subjects ran two shuttles (i.e. 40m) at each exercise intensity. This was to enable investigators to more readily identify the point at which subjects were unable to maintain the desired running speed, and thus were fatigued.

In the third study (Chapter 6) subjects ran 6 blocks of the LIST separated by the 3 min recovery periods making a fixed exercise duration of 90 min plus 15 min of rest. Following a further 3 min recovery subjects then continued to run cycles of the LIST, without recovery, to fatigue. Fatigue was defined as the inability to maintain the running speed for two consecutive shuttles at the higher running speed or a decrease in sprint performance to <95% of the mean sprints for blocks 1-3.

The final study (Chapter 7) incorporated exactly the same protocol as that utilised in the third study (Chapter 6) for the main trials. In addition, this study incorporated a glycogen depleting exercise activity 48h prior to the main trial. During the glycogen depleting protocol subjects ran 6 blocks of the LIST separated by the 3 min recovery periods making a fixed exercise duration of 90 min plus 15 min of rest.
5 min Resting Expired Air
5 min Standard Warm Up
LIST Block
Subject Resting

Figure 3.1 Schematic representation of the generic LIST protocol
3.2.5 Environmental Conditions
Dry and wet bulb temperatures were measured pre-exercise and at 15 min intervals thereafter using an aspirated psychrometer (Brannan, Thermometers Ltd. Cumberland, UK). From these values relative humidity was calculated. All experimental tests were conducted in an ambient temperature ranging from 14-20°C. Barometric pressure was measured using a wall mounted barometer (Griffin and George Ltd.)

3.2.6 Heart Rate Monitoring
For all preliminary, familiarisation and experimental trials heart rate was recorded throughout exercise and rest via coded short range telemetric transmitters and wristwatch receivers (Polar Sports Tester™, Polar Electro, Finland). Specific software (Polar HR Analysis Software, Version 5.04) enabled stored data to be downloaded onto a PC and mean heart rates for specific time periods to be displayed.

3.2.7 Subjective Ratings of Perceived Exertion (RPE)
Ratings of perceived exertion were obtained from a 15-point scale (Borg, 1973) during the last walk cycle of each 15 min LIST block. The investigator walked alongside whilst the subject was asked to point to the number or descriptor that best described their perceptions of exertion. This procedure was completed in a manner so that subjects were kept blinded to the perceptions of their running partner to prevent any bias in reporting.

3.2.8 Expired Air Collection and Analysis
Expired air was collected during the incremental treadmill test using a standard Douglas bag method and during the experimental trials by using a modified Douglas bag attached to a rucksack frame.

3.2.8.1 Standard Douglas Bag Technique
During the incremental treadmill test subjects attached a mouthpiece (Harvard Equipment Ltd.) and noseclip (Harvard Equipment Ltd.) thirty seconds prior to expired air collection to ensure evacuation of dead space with expiratory air. The mouthpiece was connected to the Douglas bag via a lightweight two-way valve, a 0.5m length of wide-bore (30mm) lightweight tubing (Fulconia) and a two-way tap (Harvard Equipment Ltd.) thus a closed circuit was formed and expired air was
collected for a 60s period during each 3 min stage and for the final one minute of exercise.

3.2.8.2 Modified Douglas Bag Technique
A 200 l capacity Douglas bag (Harvard Equipment Ltd.) was attached to the frame of a rucksack to enable expired air collection during the free running protocol. Aside from the method of conveyance of the Douglas bag the equipment was assembled as detailed above. Subjects donned the rucksack, mouthpiece and noseclip during the walk phase of cycle five of each LIST block. Subjects completed the cycle whilst wearing the apparatus with the collection valve allowing an open circuit to ensure evacuation of dead space and accustom the subject to breathing through the mouthpiece. During the next walk phase the valve was turned to complete the circuit and expired air was collected for one complete LIST cycle (~80s). Collection ceased and the apparatus was removed during the walk phase of cycle seven.

3.2.8.3 Analysis of Expired Air
The fractional oxygen (FEO₂%) and carbon dioxide (FECO₂%) contents of the expired air were measured by a single unit, incorporating both a paramagnetic oxygen analyser, operating on the basis of the susceptibility of oxygen to a paramagnetic gas, and an infra-red carbon dioxide analyser (Servomex, Model 1440C, Crowborough, Essex, UK). The analyser was accurate to ±0.01% and expressed percentages on a digital display. The analyser was calibrated against nitrogen (Air Products Ltd, Crewe, UK) a calibration gas (16.0% O₂ and 4% CO₂; Air Products Ltd, Crewe, UK) and room air immediately prior to each series of gas analyses.

A digital dry gas meter (Harvard Equipment Ltd.) was used to evacuate the Douglas bags and determine gas volumes. This had previously been calibrated using a 600 l Tissot Spirometer (Collins Ltd, USA). A thermistor probe (Edale type 2984, Model C) monitored the temperature of the expired air. This was fitted on the inner surface of the air inlet pipe connecting the Douglas bag to the gas meter.

All gas volumes were corrected to Standard Temperature and Pressure of a Dry gas (STPD). Oxygen uptake (VO₂), carbon dioxide production (VCO₂) and respiratory
exchange ratio (RER) were calculated by utilising a computerised spreadsheet (Excel, Microsoft) pre-programmed to utilise the Haldane transformation. Energy expenditure was determined by indirect calorimetry (Appendix E).

3.2.9 Subject Controls
Subjects reported for each experimental trial following a 10h overnight fast. This was to control for any intra-individual differences in blood glucose and liver glycogen stores that breakfast may have caused. Subjects were asked to refrain from physical activity in the 48h preceding each main trial. They were also asked to abstain from alcohol, caffeine and pharmacological substances for this time period. To further control for nutritional state subjects weighed and recorded their food and drink intake for the two days prior to the first experimental trial using food record diaries (Appendix F) and precision strain-gauge weight sensing scales accurate to 1g (Salter, Model 3010, England). This intake was then replicated for the corresponding period for subsequent trials. Energetic intake and nutritional content of subjects’ diets were later analysed using dietetic software (Comp-Eat, Version 5; Comp-Eat Nutrition Systems).

In Studies 1, 3 and 5 (Chapters 4, 6 and 7) subjects were required to ingest a 6.4% hypotonic carbohydrate electrolyte solution; ~118 mOsmol·kg⁻¹ (GlaxoSmithKline, UK) and a taste matched electrolyte placebo; ~25 mOsmol·kg⁻¹ (GlaxoSmithKline, UK). In addition, during Study 2 (Chapter 5) subjects were required to ingest a 6.4% isotonic carbohydrate solution; ~286 mOsmol·kg⁻¹, a commercially available sports drink (GlaxoSmithKline, UK). The composition of the test solutions, as provided by the manufacturers, is detailed in Table 3.1. Actual measured values of glucose concentration and solution osmolality are detailed in Table 3.2. The administration of solutions in each study is detailed in the relevant chapters. The prescribed volume of fluid was measured in a graduated cylinder and stored over ice in separate unmarked plastic drinking bottles. This was to ensure that the correct volume was ingested and that no spillage occurred. Subjects drank the solutions prior to the commencement of the main trial and during the 3 min rest periods between LIST blocks.
Table 3.1  Manufacturer’s guidelines on composition of test solutions

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<th>Placebo</th>
<th>Isotonic</th>
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<tbody>
<tr>
<td>Total CHO (g·100ml&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>6.4</td>
<td>0.13</td>
<td>6.4</td>
</tr>
<tr>
<td>Sodium (mg·100ml&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>50.4</td>
<td>2.0</td>
<td>49.5</td>
</tr>
<tr>
<td>Potassium (mg·100ml&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>10.3</td>
<td>11.2</td>
<td>10.3</td>
</tr>
<tr>
<td>Aspartame (mg·L&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>150.4</td>
<td>200.2</td>
<td>220</td>
</tr>
<tr>
<td>Acesulfame K (mg·L&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>75.7</td>
<td>100.1</td>
<td>109.2</td>
</tr>
<tr>
<td>Brix</td>
<td>7.4</td>
<td>0.4</td>
<td>7.0</td>
</tr>
<tr>
<td>pH</td>
<td>3.86</td>
<td>2.85</td>
<td>3.45</td>
</tr>
<tr>
<td>Acidity (%w/w CAMH)</td>
<td>0.44</td>
<td>0.29</td>
<td>0.50</td>
</tr>
<tr>
<td>Osmolality (mOsm·kg&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>118</td>
<td>25</td>
<td>286</td>
</tr>
</tbody>
</table>

Table 3.2  Measured composition of test solutions

<table>
<thead>
<tr>
<th></th>
<th>Hypotonic</th>
<th>Placebo</th>
<th>Isotonic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total CHO (g·100ml&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>6.4 ± 0.1</td>
<td>0.10 ± 0.03</td>
<td>6.3 ± 0.2</td>
</tr>
<tr>
<td>Osmolality (mOsm·kg&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>115 ± 1</td>
<td>25 ± 1</td>
<td>289 ± 2</td>
</tr>
</tbody>
</table>
3.3 Collection, Treatment, Storage and Analysis of Blood Samples

3.3.1 Sample Collection
Venous blood samples were drawn from an antecubital forearm vein using an indwelling catheter (Venflon, 16-18G, Sweden), which was kept patent by periodic infusion of sterile saline (Stenpak, Ltd. Runcorn, UK). Subjects rested in a supine position on an examination couch before local anaesthetic was administered (0.5 ml of 1% Lignocaine; Antigen Pharmaceuticals, Ireland) prior to catheter insertion. As postural changes can result in plasma volume differences of up to 10% (Rowell, 1974) all blood samples were obtained from standing subjects. After catheter insertion subjects adopted a standing position for 15 min before an 11 ml resting sample was drawn. Further 11 ml blood samples were drawn during the 3 min recovery breaks between LIST blocks and at the point of fatigue.

3.3.2 Treatment, Storage and Analysis of Venous Blood Samples
Venous blood samples were treated, stored and analysed in the following manner:

- Five ml of whole blood was dispensed into EDTA containing tubes (Sarstedt, Numbrecht, Germany) and 5ml was left to clot for 1h to obtain serum samples.

- Duplicate 20µl aliquots of whole venous blood were immediately deproteinised in 200µl of 0.38 mM perchloric acid, centrifuged (Eppendorf, Model 5414, Hamburg, Germany) and then frozen at -70°C. Blood lactate concentration was fluorometrically determined (Locarte, Model 8-9) using a method outlined by Maughan (1982).

- Triplicate 50µl samples of whole venous blood were collected using heparinised pipettes (Scientific Industries International Ltd, Leics, UK) and then micro-centrifuged (Gelman Hawksley Ltd.) for 15 min at 11 000 rpm. A sliding haematocrit reader (Gelman Hawksley Ltd.) was used to measure packed cell volume. Duplicate 20µl samples of whole venous blood were collected for the determination of haemoglobin concentration by the cyanomethaemoglobin method (Boehringer Mannheim GmbH Diagnostica,
From changes to baseline haemoglobin and haematocrit concentrations in response to exercise, percent changes in plasma volume were estimated using the formula described by Dill and Costill (1974).

- The remaining whole venous blood was centrifuged at 4°C for 15 min at 6000 rpm (Koolspin, Burkand Scientific, Uxbridge, UK). The plasma obtained was divided into smaller aliquots and stored at -70°C for later analysis of FFA, glycerol, glucose and lactate using commercially available kits (NEFA C, Wako Chemicals GmbH, UK; Colorometric method, Randox Ltd, UK; GOD-PAP method, Boehringer Mannheim, Germany and ABX Diagnostics, Montpellier, France; respectively) and an automated system (COBAS Mira Plus, Roche Diagnostics Systems, Switzerland).

- Serum was obtained by centrifuging 5ml of coagulated whole venous blood for 15 min at 6000 rpm at 4°C. The serum was divided into smaller aliquots and stored at -70°C for subsequent analysis of insulin (Coat-A-Count Insulin, Diagnostica Products Corporation DPC kit, Caernarfon, UK), cortisol (Coat-A-Count Cortisol, Diagnostica Products Corporation DPC kit, Caernarfon, UK) and prolactin (Coat-A-Count Prolactin, Diagnostica Products Corporation DPC kit, Caernarfon, UK) using commercially available kits. The hormonal assays were counted using an automated gamma counter (Packard, Cobra 5000, Pangbourne, UK).

- A coefficient of variability was performed on multiple measures of single blood samples at the upper and lower end of the physiological range for each assay; and a regression equation of correlation 0.999 on the standards was achieved before commencing sample analyses. The coefficient of variation (CV) [(Standard deviation / mean) * 100] of the blood, plasma and serum assays is shown in Table 3.3.
Table 3.3  The coefficient of variation (CV) of the blood, plasma and serum assays (n=10).

<table>
<thead>
<tr>
<th>Assay</th>
<th>CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma glucose</td>
<td>0.7</td>
</tr>
<tr>
<td>Plasma FFA</td>
<td>0.7</td>
</tr>
<tr>
<td>Plasma glycerol</td>
<td>0.7</td>
</tr>
<tr>
<td>Plasma Lactate</td>
<td>0.7</td>
</tr>
<tr>
<td>Blood lactate</td>
<td>1.6</td>
</tr>
<tr>
<td>Haematocrit</td>
<td>1.0</td>
</tr>
<tr>
<td>Haemoglobin</td>
<td>1.5</td>
</tr>
<tr>
<td>Serum insulin</td>
<td>4.5</td>
</tr>
<tr>
<td>Serum prolactin</td>
<td>4.5</td>
</tr>
<tr>
<td>Serum cortisol</td>
<td>4.5</td>
</tr>
</tbody>
</table>

Blood and plasma samples were analysed in the biochemistry laboratory situated in the School of Sports and Exercise Sciences at Loughborough University. Serum samples were analysed in the radiochemistry laboratory in the Department of Chemistry at Loughborough University.

3.4 Collection, Treatment, Storage and Analysis of Muscle Samples

3.4.1 Sample Collection

Muscle samples were obtained from the middle portion of m. vastus lateralis by the percutaneous needle biopsy technique (Bergstrom, 1962) with suction applied to maximise sample size. The m. vastus lateralis was the muscle chosen as it is easily accessible; isolated from major blood vessels and nerves; and is heavily involved during sports which are intermittent in nature. It is generally believed that m. gastrocnemius rather than quadriceps muscle is more involved during running exercise (Costill and Saltin, 1974). Costill (1988) reported greater glycogen utilisation from the plantar flexion muscles (m. gastrocnemius and m. soleus) during 2h of running (uphill, downhill and level treadmill running) than from the quadriceps.
However it has been suggested that following exhaustive road running no real difference exists in the glycogen depletion pattern of the *m. gastrocnemius* and the lateral portion of quadriceps muscles (Karlsson and Saltin, 1971). Secondly, anecdotal evidence from within our laboratory and from others (Leatt and Jacobs, 1989) proposes that biopsy-induced muscle soreness and stiffness occurs less frequently and severely in the *m. vastus lateralis* than in the *m. gastrocnemius*, especially if subjects are required to exercise immediately after the biopsy. This is pertinent since the subjects in Study 4 (Chapter 7) had to follow four of their six biopsies with high-intensity shuttle running. Furthermore, investigations into muscle soreness following intermittent running exercise (LIST) have reported significantly elevated perceptions of soreness in both the *m. gastrocnemius* and the *m. vastus lateralis* (Thompson et al., 1999; Bailey et al., 2003). Therefore, the *m. vastus lateralis* would seem to be a suitable site for analysing glycogen depletion patterns during exhaustive, high-intensity intermittent shuttle running.

Samples were taken through three separate incisions made in the skin and fascia under local anaesthetic (5ml of 1% lignocaine per site), on the central portion of the *m. vastus lateralis*, mid-way between the hip and the knee. Incisions in a particular trial were made on one leg only with the alternate leg being utilised on the subsequent trial. Three subjects had their right leg biopsied in their first trial and three their left. Coincidentally, due to the double-blind nature of the trial, this resulted in three subjects having biopsies in their right leg for the carbohydrate trial and three for the placebo trial. A ‘resting’ muscle sample was then taken through the distal incision with further samples being taken at 90 min (central incision) and at fatigue (proximal incision). Costill and colleagues (1988) reported that with multiple biopsy sampling glycogen storage was most affected in sites distal from the initial sample, for this reason it was decided that the initial sample would be taken from the distal incision site with subsequent samples becoming more proximal. It took 38s (±7) and 52s (±13) to transfer the subject from the gymnasium to the examination couch, collect the sample and store it in liquid nitrogen for the 90 min and fatigue samples respectively.
3.4.2 Treatment and Storage of Muscle Samples

After removal of the biopsy needle from the incision site it was immediately snap frozen in liquid nitrogen. The muscle sample was then removed from the needle, stored in plastic screw-top Eppendorf tubes and maintained in liquid nitrogen until all samples from the study had been collected.

Each muscle sample was divided into two portions in liquid nitrogen with one part kept in liquid nitrogen for future analysis and the other freeze-dried for 12h (Edwards, Freeze Dryer, Modulyo) and then stored in plastic screw-top Eppendorf tubes surrounded by desiccant (silica gel) at -80°C until subsequent analysis. At a later date the freeze-dried muscle was dissected free of visible blood and connective tissue and homogenised using an agate pestle and mortar. An electric balance, accurate to 0.01mg (Mettler AE240), was then used to determine the mass of the samples (range 1.01-8.64mg) which were then stored in plastic screw-top Eppendorf tubes surrounded by desiccant at -80°C.

3.4.3 Mixed Muscle Metabolite Analysis

3.4.3.1 Extraction Procedure

Mixed muscle metabolites were extracted from the powdered samples as follows: Powdered muscle samples were removed from the -80°C freezer (in batches of 8) and allowed to thaw at room temperature (~30 min) surrounded by desiccant and then kept on ice. Samples were then spun down for 60s at 14,000rpm at 0-4°C (EBA12R Hettich, Zentrifugen). A known volume (volume ml = muscle weight mg / 12.5) of 0.5mol: l⁻¹ perchloric acid (HClO₄) containing 1mmol: l⁻¹ EDTA (Ethylenediamine Tetraacetic Acid) was added to the powdered muscle sample. Samples were repeatedly vortexed very gently for 10s and then replaced on ice. This ‘vortex-ice’ procedure was continued for 10min. Samples were then spun for 180s at 14,000rpm at 0-4°C. A measured volume of the supernatant was removed by a pipette (Pipetman, Gilson, France) and placed in a new screw-top Eppendorf tube. The remaining muscle pellet was stored in plastic screw-top Eppendorf tube surrounded by desiccant at -80°C for later analysis of acid insoluble glycogen.
A known volume (25% of supernatant volume) of 2.2mol·l⁻¹ KHCO₃ was added to the supernatant. This neutralised the supernatant and CO₂ was liberated. The Eppendorf tube was vortexed gently and kept on ice for 5min with a loose top to allow the CO₂ to escape. The pH of the extract was found to be 7.0. Samples were centrifuged for 180s at 14,000rpm at 0-4°C before the clear supernatant was decanted into a new screw-top Eppendorf tube and stored at -80°C, surrounded by desiccant, for subsequent assays.

3.4.3.2 Mixed Muscle Metabolite Assays
Analysis of ATP (adenosine triphosphate), PCr (phosphocreatine) and G-6P (glucose 6-phosphate) was performed on the day of muscle extraction. Glycogen, lactate, Cr (creatine) and free glucose were assayed on subsequent days. The concentration of these metabolites has been reported to be unaffected by freeze-thaw cycles of the muscle extracts (Tsintzas, 1993). Glycogen was assayed by hydrolysis in 1mol·l⁻¹ hydrochloric acid (HCl) both on the neutralised extract (acid-soluble glycogen) and on the precipitated muscle pellet after the extraction procedure (acid-insoluble glycogen). The total mixed muscle glycogen concentration was calculated by adding the acid-soluble and acid-insoluble glycogen concentrations.

The muscle extracts were assayed for the different metabolites using modifications of the methods described by Harris et al., (1974) and Edwards et al., (1975). Duplicate analysis was performed on every sample. Detailed procedures of every assay performed are presented in Appendix G.

The buffers, co-factors (Grade-I) and enzymes used in all analyses were obtained commercially from Boehringer and Sigma. All reagents were diluted using double-distilled water on the day of analysis and kept on ice. Standards were also prepared on the day of analysis using Grade-I chemicals. The molarity of the standards was determined spectrophotometrically, based on the coefficient of extinction of NADH (Appendix G). The concentrations of metabolites were established using the standard curve regression equation (standard curves were always linear, r≥0.99), taking into account the double-distilled water blanks, extract blanks, standards, dilution factors
and extraction factors. One ml of extract was equivalent to 8mg of muscle powder. Where necessary the concentrations of the metabolites were adjusted to the true molarity of the standards and to the highest total creatine pool (PCr + Cr) in the samples of each subject. The latter correction factor was to compensate for any admixture of elements like connective tissue, fat droplets or blood that may have contaminated the samples, since total creatine content should not differ during exercise. All muscle metabolite concentrations are expressed in mmol·kg dm (dry mass)⁻¹ to avoid changes in concentration due to water shift during exercise.

