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INFLUENCE OF NUTRITION ON MUSCLE METABOLISM AND PERFORMANCE DURING PROLONGED INTERMITTENT HIGH INTENSITY SHUTTLE RUNNING IN MAN

by

Ceri Wyn Nicholas

A Doctoral Thesis

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

May 1996

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ABSTRACT

The purpose of this thesis was to study the effects of 1) carbohydrate intake, either during or in the recovery from exhaustive intermittent exercise, and 2) oral creatine supplementation for 5 days, on metabolism, performance and endurance capacity during a prolonged intermittent high intensity shuttle run test (PIHSRT).

Prolonged intermittent high intensity running is an activity pattern which is typical of the multiple sprint sports such as soccer, hockey and rugby. Understanding the physiological and metabolic responses to this type of activity can improve our understanding of the aetiology of fatigue and how training and nutritional intervention can improve performance during prolonged intermittent high intensity running. The aims of the first and second studies were to investigate the effect of increased carbohydrate availability, by ingesting additional carbohydrate in the recovery from (Chapter 4) and by drinking a carbohydrate-electrolyte solution immediately prior to, and during (Chapter 5), prolonged intermittent high intensity running.

In the first study (Chapter 4), six games players performed two prolonged intermittent high intensity shuttle run tests (PIHSRT) consisting of a fixed 70 min period of intermittent exercise (Part A) followed by high intensity intermittent running to fatigue (Part B), separated by 22 h recovery, on two occasions, separated by one week. During the 22 h recovery, subjects were prescribed their normal dietary intake of carbohydrate plus the additional energy needed to consume the equivalent of 10 g.kg\(^{-1}\)bm.day\(^{-1}\). This extra energy was consumed either in the form of carbohydrate (the CHO trial) or as fat and protein (the CON trial). No differences in sprint performance were observed 22 h following either recovery diet. High intensity running capacity was improved after 22 h in the CHO trial (T2) by 16% (P<0.01). No such improvement was observed in T2 following the CON diet. No differences were observed in the blood metabolic responses to the PIHSRT.

In the second study (Chapter 5), nine games players performed the PIHSRT on two occasions, separated by one week. Subjects were randomly assigned to ingest either a 6.9% CHO-E beverage (CHO trial) immediately prior to and at regular intervals throughout the PIHSRT or a non-CHO placebo (CON trial). The PIHSRT comprised a fixed period of 75 min of variable intensity exercise (Part A) followed by intermittent high intensity running until fatigue (Part B). Sprint performance was similar during both trials and was not affected by treatment. High intensity running capacity was improved by 33% following CHO-E ingestion (P<0.05). Blood glucose concentration was higher after 30 min (P<0.05) and after the cessation of exercise (P<0.05) in the CHO-E trial but similar concentrations were observed after 75 min of exercise, immediately prior to the exhaustive bout of high intensity running. The results of these two initial studies showed that the ergogenic effect of higher pre-exercise muscle glycogen stores (Chapter 4) and drinking a 6.9% CHO-E solution during exercise was not associated with the prevention of a declining blood glucose concentration over the duration of the exercise trials.

The hypothesis that the underlying mechanism for the increased running capacity was the sparing of muscle glycogen was investigated in the third study (Chapter 6). Six games players completed 90 min of the PIHSRT twice in a random order, separated by one week. Subjects consumed either a 6.9% CHO-E solution (CHO trial) or a non-CHO placebo (CON trial). Biopsy samples were obtained from the vastus lateralis muscle at rest and immediately following the end of exercise for the determination of mixed and single muscle fibre glycogen concentration. A 22% reduction was observed in the CHO trial compared with the CON trial [ 192.5± 26.3 mmol (kg DM)\(^{-1}\) vs. 245.3 ± 22.9 mmol (kg DM)\(^{-1}\) , respectively; P<0.05]. Single fibre analysis revealed a reduced glycogen concentration in both fibre types, but that there was a greater amount of glycogen utilised in the type II fibres compared with the type I fibres during 90 min of intermittent high intensity exercise in the CON [ 287.4± 41.2 mmol (kg DM)\(^{-1}\) vs. 182.2 ± 34.5 mmol (kg DM)\(^{-1}\) , respectively; P<0.05].
DM)·1, respectively; P<0.01]. Results indicate that there was a greater amount of glycogen utilised in both type I and type II fibres in the CON trial compared with ingesting a CHO-E solution, although no conclusive statement can be made due to the small sample size. A reduction in the amount of glycogen utilised was associated with a higher serum insulin concentration after 30 min in the CHO trial. The apparent sparing of muscle glycogen may be due to a reduction in the amount of glycogen utilised per se, or the result of glycogen resynthesis in the inactive fibres during variable intensity exercise. In support of a reduction in the breakdown of muscle glycogen was the lower blood lactate concentration observed after 30 min in the CHO trial.

The aim of the next section (Chapter 6, Part B) was to investigate whether the reduced concentration of muscle glycogen in type I and II muscle fibres following 90 min of the PIHSRT would affect muscle function. The influence of drinking a carbohydrate-electrolyte beverage during exercise, which was previously found to reduce the amount of glycogen utilised (Chapter 6, Part A), on muscle function was also examined. The same 6 subjects participated in the second part of the study as did in Part A. An assessment of muscle function was made by measuring peak torque, total work and average power at angular velocities of 60°/s and at 240°/s in the knee extensors (concentrically and eccentrically) and flexors (concentrically) before and after 90 min of the PIHSRT. Muscle function was reduced after 90 min of shuttle running at 60°/sec in both trials, although the differences were attenuated following the ingestion of a carbohydrate-electrolyte beverage at regular intervals throughout exercise.

In the previous study (Chapter 6, Part A), the post-exercise muscle concentration of PCr was lower after 90 min of shuttle running in the CON trial than the CHO trial (P<0.05). Thus, the purpose of the next study (Chapter 7) was to investigate the effect of oral creatine supplementation on sprint performance and endurance capacity during the PIHSRT. Sixteen male games players performed the PIHSRT for 75 min (Part A) followed by intermittent running to exhaustion (Part B) on two occasions, separated by one week. Following the first, pre-supplementation trial (T1) subjects were randomly assigned to either a placebo (Plac) or creatine (Cr) group in a double blind design. The supplementation regime was three 6 g doses of either 3 g of creatine monohydrate and 1.5 g maltodextrin, plus 1.5 g glucose (Cr group), or 6 g glucose (Plac group) for 5 days. The second trial (T2) was performed immediately following the end of the 5 day supplementation period. There were no differences in sprint times or running time to exhaustion between trials. Plasma ammonia concentrations were lower after 60 min and 75 min of exercise in T2 compared with T2 in the Cr group (P<0.05). Similar physiological and metabolic responses were observed between trials (within groups) for all other variables measured.

Increasing the availability of carbohydrate, either by increasing the normal carbohydrate intake to the equivalent of 10 g.kg·1·bm per day, or by ingesting a carbohydrate-electrolyte beverage which provides 47 g carbohydrate per hour immediately prior to and at regular intervals during exercise, enhances endurance running capacity following a fixed period of prolonged intermittent high intensity shuttle running. No such improvement was observed following an oral creatine supplementation period. Sprint performance during the PIHSRT did not change either following carbohydrate or creatine supplementation. The mechanism for the improved performance may be the sparing of muscle glycogen in the type I and type II fibres during intermittent high intensity exercise when a carbohydrate-electrolyte solution is ingested.
Unless otherwise indicated the work contained in this thesis is that of the author and has not been previously submitted for another degree in this or any other University.

Part of the work contained in this thesis has been published in the following scientific journals:

**Published Communications**


**Published Paper**

This thesis is dedicated to my parents,

Brian and Betty
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CHAPTER 1

INTRODUCTION

1.1 Introduction

The premature onset of fatigue limits performance in many sports, and can be delayed through appropriate training (Williams, 1990). Fatigue is considered to be the failure to maintain the required or expected force leading to a reduced performance of a given task (Edwards, 1981). Thus during dynamic physical activity, fatigue results as a consequence of the metabolic failure to provide energy at the rate at which it is required by the working muscle. The aetiology of fatigue will depend on the nature of the activity engaged in, and can occur as a result of impairment of one or several links in the chain from the central nervous system to the contractile machinery (Sahlin, 1992a). During repeated sprints, fatigue is associated with accumulation of the end products of metabolism, whereas the depletion of muscle glycogen in the active muscles coincides with fatigue in endurance events (Williams, 1990). However, many sports are comprised of a mixture of both a sprint and endurance component, thus making the identification of fatigue more problematic. Nevertheless, understanding the metabolic and physiological responses to prolonged intermittent high intensity exercise will further our knowledge of the aetiology of fatigue during this type of exercise.

High intensity intermittent exercise describes the pattern of activity seen in many ball sports, e.g., soccer, rugby, hockey, squash and tennis, where brief work periods (of less than 10 s duration) are randomly interspersed with longer periods of walking, jogging or standing still. Few studies have examined the physiological response to this activity pattern which is typically experienced by those participating in the 'multiple sprint' sports.
Early research studies which investigated the physiological and metabolic responses to intermittent exercise in the laboratory have used protocols comprised of work periods which were equal to, or less than, 100% VO$_2$ max interspersed with either active or passive recovery periods. More recent studies have investigated the physiological and metabolic responses to high intensity intermittent exercise, characterised by a similar exercise pattern, however the intensity of the exercise during the work periods was two to three times greater than 100% VO$_2$ max (Wootton and Williams, 1983).

During exercise, adenosine triphosphate (ATP) turnover increases at a rapid rate in order to fuel contractile activity. When exercise ceases, homeostasis must be restored within the muscle, and ATP turnover remains elevated during the recovery period. However, studies which have sought to investigate the relative contribution of the different energy sources to ATP resynthesis during repetitive brief high intensity exercise have documented contrasting observations. Earlier research which focused on the metabolic responses to high intensity intermittent exercise comprising brief work periods (10-15 s) interspersed with short passive recovery periods (15-40 s) reported that the contribution of glycogenolysis to the total energy demand was negligible (Margaria, 1969; Saltin and Essen, 1971) and much lower than if the same exercise intensity was performed continuously (Astrand et al., 1960a; Christensen et al., 1960a; Christensen et al., 1960b; Essen et al., 1977; Essen and Kaijser, 1978). This pioneering work concluded that lipids, particularly from intramuscular triacylglycerol stores, contributed more to substrate utilisation during intermittent than continuous exercise of the same intensity. However, the high intensity exercise referred to was that which was equivalent to or less than maximal oxygen uptake and therefore cannot be described as maximal (Lakomy, 1984; Lakomy, 1986).

Subsequent studies have shown that during a single bout of brief (<10 s) dynamic maximal exercise, energy provision is mainly provided through anaerobic pathways, that is, phosphocreatine (PCr) degradation and glycogenolysis resulting in the formation of lactate (Boobis et al., 1987; Jacobs et al., 1983; Gaitanos et al., 1993). In terms of energy provision
during maximal intermittent exercise, it has been suggested that a brief recovery duration
(30 s) between maximal intermittent exercise bouts of 6 s duration would result in reduction
of the muscle PCr content, and thus increase the reliance on glycogenolysis to provide ATP
anaerobically during subsequent bouts of exercise (Holmyard et al., 1988; Wootten and
Williams, 1983). However, blood lactate measurements were the only observations on
which this speculation was based. In contrast, the results of several subsequent studies which
measured muscle metabolite concentrations before and after repeated 30 s sprints
(McCartney et al., 1986 and Spriet et al., 1989) and repeated 6 s sprints (Gaitanos et al.,
1993) have reported a reduced anaerobic energy yield and thus a significant shift to aerobic
metabolism towards the end of exercise concomitant with the observed decrease in power
output. Consistent with this observation was the reported 500% increase in plasma glycerol
during maximal intermittent exercise in the study by McCartney and co-workers (1986),
suggesting an increased metabolism of intramuscular triacylglycerides and, possibly, plasma
free fatty acids.

Thus, PCr, carbohydrate (CHO) and fat are the main substrates for energy provision during
high intensity intermittent exercise. The relative contribution of each of these substrates will
depend on the intensity and duration of exercise, although other factors such as training
status, diet, environment and gender may also be influential (Costill, 1988; Hargreaves,
1991). Although the body's stores of CHO are limited, it is possible to increase the
concentration of liver and muscle glycogen by diet. Trained individuals are able to utilise glycogen more economically during exercise than before training. Nevertheless, prolonged high intensity intermittent exercise cannot continue indefinitely. Fatigue, or the failure to maintain the required energy output will occur and the exercising individual will have to reduce the exercise intensity. During high intensity intermittent exercise, the cause of fatigue has been associated with glycogen depletion in exercising muscles (Bangsbo, 1994). It is therefore not surprising that researchers have advocated an increased dietary CHO intake before, during and after exercise in order to ensure adequate CHO availability during exercise (Coyle, 1991; Costill and Hargreaves, 1992). Such dietary practices have been
extensively studied during prolonged continuous submaximal cycling and, more recently, running.

Other exercise protocols that have received attention in the research literature have involved prolonged intermittent or continuous cycling for a fixed duration followed by a performance or endurance capacity test. In these tests, performance was measured by completing a fixed amount of work in as short a time as possible. Endurance capacity was assessed as the time recorded to fatigue at a fixed exercise intensity. However, until recently only a few studies (Bangsbo et al., 1992b; Nevill et al., 1993) have attempted to utilise exercise protocols which attempt to replicate the demands of the ‘multiple sprint’ sports, i.e., brief periods of high intensity exercise (sprinting and striding) interspersed with longer periods of lower intensity exercise or rest (jogging, walking and standing still). Thus a form of controlled exercise which simulated the activity pattern during those sports needed to be developed. Therefore, for the purpose of this thesis, a protocol was designed which comprised of patterns of activity similar to those reported during soccer, rugby and hockey matches. That is, prolonged intermittent high intensity exercise.

The influence of nutritional intervention on performance during intermittent high intensity exercise was studied in an attempt to understand the process of fatigue during this type of activity and how it may be delayed for the benefit of sportsmen and sportswomen participating in sports demanding this type of activity pattern. Thus this thesis describes the following studies:

1) the influence of CHO supplementation in the recovery period following exercise on subsequent high intensity intermittent exercise performed 22 h later,

2) the effect of CHO ingestion during exercise on sprint performance and endurance capacity,
3) the effect of CHO ingestion during exercise on glycogen utilisation during, and on muscle function following, 90 min of intermittent high intensity shuttle running and,

4) the effect of creatine (Cr) supplementation prior to high intensity intermittent exercise on performance during this type of exercise.

The physiological and metabolic responses to high intensity intermittent exercise were studied with and without dietary manipulation in order to establish the relationship between an increased CHO availability during high intensity intermittent exercise and fatigue.

1.2 Organisation of Thesis

This thesis is presented in eight main chapters. The review of literature (Chapter 2) examines the available studies documenting the physiological and metabolic responses to high intensity intermittent exercise and the 'multiple sprint' sports. The possible mechanisms responsible for the onset of fatigue during high intensity intermittent exercise are examined. Subsequently, those studies which have investigated the effect of dietary manipulation strategies on performance during high intensity intermittent exercise are also reported.

The general methods chapter (Chapter 3) describes the high intensity intermittent exercise protocol developed for the purpose of this thesis and outlines the equipment and the testing procedures used during the studies undertaken and reported in this thesis. The procedures associated with collection and analysis of blood and muscle samples are also documented in this section.

Recovery from prolonged intense exercise is important for sportsmen and women who have to compete and train on a daily basis. Therefore, the purpose of the first study (Chapter 4) was to investigate the influence of an increased CHO intake during the recovery period after prolonged high intensity intermittent exercise on subsequent performance and metabolism.
when this exercise was repeated 22 h later.

The ingestion of CHO beverages during prolonged exercise has the twin aim of offsetting dehydration and providing a source of fuel for the exercising muscles. Thus, the purpose of the second study (Chapter 5) was to examine 1) the effect of ingestion of a carbohydrate-electrolyte (CHO-E) beverage on high intensity intermittent exercise performance and 2) describe some of the physiological and metabolic changes occurring during high intensity intermittent exercise with and without CHO ingestion.

In an attempt to explain the differences in performance observed in Chapter 5, a third study was undertaken (Chapter 6, Part A). A 90 min non-fatiguing bout of high intensity intermittent exercise was performed in order to examine the contribution of muscle glycogen to muscle metabolism during this type of prolonged exercise.

Muscle strength has been shown to decrease following glycogen depletion which may affect performance during a brief period of high intensity exercise. Thus, a sub-study (Chapter 6, Part B) was carried out to examine the effect of a 90 min bout of high intensity intermittent exercise with and without the ingestion of a CHO-E beverage on isokinetic muscle function.

Manufacturers of a Cr supplementation product have claimed that Cr ingestion is particularly useful for sports that involve intermittent high intensity efforts. Therefore the aims of the fourth study (Chapter 7) were to 1) investigate the effect of Cr supplementation on performance during the repeated sprints and at the end of exercise during the high intensity intermittent exercise protocol designed specifically for this thesis and b) determine whether Cr supplementation was more advantageous to the games player than the ingestion of a CHO-E beverage during exercise.

The final chapter (Chapter 8) provides an overview, and integrates the observations made from the studies carried out for the purpose of this thesis, in an attempt to understand the
metabolic and physiological responses to prolonged intermittent high intensity exercise. As a result, our knowledge of the aetiology of fatigue during this type of exercise will be enhanced.
CHAPTER 2

REVIEW OF LITERATURE

2.1 Introduction

The purpose of this chapter is to present and discuss the pertinent literature documented over the last few decades which has examined the physiological and metabolic responses to intermittent high intensity exercise and the influence of nutritional intervention on the development of fatigue during this type of exercise. The studies included have investigated the influence of nutritional intervention in the form of CHO intake immediately before, during and in the recovery following exercise, and also included the influence of Cr supplementation prior to intermittent high intensity exercise.

The review of literature consists of twelve main sections. The first section following the introduction (section 2.2) describes the fundamentals of intermittent exercise relating to the multiple sprint sports. Section 2.3 examines the physiological and metabolic responses to high intensity intermittent exercise and how they compare with continuous exercise. The distinction is made between intermittent exercise where the exercise intensity is equal to or less than 100% VO₂ max and that which involves maximal 'all-out' exercise. Section 2.4 reviews the studies which have examined muscle metabolism during intermittent exercise. Intermittent high intensity, or maximal, exercise is examined in section 2.5, focusing on maximal intermittent exercise of both 30 s and less than 6 s duration. The physiological and metabolic responses to intermittent high intensity exercise, with particular reference to the multiple sprint sports are reviewed in section 2.6. The next section (section 2.7) examines the possible causes of fatigue during this type of exercise, and sections 2.8 to 2.11 deal with the different nutritional strategies which have been used in order to delay the onset of fatigue. These include an increased CHO intake prior to intermittent high intensity
exercise (section 2.8), fluid and CHO ingestion during exercise, and the mechanisms responsible for the observed ergogenic effect with CHO ingestion (section 2.9), CHO supplementation during recovery from high intensity intermittent exercise (section 2.10) and Cr supplementation prior to intermittent high intensity exercise (section 2.11). Finally, the studies which have reported the physiological demands of the multiple sprint sports by time motion analysis are examined in section 2.12.

2.2 Intermittent Exercise and the Multiple Sprint Sports

Few laboratory studies to date have used exercise protocols which have attempted to replicate the demands of the multiple sprint sports (Bangsbo et al., 1992b; Nevill et al., 1993). This type of intermittent exercise comprises brief periods of intense muscular activity interspersed with periods of lower intensity exercise, or rest. The test protocol in the study of Bangsbo et al. (1992b) comprised a 46 min field test over a distance of 6856 m (Part A) followed by treadmill running to exhaustion (Parts B and C). The standard field protocol consisted of an 18 min warm-up including stretching (8 min), low (6 min) and moderate speed running (4 min) followed by two 14 min periods which were identical in nature. Activity during each of these periods alternated between 8 different activities: standing (1:35 min:s), walking (1:00 min:s), jogging (3:50 min:s), low speed running (3:15 min:s), backwards running (0:20 min:s), moderate speed running (2:55 min:s), high speed running (0:35 min:s), and sprint running (0:30 min:s). Part B consisted of seven identical intermittent exercise periods (35 min) and during Part C, treadmill speed alternated between 8 km.h⁻¹ (later 12 km.h⁻¹) and 18 km.h⁻¹ for periods of 10 and 15 s, respectively, until exhaustion. The results of this study demonstrated that when the intermittent exercise test was performed after a CHO-enriched diet (65% CHO) for 2 days, exercise capacity was improved by 0.9 km compared with a diet containing only 39% CHO.

The effect of an increased CHO intake, this time in the recovery period between exercise bouts, was studied by Nevill et al. (1993). The 60 min test protocol comprised 30
maximum 6 s sprints on a non-motorised treadmill, interspersed by a 54 s walk at 20% maximum speed followed by a 60 s jog at 40% maximum speed. Subjects were randomly assigned to either a high, normal or low CHO dietary group (79%, 47%, 12% CHO, respectively) during the 24 h recovery period before repeating the test. No differences in sprint performance were recorded between groups during the second trial. Results showed a reduced performance of, and metabolic response to, maximum intermittent exercise when the test was performed again after 24 h recovery in all 18 subjects. Further analysis by group showed that there was a tendency for sprint performance over 30 sprints to be reduced by 0.2% following the high CHO diet, by 0.5% after the normal CHO diet, and by 5% following the recovery diet low in CHO. However, several points should be made about this particular study. Firstly, the duration of the exercise test was only of 1 h, and secondly, it was a performance not a capacity test. Therefore, it would be unlikely that enhanced glycogen stores prior to the second trial would influence performance. In addition, the energy content of the recovery diet was unchanged; only the percentage of CHO intake was manipulated for the different recovery diets. Therefore, the increased energy expenditures of the subjects were not covered by their energy intake during the recovery.

In the field setting, sport-specific fitness tests have been used (Bangsbo et al., 1992b; Steininger and Wodick, 1987; Chin et al., 1995a; Chin et al., 1995b). The studies by Chin and colleagues were carried out on a badminton court. Six light bulbs were connected to a programming device causing individual bulbs to light up in a given sequence. The players were instructed to react to the flashes by running towards them, and striking shuttles mounted in the vicinity of the bulbs. The exercise intensity increased every 3 min, and was controlled by altering the interval between successive lightings. The test lasted up to a maximum of 24 min and the results obtained were used as an estimate of fitness. Heart rates and blood lactate concentrations (Mean ± SD) averaged 187 ± 8 b.min⁻¹ and 10.4 ± 2.9 mmol.l⁻¹, respectively, indicating a large physiological load. However, with this type of protocol lasting only 24 min, it is unlikely that depletion of glycogen stores would be a
factor limiting performance.

A greater understanding of the physiological and metabolic responses to intermittent high intensity exercise, such as that observed during the multiple sprint sports, and in sports-specific field studies, has come about by a continued focus on repeated exercise bouts by researchers over the last 50 years.

2.3 Physiology and Metabolism of Intermittent vs. Continuous Exercise

It is important to make a distinction between the different protocols of the initial pioneering work with regard to the comparison of continuous and intermittent exercise. Early workers on one hand studied submaximal exercise and matched the total work accomplished in repeated exercise at an intensity equal to or less than (≤) VO₂ max, with steady state continuous exercise to compare the energy cost and the cardiorespiratory responses to continuous and intermittent exercise (Astrand et al., 1960a and 1960b; Christensen, 1960a; Fox et al., 1969; Edwards et al., 1972c; Margaria et al., 1969). In addition, intermittent exercise performed at the same average power output as continuous exercise was also investigated (Astrand et al., 1960a; Edwards et al., 1971; Edwards et al., 1972c; Edwards et al., 1973). In these latter studies the exercise intensity during intermittent exercise was thus nearly twice that of continuous exercise.

The metabolic energy sources during continuous and interval running were studied by Fox and colleagues (1969). They observed that during interval running, blood lactic acid accumulation and the respiratory 'oxygen debt' (calculated by subtracting the resting oxygen consumption from that utilised during recovery) were always much lower than when the same total amount of work was performed continuously. The authors concluded that the decrease in energy from glycolysis, evident from the lower blood lactate concentration, during interval running was compensated for by a proportional increase in energy supplied via the so-called 'anaerobic' processes, including oxygen bound to
myoglobin and the resynthesis of the high-energy phosphate bonds of ATP and PCr. However, direct measurements of PCr utilisation were not made.

Other researchers had also reported an increased blood lactate concentration following continuous exercise compared with intermittent exercise of the same work rate (Edwards et al., 1971) but when performed at the same average power output or in a predetermined time, blood lactate was higher during intermittent exercise due to the higher work load required (Edwards et al., 1971; Edwards et al., 1972c).

Margaria and colleagues (1969) exercised their subjects intermittently for a 10 s period at an exercise intensity which resulted in exhaustion after 30-40 s when performed continuously. The exercise periods alternated with rest intervals of 10, 20 or 30 s on separate occasions. When the rest period was 10 s and 20 s, the total running time was increased about three and six times that of the continuous run at the same intensity, respectively. However, when the rest interval was 30 s, the exercise could continue indefinitely. Blood lactate concentration increased progressively during repeated 10 s runs, and was inversely related to the length of rest period, such that with 30 s rest it was only slightly elevated.

It was concluded that when a given quantity of work has to be done in a set time a somewhat smaller rise in blood lactate concentration is to be found if the work is performed at a continuous low rate than if performed intermittently with a higher work load (Edwards et al., 1971; Edwards and colleagues, 1972c). The metabolic response to intermittent exercise was related to that found in continuous exercise at half the work load rather than at the same work level (Saltin et al., 1976; Essen et al., 1977). This indicates that some regulatory factors, possibly citrate, are brought into play inhibiting glycolysis and enhancing fat oxidation in intermittent exercise (Essen et al., 1977).
Despite the common observation that when performed intermittently, high exercise intensities can be sustained for longer periods than if the same intensity of exercise was performed continuously, higher rates of perceived exertion (RPE) on the Borg scale (1973) are reported for intermittent exercise compared with continuous exercise at the same average power output. The difference in exercise intensity accounts for part of this difference, as does the duration of the exercise period during intermittent exercise; the longest duration resulting in the highest RPE (Saltin et al., 1976). During intermittent exercise with short exercise and rest periods, and for the longer duration exercise and rest periods with a range of ±25% absolute power output (APO), the RPE-heart rate and RPE-V̇O₂ relationships are virtually the same as in continuous exercise. However, in the long-period intermittent work with larger differences in exercise intensity a certain average heart rate or VO₂ was accompanied by higher RPE scores, possibly due to the higher exercise intensity. Lactate concentrations during intermittent exercise are sensitive to both work rate and exercise duration (Ballor and Volovsek, 1992).

Early researchers believed that the ATP required to fuel muscular contraction during brief exercise was supplied using oxygen stored with myoglobin (Astrand et al., 1960a and Astrand et al., 1960b). It was assumed that the contribution of glycogenolysis and glycolysis to energy provision was negligible. This conclusion was based on the evidence that there were only minor increases in blood lactate concentration following brief exercise and oxygen uptake was increased during both work and recovery periods (Christensen et al., 1960a). However, subsequent studies have observed that lactate can be produced in the muscle during exercise without an accompanying rise in blood lactate concentration (Hermansen and Stensvold, 1972). In addition, the role of PCr degradation in skeletal muscle metabolism during high intensity work periods, and the subsequent oxygen demand for its resynthesis during recovery periods, was not considered in these experiments carried out in the 1950's and early 60's. The first reference to PCr as a key energy substrate during high intensity intermittent exercise appeared in the late 1960's (Margaria et al., 1969; Fox et al., 1969).
2.4 Muscle Metabolism During Intermittent Exercise

The introduction of the muscle biopsy technique (Bergstrom, 1962) enabled scientists to study muscle metabolism during intermittent exercise for the first time (Saltin and Essen, 1971). Subjects performed intermittent exercise for 30 min at a supra-maximal work load of approximately 400 watts (2,400 kpm.min\(^{-1}\)). The work and rest periods during the intermittent exercise were varied in the following manner a) 10 s work, 20 s rest, b) 20 s work, 40 s rest, c) 30 s work, 60 s rest, d) 60 s work, 120 s rest. The ratio between rest and exercise was 1 : 2 and the subjects attained a total work output of approximately 4000 watts (24,000 kpm) in each experiment. Muscle biopsies were taken from the quadriceps muscle before exercise and approximately 5, 15 and 30 min after the exercise started. They were taken both immediately after an exercise period and at the end of a rest period. It was observed that heavy exercise periods which were less than 15 s duration did not result in an increase in blood lactate. However, blood lactate concentration was elevated above resting values with longer exercise bouts of 30 and 60 s duration. The ratio between rest and exercise was maintained in each experiment at 2 : 1. Muscle ATP and PCr degradation was most marked following the longer exercise periods, and there was a significant increase in these metabolites during the recovery between exercise bouts when the rest interval was greater than 20 s. The depletion of muscle glycogen during intermittent exercise was reported to be less than that observed with continuous heavy exercise. These observations confirmed the hypothesis that the myoglobin in the muscle served as an oxygen store, being utilised at the onset of exercise, and refilled during the intervening rest period. However, the results of this study did not support earlier suggestions that PCr was a crucial energy substrate during high intensity intermittent exercise.

Subsequently, Edwards and co-workers (1972b) reported that the half-time of PCr resynthesis was between 20 and 30 s, which does not appear to be consistent with the findings of Saltin and Essen (1971) that the PCr concentration in the vastus lateralis muscle
was similar after 10 s of cycle ergometer exercise and then after a 20 s recovery period. However, it is important to consider whether the PCr concentrations reported by Saltin and Essen (1971) were representative of the actual metabolic conditions in the muscle immediately following exercise and at the end of the 20 s recovery period. The muscle biopsy procedures were not documented, and as it has been subsequently demonstrated that the time delay between the cessation of exercise and freezing the muscle sample can affect PCr concentration (Soderlund and Hultman, 1986; Bogdanis et al., 1995), the results should be treated with caution.