Table 3.4 displays the coefficients of variation of the muscle metabolite assays investigated in Chapter 7. The coefficients of variation were calculated from repeated measurements on a sample obtained from human m. vastus lateralis at rest for each of the assays. All muscle metabolite assays were performed at the School of Biomedical Sciences at The Queen’s Medical Centre, Nottingham.

Table 3.4 The coefficient of variation (CV) of the muscle metabolite assays (n=10).

<table>
<thead>
<tr>
<th>Assay</th>
<th>CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acid Insoluble glycogen</td>
<td>2.8%</td>
</tr>
<tr>
<td>Acid Soluble glycogen</td>
<td>2.6%</td>
</tr>
<tr>
<td>Adenosine Triphosphate</td>
<td>2.1%</td>
</tr>
<tr>
<td>Phosphocreatine</td>
<td>1.9%</td>
</tr>
<tr>
<td>Creatine</td>
<td>2.8%</td>
</tr>
<tr>
<td>Glucose</td>
<td>2.7%</td>
</tr>
<tr>
<td>Glucose-6-Phosphate</td>
<td>2.1%</td>
</tr>
<tr>
<td>Lactate</td>
<td>1.6%</td>
</tr>
</tbody>
</table>
3.5 Statistical Analyses

Statistical comparisons of the physiological, biochemical and metabolic parameters were analysed using a two-way (treatment x time) analysis of variance for repeated measures (SPSS 11). Mauchly's test for sphericity was utilised, where asphericity was assumed the Greenhouse-Geisser correction was utilised for epsilon <0.75, if not then the Huyn-Feldt correction was used. Where significant F values were found a Holm-Bonferroni step-wise method was utilised to determine the location of the variance (Atkinson, 2002). When there were only single comparisons a student's t-test for correlated data was used to determine whether any differences between treatments existed. Null hypotheses were rejected at an alpha level of p<0.05. All data are reported as mean ± standard error of the mean (SEM).
CHAPTER 4

INFLUENCE OF INGESTING A HYPOTONIC CARBOHYDRATE ELECTROLYTE SOLUTION ON ENDURANCE CAPACITY DURING THE LIST

4.1 Introduction

A number of controlled studies conclude that the ingestion of carbohydrate-electrolyte (CHO-E) solutions at rates in excess of 45g·hr⁻¹ result in a 32-52% increase in intermittent running capacity following 75 min of prolonged high intensity intermittent exercise (Nicholas et al., 1995; Davis et al., 1999; Davis et al., 2000; Welsh et al., 2002).

These studies utilised commercially available sports drinks with osmolalities classed as isotonic. Although there appears to be agreement on the accepted range of CHO concentrations for optimal delivery of exogenous CHO (see Murray and Stofan, (2001) for a review) the ideal osmolality of such solutions is unclear. Many authors propose that energy density of the CHO-E solution defines the rate of gastric emptying rather than the solution osmolality (Murray et al., 1994; Vist and Maughan, 1994; Brouns et al., 1995; Leiper et al., 1999; Shi et al., 2000; Simpson et al., 2001) and would therefore expect similar emptying rates for isoenergetic CHO solutions regardless of osmolality. However there is evidence to suggest that by substituting glucose polymers (in the form of maltodextrins) for monomers, thereby maintaining energy density but decreasing osmolality, it is possible to increase the rate of gastric emptying (Foster et al., 1980; Vist and Maughan, 1995; Sole and Noakes, 1989).

Furthermore, although the data for intestinal absorption rates are similarly equivocal, there is some evidence to suggest that hypotonic CHO-E solutions may have a faster rate of intestinal absorption than isotonic solutions. This has been shown following the ingestion of moderately hypotonic solutions at rest (Leiper et al, 1994) although there are as yet no data from intermittent running activity. Since hypotonic CHO-E solutions may confer advantages over isotonic CHO-E solutions in terms of gastrointestinal function, and at the least will be as efficacious, this theory has had an
influence over the current formulations of sports drinks with some solutions being marketed with osmolalities in the range of 100-200 mOsmol·kg\(^{-1}\).

Preliminary data (Appendix I) suggest that hypotonic CHO-E solutions confer ergogenic benefits during treadmill running at 70%\(V\text{O}_2\text{max}\). Therefore the purpose of the present study was to determine whether the administration of a 6.4% hypotonic (118 mOsmol·kg\(^{-1}\)) CHO-E solution would improve endurance capacity during intermittent running exercise (LIST).

4.2 Methods

Twelve competitive male games players (age 20.8 years (±0.5); height 175.3 cm (±1.9); body mass 74.7 kg (±2.5); \(V\text{O}_2\text{max}\) 57.5 ml·kg\(^{-1}·\text{min}^{-1}\) (±1.3); Means (±SEM)) volunteered, with informed consent, to participate in this University Ethical Committee approved study.

Maximal oxygen uptake was determined by means of the Multistage Fitness Test (Ramsbottom et al., 1988). From the level and shuttle that the subject fatigued, maximal oxygen uptake was estimated and running speeds corresponding to 55% and 95% \(V\text{O}_2\text{max}\) were calculated, as previously described (Chapter 3). Subjects performed the LIST for 30 min to familiarise themselves with the exercise protocol and experimental procedures.

The subjects performed two experimental trials separated by at least 7 days. To eliminate any trial order effect treatments were assigned to a random crossover design. On each occasion subjects consumed either a 6.4% hypotonic (118 mOsmol·kg\(^{-1}\)) carbohydrate-electrolyte solution (CHO) or a taste-matched electrolyte placebo (PLA) free of carbohydrate. Solutions were administered in a double-blind fashion immediately prior to the trials (5ml·kg\(^{-1}\) BM) and at subsequent 15min intervals (2ml·kg\(^{-1}\) BM) for the first 75min (i.e. Part A of the LIST protocol).

Subjects refrained from strenuous exercise, caffeine, tobacco and alcohol and monitored their dietary consumption for the 48h period preceding the first trial,
replicating this approach and intake for the second trial. They reported to the laboratory after an overnight fast (≥10h) and voided prior to the measurement of nude body mass. Nude body mass was recorded pre- and post-exercise. An indwelling cannula was inserted into an antecubital vein. The subjects stood for 15 min before a resting blood sample was obtained. Resting heart rate was monitored and expired air sampled over a 5 min period. All subjects completed a standardised 15 min warm-up comprising jogging, striding, soccer specific movements and stretching. Then, immediately before commencing the LIST, the subjects consumed the prescribed solution.

The experimental design was in two parts. Part A was of a fixed duration and consisted of five 15 min exercise periods of LIST separated by 3 min recovery. Part B was designed to assess endurance capacity and comprised alternate 20m shuttles of jogging (55% VO2max) and cruising (95% VO2max) until volitional fatigue or an inability to maintain the desired running speed, at which stage the subject was withdrawn and testing ceased (Figure 4.1).

Heart rate was monitored every 15s during exercise using short-range telemetry. Expired air samples were collected during one complete cycle (~80s) of each 15 min LIST period. Subjective ratings of perceived exertion were recorded on a 15 point scale during the walk phase of the final cycle of each block.

An 11ml blood sample was taken at rest, every 15 min of Part A of the LIST and at fatigue with 4ml dispensed into an EDTA containing tube. The venous blood samples were assayed for lactate, haematocrit and haemoglobin concentrations. The remaining whole blood was centrifuged at 4°C and the plasma divided into aliquots and frozen at -20°C for later analysis of free fatty acids (FFA), glucose and glycerol. Serum was obtained by separating 4ml of coagulated whole blood at 4°C; and subsequently stored at -70°C for subsequent analysis of insulin and cortisol. All blood sampling procedures and analyses are described in more detail in Chapter 3. Blood samples for each experimental treatment were analysed in duplicate within the same assay series in order to reduce variability in the biochemical analytical procedures.
Statistical analyses were performed as detailed in Chapter 3. Null hypotheses were rejected at an alpha level of $p<0.05$. All data are reported as mean (± SEM). Blood data were unavailable for one subject due to discomfort with the cannulation process, therefore statistics are based on $n=11$ for blood analysis and $n=12$ for other variables.
Figure 4.1  Schematic representation of the experimental protocol
4.3 Results

4.3.1 Running performance

The exercise capacity in Part B for the CHO and PLA trials were 9.24 min (±1.87) and 9.24 min (±2.08) respectively (n.s; Figure 4.2). Sprint times were similar between trials and increased over time irrespective of treatment ($F_{2,23}=5.3; p=0.01$; Figure 4.3).

4.3.2 Oxygen uptake

Mean oxygen uptakes during the CHO and PLA trials for Part A were 46.8 ml·kg$^{-1}$·min$^{-1}$ (±0.6) and 46.2 ml·kg$^{-1}$·min$^{-1}$ (±0.4) respectively, representing average relative exercise intensities of 81.6% VO$_{2\text{max}}$ (±1.4) for the CHO trial and 81.1% VO$_{2\text{max}}$ (±1.8) for the PLA trial (n.s; Figure 4.4).

4.3.3 Energy expenditure and respiratory exchange ratio

There were no differences in total energy expenditure between the CHO and PLA trials for Part A of the trial (5270 kJ ± 174 and 5197 kJ ± 185 respectively). Respiratory exchange ratio (RER) had a tendency to be higher in PLA throughout exercise (Figure 4.5).

4.3.4 Plasma glucose and serum insulin

Plasma glucose concentration was maintained within the normal range in both trials; although it was higher in the CHO trial throughout exercise this did not reach statistical significance at any time point (Figure 4.6). There was a main effect of time such that glucose concentrations were higher throughout both trials compared to resting values ($F_{3,28}=11.1; p<0.001$). Serum insulin values were higher throughout exercise in the CHO trial ($F_{1,16}=8.7; p=0.015$; Figure 4.7).

4.3.5 Plasma FFA and glycerol

There were no differences in FFA concentrations between trials during exercise (Figure 4.8). Free fatty acid concentrations significantly increased with exercise irrespective of treatment ($F_{3,27}=12.9; p<0.001$). Similarly there were no treatment differences observed for plasma glycerol concentrations (Figure 4.9). There was a main effect of time with glycerol concentrations increasing with prolonged exercise ($F_{6,48}=43.2; p<0.001$).
4.3.6 Serum cortisol

Although there were no statistical differences in serum cortisol concentrations between trials there was a trend (p=0.09) for values to be higher in the PLA trial throughout exercise (Figure 4.10). Similarly there was a trend (p=0.06) for there to be a main effect of time, with concentrations increasing with exercise duration but this again failed to reach statistical significance.

4.3.7 Blood lactate, heart rate and rate of perceived exertion (RPE)

Blood lactate concentrations increased significantly with the onset of exercise although there were no observable differences between treatments, in both trials peak blood lactate concentration was observed at the point of fatigue (Figure 4.11). There were no differences in heart rates between trials; again peak values were noted at fatigue (Figure 4.12). Ratings of perceived exertion were not different between trials, increasing with exercise duration (F1,15=40.5; p<0.001; Figure 4.13).

4.3.8 Changes in plasma volume and body mass

Environmental conditions were similar during the CHO and PLA trials with dry bulb temperature and humidity approximately 15°C and 10% respectively. There were no differences between trials for changes to plasma volume. Similarly there were no differences in body mass losses between trials (~0.87 kg for both trials) which accounted for 1.2% of pre-exercise body mass. These data account for the prescribed fluid, without the fluid ingestion the exercise protocol would have led to a body mass loss of ~1.9 kg or 2.6% body mass.

4.3.9 Habitual dietary intake

Analysis of subjects' weighed dietary intake diaries (Comp-Eat 5), revealed that there were no differences between trials for subjects' habitual consumption of carbohydrate, fat, protein or total energy intake over the 48h recording period (Table 4.1).
<table>
<thead>
<tr>
<th>Daily Intake</th>
<th>Carbohydrate (g·kg⁻¹BM)</th>
<th>Fat (g·kg⁻¹BM)</th>
<th>Protein (g·kg⁻¹BM)</th>
<th>Total Energy (MJ)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HYP</td>
<td>5.6 ± 0.4</td>
<td>1.1 ± 0.1</td>
<td>1.5 ± 0.1</td>
<td>11.5 ± 1.0</td>
</tr>
<tr>
<td>PLA</td>
<td>5.2 ± 0.2</td>
<td>1.2 ± 0.2</td>
<td>1.4 ± 0.1</td>
<td>11.7 ± 0.7</td>
</tr>
</tbody>
</table>

Table 4.1  Habitual dietary intake of subjects in two days prior to main trials. Values are Mean ± SEM
Figure 4.2 Running capacity (min) for the Carbohydrate (CHO) and the Placebo (PLA) trials following 75min of LIST.

Figure 4.3 Mean 15m sprint times (s) for the Carbohydrate (CHO) and the Placebo (PLA) trials during LIST.
Figure 4.4 Exercise intensity for the Carbohydrate (CHO) and the Placebo (PLA) trials throughout trial.

Figure 4.5 RER for the Carbohydrate (CHO) and the Placebo (PLA) trials throughout trial.
**Figure 4.6** Plasma glucose concentrations (mmol·l⁻¹) for the Carbohydrate (CHO) and the Placebo (PLA) trials.
*p<0.05, at 0 min vs. all other time points

**Figure 4.7** Serum insulin concentrations (µIU·ml⁻¹) for the Carbohydrate (CHO) and the Placebo (PLA) trials.
Significant difference of treatment CHO vs. PLA (p<0.05)
Figure 4.8 Plasma FFA concentrations (mmol·l⁻¹) for the Carbohydrate (CHO) and the Placebo (PLA) trials. Significant difference of time (p<0.05)

Figure 4.9 Plasma glycerol concentrations (mmol·l⁻¹) for the Carbohydrate (CHO) and the Placebo (PLA) trials. Significant difference of time (p<0.05)
Figure 4.10 Serum cortisol concentrations (nmol·l⁻¹) for the Carbohydrate (CHO) and the Placebo (PLA) trials.

Figure 4.11 Blood lactate concentrations (mmol·l⁻¹) for the Carbohydrate (CHO) and the Placebo (PLA) trials.
Figure 4.12 Heart rate (beats·min⁻¹) for the Carbohydrate (CHO) and the Placebo (PLA) trials.

Figure 4.13 RPE for the Carbohydrate (CHO) and the Placebo (PLA) trials.
4.4 Discussion
The main finding from the present study was that prolonged, intermittent high intensity running capacity was not influenced by the ingestion of a 6.4% hypotonic CHO-E solution. Subjects ran for similar durations in the CHO trial as they did in the PLA trial. Our data are in accordance with other data on intermittent treadmill running from our laboratory (Nassis et al., 1998) that reported no benefit from the ingestion of exogenous CHO. However this is in contrast to findings of previous studies (Nicholas et al., 1995; Davis et al., 1999; 2000; Welsh et al., 2002) which all reported increases in endurance capacity in excess of 30% using a similar intermittent free running shuttle protocol (LIST).

The main difference between the current study and those of other authors is that the CHO-E solution in the present study was hypotonic. It could therefore be suggested that the osmolality of the test beverage was detrimental to the delivery of exogenous CHO. To the best of the author’s knowledge there are no data available on gastric emptying rates or intestinal absorption rates for hypotonic solutions versus isotonic solutions during intermittent shuttle running activity. However recent data suggest that the osmolalities of 6.4% CHO solutions within the range 25-390 mOsmol.kg⁻¹ (including the hypotonic solution utilised in this study) have no significant effect on gastric emptying (Simpson et al., 2002). The hypotonic solution was provided in a pre-exercise bolus (5ml·kg⁻¹BM) and serial feedings every rest period (2ml·kg⁻¹BM) so as to maintain gastric volume and maximise emptying rates. Ingestion of a 6.4% solution in these quantities provided the subjects with CHO at a rate of 48g·h⁻¹, which is similar to those trials that report improved exercise capacity.

As the hypotonic solution was delivered in similar amounts as earlier studies using isotonic solutions, and there are no apparent benefits in gastric emptying and intestinal absorption from isotonic solutions, one can assume that rates of exogenous CHO oxidation would be similar. No measures were taken in the present study; however the rate of exogenous CHO oxidation is dependent on the rate of appearance of glucose in the systemic circulation. Plasma glucose concentrations in the present study were elevated above baseline in both trials and although they were higher throughout exercise in the CHO trial this failed to reach significance. Furthermore insulin
concentrations were significantly higher in CHO compared to PLA throughout exercise.

The profile of appearance of glucose in the systemic circulation is similar in the current trial to data reported by Nicholas et al., (1995), Davis et al., (2000) and MacLaren and Close (2000). The ingestion of CHO increased the concentrations of plasma glucose to similar values reaching a peak between 15 and 30 min post-ingestion. The similarities in the profile of plasma glucose concentrations would appear to suggest that the hypotonic solution in the present study was at least as efficacious in the delivery of CHO to the systemic circulation as the isotonic solutions in the studies of Nicholas and Davis (Nicholas et al., 1995; Davis et al., 2000).

In neither trial did plasma glucose fall to hypoglycaemic concentrations, in fact plasma glucose concentrations were at a nadir at baseline and remained elevated above this level even at fatigue in both trials. The plasma glucose concentrations in the PLA trial, although within the normal physiological range, are higher than one may expect in fasted subjects even considering the stimulatory effect that exercise and catecholamine activity would have on hepatic glucose release. For this reason both solutions were analysed for glucose concentration and were found to be in agreement to the manufacturer's specifications (see Table 3.2). As hypoglycaemia has been postulated as a cause of fatigue in exercising subjects, although not in this exercise modality, the maintenance of blood-borne substrate late in exercise in the current study may serve to explain a lack of differences between running capacity between trials.

Although the running capacity in the current study was not enhanced by the ingestion of the CHO-E solution the run time in the CHO trial was comparable to the concomitant trial in earlier studies. Although there is the obvious caveat regarding comparisons of data from different studies, subjects ran for 9.2 min in the present study which is similar to the 8.9 min and 9.0-9.7 min reported in the CHO trials for Nicholas et al., (1995) and Davis et al., (1999) although it is in excess of the 3.6 min of Welsh et al., (2002) and less than the 11.1-11.2 min of Davis et al., (2000). It would therefore suggest that the CHO was offering a comparable ergogenic effect to the CHO trials of earlier studies. What is of note is that subjects in the current study
were able to run for as long (9.2 min) on the PLA, which is in excess of the 6.7 min, 6.4 min, 8.5 min and 2.6 min in the placebo trials of other authors (Figure 4.13) (Nicholas et al., 1995; Davis et al., 1999; Davis et al., 2000; Welsh et al., 2002). Thus it would appear that either the placebo was offering an enhanced ergogenic effect which we assume not to be true as it contained no ingredients purported to prolong performance or there were other factors influencing fatigue in the current study.

![Figure 4.14](image)

**Figure 4.14** A histogram to illustrate the running capacity in the Placebo trial of the present study compared to both the CHO and Placebo trials of earlier studies. (Mean data)

As glucose ingestion and the subsequent rise in serum insulin should serve to suppress fat metabolism one would expect to have seen elevated FFA concentrations and glycerol concentrations in the PLA trial. However no differences were noted, conversely the respiratory exchange ratio suggests that there was a greater reliance on CHO metabolism in the PLA trial. However these data should be treated with caution since methodological difficulties may confound interpretation of results. Due to the method of conveyance of the Douglas bag there were times when the assembled equipment was pulled loose during the high-intensity phases of the running protocol. This led to a number of missing data for subjects and subsequently it was decided not to perform statistical analyses on these results. It would appear that despite the
elevated blood glucose and serum insulin responses there were no differences in substrate metabolism between trials. The lack of difference in the blood lactate concentrations between the trials would support the notion that there were similar contributions from anaerobic glycolysis to total energy expenditure between trials.

The LIST was designed to replicate the physiological demands of soccer match play; as such subjects should be expected to be exercising at an intensity that reflects these demands. Match analysis data reports that players work at average intensities equivalent to 75%VO₂max (Ekblom, 1986; van Gool et al., 1988) and should expect to have an energy expenditure of ~55 kJ·min⁻¹ (Zelenka et al., 1967). Despite the running speeds in the trials being calculated relative to our subjects’ estimated maximal oxygen uptake values they were actually exercising at intensities equivalent to about 81%VO₂max and expending energy at rates of ~70 kJ·min⁻¹. Although these high exercise intensities could account for differing mechanisms for fatigue in the present study it must be highlighted that the heart rate response of our subjects (~170 beats·min⁻¹) during Part A and the peak values at fatigue (~180 beats·min⁻¹) were consistent with those reported from earlier studies (Nicholas et al., 1995; Davis et al., 1999, Welsh et al., 2002).

As the LIST running speeds are determined relative to a subject’s maximal oxygen uptake values the only area where an individual could theoretically increase their energy expenditure would be during the sprint phase of the protocol. Some authors have reported increased sprint performance during LIST activity with the ingestion of exogenous CHO compared to a placebo (MacLaren et al., 2000; Welsh et al., 2002; Shirreffs and Merson, 2003). In the present trial there were no differences in 15m sprint times between trials. Furthermore the sprint performances of the subjects in this study were consistently slower in both trials than those reported previously from our laboratory (Nicholas et al., 1995; McGregor et al., 1999) suggesting that maximal performance did not necessarily account for the elevated energy expenditure.

The physiological data would suggest that subjects found both trials equally challenging as no differences were noted in RPE values. Only the trend for higher serum cortisol concentrations suggests that the PLA trial may have been more
stressful. The reason posited for an increased running capacity in the studies of Nicholas et al., (1995) and the studies from Davis's lab (Davis et al., 1999; 2000; Welsh et al., 2002) was that the exogenous CHO allowed for either a resynthesis of muscle glycogen during the low intensity periods of activity or a 'sparing' of glycogen in the working muscle fibres (Nicholas et al., 1999). This did not appear to occur in the present study despite biochemical data that suggests that the delivery of CHO was efficacious. The reasons for this are not clear but may have been as a consequence of the training or nutritional status of the subjects.

Although it is difficult to compare training status of subjects between studies; the subjects in the present study had a slightly higher aerobic work capacity (~58ml·kg⁻¹·min⁻¹·BMI) compared to earlier studies (50-56ml·kg⁻¹·min⁻¹·BMI) which may suggest that they were more aerobically trained. Moreover, although nutritional intake was not reported in the earlier studies our subjects reported CHO intakes of ~5g·kg⁻¹·BM·day⁻¹ which is higher than expected for sedentary persons or those reported for soccer players (Jacobs et al., 1982; Clark, 1994; LeBlanc et al., 2002; Riach et al., 2003). The habitual dietary intake of the subjects in the present study may have served to increase the endogenous CHO stores in both the liver and the muscle. This in addition to a potential training induced enhancement of endogenous glucose production and muscle glucose uptake may have served to mask any ergogenic benefits of the CHO solution. Furthermore, studies on CHO oxidation have shown that to maximise the rate of exogenous CHO oxidation requires ingestion of 1-1.5g·min⁻¹ (Jeukendrup and Jentjens, 2000). In the present study subjects were administered CHO at a rate of 0.8g·min⁻¹ therefore in future studies it may be prudent to increase the dosage given.

In summary the ingestion of a 6.4% hypotonic CHO-E solution provided no ergogenic effect on running capacity during prolonged intermittent running (LIST) despite blood and hormonal data suggesting that the solution was efficacious in the delivery of glucose. It would appear that fatigue occurred despite normal blood glucose concentrations and regardless of the provision of exogenous CHO. The findings from the present study suggest that either hypotonic CHO-E solutions are less efficacious than isotonic solutions or that the training or nutritional status of the subject played a significant role in the oxidation of exogenous CHO.
CHAPTER 5

COMPARISONS BETWEEN HYPOTONIC AND ISOTONIC CARBOHYDRATE-ELECTROLYTE SOLUTIONS ON ENDURANCE CAPACITY DURING HIGH-INTENSITY INTERMITTENT EXERCISE

5.1 Introduction
Studies on prolonged high-intensity intermittent shuttle running have reported ergogenic benefits of ingesting isotonic carbohydrate electrolyte solutions with energy densities of approximately 6% (Nicholas et al., 1995; Davis et al., 1999; 2000; Welsh et al., 2002). In the previous study (Chapter 4) it was found that a 6.4% hypotonic glucose polymer solution did not appear to confer such exercise enhancing properties. The literature suggests that a reduced osmolality may not offer any benefits to the rate of gastric emptying; however the data regarding the effects of osmolality on intestinal absorption of both water and CHO is equivocal. Some studies suggest that absorption rates of both solute and fluid are enhanced by a reduced osmolality whereas others suggest that it is more a consequence of energy density and type of CHO. However it is generally accepted that hypotonic solutions are no less efficacious than isocaloric isotonic solutions at providing exogenous CHO to be oxidised during exercise. Therefore as the exercise protocol in the last study was similar to those of other authors who reported a benefit of CHO solutions it seems pertinent to re-investigate this issue and also to directly compare the performance and metabolic responses to both an isotonic and a hypotonic solution during intermittent shuttle running.

Subjective observations from the last study were that participants were becoming increasingly familiar with the exercise protocol and may have been averaging out the running speed during Part B of the test. That is, running slightly slower during the high intensity shuttle (95%VO_{2\text{max}}) and catching up during the lower intensity shuttle (55%VO_{2\text{max}}). This made it extremely difficult to determine the exact point of fatigue and may have affected the results of the previous study. To help tease out when subjects could no longer maintain the desired exercise intensity it was decided to increase the duration of each exercise intensity (2x20m shuttles) during Part B. Furthermore, as the literature advises a CHO intake of 1-1.5g-min^{-1} in order to maximise rates of exogenous CHO oxidation and subjects were only being provided...
with 0.8g·min⁻¹ it was felt that the volume of both the bolus and the serial feedings should be increased. Preliminary studies within our laboratory (Ali et al., 2002) reported no gastrointestinal discomfort in subjects running 90 min of the LIST following a pre-exercise bolus of 8ml·kg⁻¹BM and serial feedings every 15 min of 3ml·kg⁻¹BM. In the current exercise protocol this would enable delivery of ~1.5g·min⁻¹ for a 75kg subject.

The purpose of the present study was to compare the efficacy of a 6.4% hypotonic (118 mOsmol.kg⁻¹) CHO-E solution with an isoenergetic isotonic (286 mOsmol.kg⁻¹) CHO-E solution and the effects on endurance capacity during intermittent running exercise.
5.2 Methods

Eleven recreationally active males (age 22.7 years (±0.8); height 179.5 cm (±2.7); body mass 77.0 kg (±3.2); VO$_{2\text{max}}$ 51.0 ml.kg$^{-1}$.min$^{-1}$ (±1.2); Means (±SEM)) volunteered, with informed consent, to participate in this University Ethical Committee approved study.

Maximal oxygen uptake was determined by means of the Multistage Fitness Test (Ramsbottom et al., 1988). From the level and shuttle that the subject fatigued maximal oxygen uptake was estimated and running speeds corresponding to 55% and 95% VO$_{2\text{max}}$ were calculated, as previously described (Chapter 3). Subjects performed the LIST for 30 min to familiarise themselves with the exercise protocol and experimental procedures.

The subjects performed three experimental trials separated by at least 7 days. To eliminate any trial order effect treatments were assigned to a random crossover design. On each occasion subjects consumed either a 6.4% hypotonic carbohydrate-electrolyte solution (118 mOsmol.kg$^{-1}$), a 6.4% isotonic carbohydrate-electrolyte solution (286 mOsmol.kg$^{-1}$) (GlaxoSmithKline, UK) or a taste-matched placebo. Solutions were administered in a double-blind fashion immediately prior to the trials (8ml.kg$^{-1}$ BM) and at subsequent 15 min intervals (3ml.kg$^{-1}$ BM) until cessation of Part A of the protocol.

Subjects refrained from strenuous exercise, caffeine, tobacco and alcohol and monitored their dietary consumption for the 48h period preceding the first trial, replicating this approach and intake for the subsequent trial. They reported to the laboratory after an overnight fast (≥10h) and voided prior to the measurement of nude body mass. Nude body mass was recorded pre- and post-exercise. An indwelling cannula was inserted into an antecubital vein. The subjects stood for 15 min before a resting blood sample was obtained. Resting heart rate was monitored and expired air sampled over a 5 min period. All subjects completed a standardised 5 min warm-up comprising jogging, striding, soccer specific movements and stretching. Then, immediately before commencing the LIST, the subjects consumed the prescribed solution.
The exercise was in two parts. Part A was of a fixed duration and consisted of five 15 min exercise periods of the LIST separated by 3 min recovery. Part B was designed to facilitate glycogen depletion and comprised alternate 40m bouts (2x20m shuttles) of jogging (55% VO₂max) and cruising (95% VO₂max) until volitional fatigue or an inability to maintain the desired exercise intensity, at which stage the subject was withdrawn and testing ceased (Figure 5.1).

Heart rate was monitored every 15s during exercise using short-range telemetry. Expired air samples were collected during one complete cycle (~80s) of each 15 min LIST period. Subjective ratings of perceived exertion were recorded on a 15 point scale during the walk phase of the final cycle of each block (Borg, 1982).

An 11ml blood sample was taken at rest, every 15 min of Part A of the LIST and at fatigue with 4ml dispensed into an EDTA containing tube. The venous blood samples were assayed for lactate, haematocrit and haemoglobin concentrations. The remaining whole blood was centrifuged at 4°C and the plasma divided into aliquots and frozen at -20°C for later analysis of free fatty acids (FFA), glucose and glycerol. Serum was obtained by separating 4ml of coagulated whole blood at 4°C; and subsequently stored at -70°C for subsequent analysis of insulin and cortisol. All blood sampling procedures and analyses are described in more detail in Chapter 3. Blood samples for each experimental treatment were analysed in duplicate within the same assay series in order to reduce variability in the biochemical analytical procedures.

Statistical analyses were performed as detailed in Chapter 3. Null hypotheses were rejected at an alpha level of p<0.05. All data are reported as mean (± SEM). Blood data were unavailable for three subjects due to discomfort with the cannulation process, therefore statistics are based on n=8 for blood analysis and n=11 for other variables.
Figure 5.1 Schematic representation of the experimental protocol
5.3 Results

5.3.1 Running performance
The exercise capacity in Part B for the HYP, ISO and PLA trials were 6.8 min (±1.7), 5.3 min (±0.8) and 6.0 min (±1.8) respectively (Figure 5.2). Sprint times were similar between trials and decreased over time irrespective of treatment (F4,44=5.43 p=0.001; Figure 5.3).

5.3.2 Oxygen uptake
The mean oxygen uptakes during the HYP, ISO and PLA trials for Part A were 43.8ml.kg\(^{-1}\).min\(^{-1}\) (±1.0), 43.0ml.kg\(^{-1}\).min\(^{-1}\) (±1.0) and 43.0ml.kg\(^{-1}\).min\(^{-1}\) (±1.3) respectively, representing average relative exercise intensities of 85.4% VO\(_{2\text{max}}\) (±1.6) for the HYP trial, 84.1% VO\(_{2\text{max}}\) (±1.4) for the ISO trial and 83.9% VO\(_{2\text{max}}\) (±1.8) for the PLA trial (Figure 5.4). There were no significant differences for respiratory exchange ratio between treatments nor over time (Figure 5.5).

5.3.3 Plasma glucose and serum insulin
Plasma glucose concentration was maintained within the normal range in both trials although it was significantly higher in the HYP and ISO trials compared to the PLA trial (F2,16=9.4; p=0.002; Figure 5.6). Similarly serum insulin concentrations were higher in the HYP and ISO trials throughout exercise compared to the placebo (F2,16=13.9; p<0.001; Figure 5.7)

5.3.4 Plasma FFA and glycerol
There were no main effects of the solutions on FFA concentrations between trials during exercise however concentrations of FFA were statistically higher at 45 min in PLA compared to ISO and also at fatigue in PLA compared to HYP (F12,96=3.6; p<0.001; Figure 5.8). Similarly there were no main effects of treatment observed for plasma glycerol concentrations. There was a main effect of time with glycerol concentrations increasing with prolonged exercise (F3,48=22.2; p<0.001; Figure 5.9).

5.3.5 Serum cortisol
There were no differences in serum cortisol concentrations between trials throughout the LIST (Figure 5.10).
5.3.6 Blood lactate, heart rate and rate of perceived exertion (RPE)
Blood lactate concentrations increased significantly with the onset of exercise although there were no observable differences between treatments (Figure 5.11). There were no differences in heart rates between trials (Figure 5.12). Ratings of perceived exertion were not different between trials, but increased with exercise duration. \( F_{4,44}=31.9; p<0.001 \) (Figure 5.13).

5.3.7 Changes in plasma volume and body mass
Environmental conditions were similar during the HYP, ISO and PLA trials. There were no differences in changes to plasma volume or body mass reduction between trials.

5.3.8 Habitual dietary intake
Analysis of subjects' weighed dietary intake diaries (Comp-Eat 5), revealed that there were no differences between trials for subjects' habitual consumption of CHO, fat, protein or total energy intake over the 48h recording period (Table 5.1).

<table>
<thead>
<tr>
<th>Daily Intake</th>
<th>CHO (g·kg(^{-1})BM)</th>
<th>Fat (g·kg(^{-1})BM)</th>
<th>Protein (g·kg(^{-1})BM)</th>
<th>Total Energy (MJ)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ISO</td>
<td>3.9 ± 0.3</td>
<td>1.3 ± 0.2</td>
<td>1.4 ± 0.2</td>
<td>10.3 ± 0.8</td>
</tr>
<tr>
<td>HYP</td>
<td>4.2 ± 0.3</td>
<td>1.2 ± 0.1</td>
<td>1.2 ± 0.1</td>
<td>10.3 ± 0.6</td>
</tr>
<tr>
<td>PLA</td>
<td>4.1 ± 0.4</td>
<td>1.3 ± 0.1</td>
<td>1.2 ± 0.1</td>
<td>10.5 ± 0.5</td>
</tr>
</tbody>
</table>

Table 5.1 Habitual dietary intake of subjects in two days prior to main trials. Values are Mean ± SEM
Figure 5.2  Running capacity (min) in Isotonic (ISO), Hypotonic (HYP) and Placebo (PLA) trials following 75min of LIST.

Figure 5.3  Mean 15m sprint times (s) for each LIST block in Isotonic (ISO), Hypotonic (HYP) and Placebo (PLA) trials during 75min of LIST.
Figure 5.4 Exercise intensity (%) in Isotonic (ISO), Hypotonic (HYP) and Placebo (PLA) trials at rest and during 75min of LIST.

Figure 5.5 Respiratory Exchange Ratio in Isotonic (ISO), Hypotonic (HYP) and Placebo (PLA) trials at rest and during 75min of LIST.
Figure 5.6  Plasma glucose concentrations (mmol·l⁻¹) in Isotonic (ISO), Hypotonic (HYP) and Placebo (PLA) trials during LIST. Significant effect of treatment: ISO and HYP vs. PLA (p<0.05)

Figure 5.7  Serum insulin concentrations (µIU·ml⁻¹) in Isotonic (ISO), Hypotonic (HYP) and Placebo (PLA) trials during LIST. Significant effect of treatment: ISO and HYP vs. PLA (p<0.05)
Figure 5.8  Plasma FFA concentrations (mmol·l⁻¹) in Isotonic (ISO), Hypotonic (HYP) and Placebo (PLA) trials during LIST.
*p<0.05, ISO vs. PLA at 45 min
§p<0.05, HYP vs. PLA at fatigue

Figure 5.9  Plasma glycerol concentrations (mmol·l⁻¹) in Isotonic (ISO), Hypotonic (HYP) and Placebo (PLA) trials during LIST.
Figure 5.10  Serum cortisol concentrations (nmol·l⁻¹) in Isotonic (ISO), Hypotonic (HYP) and Placebo (PLA) trials during LIST.

Figure 5.11  Plasma lactate concentrations (mmol·l⁻¹) in Isotonic (ISO), Hypotonic (HYP) and Placebo (PLA) trials during LIST.
Figure 5.12  Heart rate (beats.min$^{-1}$) in Isotonic (ISO), Hypotonic (HYP) and Placebo (PLA) trials during LIST.

Figure 5.13  Rate of Perceived Exertion in Isotonic (ISO), Hypotonic (HYP) and Placebo (PLA) trials during LIST.
5.4 Discussion

The main finding of the present study was that there were no benefits in intermittent running capacity following the ingestion of either hypotonic or isotonic 6.4% CHO-E solutions compared to a taste-matched placebo. Performance, as measured by run time to exhaustion and sprint times, was not affected by either treatment. There was a tendency (p=0.07) for there to be a trial order effect with subjects running for longer duration in trials 2 and 3 suggesting an acute training effect from trial 1 (Trial 1: 4.3 min ± 0.8; Trial 2: 7.2 min ± 1.8; Trial 3: 6.6 min ± 1.4). Furthermore the only metabolic parameters influenced by CHO ingestion were elevated plasma glucose and serum insulin responses in both the HYP and ISO trials above those in the PLA trial. There were similar metabolic responses between the two CHO treatments and there were no differences in carbohydrate and fluid delivery as evidenced by the similarities in plasma volumes throughout exercise and also in plasma glucose and serum insulin responses. Therefore it may be presumed that the ergogenic potential of 6.4% hypotonic CHO-E solutions is similar to that of isoenergetic isotonic CHO-E solutions.

The results of the present study in addition to that conducted previously (Chapter 4) suggest that hypotonic 6.4% CHO-E solutions do not enhance running capacity in prolonged intermittent exercise (LIST). Moreover data from the current study suggests that the ergogenic properties of such hypotonic solutions are similar to those of an isoenergetic isotonic solution, which in itself did not enhance LIST capacity. This is once again in contrast to findings from previous similar investigations (Nicholas et al, 1995; Davis et al 1999; Davis et al, 2000; Welsh et al, 2002). Attributable differences between the findings of those studies and the previous study (Chapter 4) could have been due to the osmolality of the fluid utilised, however in a direct comparison study it would appear that regardless of osmolality there are no apparent benefits of a 6.4% solution on LIST performance.

One could argue that there was an increased volume of solution ingested in the present study and this could have in some manner compromised the efficacy of the solutions. However, as mentioned in the review of literature, previous studies have stated that gastric volume is the greatest factor affecting gastric emptying and that a
large pre-exercise bolus (approximately 600ml) should be encouraged followed by repeated serial feedings to maintain an elevated rate of gastric emptying. The increased volume of solution ingested in the present study is towards the optimal volume recommended in the literature (see Leiper, 2001c) and should have enhanced fluid and solute delivery.

Furthermore by increasing the volume of ingestate we were able to maximise the potential rate of CHO oxidation in both the ISO and HYP trial. It is suggested that in order to reach optimal rates of exogenous CHO oxidation (~1g·min⁻¹) it is necessary to ingest CHO at a rate of 1-1.5g·min⁻¹ (Jeukendrup and Jentjens, 2000). Our feeding schedule allowed this in comparison to a rate of about 0.8g·min⁻¹ in earlier studies (Nicholas et al., 1995; Davis et al., 1999). Subjective data from participants did not reveal any instances of gastrointestinal distress from these increased volumes in the HYP and ISO trials and therefore we would not have expected running capacity to have been compromised. Interestingly one subject reported feelings of nausea during the PLA trial and did in fact vomit causing him to cease the trial and repeat on a subsequent occasion.

The hormonal and substrate data seem to suggest that there was a large concentration of glucose available to the exercising muscles. Such elevated plasma glucose and serum insulin concentrations may facilitate the oxidation or storage of exogenous CHO by the muscles thus attenuating the depletion of muscle glycogen (Nicholas et al., 1999). It may be speculated that the supply of CHO was sufficient to promote glycogenesis, particularly during the periods of low-intensity exercise (Kuipers et al., 1987). Since both plasma glucose and serum insulin concentrations were elevated at the point of fatigue in both HYP and ISO it would suggest that inadequate CHO supply was not the cause of fatigue. Furthermore hepatic glucose release in the PLA trial was sufficient to prevent hypoglycaemia in this trial. Since subjects were fasted a possible explanation for this constant hepatic glucose output could be enhanced gluconeogenesis. There are potential gluconeogenic precursors, in the form of lactate, being made available from the high-intensity activities such as the sprints and cruises.
The habitual dietary CHO intake of subjects was approximately 4g·kg⁻¹·BM·day⁻¹. This is lower than that reported previously (Chapter 4) but higher than that expected for sedentary individuals and may provide further evidence that the subjects volunteering for experimental trials are more aware of the nutritional practices recommended for persons preparing to perform prolonged exercise. However the lower CHO intake of the subjects in the present study in addition to their lower VO₂max values compared to the subjects from the previous investigation (51 ml·kg⁻¹·min⁻¹ vs 58 ml·kg⁻¹·min⁻¹) may be a reflection of their status as recreational games players compared to the competitive games players recruited in the earlier investigation.

It could be that high endogenous glycogen concentrations could be sufficient to fuel intermittent exercise for approximately 85 min, as was seen in all three trials, and that the provision of exogenous CHO did not confer any additional benefits. A possible explanation of this is that the exogenous CHO was of a sufficient amount to suppress FFA mobilisation in both the HYP and ISO trials. This is illustrated by the trend for FFA to be higher in PLA trial post 45 min compared to the two CHO trials. Although plasma FFA concentrations were higher in PLA late in exercise this only reached significance at 45 min when compared to the ISO trial and at fatigue when compared to the HYP trial. It may be that the shift in substrate oxidation away from fat in the HYP and ISO trials was not matched by a sufficient delivery of CHO to fuel the elevated reliance on CHO as the substrate of choice. However this is merely speculation as the elevated concentration of mobilised FFA in the PLA trial merely infers that there may have been an increase in the contribution of this substrate to total substrate oxidation. There were no significant differences between trials for RER thus suggesting that there were similar contributions to substrate metabolism from both lipid and CHO for all three trials. This would appear to confirm that there was sufficient CHO available to contribute to energy expenditure in all trials thus furthering the argument that endogenous CHO stores may have served to mask any potential benefits of the exogenous CHO.