Another study by Edwards and co-workers (1973) failed to confirm the importance of PCr as an energy substrate during high intensity, intermittent exercise, as PCr concentrations in the vastus lateralis muscle were not different immediately following brief exercise (10 s) and after short recovery (30 s) periods. The first evidence that PCr concentrations increased over the duration of the recovery periods during high intensity, intermittent exercise, was reported by Essen et al. (1977). In this study, Essen et al. (1977) observed similar metabolic responses during 1 h of both intermittent intense cycle exercise (15 s exercise: 15 s rest) and continuous exercise with an almost identical average power output and oxygen uptake. The work load during intermittent exercise corresponded to that which elicited VO$_2$ max during continuous exercise. These results suggest that fat oxidation is enhanced during intermittent exercise as a consequence of some regulatory factors, possibly citrate, retarding the rate of glycolysis. The aerobic energy contribution during intermittent exercise is thought to be partly due to myoglobin functioning as an oxygen store. Glycogen depletion, muscle lactate concentration and the uptake of blood-borne substrate (FFA, glucose) was similar in both modes of exercise however, there was a lower release of lactate in continuous exercise. In intermittent exercise, the ATP and PCr concentrations increased between exercise and rest periods, which did not return to resting levels by the end of each rest period. After 5 min of the intermittent exercise, the PCr concentration was 40% of the resting level after an exercise period, and it increased to about 70% of rest in the subsequent 15 s recovery period. Similar changes were found
during the following 55 min of the intermittent exercise. It was calculated that approximately half of the oxidative metabolism during the intermittent exercise was covered by lipid substrates. This was similar to the fat oxidation during the continuous exercise at the same mean work rate.

Intermittent and continuous exercise rely on the recruitment of different types of muscle fibres. During continuous exercise at 50-60% VO₂ max it is mainly type I fibres that are activated, however both type I and II fibres are recruited during intermittent high intensity exercise (Edgerton et al., 1975; Saltin et al., 1976; Essen, 1978a).

In one of the series of studies by Essen (1978b) continuous exercise was also performed at the same power output as during the intermittent work periods (this corresponded to VO₂ max). When performed intermittently, exercise was sustained for 1 h without fatigue, but exhaustion was reached within a few min of continuous exercise. Glycogen reduction and lactate accumulation was greater, and the rate of fat oxidation lower, during continuous exercise. The differences in energy metabolism between the two modes of exercise may be partly explained by the use of intramuscular myoglobin in intermittent exercise during the work periods, which is replenished during the rest periods, thus resulting in a higher aerobic contribution during intermittent exercise.

Essen and Kaijser (1978) examined the regulation of glycolysis in intermittent exercise. Intermittent exercise (15 s exercise : 15 s rest) performed at a high workload for 60 min was compared with continuous exercise performed at an equally high work load until exhaustion, which occurred after 4-6 min. Muscle biopsies were obtained from the lateral portion of the quadriceps muscle before intermittent exercise and after the end of a work period and the end of the subsequent rest period at 5, 15, 30 and 60 min of exercise, as well as before, immediately after and about 15, 30, 60 and 180 s after continuous exercise. A lower rate of glycolysis, indicated by the lower rate in muscle glycogen utilisation, G-6-P, G-1-P, lactate and malate concentrations, was observed in intermittent compared with
continuous exercise. The reduced muscle ATP and PCr concentrations by the end of the work periods in intermittent exercise returned to almost basal levels by the end of the subsequent rest periods. At exhaustion after continuous exercise, muscle ATP and PCr concentrations decreased to lower values than those observed after intermittent exercise, but increased progressively during the 3 min recovery period. Citrate concentration increased above basal values in each rest interval between intermittent exercise bouts, and also increased, albeit at a slower rate, in the recovery period following continuous exercise. The results of this study support the assumption that glycolysis is regulated by ATP, PCr and citrate by retarding certain rate limiting steps, possibly at the phosphofructokinase (PFK) reaction. Consequently, there is a relative increase in lipid utilisation during intense intermittent compared to continuous exercise.

Thus, by the end of the 1970's, understanding of the contribution and control of metabolism during brief high intensity intermittent exercise was not complete. Compared with the magnitude of studies which had concentrated on substrate utilisation during continuous exercise, only a few had examined the contribution of substrates to energy provision during repeated bouts of high intensity exercise, predominantly due to the lack of an appropriate exercise model to measure power output during maximal exercise. It should be noted that the exercise intensities in these previous investigations were performed at a constant rate of work, related to the maximal aerobic capacity (VO₂ max) of the subjects, not to the maximal power output of the muscles involved. During brief maximal exercise, the power output is two to three times that recorded as 'maximal' during a maximal oxygen uptake test (Lakomy, 1984, 1986), and therefore the exercise intensities were considerably lower than the power output attained during maximal 'all-out' exercise.

The factors which determined power output during repeated short duration bouts of maximal exercise were investigated at the beginning of the 1980's, when an appropriate exercise model to evaluate performance during high intensity exercise was developed at the Wingate Institute (Bar-Or, 1987).
2.5 Intermittent High Intensity or Maximal Exercise

2.5.1 Physiology of Intermittent High Intensity or Maximal Exercise

High intensity, or maximal intermittent exercise describes an exercise pattern where the intensity of the exercise during the work periods is greater than 100% VO\textsubscript{2} max. Exercise protocols which reflect such an activity pattern have been used in the laboratory to investigate skeletal muscle metabolism, and hence further enhance our understanding of the aetiology of fatigue. In such protocols the exercise periods are usually of a fixed duration, lasting anywhere from a few seconds to over one min, and repeated at regular intervals. However, it must be noted that whilst the variation in intensity and duration of the exercise is regular in the laboratory, during a soccer match there is no such pattern and the changes in exercise intensity are usually more frequent. The activity pattern of many ball sports, e.g., soccer, tennis, hockey and rugby, can be characterised as intermittent high intensity exercise, where work periods of intense short duration (5-6 s) exercise are interspersed with longer recovery periods of either walking, jogging or standing still. During this form of exercise, unique demands are placed on metabolic process in the muscle where energy supply fluctuates between fuelling contractile activity and restoring resting metabolism. Performance and metabolism is ultimately influenced by the nature of previous exercise. While energy is produced primarily via anaerobic pathways during a short bout of maximal-intensity exercise (Boobis et al., 1982; Cheetham et al., 1986), energy for recovery processes appears to be derived exclusively from aerobic pathways (Harris et al., 1976).

Energy utilisation in intermittent exercise of supra maximal intensity has not been studied as extensively as other forms of exercise. This section of the review will firstly concentrate on the studies which investigated the maximal dynamic muscle power output and the associated metabolic changes in muscle during maximal intermittent exercise. Then the effect of different recovery duration on the ability to perform repeated bouts of maximal
exercise will be discussed.

2.5.2 Metabolic Responses to Maximal Intermittent Exercise (30 s duration)

The first of several studies which examined maximal dynamic muscle power output and the associated metabolic changes in muscle was reported by McCartney et al. (1986). Subjects performed four 30 s bouts of maximal isokinetic cycling at 100 rev.min\(^{-1}\) (rpm) with 4 min recovery intervals. External power and work decreased by 20% in both the second and third exercise periods, but there was no further decrement in performance in the fourth bout. Changes in muscle glycogen, lactate and glycolytic intermediates suggested rate limitation of glycogenolysis at the phosphofructokinase level during the first and second exercise periods, and phosphorylase in the third and fourth exercise periods. The inhibition of glycogenolysis, and the 485% increase in plasma glycerol from resting values, led to the speculation that intramuscular triglyceride stores could possibly function as an important substrate for energy metabolism during repeated maximal exercise.

Subsequently, Spriet et al. (1989) examined the relationships between muscle glycogenolysis, glycolysis and \(H^+\) concentration during maximal intermittent cycling. The exercise protocol comprised three 30 s bouts of maximal isokinetic cycling at 100 rpm, separated by 4 min passive recovery. Muscle biopsies were obtained prior to and following bouts 2 and 3. Total work declined progressively over the three exercise bouts. A reduced glycolytic flux was observed in bout three, although ATP and PCr degradation was similar in bouts two and three. Muscle \(H^+\) concentration \([H^+]\) was higher following bout 3 compared to bout 2, leading to the suggestion that glycogen phosphorylase activity was inhibited during the third exercise period due to the increased \([H^+]\). Another consideration is the possibility of a reduction in the \(Ca^{2+}\) activation of fast-twitch fibres (including a possible \(H^+\) effect) may contribute to the low overall glycogenolysis. Thus, during exercise bout 3, total work is maintained by a greater reliance on aerobic metabolism.
However, the studies by McCartney et al. (1986) and Spriet et al. (1989) are concerned with repeated 30 s bouts of maximal exercise. However, in terms of replicating the patterns of activity typical of the multiple sprint sports, repeated sprints of 5-6 s duration are regarded as more representative of the actual match demands during this type of exercise. Thus the attention of this review will now focus on the metabolic responses to repeated bouts of brief maximal exercise of (≤) 6 s duration.

2.5.3 Metabolic Responses to Maximal Intermittent Exercise (≤ 6 s duration)

Gaitanos et al. (1993) examined human muscle metabolism during intermittent maximal exercise. The exercise protocol consisted of ten 6 s maximal sprints on a cycle ergometer with 30 s of recovery between each sprint. Biopsies were taken from the vastus lateralis muscle before and after the first sprint and 10 s before and immediately after the tenth sprint. During the first 6 s sprint, ATP turnover was estimated to be in the region of 15 mmol (kg DM)⁻¹.s⁻¹. This value is higher than previous observations of 13 mmol (kg DM)⁻¹.s⁻¹ for an exercise bout lasting 10 s on a cycle ergometer (Jones et al., 1985) and of 6 mmol (kg DM)⁻¹.s⁻¹ reported by Spriet and colleagues (1987) during 25 s of electrical stimulation. Mean power output generated over the first 6 s sprint was sustained by an equal contribution from PCr degradation and anaerobic glycolysis. By the end of the first sprint, PCr and glycogen concentration decreased by 57% and 14% of resting values, respectively, and muscle lactate concentration increased to 28.6 mmol (kg DM)⁻¹, an indication of significant glycolytic activity. However, in the tenth sprint, mean power output was reduced to 73% of that generated in the first sprint, despite the fact that there was no change in accumulation of muscle lactate. As a consequence of the observed reduction in the contribution of anaerobic glycolysis to ATP production, it was suggested that, during the last sprint, power output was supported by energy that was mainly derived from PCr degradation and an increased aerobic metabolism.
Balsom et al. (1992a) evaluated the physiological responses to sprinting over different distances with the same recovery interval. Subjects performed three exercise protocols on different occasions, with either 15 m, 30 m or 40 m sprints repeated every 30 s. The total distance covered was 600 m during each test protocol. There was no decrement in performance between the first and last sprint over a 15 m distance. However, there was an increase in performance time when the sprints were repeated every 30 s over 30 m and 40 m. Mean plasma concentrations of hypoxanthine and uric acid increased over the duration of the 30 m and 40 m sprint protocols. In all three conditions, post-exercise blood lactate concentrations were higher than pre-exercise values, the longer the sprint, the greater the increase. The increase in sprint times over 30 m and 40 m was thus associated with a net loss to the adenine nucleotide pool.

2.5.3.1 Hormonal Responses to Maximal Intermittent Exercise

Sprinting is accompanied by a high rate of energy production and endocrine response. For example, Brooks et al. (1990) studied the hormonal responses of male and female athletes to repetitive brief maximal exercise. The exercise protocol consisted of ten 6 s sprints with 30 s recovery between sprints on a non-motorised treadmill. The males performed greater amount of total work than the females, but this was not associated with the higher blood lactate concentrations in the men. Plasma adrenaline increased 18-fold, with the peak concentration observed after five sprints. The peak adrenaline concentration was larger in the men than the women. The maximum noradrenaline concentration occurred after 10 sprints in the males and after five sprints in the females. Thus the results indicate differences between men and women in performance and physiological responses to intermittent high intensity exercise.

Similar catecholamine responses were reported in response to ten 6 s sprints repeated at 30 s intervals on a cycle ergometer (Gaitanos et al., 1993). Plasma adrenaline increased fivefold after the first sprint from 0.4 nmol.l⁻¹ at rest to 1.9 nmol.l⁻¹ and plasma
noradrenaline increased twofold from 1.7 nmol.l\(^{-1}\) at rest to 3.3 nmol.l\(^{-1}\). Peak values were observed after the ninth sprint, increasing 13-fold from resting values for both adrenaline (5.1 nmol.l\(^{-1}\)) and noradrenaline (22.3 nmol.l\(^{-1}\)).

The catecholamine response to two exhaustive exercise bouts of 13 min duration, separated by 1 h, were reported in a study by Marliss et al. (1991). Similar increases in plasma noradrenaline (18-fold) and adrenaline (14-fold) were reported with both exercise bouts. The peak values which occurred at exhaustion (~2.9 nmol.l\(^{-1}\) for adrenaline and ~23 nmol.l\(^{-1}\) for noradrenaline) were of similar magnitude to those reported for those previously reported for ten 6 s sprints.

2.5.4 Training Status and Maximal Intermittent Exercise

Physiological responses to maximal intermittent exercise were also influenced by training status (Hamilton et al., 1991). Ten 6 s sprints, separated by a 30 s recovery period, were performed on a non-motorised treadmill by subjects who were either games players or endurance-trained runners. Games players tended to produce higher peak power outputs and higher peak speeds, but had a greater decrement in mean power output than endurance-trained runners. Blood lactate was higher for the games players after the exercise, but there was a similar decrease in pH for both groups. Endurance-trained athletes showed a greater increase in oxygen uptake above pre-exercise levels during the sprint test than games players. Thus, the greater decrement in performance observed in the games players could possibly be related to higher glycolytic rates, as reflected by higher lactate concentrations, and to their lower oxygen uptake over the duration of the 10 sprints.

2.5.5 Maximal Intermittent Exercise and Recovery Duration

Wootton and Williams (1983) examined the ability to perform repeated bouts of maximal dynamic exercise on a cycle ergometer, with different recovery duration. The exercise
protocol consisted of five 6 s maximal sprint bouts separated by either 30 s or 60 s passive recovery. Results showed that the capacity to perform repeated 6 s bouts of maximal exercise is markedly influenced by the preceding number of sprint bouts and the recovery duration. Greater decrements in performance were observed during the 30 s recovery protocol. Blood lactate concentrations increased considerably over the five sprint bouts, although the increase was lower with 60 s recovery compared with 30 s. The authors concluded that the greater decrements in performance and increases in blood lactate concentration during the 30 s recovery protocol was a reflection of the earlier and more pronounced reduction in PCr stores than occurs during the 60 s recovery protocol. Therefore, the contribution of glycolysis to the rapid replenishment of ATP during high intensity exercise would increase.

Holmyard et al. (1988) investigated the influence of recovery duration and the preceding number of exercise bouts on the power output during sprint running on a non-motorised treadmill. The two treadmill running tests consisted of ten 6 s sprints either with a 30 s recovery or a 60 s recovery between each sprint. Peak and mean power output decreased by 13.2% and 21.4%, respectively, between sprints 1 and 10 with the 30 s recovery protocol. However, during the 60 s recovery protocol, the decrement in performance was reduced by 3% and 4.2% for peak and mean power outputs, respectively. With the 30 s recovery only 5 sprints could be performed before fatigue reduced mean power output, whereas with the 60 s recovery the power output was maintained throughout the test. Post- exercise blood lactate and glucose concentrations after 5 min and 1 min, respectively, were lower after the 60 s test than the 30 s test. From these findings it was clear that anaerobic energy production from glycolysis, leading to the production of lactate, had made a significant contribution to the total energy demand. The larger decrements in performance with the 30 s recovery interval were probably due to an incomplete resynthesis of PCr and also due to greater acidosis, because of the limited time for translocation of H+ from muscle to blood.
Recovery duration was also manipulated, this time during running, in a study by Balsom and colleagues (1992b). Subjects performed 15 x 40 m sprints on a synthetic indoor running track on 3 separate occasions. The rest interval between each sprint varied for each test and was either 30 s, 60 s, or 120 s duration. Post-exercise blood lactate concentration was higher after the shortest rest periods, but there were no differences between trials after 6 sprints. Running speed declined over the last 10 min all trials, however, acceleration speed over the first 15 m was only affected in the 30 s recovery trial. The authors concluded that 40 m sprint performance was better maintained with the longer rest period between sprints, although the associated net loss to the total adenine nucleotide pool, measured by muscle concentration of uric acid, was unchanged.

In a laboratory controlled environment, intermittent exercise comprises fixed patterns of exercise and rest periods or consists of a fixed rate of work. However, during the multiple sprint sports, there are no definitive patterns of exercise intensity or duration or fixed periods of rest. Thus the measurement of the physiological and metabolic responses to actual match play in the field environment is problematic. However, these observations made do provide some useful information regarding match demands.

2.6 Physiological and Metabolic Responses to Intermittent High Intensity Exercise With Reference to Multiple Sprint Sports

2.6.1 Soccer

2.6.1.1 Exercise Intensity

The heart rates and oxygen uptakes recorded during a soccer game have been used to estimate the energy expenditure and intensity of the game. Results indicate that the aerobic contribution to energy supply is very high, and that many players are performing at an exercise intensity close to their maximal heart rates for long durations and the average
oxygen consumption during a normal game is close to 80% of VO₂ max (Ekblom, 1986). Indeed, Smolak (1978) reported that for two thirds of a game, heart rate was approximately 85% of maximum. Ali and Farrally (1991) recorded the heart rates of soccer players every 5 s during a soccer game by short-range radio telemetry. The mean heart rates recorded were 171, 167 and 168 b.min⁻¹ for semi-professional, university and recreational soccer players, respectively. Unfortunately, the maximum heart rates of the soccer players were not given, but based on age, these heart rates correspond to 88%, 84% and 87% of maximum heart rate, respectively. Analysis of heart rate by position revealed that midfield and forward players had a greater mean heart rate during a game than defensive players, and that the heart rate was lower during the second compared with the first half for all three playing standards studied.

2.6.1.2 Blood Lactate and Glucose Concentrations

The blood lactate concentrations recorded during a soccer game are an indication of the contribution of anaerobic glycolysis to total energy production. However, the interpretation of concentrations recorded is limited to giving an indication of the type of activity performed a few minutes prior to sampling (Bangsbo et al., 1991). Peak values of 12 mmol.l⁻¹ have been recorded, and the concentrations are greater, the higher the standard of soccer (Ekblom, 1986; Smith et al., 1993). Even during recreational games, lactate concentrations of between 8 - 10 mmol.l⁻¹ have been recorded for groups of players aged 25 yr and 36-40 yr (Ekblom et al., 1981, cited in Ekblom, 1986). Post-game lactate concentrations of 4.4 mmol.l⁻¹ were measured by Bangsbo et al. (1991) in a study of Danish professional first division soccer players. The mean lactate concentration during the first half of the match was higher than during the second half, and a significant relationship was found between blood lactate concentration and the duration of high intensity activities just prior to sampling during the match (Bangsbo et al., 1991).
In a study of Canadian national soccer players, Leatt and Jacobs (1989) observed a rise of blood glucose from the pre-game level of 4.96 mmol.l\(^{-1}\) to 5.67 mmol.l\(^{-1}\) at half-time, which then declined to 4.74 mmol.l\(^{-1}\) by the end of the game. Lower average values of 3.8 mmol.l\(^{-1}\) have been observed at the end of a soccer match (Ekblom, 1986).

### 2.6.1.3 Muscle Glycogen

Studies have shown that the pre-game levels of liver and muscle glycogen are important for performance and endurance, particularly in the second half of the game. Glycogen concentration decreases rapidly during a game, and depending on the level of competition and the extent of initial reserves, different authors have reported 20-90% reduction of intramuscular glycogen stores during competitive soccer matches (Karlsson, 1969 cited in Ekblom, 1986; Jacobs et al., 1982b; Leatt and Jacobs, 1989).

Field studies on other multiple sprint sports which have not received as much attention as soccer, include the sports of rugby union and field hockey.

### 2.6.2 Rugby Union

Mean and maximal heart rates of 161 and 184 b.min\(^{-1}\), respectively, have been recorded during a rugby game (Maud, 1983; Morton, 1978). The highest blood lactate concentrations ranged from 5.8 to 9.8 mmol.l\(^{-1}\) were reported by McLean (1992) and mean concentrations of 6 to 12 mmol.l\(^{-1}\) have also been documented (Menchinelli et al., 1992).

### 2.6.3 Field Hockey

The heart rates of nine international male hockey players were recorded by short range radio telemetry during a club hockey match on an artificial playing surface, and compared with the heart rate and oxygen uptake data measured in the laboratory (Boyle et al., 1994).
Regression equations were calculated for individual players, from which energy expenditure was estimated during competitive match-play. Mean heart rate during competition was 159 ± 8 b.min⁻¹ (midfield players 165 ± 6 b.min⁻¹ and forward and defensive players 154 ± 5 b.min⁻¹) and the mean estimated oxygen uptake during competition was 48.2 ± 5.2 ml.kg⁻¹.min⁻¹, equivalent to 78% of the group's mean VO₂ max value.

The energy demands during intermittent high intensity exercise and the degree of fatigue experienced as a consequence of lactic acid accumulation and glycogen depletion will be dependent on a number of factors. Namely, (i) the intensity or velocity of running; (ii) the duration; (iii) the time allowed for recovery, in addition to the exercise intensity during recovery; and (iv) the number of times these work recovery cycles are repeated (Coyle, 1993). Games players are required to perform intermittent sprinting at speeds between 75-100% of maximum sprinting velocity (i.e., 125-175% VO₂ max) during competitive games (Coyle, 1993).

The physiological and metabolic responses to intermittent high intensity exercise and the mechanisms responsible for the onset of fatigue must be understood before any intervention procedures to delay the premature onset of fatigue can be investigated. Muscle fatigue during voluntary contraction is defined as a failure to maintain the required or expected force (Edwards, 1981), and may be peripheral or central in origin. The cause and extent of muscle fatigue is dependent on the duration, intensity and nature of the exercise, fibre type composition of the muscle, individual level of fitness, as well as numerous environmental factors. Clearly, the demands of prolonged intermittent high intensity exercise would suggest that fatigue during this type of activity is similar to that observed during both short duration, maximal exercise and prolonged exercise.
2.7 Mechanisms of Fatigue

2.7.1 Short Duration (< 5 min), High Intensity Exercise

Intense exercise requires an energy demand which exceeds the individual's maximal aerobic power, and thus requires a high level of anaerobic metabolism. As a consequence, the concentrations of ATP and PCr decrease, inorganic phosphate, ADP, lactate and H+ concentration increase, resulting in an elevated acidity within the exercising muscles. All of these factors have been suggested as possible fatiguing agents (Fitts and Metzger, 1993; Simonson, 1971; MacLaren et al., 1989; Newsholme et al., 1992). Decreases in muscle pH from about 7.1 to 6.5-6.8 are often observed during intense exhaustive exercise (Sahlin and Henriksson, 1984), and based on NMR studies it has been suggested that pH in individual fibres can be even lower (Wilson et al., 1988; Vandenborne et al., 1991). A low pH may have an inhibitory effect on various functions within the muscle cell, such as the activity of phosphorylase and PFK, the excitation-contraction coupling, the affinity for Ca²⁺ to bind to troponin, the coupling between the contractile elements and the re-uptake of Ca²⁺ in the sarcoplasmic reticulum (Cooke and Pate, 1990; Donaldson, 1990; Hultman et al., 1990; Edman, 1992).

However, elevated lactate concentration and lowered muscle pH are not necessarily critical in the development of fatigue (Sahlin and Ren, 1989; Sahlin and Henriksson, 1984), nor does fatigue appear to be related to energy depletion, since muscle ATP is found to be relatively high at exhaustion during intense voluntary exercise (Edwards, 1981; Bangsbo et al., 1992a; Bangsbo et al., 1992b; Bangsbo, 1993). Fatigue may be caused by a decreased capacity to re-phosphorylate ADP in combination with a high rate of ATP turnover (Sahlin, 1986), an energetic deficiency, with a concomitant accumulation of IMP (Sahlin, 1992a), related to a failure in the coupling between T-tubular depolarisation and Ca²⁺ release from the SR, or possible failure of the calcium pumps to return calcium to the sarcoplasmic reticulum, thereby slowing the rate of relaxation (MacLaren et al., 1989; Donaldson, 1990).
Fatigue may also be caused by a reduced neural activation of the muscle (Bigland-Ritchie and Woods, 1984) as a result of an impaired surface membrane potential transmission, possibly due to accumulation of potassium and/or a reduction in sodium in the interstitium (Bigland-Ritchie et al., 1986; Edwards et al., 1977).

2.7.2 Prolonged Intermittent High Intensity Exercise

Numerous factors have been linked to fatigue resulting from prolonged endurance exercise, namely a reduction in muscle and liver glycogen, decreases in blood glucose, dehydration and increases in body temperature (Fitts et al., 1981). Each of these factors contributes to fatigue during prolonged exercise of an intermittent nature, their relative contribution varying according to environmental conditions and the intensity of the activity. However, the depletion of muscle glycogen has been reported to be associated with the onset of fatigue during prolonged intermittent exercise (Bangsbo, 1994). Those fibres which are recruited most frequently and have the lowest capacity to re-synthesise glycogen during the periods of recovery or lower intensity exercise may become depleted of glycogen (Essen, 1978a). Thus the potential number of fibres that can be recruited to compensate for a loss in muscle force is reduced, and the muscles may not be able to generate the required tension during the high intensity exercise periods. The close relationship between muscle glycogen depletion and fatigue appears to be due to the inability of glycogen depleted muscle cells to maintain a sufficient rate of ATP resynthesis. It has been recently reported that in glycogen depleted muscles, there is an increase in the ATP breakdown products IMP and ammonia (Broberg and Sahlin, 1989; Norman et al., 1988). In addition, a further consequence of a reduced availability of muscle glycogen is the loss of substrate, namely pyruvate, for anaplerotic reactions that provide Krebs cycle intermediates necessary for the continued oxidation of acetyl units derived from other substrates (Sahlin et al., 1990). However, muscle glycogen depletion may not exclusively be associated with fatigue during prolonged exercise (Madsen et al., 1990; Sejersted and Vollestad, 1992). Fatigue during prolonged intermittent exercise may also be caused by dehydration (Maughan and Noakes,
disruption of important intracellular organelles such as the mitochondria, the SR-system or the myofilaments (Fitts and Metzger, 1993).

In summary, fatigue during prolonged intense intermittent exercise is primarily associated with the depletion of muscle and liver glycogen which will limit performance both in competition and training. Thus, a high CHO diet is advocated at all times before, during and in the recovery from exercise (for review see Coyle, 1991). The basic strategy is to optimise the availability of liver and muscle glycogen and blood glucose in order to maintain CHO availability and oxidation during exercise (Costill and Hargreaves, 1992).

2.8 Carbohydrate Intake and Intermittent High Intensity Exercise Performance

Carbohydrates are consumed before, during and following exercise for the primary purpose of providing glucose (the sole type of CHO readily metabolised) to skeletal muscle. In fact, CHO is the single most important nutrient, being the predominant source of energy for muscles exercising intensely for at least 1 h (Coyle, 1993).

2.8.1 Carbohydrate Metabolism During Exercise

2.8.1.1 Endogenous Carbohydrate Metabolism

2.8.1.1.1 Muscle Glycogen Utilisation

At the onset of exercise, muscle glycogen is the primary source of CHO used for energy. The rate of muscle glycogen depletion will be influenced by a number of factors, including exercise intensity, physical conditioning, exercise mode, environmental factors and the pre-exercise diet (Costill, 1988). An exponential relationship has been demonstrated between the rate of muscle glycogen utilisation and exercise intensity, and it is the main fuel for working muscle at work intensities above 75% \( \text{VO}_2 \text{ max} \) (Saltin and Karlsson, 1971).
2.8.1.1.2 Regulation of Glycogenolysis

Several regulatory mechanisms are involved in the control of skeletal muscle glycogenolysis during exercise, including both local and systemic factors such as calcium and cyclic AMP-mediated transformation of phosphorylase from an inactive \( b \) form to the active \( a \) form, allosteric activation of phosphorylase by exercise-induced alterations in metabolite levels, and changes in substrate levels (Chasiotis et al., 1982; for reviews see Hargreaves and Richter, 1988; Bonen et al., 1989). Both mechanisms of phosphorylase activation are important for skeletal muscle glycogenolysis during exercise (Issekutz, 1984; Richter et al., 1982).

The elevation in circulating catecholamines during higher exercise intensities (Galbo, 1981) may also play a regulatory role because adrenaline stimulates muscle glycogenolysis during exercise (Jansson et al., 1986). However, the importance of adrenaline and contractions upon glycogenolysis varies depending upon the type of muscle and the intensity of the contraction in specific muscles (Bonen et al., 1989).

2.8.1.1.3 Regulation of Glycolysis

The rate limiting enzyme in the glycolytic pathway is thought to be PFK, and is thus considered to be central to the regulation of CHO metabolism. The hypotheses proposed to explain how PFK is regulated (Bonen et al., 1989) indicate that the regulation of glycolytic flux through PFK may involve a complex set of interactions of effectors, which may assume varied potency depending on the duration and rate of glycolytic flux necessary, and that the PFK reaction is not the exclusive rate-limiting step in the glycolytic pathway.
2.8.1.1.5 Blood Glucose Utilisation During Prolonged Intermittent High Intensity Exercise

Muscle glycogen alone cannot provide all the CHO needed for exercise lasting several hours. Although muscle glycogen is the predominant CHO energy source during the early stages of exercise, blood glucose becomes more important as exercise continues (Wahren et al., 1971). Indeed, compared with resting levels, muscle glucose uptake can increase 30-40 fold during exercise, depending on intensity and duration (Katz et al., 1986; Wahren et al., 1971). In the later stages of an endurance event, the fraction of energy derived from blood glucose may account for 75-90% of the muscle's CHO metabolism (Wahren et al., 1971). This large drain on blood glucose necessitates a concomitant increase in hepatic glucose output to keep pace with the increased glucose uptake by the muscles. Glucose uptake is increased by the activation of the membrane mechanisms associated with glucose transport and the enzymes responsible for glucose disposal. These include the number and activity of membrane glucose transporters, sarcoplasmic calcium, circulating insulin levels, intramuscular and blood-borne substrate levels and glucose availability (for review see Hargreaves, 1990; Coggan, and Coyle, 1991).