Aside from the subject who experienced gastrointestinal distress during the placebo trial necessitating him to repeat the trial, it should be noted that all subjects, in all trials were able to complete the 75 min LIST protocol that comprised Part A of the trial. Furthermore all subjects in all trials fatigued within 10 min of the higher
intensity Part B of the experimental trial. Nicholas et al., (1999) reported muscle glycogen concentrations of approximately 180mmol glucosyl units kg⁻¹DM following 90 min of the LIST, which all of their subjects were able to complete. As the subjects in the present study had only run for 75 min, and are presumed to have higher habitual CHO intakes it would be pertinent to assume that they had muscle glycogen concentrations that were at least similar to those reported by Nicholas and colleagues, if not slightly elevated prior to Part B of the run. Since fatigue occurred in these subjects within 10 min of beginning the higher intensity Part B of the protocol it could be argued that this fatigue was not caused by depletion of muscle glycogen as posited by earlier authors. Jacobs et al., (1982) report a potential critical threshold for muscle glycogen, below which fatigue occurs. As fatigue in this protocol does not appear to relate to either muscle glycogen concentrations or low blood glucose levels and since Part B is not consistent with the running protocol utilised in intermittent activity it may be more pertinent to examine a different protocol when investigating the efficacy of CHO-E solutions to prolong exercise capacity.
CHAPTER 6

INFLUENCE OF INGESTING A HYPOTONIC CARBOHYDRATE-ELECTROLYTE SOLUTION ON ENDURANCE CAPACITY DURING PROLONGED HIGH-INTENSITY INTERMITTENT EXERCISE

6.1 Introduction
Earlier studies using the LIST suggest that participants in sports such as soccer, hockey and rugby may benefit from the ingestion of isotonic CHO-E solutions during exercise. However evidence from more recent studies, as presented in Chapters 4 and 5, do not concur with these findings. This may be in part due to the habitual dietary practices of the subjects but also may be the nature of the experimental design used in these investigations. The exercise protocols utilised in these studies were such that they comprised 75 min of intermittent variable-intensity shuttle running followed by an exhaustive high-intensity shuttle run contrived to elicit fatigue within 15 min. As fatigue occurred in all studies within 11 min of the commencement of the higher intensity Part B of the protocol (Nicholas et al., 1995; Davis et al, 1999; 2000) it may well be that the failure to maintain the desired exercise intensity was not as a consequence of substrate availability, either in the form of blood-borne substrate or muscle glycogen, as discussed previously. If fatigue in these studies was not due to provision of substrate then nutritional intervention, in the form of a CHO-E solution, whether isotonic or hypotonic, would not be expected to prolong exercise duration.

The type of running protocol used in these studies has relevance for the state of soccer players' physical reserves during the final moments of a match; however the physiological and performance data from which the LIST was devised was based on match analysis data from 90 min soccer matches (Nicholas et al., 2000). In studies from within our laboratory subjects are able to complete the 90 min LIST running protocol even without nutritional intervention (Nicholas et al., 1999; McGregor et al., 1999), and with deliberately lowered pre-exercise muscle glycogen concentrations (Ali et al., 2002), whereas in the studies cited above fatigue occurred before 90 min had passed. Furthermore, muscle biopsy data suggest that following 90 min of LIST subjects still had muscle glycogen concentrations (~180mmol glucosyl units·kg⁻¹DM)
that would allow for continued high intensity exercise (Nicholas et al., 1999). Data from exhaustive treadmill running at slightly lower overall exercise intensities (70% VO2max) found that subjects ran for 104-132 min and fatigued at muscle glycogen concentrations of 50-60mmol glucosyl units·kg⁻¹·DM (Tsintzas et al., 1996). The intermittent nature of the LIST protocol compared to continuous treadmill running may explain the differences between muscle glycogen concentrations at the point of fatigue. However it may be that the higher intensity Part B of the study elicits fatigue irrespective of endogenous glycogen concentrations or exogenous provision and that it may be more pertinent to maintain the simulated soccer activity rather than adopt Part B of the protocol. The studies presented in Chapters 4 and 5, in addition to those of Nicholas et al., (1995) and Davis et al., (1999; 2000), examined the high-intensity exercise capacity of players during the final stages of a match (i.e. post 75 min). A more important investigation may be to examine whether nutritional intervention can allow players to maintain the desired intensity up to and beyond 90 min and into extra-time.

Therefore the aim of the study was to continue with the variable-intensity shuttle running (i.e. the simulated soccer activity), to volitional fatigue rather than modify the running intensities post-75 min as in previous studies and determine whether the administration of a hypotonic CHO-E solution would delay fatigue and allow soccer players to maintain the desired physiological performance up to and beyond 90 min of intermittent exercise.
6.2 Methods

Nine trained male University soccer players (mean (±SEM); age 21.0 years (±0.4); height 179.0 cm (±2.0); body mass 75.7 kg (±2.7); VO$_{2\text{max}}$ 61.9 ml.kg$^{-1}$.min$^{-1}$ (±0.7)) volunteered, with informed consent, to participate in this University Ethical Committee approved study.

Maximal oxygen uptake values were determined by means of a continuous incremental running test (Taylor et al., 1955) on a motorised treadmill (Runrace HC1200, Technogym, Italy). From these values running speeds corresponding to 55% and 95% VO$_{2\text{max}}$ were calculated. We used the treadmill protocol for assessing subjects’ VO$_{2\text{max}}$ rather than the multi-stage shuttle run test that we have used in previous studies (Chapters 4 and 5) because our subjects were no longer naïve to the rationale for the preliminary tests. There was some suggestion from other studies within the laboratory that subjects would not perform maximally during the multi-stage shuttle run test as they realised that this would then influence their exercise intensity during the experimental trials. It was felt that subjects were not able to contain their efforts as easily on the treadmill test and subsequently their results would be a better reflection of the aerobic capacity. Subjects performed the LIST for 30 min to familiarise themselves with the exercise protocol and experimental procedures.

The subjects performed two experimental trials separated by at least 7 days. To eliminate any trial order effect treatments were assigned randomly in a crossover design. On each occasion subjects consumed either a 6.4% hypotonic carbohydrate-electrolyte solution (118 mOsmol.kg$^{-1}$) (CHO) or a taste-matched placebo (PLA) free of carbohydrate. Solutions were administered ‘double-blind’ immediately prior to the trials (5ml·kg$^{-1}$ BM) and at subsequent 15 min intervals (2ml·kg$^{-1}$ BM) until cessation of the exercise.

Subjects refrained from strenuous exercise, caffeine, tobacco and alcohol and monitored their dietary consumption for 48h preceding the first trial, replicating this approach and intake for the second trial. They reported to the laboratory after an overnight fast (≥10h) and voided prior to the measurement of nude body mass. An indwelling cannula was inserted into an antecubital vein and kept patent with infusion
of sterile saline. Subjects stood for 15 min before a resting blood sample was obtained. Resting heart rate was monitored and expired air sampled over a 5min period. All subjects completed a standardised 5 min warm-up comprising jogging, striding, soccer specific movements and stretching. Immediately before commencing the LIST to fatigue the subjects consumed the prescribed solution.

Subjects ran the LIST (see Chapter 3) for 90 min in six blocks of 15 min separated by 3 min rest periods. Post-90 min subjects continued with this pattern of intermittent shuttle running, this time with no rest breaks, until fatigue (Figure 6.1). Fluid was administered during the rest phases for the first 90 min and then at 15 min intervals during the walking phases of the continuous protocol. Fatigue was defined as the inability to maintain the running speed for consecutive shuttles or a decrease in sprint performance to <90% of the mean sprints for blocks 1-3.

Heart rate was monitored every 15s during exercise using short-range telemetry (Polar Electro Sports Testers PE3000, Polar Electro, Kempele, Finland), and the mean was recorded for each 15 min exercise period. Expired air samples were collected during exercise using a Douglas bag (Harvard), attached to a rucksack frame, for one complete cycle (~80s) of each 15 min LIST period. Subjective ratings of perceived exertion were recorded on a 15 point scale (Borg, 1973) during the walk phase of the final cycle of each block.

An 11ml blood sample was taken at rest, after 30, 60 and 90 min of exercise and at fatigue with 4ml dispensed into an EDTA containing tube. The venous blood samples were assayed for lactate, haematocrit and haemoglobin concentrations. The remaining whole blood was centrifuged at 4°C and the plasma divided into aliquots and frozen at -20°C for later analysis of free fatty acids (FFA), glucose and glycerol. Serum was obtained by separating 4ml of coagulated whole blood at 4°C; and subsequently stored at -70°C for subsequent analysis of insulin, prolactin and cortisol. All blood sampling procedures and analyses are described in more detail in Chapter 3. Blood samples for each experimental treatment were analysed in duplicate within the same assay series in order to reduce variability in the biochemical analytical procedures.
Statistical analyses were performed as detailed in Chapter 3. Null hypotheses were rejected at an alpha level of $p<0.05$. All data are reported as mean ($\pm$ SEM). Blood data were unavailable for one subject due to discomfort with the cannulation process, therefore statistics are based on $n=8$ for blood analysis and $n=9$ for other variables.
Figure 6.1  Schematic representation of the experimental protocol.
6.3 Results

6.3.1 Running performance
The run times to fatigue for the CHO and PLA trials were 106.6 min (±5.9) and 103.0 min (±6.8) respectively (n.s; Figure 6.2). Sprint times were similar between trials and increased over time irrespective of treatment (F₁,₂, =12.68; p<0.01; Figure 6.3). The sprint times post-90 min were significantly slower than those during the first hour of exercise (F₁,₂, =12.68; p<0.01). However this decrease in performance was not sufficient to withdraw subjects from the test because their speeds did not fall below 96% of the mean speed of blocks 1-3. There were no trial order effects on running performance or sprint times.

6.3.2 Oxygen uptake
The mean oxygen uptake during the CHO and PLA trials were 46.0ml.kg⁻¹.min⁻¹ (±0.9) and 45.7ml.kg⁻¹.min⁻¹ (±0.8) respectively, representing average relative exercise intensities of 74% VO₂max (±3) for the CHO trial and 74% VO₂max (±3) for the PLA trial (n.s; Figure 6.4). Oxygen uptake decreased throughout exercise in both trials reflecting the decrease in sprint performance over time (F₁,₅, =13.68; p<0.01).

6.3.3 Energy expenditure and respiratory exchange ratio
There were no differences in total energy expenditure or metabolic rate between the CHO and PLA trials. Similarly there were no differences between treatments for the respiratory exchange ratio (RER). There were trends in both the CHO and PLA trials for RER to decrease slightly as exercise duration progressed but this failed to reach statistical significance (Figure 6.5). There were further trends for the rate of carbohydrate oxidation and the total carbohydrate oxidised to be higher in the CHO trial but again this failed to reach statistical significance (Table 6.1).

6.3.4 Plasma glucose and serum insulin
Plasma glucose concentration was maintained within the normal range in both trials although it was significantly higher in the CHO trial throughout exercise (F₁,₇, =8.09; p<0.05; Figure 6.6). There were main effects of time with plasma glucose concentrations higher at 30 and 60 min than at rest (F₁,₆,₁₁,₃, =8.54; p<0.01). Serum insulin values were higher in the CHO trial (F₁,₄, =8.23; p<0.05; Figure 6.7).
6.3.5 Plasma FFA and glycerol

There were no differences in FFA concentrations between trials during exercise. Free fatty acid concentrations increased with exercise irrespective of treatment and were significantly higher post 60 min than they were at 30 min ($F_{1,2} = 16.66; p<0.01$; Figure 6.8). Similarly there were no treatment differences observed for plasma glycerol concentrations. There was a main effect of time with glycerol concentrations increasing with prolonged exercise from a resting concentration of $\sim 35\mu\text{mol.l}^{-1}$ to $\sim 270\mu\text{mol.l}^{-1}$ at fatigue ($F_{1,3} = 46.31; p<0.01$; Figure 6.9).

6.3.6 Serum prolactin and cortisol

There were no differences in serum prolactin values between trials during exercise. They increased with exercise irrespective of treatment and were significantly higher at fatigue than at any other time point ($F_{1,2} = 9.05; p<0.01$; Figure 6.10). Similarly there were no differences in serum cortisol concentrations between trials throughout the LIST. They increased with exercise irrespective of treatment and were significantly elevated post 60 min than during the first 30 min of exercise ($F_{1,3} = 21.10; p<0.01$) and were higher at fatigue than at any other time point ($F_{1,3} = 21.10; p<0.01$; Figure 6.11).

6.3.7 Blood lactate, heart rate and rate of perceived exertion (RPE)

Blood lactate concentrations increased significantly with the onset of exercise from a baseline of $\sim 1\text{mmol.l}^{-1}$ to an exercising plateau of $\sim 5.5\text{mmol.l}^{-1}$ ($F_{1,2} = 27.27; p<0.01$; Figure 6.12) although there were no observable differences between treatments. Heart rates were higher throughout the CHO trial than PLA trial (172bpm ($\pm 3$) vs 166bpm ($\pm 3$); $F_{1,3} = 12.30; p<0.01$; Figure 6.13). There was also a main effect of time with lower heart rates during the first 45 min than post 90 min. ($F_{1,3} = 11.97; p<0.01$), and lowest during the first 15 min compared to all other time points ($F_{1,3} = 11.97; p<0.01$). Although ratings of perceived exertion were not different between trials they increased with exercise duration (Figure 6.14).
6.3.8 Changes in plasma volume and body mass

Environmental conditions were similar during the CHO and PLA trials with dry bulb temperature and humidity approximately 18.5°C and 57% respectively. There were no differences between trials for changes in plasma volume from rest to fatigue (approx. –3.6%) or body mass reduction during each trial.
Figure 6.2  Running capacity (min) for the Carbohydrate (CHO) and the Placebo (PLA) trials during LIST.

Figure 6.3  Mean sprint times (s) for the Carbohydrate (CHO) and the Placebo (PLA) trials during LIST to fatigue. * p<0.05; at 15, 30, 45 and 60 min vs. Post 90 min
Figure 6.4  Exercise intensity (\%VO_{2max}) for the Carbohydrate (CHO) and the Placebo (PLA) trials.

Figure 6.5  Respiratory exchange ratio for the Carbohydrate (CHO) and the Placebo (PLA) trials.
Figure 6.6 Plasma glucose concentrations (mmol·L⁻¹) for the Carbohydrate (CHO) and the Placebo (PLA) trials.
* p<0.05, CHO vs. PLA; § p<0.05, at 30 and 60 min vs. 0 min

Figure 6.7 Serum insulin concentrations (µIU·ml⁻¹) for the Carbohydrate (CHO) and the Placebo (PLA) trials.
Significant effect of treatment: CHO vs. PLA (p<0.05)
Figure 6.8  Plasma FFA concentrations (mmol·l⁻¹) for the Carbohydrate (CHO) and the Placebo (PLA) trials.
*p<0.05; at 60 min, 90 min and fatigue vs. 30 min

Figure 6.9  Plasma glycerol concentrations (mmol·l⁻¹) for the Carbohydrate (CHO) and the Placebo (PLA) trials.
Significant effect of time: p<0.001
Figure 6.10  Serum prolactin concentrations (ng·ml⁻¹) for the Carbohydrate (CHO) and the Placebo (PLA) trials. *p<0.05, at fatigue vs. all other time points

Figure 6.11  Serum cortisol concentrations (nmol·l⁻¹) for the Carbohydrate (CHO) and the Placebo (PLA) trials. * p<0.05, at 60 min, 90 min and fatigue vs. 0 min and 30 min § p<0.05, at fatigue vs. all other time points
Figure 6.12 Blood lactate concentrations (mmol·l⁻¹) for the Carbohydrate (CHO) and the Placebo (PLA) trials.

Figure 6.13 Heart rate (beats·min⁻¹) for the Carbohydrate (CHO) and the Placebo (PLA) trials.
Significant effect of treatment: CHO vs. PLA (p<0.05)
†p<0.05, at 15 min vs. all other time points
§ p<0.05, at 90 min and fatigue vs. pre-45 min
Figure 6.14  Rate of perceived exertion for the Carbohydrate (CHO) and the Placebo (PLA) trials. Significant effect of time: (p<0.001)

Table 6.1  Energy expenditure and substrate utilisation during LIST activity

<table>
<thead>
<tr>
<th></th>
<th>Total Energy Expenditure (MJ)</th>
<th>RER</th>
<th>CHO Oxidation (g.min⁻¹)</th>
<th>Total CHO oxidised (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CHO</td>
<td>7.6 (+0.6)</td>
<td>0.99 (+0.01)</td>
<td>4.0 (+0.2)</td>
<td>426.7 (+32.2)</td>
</tr>
<tr>
<td>PLA</td>
<td>7.4 (+0.7)</td>
<td>0.97 (+0.01)</td>
<td>3.7 (+0.2)</td>
<td>393.4 (+46.8)</td>
</tr>
</tbody>
</table>
6.4 Discussion

The main finding from the present study was that prolonged, intermittent high-intensity running capacity was not influenced by the ingestion of a 6.4% hypotonic carbohydrate-electrolyte solution. Subjects did run for longer (~17 min) following the consumption of the CHO-E beverage compared to a placebo (~13 min). This increase in endurance capacity was of similar magnitude to the range (32-52%) reported by other authors (Nicholas et al., 1995; Davis et al., 1999; 2000), however the improvements in capacity were not shown by all subjects. Of the 9 subjects only 5 performed better in the CHO-E trial, whereas the studies cited above report improved exercise capacity in all of their subjects following CHO-E ingestion.

In these earlier studies the authors did not attribute the increased performance to the elevated blood glucose concentrations, per se, but rather to a resynthesis or sparing of muscle glycogen. Thus it can be argued that for fatigue to be delayed by carbohydrate supplementation then the experimental protocol must be carbohydrate demanding in some, or all, of the exercising muscle fibres. Each of the studies that report improvements in intermittent running capacity following carbohydrate ingestion have utilised protocols that elicit a set period of intermittent activity followed by an increased intensity capacity test (Nicholas et al., 1995; Walton and Rhodes, 1997; Davis et al., 1999; Quanz, 1999; Davis et al., 2000; Ingle et al., 2000). Although 90 min of the LIST has been shown to decrease muscle glycogen in mixed muscle fibres by 21% (Nicholas et al., 1999) it has not been established whether this reduction is sufficient to cause fatigue in the exercising subject. In the present study subjects were able to exceed 90 min, however it should be noted that they were only able to continue with this simulated soccer activity for approximately 15 min.

The carbohydrate content of the subjects' diets in the days preceding the main trials was approximately 5g·kg BM⁻¹. Burke et al., (2000) found no differences between a moderate (6g·kg BM⁻¹) and a high carbohydrate dietary intake (9g·kg BM⁻¹) on performance nor on muscle glycogen utilisation during a 100km cycle time trial, despite higher pre-exercise muscle glycogen concentrations in the high carbohydrate trial. It may have been that the moderate carbohydrate intake in the study of Burke et al., (2000) led to muscle glycogen concentrations sufficient to meet the demands of the time trial. Nicholas and colleagues (1995), who reported a positive finding from
the provision of exogenous carbohydrate during a similar intermittent running protocol, did not report their subjects’ pre-exercise dietary carbohydrate intakes. It is interesting to note however that the resting muscle glycogen concentrations of their participants in a subsequent study (Nicholas et al., 1999) are about 20% lower than those in the moderate carbohydrate intake trial in Burke’s study. Thus it is not unreasonable to suggest that the dietary carbohydrate intake of our subjects may have produced resting muscle and hepatic glycogen concentrations high enough to mask any ergogenic benefits of carbohydrate ingestion during the LIST. Furthermore a recent study re-investigating the optimal protocol for pre-competition carbohydrate loading (Bussau et al., 2002) found that a diet high in carbohydrate (10g·kg BM\(^{-1}\)) led to a ‘super-compensation’ in muscle glycogen concentrations within 24h. The authors reported no further increases in endogenous stores when this diet was maintained for 48h or 72h. These data suggest that the habitual consumption of 10g·kg BM\(^{-1}\) CHO for 24h is sufficient to maximise glycogen stores. It is therefore not unreasonable to suggest that if our subjects consumed >5g·kg BM\(^{-1}\)·day\(^{-1}\) CHO for 48h whilst adhering to an inactive lifestyle for that time period that they may have elevated their muscle glycogen concentrations to a near optimal content.