The factors which affect the rate of blood glucose oxidation during exercise include intensity, duration, muscle mass involved, muscle glycogen concentration, training status, environment, age and gender (Hargreaves, 1991; Kjaer et al., 1991). However, Hargreaves et al. (1995) reported that pre-exercise muscle glycogen availability had no effect on glucose uptake during exercise but only influenced the rate of muscle glycogenolysis.

2.8.1.1.5 Regulation of Hepatic Glucose Production

An increased hepatic glucose output is observed concomitant with the peripheral increase in glucose utilisation, determined by the intensity and duration of exercise. This is due to an accelerated glycogenolysis at first, but gluconeogenesis becomes more important as
exercise continues with lactate, pyruvate, glycerol and alanine being the major gluconeogenic precursors (Felig and Wahren, 1975). Several mechanisms have been suggested for the regulation of hepatic glucose release. At the onset of exercise, the resulting changes in the concentration of circulating hormones which ensure an adequate supply of fuels for the working muscle (plasma insulin decreases; glucagon, growth hormone, adrenaline and noradrenaline increase during exercise) in addition to the possible neural activation of the hepatocyte are considered to be the primary regulatory factors responsible for control of hepatic glucose production during exercise (for review see Wahren and Katz, 1992 and Winder, 1985).

2.8.1.1.6 Contribution of Endogenous Carbohydrate and Fat To Metabolism

The contribution of endogenous carbohydrate and fat to metabolism in relation to exercise intensity and duration was reported in a study by Romijn and co-workers (1993) using stable isotope tracers and indirect calorimetry. Plasma glucose tissue uptake and muscle glycogen oxidation increased whilst fatty acid release into plasma decreased as exercise intensity increased. Conversely, at the lowest exercise intensity, maximal stimulation of peripheral lipolysis was observed. Muscle triglyceride lipolysis was initiated only at the higher exercise intensities. With regard to the duration of exercise, intramuscular oxidation of both glycogen and triglycerides decreased, whilst plasma-derived substrate oxidation increased, over time at 65% VO2 max.

2.8.1.2 Exogenous Carbohydrate Metabolism

Recently, studies investigating the fate of exogenous glucose have utilised labelled exogenous CHO (13C) which permits the ingestion of glucose and estimation of its metabolism by measuring 13CO2 production. The fate of exogenous glucose during exercise of different intensities was investigated by Pirnay et al. (1982) who administered 100 g naturally labelled [13C]glucose to subjects 15 min after commencing a 105-min
treadmill exercise at 22, 39, 51 and 64% of VO₂ max. Between 22 and 51% VO₂ max, total
CHO, lipid oxidation, and exogenous glucose oxidation were linearly correlated with the
relative workload. Between 51 and 64% VO₂ max, exogenous glucose oxidation reached a
plateau, whereas endogenous CHO oxidation was markedly enhanced. The lesser
contribution of exogenous glucose during the most intense exercise may be due to a
decrease in the oxidation in the muscles or to a lesser availability of this exogenous
glucose.

Krzentowski et al. (1984) studied the effect of timing of glucose ingestion during exercise
on exogenous glucose disposal. Exercise consisted of 4 h of treadmill exercise at
approximately 45% of VO₂ max. A 100 g load of naturally labelled [¹³C]glucose was
administered orally after 120 min or 15 min of exercise. In the 2 h after glucose ingestion,
total CHO oxidation, exogenous glucose oxidation and lipid oxidation were similar in both
groups. Of the total 100 g oral glucose load, 55% was recovered as expired CO₂ within 2 h
of ingestion. These results demonstrate that glucose ingestion during prolonged exercise of
moderate intensity is effectively oxidised and the rate of oxidation is similar when
ingestion takes place 15 or 120 min after initiation of exercise.

2.8.2 Protein Metabolism During Intermittent High Intensity Exercise

The contribution of protein to metabolism during intermittent high intensity exercise is
uncertain, although studies during prolonged submaximal, continuous exercise have shown
that protein oxidation may contribute less than 10% of the total energy production
(Wagenmakers et al., 1989; Wagenmakers et al., 1990). Protein degradation has also been
shown to be related to exercise intensity (Lemon et al., 1982), and carbohydrate availability
(Lemon and Mullin, 1980). Thus, intermittent high intensity exercise involves a
significant amount of amino acid oxidation (Lemon, 1994).
2.8.3 Pre-exercise Nutritional Strategies and Intermittent High Intensity Exercise Performance

2.8.3.1 Short duration Intermittent High Intensity Exercise

The effect of a normal and a low CHO diet on repeated bouts of supra-maximal exercise on the cycle ergometer was investigated by Fulcher and Williams (1992). The test involved five sets of five all-out sprints of pre-determined work with 30 s recovery (65 g.kg\(^{-1}\) body mass applied load) separated by 5 min of cycling at 60 rev min\(^{-1}\) (30 W). The sixth set involved 10 x 6 s fixed time sprints, again with 30 s recovery. The control group (normal diet) showed a significant improvement on test 2 compared with test 1 both in terms of time taken to the pre-set work level and peak power output in the five sets of sprints, whereas the experimental group showed no improvement on test 1 performance. These results demonstrate the importance of optimal glycogen stores prior to the performance of repeated maximal exercise. It is possible that the adaptive response in muscle fibre recruitment may be adversely affected by low muscle glycogen concentrations in the active muscles as a consequence of the low CHO diet.

Jenkins et al. (1993) examined the influence of dietary CHO on the performance of supra-maximal intermittent exercise. Subjects performed two identical maximal interval tests separated by 3 days. The interval tests comprised five 60 s 'all-out' cycling bouts working against a resistance of 74 g.kg\(^{-1}\) body mass; each bout was separated by 5 min of passive recovery. For 3 days preceding the first interval test, all subjects followed a 'moderate' CHO diet (55.3% of energy intake as CHO) and were then randomly assigned to follow either a high CHO (83%), moderate CHO (58%) or a low CHO (12%) diet for the three days separating the two maximal interval tests. Total work done during the second interval test was improved by 5.6% and 2.3% after the high and moderate CHO diets, respectively. These improvements were significantly different to the 5.4% decrease in performance in the low CHO group. These results emphasise the importance of dietary CHO for optimal
performance during repeated periods of very high intensity exercise. However it still remains to be established whether a diet containing higher than normal amounts of \( \text{CHO} \) is of any greater benefit during this type of exercise.

However, not all studies have observed performance improvements with pre-exercise \( \text{CHO} \) intake. Snyder and colleagues (1993) investigated the effect on performance of ingesting a carbohydrate beverage prior to repeated bouts of high-intensity, short-duration exercise. Ten well-trained male cyclists performed two experimental rides, one 15 min after consumption of 5.0 ml.kg\(^{-1}\) bm of a 19.7% carbohydrate drink and one following a placebo. The experimental ride consisted of four 1.6 km timed performance rides separated by 4.8 km steady state rides at 80\% \( \text{VO}_2 \) max (between the last two performance rides the steady-state rides were 1.6 km at 80\% and 1.6 km at 90\%). Total exercise time was not different between conditions. These data support findings that where normal or supra-normal concentrations of muscle glycogen are observed pre-exercise, the glycogenolytic rate during high intensity exercise is not affected, irrespective of the pre-glycogen level (Bangsbo et al., 1992a; Ren et al., 1990). There is evidence to suggest that \( \text{CHO} \) ingestion prior to cycling exercise affects performance only after a significant reduction in muscle glycogen has taken place (Coyle et al., 1986; Hargreaves and Briggs, 1988). Studies that have failed to show an ergogenic effect of \( \text{CHO} \) ingestion on performance have used protocols of less than 30 min duration (Davis et al., 1988a). Also, the administration of additional \( \text{CHO} \) prior to high intensity exercise may not have been available due to a reduction in gastric emptying during exercise exceeding 70\% \( \text{VO}_2 \) max (Fortrand et al., 1967).

The effect of pre-exercise consumption of glucose on supra-maximal intermittent exercise performance was examined on subjects who had previously adhered to a 3-day low carbohydrate or normal \( \text{CHO} \) diet (Jenkins et al., 1994). Subjects performed two identical maximal intermittent cycling tests separated by 3 days. The intermittent tests comprised five 60 s 'all-out' cycling bouts working against a resistance of 75 g.kg\(^{-1}\) bm; each bout was
separated by 5 min of passive recovery. For 3 days preceding the first interval test, all subjects followed a 'moderate' CHO diet which comprised 59.1% (approximately 4.1 g.kg\(^{-1}\) bm) of the daily energy intake as CHO. Following the first and for 3 days prior to the second interval test, subjects were randomly assigned to follow either a moderate CHO diet (60.8%) or a low CHO diet (14.4% or 1.1 g.kg\(^{-1}\) bm). One hour before the second interval test, eight subjects were administered a 15% glucose solution (1 g.kg\(^{-1}\) bm) while the other eight subjects consumed a low-energy sweetened placebo. No changes in performance between the two interval tests across conditions were found. However, less oxygen was consumed during exercise in those subjects who consumed the glucose solution, compared with those who had been given the placebo solution, immediately prior to the second interval test, irrespective of their dietary CHO intake. The results further demonstrate that additional CHO ingested prior to repeated maximal exercise has no effect on performance, possibly due to a reduced availability as previously mentioned, or because the extra available blood glucose is not taken up by the muscle. It has been shown that an inverse relationship exists between glucose uptake during exercise and muscle glycogen content (Richter and Galbo, 1986).

2.8.3.2 Prolonged Intermittent High Intensity Exercise

As previously mentioned, the only study to look at the effect of a high CHO diet on the performance of prolonged intermittent high intensity exercise under controlled conditions was reported by Bangsbo et al. (1992b). They observed that performance during a prolonged intermittent running test was enhanced in professional soccer players following the consumption of a CHO enriched diet for two days. The standardised test comprised a field part (6856 m) followed by intermittent treadmill running to exhaustion. The players ingested a diet containing either 39% CHO (Control diet) or 65% CHO (CHO diet) during the two days prior to each test. The total mean running distance after the CHO-diet was 17.1 km, which was 0.9 km longer than after the control diet.
2.8.3.3 Multiple Sprint Sports

Saltin (1973) reported the results of a study which investigated the effect of muscle glycogen concentration on performance during a soccer game. Players were divided into two groups, those with either normal muscle glycogen or half the levels of the normal group. Running distances during the game were estimated from filming segments of the game. The total distance run was 24% less in the low glycogen-level group and the distance covered at a walk was 50% of the total distance. Post-game biopsies revealed that those players who began the match with sub-normal levels of muscle glycogen were glycogen depleted, and those with normal pre-game concentrations had only 10% of their total pre-game muscle glycogen remaining at the end of the match.

Thus a high CHO diet prior to performance of prolonged intermittent high intensity exercise is important in order to optimise the availability of muscle and liver glycogen and blood glucose. This is also the rationale behind CHO ingestion during the exercise period.

2.9 Fluid Ingestion and Performance During Intermittent High Intensity Exercise

Fluid ingestion during exercise serves a dual function; to maintain the fluid balance of the body and to provide CHO which the body can oxidise to provide energy. Hydration during prolonged endurance exercise is of particular importance because, not only does a negative fluid balance have a detrimental effect on performance (Saltin, 1964; Armstrong et al., 1985; Sawka and Pandolf, 1990), it poses a potentially serious health risk (Wyndham, 1977). Limited CHO availability does not pose such a life-threatening hazard, although it can limit performance.

When muscles contract, chemical energy is converted to mechanical energy, total metabolic rate is increased and heat is liberated. The higher the exercise intensity, the greater is the increase in metabolic rate, heat production and rise in core body temperature.
Nadel et al., 1977). Heat produced is dissipated by increasing the circulation to the periphery and then sweat production cools the body by evaporation. Sweat rate increases with increasing exercise intensity (Greenhaff and Clough, 1989; Maughan, 1985) and during prolonged physical exercise, sweat rates varying between 1.0 and 3.7 l.h⁻¹ have been reported (Murray, 1987; Armstrong et al., 1986; Sawka, 1992). Loss of body mass during a soccer, hockey or rugby game is primarily due to sweating, the rate of which will vary according to both the intensity of the game and the climatic conditions. Previous observations during soccer matches have reported reduction in body mass to be in the range 1 to 2.5 kg in temperate climates (Ekblom, 1986; Leatt and Jacobs, 1989). Higher losses of up to 4-5 kg have been recorded when the air temperature is increased (Mustafa and Mahmoud, 1979). This will have adverse effects on performance when dehydration exceeds 2% of body mass (Saltin, 1964; Saltin et al., 1972; Costill and Miller, 1980) due to a decrease in blood volume, stroke volume, circulatory capacity, and a decreased capacity to cool the body.

When the effect on performance is considered, the provision of water may not necessarily take preference over CHO intake. Soccer is a game that can place heavy demands on both CHO and fluid reserves (Shephard and Leatt, 1987), and the relative needs of CHO and water will be determined by the climatic conditions, exercise intensity and duration, and the individual's particular body composition, size and physiology. Consideration must also be given to the limiting factors placed by ingestion and gastrointestinal function when aiming to optimise fluid intake. Any benefits from supplementation of an exogenous beverage are partially dependent on the rate at which the ingested fluid leaves the stomach (gastric emptying) and is absorbed from the small intestine into the circulation (intestinal absorption).
2.9.1 Factors Affecting Gastric Emptying

The factors influencing the rate of gastric emptying of CHO-containing solutions during exercise include volume, energy density, electrolyte content, osmolality, temperature and pH of the ingested solution, intensity and mode of exercise, and the level of hydration and specific characteristics of the individual concerned (Noakes et al., 1991a; for reviews see Brouns et al., 1993 and Murray, 1987). Of these, volume and constituents of the ingested solution (energy content and osmolality) and the type/intensity of exercise appear to be the main determinants of gastric emptying rate (Noakes et al., 1991b).

2.9.1.1 Gastric Volume

The maximum rate at which CHO and water can be delivered from an ingested solution is very strongly influenced by the average volume of fluid in the stomach, which is determined by the volume ingested and the drinking pattern employed (Duchman et al., 1990; Mitchell and Voss, 1991; Rehrer et al., 1989 and Rehrer et al., 1990). Larger volumes are emptied faster, presumably due to the elevation of intragastric pressure (Noakes et al., 1991b). Thus, the greater distension of the stomach that can be tolerated by an individual, the more CHO and water will be delivered to the intestine from any ingested solution. A repeated drinking pattern increases the gastric emptying rate by maintaining a higher average volume of fluid in the stomach.

2.9.1.2 Carbohydrate Concentration

Carbohydrate solutions which have a concentration of between 5 and 7.5% have similar GE rates to that of water when administered in serial feedings at 15-20 min intervals (Mitchell et al., 1988; Mitchell et al., 1989b; Ryan et al., 1989). When given as one large bolus (≥400 ml) or as one large bolus followed by serial boli drinking (~200 ml), iso- or hypotonic CHO solutions (≤7% CHO) empty at a similar rate as water (Rehrer et al., 1990;
Rehrer et al., 1989; Seiple et al., 1983). As a rule, as the CHO concentration of a solution increases, the GE rate decreases. Nevertheless, with one bolus ingestion of 400-600 ml, although there is a decreased rate of fluid delivery, the rate of CHO delivery increases, at least up to a concentration of 18% (Mitchell et al., 1988 and Mitchell et al., 1989b; Rehrer et al., 1989). However, when smaller volumes of fluid (200 ml) are ingested, water is observed to empty at a faster rate than a 5% glucose solution (Maughan and Leiper, 1990). This is probably due to the inhibitory effect of glucose concentration taking precedence over the stimulatory effect of volume.

It is important to emphasise that the rate of GE (expressed as % emptied per unit time) is influenced by the CHO content of the solution, but the volume of the solution emptied is determined by the gastric volume, which is a function of the drinking pattern (Rehrer et al., 1989).

2.9.1.3 Osmolality

Foster and co-workers (1980) observed an increase in GE of a glucose polymer solution (75 mosm.l⁻¹) compared with a free glucose solution (266 mosm.l⁻¹), when residual gastric volumes were compared 30 min after ingestion. Sole and Noakes (1989) also observed that a glucose polymer solution (117 mosm.l⁻¹) emptied faster than a free glucose solution (739 mosm.l⁻¹) when only the residual volumes were compared 15 min after ingestion. This is possibly due to increased gastric secretions with the glucose solution compared with the glucose polymer (Foster et al., 1980; Rehrer et al., 1989; Sole and Noakes, 1989). However only small differences were observed between 2 solutions varying in osmolality (444 and 1,060 mosm.l⁻¹) but of a similar glucose concentration (Rehrer et al., 1989).
2.9.1.4 Type and Intensity of Exercise

The gastric emptying rate of a glucose solution is not different from rest during cycling at ≤ 70% VO$_2$ max (Costill and Saltin, 1974; Mitchell et al., 1989b) and the rate during intermittent exercise is similar to that observed during continuous exercise (Mitchell et al., 1989b). Houmard et al. (1991) observed that there were no differences in the GE rate of a 7% CHO solution between running and cycling at 75% VO$_2$ max for 1 h. Above this rate, GE was significantly affected during both cycling (Costill and Saltin, 1974; Rehrer et al., 1989) and running (Sole and Noakes, 1989) compared with at rest, possibly due to increased sympathetic tone and catecholamine release (Galbo et al., 1975; Galbo et al., 1977). In addition, the effect may be greater on water than on CHO-containing solutions, so that at higher exercise intensities, the magnitude of the difference in GE rates for water and CHO solutions may be considerably reduced (Foster, 1990; Sole and Noakes, 1989) and may even disappear at exercise intensities ≥ 90% VO$_2$ max (Foster, 1990) In contrast, Neufer et al. (1986) and Neufer et al. (1989) demonstrated that running at 50-70% VO$_2$ max accelerated the GE rate of various CHO solutions and water as compared with rest, while GE during running at 75% VO$_2$ max and at rest were similar.

2.9.2 Factors Affecting Intestinal Absorption

The amount of glucose delivered to the small intestine will be greater the more concentrated the ingested solution (Rehrer et al., 1992; Vist and Maughan, 1993). Intestinal absorption is mainly a passive process, made possible by local osmotic gradients which increase the lumen-to-mucosal flux of water. One of the mechanisms known to establish suitable osmotic gradients is the active co-transportation of glucose with sodium by the mucosa. Previous studies which have observed that the amount of exogenous glucose oxidised by the muscles is less than the amount of CHO delivered suggest that it is CHO absorption rather than GE rate which determines its rate of delivery to the muscles (Hawley et al., 1992). However, since the rate of glucose absorption increases with increasing
delivery of glucose into the small intestine (Rehrer et al., 1992), it is unlikely that the rate of exogenous glucose oxidation is limited by intestinal absorption. Thus, despite the slower GE rate of CHO solutions compared with plain water, their absorption in the small intestine is higher due to the stimulating effect of glucose and sodium (Murray, 1987).

Thus results seem to indicate that the ingestion of low concentrations of CHO solutions would provide the muscles with glucose without compromising fluid availability. This is very important since the two main aims of ingesting a CHO solution during prolonged exercise are to provide an alternative source of energy to the muscles and offset dehydration and the associated decrease in performance. It is recommended that fluid replacement during exercise should be equal to the body weight loss in order to prevent dehydration (Coyle and Montain, 1992). This may be difficult to achieve since thirst provides a poor indicator of whole body hydration status and ad libitum drinking results in incomplete fluid replacement or 'involuntary dehydration' (Hubbard et al., 1984; Greenleaf, 1992). However, a fluid intake of 0.5 l.h⁻¹ has been recommended (Noakes et al., 1988), making allowances for the fact that metabolic water stored with glycogen is released during exercise.

2.9.3 Carbohydrate Ingestion During Intermittent High Intensity Exercise

After 1-3 h of continuous exercise at 60-80% VO₂ max, it is well documented that fatigue is associated with glycogen depletion in the exercising muscles. Carbohydrate feedings during exercise have been shown to delay fatigue during cycling by 30-60 min (Coyle et al., 1983; Coyle et al., 1986; Coggan and Coyle, 1987) and running by 28 min (Tsintzas et al., 1995a). During cycling, this ergogenic effect has been associated with either the maintenance of a high CHO oxidation rate late in exercise (Coyle et al., 1986; Fielding et al., 1985), or the sparing of muscle glycogen use during exercise (Bjorkman et al., 1984; Erickson et al., 1987). In two of the few studies which have investigated the influence of carbohydrate feedings on muscle glycogen utilisation during running as the mode of
exercise, glycogen sparing was reported in the type I fibres during continuous treadmill running at 70% VO\textsubscript{2} max for a fixed 60 min period (Tsintzas et al., 1993b) and to exhaustion (Tsintzas et al., 1995a).

Carbohydrate feedings have shown to enhance intermittent exercise performance, possibly due to glycogen resynthesis within the non-active muscle fibres with low glycogen concentration, previously demonstrated in rats and man (Constable et al., 1984; Kuipers et al., 1987; Hargreaves et al., 1984; Yaspelkis et al., 1993), although not always (Kuipers et al., 1989).

Thus it is well established that CHO ingestion during exercise limited by carbohydrate availability improves endurance capacity and performance (for review see Coggan and Coyle, 1991). The oxidation of exogenous glucose is very important for energy metabolism during prolonged exercise, during the latter stages directly (Coyle and Coggan, 1984), and in the sparing of muscle glycogen during exercise (Hargreaves et al., 1984; Yaspelkis et al., 1993).

2.9.4 Nutritional Strategies During Intermittent High Intensity Exercise; Effect on Performance

2.9.4.1 Prolonged Intermittent High Intensity Exercise

Nassis (1991, unpublished MSc thesis) examined the effect of a carbohydrate-electrolyte (CHO-E) drink on endurance capacity during prolonged intermittent running. Subjects ran to exhaustion on a motorised treadmill on two occasions, separated by 10 days. The protocol consisted of repeated 15 s bouts of fast running (i.e., at 80% VO\textsubscript{2} max for 60 min and at 85% VO\textsubscript{2} max from 60 to 100 min, and then at 90% VO\textsubscript{2} max until exhaustion) separated by 10 s of slow running (i.e., at 45% VO\textsubscript{2} max). On each occasion they drank either a placebo or a 6.9% CHO-E solution immediately prior to the run (3 ml.kg\textsuperscript{-1}bm\textsuperscript{-1})
and every 20 min thereafter (2 ml.kg\(^{-1}\)bm\(^{-1}\)). Performance times were not different between the two trials.

Coggan and Coyle (1988) administered CHO supplements to subjects during cycling exercise that alternated every 15 min between moderate (60%) \(\text{VO}_2\) max and high (85% \(\text{VO}_2\) max) intensity. Results showed that blood glucose levels and CHO oxidation was maintained with CHO feedings, but this was not sufficient to support exercise at an intensity > 75% \(\text{VO}_2\) max late in exercise.

### 2.9.4.2 Prolonged Intermittent Exercise

Mitchell et al. (1988) investigated the effects of different concentrations of CHO solution on gastric emptying and intermittent exercise performance. Subjects performed 4 trials of intermittent (7 x 12 min bout, separated by 3 min ) cycling at 70% \(\text{VO}_2\) max. A final 12 min self-paced performance ride was then completed on an isokinetic ergometer interfaced with a computer to provide total work output. During the 3 min rest intervals between exercise bouts, subjects consumed a drink (8.5 ml.kg\(^{-1}\).h\(^{-1}\); mean total = 1.336 ml. h\(^{-1}\)). Only the volume of solution emptied in the 5% CHO trial was significantly less than the water placebo trial. In all of the CHO trials, significantly more work was produced compared to the water placebo trial. There were no performance differences between any of the CHO trials. Blood glucose was significantly lower in the water trial than in the CHO trials throughout exercise, which was possibly the mechanism responsible for the enhanced performance in the CHO trials.

The effect of ingesting water or CHO solutions during 1.6 h of intermittent cycling exercise on physiological function and performance in a warm environment was studied by Murray and co-workers (1987). The protocol comprised 4 trials, each ride consisted of intermittent steady-state cycling (at 55 and 65% \(\text{VO}_2\) max) interspersed with 5 rest periods. A timed 480 revolution cycling task completed each experimental session. During each rest
interval, subjects drank 2 ml.kg⁻¹ bm of water placebo or solutions of 5% glucose polymer, 6% sucrose/glucose, or 7% glucose polymer/fructose. No differences were observed in the physiological responses to intermittent exercise, with the exception of higher plasma glucose values after 1 h of cycling. However, performance time was improved in all CHO trials compared to the water trial.

Thus the time to complete a high intensity performance bout, or the total work output recorded over a set duration, at the end of prolonged intermittent cycling exercise is improved when carbohydrate is ingested as opposed to water during exercise (Table 2.1).

Table 2.1: CHO ingestion during intermittent exercise varying between 45 and 100% VO₂ max and subsequent performance of brief high intensity exercise.

<table>
<thead>
<tr>
<th>Study</th>
<th>N</th>
<th>Type of CHO ingested</th>
<th>Amount of CHO ingested (g.CHO.h⁻¹)</th>
<th>Improvement</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mitchell et al., 1988</td>
<td>8</td>
<td>5% M + G</td>
<td>33.5</td>
<td>8%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6% M + F + S</td>
<td>39.4</td>
<td>7%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>7.5% M + F</td>
<td>50.1</td>
<td>12%</td>
</tr>
<tr>
<td>Murray et al., 1987</td>
<td>13</td>
<td>5% GP</td>
<td>24.4</td>
<td>8%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6% S + G</td>
<td>29.3</td>
<td>13%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>7% GP + F</td>
<td>34.1</td>
<td>15%</td>
</tr>
<tr>
<td>Murray et al., 1991</td>
<td>10</td>
<td>G</td>
<td>26</td>
<td>6%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>G</td>
<td>52</td>
<td>4% ns</td>
</tr>
<tr>
<td></td>
<td></td>
<td>G</td>
<td>78</td>
<td>6%</td>
</tr>
<tr>
<td>Murray et al., 1989</td>
<td>12</td>
<td>6% G</td>
<td>39.8</td>
<td>5% vs. F</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6% F</td>
<td>39.8</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6% S</td>
<td>39.8</td>
<td>17% vs. F</td>
</tr>
<tr>
<td>Davis et al., 1988</td>
<td>19</td>
<td>S + G</td>
<td>149 g (Total)</td>
<td>9%</td>
</tr>
<tr>
<td>Kingwell et al., 1989</td>
<td>9</td>
<td>GP</td>
<td>160 g (Total)</td>
<td>ns</td>
</tr>
<tr>
<td>Hargreaves et al., 1984</td>
<td>10</td>
<td>S</td>
<td>43</td>
<td>45%</td>
</tr>
<tr>
<td>Fielding et al., 1985</td>
<td>9</td>
<td>S</td>
<td>21.5</td>
<td>49%</td>
</tr>
<tr>
<td>Yaspelkis et al., 1993</td>
<td>7</td>
<td>10% LCP</td>
<td>54</td>
<td>14%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Solid CHO</td>
<td>50</td>
<td>11%</td>
</tr>
</tbody>
</table>

M=maltodextrin, G=glucose, F=fructose, S=sucrose, WP=water placebo, GP=glucose polymer, ns=not significant, LCP=Liquid carbohydrate polymer, CHO=carbohydrate.
The results of these studies indicate that a dose-response relationship does not exist between the amount of CHO consumed during exercise and exercise performance. However, there does appear that in order to elicit performance improvements during this mode of exercise, a minimum CHO feeding rate of 21.5 g·h⁻¹ is necessary.

2.9.4.3 Multiple Sprint Sports

Leatt and Jacobs (1989) examined the effect of glucose polymer ingestion on glycogen depletion during a soccer match. Players ingested either 0.5 L of a 7% glucose polymer solution or a placebo 10 min before the start of the game and at half-time. Biopsies were taken pre- and post-game. Glycogen depletion was significantly greater in the placebo group compared with the polymer group, demonstrating that glucose ingestion reduces net muscle glycogen utilisation during soccer match play.

Kirkendall et al. (1988) investigated the effect of a glucose polymer supplement on work rate during soccer matches as estimated from video tape recordings. Players ingested either 400 ml of the polymer or placebo pre-game and at half-time. Conditions were reversed for a second game. During the first half, work volume and rate were not different between conditions. However, work volume was increased by 25%, and distance covered 'at speed' was increased by 40% in the second half when players consumed the polymer.

Foster et al. (1986) examined the effects of glucose polymer (25% CHO) ingestion on performance in successive simulated indoor soccer tournaments. Players competed in one 50 min game, then rested for one h before playing again. During the interval, players ingested either a polymer or placebo solution. Video analysis revealed that players who had consumed the polymer ran significantly further and faster in the second game compared with following placebo ingestion.
2.9.5 Mechanism(s) by which CHO Ingestion Exerts its Ergogenic Effect

Substantial evidence exists that suggests that when CHO is ingested during prolonged endurance exercise, continuous or discontinuous, fatigue can be delayed. During prolonged, continuous moderate-intensity exercise, this ergogenic effect has been reported to be achieved by preventing hypoglycaemia and maintaining the rate of CHO oxidation as opposed to reducing the rate of muscle glycogen utilisation (Coyle et al., 1986; Hargreaves and Briggs, 1988). However, muscle glycogen sparing as a consequence of CHO ingestion during exercise has also been reported (Hargreaves et al., 1984; Erickson et al., 1987; Tsintzas et al., 1993b; Tsintzas et al., 1995a; Yaspelkis and Ivy, 1991). The variation in muscle glycogen utilisation with CHO feedings during moderate- and low-intensity steady-state exercise may be due to the differences observed in the plasma glucose and insulin responses.