There was a trend for higher carbohydrate oxidation rates and RER values in the CHO trial suggesting that the ingestion of exogenous carbohydrate led to a suppression of fat metabolism and a shift in substrate oxidation. Unless the exogenous carbohydrate delivery or the endogenous carbohydrate stores were sufficient to compensate for this reduction in fat metabolism then it would be pertinent to suggest that running capacity may not be enhanced. Although the methods for measuring respiratory exchange ratio and for estimating substrate oxidation rates, using indirect calorimetry, are well established in steady-state exercise they are not so in intermittent running. The high-intensity bouts integral to the LIST and the subsequent increased lactate production may confound the assumptions of a stable HCO\(_3\) pool necessary for a reliable estimation of tissue VCO\(_2\) from expired VCO\(_2\). However studies by Christmass et al (1999a; 1999b) report constant lactate concentrations, of similar values to the present study, and stable HCO\(_3\) pools during intermittent running at similar overall exercise intensities to the present study and the authors therefore support the validity of indirect calorimetry for the determination of substrate oxidation rates during sustained repeated intermittent exercise bouts.
Early data from cycling studies found that those subjects who exhibited marked decreases in blood glucose concentration during prolonged exercise benefited most from carbohydrate ingestion (Coyle et al., 1983; Bjorkman et al., 1984). Although plasma glucose concentrations were significantly lower in the PLA trial in the present study they did not reach hypoglycaemic values even at fatigue. This finding is similar to previous data reported in this thesis and also data reported by other authors utilising a similar intermittent exercise protocol (Nicholas et al., 1995; 1999; Davis et al., 1999; 2000). There was a trend for higher plasma FFA and glycerol concentrations in the PLA trial however these failed to reach statistical significance. These data further support the argument that an inadequate provision of substrate to the working muscles was not necessarily the causative factor for fatigue.

It has been postulated that central fatigue may be an important limiting factor in exhaustive exercise (Davis and Bailey, 1997). This mechanism has not been investigated in the previous two studies (Chapters 4 and 5), although it was indirectly measured by Davis and colleagues (1999) by including a third trial to their design where they supplemented with BCAA in addition to CHO. These authors found no difference in capacity between the CHO trial and the CHO+BCAA trial although they did not report any data from markers of central fatigue. In the present study we determined the changes in serum prolactin as a surrogate measure of serotonergic activity. No differences were noted thus suggesting that serotonin concentrations may not have differed between trials. Furthermore subjective ratings of perceived exertion were similar between trials, which although contrasting with suggestions that carbohydrate ingestion mediates the intensity of emotional perceptions (Utter et al., 1997) appears to suggest that central fatigue was not a limiting factor in this exercise mode.

A further mechanism often responsible for fatigue in high intensity activity is metabolic acidosis brought about by increases in glycogenolysis. Although there were marked rises in plasma lactate with the onset of exercise there were no differences between trials and values levelled off at ~5mmol.l⁻¹, similar to other trials from our laboratory (Nicholas et al., 1995; Nicholas et al., 1999).
It appears that the physiological demands of intermittent activity employed in the present study, although glycogen reducing (Nicholas et al., 1999), are not sufficient to elicit fatigue during normal match play (ie 90 min). Since fatigue occurred at similar time points regardless of the type of fluid ingested it would suggest that glycogen unavailability was not the causative factor. Although the mechanisms responsible for fatigue are unclear it would appear that exercise capacity in such activities might not be enhanced by the ingestion of a 6.4% hypotonic carbohydrate-electrolyte solution, especially when players have moderate pre-exercise muscle glycogen concentrations possibly due to the provision of carbohydrate being insufficient to compensate for a shift in substrate metabolism.
CHAPTER 7

INFLUENCE OF A HYPOTONIC CHO-E SOLUTION DURING HIGH INTENSITY SHUTTLE RUNNING ON TRAINED SUBJECTS WITH HIGH PRE-EXERCISE MUSCLE GLYCOGEN CONCENTRATIONS

7.1 Introduction

The main mechanism postulated for improving exercise capacity during prolonged running, both intermittent and continuous, following the ingestion of CHO-E solutions has been through glycogen sparing (Tsintzas et al., 1995; Tsintzas and Williams, 1998; Nicholas et al., 1999). Although some intermittent running studies have reported enhanced running capacity following CHO ingestion (Nicholas et al., 1995; Davis et al., 1999; 2000; Welsh et al., 2002) and attribute the ergogenic benefits to glycogen sparing or an increased glycogenesis none of these studies have actually measured muscle glycogen concentrations. The one study that reported a sparing of muscle glycogen during intermittent running activity was not a capacity test but a set 90 min intermittent run (Nicholas et al., 1999). The authors reported a 22% lower muscle glycogen utilisation following ingestion of a 6.9% CHO-E solution compared to a placebo, however they did not examine whether this lower glycogen utilisation would have led to an increased exercise capacity.

Nicholas et al., (1999) ran their subjects intermittently for 90 min and even in the PLA trial all subjects completed the test. Conversely, in the studies that reported increased running capacities subjects ran at a higher intensity post 75 min and all reported fatigue occurring before 90 min. Furthermore although there were statistical differences in the amount of muscle glycogen utilised there were no differences in the actual muscle glycogen concentrations at 90 min. At the end of 90 min subjects in both trials had muscle glycogen concentrations of ~180mmol glucosyl units·kg⁻¹ DM, Thus suggesting that if there was a critical threshold of muscle glycogen concentration below which running capacity was impaired (Jacobs et al., 1982) then one would have expected subjects to have been able to run for additional minutes in both trials.

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Since the ability to maintain high intensity exercise for prolonged durations is dependent upon the ability to sustain a high rate of CHO oxidation, which in itself is dependent upon endogenous CHO stores and the provision of exogenous CHO, many authors have investigated the effect of dietary manipulation on exercise capacity. A diet high in CHO in the days preceding the main trials will lead to an increase in endogenous CHO stores both in the liver and the muscle. Pre-exercise diets are not reported in any of the studies on intermittent running capacity however in the previous investigations it has been noted that subjects were ingesting on average slightly above 5g·kg⁻¹·BM·day⁻¹ CHO in the days preceding the trials. Although this is not as high as that recommended for endurance athletes (8-10g·kg⁻¹·BM·day⁻¹) it is higher than what one would expect from a sedentary individual (3g·kg⁻¹·BM·day⁻¹). Furthermore recent evidence suggests that 10g·kg⁻¹·BM·day⁻¹ CHO for 24h is sufficient to 'supercompensate' muscle glycogen stores (Bussau et al, 2000). Therefore if subjects were inactive for the two day period prior to testing, and ate 5g·kg⁻¹·BM·day⁻¹ CHO it is quite possible that they would have exhibited high endogenous glycogen stores. This above average dietary CHO intake could have masked any ergogenic benefits of exogenous CHO ingestion.

Thus the purpose of the present study is to examine the effect of exogenous CHO ingestion on muscle glycogen concentrations and intermittent running capacity on subjects with high pre-exercise muscle glycogen concentrations.
7.2 Methods

Six recreationally active males (age 21.0 years (±0.4); body mass 75.0 kg (±1.2); VO₂ max 60.0 ml·kg⁻¹·min⁻¹ (±0.7)) volunteered, with informed consent, to participate in this University Ethical Committee approved study.

Maximal oxygen uptake values were estimated from performance in the Multistage Fitness Test. From these values running speeds corresponding to 55% and 95% VO₂ max were calculated. Subjects performed the LIST for 45 min to familiarise themselves with the exercise protocol and experimental procedures.

The subjects performed two experimental trials separated by 14 days. To eliminate any trial order effect treatments were assigned randomly in a crossover design. On each occasion subjects consumed either a 6.4% hypotonic carbohydrate-electrolyte solution (CHO; GlaxoSmithKline, UK) or a taste-matched placebo (PLA) free of carbohydrate. Solutions were administered in a double-blind fashion immediately prior to the trials (8ml·kg⁻¹ BM) and at subsequent 15 min intervals (3ml·kg⁻¹ BM) until cessation of the exercise.

Subjects performed a glycogen reducing trial comprising 90 min of LIST activity 48h prior to the experimental trials and refrained from strenuous exercise, caffeine, tobacco and alcohol during the recovery period. Following the glycogen reducing trial subjects were administered a carbohydrate rich (~10g·kg⁻¹BM·day⁻¹) test diet for 48h (Appendix H). They reported to the laboratory for the experimental trial following an overnight fast (≥10h) and voided prior to the measurement of nude body mass. A urine sample was collected pre-exercise and at fatigue and analysed for osmolality using a cryoscopic osmometer (Osmomat 030). An indwelling cannula (Venflon, 16-18G, Ohmeda, Hatfield, Herts, UK) was inserted into an antecubital vein and kept patent with infusion of sterile saline. A resting muscle sample was then obtained from the m. vastus lateralis by needle biopsy. Each biopsy sample was taken from the central portion of the m. vastus lateralis muscle midway between the hip and the knee. Subjects stood for 15 min before a resting blood sample was obtained. Resting heart rate was monitored over a 5 min period. Ten minutes before commencing the LIST, the subjects consumed the prescribed solution and stretched and warmed up.
On completion of the 90 min of shuttle running a second muscle sample was obtained after the subject had been transferred to an examination couch in the laboratory adjacent to the sports hall. Following this sample subjects ran to volitional fatigue, or until they were withdrawn by the experimenters, at which point a final muscle sample was obtained.

Subjects ran the LIST (see Chapter 3) for 90 min in six blocks of 15 min separated by 3 min rest periods. Post-90 min subjects continued with this pattern of intermittent shuttle running, this time with no rest breaks, until fatigue (Figure 7.1). Fluid was administered during the rest phases for the first 90 min and then at 15 min intervals during the walking phases of the continuous protocol. Fatigue was defined as the inability to maintain the running speed for consecutive shuttles or a decrease in sprint performance to <95% of the mean sprints for blocks 1-3.

Heart rate was monitored every 15s during exercise using short-range telemetry (Polar Model 810, Kempele, Finland), and the mean was recorded for each 15 min exercise period and the run to fatigue. Subjective ratings of perceived exertion were recorded on a 15 point scale (Borg, 1973) during the walk phase of the final cycle of each block.

An 11ml blood sample was taken at rest, after 30, 60 and 90 min of exercise and at fatigue with 4ml dispensed into an EDTA containing tube. The venous blood samples were assayed for lactate, haematocrit and haemoglobin concentrations. The remaining whole blood was centrifuged at 4°C and the plasma divided into aliquots and frozen at -20°C for later analysis of free fatty acids (FFA), glucose and glycerol. Serum was obtained by separating 4ml of coagulated whole blood at 4°C; and subsequently stored at -70°C for subsequent analysis of insulin and cortisol. All blood sampling procedures and analyses are described in more detail in Chapter 3. Blood samples for each experimental treatment were analysed in duplicate within the same assay series in order to reduce variability in the biochemical analytical procedures.

Muscle samples were obtained through separate incisions from the lateral portion of the vastus lateralis muscle using the needle biopsy procedure (Bergstrom, 1962) with
suction being applied. All incisions were made through the skin and muscle fascia under local anaesthetic (2-3mL of 1% Lignocaine) before the start of exercise while the subjects were lying supine on an examination couch. All muscle samples were snap-frozen in liquid nitrogen; freeze dried and stored at -85°C. The methodology for sample collection procedures and analyses are detailed in Chapter 3.

Statistical analyses were performed as detailed in Chapter 3. Furthermore a Shapiro-Wilks test for normality was utilised to test whether differences in data were normally distributed, if this was found to be significant (as was the case for exercise capacity) then a non-parametric Wilcoxon Signed ranks test was utilised instead of the Student's t-test. Null hypotheses were rejected at an alpha level of p<0.05. All data are reported as mean ± standard error of the mean (SEM).
Figure 7.1 Schematic representation of experimental protocol
7.3 Results

7.3.1 Running performance
Running capacity was increased in the CHO trial compared to the PLA trial (Figure 7.2). Subjects ran for 158.0 min ± 11.6 on the CHO trial compared to 131.0 min ± 8.0 on the PLA trial, an approximate 21% longer total running duration. Furthermore all of the six subjects ran for longer on the CHO trial. Sprint times were similar between trials and decreased over time irrespective of treatment ($F_{6,30}=4.1; p=0.004$; Figure 7.3).

7.3.2 Muscle metabolites
There were no differences in mixed muscle glycogen concentrations between trials (Figure 7.4). There was a similar rate of mixed muscle glycogen utilisation between trials from pre-exercise to 90 min (~2mmol glucosyl units·kg⁻¹DM) but there was a trend ($p=0.1$) for mixed muscle glycogen utilisation to be higher from 90 min to fatigue in the PLA trial (4.2 ± 1.2 mmol glucosyl units·kg⁻¹DM) compared to the CHO trial (2.5 ± 0.3 mmol glucosyl units·kg⁻¹DM; Figure 7.5). Interestingly fatigue occurred at similar mixed muscle glycogen concentrations in both trials (~200 mmol glucosyl units·kg⁻¹DM). There were no differences between trials for muscle concentrations of phosphocreatine, ATP, G6P, lactate and creatine (Table 7.1). There were no differences in the delay in scheduled biopsy sampling time-point and actual sampling time-point at either 90 min (CHO 41s ± 2 vs PLA 36s ± 3) or Fatigue (CHO 54s ± 7 vs PLA 51s ± 4).

7.3.3 Plasma glucose and serum insulin
Plasma glucose concentration was maintained within the normal range in both trials although it was significantly elevated at fatigue in the CHO trial compared to the PLA trial ($F_{4,20}=9.1; p<0.001$; Figure 7.6). Plasma glucose concentrations also appeared higher in the CHO at 30 min although this failed to reach significance. Serum insulin concentrations were significantly higher throughout exercise in CHO compared to PLA ($F_{1,5}=29.1; p=0.003$; Figure 7.7)
7.3.4 Plasma FFA and glycerol

There were main effects of the solutions on FFA concentrations between trials during exercise ($F_{1,5}=10.3$; $p=0.024$), there was also a main effect of time with concentrations increasing with exercise duration ($F_{1,2,6,1}=26.1$; $p<0.001$; Figure 7.8). Similarly there were main effects of treatment observed for plasma glycerol concentrations ($F_{1,5}=13.8$; $p=0.014$). There was also a main effect of time with glycerol concentrations increasing with prolonged exercise ($F_{1,6,7,8}=35.1$; $p<0.001$; Figure 7.9).

7.3.5 Serum cortisol

There was a main effect of time in serum cortisol concentrations with values being higher in both trials at fatigue than at 30 min, 60 min and 90 min of exercise ($F_{1,4,7}=12.5$; $p=0.007$; Figure 7.10)

7.3.6 Blood lactate, heart rate and ratings of perceived exertion (RPE)

Blood lactate concentrations increased significantly with the onset of exercise although there were no observable differences between treatments ($F_{4,20}=40.5$; $p<0.001$; Figure 7.11). There were no differences in heart rates between trials ($F_{7,35}=485$; $p<0.001$; Figure 7.12), similarly ratings of perceived exertion were not different between trials, but they did increase with exercise duration ($F_{5,25}=10.8$; $p<0.001$; Figure 7.13).

7.3.7 Changes in urine osmolality, plasma volume and body mass

Environmental conditions were similar during both trials. There were no differences in urine osmolality pre- and post trials in either condition. Subjects presented at the laboratory with urine osmolalities averaging within the range 500-600 mOsm·kg$^{-1}$ and these were maintained by the end of the main trials. Furthermore there were no body mass losses within or between trials suggesting fluid homeostasis regardless of solution ingested. However there were statistical differences in changes to plasma volume with the CHO trial allowing better maintenance of pre-exercise volumes (Figure 7.14).
Figure 7.2  Running capacity (min) for the Carbohydrate (CHO) and the Placebo (PLA) trials following 90min of LIST.
* p<0.05, CHO vs. PLA

Figure 7.3  Mean sprint times (s) for the Carbohydrate (CHO) and the Placebo (PLA) trials during LIST to fatigue.
Figure 7.4 Muscle glycogen concentrations (mmol·kg⁻¹DM) for the Carbohydrate (CHO) and the Placebo (PLA) trials.

Figure 7.5 Muscle glycogen utilisation (mmol·kg⁻¹ DM ·min⁻¹) for the Carbohydrate (CHO) and the Placebo (PLA) trials.
Table 7.1 Mixed muscle metabolite concentrations pre-exercise, at 90min and at fatigue. Values are mean ± SE

<table>
<thead>
<tr>
<th></th>
<th>Pre-exercise (mmol·kg⁻¹ DM)</th>
<th>90min (mmol·kg⁻¹ DM)</th>
<th>Fatigue (mmol·kg⁻¹ DM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CHO</td>
<td>PLA</td>
<td>CHO</td>
</tr>
<tr>
<td>ATP</td>
<td>28 ± 4</td>
<td>23 ± 6</td>
<td>24 ± 6</td>
</tr>
<tr>
<td>PCr</td>
<td>89 ± 5</td>
<td>89 ± 4</td>
<td>82 ± 2</td>
</tr>
<tr>
<td>Creatine</td>
<td>35 ± 7</td>
<td>35 ± 6</td>
<td>42 ± 2</td>
</tr>
<tr>
<td>Glucose</td>
<td>3.9 ± 1.1</td>
<td>3.7 ± 1.1</td>
<td>2.6 ± 0.5</td>
</tr>
<tr>
<td>G6P</td>
<td>3.8 ± 1.6</td>
<td>3.0 ± 0.9</td>
<td>4.1 ± 0.8</td>
</tr>
<tr>
<td>Lactate</td>
<td>8.3 ± 1.4</td>
<td>5.2 ± 1.4</td>
<td>11.6 ± 2.9</td>
</tr>
</tbody>
</table>

Figure 7.6 Plasma glucose concentrations (mmol·l⁻¹) for the Carbohydrate (CHO) and the Placebo (PLA) trials. * p<0.001, CHO vs. PLA at fatigue
Figure 7.7 Serum insulin concentrations (\( \mu \text{IU.mL}^{-1} \)) for the Carbohydrate (CHO) and the Placebo (PLA) trials.
* \( p<0.05 \), CHO vs. PLA

Figure 7.8 Plasma FFA concentrations (mmol.L\(^{-1}\)) for the Carbohydrate (CHO) and the Placebo (PLA) trials.
* \( p<0.05 \), CHO vs. PLA
Figure 7.9 Plasma glycerol concentrations (mmol·l⁻¹) for the Carbohydrate (CHO) and the Placebo (PLA) trials. * p<0.05, CHO vs. PLA

Figure 7.10 Serum cortisol concentrations (nmol·l⁻¹) for the Carbohydrate (CHO) and the Placebo (PLA) trials. * p<0.05, at 30, 60 and 90 min vs. fatigue
Figure 7.11 Blood lactate concentrations (mmol·L⁻¹) for the Carbohydrate (CHO) and the Placebo (PLA) trials.

Figure 7.12 Heart rate (beats·min⁻¹) for the Carbohydrate (CHO) and the Placebo (PLA) trials.
Figure 7.13  Rate of perceived exertion for the Carbohydrate (CHO) and the Placebo (PLA) trials.

Figure 7.14  Changes to plasma volume for the Carbohydrate (CHO) and the Placebo (PLA) trials.
* p<0.05; CHO vs. PLA
7.4 Discussion

The main finding from this study is that the combination of a high CHO recovery diet (10g·kg⁻¹·BM·day⁻¹ CHO) with exogenous CHO supplementation (90g·h⁻¹) in the form of a 6.4% hypotonic CHO-E solution led to an improvement in intermittent shuttle running (LIST) capacity. Subjects were able to run for approximately 21% longer in the CHO trial (158.0 min ± 11.6) compared to the PLA trial (131.0 min ± 8.0). When calculated as improvements to capacity in Part B of the protocol as earlier authors have done (Nicholas et al., 1995; Davis et al., 1999; 2000) this amounts to a 66% improved exercise capacity. Moreover this enhanced endurance capacity was displayed in all six subjects. This finding contradicts the initial hypothesis where it was postulated that high resting muscle glycogen concentrations could serve to mask any ergogenic benefits of CHO supplementation during exercise.

The glycogen loading protocol utilised in the present study appears to have been effective. Following the glycogen depleting protocol and the 48h high CHO recovery diet subjects exhibited similar high muscle glycogen concentrations pre-exercise in both the CHO (533 mmol glucosyl units·kg⁻¹·DM ± 31) and PLA (512 mmol glucosyl units·kg⁻¹·DM ± 42) trials. These values are higher than those reported by Nicholas et al., (1999) (350-400 mmol glucosyl units·kg⁻¹·DM) who reported glycogen sparing after completing 90 min of the LIST. The elevated muscle glycogen concentrations in the present study may serve to explain the different results between these two studies as they followed similar protocols up to 90 min.

Nicholas et al., (1999) report a greater net muscle glycogen utilisation following 90 min of LIST in the control trial compared to the CHO trial. This apparent glycogen sparing was then used to explain the mechanism for their earlier findings (Nicholas et al., 1995) that exogenous CHO led to increased exercise capacity during LIST. This data was also used as evidence to explain the similar findings of Davis et al., (1999), Davis et al., (2000) and Welsh et al., (2002) although these studies failed to measure muscle glycogen concentrations. Nicholas et al., (1999) report no differences between muscle glycogen concentrations pre-exercise but there does appear to be a higher glycogen content in the control trial and this in itself could explain why there was a greater utilisation in this trial as initial muscle glycogen concentrations have been shown to effect muscle glycogen utilisation (Rico-Sanz et al., 1999). Studies that have
manipulated pre-trial exercise and pre-trial diet (Gollnick et al., 1972; Hargreaves et al., 1995) have shown that higher initial muscle glycogen concentrations lead to greater net muscle glycogen utilisation. Similar findings were reported from investigations comparing exercised limbs with their contra-lateral rested limbs (Blomstrand and Saltin, 1999). Therefore the data of Nicholas and colleagues may not actually reflect muscle glycogen sparing per se but rather a higher glycogen utilisation in their control trial due to higher initial glycogen concentrations.