It has been previously reported that blood glucose oxidation cannot provide the energy required to support high-intensity work late in exercise (Coggan and Coyle, 1988). Therefore, it is a logical suggestion that adequate muscle glycogen stores must be available to support high intensity exercise. In those studies where high intensity performance is enhanced at the end of a fixed bout of intermittent exercise following CHO ingestion throughout exercise, the mechanism is suggested to be a higher muscle glycogen concentration prior to the performance task. Indeed, some (Hargreaves et al., 1984; Yaspelkis et al., 1993), but not all (Fielding et al., 1985) have reported a reduced glycogen utilisation when CHO feedings were administered during this mode of exercise. These discrepancies in muscle metabolism may be due to the differences in plasma glucose and insulin concentrations during exercise. Although insulin concentrations were not measured in the studies by Fielding et al. (1985) and Hargreaves et al. (1984), plasma glucose and insulin levels rapidly increased by 30 min of exercise and continued to be significantly higher than those of the placebo treatment throughout the remainder of the exercise in the glycogen sparing study by Yaspelkis et al. (1993). Another possibility is that the exogenous
CHO contributed to glycogen synthesis in depleted non-active muscle fibres (i.e. fast twitch fibres) during the low-intensity phases during intermittent exercise (Constable et al., 1984; Kuipers et al., 1987).

2.10 Carbohydrate Intake During Recovery From Intermittent High Intensity Exercise

Endurance athletes and games players regularly undertake daily training or participate in competitive events that significantly reduce the limited muscle glycogen stores. Replenishment of both liver and muscle glycogen in the period following such intense exercise is thus necessary in order to make sure that athletes can perform optimally during subsequent exercise bouts. It is assumed that nutritional strategies which result in greater glycogen resynthesis in the liver and muscles will ensure a greater recovery of exercise capacity between training sessions or competitions than nutritional strategies that result in slower liver and/or muscle glycogen synthesis.

2.10.1 Liver and Muscle Glycogen Resynthesis After Exercise

After only 2 h of exercise at 70% VO₂ max, liver glycogen is almost totally depleted, but its repletion to pre-exercise levels can be achieved within 24 h provided adequate CHO is consumed (Hultman, 1978). During recovery from exercise, splanchnic glucose release is enhanced by the post-exercise increase in glucagon and reduction in insulin (Krzentowski et al., 1982). In addition, most of a glucose load ingested after exercise contributes to glycogen resynthesis and not to replenishing liver glycogen (Maehlum et al., 1978).

Muscle glycogen is re-synthesised to normal levels at a rate of approximately 5 mmol.kg⁻¹.kg⁻¹ h⁻¹, corresponding to a rate of about 5% per h and is determined by the glycogen synthase activity of the muscle, dietary CHO content, and the degree of muscle trauma experienced during the exercise (O'Reilly et al., 1987; Piehl, 1974; Sherman et al., 1983).
Thus, one crucial determinant of muscle glycogen restoration after a match or heavy training is the time of recovery between successive training periods and matches. Approximately 20 h are necessary to restore muscle glycogen stores, provided the dietary intake is optimal. The important dietary considerations are 1) the rate of carbohydrate ingestion, 2) carbohydrate type, and 3) timing of carbohydrate ingestion after exercise.

2.10.1.1 Amount of Carbohydrate Ingested

The optimal CHO intake in the early post-exercise period appears to be 50 g every 2 h (Blom et al., 1987b; Ivy et al., 1988a; Ivy et al., 1988b), with the aim of ingesting a total of 600 g of CHO. This recommendation is based on observations that glycogen synthesis increased from 2% per h (i.e. 2 mmol.kg⁻¹h⁻¹) when 25 g of a high glycemic CHO was ingested every 2 h for the first 6 h after exercise to 5-6% (i.e. 5-6 mmol.kg⁻¹h⁻¹) when 50 g was ingested every 2 h. However, there was no further increase in the rate of glycogen resynthesis when 100, 112 or 225 g were ingested every 2 h. In agreement with these results are the reports by Reed et al. (1989) that intravenous glucose infusion at about 100 g every 2 h also failed to increase the rate of muscle glycogen resynthesis above 7-8 mmol.kg⁻¹h⁻¹, despite the greater increases in blood glucose and plasma insulin concentration with increased CHO administration.

2.10.1.2 Carbohydrate Type and Glycogen Resynthesis

The rate of glycogen synthesis after exercise and the ingestion of glucose or other high glycemic foods is 5-6% per h (i.e. 5-6 mmol.kg⁻¹h⁻¹) (Blom et al., 1987b; Ivy et al., 1988a; Ivy et al., 1988b; Reed et al., 1989). When simple CHO is ingested, a greater muscle glycogen storage is observed in the first 6 hours, compared with complex CHO (Kiens et al., 1990), although the amount of glycogen resynthesised after 24 h was similar, regardless of the type of CHO ingested (Costill et al., 1981; Kiens et al., 1990). However, this simplistic approach of categorising foods as 'simple' or 'complex' CHO based on their
chemical composition is not very useful as the blood glucose response to each CHO food is unpredictable (Burke et al., 1993). The concept of high, medium and low glycemic foods is a more helpful one in terms of comparison. When CHO foods of a high or moderate glycemic index are ingested, it makes little difference if the CHO is in liquid or solid form (Keizer et al., 1986; Reed et al., 1989). The ingestion of high glycemic CHO foods in the 24 h recovery period from prolonged, submaximal exercise, results in a greater amount of glycogen repletion being observed compared to equal quantities of CHO foods with a low glycemic index (Burke et al., 1993). Glucose and sucrose result in a faster muscle glycogen resynthesis than fructose (Blom et al., 1987b), although fructose may be of more benefit in liver glycogen repletion.

2.10.1.3 Timing of Carbohydrate Ingestion After Exercise

The rate of glycogen resynthesis is faster (7-8% per h) during the first 2 h immediately following exercise, compared with the normal rate of 5-6% per h (Ivy et al., 1988a). Thus it is important that an athlete recovering from exercise should ingest sufficient CHO as soon after exercise as is practical in order to take advantage of the faster resynthesis rate in the 2 h following exercise and to provide more time for glycogen repletion. Thereafter, it is important that the athlete ingests the equivalent of 8-10 g.CHO.kg⁻¹bm within 24 h. The strategy for CHO intake does not matter as the rate of muscle glycogen synthesis is similar when two large meals were eaten as compared to when seven smaller meals were consumed (Costill et al., 1981).

In summary, in order to optimise muscle glycogen repletion following prolonged, intense training or competition, and hence recovery, it is recommended that CHO intake should average 50 g per 2 h of mostly moderate and high glycemic CHO foods, beginning as soon after exercise as is practical (Coyle, 1991). The aim should be to ingest a total of 600 g, or 8-10 g.kg⁻¹bm.d⁻¹, of CHO within 24 h of exercise cessation (Coyle, 1991; Fogelholm et al., 1989; Hargreaves, 1991; Costill and Hargreaves, 1992).
2.10.2 Nutritional Strategies During Recovery From Intermittent High Intensity Exercise

2.10.2.1 Short Duration Intermittent High Intensity Exercise

In a study carried out by MacDougall and co-workers (1977), six subjects exercised to exhaustion on a cycle ergometer at an intensity corresponding to approximately 140% of their maximal aerobic power. Protocol consisted of 1 min exercise bouts separated by 3 min of recovery, continued until 30 s of exercise could no longer be maintained. Muscle biopsies were taken from the quadriceps before and immediately after exercise and at 2, 5, 12 and 24 h intervals thereafter and analysed for glycogen concentration. Subjects were assigned to consume either a mixed normal diet, or the same diet with an additional 10.5 MJ of carbohydrate to be consumed for lunch, supper and breakfast in the post-exercise period. The accumulated exercise times until the defined point of fatigue ranged from approximately 6 to 17 min and immediately after the termination of exercise, muscle glycogen concentration had decreased to 28% of its pre-exercise value. Within 24 h after exercise, glycogen had returned to pre-exercise concentrations, and there was no difference in the resynthesis rates between the two groups. It was concluded that within 24 h following glycogen depletion through intense intermittent exercise, muscle glycogen can be replenished to pre-exercise values; and within this time period, CHO intake in addition to a normal mixed diet does not enhance the resynthesis rate. Similar glycogen resynthesis rates following short term high intensity exercise, regardless of CHO intake, may be expected as a number of factors in the post-exercise recovery may account for these results. The resynthesis rates after short term high intensity exercise (15.1 to 33.6 mmol.kg\(^{-1}\).h\(^{-1}\)) are much higher than those following prolonged sub-maximal exercise (~8 mmol.kg\(^{-1}\).h\(^{-1}\)) due to the different post-exercise conditions (Pascoe and Gladden, 1996). These include higher concentrations of blood glucose, plasma insulin, glycolytic intermediates in muscle and blood and muscle lactate during the recovery from short duration high intensity exercise. Also, the recruitment of predominantly fast-twitch fibres
during high intensity exercise results in greater glycogen depletion in these fibres which have a higher level of glycogen synthase activity than slow-twitch fibres (Pascoe and Gladden, 1996). Thus these conditions are conducive to a fast rate of glycogen resynthesis.

2.10.2.2 Prolonged Intermittent High Intensity Exercise

Keizer et al. (1986) investigated the effect of prescribed liquid or solid CHO feedings and CHO intake ad libitum on glycogen synthesis after intense exhaustive intermittent exercise designed to deplete muscle glycogen stores. In addition, the relationship between restoration of muscle glycogen and maximal physical working capacity (MPWC) was examined. Muscle glycogen was determined before, immediately after, then 5 h and 22 h following the cessation of exercise. Glycogen synthesis was lower in those subjects who consumed CHO ad libitum compared to those prescribed CHO. In the prescribed liquid and solid CHO fed groups, glycogen restoration was complete within 22 h of exercise cessation. However, despite glycogen repletion to pre-exercise values, MPWC was significantly decreased by approximately 7% in both experiments, suggesting that there are factors other than the repletion of muscle glycogen which are important in the recovery of physical performance.

The influence of manipulating CHO intake on performance 24 h after intermittent sprint exercise was examined in a study by Nevill et al. (1993). Games players performed 30 maximum 6 s sprints on a non-motorised treadmill. Sprints were repeated every 2 min, and followed 60 s jog at 40% maximum speed and a 54 s walk at 20% maximum speed. Subjects were randomly assigned to either a high, (79% CHO) normal (47% CHO) or low (12% CHO) carbohydrate diet and repeated the 1 h intermittent sprint protocol 24 h later. Mean power output for each 6 s sprint declined over the course of the 30 sprints during trial 1 and power output was lower during trial 2 compared with trial 1. When the first nine sprints were compared between trials, a difference in sprint performance was observed between the high CHO group and the low CHO group. The metabolic responses to
maximal intermittent exercise were also reduced during trial 2, and were significantly altered by dietary manipulation in the recovery period between trials.

2.10.2.3 Multiple Sprint Sports

Leatt and Jacobs (1988) looked at glycogen repletion of soccer players when the normal diet was supplemented with a glucose polymer. Players were given a polymer or placebo to consume under no time constraints. Muscle biopsies were taken immediately after the game and then 19 h later. There was no difference in glycogen storage patterns between the two groups, although the amount of glycogen synthesised was very small [50 and 18 mmol (kg DM)^{-1}] in the experimental and control groups, respectively. The amount of glycogen resynthesis is inversely proportional to the post-exercise concentration. Thus, if the amount of glycogen used during the game was not great, then the stimulus for repletion will be low. If both groups consumed their normal diet then the CHO intake would be sufficient to cover the glycogen synthesis needs of the muscle.

Muscle glycogen resynthesis is reduced following eccentric exercise (Costill et al., 1990a; O'Reilly et al., 1987) and exhaustive running (Blom et al., 1987a; Sherman et al., 1983) or any activity which produces muscle damage and soreness. This is due to the increased glucose oxidation by the inflammatory cells present in the damaged muscle. Thus less glucose is available for glycogen-depleted muscle cells (Costill et al., 1990a). In addition, mechanical trauma due to ultra structural changes mainly in the Z-bands of the myofibril (Friden et al., 1981) may interfere with the membrane structures involved in insulin binding and glucose transport. These detrimental effects may be partially overcome by the ingestion of increased amounts of CHO (Costill et al., 1990).

Thus the importance of CHO before, during and in the recovery from prolonged, intermittent high intensity exercise is well established. However, other nutritional strategies may have an ergogenic effect during the brief periods of maximal exercise and
may improve performance and delay fatigue over the duration of the prolonged intermittent exercise bout. The decline in the force-generating capacity of the muscle during short-term high intensity exercise has often been attributed to reductions in the rate of ADP rephosphorylation as a consequence of the depletion of intramuscular PCr (Hultman et al., 1990), concomitant with an increased intramuscular acidosis (Costill et al., 1984; Hultman and Sahlin, 1980; Sahlin, 1978) which possibly affects the rate of glycolysis and glycogenolysis (Spriet et al., 1986; Gaitanos et al., 1993), and/or displacement of the creatine kinase equilibrium (Harris et al., 1977). Therefore, ways of increasing the PCr stores have been examined. Results have shown that the principal method is by Cr supplementation (Harris et al., 1992). Thus the efficacy of Cr administration prior to repeated bouts of maximal intensity exercise has also been examined.

2.11 Creatine Supplementation and Intermittent High Intensity Exercise Performance

The ingestion of Cr for 5 days at a rate of 4 x 5 g per day has been shown to increase resting muscle concentrations of total Cr (Harris et al., 1992) and facilitates PCr resynthesis during recovery (Greenhaff et al., 1992; Greenhaff et al., 1994a). Consequently, the depletion of muscle PCr stores during maximal exercise is delayed and performance enhanced during successive exercise bouts (Greenhaff et al., 1993; Balsom et al., 1993a; Birch et al., 1994; Harris et al., 1993; Bogdanis et al., 1996) due to the maintenance of the required ATP turnover.

Greenhaff et al. (1993) investigated the influence of oral Cr supplementation (4 x 5 g Cr plus 1 g of glucose/day for 5 days) on muscle torque production during 5 bouts of 30 maximal voluntary isokinetic contractions, separated by recovery periods of 1 min. In comparison with pre-supplementation values, following Cr supplementation, muscle peak torque production was enhanced during the last 10 contractions of exercise period 1, for the entire exercise in periods 2, 3 and 4, and during contractions 11-20 of the final exercise period. The mechanism for this improvement in performance was suggested to be due to an
acceleration of PCr resynthesis, induced by the additional Cr available. This may increase the ability to maintain the required rate of ATP resynthesis, and would explain the lower plasma ammonia concentrations observed during exercise following Cr supplementation.

Balsom and colleagues (1993) reported that after Cr supplementation, performance was enhanced during repeated bouts of high intensity exercise. Two intermittent exercise protocols (ten 6 s exercise periods interspersed with 30 s recovery, each at different intensities) were performed before and after Cr supplementation (5 x 5 g Cr plus 1 g glucose for 6 days). The intensity of the first bout was the equivalent of 820 W and was not designed to induce fatigue. However, the intensity of the second bout was the equivalent of 880 W which was chosen to induce fatigue over the course of the 10 exercise periods. The underlying mechanisms for this improvement were suggested to be a greater pre-exercise Cr availability in addition to an enhanced rate of PCr resynthesis in the recovery periods. The lower blood lactate and hypoxanthine accumulation after Cr supplementation would also support this explanation.

The effect of a period of Cr administration (4 x 75 mg.kg⁻¹ bm of Cr plus 1 g glucose for 5 days) on sprint running was examined in a study by Bogdanis et al. (1996). The exercise protocol was repeated twice, before and after 5 days of Cr supplementation, and consisted of six 10 s maximal sprints on a non-motorised treadmill, separated by 30 s of passive rest. Following Cr supplementation, power output and distance run were significantly increased in the sixth sprint by 10.8% and 6.9%, respectively. Plasma ammonia during exercise was lower following the period of Cr administration, possibly as a consequence of a reduction in AMP deamination and ammonia production due to the increased availability of PCr and hence the maintenance of a higher ATP turnover. In the same study, it was shown that Cr supplementation had no effect on VO₂ max or VO₂ and blood lactate concentration during submaximal exercise. Other studies have also shown that Cr supplementation has no effect on performance or metabolism during submaximal exercise (Green et al., 1994; Balsom et al., 1993; Stroud et al., 1994).
However, there are reports that Cr ingestion has no effect on peak power during repeated bouts of maximal exercise (Earnest et al., 1994) nor on a single bout of maximal exercise (Odland et al., 1994). Nevertheless, it may be suggested that in the multiple sprint sports, where repeated sprints are performed intermittently over a prolonged period, Cr supplementation may be a useful ergogenic aid if the availability of PCr in the working muscles becomes limiting.

2.12 The Prolonged Intermittent High Intensity Shuttle Run Test (PIHSRT)

Few laboratory studies to date have used exercise protocols which have attempted to replicate the demands of the multiple sprint sports (Bangsbo et al., 1992b; Nevill et al., 1993). This type of intermittent exercise comprises brief periods of intense muscular activity interspersed with periods of lower intensity exercise, or rest. Thus, for the purpose of this thesis, a protocol which would mimic the activity pattern of sports such as soccer, hockey, and rugby was designed. The activity patterns, physiological demands, and metabolic responses to these games were therefore reviewed in order to design an appropriate protocol.

2.12.1 Time Motion Analysis and Activity Patterns of the Multiple Sprint Sports

Exercise during the multiple sprint sports such as a soccer, rugby or hockey match is of variable intensity and duration, and types of activity range from standing still to maximal sprinting. Each sport also has its own specific activities, such as heading the ball (soccer), dribbling the ball (hockey and soccer) and scrummaging (rugby). Thus, although the physiological demands of each sport vary in terms of distance covered, average intensity and percentage time engaged in different activities, the pattern of variable intensity activity is common to all of them.
2.12.1.1 Soccer

The distance a player covers will vary, depending on position played, the quality of the opposition, tactical considerations and the importance of the game (Bangsbo et al., 1991). Large intraindividual differences between studies in distances covered reported in the literature may be due to different methods of observation and determination of distance covered during a match. Total distance covered and relative distance covered in each mode of activity are shown in Table 2.2.

Table 2.2: Distance (km) covered by male players in a soccer match and distances covered in each movement mode as a % of total distance.

<table>
<thead>
<tr>
<th>Study</th>
<th>TOTAL</th>
<th>Walk</th>
<th>Side/Back</th>
<th>Jog</th>
<th>Stride</th>
<th>Sprint</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Withers et al. (1982)</td>
<td>11.2</td>
<td>26.3</td>
<td>-</td>
<td>44.6</td>
<td>18.9</td>
<td></td>
</tr>
<tr>
<td>Saltin (1973)</td>
<td>11.5</td>
<td>3.1</td>
<td>5.6</td>
<td>2.6</td>
<td>0.5</td>
<td></td>
</tr>
<tr>
<td>Knowles and Brooke (1974)</td>
<td>4.8</td>
<td>1.7</td>
<td>2.6</td>
<td>0.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reilly and Thomas (1976)</td>
<td>8.7</td>
<td>2.2</td>
<td>3.7</td>
<td>2.8</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>Van Gool et al. (1988)</td>
<td>10.3</td>
<td>4.4</td>
<td>4.4</td>
<td>4.4</td>
<td>0.8</td>
<td></td>
</tr>
</tbody>
</table>

LIR=Low Intensity Running; HIR=High Intensity Running.

Winterbottom (1952), one of the first to analyse the game of soccer systematically by tracking players' movements on a scale plan of the pitch, estimated that players covered a distance of 3361 m. Speed running made up 1015 m, while walking and jogging accounted for 2347 m of the total. Wade (1962) reported that professional soccer players covered a total distance which ranged between 1.6 km to 5.5 km. Walking/jogging distance ranged 1.4 to 3.7 km, and speed running ranged 0.2 to 1.8 km.
Reilly and Thomas (1976) studied English First Division players for a whole playing season. Running performance was estimated from tracking individual players during 51 games, and speeds were divided into walking, jogging, backing, cruising and sprinting. Players were also divided according to position (forward, midfielders, backs and centre backs). Individual movements were noted (jumps, falls, distance dribbling the ball, head ball contacts, pauses). The total amount of distance covered was just short of 9 km with approximately 60% of that distance covered at a walk and a jog. Sprints occurred every 90 s, were typically 10-40 m in length and totalled nearly 800 m. There were approximately 1000 playing activity changes, each activity lasting 4-5 s.

The distance covered at an individual's top speed was between 7 and 10% (Ekblom, 1986; Yamanka et al., 1988). The distance covered in possession of the ball has been reported as 1.7% (Reilly and Thomas, 1976). In addition to the profile of these latter activities, accelerations, decelerations, angled runs, backward running and game-related activities such as jumping, tackling contribute to the overall match demands (Reilly, 1993).

The relative time spent engaged in the various activity modes is shown in Table 2.3. In a study of elite Danish soccer players (Bangsbo et al., 1991) in which each player was observed in at least two matches, the various activities and average duration expressed as a percentage of the total playing time included standing still (17.1%), walking (40.4%), low intensity activities (jogging, low speed running, backwards running) (5.3%), moderate speed running (5.3%), high speed running (2.1%) and sprint running (0.7%). There were 1179 changes in playing activities during a match, of a mean duration of 4.5 s. Players sprinted about 17 m (lasting 2 s) approximately every 4-5 min. When expressed in terms of total playing time, the mean ratio between high speed running, low speed running and standing/walking for the entire match was observed to be 1: 4.3 : 7.1 for the elite Danish soccer players.
Mayhew and Wenger (1985) reported that American professional soccer players performed high intensity exercise every 39 s. High intensity activity was of 4.4 s duration, making up 11.3% of the total playing time (Mayhew and Wenger, 1985). The total time spent in possession of the ball has been reported as 1.5% (Treadwell, 1988).

Table 2.3: Time spent in each movement mode as a % of total time.

<table>
<thead>
<tr>
<th>Study</th>
<th>Walk</th>
<th>Standing</th>
<th>Jog (LIR)</th>
<th>Striding/Running (HIR)</th>
<th>Sprint</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mayhew and Wenger (1985)</td>
<td>46.4</td>
<td>2.3</td>
<td>38</td>
<td>11.3</td>
<td>-</td>
</tr>
<tr>
<td>Yamanka et al. (1988)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Europeans</td>
<td>55</td>
<td>4</td>
<td>33</td>
<td>6</td>
<td>2</td>
</tr>
<tr>
<td>Japanese</td>
<td>48</td>
<td>5</td>
<td>38</td>
<td>7</td>
<td>5</td>
</tr>
<tr>
<td>Japanese collegiate</td>
<td>48</td>
<td>7</td>
<td>35</td>
<td>8</td>
<td>3</td>
</tr>
<tr>
<td>Bangsbo et al. (1991)</td>
<td>40.4</td>
<td>17.1</td>
<td>35.1</td>
<td>7.4</td>
<td>0.7</td>
</tr>
</tbody>
</table>

LIR=Low Intensity Running; HIR=High Intensity Running.

2.12.1.2 Rugby Union

On average, a rugby match has 40 scrums (20 s duration), 50-60 line-outs and 60-80 rucks and mauls (Rugby Football Union, 1978). Treadwell (1988) reported data describing the relative time engaged in various activities for rugby forwards and the backs. Non-purposeful running accounted for 63.8 and 73.2% for forwards and backs, respectively. Purposeful running, sprinting and time spent with the ball made up 19.2, 2.3 and 1.9%, respectively, of the total time for the backs. Time spent by the forwards engaged in purposeful running, scrumming and rucks and mauls was 21.5, 9.2 and 6.3%, respectively.
McLean (1992) investigated the physical demands of international rugby union over 5 games and reported that when the ball was in play, the average running pace of players central to action ranged from 5-8 m.s\(^{-1}\). In addition to this, scrum, line-out, ruck and maul was classified as high-intensity exercise. The most frequent work to rest ratio was from 1:1 to 1:1.9 and the mean duration of the work periods was 19 s. On average, a scrum, lineout, ruck or maul occurred every 33 s. The ball was in play for an average of 29 min during a scheduled time of play of 80 min.

Docherty et al. (1988) reported that the duration of work periods during a rugby match was not greater than 8.6 s. Of the total time spent, 47% was engaged in low intensity activity (walking and jogging), 6% in intense activity (striding and sprinting), 9% in non-running activities (e.g., tackling) and 38% of the time was spent standing.

Thus the multiple sprint sports, such as soccer, can be viewed as games of hybrid movement forms: numerous short runs that change frequently and accumulate a reasonably high volume of work (Kirkendall, 1993).

2.13 Summary

The physiology of maximal intensity intermittent exercise is extremely diverse as patterns of time relationships between work and recovery periods have no defined limits (Balsam, 1992a). Earlier studies, describing physiological responses to dynamic high intensity intermittent exercise, have standardised exercise intensity by using either a selected power output on a cycle ergometer (Saltin et al., 1976; Essen, 1978) or a given speed and gradient on motorised treadmills (Christensen et al., 1960; Astrand et al., 1960; Margaria et al., 1969). More recently, research has concentrated on the physiological responses to intermittent high intensity exercise where the exercise bouts are maximal or ‘all-out’ in nature. This type of exercise pattern, where maximal intensity short sprints are interspersed with periods of lower intensity exercise or rest is characteristic of the physiology of
multiple sprint sports (e.g., soccer, rugby, and field hockey) (Williams, 1990). Laboratory and field studies have previously described the physiological responses to repeated 6 s exercise periods on a cycle ergometer (Williams and Wootton, 1983) and on a non-motorised treadmill (Holmyard et al., 1988; Brooks et al., 1990). Responses to shorter periods of maximal intensity sprint exercise have also been reported (Balsom et al., 1992a; Balsom et al., 1992b). However, the physiological and metabolic responses to intermittent high intensity exercise designed to replicate the demands of a soccer, rugby or hockey match has yet to be evaluated in a controlled environment. Thus a prolonged intermittent high intensity shuttle run test (PHSRT) was designed for the purpose of this thesis based on the activity patterns described in the multiple sprint sports.

Creatine supplementation has been shown to improve performance during repeated bouts of maximal exercise (Greenhaff et al., 1993; Harris et al., 1992). However, whether performance can be enhanced during intermittent high intensity exercise where brief periods of sprinting or high intensity (< 10 s) are interspersed with longer periods of rest or lower intensity activity (~ 40-90 s), an exercise pattern typical of a soccer or rugby match (Reilly and Thomas, 1976; Dufour, 1993; Morton, 1978), has yet to be evaluated. Fatigue during prolonged intense intermittent exercise is primarily due to depletion of muscle and liver glycogen which will limit performance both in competition and training. Thus, a high CHO diet is advocated at all times before, during and in the recovery from exercise. The basic strategy is to optimise the availability of liver and muscle glycogen and blood glucose in order to maintain CHO availability and oxidation during exercise (Costill and Hargreaves, 1992).
CHAPTER 3

GENERAL METHODS

3.1 Introduction

The specific experimental procedures pertaining to each study will be described within the methods section of each experimental chapter. The methodological procedures which are common to each study are described below.

Ethical approval was obtained from the Loughborough University Ethical Advisory Committee for all procedures subsequently outlined. The 'Code of Practice for Workers having Contact with Body Fluids' was strictly adhered to. Subjects were informed of the physical demands of each experiment and any potential risks and discomforst they may be exposed to before written informed consent was obtained. Subjects were also required to complete a questionnaire providing both information of their medical history and their current training status, and frequency and level of participation in sport (Appendix A). Individuals with diabetes mellitus, or any other medical condition were excluded.

This chapter consists of 3 sub-sections. The first section outlines the apparatus and procedures employed during the administration of the preliminary and main experimental tests. The second and third sections describe the procedures concerned with the collection, treatment, storage and analysis of blood and muscle samples, respectively.
3.2 Experimental Testing Procedures

3.2.1 Preliminary Measurement - Estimation of Maximal Oxygen Uptake (VO\textsubscript{2} max)

Maximal oxygen uptake was estimated during a progressive shuttle run test (Ramsbottom et al., 1988), modified from the original protocol (Leger and Lambert, 1982). From this estimate of VO\textsubscript{2} max, running speeds corresponding to 55% and 95% VO\textsubscript{2} max were calculated using the tables for predicted VO\textsubscript{2} max values (Ramsbottom et al., 1988; Appendix B). The actual speeds run during the PIHSRT was not a critical factor, but using shuttle speeds corresponding to percentages of predicted VO\textsubscript{2} max was a method of ensuring that subjects were exercising at intensities relative to their aerobic capacity. The important point was that identical speeds were run on the two experimental occasions. In Chapters 5 - 7, subjects were then matched for VO\textsubscript{2} max values, endurance fitness, height and weight. This was in order to administer the experiments using pairs of subjects, for motivational purposes.

All subjects were then familiarised with testing procedures. This required them to get used to the pace required during the different exercise intensities within one exercise cycle, the ingestion of fluid between exercise bouts, heart rate telemetry, and being introduced to the Borg Scale of perceived exertion, and the Gut Fullness Scale (Chapters 4 and 5).

3.2.2 Apparatus and Instrumentation

Experimental protocols were conducted in the Sports Hall at Loughborough University of Technology, which had a sprung wooden floor. Subjects were required to perform continuous 20 m shuttles, identified by cones and floor markings, at various speeds related to estimated individual maximal oxygen uptake (VO\textsubscript{2} max) values in 20 min, 30 min (Chapter 4) or 15 min (Chapters 5-7) exercise blocks (Fig. 3.1). The combination of shuttle speeds were designed to mimic the activity pattern typically recorded for soccer match play.
(Reilly and Thomas, 1976; Withers et al., 1982) and consisted of the following exercise intensities:

**One Exercise Cycle**

- 3 x 20 m at walking pace,
- 1 x 20 m at maximal speed,
- 3 x 20 m at a speed corresponding to 55% of individual VO$_2$ max,
- 3 x 20 m at a speed corresponding to 95% of individual VO$_2$ max.

![Diagram of shuttle protocol](image)

**Distance = 20 m**

*Fig. 3.1: Schematic illustration of the shuttle protocol.*
The time spent in each movement mode expressed relatively to the total time of the PIHSRT is shown in Table 3.1. Comparison with the time motion analysis studies of soccer shows that the relative time spent walking and sprinting during the PIHSRT is similar to that of a soccer match (Chapter 2; Table 2.3). However, the percentage time engaged in high intensity activity was greater, and in low intensity activity less, during the PIHSRT compared with a soccer match. The increase in time spent performing high intensity exercise was designed to ensure that the PIHSRT put the subjects under a greater physiological stress than during a match.

Table 3.1: Time spent in each movement mode during the PIHSRT as a % of total time.

<table>
<thead>
<tr>
<th>Activity</th>
<th>% Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Walk</td>
<td>49.8</td>
</tr>
<tr>
<td>Jog</td>
<td>26.4</td>
</tr>
<tr>
<td>Cruise (Striding)</td>
<td>20.7</td>
</tr>
<tr>
<td>Sprint</td>
<td>3.1</td>
</tr>
</tbody>
</table>

In the first study (Chapter 4) this cycle was repeated continuously for 20 min before a rest period of 2.5 min was introduced during which blood samples were collected. This pattern of intermittent exercise was continued for a further 20 min and then followed with a 5 min rest period. Finally, a 30 min, non-fatiguing exercise bout was completed. Thus the total fixed duration of exercise was 70 min.
In Studies 2 and 4 (Chapters 5 and 7), the exercise blocks were reduced to 15 min duration, interspersed by 3 min rest periods (in order to collect blood samples and to allow for the administration of fluid ingestion, where appropriate), repeated 5 times. Thus the total fixed duration of exercise was 75 min. Following the fixed period of intermittent high intensity exercise in Studies 1, 2 and 4 (Chapters 4, 5 and 7), subsequently referred to as 'Part A', subjects performed a timed shuttle run to fatigue ('Part B'). This consisted of shuttle speeds alternating between 55% and 95% VO₂ max, repeated continuously until subjects could no longer maintain the required speed. The time taken to reach fatigue was recorded as a measure of their endurance capacity.

In Study 3 (Chapter 6, Parts A and B), the experimental protocol was designed to investigate the metabolic responses during (Chapter 6, Part A) and muscle function following (Chapter 6, Part B) prolonged intermittent high intensity exercise. Thus a fixed 90 min (6 x 15 min exercise) bout of non-fatiguing intermittent high intensity exercise was used.

In all studies, the specific intermittent exercise protocol employed is subsequently referred to as a prolonged intermittent high intensity shuttle run test (PIHSRT). Two experimental models were used in this thesis. To summarise, this was a fixed bout of intermittent high intensity exercise (Part A) followed by an open ended run to fatigue (Part B) in studies 1, 2 and 4 (Chapters 4, 5 and 7). Sprint times and the running time to exhaustion were recorded as the performance measures.

In study 3 (Chapter 6, Parts A and B), the test protocol was of a fixed duration rather than exercise to exhaustion. Muscle and blood metabolic responses were the primary concern and sprint times over 15 m constituted the performance measure.

The running and walking speeds during each 20 m of the PIHSRT were dictated by an audio signal from a microcomputer (BBC Master Series) using software developed in the
department (© Henryk K. A. Lakomy, Department of Physical Education, Sports Science and Recreation Management, 1992). Sprint times were measured in one direction over the first 15 m by 2 infra-red photoelectric cells (RS Components Ltd), interfaced with the microcomputer.

3.2.3 Environmental Conditions

Dry and wet bulb temperatures were measured at 15 min intervals during exercise using a whirling hydrometer (Brannan Thermometers Ltd). From these values, relative humidity was calculated using a sliding scale. All experimental tests were conducted in an ambient temperature ranging between 17 - 22 °C.

3.2.4 Heart Rate Monitoring

In all preliminary and experimental tests, heart rate was monitored throughout exercise by short range telemetry (Polar Electro Sports Tester, PE3000) and stored in memory mode. Heart rate data were later retrieved by interfacing the watch with the microcomputer (BBC Master Series).

3.2.5 Subjective Ratings of Exertion (RPE)

Ratings of perceived fatigue during the preliminary tests and experimental trials were measured using the fifteen point scale (Borg, 1973) in Chapters 4 and 7. In Chapters 5 and 6, a category scale with ratio properties was used, as it has been suggested to be appropriate for determining subjective symptoms, such as breathing difficulties, aches and pain (Borg, 1982). Subjects were shown the category ratio scale once during each 15 min exercise bout, and at the cessation of exercise, and their responses recorded.
3.2.6 Body Mass and Height

In all studies nude body mass (kg) was determined using balance scales (Avery Ltd, Model 3306B V), accurate to ± 50 g. Body mass was recorded before exercise, after the subject had voided, and immediately post-exercise, after any sweat had been removed from the skin. Height (cm) was measured using a wall mounted stadiometer (Holtain Ltd), accurate to ± 0.1 cm.

3.2.7 Nutritional Control

In all studies, the experimental trials began between 8 and 10 o'clock in the morning following a 10 - 12 h overnight fast. This ensured that subjects began each trial with an empty stomach, thus eliminating any negative effect a previous meal might have both on exercise metabolism, and on the gastric emptying rate of the test solutions. In addition, during the 2 days preceding each trial, subjects refrained from any strenuous physical activity, and consumed their normal diet in an effort to control for individual variation in muscle and liver glycogen stores. Subjects recorded everything they ate and drank during the 2 days before the first trial, and then replicated the same diet for the corresponding period prior to the second trial. During the initial 5 days, 7 days prior to each trial, subjects maintained the same training schedule.

In Study 1 (Chapter 4), the subjects' food record diaries were used to analyse the nutritional content of their normal diet (Paul and Southgate, 1978). From this dietary information, the appropriate nutritional prescriptions were made during the 22 h recovery period between trials.

In Chapters 4, 5 and 6, subjects were required to ingest a 6.9% CHO-E solution, a commercially available sports drink (Lucozade Sport, SmithKline Beecham), the composition of which is listed in Appendix C. In Chapter 4, subjects drank the equivalent
of 1g.kg\(^{-1}\)bm of the 6.9% CHO-E solution within 20 min of completing both the CHO and CON trials. This had the dual purpose of contributing to both rehydration and maximising muscle glycogen resynthesis in the immediate post-exercise period. In Chapters 5 and 6, an artificially sweetened placebo was ingested during the control trial, whereas the 6.9% CHO-E solution was ingested during the other trial. The administration of drinks in each study is described in the relevant chapters. The volume of fluid to be ingested was measured out using a metric measuring cylinder and stored in separate plastic drinking bottles, in order to avoid spillage and to ensure that the correct amount was consumed. Fluid ingestion took place in the 3 min recovery periods between exercise bouts. At the end of each trial, subjects were requested to complete a questionnaire in order to obtain subjective opinions regarding the taste and palatability of the ingested solution, any abdominal discomfort or gastrointestinal distress suffered whilst exercising, hypoglycaemic symptoms, as well as muscle cramps, pain and soreness.

### 3.2.8 Reproducibility Study of the Experimental Protocol

The test-retest reliability of the prolonged intermittent high intensity shuttle run test model (Parts A and B) was examined.

#### 3.2.8.1 Subjects

Eight male games players (age 21.5 ± 3 yr; body mass 77.3 ± 5.8 kg; height 177.3 ± 6.5 cm; VO\(_2\) max 57.5 ± 4.4 ml.kg\(^{-1}\)min\(^{-1}\)) volunteered and gave their informed consent to participate in this reproducibility study.

#### 3.2.8.2 Methods

The VO\(_2\) max of the subjects was estimated during a progressive shuttle run test (Ramsbottom et al., 1988), modified from the original protocol (Leger and Lambert, 1982).
From this estimate of VO₂ max, running speeds corresponding to 55% and 95% VO₂ max were calculated using the tables for predicted VO₂ max values (Ramsbottom et al., 1988). Subjects were then familiarised with the exercise pattern during the PIHSRT for 15 min.

Subjects performed the PIHSRT on two occasions, separated by one week (Trial A and Trial B). The PIHSRT comprised of Part A (5 x 15 min periods of variable intensity exercise) and Part B (shuttle running to fatigue). Sprint times during Part A and exercise time to fatigue during Part B were the performance criterion measurements, and were compared between trials by Student's t-test for correlated data.

3.2.8.3 Results

The sprint times for Trials A and B are shown in Table 3.2. Part B run times were 6.3 ± 2 min and 6.1 ± 1.3 min for Trials A and B, respectively. Individual running times during Part B are shown in Table 3.3. There were no differences between trials for any performance measurement.

3.2.8.4 Conclusion

The PIHSRT reported in this thesis provides a reliable and reproducible measure for investigating the influence of nutritional strategies on prolonged intermittent high intensity exercise performance.
Table 3.2: Sprint times over first 75 min during Trials A and B of the PIHSRT (n=8).

<table>
<thead>
<tr>
<th>Sprint Set</th>
<th>Trial A Mean</th>
<th>Trial A SEM</th>
<th>Trial B Mean</th>
<th>Trial B SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2.38</td>
<td>0.03</td>
<td>2.39</td>
<td>0.04</td>
</tr>
<tr>
<td>2</td>
<td>2.41</td>
<td>0.03</td>
<td>2.39</td>
<td>0.04</td>
</tr>
<tr>
<td>3</td>
<td>2.43</td>
<td>0.04</td>
<td>2.44</td>
<td>0.04</td>
</tr>
<tr>
<td>4</td>
<td>2.45</td>
<td>0.03</td>
<td>2.45</td>
<td>0.04</td>
</tr>
<tr>
<td>5</td>
<td>2.45</td>
<td>0.05</td>
<td>2.46</td>
<td>0.03</td>
</tr>
</tbody>
</table>

Table 3.3: Individual Part B running times during Trials A and B of the PIHSRT (n=8).

<table>
<thead>
<tr>
<th>SUBJECT</th>
<th>Trial A Time (min)</th>
<th>Trial B Time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>20.0</td>
<td>14.3</td>
</tr>
<tr>
<td>2</td>
<td>2.1</td>
<td>4.4</td>
</tr>
<tr>
<td>3</td>
<td>5.2</td>
<td>6.1</td>
</tr>
<tr>
<td>4</td>
<td>6.2</td>
<td>7.5</td>
</tr>
<tr>
<td>5</td>
<td>3.4</td>
<td>3.4</td>
</tr>
<tr>
<td>6</td>
<td>3.8</td>
<td>2.4</td>
</tr>
<tr>
<td>7</td>
<td>3.2</td>
<td>4.7</td>
</tr>
<tr>
<td>8</td>
<td>6.7</td>
<td>5.7</td>
</tr>
</tbody>
</table>

| Mean ± SE | 6.3 ± 2 | 6.1 ± 1.3 |
3.2.9 Statistical Analyses

Student's t-test for independent samples, Student's t-test for correlated samples, one-way analysis of variance (ANOVA), or two-way ANOVA for repeated measures on both factors were used, where appropriate, for statistical analysis. Where significant differences were found using ANOVA, a post-hoc Scheffe test was used to compare means. The level of significance was accepted at $P<0.05$. Results are reported as mean ± standard error (SEM).
3.3 Collection, Treatment, Storage and Analysis of Blood Samples

3.3.1 Sample Collection

In Study 1 (Chapter 4), capillary blood samples (20 μl) were taken in duplicate from the thumb using micro-pipettes. For collections at rest, the hand was initially pre-warmed in order to arterialise the capillary blood. Blood samples were immediately deproteinised in 200 μl of cool 0.38 mM perchloric acid, centrifuged (Eppendorf, Model 5414) and then frozen at -20°C for subsequent analysis of blood lactate and glucose concentrations. Blood glucose concentration was determined by photometric analysis using the glucose oxidase method as previously described by Werner et al. (1970). Blood lactate concentration was determined fluorimetrically using a method modified from that described by Maughan (1982).

In studies 2, 3 and 4 (Chapters 5, 6 and 7), venous blood samples were drawn from an antecubital vein in the forearm using an indwelling catheter (Venflon, 16-18G), and was kept patent by infusion of sterile saline (10 units of heparin per ml). Subjects rested on an examination couch for 15-20 min before local anaesthetic was administered (0.5 ml of 1% lignocaine) prior to catheter insertion. Subjects then assumed a standing position for 15-20 min before a 10 ml resting blood sample was drawn. Further blood samples were obtained throughout exercise and during recovery (Studies 2 and 4; Chapters 5 and 7) from exercise at pre-determined times.

3.3.2 Methodological Considerations on Estimating Changes in Plasma Volume

Changes in body posture from lying to standing can result in decreases in plasma volume of up to 10% (Rowell, 1993). Therefore all blood samples were taken while subjects were standing. Hence subjects assumed a standing position for 15-20 min before a 10 ml resting blood sample was drawn.
3.3.3 Treatment, Storage and Analysis of Venous Blood Samples

Venous blood samples were treated, stored and analysed in the following manner:

• One ml of whole blood was immediately dispensed into a calcium-heparinised Eppendorf tube (50 units per tube) and centrifuged for 5 min at 1300 rev.min⁻¹ (Eppendorf, Model 5414). The plasma obtained was stored at -70 °C, and analysed for ammonia concentration within 48 h using a commercially available kit (Boeringher Mannheim) (Studies 2 and 4; Chapters 5 and 7).

• Of the remaining whole blood, 5 ml was dispensed into lithium-heparinised tubes and 3 ml was left to clot for 1 h to obtain serum.

• Duplicate 20 μl aliquots of the whole venous blood were immediately deproteinised in 200 μl of cool 0.38 mM perchloric acid, centrifuged (Eppendorf, Model 5414) and then frozen at -20°C for subsequent analysis of blood lactate and glucose concentrations. Blood glucose concentration was determined by photometric analysis using the glucose oxidase method as previously described by Werner et al. (1970). Blood lactate concentration was determined fluorimetrically using a method modified from Maughan (1982).

• Haemoglobin concentration was determined in duplicate (2 x 20 μl) by the cyanomethaemoglobin method (Boeringher Mannheim GmbH Diagnostica). Triplicate 50 μl samples of whole venous blood were collected using heparinised pipettes and then micro-centrifuged (Hawksley Ltd) for 15 min at 11 000 rpm. Packed cell volume was measured using a sliding haematocrit reader (Hawksley Ltd). From the changes in haematocrit and haemoglobin concentrations from rest to the end of 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 rev.min\(^{-1}\) (Burkard μP Koolspin). The plasma obtained was divided into smaller aliquots and stored at -20°C for later analysis of FFA, using a commercially available kit (Waiko Ltd) and glycerol (Laurell and Tibbling, 1966). In Study 2 (Chapter 5), one aliquot of plasma containing anti-oxidant was stored at -70°C for later analysis of adrenaline and nor-adrenaline concentrations (Forster et al., 1991).

• Serum was obtained by centrifuging 4 ml of coagulated whole venous blood for 15 min at 6000 rev.min\(^{-1}\) at 4°C. It was then stored at -70°C for subsequent analysis of insulin by radio-immunoassay (Soeldner and Sloane, 1965) and sodium and potassium concentrations by flame photometry (Ciba Corning, Model M435).

The coefficient of variation [((Standard Deviation/Mean) × 100)] of the blood, plasma and serum assays on exercise samples is shown in Table 3.4.

**Table 3.4: Coefficient of variation (CV, %) for blood, plasma and serum assays (n=20).**

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>CV(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood lactate</td>
<td>1.3</td>
</tr>
<tr>
<td>Blood glucose</td>
<td>1.5</td>
</tr>
<tr>
<td>Plasma FFA</td>
<td>1.0</td>
</tr>
<tr>
<td>Plasma glycerol</td>
<td>3.1</td>
</tr>
<tr>
<td>Plasma ammonia</td>
<td>2.1</td>
</tr>
<tr>
<td>Serum insulin</td>
<td>5.6</td>
</tr>
<tr>
<td>Serum sodium</td>
<td>0.7</td>
</tr>
<tr>
<td>Serum potassium</td>
<td>2.3</td>
</tr>
</tbody>
</table>
3.4 Sampling, Treatment, Storage and Analysis of Muscle Tissue

3.4.1 Tissue Sampling

Muscle biopsies were taken from the middle portion of vastus lateralis by the percutaneous needle biopsy technique (Bergstrom, 1962), with suction applied to maximise sample size (Evans et al., 1982). The pre- and post-exercise samples (size ranging between 30 and 100 mg wet weight) were obtained through separate incisions made in the skin and fascia over the vastus lateralis, made under local anaesthetic (2-3 ml of 1% lignocaine). The vastus lateralis was the preferred muscle because not only is it easily accessible and isolated from major vessels and nerves, it is heavily involved during sports which are intermittent in nature, such as soccer (Agnevik, 1970 and Karlsson, 1969, cited by Ekblom, 1986).

3.4.2 Treatment and Storage of Muscle Samples

The time delay between the cessation of the PIHSRT and the post-exercise muscle biopsy was, on average, less than 1 min. Within 2-3 s of sampling, the muscle tissue was immersed in liquid nitrogen. Muscle samples were subsequently placed in plastic Eppendorf tubes and stored in liquid nitrogen for 12-24 h and then they were freeze dried for 12 h.

Each muscle sample was then divided into two portions and stored separately at -70°C in plastic screw-top Eppendorf tubes. One part of the freeze-dried muscle sample was stored for single fibre analysis at a later date, and the other was powdered for subsequent analysis of metabolites. Firstly, the freeze-dried muscle was washed twice with 1 ml petroleum ether in order to extract the fat from the muscle tissue. On both occasions the sample was mixed and centrifuged before the petroleum ether was removed by pipette. Finally, after the ether was removed for the second time, the samples were left to dry completely in the fume cupboard. Once the fat had been removed, each freeze-dried muscle sample was dissected
free of any obvious blood and connective tissue and homogenised using an agate pestle and mortar, weighed on an electrical balance, accurate to 5 decimal places, and stored in a screw-top Eppendorf tube at -70°C. The powdered muscle samples were analysed for metabolites at a later date.

3.4.3 Analysis for Mixed Muscle Metabolites

3.4.3.1 Extraction Procedure

Mixed muscle metabolites were extracted from the muscle powder on the same day as the analysis procedures by the following method (Harris et al., 1974). Firstly, the powdered muscle samples were removed from the freezer and left to stand at room temperature for approximately 1 h. Each sample was then centrifuged (Eppendorf, Model 5414) for 3 min at 13000 rev.min⁻¹ in order to be certain that the muscle powder was at the bottom of the tube. The tip of the Eppendorf tube containing the powdered muscle was dipped in liquid nitrogen and then 100 μl of 0.5 mmol.l⁻¹ perchloric acid (HClO₄), containing 1 mmol.l⁻¹ EDTA.Na₂ (ethylenediamine tetra-acetic sodium salt), was added per mg of dry muscle powder. This ensured that the extracting solution froze immediately it was dispensed into the eppendorf tube. Each sample was then immediately placed in the freezer at -20°C for a few minutes for the extracting solution to thaw until all samples were ready to be agitated. The samples were then placed on ice in a box, sealed tightly, and agitated using a Stuart flask shaker for 30 min. Samples were then centrifuged at 6000 rev.min⁻¹ for 10 min at 3-4°C (Burkard Koolspin, μP refrigerated centrifuge). The supernatant was then removed using a pipette, carefully measured (approximately 10-20 μl less than the volume of HClO₄ originally added), placed into clean pre-cooled screw-top Eppendorf tubes, and neutralised by adding 25 μl (that is, one-fourth volume) of 2.1 mol.l⁻¹ potassium bicarbonate (KHCO₃) per 100 μl of supernatant removed. The samples were then mixed and left in the fridge on ice at 3-4°C with caps unscrewed and loose in order for the CO₂ formed to escape. They were then centrifuged at 6000 rev.min⁻¹ for 5 min at 3-4°C. Finally, the
supernatant was removed, placed into clean pre-cooled screw-top eppendorf tubes and stored on ice. The pH of the extract was measured at 7.0 (neutralised). One ml of the extract was equivalent to 8 mg of muscle powder.

3.4.3.2 Mixed Muscle Metabolite Assays

All assays for each neutralised muscle extract were performed within two days. The analysis of ATP, PCr and Cr was performed on the first day immediately following the extraction procedure. Later in the day, free glucose, G-6-P and glycogen were assayed. The remaining extract was stored at -70°C and muscle lactate was assayed on the following day. Glycogen was assayed both on the neutralised extract (acid-soluble glycogen fraction) and on the remaining muscle pellet following prior hydrolysis in 1 mol.l⁻¹ hydrochloric acid (HCl) (acid-insoluble glycogen fraction), by determining the glucosyl units obtained after the glycogen was acid hydrolysed (Jansson, 1981). 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 Lowry and Passonneau (1972). Acid insoluble glycogen was assayed photometrically (Cecil, CE 393) for glucose using a commercially available glucose kit (Boeringher Mannheim- Glucose test combination, GOD/Perid method). Phosphocreatine Cr, ATP, free glucose, G-6-P and lactate were assayed flurometrically (Locarte, model 8-9). Their analysis was based on enzyme catalysed reactions, the coenzymes NAD⁺ and NADP⁺ being simultaneously reduced to NADH and NADPH, respectively. Duplicate analysis was performed on every sample. Detailed procedures of every assay performed are presented in Appendix D.
Buffers, co-factors (Grade-I) and enzymes were obtained as standard commercial items from Boehringer and Sigma. All reagents were made up with double-distilled water the day before the analysis and kept at 4°C. Standards were prepared on the day of the analysis using Grade-I chemicals. The molarity of the standards was checked on the same day spectrophotometrically using the coefficient of extinction of NADH/NADPH (Appendix E). The standard curves were always linear (r=0.999 to r=1.000). Metabolite concentrations [expressed in mmol.kg dry muscle (dm)⁻¹] were calculated using the resulting standard regression equation, and taking into account double-distilled water blanks, extract blanks, standards, dilution factors and the extraction factor. Where necessary, the concentrations of the metabolites were adjusted to the true molarity of the standards as calculated from the coefficient of extinction of NADH/NADPH, and to the highest content of total Cr (PCr + Cr) in the samples of each subject. The aim of the latter adjustment was to act as an internal reference in order to account for any errors in muscle metabolite concentrations as a consequence of any remaining connective tissue, blood, and fat droplets (Harris et al., 1974).

The coefficient of variation for all the assays using exercise samples is shown in Table 3.5. All muscle metabolite concentrations were determined in dry weight to avoid changes in concentration due to water shift during exercise.

3.4.4 Single Muscle Fibre Analysis

Single muscle fibre analysis was performed by Dr. O.K. Tsintzas at the Department of Physiology and Pharmacology, Medical School, Nottingham University. Details of the separation and identification of individual fibres, the extraction procedure, and the glycogen assay employed can be found in Appendix F, and have been reported elsewhere (Tsintzas, unpublished Doctoral thesis).
<table>
<thead>
<tr>
<th>Metabolite</th>
<th>CV(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acid insoluble glycogen</td>
<td>2.3</td>
</tr>
<tr>
<td>Acid soluble glycogen</td>
<td>4.1</td>
</tr>
<tr>
<td>ATP</td>
<td>2.5</td>
</tr>
<tr>
<td>PCr</td>
<td>2.4</td>
</tr>
<tr>
<td>Cr</td>
<td>2.8</td>
</tr>
<tr>
<td>Glucose</td>
<td>3.8</td>
</tr>
<tr>
<td>G-6-P</td>
<td>3.3</td>
</tr>
<tr>
<td>Lactate</td>
<td>1.5</td>
</tr>
</tbody>
</table>
CHAPTER 4

CARBOHYDRATE INTAKE AND RECOVERY
OF INTERMITTENT RUNNING CAPACITY

4.1 INTRODUCTION

It has been well established that the ability to sustain prolonged exercise at intensities ranging from 65 to 85 % $\text{VO}_2 \text{max}$ is related to the pre-exercise concentration of muscle glycogen (Bergstrom et al., 1967; Hermansen et al., 1967). Fatigue during such exercise is associated with the depletion of the muscle's limited glycogen stores. The replenishment of muscle glycogen stores may take 24 h or longer following intense, intermittent, exhaustive cycling exercise (MacDougall et al., 1977; Keizer et al., 1986), or up to 46 h (Piehl, 1974), and is dependent upon the CHO intake during the post-exercise period (Bergstrom et al., 1967; Costill et al., 1981; Blom et al., 1987b; Ivy et al., 1988a; Ivy et al., 1988b). However, the interest in the time course of glycogen repletion is not the exclusive focus of attention, but the recovery of functional fitness.

Although there is now a better understanding of the factors which contribute to the restoration of muscle glycogen stores within 24 h, there is relatively little information on the recovery of functional fitness. In one of the few studies on recovery, Fallowfield and Williams (1993) reported that endurance running capacity could be restored within 24 h after appropriate nutritional intervention. However, during high intensity exercise, Keizer et al. (1986) reported that maximal physical working capacity (MPWC) was decreased 22 h after exhaustive interval exercise, despite the restoration of muscle glycogen concentrations.

Few studies have documented the effects of dietary intervention on intermittent exercise, designed to replicate the activity pattern typical of the multiple sprint sports. Bangsbo et al. (1992b) reported that a CHO enriched diet improved intermittent high intensity running
capacity. Indeed, only one study has been concerned with the recovery of functional fitness during maximal intermittent sprinting. Nevill et al. (1993) observed no differences in power output between sprint performance during 1h of intermittent exercise on a non-motorised treadmill when repeated 24 h later after either a high, normal or low CHO intake. The protocol in this study comprised 30 maximal 6 s sprints interspersed with a 54 s walk at 20% of maximal speed and a 60 s jog at 40% of maximal speed, thus replicating the activities commonly observed in the multiple sprint sports. However, it is possible that the differences in power output may have been observed if the duration of the trial was increased and/or if the energy intake of the different groups was increased, the groups still consuming relatively high, normal and low amounts of CHO. This may have been preferable to manipulating the relative amounts of CHO based on normal energy intake values. In addition, the type of CHO consumed in the 24 h recovery period may have resulted in different rates of glycogen repletion which could have affected the results. It has been shown that the most rapid increase in muscle glycogen concentration during the 24 h post-exercise period occurs following the consumption of foods with a high glycemic index (Burke et al., 1993).

Thus, little information exists regarding the effect of nutritional intervention on the recovery from prolonged, maximal, intermittent exercise. Therefore the purpose of the present study was to examine the effect of providing additional energy, either as CHO or fat and protein, on the recovery of maximal, intermittent sprint performance and running capacity 22 h following intermittent running to exhaustion.
4.2 METHODS

4.2.1 Subjects

Six healthy male games players (age 21.8 ± 0.7 yr; height 174.4 ± 4.0 cm; body mass 70.5 ± 4.2 kg; maximal oxygen uptake 56.0 ± 0.9 ml.kg⁻¹.min⁻¹; means ± SEM) volunteered and gave their informed consent to take part in this study. Each subject had at least 5 years playing experience in soccer, rugby, hockey or basketball. Weekly training and playing time ranged from 5 to 8 h.

4.2.2 Experimental Design

The study consisted of a cross-over design (n=6). Subjects were required to attend the laboratory on 4 occasions and performed a total of 4 trials, under 2 dietary conditions. Trial 1 (T1) and trial 2 (T2) were performed 22 h apart and then 1 week later, trial 3 (T3) and trial 4 (T4) were performed on 2 consecutive mornings, 22 h apart. Trials 2 and 4 were performed following dietary manipulation of either a carbohydrate (CHO) or control (CON) condition (Fig. 4.1). The order of dietary condition, CHO or CON, was randomly assigned.
Fig 4.1: Schematic diagram illustrating the experimental design.

4.2.3 Experimental Procedures

Subjects performed a couple of preliminary tests to predict VO$_2$ max in order to calculate the relative exercise intensities as previously described (Chapter 3) and to familiarise themselves with the experimental procedures.

For the 2 days prior to trials 1 and 3, and during the 22 h recovery periods, subjects were requested to abstain from consuming alcohol and from training, in order to avoid the possible effects of exercise on muscle glycogen stores prior to the experimental trials. Subjects arrived in the laboratory in the morning following an overnight fast of approximately 10 h. They then voided before the measurement of nude body mass was taken. Nude body mass was recorded pre- and post-exercise. Heart rate was monitored by short-range telemetry (Sport Tester PE3000) every 15 s during exercise. Subjects sat relaxed for approximately 10 min,
during which time, resting heart rate was recorded and an estimation of gut fullness was made using a questionnaire. Duplicate 20 µl capillary blood samples were obtained at rest from the thumb of a pre-warmed hand. Capillary blood samples were also taken after 20, 40 and 70 min of exercise, at exhaustion and 5 min post-exhaustion. At each sampling point, duplicate 20 µl samples were deproteinised in 200 µl of cooled 0.4 M perchloric acid, mixed, centrifuged and stored at -20°C until analysed for concentration of blood lactate and glucose (Maughan, 1982).

A standardised warm up consisting of jogging, stretching and striding was then performed by each subject for 20 min. The test protocol consisted of 2 parts (Fig. 4.2) and has been previously described (Chapter 3). Part A was a fixed 70 min period of variable intensity exercise. In Part B, subjects ran intermittently between low (55% $\text{VO}_2\text{max}$) and high (95% $\text{VO}_2\text{max}$) exercise intensities, until they could no longer maintain the higher intensity running speed required.

Subjective ratings of perceived exertion (Borg, 1973) were obtained after every 5 completed exercise units during Part A and the mean values during 0-20, 20-40 and 40-70 min of exercise, and at exhaustion were recorded. The temperature of the gymnasium was maintained at ≤ 20°C by adjusting the thermostatically controlled radiators and by opening and closing doors and windows. Dry and wet bulb temperatures were recorded at 0, 20, 40, 70 min of exercise, and at exhaustion using a whirling hydrometer (Brannan Thermometers Ltd.), from which relative humidity was calculated. The ingestion of water during each trial was allowed ab libitum. The total amount ingested for the duration of each trial was recorded and accounted for when calculating the changes in body weights of the subjects from their pre-exercise values.
Figure 4.2: Schematic illustration of the PIHSRT protocol and experimental design.
4.2.4 Dietary Regimen and Analysis

Subjects weighed and recorded all food and drink consumed in their normal diet for 2 days prior to T1. From these food diaries, the average daily energy intake and amount of CHO ingested was recorded for each individual. The amount of energy needed to increase individual daily CHO consumption to 10 g.kg\(^{-1}\) bm was calculated. For the 22 h recovery period, subjects were prescribed a recovery diet which included this energy in addition to the normal dietary intake, either in the form of carbohydrate (CHO group) or fat and protein (CON group). Recovery diet was prescribed by a dietician using a food exchange system, which was consistent with the foods normally consumed by the subjects. Subjects were also required to eat their normal diet for the 2 days prior to T3.