The data from the current study do not support the concept of glycogen sparing occurring during 90 min of the LIST. There were no differences in muscle glycogen concentrations between trials at 90 min nor were there any differences in muscle glycogen utilisation for the first 90 min (~2 mmol glucosyl units·kg⁻¹·min⁻¹·DM). The data from the present study are similar to those of Chyssanthopolous et al., (2002) who found no differences in muscle glycogen utilisation between a CHO solution and water during the first 60 min of treadmill running at 70% VO₂ max when subjects had eaten a high CHO breakfast 3h prior to exercise. What is interesting to note is that in an earlier study with a similar feeding protocol, but this time with exercise to exhaustion, the same authors (Chryssanthopolous et al., 1994) reported an enhanced exercise capacity in the trial where subjects were fed a CHO solution in addition to the breakfast. Although the nature of the running protocol of Chryssanthopolous and colleagues’ studies differs from the present study it is evident that the pre-exercise dietary practice is affecting the timing of any apparent glycogen sparing.

What is apparent in the present study is that beyond 90 min, as subjects ran to exhaustion, there was a trend for higher net muscle glycogen utilisation in the PLA trial (~4 mmol glucosyl units·kg⁻¹·min⁻¹·DM) compared to the CHO trial (~2.5 mmol glucosyl units·kg⁻¹·min⁻¹·DM). This suggests that there is either a greater reliance on the exogenous CHO late in exercise allowing for the prolonged exercise duration or that the exogenous CHO allowed for a greater muscle glycogen resynthesis later in exercise which reduced the net muscle glycogen utilisation. As fatigue occurred at similar muscle glycogen concentrations it would appear that whichever mechanism is responsible for delaying the utilisation of muscle glycogen to this critical threshold contributes to the increased exercise capacity.
Interestingly fatigue occurred in both trials when plasma glucose concentrations were still above baseline values. This is similar to the findings from each of the studies presented in this thesis and suggests that exercise capacity in intermittent shuttle running is not determined by plasma glucose concentrations per se. It would appear that the elevated plasma glucose concentrations serve to indirectly effect exercise duration by delaying the rate of muscle glycogen utilisation. Furthermore it seems that once muscle glycogen concentrations have fallen to an individual’s critical level fatigue will ensue regardless of plasma glucose concentrations. The results from studies on cycling to fatigue suggest that capacity is increased through the maintenance of plasma glucose concentrations (Coggan and Coyle, 1991). However even in this exercise modality it has been shown that once fatigue is impending the delivery of exogenous glucose cannot sustain exercise at a high intensity (>75% VO2max) (Coggan and Coyle, 1988). This supports the view that muscle glycogen is the substrate that dictates exercise capacity. Interestingly the muscle glycogen concentrations reported at fatigue in this study and after 90 min of LIST running (Nicholas et al., 1999) are higher than those reported in players following actual soccer match play (Saltin, 1973). During exhaustive LIST running players are either removed from the protocol due to an inability to maintain the high intensity bouts of exercise or retire due to volitional fatigue. This would suggest a fibre-type depletion of muscle glycogen, namely Type II fibres (Nicholas et al., 1999). However during actual soccer match play players are not necessarily removed from the field of play once they have depleted their type II muscle fibres. They may be unable to maintain the high intensity bursts of activity but still may continue to play at a lower exercise intensity, thus further depleting their type I muscle fibres and consequently displaying a lower mixed muscle fibre glycogen concentration.

One limitation of the design of the present study is that only three biopsy samples were included per trial. This limited number of sampling points provided less than complete descriptions of the rate of glycogen degradation near the onset of fatigue. With only three biopsy samples per trial one must extrapolate the changes that are occurring to muscle glycogen concentrations between these points. It was decided to take a sample at 90 min so that direct comparisons could be made with the data of Nicholas and colleagues (1999). Ideally an additional sample would have been obtained in the CHO trial that would have coincided with the point of fatigue in the
PLA trial to allow direct comparisons between the two treatments at that same time point. This was attempted successfully by Tsintzas and colleagues (1996) in a study on continuous running. However the inherent difficulties to the design in the present study were three-fold. Firstly it would have necessitated the PLA trial being completed first thus compromising the experimental integrity of the investigation by introducing a potential trial-order effect and also removing the blind aspect of the study. Secondly the initial hypothesis was that there would be no difference between the trials due to the effect of the dietary manipulation and glycogen depleting exercise. Therefore subjects would not have been expected to fatigue earlier in the PLA trial. Finally the study of Tsintzas and colleagues (1996) only required 5 biopsies in total from the subject, for the present study to include an additional sample point would have required 7 biopsies for each subject.

As there are no muscle metabolite data for the CHO trial that corresponds to the fatigue sample in the PLA trial it is merely supposition to suggest that the additional exercise capacity in the CHO trial was due to a reduced glycogen utilisation post-90 min, or an increased muscle glycogen resynthesis. It may be speculated that at this time point (131 min ± 8) muscle glycogen concentration in both trials was at a critical point at which one would expect fatigue to occur. The extended exercise capacity in the CHO trial may have been attributable to the elevated plasma glucose concentrations. This is unlikely however since fatigue still occurred in this trial eventually despite continued elevated plasma glucose concentrations. Furthermore as already mentioned earlier studies have demonstrated that plasma glucose alone cannot sustain exercise at high intensities.

As there were no differences in any other muscle metabolites one should rule out the possibility of fatigue being caused by insufficient PCr resynthesis or acid imbalances within the muscle between the two trials. This appears to be confirmed by the sprint data. Although there were decreases in sprint performance over time there were no differences in sprint performance between trials at the point of fatigue, nor indeed at the time point in the CHO trial that coincided with the point of fatigue in the PLA trial. However it would be prudent to view these metabolic data with caution since the logistics of the protocol necessitated a short delay between the point of fatigue and the final biopsy sample being obtained as the subject had to be move from the testing hall.
to an examination couch in an adjacent room. Although this was done expediently and the samples were taken by an experienced and efficient surgeon there was still a delay of 45s (±12) between the planned sample time and the actual sample being taken. As the half time of PCr resynthesis and the clearance of lactate from the muscle cell can both be measured in seconds the muscle metabolite data may not truly reflect the exact scenario within the muscle cell at the actual point of fatigue.

Contrary to the hypothesis outlined earlier in this chapter it would appear that the high CHO intake in the days prior to the main trials did not serve to mask any ergogenic effects of the exogenous CHO solution. The results, with regards to exercise capacity, in the present study contradict those of the earlier studies (Chapters 4-6) which found no differences in exercise capacity between the two treatments. There are two possible explanations for this. Firstly it may be that the protocol of the present study allowed for a higher level of control of extraneous variables in the two days preceding the main trials. By having the subjects perform 90 min of LIST activity and then presenting them with post-exercise lunches and pre-weighed meal packages it is more likely that subjects would both adhere to the protocol and also that their dietary intake would be more accurately controlled. There is evidence to suggest that weighed food diaries or dietary recall methods lead to under-reporting when compared to actual energy intakes (see Hill and Davies, 2001 for a review), moreover this has been shown to occur specifically in soccer players (Ebine et al., 2002). Therefore the subjects in the earlier studies may have misreported their actual dietary intakes. However, this may not be the case as Black and colleagues (1993) report that normal-weight, highly motivated subjects, as were recruited in all of the studies in this thesis, generally display a fairly accurate self-report of dietary intake. Furthermore it is a crucial aspect of human physiological testing that one trusts in the probity and adherence to protocol of one’s subjects.

The second, and most likely, explanation may be that the large CHO diet in the present study initiated a greater elevation in endogenous glycogen stores compared to the moderate CHO intake of the earlier studies. The high CHO recovery diet was effective in elevating muscle glycogen and the data show that the pre-exercise concentrations were considerably higher than those reported by Nicholas and colleagues. It would appear to suggest that with low muscle glycogen concentrations
there is an ergogenic effect of exogenous CHO provision. This effect appears to be negated when the habitual CHO intake is moderate, possibly as a consequence of the additional CHO causing a greater shift in substrate metabolism without a compensatory increase in CHO provision, whereas a high CHO diet appears to offer substantial endogenous and exogenous CHO to allow for a greater CHO oxidation throughout exercise.

The results from the present study illustrate that in subjects with high endogenous muscle glycogen concentrations, brought about by glycogen depleting exercise and a high CHO recovery diet, the provision of a 6.4% hypotonic carbohydrate-electrolyte solution during exercise can enhance intermittent shuttle running capacity to the magnitude of 21% greater than that of a placebo solution.
CHAPTER 8

GENERAL DISCUSSION

8.1 Introduction and key findings

The research investigations presented in the preceding four chapters of this thesis have attempted to examine the influence of a hypotonic carbohydrate-electrolyte solution on exercise capacity during high intensity intermittent running similar to that exhibited during the multiple sprint sports such as soccer, hockey and rugby. Furthermore the investigations within this thesis have examined the metabolic and physiological responses to the provision of carbohydrate during this type of exercise. The main findings are summarised below:

1. From the results presented in Chapters 4, 5 and 6 it appears that there are no ergogenic benefits from ingesting a 6.4% hypotonic CHO-E solution, compared to a taste-matched placebo, during high-intensity shuttle running in subjects with a moderate habitual CHO intake (~5g CHO·kg⁻¹·BM·d⁻¹). (Chapters 4 and 5 had subjects perform high-intensity shuttle running to exhaustion following a 75 min bout of intermittent shuttle running (LIST) whilst Chapter 6 had subjects perform LIST to exhaustion.)

2. Earlier studies that showed improved LIST capacity used isotonic CHO-E solutions. It is clear from the results presented in Chapter 5 that there are no physiological or performance differences following the ingestion of a 6.4% isotonic CHO-E solution or a 6.4% hypotonic CHO-E solution during intermittent shuttle running (LIST).

3. Data from Chapters 4, 5 and 6 suggest that there was an enhanced exercise capacity during the control (placebo) trials in the present investigations compared to earlier studies, rather than a lack of an ergogenic effect from the CHO-E solution per se.
4. It is evident from the results presented in Chapter 7 that in subjects with elevated muscle glycogen concentrations, induced by glycogen depleting activity and a high CHO recovery diet (10g CHO·kg\(^{-1}\) BM·day\(^{-1}\)), the ingestion of a 6.4% hypotonic CHO-E solution led to improved exercise capacity during intermittent shuttle running (LIST) to exhaustion. This enhanced exercise capacity was the result of a decreased rate of muscle glycogen utilisation late in exercise, possibly due to an elevated plasma glucose allowing for an increased rate of muscle glycogen resynthesis.

5. Interpretation of the data from all studies suggests that increases in exercise capacity during LIST are dependent upon habitual CHO intake and exogenous CHO provision. In Chapter 7 it appears that these ergogenic benefits are additive, however in Chapters 4, 5 and 6 there is some suggestion that the moderate CHO intake and the resultant endogenous glycogen concentrations mask any effects of exogenous CHO ingestion in the form of a CHO-E solution.

The following discussion will try to analyse these results with respect to other research studies in an attempt to explain these apparently conflicting findings.

As fatigue occurs during intermittent high-intensity shuttle running, despite moderate muscle glycogen concentrations and the maintenance of euglycaemia, alternative mechanisms contributing to fatigue will be examined. A graphical depiction of these potential mechanisms is presented in Figure 8.1 and the following subsections will address these factors.

8.2 Carbohydrate availability

8.2.1 Blood glucose

The results from Chapters 4, 5 and 6 showed that there were no benefits of a hypotonic (and isotonic in Chapter 5) CHO-E solution on either exercise capacity or 15m sprint performance. This suggests that CHO availability was not a contributory factor in the onset of fatigue. Substrate and hormonal responses in each of these investigations support the notion that these CHO-E solutions were being delivered to
the systemic circulation. However it would appear that the increased glucose delivery in the CHO trials did not elevate the total CHO oxidation rate as judged from the RER values.

The increased insulinaemic response associated with a rise in blood glucose concentration following CHO ingestion causes hepatic glucose production to fall (Vella et al., 2003). The ingestion of moderate amounts of glucose (35g·h⁻¹) during prolonged cycling at 50%VO₂max has been shown to lead to partial suppression of hepatic glucose production whilst higher amounts of glucose ingestion (175g·h⁻¹) completely suppress hepatic glucose production (Jeukendrup et al., 1999). Subjects in the present investigations ingested CHO at rates of 48-90g·h⁻¹ thus suggesting that there would be a degree of suppression of hepatic glucose production occurring in the CHO trials. The elevated insulin concentrations evident in the CHO trials in the present studies would have led to a reduced hepatic glycogenolysis and a sparing of liver glycogen. As ethical considerations prevent us from assessing liver glycogen one can merely speculate on the effect of exogenous CHO and hepatic glycogenolysis. However in each of the investigations in the present thesis and also in the data reported by others (Nicholas et al 1995; Davis et al 1999; 2000; MacLaren and Close 2000; Welsh et al 2002) there are no reports of hypoglycaemia from subjects even in the control trials. In fact in the control trials blood glucose is reported as being maintained at euglycaemic concentrations throughout exercise even at the point of fatigue. Although each of these studies followed an overnight fast these results would suggest that there is sufficient liver glycogen at the onset of exercise to maintain blood glucose at euglycaemic concentrations.

8.2.2 Muscle glycogen

Blood glucose did not fall below baseline values even at the point of fatigue and total CHO oxidation rate was maintained therefore any differences in exercise capacity may be explained by the contribution of muscle glycogen to energy metabolism. Thus the availability of muscle glycogen is the factor underpinning exercise capacity rather than the availability of CHO per se. Tsintzas and Williams (1998) in an examination of the differing blood glucose responses to continuous running and cycling (ie hypoglycaemia evident in the latter but not the former) attributed these to the larger muscle masses involved in running and the inverse relationship between blood
glucose uptake and muscle mass involvement (as reported by Richter et al., 1988). The nature of the LIST is such that participants perform sprints, accelerate, decelerate and turn many times. This combination of activities may demand a greater muscle mass than is recruited during constant pace submaximal running. As such the uptake of blood glucose may be even lower than in continuous running or cycling.

Therefore in LIST exercise the role of CHO-E solutions would be to supplement the limited endogenous glycogen stores rather than to prevent a decline in blood glucose to hypoglycaemic levels as reported in cycling studies. This would serve to explain the findings of Nicholas et al., (1999) who reported that exogenous CHO led to glycogen sparing as evidenced by a 22% lower muscle glycogen utilisation in their CHO trial. This finding enabled the authors to attribute the findings of their earlier performance study to a 'glycogen sparing' effect (Nicholas et al., 1995). Other authors also used these results to explain improved LIST capacity following ingestion of CHO-E solutions (Davis et al., 1999; 2000; Welsh et al., 2002).

The reported muscle glycogen concentrations of Nicholas and colleagues (1999) are, however, much lower than those reported for athletes who consume a CHO-rich diet. The low pre-exercise muscle glycogen concentrations, reported by Nicholas and co-workers' for their subjects could have effected both muscle glycogen utilisation and glucose uptake. Hargreaves and colleagues (1995) suggested that there is a potential stimulatory effect of reduced muscle glycogen availability on muscle glucose uptake. Moreover Blomstrand and Saltin (1999) reported a 60% lower muscle glycogen utilisation and a 30% greater glucose uptake in the low glycogen state versus a more moderate state when comparing contra-lateral legs during exercise. Therefore, although one cannot refute the effect of the hormonal milieu associated with CHO ingestion, it is plausible to suggest that in subjects with low pre-exercise muscle glycogen concentrations the muscle glycogen utilisation in both the CHO and PLA trials would be lower and the blood glucose uptake greater than if the subjects had higher pre-exercise muscle glycogen concentrations. In short the ergogenic effect of exogenous CHO provision would be more pronounced in such situations.
8.2.3 Dietary carbohydrate

As the findings from Chapter 5 discount any differences in efficacy between the hypotonic and isotonic CHO-E solutions in their delivery of CHO, it is pertinent to suggest that the pre-exercise nutritional state of the subjects may have had a consequential impact on their subsequent exercise capacity. In Chapters 4, 5 and 6 dietary feedback diaries demonstrated that subjects were habitually consuming moderate amounts of CHO (5g CHO·kg⁻¹ BM·day⁻¹) whilst pursuing an inactive lifestyle for at least 48h. If one speculates that this may have led to elevated muscle glycogen concentrations then this could have served to mask any potential ergogenic effect of the CHO-E solution.

It is well established that there is a direct correlation between muscle glycogen concentration and the rate of muscle glycogen utilisation (Gollnick et al., 1972; Hargreaves et al., 1995; Blomstrand and Saltin, 1999). Higher rates of muscle glycogen utilisation would lead to an increased concentration of glucose-6-phosphate (G6P) within the muscle. As G6P concentration is the main inhibitor of hexokinase (HK) in human skeletal muscle then one would expect a decrease in the phosphorylation of glucose to G6P and hence a reduced glucose uptake. Therefore if there were high pre-exercise muscle glycogen concentrations in subjects in the investigations presented in Chapters 4, 5 and 6 it may serve to explain why there was a lower influence of the exogenous CHO on exercise capacity.

As fatigue occurred in these studies in the presence of euglycaemia it would suggest that glycogen depletion was the factor determining CHO availability. Thus the common finding from Chapters 4, 5 and 6: that capacity in the PLA trials appears to be greater than in earlier studies, rather than no improvement in capacity in the CHO-E trials suggests that pre-exercise nutritional state has a greater impact on LIST capacity than exogenous CHO provision. This is most evident in Chapter 4 (see Figure 4.13) but less so in Chapter 5 possibly due to the training status of the subjects and the apparent trial order (or acute training) effect. Unfortunately the results from the study discussed in Chapter 6 cannot be compared directly because different protocols were used in the assessment of endurance capacity.
The results presented in Chapter 7, however, appear to contradict those from the earlier studies presented in this thesis and subsequently cast doubts over the above hypothesis. In Chapter 7 subjects undertook a glycogen depleting activity and a high CHO recovery diet before the main trials in a deliberate attempt to increase the pre-exercise muscle glycogen stores to above normal values. This was confirmed from analysis of muscle biopsy samples for muscle glycogen concentrations. Thus subjects began exercise with muscle glycogen concentrations that were higher than those reported by Nicholas and colleagues (1999). As such, if the above hypothesis were to hold true one would have expected these elevated muscle glycogen concentrations to have negated any ergogenic potential of the exogenous CHO. In reality all six subjects ran longer following CHO ingestion and their improved endurance capacity suggests that there was an additive effect of the high CHO diet and the provision of CHO from the hypotonic CHO-E solution. This additive ergogenic effect of dietary CHO and exogenous CHO during exercise has been reported previously for continuous treadmill running at 70%VO2max (Chryssanthopolous et al., 1994). From an analysis of the studies on the effects of exogenous and endogenous CHO on intermittent running capacity it suggests that when pre-exercise muscle glycogen concentrations are low there is an ergogenic effect of exogenous CHO provision. This effect appears to be negated when the habitual CHO intake is moderate, but the ergogenic effect is evident again when the habitual CHO intake is high and muscle glycogen concentrations are elevated.

Interestingly in the carbohydrate-loading study reported in Chapter 7 the enhanced exercise capacity in the CHO trial appeared to be as a consequence of a reduced muscle glycogen utilisation late in exercise rather than a glycogen sparing effect throughout exercise as posited by Nicholas and colleagues (1999). In fact the subjects in the current study had similar muscle glycogen concentrations, following 90 min of exercise, as Nicholas and co-workers' subjects' had at rest, prior to exercise. Although speculative, this may suggest that there is a muscle glycogen concentration at which glucose uptake into the muscle is maximised and when muscle glycogen concentrations are above this value, muscle glycogen is the metabolic substrate of choice. Only when concentrations fall below this optimal value is glucose uptake from the blood an important substrate. This could potentially explain why exogenous CHO provision had an ergogenic effect in the studies of Nicholas et al., (1995) and
Davis et al., (1999; 2000) but failed to have an effect in the investigations presented in Chapters 4, 5 and 6 where we speculate that subjects had higher initial muscle glycogen concentrations. It may also serve to explain the apparent muscle glycogen sparing during 90 min of LIST attributed to exogenous CHO in the study of Nicholas et al., (1999) but the lack of any evidence of sparing in the first 90 min of LIST in the study presented in Chapter 7, again where subjects had elevated pre-exercise muscle glycogen concentrations.