Within 20 min of the termination of Trials 1 and 3 (the pre-dietary manipulation trials), all subjects were provided with 1 g.kg\(^{-1}\) of a 6.9% CHO-E drink (Lucozade Sport, SmithKline Beecham) and breakfast. For the CHO group, this consisted of 60 g cornflakes, 20 g raisins, 250 g semi-skimmed milk, 60 g wholemeal bread and 30 g jam (122 g CHO; 603 kcal). Breakfast for the control group consisted of 105 g scrambled egg, 40 g cheddar cheese, 100 g semi-skimmed milk, 30 g wholemeal bread and 10 g butter (18 g CHO; 607 kcal).

4.2.5 Statistical Analyses

Performance times and the physiological responses of subjects following dietary manipulation during trials were analysed by means of a two-way analysis of variance (2-WAY ANOVA) for repeated measures on two factors and Student's paired t-test, where applicable. Where differences emerged, a post-hoc Tukey test was used to identify where the differences occurred. Statistical significance was accepted at the P<0.05 level. Values are reported as mean ± SEM.
4.3 RESULTS

4.3.1 Performance Data

During Part B of T1, prior to dietary manipulation, subjects ran for 17.2 ± 2.8 min and 15.6 ± 2.0 min in the CHO and CON trials, respectively (ns). In the CHO T2, subjects ran for 3.3 ± 1.1 min longer than in Part B during T1 (P<0.05) and 7.4 min longer than their relative performance during T2 following the CON recovery diet (P<0.01). When delta T1 and T2 differences in performance were compared between experimental conditions, subjects ran for 5.8 ± 1.7 min longer in the CHO trial (P<0.05). This difference in time corresponded to a distance of 1088 ± 316 m (P<0.05). For Part A, the distance covered was similar for all trials, ranging between 9919 to 9928 m. For the CHO trial, subjects covered a greater total distance in trial 1 (13.2 km) than trial 2 (13.8 km; P<0.05). In the CON trial, the total distance was 12.9 km for T1 and 12.4 km for T2 (ns) (Fig. 4.3). In essence, not only was intermittent running capacity restored in the 22 h recovery period, it was also improved. No such improvement was observed in the CON group.

The sprint times during Part A are shown in Table 4.1. Similar sprint times were recorded between trials and also within each trial over the 70 min. However, there was a tendency for the fastest sprints to be recorded in the first 20 min of exercise.

4.3.2 Blood Glucose and Lactate Concentrations

Blood glucose concentrations remained within the normal range during all trials (Fig. 4.4). No differences were observed between trials or groups, although there was a tendency for blood glucose concentration to be lower during T2 than T1, under both dietary conditions.

No differences in blood lactate concentrations were observed between experimental trials (Fig. 4.5). The highest concentration was reached at the end of exercise, being higher than
resting values in all trials (P<0.01) and after 20 and 40 min of exercise in T2 in both CHO (P<0.01 and P<0.05, respectively) and CON groups (P<0.05).

4.3.3 Changes in Body Mass and Fluid Consumption

Similar reductions in body mass of 2.2% were observed during all trials. Fluid ingestion of 524 ± 85 ml and 536 ± 74 ml (ns) are respectively accounted for in T1 and T2 (CHO). Fluid intake of 520 ± 69 ml for T1 and 657 ± 97 ml for T2 (ns) was accounted for under CON condition. Body weight was restored to T1 pre-exercise values during the 22 h recovery following dietary control in both groups.

4.3.4 Heart Rate

Heart rate values are shown in Figure 4.6. No differences were observed between trials or conditions. During the first 20 min of exercise, mean heart rate increased from resting values (P<0.01) and were then maintained within a range of 3 b.min⁻¹ for the subsequent 20, and further 30 min of exercise during Part A of the PIHSRT for all trials. Heart rate increased during Part B (P<0.01) during all trials, reaching maximal values immediately prior to the cessation of exercise.

4.3.5 Energy and CHO Intake In Normal and Recovery Diets

The total energy consumed and the CHO intake in the 22 h recovery period for both dietary conditions is shown in Table 4.2. In the CHO trial, a greater amount of CHO was consumed during recovery compared with the normal dietary intake of CHO in the CON trial (P<0.01). The corresponding increase in energy consumed was significantly greater than their normal food intake during both prescribed recovery diets (P<0.01), but there was no difference between the CHO and CON trials.
Figure 4.3: Distance run (m) during intermittent exercise trials
(* P<0.05 : CHO T2 vs. T1; CHO T2 vs. CON T2).

Figure 4.4: Blood glucose concentration (mmol.l⁻¹) during intermittent exercise trials.
Figure 4.5: Blood lactate concentration (mmol.l\(^{-1}\)) during intermittent exercise trials.

Figure 4.6: Heart rate response during intermittent exercise to exhaustion (\(\ast\ast P<0.01\) : Part B and End vs. Part A).
Table 4.1: Mean sprint times (s) for each exercise period.

<table>
<thead>
<tr>
<th>Trial</th>
<th>EXERCISE TIME (min)</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0-20</td>
<td>20-40</td>
<td>40-70</td>
<td>Trial Mean</td>
</tr>
<tr>
<td>CHOT1</td>
<td>2.50 ± .05</td>
<td>2.51 ± .07</td>
<td>2.54 ± .09</td>
<td>2.52 ± .07</td>
</tr>
<tr>
<td>CHOT2</td>
<td>2.52 ± .06</td>
<td>2.59 ± .08</td>
<td>2.59 ± .07</td>
<td>2.57 ± .07</td>
</tr>
<tr>
<td>CONT1</td>
<td>2.48 ± .05</td>
<td>2.48 ± .05</td>
<td>2.49 ± .06</td>
<td>2.48 ± .05</td>
</tr>
<tr>
<td>CONT2</td>
<td>2.55 ± .07</td>
<td>2.56 ± .07</td>
<td>2.55 ± .08</td>
<td>2.55 ± .07</td>
</tr>
</tbody>
</table>

Table 4.2: Composition of normal and recovery diets.

<table>
<thead>
<tr>
<th>DIET</th>
<th>ENERGY (MJ)</th>
<th>CHO (g)</th>
<th>CHO (g.kg⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NORMAL</td>
<td>10.7 ± 0.7</td>
<td>381 ± 23</td>
<td>5.4</td>
</tr>
<tr>
<td>RECOVERY(CHO)</td>
<td>15.6** ± 1.0</td>
<td>705** ± 42</td>
<td>10.0**</td>
</tr>
<tr>
<td>RECOVERY(CON)</td>
<td>15.6** ± 1.0</td>
<td>381 ± 23</td>
<td>5.4</td>
</tr>
</tbody>
</table>

**P<0.01; Normal vs. Recovery Diet.
The main finding in this study was that a normal diet supplemented with additional CHO to the order of 10 g.kg\(^{-1}\) bm.day\(^{-1}\) restored and improved intermittent running capacity after a recovery of 22 h. An equivalent return in exercise performance did not occur when subjects consumed an isocaloric diet containing additional fat and protein during the same period of recovery.

The CHO intake over the 22 h recovery period was 705 ± 42 g (10 g.kg\(^{-1}\)bm.day\(^{-1}\)) and 381 ± 23 g (5.4 g.kg\(^{-1}\)bm.day\(^{-1}\)) for the CHO and CON trials, respectively. Performance times were similar during trial 1 for both experimental conditions, however, in the CHO trial 2, subjects were able to run 1.1 km further than in the CON trial 2. This increase in running capacity is similar to the 0.7 km improvement reported in soccer players during a field test of 45 min duration and then intermittent treadmill running to exhaustion, following the ingestion of a CHO enriched diet for 2 days (Bangsbo et al., 1992b). Thus the equivalent of 10 g.kg\(^{-1}\)bm.day\(^{-1}\) of CHO restored and enhanced intermittent running capacity in the present study, which is consistent with previous recommendations (Costill et al., 1981; Keizer et al., 1986; Fogelholm et al., 1989; Coyle, 1991; Friedman et al., 1991). Fallowfield and Williams (1993) investigated the influence of an increased CHO intake on endurance running capacity 22.5 h following recovery from 90 min of prolonged treadmill running at 70% \(\text{VO}_{2}\max\). A high CHO diet (8.8 g.kg\(^{-1}\)bm.day\(^{-1}\)) resulted in the restoration of endurance capacity, whereas exercise capacity was not restored following an isocaloric diet with additional energy provided in the form of fat and protein.

Muscle glycogen stores can be replenished within 24 h, but there is an optimal CHO intake which is relative to the intensity, duration, frequency and mode of exercise (for reviews, see Robergs, 1991; Sherman, 1992; Doyle et al., 1993; Pascoe and Gladden, 1996). The type of CHO intake during the recovery period is also important, as high glycemic foods result in a more rapid rate of muscle glycogen resynthesis 24 h after the cessation of 2 h cycling at 75%
VO₂ max followed by four 30 s sprints (Burke et al., 1993). Where CHO intake matches CHO utilisation, it may be possible to maintain resting glycogen stores and training load (Costill et al., 1988). For example, following short duration, intense intermittent cycling, a CHO intake of 4.8 g·kg⁻¹·bm⁻¹·day⁻¹ was sufficient to replenish resting muscle glycogen levels, whilst additional CHO did not accelerate the rate of glycogen resynthesis (MacDougall et al., 1977). In contrast, Pascoe et al. (1990) reported that a CHO intake of 5 g·kg⁻¹·bm⁻¹·day⁻¹ did not prevent a ~13% reduction in muscle glycogen over a 3 day training period.

Furthermore, Kirwan et al. (1988) found that 8.0 g·kg⁻¹·bm⁻¹·day⁻¹ was not sufficient to restore muscle glycogen levels daily in subjects running 20 km at 80% VO₂ max on 5 successive days. The possible reasons for the inadequate repletion of glycogen stores is that either training on consecutive days requires a greater CHO intake than previously calculated, or that micro-trauma experienced as a consequence of the eccentric component of running delays glycogen resynthesis (Kirwan et al., 1988; Costill et al., 1990; Doyle et al., 1993).

However, an increased CHO intake that restores muscle glycogen levels to pre-exercise values may not result in the restoration of maximal performance within 24 h. Keizer et al. (1986) reported that MPWC was reduced by 7.2%, 22 h after the cessation of exhaustive interval exercise, despite the complete restoration of muscle glycogen. Sherman et al. (1983) and Sherman et al. (1984) reported that 7 days following marathon running, isokinetic strength remained significantly reduced compared with pre-marathon values, despite the restoration of normal muscle glycogen levels. Thus the recovery of maximal physical performance following exhaustive exercise may be influenced by the degree of microtrauma as a result of exercise, and may require factors in addition to muscle glycogen restoration. Also, MPWC may have been affected by the reduction in muscle fibre recruitment as a result of muscle damage caused by biopsy sampling (Costill et al., 1988).

In the present study, similar sprint times were observed over the duration of each trial and between trials. These findings are consistent with those of Nevill et al. (1993), who reported no differences in mean power output during sprinting on a non-motorised treadmill between
subjects following 24 h recovery and dietary intervention. The possible explanation for the fact that no differences were observed was that the energy intake was not increased over the 24 h recovery period; only the relative CHO content was altered. In addition, the test was of a fixed 1 h duration which may not have challenged the body's energy reserves sufficiently. Phosphocreatine availability is crucial for maximal sprint performance (Greenhaff et al., 1994c), and thus, sprint performance was well maintained over the course of thirty, 6 s sprints, because the 114 s recovery between sprints was adequate for PCr resynthesis. In the present study, it is likely that PCr may be almost completely restored during the ~80 s recovery between sprints.

The cause of fatigue during exercise in Part B may possibly be due to muscle glycogen depletion in a specific fibre population. Blood glucose concentrations were maintained within the normal range for the duration of each trial and were similar between trials and between dietary groups. Thus, hypoglycaemia was not responsible for the onset of fatigue during prolonged intermittent exercise in the present study. Intermittent exercise and sprinting results in a marked reduction in muscle glycogen concentration in both type I and type II fibres (Karlsson and Saltin, 1971; Jacobs et al., 1982b; Keizer et al., 1986; Greenhaff et al., 1994c). It is postulated that the prolonged intermittent, high intensity exercise protocol used in the present study depleted muscle glycogen, thereby contributing to the onset of fatigue. In addition, the alternate exercise intensities at 95% and 55% Vmax may have caused a significant reduction in the PCR content of the exercising muscles which could not be restored during exercise which, although intermittent, was performed continuously. This would increase the reliance on glycogen as the substrate. Thus it proposed that the additional CHO, provided during the recovery duration of 22 h between exercise trials, delayed the onset of fatigue and allowed subjects to perform intermittent, high intensity exercise for longer during the second exercise trial.

In summary, increasing the CHO intake of a normal diet to 10g.kg⁻¹bw.day⁻¹ improves intermittent running capacity after 22 h of recovery.
CHAPTER 5

INFLUENCE OF INGESTING A CARBOHYDRATE ELECTROLYTE SOLUTION ON ENDURANCE CAPACITY DURING PROLONGED INTERMITTENT SHUTTLE RUNNING

5.1 INTRODUCTION

Increased CHO intake over a 22 h recovery from exhaustive intermittent high intensity exercise has been shown to enhance subsequent endurance capacity (Chapter 4). In addition, the ingestion of a CHO solution immediately prior to, and at frequent intervals during exercise delays fatigue and so increases exercise capacity (Coyle et al., 1983; Hargreaves et al., 1984; Yaspelkis et al., 1993) and improves exercise performance (Murray et al., 1987; Mitchell et al., 1988; Tsintzas et al., 1993a; Tsintzas et al., 1995a; Tsintzas et al., 1995b). This ergogenic effect may be due to the maintenance of blood glucose (Coyle et al., 1983; Mitchell et al., 1988) or a reduction in the rate of muscle glycogen utilisation (Hargreaves et al., 1984; Tsintzas et al., 1995a; Yaspelkis et al., 1993).

Few studies (Kirkendall et al., 1988; Leatt and Jacobs, 1989) have investigated the effect of CHO feedings on performance during intermittent running which is typical of the activity pattern observed in many competitive and recreational sports. Thus the purpose of this study was to examine the influence of ingesting a commonly available sports drink (6.9 % CHO-E solution) on sprint performance and running capacity during the PIHSRT.
5.2 METHODS

5.2.1 Subjects

Nine trained, healthy male games players (age 24.8 ± 0.6 yr; height 179.6 ± 1.9 cm; body mass 80.7 ± 3.2 kg; VO₂ max 56.3 ± 1.3 ml.kg⁻¹.min⁻¹) volunteered and gave their informed consent to participate in this study, which had University Ethical Committee approval.

5.2.2 Preliminary Measurements

The speeds at which each subject was required to run during the PIHSRT were determined from predicted VO₂ max values, as previously described (Chapter 3). Subjects then performed the PIHSRT for a 30 min period in order to familiarise themselves with the required running speeds and experimental procedures.

5.2.3 Experimental Design

Subjects completed two exercise trials separated by at least 7 days. The trials were randomised in order to offset any training or order effects. On each occasion, they consumed either a 6.9% CHO-E solution (Lucozade Sport, SmithKline Beecham, Coleford, UK) (the CHO trial) or a non-CHO placebo, artificially sweetened with aspartame (SmithKline Beecham, Coleford, UK) (the CON trial), immediately before the start of exercise (5ml.kg⁻¹bm⁻¹), and every 15 min thereafter (2ml.kg⁻¹bm⁻¹). The solutions ingested were of the same colour, texture and taste, and were administered in a double-blind design.

During the 2 days preceding each trial, subjects refrained from any strenuous physical activity, and consumed their normal diet. They reported to the laboratory after an overnight fast of approximately 10 h, and then voided before the measurement of nude body mass was made. Nude body mass was recorded pre- and post-exercise. An indwelling catheter
(Venflon, 16-18G) was inserted into an antecubital vein and kept patent with infusion of sterile saline (10 units of heparin per ml). Subjects assumed a standing position for 15 min before a resting blood sample was obtained.

A standardised warm up consisting of jogging, stretching and striding was then performed by each subject for 15 min. Then, immediately before starting exercise, subjects consumed the prescribed solution. The set pattern of intermittent exercise (previously described in Chapter 3) during the PIHSRT was performed for 75 min (Part A) before a timed run to exhaustion (Part B)(Fig. 5.1). During Part B, subjects ran at speeds corresponding to 55% and 95% of their predetermined VO$_2$ max, alternating every 20 m, until fatigue. Fatigue was defined as the inability to maintain the prescribed running speed for 2 consecutive 20 m at the higher running speed. Subjects were verbally encouraged throughout each trial, and were not aware of their performance time during Part B.

Heart rate was continuously recorded every 15 s throughout each exercise trial using short-range telemetry (Sports Tester PE 3000, Polar Electro, Finland) and stored in memory mode. Subjective ratings of perceived exertion (Borg, 1982) and gut fullness (determined by a linear scale questionnaire) were obtained at 15 min intervals during Part A, and at fatigue. Dry and wet bulb temperatures were recorded every 15 min using a whirling hydrometer (Brannan Thermometers, Cumberland, UK), and the temperature of the gymnasium was maintained at $\leq 20 ^\circ C$.

5.2.4 Blood Sampling and Analysis

Ten ml of blood was withdrawn at rest, after 15, 30, 45, 60, 75 min of exercise, at exhaustion, and at 15, 30 and 45 min post-exercise. The blood was dispensed, treated and stored as previously described (Chapter 3). Serum was analysed for insulin and electrolyte concentrations and plasma was analysed for ammonia, FFA, glycerol, adrenaline and noradrenaline concentrations. Whole blood was assayed for lactate and glucose
concentrations and plasma volume changes, using methods which have been previously described (Chapter 3).

5.2.5 Statistical Analyses

The performance times of the two groups were compared using Student's t-test for correlated data. Physiological and blood biochemical responses in both trials were analysed using a two-way (treatment by time) analysis of variance for repeated measures. Significant differences between means were identified using a Scheffe post-hoc test. The level of significance was accepted at P<0.05. All data are reported as mean ± SEM.
Figure 5.1: Schematic illustration of the PIHSRT protocol and experimental design.
5.3 RESULTS

5.3.1 Exercise Times to Fatigue

The run times of the CHO and CON groups during Part B, after the fixed 75 min period of intermittent exercise, were 8.9 ± 1.5 min and 6.7 ± 1.0 min, respectively. Thus, when subjects ingested a CHO-E solution, they ran for 2.2 min longer than when drinking a placebo solution (P<0.05). The distance covered during Part A in both trials was 10.8 ± 0.2 km, whereas in Part B the distance covered was 1.7 ± 0.3 km and 1.3 ± 0.2 km (P<0.05), for the CHO and CON trials, respectively. Similar run times to exhaustion and distance covered were recorded when trials were compared by order, irrespective of treatments. Thus there was no effect of trial order on intermittent running capacity.

5.3.2 Sprint Times

For clarity of presentation, the mean time for every 6 sprints was recorded. Sprint times were similar between trials and over the duration of each trial. Times for 15 m sprints during Part A for the CHO and CON trials were 2.33 ± 0.03 s and 2.43 ± 0.1 s, respectively.

5.3.4 Blood Glucose and Serum Insulin

Blood glucose concentrations were maintained within the normal range in both trials (Fig. 5.2), but there was a tendency for values to be higher when the CHO beverage was ingested compared with a placebo solution. Differences in blood glucose concentrations between trials occurred at 30 min during exercise, at exhaustion (P<0.05), and at 15, 30 (P<0.01) and 45 min after exercise (P<0.05). Serum insulin values tended to be higher at each sampling point (Fig. 5.3), but only reached statistical significance at 15, 30 and 45 min post-exercise (P<0.05). In the CHO trial, insulin concentrations were similar during exercise, but increased after 15 min of recovery (P<0.05), and remained elevated above exercise values at
During the CON trial, insulin concentrations declined from resting values and were lower than resting at 60 min (P<0.05), at 75 min (P<0.01) and at the end of exercise (P<0.05).

5.3.4 Plasma FFA and Glycerol

There were no differences in plasma FFA concentrations between trials during exercise (Fig. 5.4). However, post-exercise values were higher in the CON trial than the CHO trial at +30 min (P<0.05) and at +45 min (P<0.01). Post-exercise plasma FFA concentrations were higher than resting and all exercise values in the CON trial (P<0.01). In the CHO trial, plasma FFA concentrations at 15 min post-exercise were also higher than at resting, as well as during 15, 30, 45, 60 and 75 min of exercise (P<0.01), and at exhaustion (P<0.05). Similarly, the only differences found between trials for plasma glycerol was 45 min after exercise (P<0.05; Fig. 5.5). Plasma glycerol concentrations increased from resting to the end of exercise under both conditions (P<0.01) and then fell to near-resting values 45 min after exercise (P<0.01).

5.3.5 Plasma Ammonia and Serum Electrolytes

Plasma ammonia increased in both groups over the duration of exercise (P<0.01). Similar concentrations were observed between trials. Mean resting values were 27 ± 3 μmol.l⁻¹ and 33 ± 6 μmol.l⁻¹ for the CHO and CON groups, respectively, rising to 110 ± 15 μmol.l⁻¹ and 112 ± 19 μmol.l⁻¹ post-exercise.

There were no differences in serum sodium and potassium concentrations between the two groups. During each trial, serum concentrations of sodium and potassium increased from resting values to exhaustion (Table 5.1; P<0.01).
5.3.6 Blood Lactate, Heart Rate and Perceived Rate of Exertion

There were no differences in either blood lactate concentrations or heart rate response between trials (Table 5.2). Blood lactate concentrations increased over the duration of each trial from resting values of $1.1 \pm 0.1 \text{ mmol.l}^{-1}$ and $1.2 \pm 0.1 \text{ mmol.l}^{-1}$ for the CHO and CON trials, respectively to $8.9 \pm 1.1 \text{ mmol.l}^{-1}$ and $7.7 \pm 0.9 \text{ mmol.l}^{-1}$, respectively, at the end of exercise ($P<0.01$). Ratings of perceived exertion were not different between trials, rising to $9.5 \pm 0.2$ and $9.7 \pm 0.2$ at exhaustion in the CHO and CON trials, respectively.

5.3.7 Plasma Noradrenaline and Adrenaline

The catecholamine responses to fluid ingestion during the PIHSRT are shown in Fig. 5.6 (noradrenaline) and Fig. 5.7 (adrenaline). Plasma noradrenaline and adrenaline peaked at the end of exercise in both trials and were higher than resting values ($P<0.01$). The only difference observed in the catecholamine response between trials was that of noradrenaline, which was greater in the CHO trial after 15 min ($P<0.05$).

5.3.8 Changes in Plasma Volume and Body Weight

Environmental conditions were similar during the CHO and CON trials. There were no differences between trials for changes in plasma volume from resting to the end of exercise (CHO, -4.5%; CON, -1.4%) or body mass reduction during each trial (CHO, 2.4 kg; CON, 2.4 kg). Thus differences in body mass after exercise represented a 3% loss in both trials (the ingested fluid during exercise was accounted for in these calculations).
Fig. 5.2: Blood glucose concentration (mmol.l\(^{-1}\)) in the CHO and CON trials during the PIHSRT. * and ** denotes significantly different from CON trial, \(P<0.05\) and \(P<0.01\), respectively.

Fig. 5.3: Serum insulin concentration (\(\mu\)IU.ml\(^{-1}\)) in the CHO and CON trials during the PIHSRT. * denotes significantly different from CON trial \((P<0.05)\).
Fig. 5.4: Plasma FFA concentration (mmol.1⁻¹) in the CHO and CON trials during the PIHSRT. * and ** denotes significantly different from CON trial, P<0.05 and P<0.01, respectively.

Fig. 5.5: Plasma glycerol concentration (mmol.1⁻¹) in the CHO and CON trials during the PIHSRT. * denotes significantly different from CON trial (P<0.05).
Fig. 5.6: Plasma noradrenaline concentration (nmol.l\(^{-1}\)) in the CHO and CON trials during the PIHSRT. * denotes significantly different from CON trial (P<0.05).

Fig. 5.7: Plasma adrenaline concentration (nmol.l\(^{-1}\)) in the CHO and CON trials during the PIHSRT.
Table 5.1: Serum sodium and potassium concentration (mmol.l⁻¹) in CHO and CON trials during the PIHSRT (n=9).

<table>
<thead>
<tr>
<th>Serum Electrolyte</th>
<th>Rest Mean</th>
<th>Rest SEM</th>
<th>End Mean</th>
<th>End SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium CHO</td>
<td>140</td>
<td>1</td>
<td>144</td>
<td>1 **</td>
</tr>
<tr>
<td>Sodium CON</td>
<td>141</td>
<td>1</td>
<td>143</td>
<td>2</td>
</tr>
<tr>
<td>Potassium CHO</td>
<td>4.2</td>
<td>0.1</td>
<td>4.8</td>
<td>0.1 **</td>
</tr>
<tr>
<td>Potassium CON</td>
<td>4.2</td>
<td>0.1</td>
<td>4.9</td>
<td>0.1 **</td>
</tr>
</tbody>
</table>

**P<0.01 from rest.

Table 5.2: Blood lactate concentration and heart rate response in the CHO and CON trials during the PIHSRT (n=9).

<table>
<thead>
<tr>
<th>EXERCISE TIME (min)</th>
<th>TRIAL</th>
<th>Part A</th>
<th>Part B</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Rest</td>
<td>15</td>
<td>30</td>
</tr>
<tr>
<td>Blood Lactate Concentration (mmol.l⁻¹)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CHO</td>
<td>1.1 ± 0.1**</td>
<td>7.0 ± 1.0</td>
<td>6.5 ± 0.7</td>
</tr>
<tr>
<td>CON</td>
<td>1.2 ± 0.1**</td>
<td>6.7 ± 0.9</td>
<td>7.0 ± 1.0</td>
</tr>
<tr>
<td>Heart Rate Response (beats.min⁻¹)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CHO</td>
<td>91 ± 4**</td>
<td>163 ± 3</td>
<td>169 ± 3</td>
</tr>
<tr>
<td>CON</td>
<td>92 ± 3**</td>
<td>163 ± 3</td>
<td>171 ± 3</td>
</tr>
</tbody>
</table>

**P<0.01 from all subsequent values.
††P<0.01 from Part A measurements.
5.4 DISCUSSION

The main finding of this study was that a CHO-E solution consumed immediately before, and throughout intermittent, high intensity exercise, delayed fatigue and improved endurance running capacity by 33%, when compared with the same volume of a non-CHO placebo. This improvement in high intensity endurance capacity following a fixed amount of intermittent exercise is consistent with observations in previous studies using cycling as the mode of exercise (Fielding et al., 1985; Hargreaves et al., 1984; Yaspelkis et al., 1993).

The intermittent exercise protocol used in the present study consisted of a fixed bout of variable intensity running, followed by a combination of high and low speed intermittent running until volitional fatigue. Other laboratory studies which have also shown improvements in exercise capacity after ingesting CHO during exercise have utilised different intermittent exercise protocols during cycling. Hargreaves and co-workers fed subjects 43g of solid CHO along with 400 ml of water prior to, and then every hour during 4 h of intermittent cycling (Hargreaves et al., 1984). The experimental protocol for each 30 min consisted of 20 min of moderate-intensity cycling at 50% VO₂ max, and then 10 min of intense, intermittent exercise (30 s cycling at 100% VO₂ max followed by 2 min of rest). After 4 h, subjects fed solid CHO improved sprint time at 100% VO₂ max to exhaustion compared with the placebo treatment. Yaspelkis and colleagues (Yaspelkis et al., 1993) observed that providing their subjects with liquid CHO at an ingestion rate of 18 g.h⁻¹ enhanced endurance cycling capacity following 190 min of intermittent cycling, which varied between low (45% VO₂ max) and moderate intensity (75% VO₂ max).

Ingesting CHO solutions providing approximately 19-25 g.CHO.h⁻¹ improves prolonged, submaximal running performance (Tsintzas et al., 1993a; Williams et al., 1990) and enhances endurance running capacity (Wilber and Moffatt, 1992). However, these latter studies employed continuous, submaximal running, and not running at variable exercise intensities. The benefits of CHO ingestion during intermittent, high intensity running are less
well documented. Leatt and Jacobs (1989) found that the ingestion of a 7% glucose solution reduced net muscle glycogen utilisation during a game of soccer, but did not comment on any improvement in performance. However, other investigators have reported that 25% greater distance was covered by soccer players during a game when fed a glucose polymer solution than when fasted (Kirkendall et al., 1988). Furthermore, Bangsbo and co-workers observed that endurance capacity was improved during intermittent high speed treadmill running, following the ingestion of a CHO enriched diet for 2 days prior to the exercise tests (Bangsbo et al., 1992b).

The mechanisms responsible for improving endurance capacity have been linked with the prevention of hypoglycaemia, maintaining a high rate of CHO oxidation (Bangsbo et al., 1992b; Coggan and Coyle, 1989) and, in some studies, glycogen sparing (Tsintzas et al., 1993b; Tsintzas et al., 1995a; Yaspelkis et al., 1993), all as a consequence of ingesting CHO-E solutions throughout exercise. It has previously been reported that blood glucose oxidation cannot support exercise at an intensity >75% VO\textsubscript{2} max late in exercise (Coggan and Coyle, 1988). Thus, it is unlikely that the provision of exogenous CHO would have been capable of significantly extending time to fatigue during exercise intensities which varied between 55% and 95% VO\textsubscript{2} max, without adequate muscle glycogen stores. In addition, the decline in the CHO oxidation rate observed during prolonged cycling exercise (Coggan and Coyle, 1988) has not been reported during running (Williams et al., 1990; Tsintzas et al., 1993a). Thus the 33% increase in endurance capacity in the present study is suggested to be due to a reduced muscle glycogen utilisation during the first 75 min of exercise in the CHO trial.

Fatigue during prolonged exercise is the result of a reduction, if not depletion, of the limited glycogen stores in working muscles (Costill, 1988; Hermansen et al., 1967) and this may be compounded by dehydration. In the present study, the differences in the performances during the placebo and CHO trials could not be attributed to differences in the degree of dehydration experienced by the subjects. This assumption is based on the observations that
changes in body mass, plasma volumes or serum electrolyte concentrations were not different between trials. Therefore, it is reasonable to conclude that it was the provision of additional CHO, in the form of a 6.9% CHO-E solution, which enabled the subjects to run for longer at speeds varying between low (55% VO_{2max}) and high intensity (95% VO_{2max}). Thus the ingestion of a CHO-E solution throughout 75 min of intermittent, high intensity running, which provided CHO at a rate of 47 g.h^{-1}, appears to have been able to complement the existing CHO stores of the exercising subjects and thus delay the onset of fatigue experienced in the placebo trial.

Whereas endurance running capacity increased as a consequence of CHO ingestion, sprint performance did not. Nevill and co-workers (1993) also observed that increasing the CHO content of the normal diet did not improve sprint performance during 1 h of maximal, intermittent exercise (Nevill et al., 1993). This is due to the fact that factors other than CHO availability dictate sprint performance. Phosphocreatine availability, and thus its rate of resynthesis between sprints, determines maximal sprinting performance (Greenhaff et al., 1994c). Complete resynthesis of PCr requires in excess of 4 min recovery (Sahlin et al., 1979). In the present study, approximately 55 sprints were performed in total, with approximately 80 s period of lower intensity exercise separating them. Thus, the sprint performance data suggest that there was an adequate resynthesis of PCr during the intervening recovery periods. This is supported by the observations of Balsom et al. (1992a) who reported that 15 m sprints could be repeated every 30 s without decreases in performance, indicating that the PCr-Cr phosphokinase system was able to buffer energy demands during each exercise period.

In summary, the ingestion of a 6.9% CHO solution which provided 47 g.CHO.h^{-1} during 75 min of high intensity, intermittent running, improved endurance running capacity by 33%, compared with drinking a placebo.
CHAPTER 6

PART A

THE INFLUENCE OF A CARBOHYDRATE-ELECTROLYTE BEVERAGE ON MUSCLE GLYCOGEN UTILISATION DURING PROLONGED, INTERMITTENT HIGH INTENSITY SHUTTLE RUNNING

6.2.1 INTRODUCTION

In the previous study we found that drinking a CHO-E drink during prolonged intermittent high intensity exercise improved endurance capacity. However, the underlying mechanism for this ergogenic effect has not been clearly identified. Some investigators have reported that performance benefits are as a consequence of a reduction in the rate of muscle glycogen utilisation (Galbo et al., 1977; Bagby et al., 1978; Felig et al., 1982; Hargreaves et al., 1984; Yaspelkis et al., 1993), whilst others have observed that improvement is due to the maintenance of high blood glucose concentrations and thus utilisation late in exercise (Coyle et al., 1986; Hargreaves and Briggs, 1988; Mitchell et al., 1989a; Neufer et al., 1987). In the previous study (Chapter 5), there were no differences in blood glucose concentrations between the CHO and placebo trials. The difference in performance may have been a consequence of different rates of glycogen utilisation as a result of ingesting the CHO-E solution.

Therefore, the aim of the present study was to examine the changes in muscle glycogen before and after 90 min of the prolonged, intermittent high intensity, shuttle running test (PIHSRT) after subjects ingested the same 6.9 % CHO-E and placebo solutions as in the previous study described in Chapter 5.
6.A.2 METHODS

6.A.2.1 Subjects

Six, trained, healthy male games players (age 24.6 ± 2.2 yr; height 179.6 ± 1.9 cm; body mass 74.5 ± 2.0 kg; VO₂ max 56.3 ± 1.3 ml.kg⁻¹.min⁻¹) volunteered and gave their informed consent to participate in this study, which had University Ethical Committee approval.

6.A.2.2 Preliminary Testing

From the predicted measure of VO₂ max, running speeds corresponding to 55% and 95% VO₂ max were calculated as previously described (Chapter 3). Subjects then performed the prolonged, intermittent high intensity, shuttle running test (PIHSRT) for one h in order to familiarise themselves with the required running speeds and experimental procedures.

6.A.2.3 Experimental Design

Subjects completed two exercise trials separated by at least 7 days. The trials were randomised in order to offset any training or order effects. On each occasion, they consumed either a 6.9% CHO-E solution (Lucozade Sport, SmithKline Beecham) (the CHO trial) or a non-CHO placebo (the CON trial) immediately before exercise (5ml.kg⁻¹ bm), and every 15 min thereafter (2ml.kg⁻¹bm). The sodium and potassium contents of the CHO-E solution was 55 and 25 mg.100⁻¹ ml, respectively. The solutions ingested were of the same colour, texture and taste, and were administered in a double-blind design.

During the 2 days preceding each trial, subjects refrained from any strenuous physical activity, and consumed their normal diet in an effort to control for individual variation in muscle and liver glycogen stores.
Subjects reported to the laboratory after an overnight fast of approximately 10 h, and then voided before the measurement of nude body mass was made. Nude body mass was recorded to the nearest 50 g pre- and post-exercise. An indwelling catheter (Venflon, 16-18G) was inserted into an antecubital vein and kept patent with frequent flushing with sterile saline (10 units of heparin per ml).

Muscle samples were obtained from the vastus lateralis by needle biopsy at rest prior to the warm up preceding the PIHSRT, and within 1 min following the completion of 90 min of exercise.

A standardised warm up consisting of jogging, stretching and striding was then performed by each subject for 15 min. Then, immediately before starting exercise, subjects consumed the prescribed solution. The PIHSRT was performed for 90 min, as described elsewhere (Chapter 3) and consisted of six, 15 min exercise bouts separated by a 3 min rest period (Fig. 6.1).

Heart rate was continuously recorded every 15 s throughout each exercise trial using short-range telemetry (Sports Tester PE 3000) and stored in memory mode. Subjective ratings of perceived exertion using Borg’s 10 point scale (Borg, 1982) and gut fullness by a simple linear scale were obtained at 15 min intervals. Dry and wet bulb temperatures were recorded every 15 min using a whirling hydrometer (Brannan Thermometers Ltd), for the calculation of relative humidity. The temperature of the gymnasium was maintained between a range of 17 - 21 °C.

6.A.2.4 Blood Sampling and Analysis

Ten ml of blood was withdrawn at rest, and after 30, 60, and 90 min of exercise. The blood was dispensed, treated and stored as previously described (Chapter 3). Serum was analysed for insulin concentration; plasma was analysed for FFA and glycerol concentrations and
whole blood for lactate and glucose concentrations and plasma volume changes, using methods which have been previously described (Chapter 3).

6.A.2.5 Muscle Sampling and Analysis

Muscle samples were obtained from the vastus lateralis by needle biopsy at rest prior to, and within 1 min following the completion of 90 min of exercise. They were snap-frozen, freeze-dried and divided into 2 portions before being stored at -70°C. At a later date, one part of the freeze-dried muscle was treated and analysed enzymatically for glycogen, glucose, G-6-P, lactate, ATP and PCr using modifications of the methods described by Harris et al. (1974) and Lowry and Passonneau (1972), as previously described (Chapter 3).

The second part of the freeze-dried muscle was treated and analysed for glycogen content of single muscle fibres as documented previously (Chapter 3). Single fibre analysis was performed on the biopsy samples of five subjects during the CON trial and three subjects for both the CHO and CON trials.

6.A.2.5 Statistical Analyses

Muscle metabolites, physiological and blood biochemical responses in both trials were analysed using a two-way (treatment by time) analysis of variance for repeated measures. Changes (delta) in muscle glycogen concentration for each trial were compared using Student's t-test for correlated data. Significant differences between means were identified using a Scheffe post-hoc test. The level of significance was accepted at P<0.05. All data are reported as mean ± SEM.
PROLONGED INTERMITTENT HIGH INTENSITY SHUTTLE RUN TEST (PIHSRT)

(TOTAL TIME = 105 min: 90 min exercise + 15 min rest)

-30 -15 0 15 15 30 30 45 45 60 60 75 75 90

Figure 6.1: Schematic illustration of the PIHSRT and experimental design.
6.A.3 RESULTS

6.A.3.1 Perceived Rate of Exertion and Heart Rate

There were no differences in either ratings of perceived exertion or heart rate response between trials during 90 min of intermittent, high intensity shuttle running, indicating that the physical stress imposed by the protocol was similar in both trials. Ratings of perceived exertion increased from initial values of 5 ± 1 to 7 ± 1 during the final 15 min of exercise in the CHO (P<0.01) and CON (P<0.05) trials. Mean heart rates were 169 ± 2 b.min⁻¹ in CHO and 171 ± 2 b.min⁻¹ in CON.

6.A.3.2 Sprint Times

For clarity of presentation, the mean time for every 6 sprints was recorded. Sprint times were similar between trials and over the duration of each trial. Average times for 15 m sprints during the CHO and CON trials were 2.44 ± 0.04 s and 2.42 ± 0.06 s, respectively.

6.A.3.3 Blood Glucose and Serum Insulin

Blood glucose concentrations were similar in each trial, although there was a tendency for values to be higher when fed CHO than when fasted (Figure 6.2). In both trials, blood glucose was maintained within the normal range and after an initial rise after 30 min (P<0.01) of exercise, declined over the duration of exercise (P<0.05) in the CON trial. Higher serum insulin values were also observed at each sampling point (Figure 6.3), although the difference was only significant after 30 min (P<0.05).
6.A.3.4 Plasma FFA and Glycerol

There were no differences in plasma FFA or glycerol concentrations between trials during exercise. In both trials, plasma FFA concentration was suppressed after 30 min of exercise before steadily increasing after 60 and 90 min (Figure 6.4). In the CHO trial, FFA concentration at the end of exercise was greater than at 30 min (P<0.05). In the CON trial, FFA concentration at the end of 90 min exercise was greater than at rest (P<0.05) and at 30 and 60 min during exercise (P<0.01). Plasma glycerol concentrations increased from resting to the end of exercise under both conditions (Figure 6.5). In the CHO trial, post-exercise glycerol concentrations were significantly greater than at rest (P<0.05).

6.A.3.5 Blood Lactate

Thirty minutes after the start of exercise, blood lactate concentration was significantly higher in CON than CHO (CON = 6.3 ± 1.1 mmol.l⁻¹ vs CHO = 4.8 ± 0.8 mmol.l⁻¹; P=0.05). At this point in each trial, blood lactate concentrations reached a peak before steadily declining over the remainder of each trial. Similar concentrations were observed after 60 and 90 min, averaging 3.5 mmol.l⁻¹ and 4.3 mmol.l⁻¹, respectively (Figure 6.6).

6.A.3.6 Changes in Plasma Volume and Body Mass

There were no differences between trials in plasma volume changes from resting to the end of exercise (CHO, 1.8 ± 1.8%; CON, -2.3 ± 1.4%), or loss of body mass during each trial (CHO, 2.8 ± 0.5 kg; CON, 2.5 ± 0.3 kg). Differences in body mass after exercise represented a 3.7% and 3.3% weight loss, respectively, in each trial.
6.A.3.7 Mixed Muscle Metabolites

Mixed muscle glycogen concentration is shown in Fig. 6.7. Total glycogen utilisation was lower (P<0.05) during CHO [192.5 ± 26.3 mmol (kg DM)⁻¹] than CON [245.3 ± 22.9 mmol (kg DM)⁻¹]. Despite the cross-over design, initial glycogen levels were higher prior to the CON trial than the CHO trial, although the difference was not statistically significant. Mixed muscle metabolite concentrations of ATP, free glucose, PCr, G-6-P and lactate are shown in Table 6.1. The concentration of PCr in the muscle after 90 min of exercise was lower in CON than CHO (P<0.05). Similar concentrations of ATP, free glucose, G-6-P and lactate were observed between trials. However in each trial, differences were observed between resting and post-exercise metabolite concentrations of glycogen, glucose, G-6-P and lactate (P<0.05).

6.A.3.8 Single Muscle Fibre Glycogen Concentration

The concentration of muscle glycogen in type I and type II fibres before and after prolonged shuttle running with placebo ingestion is shown in Table 6.2. There was a greater amount of glycogen utilised in type II fibres compared with type I (P<0.01). The resting muscle glycogen concentration was higher in type II fibres (P<0.01). In addition, the glycogen concentration for 3 subjects was analysed during the CHO trial and the results of both trials are shown in Table 6.3. No statistical analysis was performed on these data due to the small sample (n=3), although there was clear evidence to suggest that glycogen sparing had occurred in these 3 subjects during the CHO trial (individual results are shown in Figs. 6.8 and 6.9).
Fig 6.2: Blood glucose concentration (mmol.l\(^{-1}\)) in the CHO and CON trials during the PIHSRT.

Fig 6.3: Serum insulin concentration (\(\mu\text{IU.ml}\^{-1}\)) in the CHO and CON trials during the PIHSRT.
**Fig 6.4:** Plasma FFA concentration (mmol.l⁻¹) in the CHO and CON trials during the PIHSRT.

**Fig 6.5:** Plasma glycerol concentration (mmol.l⁻¹) in the CHO and CON trials during the PIHSRT.
Fig 6.6: Blood lactate concentration (mmol.l^{-1}) in the CHO and CON trials during the PIHSRT.

Fig 6.7: Mixed muscle glycogen concentration and utilisation [mmol glucosyl units (kg DM)^{-1}] during the CHO and CON trials (n=6).
**Fig 6.8:** Muscle glycogen utilisation [mmol glucosyl units (kg DM)$^{-1}$] in type I fibres in the CHO and CON trials (n=3).

**Fig 6.9:** Muscle glycogen utilisation [mmol glucosyl units (kg DM)$^{-1}$] in type II fibres in the CHO and CON trials (n=3).
Table 6.1: Mixed muscle metabolites [mmol glucosyl units (kg DM)⁻¹] at rest and after 90 min of intermittent high intensity shuttle running in the CHO and CON trials (n=6).

<table>
<thead>
<tr>
<th>METABOLITE</th>
<th>CHO Pre-Exercise</th>
<th>CHO Post-exercise</th>
<th>CON Pre-Exercise</th>
<th>CON Post-Exercise</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATP</td>
<td>25.3 ± 0.8</td>
<td>26.0 ± 1.2</td>
<td>24.7 ± 1.3</td>
<td>23.3 ± 0.6</td>
</tr>
<tr>
<td>PCr</td>
<td>75.5 ± 2.6</td>
<td>79.5 ± 2.7</td>
<td>76.3 ± 2.7</td>
<td>69.6 ± 1.1*</td>
</tr>
<tr>
<td>GLUCOSE</td>
<td>1.3 ± 0.2</td>
<td>2.7 ± 0.4†</td>
<td>1.2 ± 0.3</td>
<td>2.5 ± 0.3†</td>
</tr>
<tr>
<td>G-6-P</td>
<td>1.8 ± 0.3</td>
<td>3.1 ± 0.4</td>
<td>1.8 ± 0.3</td>
<td>3.1 ± 0.2†</td>
</tr>
<tr>
<td>LACTATE</td>
<td>4.9 ± 0.9</td>
<td>14.3 ± 2.2†</td>
<td>5.4 ± 1.2</td>
<td>15.9 ± 3.3†</td>
</tr>
</tbody>
</table>

*P<0.05, CHO vs. CON; †P<0.01, Pre- vs. Post-exercise.

Table 6.2: Muscle glycogen concentration [mmol glucosyl units (kg DM)⁻¹] in type I and type II fibres at rest and after 90 min of intermittent high intensity shuttle running in the CON trial (n=5).

<table>
<thead>
<tr>
<th>CONTROL TRIAL</th>
<th></th>
<th></th>
<th>Δ VALUES</th>
</tr>
</thead>
<tbody>
<tr>
<td>PRE-EXERCISE</td>
<td>POST-EXERCISE</td>
<td>TYPE I</td>
<td>TYPE II</td>
</tr>
<tr>
<td>TYPE I</td>
<td>TYPE II</td>
<td>TYPE I</td>
<td>TYPE II</td>
</tr>
<tr>
<td>316.0ᵃ</td>
<td>402.0ᵇ</td>
<td>133.8</td>
<td>114.6</td>
</tr>
<tr>
<td>± 22.3</td>
<td>± 26.5</td>
<td>± 31.4</td>
<td>± 29.9</td>
</tr>
</tbody>
</table>

ᵃP<0.01 from type II at rest and type I post-exercise;ᵇP<0.01 from type II post-exercise;  
*P<0.01 from type I.
Table 6.3: Muscle glycogen concentration [mmol glucosyl units (kg DM)^{-1}] in type I and type II fibres at rest and after 90 min in CHO and CON trials (Mean ± SEM; n=3).

<table>
<thead>
<tr>
<th></th>
<th>CHO TRIAL</th>
<th></th>
<th>CONTROL TRIAL</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PRE-EXERCISE</td>
<td>POST-EXERCISE</td>
<td>D VALUES</td>
<td>PRE-EXERCISE</td>
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<tr>
<td>TYPE I</td>
<td>315.3</td>
<td>157.6</td>
<td>165.6</td>
<td>157.7</td>
</tr>
<tr>
<td>TYPE II</td>
<td>387.0</td>
<td>± 32.6</td>
<td>± 13.5</td>
<td>± 41.6</td>
</tr>
</tbody>
</table>
The main finding in the present study was that a CHO-E solution consumed immediately before, and at frequent intervals during prolonged, intermittent high intensity exercise, reduced the amount of muscle glycogen utilised. Analysis of the muscle glycogen data was complicated by the higher pre-exercise concentration for the CON trial. Previous research has shown that the initial muscle glycogen concentration in the exercising muscle influences its subsequent use (Galbo et al., 1979; Sherman et al., 1981). However, the differences in the pre-exercise concentrations in the present study were not statistically significant, and thus it was concluded that these initial differences were not sufficiently large to cause significant differences in the rate of glycogenolysis under the two conditions. Thus CHO-E feedings reduce muscle glycogen utilisation during prolonged, intermittent high intensity, shuttle running.

This apparent sparing of muscle glycogen in the CHO trial compared with the CON trial is consistent with observations in previous studies examining CHO feedings during prolonged, submaximal running (Tsintzas et al., 1993a; Tsintzas et al., 1995a) and cycling (Bjorkman et al., 1984; Hargreaves et al., 1984; Erickson et al., 1987). A decrease in muscle glycogen utilisation has also been observed when glucose was administered intravenously in humans (Hultman, 1967) and rats (Bagby et al., 1978; Kuipers et al., 1986).

In contrast, several studies which have observed that CHO feedings delay fatigue and improve performance have failed to observe this glycogen sparing effect (Coyle et al., 1986; Flynn et al., 1987; Hargreaves and Briggs, 1988). In these studies it was suggested that CHO feedings exerted an ergogenic effect by maintaining elevated blood glucose levels, and sustaining a high rate of CHO oxidation. Thus, when muscle glycogen levels are low in the latter stages of prolonged exercise, blood glucose makes an ever increasing contribution to CHO metabolism in exercising muscle (Coyle et al., 1986; Coggan and Coyle, 1987), thereby allowing exercise to continue (Coyle et al., 1983; Coyle et al., 1986; Coggan and
Coyle, 1987). The difference in exercise protocols may help explain, in part, these different results. It is possible that during the rest and lower intensity exercise periods (a total of 58 min, or 55% of the total exercise duration) in the present study and other intermittent exercise protocols, the elevated blood glucose and serum insulin levels led to glycogen resynthesis (Constable et al., 1984; Kuipers et al., 1986). Thus the net glycogen use over the duration of exercise would be reduced. During more intense continuous exercise (70-75% VO$_2$ max) a glycogen-sparing effect was not seen during cycling (Coyle et al., 1986) but was reported during running (Tsintzas et al., 1993a; Tsintzas et al., 1995a).

The intermittent exercise protocol used in the present study recruited both type I and type II fibre populations and this is consistent with the observations of previous studies which used intermittent exercise as the exercise model (Edgerton et al., 1975; Saltin et al., 1976; Essen, 1978a). The greater amount of glycogen utilised in the type II compared with the type I fibres during the PIHSRT illustrates the high intensity nature of the exercise protocol.

It has been suggested that the main benefit of glucose feeding in trained athletes is the maintenance of liver glycogen stores (Van Handel et al., 1980). However, studies using naturally labelled CHO have shown that the oral intake of glucose could contribute up to 55% of the total CHO metabolism (Pirmay et al., 1977a; Krzentowski et al., 1984). Indeed, CHO ingestion during prolonged exercise results in the suppression of hepatic glucose production and increased glucose uptake, possibly due to increased plasma glucose and insulin levels (Yaspelkis et al., 1993; McConell et al., 1994). Thus it is likely that a large proportion of the ingested CHO was metabolised during intermittent exercise in the present study, thus elevating the extramuscular CHO stores, and reducing the contribution of intramuscular glycogen stores. It is well documented that exogenous glucose feeding leads to a CHO utilisation rate of around 1 g.min$^{-1}$ (Hawley et al., 1992). In addition, it is possible that during rest and the less intense exercise periods, the exogenous CHO contributed to glycogen synthesis within the working muscle. During these intervals when there is a reduction in the contribution of muscle glycogen, elevated blood glucose levels may accelerate the rate of
glycogen resynthesis from blood glucose (Neufer et al., 1987), thus reducing the 'net' glycogen breakdown. The ingestion of glucose during exercise increases its uptake by the working muscles even though exercise suppresses plasma insulin concentration to values below what they would be at rest (Ahlborg and Felig, 1976). As anticipated, the insulin levels in the present study had a tendency to be higher during the course of the CHO trial, differences being significant after 30 min of exercise.

There was a small, but none the less significant, difference in the post-exercise PCr values between trials. In the CON trial, the post-exercise PCr concentration was 91% of its initial resting value. This is in contrast to the multiple sprint studies on the cycle ergometer, where the PCr concentration has been reported to fall by 57% after the first sprint, and 84% after the tenth sprint (Gaitanos et al., 1993). The degradation of PCr observed in the present study was more comparable with the concentrations reported following 60 min of constant pace running (Tsintzas et al., 1993a). This decrease in PCr during prolonged exercise may possibly be associated with glycogen depletion as a consequence of a reduced CHO availability (Sahlin et al., 1990).

Similar concentrations of plasma glycerol and FFA were observed between trials, which suggests that fat utilisation was also similar, as the arterial FFA concentration determines FFA uptake by the active muscle (Hagenfeldt and Wahren., 1968). The increase in plasma FFA and glycerol concentrations is similar to that observed during constant paced running (Tsintzas et al., 1995a). However, the absence of the differences in plasma FFA and glycerol concentrations between trials which have been observed in constant paced, submaximal running exercise may be explained by the variable exercise intensities in the present study. Indeed, the blood and plasma metabolite and hormone concentrations in addition to the plasma volume changes observed in the present study are similar to those reported in the previous study (Chapter 5) investigating the effect of CHO-E ingestion on performance during prolonged, intermittent high intensity, shuttle running (Nicholas et al., 1995).
The results of the present study show that the ingestion of 47 g.CHO.h\(^{-1}\) was adequate to elicit a reduction in the amount of glycogen utilised during 90 min of intermittent, high intensity shuttle running. Similar amounts of CHO administered before 1 h of high-intensity cycling exercise (Neufer et al., 1987) and during 4 h of intermittent cycling (Hargreaves et al., 1984) have also demonstrated an ergogenic effect. Smaller doses (33g and 39g) during intermittent cycling also improved performance (Mitchell et al., 1988). However, in a subsequent study by Mitchell et al. (1989a), a dose of 37g.h\(^{-1}\) was insufficient to improve performance following prolonged submaximal cycling. Thus, depending on the exercise protocol, intensity and duration, there may be a threshold dose, below which, no improvements in performance will be observed (Mitchell et al., 1989a).

Plasma volume changes, weight loss and plasma sodium and potassium changes over time observed in this study were not different between trials, indicating that a 6.9% CHO-E solution was as effective as a water placebo in terms of fluid replacement and physiological function. Other investigators have reported similar findings (Candas et al., 1986; Murray et al., 1987; Owen et al., 1986). Fluid ingestion during exercise can attenuate the rise in core body temperature by 0.5-0.8°C and cardiovascular drift due to a higher skin blood flow (Montain and Coyle, 1992). However, dehydration was observed in both trials, indicated by a weight loss greater than 1-2 kg (Shephard, 1990), which may have had a detrimental effect on performance during the PIHSRT (Sawka, 1992; Armstrong et al., 1985). Increasing the volume of solution given may offset any impairment due to weight loss in excess of 2%, although consideration must be given to abdominal discomfort and its effect on subjective perception of fatigue (Millard-Stafford, 1992).

In summary, the ingestion of a 6.9% CHO-E solution during 90 min of intermittent high intensity, shuttle running, resulted in a 22% reduction in the amount of muscle glycogen utilised, compared with drinking an equal volume of a non-CHO placebo solution.
CHAPTER 6

PART B

EFFECT OF 90 MINUTES INTERMITTENT HIGH INTENSITY RUNNING AND CARBOHYDRATE-ELECTROLYTE INGESTION ON MUSCLE FUNCTION

6.B.1 INTRODUCTION

In addition to investigating muscle glycogen utilisation during prolonged intermittent high intensity running, a sub-study was carried out in order to measure muscle function pre- and post exercise with and without CHO-E supplementation.

There is some information to suggest that the force generating capacity of the muscle is reduced following prolonged exercise and not necessarily as a result of low muscle glycogen *per se*, but as a direct consequence of the previous prolonged activity (Young and Davies, 1984; Jacobs et al., 1981; Jacobs et al., 1982a; Sherman et al., 1984). In the main study of this section, we found that muscle glycogen concentration was reduced to $170.2 \pm 23.6$ and $159.5 \pm 14.9$ mmol (kg DM)$^{-1}$ in the CHO and CON trials, respectively.

The purpose of this sub-study is to examine whether or not muscle function is altered following 90 min of intermittent high intensity exercise, and to observe whether drinking a CHO-E beverage during exercise affects muscle function post-exercise.
6.B.2 METHODS

6.B.2.1 Subjects

Informed written consent was obtained from the six males who volunteered as subjects in the main trial (Chapter 6, Part A). Height, body mass, age, and predicted VO\textsubscript{2} max were 179.6 ± 1.9 cm; 74.5 ± 2.0 kg; 24.6 ± 2.2 yr and 56.3 ± 1.3 ml.kg\textsuperscript{-1}.min\textsuperscript{-1}, respectively.

6.B.2.2 Experimental Design

Each subject participated in two conditions, separated by one week. On both occasions, subjects performed a muscle function test (MFT) before and after 90 min of intermittent high intensity shuttle running, as described previously in Chapter 6, Part A (Fig. 6.10).

6.B.2.3 Preliminary Testing

In addition to the preliminary tests and familiarisation periods for the PIHSRT, subjects were familiarised with the isokinetic dynamometer on several occasions until consistent measurements of peak torque were obtained on all tests. Kues et al. (1992) reported that 1-2 days of familiarisation and practice enhanced measurement reliability.

6.B.3 Muscle Function Test (MFT) on the Isokinetic Dynamometer

Muscle function during knee extension and flexion was measured concentrically and eccentrically using an isokinetic dynamometer (Cybex 6000). Subjects performed the test in a seated position, strapped at the chest and waist and with arms folded, in order to eliminate contribution from the upper extremities. Stabilisation of the thigh and shin ensured that the
target muscle groups were isolated, any adjustments being made for variations in individual limb length. The axis of rotation of the lever arm was aligned with the anatomical axis of the joint being tested and its position checked by moving the joint to be tested through the active range of motion. The position of anatomical zero at full knee extension and the range of motion stops were set for each subject prior to commencing the test.

Knee extension and flexion was tested concentrically and eccentrically in the extensors and concentrically in both the flexors and extensors. The leg tested was randomly assigned for each trial condition and movement was tested at both slow and fast velocities (60 °/s and 240 °/s, respectively). In order to ensure valid and reliable data, the following guidelines (Perrin, 1993) were strictly adhered to. At each velocity the subject performed 3 submaximal warm-up trials and 3 maximal warm-up trials before performing 3 maximal trials one min later. It has been previously reported that 3-4 repetitions are necessary to achieve measurement of peak torque. The slower velocity was performed first, with recovery periods of 90 s and 3 min between the two different velocities, and the concentric-eccentric and concentric contractions, respectively. Consistent verbal encouragement and visual feedback were provided for each subject.

6.B.4 Measurements and Definitions

The measurements recorded by computer were absolute values of Peak Torque (PT in Nm), Total Work (TW in J), Average Power (AP in W) and angle of PT at velocities of 60 and 240 °/s. Peak Torque refers to the single highest torque output of the joint produced by muscular contraction as the limb moves through the range of motion (Kannus, 1994). Muscular work is defined and measured as output of mechanical energy; that is, externally applied force multiplied by the distance through which it is applied (Sahega, 1990). In isokinetics, work is defined as the area under the torque versus angular displacement (degrees) curve (work =
torque x angle) (Perrin et al., 1987). Total work is the sum of the work performed in all test repetitions (Kannus, 1994). Muscular power refers to the rate of muscular work output, expressed in units of work per unit time (Rothstein et al., 1983). Average power is defined as the total work of the given contractions divided by the actual total movement time (Kannus, 1994). In addition, the ratios of concentric to eccentric contraction of the knee extensors and knee flexors to extensors when contracting concentrically and the average range of movement (ROM) were also measured.

On each testing occasion the MFf was performed twice - initially in the morning following an overnight fast, 45 min prior to performing 90 min of intermittent high intensity exercise and then 15 min after the completion of the PIHSRT. Thus the tests were separated by a period of 2.5 h.

6.B.5 Muscle Sampling and Analysis

Muscle samples were obtained from the vastus lateralis by needle biopsy at rest prior to the MFf and within 1 min following the completion of the 90 min exercise bout. They were treated and analysed as described previously (Chapter 6, Part A).

6.B.6 Statistical Analyses

Isokinetic strength data in both trials were compared using a two-way (treatment by time) analysis of variance for repeated measures. Changes (delta) in muscle strength for each trial were compared using Student's t-test for correlated data. Significant differences between means were identified using a Scheffe post-hoc test. The level of significance was accepted at P<0.05. All data are reported as mean ± SEM.
PROLONGED INTERMITTENT HIGH INTENSITY SHUTTLE RUN TEST (PHSRT)

(TOTAL TIME = 105 min: 90 min exercise + 15 min rest)

Figure 6.10: Schematic illustration of the intermittent shuttle run protocol and experimental design.
6.B.3 RESULTS

There were no differences in either pre- or post-exercise measurements of muscle function between trials. The only differences observed were within each trial between pre- and post-exercise measurements and between the two different angular velocities.