As for the apparent contrasting results presented in this thesis i.e. the similarity in exercise capacity regardless of ingested solutions (Chapters 4, 5 and 6) and the increased exercise capacity attributable to CHO ingestion (Chapter 7) the probable reason is the extent of the increase in pre-exercise muscle glycogen concentrations. The habitual CHO intake in the final investigation (Chapter 7) was double that of the earlier investigations, this would have led to a concomitant increase in muscle glycogen to values that were greater than those in the subjects who participated in earlier studies. If this hypothesis holds true in that there is an optimal muscle glycogen concentration above which there is no evidence of glycogen sparing then it may well be that the exercise intensity, and therefore duration, played an important factor in the determinant of fatigue time.

In Chapter 7 subjects ran for over 2h in both trials which is longer than in any previous investigation utilising a similar protocol. It is generally agreed that liver glycogenolysis can maintain euglycaemia during exercise at ~75% VO\textsubscript{2max} for about 2h and therefore in the studies presented in Chapters 4-6 there is evidence to suggest that subjects fatigued before the elevated blood glucose in the CHO trial had time to enhance muscle glycogen resynthesis rates or have a glycogen sparing effect. In contrast in Chapter 7, the greater running times of the subjects suggest that the exogenous CHO was able to offer an ergogenic role by reducing the rate of muscle glycogen utilisation from ~4 mmol·kg\textsuperscript{-1}·min\textsuperscript{-1} in the PLA trial to ~2.5 mmol·kg\textsuperscript{-1}·min\textsuperscript{-1}. This was achieved through an increased oxidation of exogenous CHO or through an enhanced resynthesis of muscle glycogen. Interestingly this investigation (Chapter 7) appears to be the only study using this exercise modality that has shown a significantly elevated plasma glucose concentration in the CHO trial at the point of
fatigue. Unfortunately methodological considerations prevented measurement of either plasma glucose or muscle glycogen concentrations in the CHO trial at the point of fatigue in the PLA trial thus preventing further expansion on the actual mechanisms underlying the enhanced energetic provision.

A further possible explanation for the differences in the results of the studies reported in this thesis and those in the research literature is the effects of the ingested CHO on substrate metabolism. In the first 3 studies (Chapters 4-6) there appears to be a suppression of lipid metabolism in the CHO trials. This conclusion is based on the assumption of a trend towards lower plasma FFA and glycerol concentrations in the CHO trials compared with the PLA trials. Although these differences do not reach statistical significance this may well be as a consequence of the low sample size in each of the investigations. If the exogenous CHO did suppress lipid mobilisation and their subsequent contribution to metabolic processes without offering sufficient CHO to compensate for this shift in substrate utilisation then the deficit would be covered by an increased CHO metabolism. This may therefore mask any ergogenic potential of the ingested CHO. However the respiratory exchange ratio (RER) data from these studies fails to support this observation as no differences are evident between solutions however this may be due to limitations in the methods for measuring respiratory exchange ratio under these circumstances. Although Christmass et al., (1999a; 1999b) support the validity of indirect calorimetry for the determination of substrate oxidation rates during sustained repeated intermittent exercise bouts the protocol utilised in their studies differs somewhat from the LIST. Christmass and colleagues (1999a; 1999b) had subjects perform interval type exercise comprising supra-maximal exercise (109-120% VO_2peak) of short duration (6-24s) followed by short rest periods (9-36s). During the LIST subjects perform maximal sprints every ~80s, followed by high intensity running before they experience an active recovery period. It may be that the maximal bouts of sprinting and the associated disturbances to the respiratory pattern and the HCO₃ pools confound the interpretation of the respiratory data and thus the RER value.
8.3 Other factors that may contribute to fatigue

8.3.1 Dehydration

Although dehydration has long been recognised as a primary mechanism responsible for fatigue in prolonged exercise (see Convertino et al., 1996 for ACSM Position Stand) it is unlikely to have had a marked effect in the studies presented in the present thesis. Firstly the ambient temperature during all of the trials was moderate and thus the thermoregulatory strain on the subjects would have been moderate and tolerable. Furthermore the control trial in all investigations was an isovolumic placebo thus the fluid replacement in each trial was matched. Although there is evidence that supports a faster intestinal absorption of CHO-E solutions due to the co-transport of substrate and fluid and also the associated solvent drag (see Chapter 2) data on plasma volume and body mass losses show that there were no post-exercise differences between solutions in all trials.

8.3.2 Central factors

In Chapter 6 there were no differences in prolactin concentrations between the CHO and PLA trials throughout LIST exercise to fatigue. Accepting that prolactin serves as a surrogate measure of serotonin this suggests that there were no potential differences in serotonergic activity following CHO ingestion. Moreover branched-chain amino acid supplementation failed to improve LIST capacity (Davis et al., 1999) and euglycaemia was maintained in all trials. These findings appear to confirm that central fatigue was not a determining factor in exercise capacity during the LIST. Furthermore ratings of perceived exertion did not differ between treatments in any of the trials suggesting that subjects did not perceive there to be any advantages attributable to any of the solutions.

8.3.3 Skeletal muscle fatigue

From the muscle metabolite data in Chapter 7 it is not possible to examine whether changes in PCr resynthesis or muscular acidosis were responsible for fatigue. There were no differences for any muscle metabolites between trials but this is hardly surprising since in both trials the muscle was sampled at the point of fatigue therefore one would expect similar results between trials whether metabolic data was responsible for fatigue or not. Unfortunately there was not a sample taken in the CHO trial at the time point that coincided with the point of fatigue in the PLA trial to allow
Figure 8.1 Potential mechanisms underpinning fatigue during prolonged high-intensity intermittent exercise.
such comparisons. There were significant differences within trials for sprint performance, with sprints slowing as exercise duration increased. However there were no differences between trials thus suggesting that the ability to repeatedly perform maximal sprints was effected similarly regardless of the solution ingested. Furthermore although subjects in both Chapters 6 and 7 received warnings when they slowed down during the sprints in the LIST capacity test post-90 min none of the subjects were withdrawn because of a failure to maintain overall sprint speed. This would suggest that although changes to muscle metabolites and muscular acidosis may have effected sprinting ability as LIST duration progressed the effect was both similar regardless of the solution ingested and ultimately not the cause of fatigue.

8.3.4 Experimental model

It should be noted that the exercise models used throughout this thesis were modified versions of the original LIST protocol (Nicholas et al., 19995; 2000) and as such have not been validated for use as reliable experimental protocols. In the first two studies (Chapters 4 and 5) the order of the cruise and jog cycles of the LIST were switched. This was in order to facilitate a greater adherence to the actual running intensities as explained in the appropriate methodologies. There were no changes to the amount of time/distance spent at the relative intensities and as such the overall exercise intensity and energy expenditure for this protocol should reflect those of the earlier protocol. In the subsequent two studies (Chapters 6 and 7) the LIST protocol was extended beyond 75 min to volitional exhaustion. Although this protocol has not been validated as an exhaustive test Nicholas et al., (2000) demonstrated that the performance and physiological responses to 90 min of LIST activity was reproducible. As the latter two studies in the current thesis merely extended this exercise protocol it is reasonable to assume that the performance and physiological responses would continue to be reproducible. However it should be noted that the differences (or lack thereof) in exercise capacity presented in this thesis could have been as a consequence of the exercise protocol utilised rather than the experimental interventions.

8.4 Conclusion

In summary the data presented in this thesis demonstrate that there are potential ergogenic properties of a 6.4% hypotonic carbohydrate-electrolyte solution for enhanced capacity during prolonged high-intensity intermittent running exercise
similar to that displayed in sports like soccer, hockey and rugby. However the data suggest that the ergogenic role of the CHO-E solution may not be taken in isolation and that the pre-exercise nutritional state of the subject plays an integral role in prolonging performance. This is due to the inverse relationship between the amount of exogenous glucose consumed and hepatic glucose production in addition to the relationship between high muscle glycogen concentrations and glucose uptake. The results from the studies presented in this thesis suggest that the optimal nutritional strategy for performers in high-intensity intermittent exercise is to consume a high CHO diet before exercise and to supplement with exogenous CHO during activity in order to prolong performance and also promote recovery.

8.5 Directions for future research

➢ Determine experimental validity of utilising the modified LIST protocols as models for measuring exhaustive capacity during intermittent running

➢ Compare muscle metabolites in a CHO trial at a time-point that coincides with fatigue in a PLA trial during exhaustive LIST running in order to further understand metabolic data at the point of exhaustion

➢ Further investigate the accumulative effect of hepatic and exogenous CHO on LIST capacity by comparing individuals with low and high pre-exercise muscle glycogen concentrations both with and without CHO supplementation

➢ Investigate the effect of exhaustive LIST running on muscle metabolites in single muscle fibres

➢ Determine experimental validity of utilising indirect calorimetry to estimate energy expenditure and RER during LIST

➢ If above measures are deemed to be valid then examine the contribution of endogenous and exogenous CHO to total CHO oxidation during LIST activity using labelled isotopes
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APPENDICES
APPENDIX A

The influence of a hypotonic carbohydrate-electrolyte solution on exercise metabolism and performance during prolonged, high-intensity intermittent shuttle running

STATEMENT OF INFORMED CONSENT

I have read the subject information sheet, detailing the procedure and requirements which are involved with this study and I fully understand what is required of me. I have had an opportunity to ask for further information and clarification of the demands of each of the procedures. I am aware that I have the right to withdraw at any time with no obligation to give reasons for my decision.

I agree to take part in the study.

Name ___________________________ Phone No. ___________________________

Age _______ Date of Birth _______________

Contact Address _______________________________________________________

Signed ___________________________ Witnessed by _______________________

Date _______________________________
HEALTH SCREEN FOR STUDY VOLUNTEERS

Name or Number .................

It is important that volunteers participating in research studies are currently in good health and have had no significant medical problems in the past. This is to ensure (i) their own continuing well-being and (ii) to avoid the possibility of individual health issues confounding study outcomes.

Please complete this brief questionnaire to confirm fitness to participate:

1. At present, do you have any health problem for which you are:
   (a) on medication, prescribed or otherwise .............................................. Yes □ No □
   (b) attending your general practitioner ................................................. Yes □ No □
   (c) on a hospital waiting list .................................................................. Yes □ No □

2. In the past two years, have you had any illness which require you to:
   (a) consult your GP ........................................................................ Yes □ No □
   (b) attend a hospital outpatient department ...................................... Yes □ No □
   (c) be admitted to hospital .................................................................. Yes □ No □

3. Have you ever had any of the following:
   (a) Convulsions/epilepsy ................................................................ Yes □ No □
   (b) Asthma ..................................................................................... Yes □ No □
   (c) Eczema .................................................................................... Yes □ No □
   (d) Diabetes ................................................................................... Yes □ No □
   (e) A blood disorder ........................................................................ Yes □ No □
   (f) Head injury ................................................................................ Yes □ No □
   (g) Digestive problems ..................................................................... Yes □ No □
   (h) Heart problems .......................................................................... Yes □ No □
   (i) Problems with bones or joints ...................................................... Yes □ No □
   (j) Disturbance of balance/coordination .......................................... Yes □ No □
   (k) Numbness in hands or feet .......................................................... Yes □ No □
   (l) Disturbance of vision ................................................................... Yes □ No □
   (m) Ear / hearing problems ............................................................... Yes □ No □
   (n) Thyroid problems ........................................................................ Yes □ No □
   (o) Kidney or liver problems ............................................................. Yes □ No □
   (p) Allergy to nuts ............................................................................ Yes □ No □

4. Has any, otherwise healthy, member of your family under the age of 35 died suddenly during or soon after exercise? ............. Yes □ No □

If YES to any question, please describe briefly if you wish (eg to confirm problem was/is short-lived, insignificant or well controlled.) ........................................................................................................

Thank you for your cooperation!
### PHYSICAL ACTIVITY QUESTIONNAIRE

<table>
<thead>
<tr>
<th>NAME</th>
</tr>
</thead>
<tbody>
<tr>
<td>SPORT</td>
</tr>
<tr>
<td>EVENTS</td>
</tr>
<tr>
<td>LEVEL</td>
</tr>
<tr>
<td>TRAINING STATUS</td>
</tr>
</tbody>
</table>

**How Many Days of the Week Do You Usually Train?**

**Does Your Training Include Light and Hard Days?**
- If yes, how many of each per week?
  - Hard: _______________________
  - Light: _______________________

**Do You Practice Endurance Training?**
- If YES, how many times per week?
- How many minutes does each session last?
  
**Do You Practice Interval Training?**
- If YES, how many times per week?
- How many minutes does each session last?

**What Time of the Day Do You Usually Train?**
(Please circle all that are appropriate)
- Mornings
- Lunchtimes
- Evenings
The influence of a hypotonic carbohydrate-electrolyte solution on exercise metabolism and performance during prolonged, high-intensity intermittent shuttle running

Health Questionnaire

Please complete the following brief questions to confirm your fitness to participate:
At present do you have any health problems for which you are:

1) On medication, prescribed or otherwise  YES ☐ NO ☐.
2) Attending your general practitioner  YES ☐ NO ☐.

Have you any symptoms of ill health, such as those associated with a cold or other common infection?  YES ☐ NO ☐.

If you have answered yes to any of the above questions please give more details below:

Do you want to take part in today's experiments?  YES ☐ NO ☐.

Signature:__________________________ Date:_____________
APPENDIX E

Estimation of energy expenditure from indirect calorimetry.

The contribution of CHO and fat to metabolism were estimated from the non-protein respiratory exchange ratio (RER) value. This assumes that the contribution of protein to energy metabolism is both negligible and relatively constant (Consolazio et al, 1963).

The following method for calculating energy expenditure by indirect calorimetry is adapted from McArdle et al (1991).

Oxidising 1g CHO uses 0.828 l of O₂ and produces 0.828 l of CO₂ and 17kJ of energy

Oxidising 1g FAT uses 1.989 l of O₂ and produces 1.419 l of CO₂ and 39kJ of energy

If parallel, steady state values for VO₂ and VCO₂ are known the quantities of FAT and CHO oxidised per minute may be calculated using simultaneous equations.

Let x be g of CHO and y be g of FAT oxidised per minute.

Then

\[ \text{VO}_2 = 0.828x + 1.989y \]  \hspace{1cm} (i)
\[ \text{VCO}_2 = 0.828x + 1.419y \]  \hspace{1cm} (ii)

Solving x and y by subtracting (ii) from (i) gives rise to the following:

\[ \text{VO}_2 - \text{VCO}_2 = 0.57y \]

Substituting the value for y back into (i) or (ii) gives rise to x

Thus total energy expenditure is given by:

\[ \text{Energy expenditure} = (17x + 39y) \text{kJ} \cdot \text{min}^{-1} \]
APPENDIX F

Food Record Diaries

Name: 

Dates: Trial 1: / / Trial 2: / / Trial 3: / / 

Please complete the following 2-day weighed intake record. Your help is very much appreciated.

The following instructions may be useful:

- Record your intake the two days before the main trials
- Do not change what you eat because it is easier to weigh, or choose 'healthier' foods than you would normally consume
- Record foods eaten at the time that you eat them
- Please give the most accurate description of the foods as possible (e.g. Heinz reduced salt and sugar baked beans)
- Include any comments and information about vitamin supplements etc that you feel are relevant in the notes section at the bottom of the sheet and on the back
- Please refrain from alcohol during the 2-day weighed record intake
- Please record all food wasted e.g. weigh the apple and then weigh the core and record both
- Use as many diet sheets as you need
- Please bring this sheet with you every time you attend the laboratory

Please refer to the example provided if unsure what to do. Furthermore, feel free to contact Andy 228183 (office) if you have any problems.

Thank you.
APPENDIX F

NAME

Date:   /   /   Trial Number:   

Please use a separate line for each item eaten; leave a line between different meal entries.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th>Weight Served</th>
<th>Weight of Leftovers</th>
<th>Actual Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Time</td>
<td>Brand name of each item (except fresh food)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>am/pm</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Full description of each item including:
-whether fresh, frozen, dried, canned
-cooked: boiled, grilled, fried, roasted.
-what type of fat food fried in

GENERAL COMMENTS:

---

(gms) (gms) (gms)
APPENDIX G

Measurement of Glucose (Fluorometric method)

Principle:

\[ \text{Glucose} + \text{ATP} \xrightarrow{HK} \text{G6P} + \text{ADP} \]

\[ \text{G6P} + \text{NADP}^+ \xrightarrow{\text{G6PDH}} 6\text{-P-Gluconolactone} + \text{NADPH} + \text{H}^+ \]

Acid Insoluble Glycogen (Proglycogen) – on muscle pellet

Method:

1. Make up the following reagent mixture using these quantities per cuvette

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Quantity Per Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>Buffer (Tris 100 mmol)</td>
<td>5ml</td>
</tr>
<tr>
<td>NADP 5mM (3.937 mg·ml(^{-1}))</td>
<td>30µl</td>
</tr>
<tr>
<td>ATP 200mM (121.04 mg·ml(^{-1}))</td>
<td>7.5µl</td>
</tr>
<tr>
<td>MgCl(_2) 100mM (2.0331 g·100ml(^{-1}))</td>
<td>50µl</td>
</tr>
<tr>
<td>EDTA 100mM (37.224 mg·ml(^{-1}))</td>
<td>25µl</td>
</tr>
<tr>
<td>DTT 50mM (7.715 mg·ml(^{-1}))</td>
<td>50µl</td>
</tr>
</tbody>
</table>

Add enzymes last

\[ \text{G6PDH} (7U/ml) (20µl + 980 µl of dist H\(_2\)O) \]

\[ \text{HK} (28U/ml) (20µl + 980 µl of dist H\(_2\)O) \]

2. Use hydrolysed extract from pellet diluted with water (1:49 eg 20µl with 980µl of distilled H\(_2\)O)

3. Make up standards as below

4. Add 20µl of diluted extract/standards to fluorometric cuvettes

5. Add 200µl of reagent to samples every 30s (time not important as long as it is constant)

6. Incubate for >30min at room temperature

7. Add 1ml of carbonate buffer (again every 30s)

8. Read fluorescence (F1) (every 30s)

Standards

- Use 0, 20, 50, 100 and 200 µmol·l\(^{-1}\) glucose standards (beyond this range line is not linear).
- Roche 5.55mmol·l\(^{-1}\) – dilute 360µl: 10ml dist H\(_2\)O to make 200µM stock

<table>
<thead>
<tr>
<th>Conc (µmol·l(^{-1}))</th>
<th>0</th>
<th>20</th>
<th>50</th>
<th>100</th>
<th>200</th>
</tr>
</thead>
<tbody>
<tr>
<td>Std (0.505mmol·l(^{-1}))</td>
<td>0</td>
<td>20</td>
<td>50</td>
<td>100</td>
<td>200</td>
</tr>
<tr>
<td>Dist H(_2)O</td>
<td>200</td>
<td>180</td>
<td>150</td>
<td>100</td>
<td>0</td>
</tr>
</tbody>
</table>

Acid insoluble glycogen concentration (as glucosyl units) was calculated from the above standard curve. The resulting value was multiplied by a hydrolysis factor (50) and a dilution factor (100) to give a corrected true acid insoluble concentration.
Acid Soluble Glycogen (Macro2lyco2en) — on hydrolysed extract

Method:

1. Make up the following reagent mixture using these quantities per cuvette

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Quantity</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Buffer (Tris 100 mmol)</td>
<td>5 ml</td>
<td></td>
</tr>
<tr>
<td>NADP 5mM (3.937 mg·ml⁻¹)</td>
<td>30 µl</td>
<td>(3.937 mg·ml⁻¹)</td>
</tr>
<tr>
<td>ATP 200mM (121.04 mg·ml⁻¹)</td>
<td>7.5 µl</td>
<td>(121.04 mg·ml⁻¹)</td>
</tr>
<tr>
<td>MgCl₂ 100mM (2.0331 g·100 ml⁻¹)</td>
<td>50 µl</td>
<td>(2.0331 g·100 ml⁻¹)</td>
</tr>
<tr>
<td>EDTA 100mM (37.224 mg·ml⁻¹)</td>
<td>25 µl</td>
<td>(37.224 mg·ml⁻¹)</td>
</tr>
<tr>
<td>DTT 50mM (7.715 mg·ml⁻¹)</td>
<td>50 µl</td>
<td>(7.715 mg·ml⁻¹)</td>
</tr>
</tbody>
</table>

Add enzymes last

G6PDH (7U·ml⁻¹) (20 µl + 980 µl of dist H₂O) | 15 µl
HK (28U·ml⁻¹) (20 µl + 980 µl of dist H₂O) | 25 µl

2. Make up standards as below
3. Add 20 µl of diluted extract/standards to fluometric cuvettes
4. Add 200 µl of reagent to samples every 30s (time not important as long as it is constant)
5. Incubate for >30 min at room temperature
6. Add 1 ml of carbonate buffer (again every 30s)
7. Read fluorescence (Fl) (every 30s)

Standards
- Use 0, 20, 50, 100 and 200 µmol·l⁻¹ glucose standards (beyond this range line is not linear).
- Roche 5.55 mmol·l⁻¹ — dilute 360 µl: 10 ml dist H₂O to make 200 µM stock

<table>
<thead>
<tr>
<th>Conc (µmol·l⁻¹)</th>
<th>0</th>
<th>20</th>
<th>50</th>
<th>100</th>
<th>200</th>
</tr>
</thead>
<tbody>
<tr>
<td>Std (0.505 mmol·l⁻¹)</td>
<td>0</td>
<td>20</td>
<td>50</td>
<td>100</td>
<td>200</td>
</tr>
<tr>
<td>Dist H₂O</td>
<td>200</td>
<td>180</td>
<td>150</td>
<td>100</td>
<td>0</td>
</tr>
</tbody>
</table>

Acid soluble glycogen concentration (as glucosyl units) was calculated from the above standard curve. The resulting value was multiplied by a hydrolysis factor (6.75) and an extraction factor (80) to give a corrected true acid soluble concentration.
Free Glucose

Method:

1. Make up the following reagent mixture using these quantities per cuvette

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Quantity</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Buffer (Tris 100 mmol)</td>
<td>5ml</td>
<td></td>
</tr>
<tr>
<td>NADP 5mM (3.937mg·ml⁻¹)</td>
<td>30µl</td>
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<td>ATP 200mM (121.04 mg·ml⁻¹)</td>
<td>7.5µl</td>
<td></td>
</tr>
<tr>
<td>MgCl₂ 100mM (2.0331 g·100ml⁻¹)</td>
<td>50µl</td>
<td></td>
</tr>
<tr>
<td>EDTA 100mM (37.224 mg·ml⁻¹)</td>
<td>25µl</td>
<td></td>
</tr>
<tr>
<td>DTT 50mM (7.715 mg·ml⁻¹)</td>
<td>50µl</td>
<td></td>
</tr>
<tr>
<td>G6PDH (7U·ml⁻¹) (20µl + 980 µl of dist H₂O)</td>
<td>15µl</td>
<td></td>
</tr>
<tr>
<td>HK (28U·ml⁻¹) (20µl + 980 µl of dist H₂O)</td>
<td>25µl</td>
<td></td>
</tr>
</tbody>
</table>

Add enzymes last

2. Make up standards as below
3. Add 20µl of diluted extract/standards to fluometric cuvettes
4. Add 200µl of reagent to samples every 30s (time not important as long as it is constant)
5. Incubate for >30min at room temperature
6. Add 1ml of carbonate buffer (again every 30s)
7. Read fluorescence (F1) (every 30s)

Standards

- Use 0, 20, 50, 100 and 200 µmol/l glucose standards (beyond this range line is not linear).
- Roche 5.55mmol/l – dilute 360µl: 10ml dist H₂O to make 200µM stock

<table>
<thead>
<tr>
<th>Conc (µmol·l⁻¹)</th>
<th>0</th>
<th>20</th>
<th>50</th>
<th>100</th>
<th>200</th>
</tr>
</thead>
<tbody>
<tr>
<td>Std (0.505mmol·l⁻¹)</td>
<td>0</td>
<td>20</td>
<td>50</td>
<td>100</td>
<td>200</td>
</tr>
<tr>
<td>Dist H₂O</td>
<td>200</td>
<td>180</td>
<td>150</td>
<td>100</td>
<td>0</td>
</tr>
</tbody>
</table>

Free glucose concentration (as glucosyl units) was calculated from the above standard curve. The resulting value was multiplied by a hydrolysis factor (100) to give a corrected true free glucose concentration.
APPENDIX G

Measurement of ATP, PCr and G6P

Principle:

\[
\text{Glu-6-P} + \text{NADP} \xrightarrow{\text{G-6-PDH}} \text{P-gluconolactate} + \text{NADPH}
\]

\[
\text{ATP} + \text{Glucose} \xrightarrow{\text{HK}} \text{ADP} \xrightarrow{\text{CPK}} \text{Cr} + \text{ATP}
\]

Method:

1. Make up the following reagent mixture using these quantities per cuvette:

<table>
<thead>
<tr>
<th>Component</th>
<th>Quantities</th>
<th>Spectro method</th>
<th>Plate Reader</th>
</tr>
</thead>
<tbody>
<tr>
<td>D1 (TEA pH 7.5-7.6 buffer)</td>
<td>37.5µl</td>
<td>22.2µl</td>
<td></td>
</tr>
<tr>
<td>DTT (7.8mg·ml⁻¹)</td>
<td>7.5µl</td>
<td>4.4µl</td>
<td></td>
</tr>
<tr>
<td>NADP (20.9 mg·ml⁻¹)</td>
<td>15µl</td>
<td>8.9µl</td>
<td></td>
</tr>
<tr>
<td>ADP (5.1 mg·ml⁻¹)</td>
<td>1.5µl</td>
<td>0.9µl</td>
<td></td>
</tr>
<tr>
<td>Glucose (22.5 mg·ml⁻¹)</td>
<td>15µl</td>
<td>8.9µl</td>
<td></td>
</tr>
<tr>
<td>Dist H₂O</td>
<td>261µl</td>
<td>154.7µl</td>
<td></td>
</tr>
</tbody>
</table>

Vortex and keep on ice

2. Make standards (see below). Combine standards into one tube.

3. Dilute the following enzymes:
   a) Glucose-6-Phosphate-Dehydrogenase (Boeh) (G6PDH) (fridge). Need 3µl per cuvette for spectro and 2µl per sample for plate reader.
   b) Hexokinase (HK) (Boeh) (fridge). Dilute 1 part enzyme to 1 part Dist H₂O. Need 5µl per cuvette for spectro and 2µl per sample for plate reader.
   c) Creatine phosphokinase (CPK) (sigma) (-20). Dissolve 15mg·ml⁻¹ in 0.5% NaHCO₃ + 0.05% BSA (D5). Dilute 2 parts enzyme to 1 part Dist H₂O. Need 5µl per cuvette for spectro and 2µl per sample for plate reader.

Vortex and keep on ice.

4. Defrost samples quickly in hot water, vortex and spin down (14000rpm, 3mins)

5. Set the plate reader to read samples at 340nm.

6. Pipette 200µl of the reagent mix into each well of the plate reader.

7. Add 20µl of water/standard/sample.

8. Read absorbance.

10. Read absorbance.

11. Add 2µl of the HK. Incubate and agitate for 15mins.

12. Read absorbance.

13. Add 2µl of the CPK. Incubate and agitate for 30mins.

Standards:

ATP, PCr and G6P
- From MW calculate how many mg to add to ml (H₂O) to make a 1Mol solution.
- Add 6.6µl of each to make a 20µl standard.

Calculations: (units = mmol·kg⁻¹·dm)

\[
\text{Concentration} = \frac{((\text{final vol} \times (\text{Ab}_{\text{sample}} - \text{Ab}_{\text{blank}})) - (\text{vol before enzyme} \times (\text{Ab}_{\text{enzyme}} - \text{Ab}_{\text{blank}}))) \times \text{ext factor} \times 1.25 \times \text{dil factor}}{6.22 \times \text{volume of sample}}
\]

Notes:
- Remember that volumes increase as each subsequent enzyme is added.
- Ext factor is extraction factor
- Dil factor is dilution factor
- 6.22 is mmolar extinction coefficient for NADH/NADPH at 340nm
APPENDIX G

Measurement of Lactate

Principle:

Lactate + NAD $\text{L DH} \rightarrow$ Pyruvate + NADH

Method:

1) Make up the following reagent mixture with these amounts per cuvette:

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>D2 (glycine buffer)</td>
<td>150µl</td>
</tr>
<tr>
<td>NAD (16.6 mg·ml⁻¹)</td>
<td>50µl</td>
</tr>
<tr>
<td>H2O</td>
<td>200µl</td>
</tr>
</tbody>
</table>

Vortex and keep on ice

2) Set spectrophotometer to read at 340nm for 1200sec using the ‘METABOLI’ method with the absorbance scale set from 0 to 1.2 units.

3) Pipette 400µl of reagent into each cuvette

4) Defrost the samples quickly in hot water.

5) Vortex and spin for 3 min at 14000rpm.

6. Pipette 50µl of water/sample/blank into each cuvette. Mix and start reading absorbance (A1).

7. Add 3µl of LDH to each cuvette. Agitate to mix. Continue reading for 20 minutes

8. Read final absorbance (A2).

Calculation:

Conc. Lactate = $\frac{453 \times (A2-B1) - 450 \times (A1-B1) \times \text{Ext Fac} \times 1.25}{50 \times 6.22}$

Units = mmol·kg⁻¹ DM
APPENDIX G

Measurement of Creatine

Principle:

Pyruvate + NADH \( \text{LDH} \) Lactate + NAD

ADP + PEP \( \text{PK} \) ATP + Pyruvate

Cr + ATP \( \text{CPK} \) PCr + ADP

Method:

1. Make up the following reagent mixture with these amounts per cuvette:

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>D4 (Glycine buffer)</td>
<td>150µl</td>
</tr>
<tr>
<td>D3 (KCl 15g·100ml(^{-1}))</td>
<td>7.5µl</td>
</tr>
<tr>
<td>ATP Na(_2)H(_2)O (15.4mg·ml(^{-1}))</td>
<td>30µl</td>
</tr>
<tr>
<td>PEP (11.6mg·ml(^{-1}))</td>
<td>22.5µl</td>
</tr>
<tr>
<td>NADH (9mg·ml(^{-1})) (F)</td>
<td>7.5µl</td>
</tr>
<tr>
<td>LDH (Boeh. 107034) (F)</td>
<td>0.75µl</td>
</tr>
<tr>
<td>PK (Boeh. 128155) (F)</td>
<td>0.75µl</td>
</tr>
<tr>
<td>H(_2)O</td>
<td>232.5µl</td>
</tr>
</tbody>
</table>

Vortex and keep on ice.

2. Prepare the enzyme, CPK (Sigma C-3755 (-20)). Dissolve 15mg·ml\(^{-1}\) in 0.5% NaHCO\(_3\) + 0.05% BSA (D5). Use 6µl per cuvette. Vortex and keep on ice.

3. Set spectrophotometer to read at 340nm for 1200sec using the ‘METABOLI’ method with the absorbance scale set from 0 to 1.2 units.

4. Pipette 450µl of the reagent mix into each cuvette.

5. Defrost the samples quickly in hot water, vortex and spin for 3 min at 14000rpm.


7. Add 6µl of CPK to each cuvette. Agitate to mix. Continue reading for 20 minutes

8. Read final absorbance (A2).

Calculation:

\[
\text{Conc. Cr} = \frac{-486 \times (A2-B12) - 480 \times (A1-B11) \times \text{Ext Fac} \times 1.25}{30 \times 6.22}
\]

Units = mmol·kg\(^{-1}\) DM
Enter consumed portion as multiple of 100g for the day

<table>
<thead>
<tr>
<th>FOOD</th>
<th>70kg</th>
<th>75kg</th>
<th>80kg</th>
<th>87kg</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Breakfast</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kellogg’s Start</td>
<td>0.5</td>
<td>0.5</td>
<td>0.6</td>
<td>0.7</td>
</tr>
<tr>
<td>Semi Skimmed Milk</td>
<td>1.0</td>
<td>1.1</td>
<td>1.1</td>
<td>1.3</td>
</tr>
<tr>
<td>Bread Sainsbury’s White</td>
<td>0.9</td>
<td>0.9</td>
<td>0.9</td>
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<tr>
<td>Flora Margarine</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>Jam S’bury Strawberry</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>Pure OJ (s’bury’s)</td>
<td>2.5</td>
<td>2.7</td>
<td>2.9</td>
<td>3.3</td>
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<tr>
<td><strong>Dinner</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pasta S’burys Fusilli White</td>
<td>1.5</td>
<td>1.6</td>
<td>1.7</td>
<td>2.0</td>
</tr>
<tr>
<td>Tuna</td>
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<td>2.3</td>
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<tr>
<td>Broccoli</td>
<td>1.2</td>
<td>1.3</td>
<td>1.4</td>
<td>1.6</td>
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<tr>
<td>S’burys hot mixed pepper</td>
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<td>2.7</td>
<td>2.9</td>
<td>3.3</td>
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<tr>
<td>Turkey steaks</td>
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<td>2.5</td>
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<tr>
<td>Salad</td>
<td>1.5</td>
<td>1.5</td>
<td>1.5</td>
<td>1.5</td>
</tr>
<tr>
<td>Rice Pudding (Ambrosia Low fat)</td>
<td>2.2</td>
<td>2.3</td>
<td>2.5</td>
<td>2.9</td>
</tr>
<tr>
<td>Jam S’bury Strawberry</td>
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<tr>
<td><strong>Supper</strong></td>
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<tr>
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<tr>
<td>Baked Beans (Heinz)</td>
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<tr>
<td>Flora Margarine</td>
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<tr>
<td>Yorkie</td>
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<tr>
<td>Jaffa Cakes</td>
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<td>Banana</td>
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<td>Muller Light yoghurt vanilla</td>
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<tr>
<td>Walkers (ready salted)</td>
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<tr>
<td>Lucozade Sport (orange)</td>
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<td>10.7</td>
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<tr>
<td>Lucozade original</td>
<td>4.0</td>
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**kcal**

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**Percentage**

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<tr>
<td>CHO</td>
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**Grams**

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<tr>
<td><strong>Breakdown</strong></td>
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<tr>
<td>CHO</td>
<td>677</td>
<td>724</td>
<td>770</td>
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<td>Protein</td>
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<td>Fat</td>
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**g.kg-1**

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<tr>
<td>CHO</td>
<td>9.7</td>
<td>9.7</td>
<td>9.6</td>
<td>10.0</td>
</tr>
<tr>
<td>Protein</td>
<td>2.1</td>
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<td>2.0</td>
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<tr>
<td>Fat</td>
<td>0.9</td>
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</table>

High carbohydrate recovery diet for subjects in Study 4 (Chapter 7)
APPENDIX I

Preliminary study into the ergogenic effect of a 6.4% hypotonic CHO-E solution. Treadmill running at 70%VO$_{2\text{max}}$ to volitional fatigue.

Introduction
A pilot study was performed to see if there were any differences in substrate utilisation, exercise capacity, heart rate and ratings of perceived exertion (RPE) during continuous steady-state running on a treadmill between a hypotonic CHO solution and a taste placebo.

Methods
Eleven male participants (4 competitive distance athletes; 7 recreational athletes) volunteered for the study. The mean (±SEM) age, body mass (BM) and maximal oxygen uptake (VO$_{2\text{max}}$) for the group were 20.3 years ± 0.7, 72.6 kg ± 3.8 and 62.92 ml.kg$^{-1}$.min$^{-1}$ ± 2.14 respectively.

Preliminary Tests
Subjects performed preliminary tests to establish the treadmill speed-VO$_2$ relationship and estimate VO$_{2\text{max}}$ in order to calculate the relative treadmill speed to elicit the desired submaximal exercise intensity. Subjects also performed a trial where they ran for 60min at the determined exercise intensity in order to familiarise themselves with the running speed and all experimental measures.

Experimental Design
Subjects acted as their own controls in a repeated measures cross-over design. They completed two runs to exhaustion at 70%VO$_{2\text{max}}$ on two separate occasions, the order of which was randomised. Each trial was separated by at least seven days. In the two days prior to the main trials subjects avoided alcohol, caffeine and strenuous exercise. They also monitored their dietary intake for the two days prior to trial one and replicated this intake prior to trial two. Participants reported to the laboratory for the main trials after an overnight fast (>10h). They then voided before the measurement of nude body mass which was determined to the nearest 0.1 kg using a beam balance. An indwelling cannula was inserted into an antecubital forearm vein (see Chapter 3 for detailed methodology).
Subjects ran at 60% VO\(_{2\text{max}}\) for 5min as a warm up and were then given 5min to stretch. They then ran at 70% VO\(_{2\text{max}}\) until volitional fatigue, at this point the treadmill speed was reduced to a walk (4km.h\(^{-1}\)) for 2min and then returned to the exercising speed. This reduction in intensity occurred twice; on the third occasion that the subject denoted that they could no longer maintain the required running intensity the trial ceased.

At rest and at 20 min intervals throughout exercise RPE was obtained, 10ml of venous blood was drawn and expired air was collected by the standard Douglas bag method. Heart rate was recorded every 15s during the trials by short-range telemetry. Dry-bulb and wet-bulb temperatures were measured every 15min using a whirling hygrometer and ambient temperature was maintained within the range 15-20°C by the use of portable fans. Expired air and blood were analysed and statistical procedures performed as detailed in Chapter 3.

Results

**Endurance**

There was a tendency for subjects to run for longer when fed the hypotonic carbohydrate solution compared to the placebo (151.7 min ± 9.3 vs 130.89 min ± 9.9; p=0.1) although this failed to reach significance (Figure 1).

**Blood Analyses**

Following ingestion of the hypotonic solution there was an initial decrease in plasma glucose with concentrations being significantly lower at 20min compared to the placebo trial (F\(_{3,1}\) = 4.7; p<0.05; Figure 2). After this time-point plasma glucose concentrations rose in the hypotonic trial and there was a trend for them to be maintained higher than in the placebo trial but this only reached significance at 80min (F\(_{3,1}\) = 4.7; p<0.05). There were no differences in serum insulin concentrations regardless of solution ingested. There was a tendency higher FFA concentrations in the placebo trial (p=0.07) compared to the hypotonic trial (Figure 3). There was also an effect of time with concentrations increasing with exercise duration (F\(_{1,14}\) = 47.59; p<0.05). There were no differences between trials for blood lactate values although there was an effect of exercise with concentrations rising from a baseline level of
APPENDIX I

~1.3mmol.l⁻¹ and plateauing during exercise at ~2.3mmol.l⁻¹. There were no trial differences in serum prolactin although there was an effect of time with concentrations increasing with exercise duration ($F_{1,8} = 6.64; p<0.05$). There was a tendency for serum cortisol concentrations to be higher in the placebo trial during exercise but this only reached significance at 60min (Figure 4). Cortisol concentrations were no different from baseline and plateaued through exercise but increased to a peak at the onset of fatigue ($F_{2,15} = 10.182; p<0.05$).

**Physiological Variables**

There were no differences between treatments for any of the following physiological variables: respiratory exchange ratio, ratings of perceived exertion, oxygen uptake and heart rate.

**Experimental Variables**

There were no trial order effects nor were there any environmental differences between trials.
Figure 1  Running capacity (min) during exhaustive treadmill running at 70%VO_{2max}. (Mean ± SEM)

Figure 2  Plasma Glucose concentrations (mmol·l^{-1}) during exhaustive treadmill running at 70%VO_{2max} (Mean ± SEM). *p<0.05, CHO vs. PLA at 20 min and 80 min
APPENDIX I

Figure 3  Plasma FFA concentrations (mmol·l⁻¹) during exhaustive treadmill running at 70%VO₂max (Mean ± SEM)

Figure 4  Serum Cortisol concentrations (mmol·l⁻¹) during exhaustive treadmill running at 70%VO₂max (Mean ± SEM)
* p<0.05, CHO vs. PLA at 60 min; § p<0.05, fatigue vs. others
Results from this preliminary study suggest that a 6.4% hypotonic CHO-E solution may confer ergogenic benefits over a taste-matched placebo during continuous treadmill running to fatigue. Although the data failed to reach statistical significance this was probably due to the limited number of subjects. An a priori power analysis revealed that to achieve a significant difference ($p<0.05$) between treatments whilst maintaining the same power and effect size as in the present study would require 22 subjects to be tested. The lack of treatment effect was further exacerbated by the inter-individual differences between subjects which were a consequence of using both elite and recreational athletes. Despite the lack of statistical significance the difference between the two treatments amounts to an improvement in performance in the magnitude of 16% in the hypotonic trial. This is similar to data from Tsintzas et al (1996) who report an 11% and 14% increase in treadmill running capacity at 70%$VO_2$max when fed 6.9% and 5.5% isotonic CHO-E solutions, respectively, compared to water. It should be noted that the isotonic CHO-E solutions in Tsintzas and colleagues study were only administered for the first 60min of exercise with subjects consuming water for the remaining duration in all trials.

Blood data from the present study reveal that plasma glucose concentrations in the hypotonic trial, despite an initial decrease below baseline values, were elevated above those of the placebo trial throughout exercise reaching significance at 80min. Although no difference was noted between treatments for insulin concentrations, which is again in agreement with Tsintzas et al (1996), there was a trend for FFA concentrations to be higher throughout the placebo trial. This suggests an increased mobilisation of this substrate for utilisation during exercise. Unfortunately respiratory exchange ratio data were unable to confirm an increased dependence on lipid utilisation in the placebo trial during the run to fatigue.

Despite the lack of definitive findings in this preliminary study the results would suggest that a 6.4% hypotonic CHO-E solution is effective at delivering glucose to the systemic circulation and has a suppressive effect on FFA mobilisation. Furthermore the results suggest that such a solution would enhance exercise capacity at 70%$VO_2$max. Therefore it is warranted to investigate the efficacy of such solutions during intermittent exercise at a similar intensity.