Following the 90 min intermittent high intensity shuttle running test, PT, TW and AP was significantly reduced at the angular velocity of 60 °/s in the CON trial (Figs. 6.14 and 6.12; Table 6.5). This decrease in muscle function recorded in the CON trial was not observed to the same magnitude or extent in the CHO trial (Table 6.4). There were no changes in PT in the CHO trial following the PIHSRT. However, TW and AP were reduced following the CHO trial in the concentric extensors of knee extension and flexion, whereas only the TW decreased when the knee extensors were contracting eccentrically (Fig. 6.15; Table 6.4). This decrease in muscle function was predominantly observed at the angular velocity of 60 °/s in both trials. However, in the CHO trial, TW and AP at the angular velocity of 240 °/s was reduced after exercise in the extensors acting concentrically only (P<0.05).

Under both pre- and post-exercise conditions, PT and TW were greater at 60°/s than 240°/s during concentric contraction of the knee extensors and flexors in both trials (P<0.01; Figs. 6.11 and 6.12, respectively). In addition, PT in the eccentric extensors was greater pre- and post-exercise at the angular velocity of 60 °/s in the CON trial (P<0.05; Fig. 6.11). Average power was greater at 240°/s during both concentric and eccentric contractions, pre- and post-exercise and in both the CHO and CON trials (P<0.01; Fig. 6.13).

Eccentric contraction of the knee extensors produced a higher PT, TW and AP than concentric contraction (Tables 6.4 and 6.5). During eccentric contraction, PT was higher at
60°/s than at 240°/s, however TW and AP were greater at the higher velocity before and after the PIHSRT in the two trials (Tables 6.4 and 6.5).
Fig. 6.11: Pre-exercise peak torque in CHO and CON trials
(**P<0.01; *P<0.05 for 60°/s vs. 240°/s).
Fig. 6.12: Pre-exercise total work in CHO and CON trials

(**P<0.01; 60 °/s vs. 240 °/s).
Fig. 6.13: Pre-exercise average power in the CHO and CON trials
(**P<0.01; 60 °/s vs. 240 °/s).
Fig. 6.14: Peak torque pre- and post-exercise in the CON trial at an angular velocity of 60 °/s.
Fig. 6.15: Total work pre- and post-exercise in the CHO and CON trials at an angular velocity of 60 °/s.
Table 6.4: Isokinetic knee extension measurements before and after 90 min of intermittent high intensity exercise in the CHO trial (n=6).

<table>
<thead>
<tr>
<th>Muscle Action</th>
<th>SPEED OF MOVEMENT (deg/s)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PRE-EXERCISE</td>
</tr>
<tr>
<td></td>
<td>60°/s 240°/s 60°/s 240°/s</td>
</tr>
</tbody>
</table>

1. **Knee extension/flexion; Concentric/eccentric**

**Concentric Extensors**
- **PT (Nm)**: 209.6 ± 3.0†† 133.8 ± 4.6 199.0 ± 6.6†† 135.2 ± 3.7
- **TW (BWR) (J)**: 235.0 ± 6.1*†† 146.4 ± 7.5 210.8 ± 7.8†† 135.8 ± 6.7
- **AP (BWR) (W)**: 145.6 ± 3.0*†† 338.6 ± 16.6 124.3 ± 7.3†† 302.3 ± 24.6

**Eccentric Extensors**
- **PT (Nm)**: -294.4 ± 16.6 -272.2 ± 25.2 -278.0 ± 19.6 -274.5 ± 21.2
- **TW (BWR) (J)**: -296.0 ± 22.5* -308.0 ± 28.0 -270.2 ± 22.9 -287.7 ± 25.4
- **AP (BWR) (W)**: -178.8 ± 15.1†† -640.2 ± 51.3 -166.8 ± 15.3†† -589.8 ± 46.7
- **RATIO C/E PT**: -71.6 ± 4.5†† -50.2 ± 4.4 -72.7 ± 4.5†† -50.0 ± 3.3
- **RATIO C/E TW**: -80.4 ± 5.3†† -48.2 ± 3.8 -80.7 ± 8.6† -48.0 ± 3.4
- **Avg ROM (96)**: 92.8 ± 1.9† 94.0 ± 1.9 92.0 ± 1.6†† 93.2 ± 1.6

2. **Knee extension/flexion; Concentric/concentric**

**Concentric Flexors**
- **PT (Nm)**: 132.0 ± 6.2†† 96.3 ± 2.3 124.0 ± 6.7†† 94.2 ± 5.5
- **TW (BWR) (J)**: 154.2 ± 6.1†† 106.5 ± 5.2 134.5 ± 12.4†† 100.8 ± 7.4
- **AP (BWR) (W)**: 100.2 ± 5.9†† 256.0 ± 12.8 90.7 ± 7.7†† 247.5 ± 16.1
Table 6.4 cont.

<table>
<thead>
<tr>
<th>Muscle Action</th>
<th>PT (Nm)</th>
<th>TW (BWR) (J)</th>
<th>AP (BWR) (W)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>217.8 ± 5.1††</td>
<td>139.3 ± 4.9</td>
<td>207.2 ± 6.8††</td>
</tr>
<tr>
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<td>236.5 ± 8.7††</td>
<td>156.3 ± 6.4*</td>
<td>225.7 ± 10.6††</td>
</tr>
<tr>
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<tr>
<td>RATIO F/E TW</td>
<td>65.3 ± 3.4</td>
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<td>59.3 ± 5.2</td>
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<td>Avg ROM (97)</td>
<td>88.3 ± 2.2††</td>
<td>91.0 ± 1.7</td>
<td>89.0 ± 2.1</td>
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</table>

*P<0.05 pre- vs. post-exercise; ††P<0.01 and †P<0.05; 60 °/s vs. 240 °/s.

Table 6.5: Isokinetic knee extension measurements before and after 90 min of intermittent high intensity exercise in the CON trial (n=6).

<table>
<thead>
<tr>
<th>Muscle Action</th>
<th>SPEED OF MOVEMENT (deg/s)</th>
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</thead>
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<td></td>
<td>PRE-EXERCISE</td>
</tr>
<tr>
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<td>60°/s</td>
</tr>
<tr>
<td>1. Knee extension/flexion; Concentric/eccentric Concentric Extensors</td>
<td></td>
</tr>
<tr>
<td>PT (Nm)</td>
<td>236.2 ± 9.6*††</td>
</tr>
<tr>
<td>TW (BWR) (J)</td>
<td>248.2 ± 13.3**††</td>
</tr>
<tr>
<td>AP (BWR) (W)</td>
<td>154.0 ± 7.3**††</td>
</tr>
</tbody>
</table>
Table 6.5 cont.

### Eccentric Extensors

<table>
<thead>
<tr>
<th></th>
<th>PT (Nm)</th>
<th>TW (BWR) (J)</th>
<th>AP (BWR) (W)</th>
<th>Ratio C/E PT</th>
<th>Ratio C/E TW</th>
<th>Avg ROM (96)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eccentric Extensors</td>
<td>-306.8 ± 17.3*†</td>
<td>-319.2 ± 23.2**</td>
<td>-190.8 ± 14.4**††</td>
<td>-77.0 ± 2.8††</td>
<td>-78.8 ± 6.4**††</td>
<td>93.8 ± 2.4†</td>
</tr>
<tr>
<td></td>
<td>-278.0 ± 25.8</td>
<td>-308.4 ± 22.4</td>
<td>-638.2 ± 44.9</td>
<td>-50.6 ± 3.6</td>
<td>-46.6 ± 2.6</td>
<td>94.4 ± 2.4</td>
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<tr>
<td></td>
<td>-282.8 ± 12.0†</td>
<td>-276.2 ± 20.3</td>
<td>-165.6 ± 11.2††</td>
<td>-77.8 ± 2.0††</td>
<td>-83.6 ± 6.0††</td>
<td>93.6 ± 2.4††</td>
</tr>
<tr>
<td></td>
<td>-264.0 ± 18.2</td>
<td>-278.2 ± 8.5</td>
<td>-559.4 ± 22.4</td>
<td>-53.2 ± 1.9</td>
<td>-50.0 ± 1.3</td>
<td>94.4 ± 2.4</td>
</tr>
</tbody>
</table>

### 2. Knee extension/flexion; Concentric/concentric

### Concentric Flexors

<table>
<thead>
<tr>
<th></th>
<th>PT (Nm)</th>
<th>TW (BWR) (J)</th>
<th>AP (BWR) (W)</th>
<th>Ratio F/E PT</th>
<th>Ratio F/E TW</th>
<th>Avg ROM (96)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concentric Flexors</td>
<td>133.0 ± 14.6*††</td>
<td>162.2 ± 17.0*††</td>
<td>104.6 ± 11.1*††</td>
<td>56.4 ± 5.0†</td>
<td>62.6 ± 4.5</td>
<td>90.4 ± 1.6</td>
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<tr>
<td></td>
<td>91.8 ± 10.1</td>
<td>103.2 ± 9.6</td>
<td>248.2 ± 27.7</td>
<td>62.2 ± 5.1</td>
<td>62.4 ± 3.0</td>
<td>92.6 ± 2.7</td>
</tr>
<tr>
<td></td>
<td>123.2 ± 11.0††</td>
<td>145.6 ± 14.0††</td>
<td>96.2 ± 9.6††</td>
<td>56.0 ± 5.7</td>
<td>62.2 ± 5.2</td>
<td>92.8 ± 2.7</td>
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<tr>
<td></td>
<td>92.8 ± 9.7</td>
<td>98.6 ± 9.9</td>
<td>241.2 ± 26.5</td>
<td>62.2 ± 5.2</td>
<td>62.4 ± 3.9</td>
<td>92.8 ± 2.7</td>
</tr>
</tbody>
</table>

*P<0.05 and **P<0.01; pre- vs. post-exercise;
††P<0.01 and †P<0.05; 60 °/s vs. 240 °/s.
6.B.4 DISCUSSION

The main finding of this study was that muscle function, as measured by changes in PT, TW and AP of the extensors and flexors during knee extension, was impaired following 90 min of intermittent, high intensity exercise. This decrease in muscle force was greater at 60°/s compared with 240°/s. However, the ingestion of a CHO-E drink immediately before and during exercise attenuated these differences.

Other investigators have also observed an acute loss of muscular strength after prolonged exercise (Forsberg et al., 1978; Jacobs et al., 1981; Jacobs et al., 1982a; Sherman et al., 1984; Young and Davies, 1984; Symons and Jacobs, 1989). Forsberg and colleagues (1978) studied the changes in strength performance after an 85 km cross-country ski race. Maximal peak torque (MPT) during knee extension at 30 °/s (0.53 rad/s) was reduced 28%, but MPT at 180 °/s (3.2 rad/s) was reduced only 12%. There was a greater glycogen depletion in the type I fibres than in type II fibres which may have caused the pronounced decrease in slow isokinetic contractions.

Jacobs et al. (1981) reported that when both the type I and type II fibres were depleted of muscle glycogen by a combination of prolonged running, sprints, and maximal isokinetic contractions, MPT at a relatively fast velocity of contraction (180°/s) was significantly impaired. In this study, however, it is impossible to determine the separate effect of the exercise bouts used to deplete muscle glycogen and glycogen depletion per se on the measurement of MPT. However, in a subsequent study, Symons and Jacobs (1989) concluded that short-term high intensity exercise performance (maximum isokinetic strength and endurance, isometric strength and electrically evoked force of the leg extensors) was not affected when intramuscular glycogen concentrations were below 220 mmol glucose units (kg DM)⁻¹.
Jacobs et al. (1982a) examined the effects of glycogen depleting exercise, designed to deplete both main fibre types, on performance of short-term activities such as single maximal contractions and exhaustive, repeated maximal contractions at an angular velocity of 180°/s. Maximal strength, expressed as peak torque at an angular velocity of 180°/s was significantly reduced in all subjects following the exercise programme, and the decrease in maximal torque production was more pronounced in those subjects with a high proportion of type II fibres than in subjects with a high proportion of type I fibres following glycogen depletion.

Sherman et al. (1984) investigated the effect of running 42.2 km and subsequent rest on muscular strength and work capacity. Muscle glycogen was depleted in both type I and type II (oxidative glycolytic) muscle fibres in the subjects after the marathon, and MPT was impaired not only at 180°/s (3.2 rad/s), but also at 300°/s and 60°/s (5.3 and 1.1 rad/s, respectively).

Other investigators have also reported that the loss of force produced at the slower velocity is greater following exercise under low CHO than high CHO conditions (Young and Davies, 1984), which suggests that the level of stored glycogen within the musculature directly affects its ability to generate force. Changes in muscle glycogen alone did not alter the isometric force generating capacity of human muscle, but when combined with prolonged dynamic exercise, exercise-induced muscle weakness is significantly greater under conditions of low CHO. It should be noted that it is not possible to achieve low muscle glycogen concentrations without exercise. In addition, the effects of prolonged exercise cannot be achieved without low glycogen concentrations.

In the present study, PT and TW, expressed in both absolute and relative terms, were greatest at the slower velocity during concentric contraction of the knee extensors and
flexors. This is in agreement with several other investigations which have reported that the ability of a muscle to generate concentric force is greatest at slow isokinetic velocities and decreases linearly as the test velocity increases (Coyle et al., 1979; Westing et al., 1991; Perrin, 1993). This decline in peak torque is a reflection of the muscle's inability to develop its maximum tension before it passes the optimal joint angle and is simply a function of the force-velocity relationship of muscle. Peak Torque stays almost unchanged between the angular velocities of 0 and 60°/s but shows thereafter almost a linear decline with increasing velocity (the torque-velocity relationship). The reason for such a decline is the different recruitment capability of different muscle fibres, motor unit recruitment being dependent on exercise intensity (Secher and Nygaard Jensen, 1976; Thompson et al., 1979). During leg extension at an angular velocity of 60°/s, the activation of type I fibres predominates (Burke and Edgerton, 1975). At these lower speeds, both type I and II fibres can be maximally activated while with increasing angular velocity during maximal exercise all muscle fibre types are recruited (Gollnick et al. 1973; Greenhaff et al., 1994).

However, AP was greatest at the higher velocity during both concentric and eccentric contraction of the knee extensors and flexors. This is due to the time factor of contraction at higher velocities when compared with slower velocities. In contrast with torque, power production in isokinetic exercise rises as angular velocity increases (Osternig, 1986). This means that the magnitude of the torque drop is not sufficient to offset the effect of speed increase (decrease in movement time) in continuing to produce a rise in power as angular velocity increases.

During eccentric contraction of the knee extensors in the present study, PT was higher at 60°/s than at 240°/s, however TW and AP were higher at 240°/s in both pre- and post-exercise conditions. Eccentric contraction also produced higher peak torque, total work output and average power than concentric contraction. These findings are similar to
observations in previous studies (Seliger et al., 1980; Westing et al., 1991). The physiological mechanism for the discrepancy in the concentric and eccentric force-velocity relationship appears to be related to differences in the binding and interaction of actin and myosin within the muscle sarcomere. Upon activation of the excitation-contraction coupling mechanism, myosin binds to actin as inhibitory factors on the actin binding site are removed by the release of calcium. Once attachment has occurred, the potential energy stored in the myosin filament is transformed into the mechanical events of the cross-bridge action. This produces tension, or concentric shortening of the muscle. If the external resistance exceeds the cross-bridge ability to shorten (eccentric contraction), the actin-myosin bond is broken before transduction of energy can occur. As the external force continues, the energised myosin is repeatedly reattached and pulled apart from the actin without transduction of energy. Not only does this process produce greater tension at a given sarcomere length than does shortening (concentric) contraction, it is also independent of velocity until the velocity of lengthening exceeds the binding rate of the actin and myosin. The practical application is that as velocity of concentric contraction increases, fewer cross bridges are formed and thus less force is produced. In contrast, the cross bridge is not required to undergo the complete series of chemical events during eccentric contraction, and so the ability to generate tension at higher velocities is not adversely affected (Perrin, 1993).

This particular method for muscle function testing was used as it is has been proven as a valid and reliable test of muscle force, sensitive to muscle glycogen concentration in either type II or type I fibres, or both (Thorstensson et al., 1976; Jacobs et al., 1981; Jacobs et al., 1982a). Peak torque is consistently favoured as a measure of isokinetic leg strength (Burnie and Brodie, 1986; Kannus and Jarvinen, 1989), referring to the highest torque output of the joint produced by muscular contraction as the limb moves through a range of motion (Gleeson and Mercer, 1992). Peak torque has become a gold standard and reference point in all isokinetic measurements against which accuracy, precision, and clinical relevance of all
other parameters should be compared (Kannus, 1994). Three sub-maximal and 3 maximal repetitions are necessary before stability of measurement and reliable measurements of isokinetic peak torque, work and power can be obtained during isokinetic assessment of knee extensor peak torque (Perrin, 1986).

The impairment of muscular strength at the slower velocity following 90 min of intermittent exercise may be related to the pattern of glycogen depletion of the type II and type I muscle fibres. Previous studies have shown that peak torque production at an angular velocity of 180 °/s was impaired in subjects following an exercise protocol designed to induce severe glycogen depletion from both the type II and type I muscle fibres (Jacobs et al., 1981). There was no such detriment in performance following exercise which predominantly emptied the type I fibres. Thus the fact that in the present study, the observed decrease in muscle force was mainly apparent at a slower angular velocity of 60°/s and not at 240 °/s may suggest that glycogen depletion during 90 min of intermittent high intensity exercise was mainly confined to the type I muscle fibres. A high proportion of type II fibres is a prerequisite for performance at high speeds of contraction (Thorstensson et al., 1976; Tesch et al., 1978), conversely type I motor unit recruitment is critical to force production at 60 °/s (Burke and Edgerton, 1975). Consequently, impaired type II and type I fibre function impairs performance capacity at high and low rates of contraction, respectively. Even though glycogen utilisation was evident in both type I and type II fibres, there was a more pronounced degradation in the type II fibres in the present study, which may appear to contradict the strength decrements observed at the slower angular velocity. However, an angular velocity of 240°/s and the performance of only 3 repetitions may not have been a sensitive enough criterion measurement to reflect glycogen depletion in the different fibre populations. An angular velocity of 180°/s would have been a more appropriate choice, as PT has been shown to be reduced at this angular velocity following glycogen depleting in type I and type II fibres (Jacobs et al., 1982a). In addition, more pronounced strength impairments
were observed in the CON group, and this may be due to the fact that following the CON trial, subjects were more glycogen depleted than after the CHO trial. Indeed, a previous study from this laboratory has shown that the ingestion of a CHO-E drink during exercise results in a reduction in the amount of glycogen utilised during 90 min of intermittent high intensity exercise (Chapter 6, Part A). This may be due to a reduced rate of utilisation per se, or due to the ingestion of glucose facilitating glycogen resynthesis during the rest and lower intensity periods (Constable et al., 1984; Kuipers et al., 1987).

The values of PT, TW and AP obtained in the present study are difficult to compare with results from other studies. Normative data collected in various studies are always dynamometer-specific and thus not directly applicable to other testing systems, due to many differences in instrumentation, testing protocols, data reduction, and output (Francis and Hoobler, 1987; Kannus et al., 1992; Wilke et al., 1987). Nevertheless, measurements for pre-exercise PT at 240°/s in the present study are similar to those recorded by Jacobs et al. (1981) at an angular velocity of 180°/s. Peak Torque recorded in the present study was greater than that reported by several other investigators (Jacobs et al., 1982a; Sherman et al., 1984; Gleeson and Mercer, 1992). However, when making comparisons with a previously collected population data base, one should keep in mind that there is substantial variation in muscular performance with age, sex, body mass, activity level, and sports event, and therefore, the data base used may give a misleading idea of how good (or bad) the test result is (Kannus, 1994).

Although the changes observed in muscle function coincided with the glycogen depletion of the contracting fibres, the causal factor might involve other neural and/or biochemical phenomena. Sherman et al. (1984) reported muscle strength impairments following a marathon concomitant with depleted muscle glycogen stores. However, when muscle glycogen returned to normal levels 1-7 days post-marathon, MPT was still reduced.
throughout the week post-marathon. Thus muscle glycogen levels could not account for the reduced MPT directly after the marathon and during the week post-marathon. In agreement with these findings, Keizer et al. (1986) reported that maximal work capacity was reduced by approximately 7%, 22 h following interval exercise to exhaustion, despite the restoration of muscle glycogen stores to pre-exercise values.

In conclusion, prolonged intermittent high intensity exercise inducing glycogen utilisation in both type I and type II muscle fibres has been shown to be associated with impaired maximal muscular torque produced during a single dynamic contraction. It is not known whether this reduction in performance was due to a reduced glycogen availability in the type I and type II fibres, or as a direct consequence of the exercise per se. However, the decrease in muscle function was more pronounced in the CON trial, and was essentially limited to the impairment of force generation at an angular velocity of 60°/s. Thus the ingestion of a CHO-E drink during prolonged intermittent high intensity exercise will enhance the ability to perform subsequent short term (≤10 s) maximal exercise compared with the ingestion of a non-CHO placebo solution.
CHAPTER 7

THE INFLUENCE OF ORAL CREATINE SUPPLEMENTATION ON PERFORMANCE DURING PROLONGED INTERMITTENT HIGH INTENSITY SHUTTLE RUNNING

7.1 INTRODUCTION

In the previous studies it was shown that endurance capacity at the end of a fixed bout of intermittent high intensity exercise was improved when subjects ingested a CHO-E solution (Chapter 5) and that there is evidence that the mechanism involves the sparing of muscle glycogen in the exercising muscles (Chapter 6). It was also found that PCr concentration was lower after 90 min of exercise in the control trial compared with the CHO trial.

Oral Cr supplementation at a rate of 20g.day\(^{-1}\) for 5 days has been shown to increase the total Cr content of human quadriceps femoris muscle (Harris et al., 1992), and to increase the amount of work performed during repeated bouts of maximal exercise (Greenhaff et al., 1993, Balsom et al., 1993a; Harris et al., 1993, Birch et al., 1994). No performance benefit has been observed during submaximal exercise following Cr ingestion (Balsom et al., 1993; Bogdanis et al., 1996).

Although the manufacturers of an oral Cr supplement (Ergomax\textsuperscript{TM}) claim that their product is 'particularly useful for sports that involve intermittent high intensity efforts,' little is known about the effects of oral Cr supplementation on performance during maximal intensity, intermittent running, the pattern of exercise typical of the 'multiple sprint' sports such as soccer, rugby and hockey.

Therefore, the purpose of this investigation was to examine the efficacy of the oral Cr supplementation regimen prescribed by the manufacturers of Ergomax\textsuperscript{TM} C150 (3g, 3
times per day for 5 days) on sprint performance and intermittent endurance running capacity during a prolonged intermittent high intensity shuttle run test (PIHSRT).

7.2 METHODS

7.2.1 Subjects

Sixteen male games players volunteered and gave their informed consent to participate in this study, which had University Ethical Committee approval. The subjects, who had not hitherto used an oral Cr supplement, were randomly assigned to either a placebo (Plac) or creatine (Cr) group in a double blind design. Their physical characteristics are described in Table 7.1.

7.2.2 Preliminary Measurements

Percentage body fat was measured in this study because previous observations have reported that body mass was increased following Cr supplementation (Balsom et al., 1993). Skinfold measurements were taken at the biceps, triceps, subscapular and suprailiac sites according to the methods described by Weiner and Lourie (1969). The sum of the skinfolds was used to calculate body density (Durnin and Rahaman, 1967) and body fat was calculated using Siri's (1956) formula. The speeds that each subject was required to run during the PIHSRT were determined from the estimated VO₂ max values, as described elsewhere (Chapter 3). Subjects were then familiarised with the required running speeds, activity pattern and experimental procedures of the PIHSRT.

7.2.3 Experimental Design and Procedures

Subjects completed two exercise trials separated by at least 7, and no more than 9 days. The first pre-supplementation visit provided a baseline performance for all subjects. (trial
The second trial was performed immediately following a 5 day period of Cr or Plac supplementation regimen (trial 2, T2).

The Cr administration regimen consisted of three 6 g doses of either 3 g of creatine monohydrate and 1.5 g maltodextrin (Ergomax™), plus 1.5 g glucose (Cr group) or 6 g of glucose (Plac group) per day. They were instructed to mix each dose, a pre-measured packet of powdered supplement, in warm-hot tea or coffee, for immediate consumption at 9:00, 15:00 and 21:00 h. A verbal check was made to ensure that subjects had complied with these procedures after the supplementation period.

During the 2 days preceding each trial, subjects refrained from any strenuous physical activity, and consumed their normal diet. They maintained a Food Record Diary for 3 days prior to trial 1 in order that the same dietary intake could be repeated for the 3 days prior to trial 2. Subjects reported to the laboratory after an overnight fast of approximately 10 h, and then voided before the measurement of nude body mass was made. Nude body mass was determined before and immediately after each trial.

A standardised warm up consisting of jogging, stretching and striding was performed by each subject for 15 min prior to the PIHSRT. The PIHSRT was comprised of 2 parts (Fig. 7.1). Part A consisted of five 15 min exercise bouts separated by 3 min recovery. The activity pattern has been described previously (Chapter 3). Part B immediately followed Part A and continued until volitional fatigue, that is, when the subject was no longer able to maintain the required pace. Subjects were verbally encouraged throughout each trial, and were not aware of their performance time during Part B.

Total running time for Part B was recorded to the nearest second. Subjects were allowed to drink water ad libitum throughout trial 1, and they were subsequently encouraged to consume an equivalent amount during trial 2. Heart rate was continuously recorded every 15 s throughout each exercise trial using short-range telemetry (Sports Tester PE 3000,
Polar Electro, Finland) and stored in memory mode. Subjective ratings of perceived exertion using Borg's 6 - 20 scale (Borg, 1973) were obtained at 15 min intervals during Part A, and at fatigue. Dry and wet bulb temperatures were recorded every 15 min using a whirling hydrometer (Brannan Thermometers, Cumberland, UK), and the temperature of the gymnasium was maintained at ≤ 20°C.

7.2.4 Blood Sampling and Analysis

An indwelling catheter (Venflon, 16-18G) was inserted into an antecubital vein and kept patent with infusion of sterile saline (10 units of heparin per ml). Subjects assumed a standing position for 15 min before a resting blood sample was obtained. Venous blood samples were obtained at rest, after each 15 min exercise period of Part A, and after 3 and 10 min following the completion of Part B and dispensed as previously described (Chapter 3). Plasma was later analysed for ammonia within 48 h, FFA and glycerol; whole blood was analysed for lactate, haematocrit and haemoglobin using methods which have been reported elsewhere (Chapter 3) and glucose (Roche Unimate and GLUC GDH kit). Changes in plasma volume were subsequently calculated (Dill and Costill, 1974).

7.2.5 Statistical Analyses

The results are reported as means (± SEM). Performance data were compared using a three way ANOVA for repeated measures. A one way ANOVA using Scheffe's post hoc analysis was used to determine differences. The level of significance was accepted at P<0.05.
Fig. 7.1: Schematic illustration of the PIHSRT protocol and experimental design.
7.3 RESULTS

7.3.1 Performance Times

Similar mean sprint times were recorded between trials and over the duration of each trial. (Table 7.2) Part B run times to exhaustion are shown in Fig. 7.2. Despite running for 92 s longer in trial 2, following Cr supplementation, this improvement was not significant.

7.3.2 Blood Glucose, Blood Lactate Concentration and Heart Rate Response

Blood glucose concentrations demonstrated a similar pattern of change throughout each trial. Peak concentration occurred after 30 min of intermittent shuttle running, and then declined thereafter to the end of the recovery period, with the exception of a small rise in concentration after 3 min recovery in PlacT2, CrT1 and CrT2 (Figs. 7.3a and 7.3b). Blood lactate concentrations markedly increased from rest after 15 min of exercise. Similar concentrations were measured over the course of each trial, and although the blood lactate values during trial 2 tended to be higher in the Plac group and lower in the Cr group following supplementation, none of the differences within groups were significant (Figs. 7.4a and 7.4b).

Heart rate responses before and after supplementation were similar in both groups (Table 7.3).

7.3.3 Plasma FFA and Glycerol Concentration

Plasma FFA concentrations declined from initial resting levels after 15 min of exercise in all groups and increased gradually, reaching peak concentrations at the end of the recovery period (Figs. 7.5a and 7.5b). Following Cr supplementation, plasma FFA concentrations
were higher in the Cr group after 75 min of intermittent shuttle running and after 3 and 10 min of recovery than at the corresponding time points in the Plac group. Plasma glycerol concentrations increased over the duration of each trial, peaking after 60 to 75 min of exercise before a slight decrease during the recovery period (Figs. 7.6a and 7.6b). Similar concentrations were measured between and among groups except after 60 min of exercise, glycerol concentration was higher in the Cr group during trial 2 (P<0.05).

7.3.4 Plasma Ammonia Concentration

Similar plasma ammonia concentrations were observed between trials 1 and 2 in the Plac group and trial 1 in the Cr group (Fig. 7.7). However in the Cr group following supplementation, plasma ammonia concentrations were lower in Trial 2 compared with trial 1 after 60 and 75 min of intermittent shuttle running and during the recovery measurements following exhaustive running (P<0.05).

7.3.5 Body Mass and Plasma Volume Changes

Body mass and % body fat was unchanged following the 5 day supplementation period (Table 7.4). Similar changes in body mass were observed during each trial for both groups (T1 Plac 2.1±0.1 kg, T2 Plac 2.3±0.1 kg; T1 Cr 2.0±0.1 kg, T2 Cr 2.0±0.1 kg). This represented an average 2.8% and 2.6% loss in the Plac and Cr groups, respectively. The volume of water consumed which was similar for each trial was accounted for in these calculations (1317± 233 ml and 1139± 132 ml for the Plac and Cr groups, respectively), and there were no differences in plasma volume changes pre- and post-exercise between groups or trials.
Fig. 7.2 Part B run times to exhaustion during trials 1 and 2 for both groups.

Fig. 7.3a Blood glucose concentration during trials 1 and 2 for the Cr group.
Fig. 7.3b Blood glucose concentration during trials 1 and 2 for the Plac group.

Fig. 7.4a Blood lactate concentration during trials 1 and 2 for the Cr group.
Fig. 7.4b Blood lactate concentration during trials 1 and 2 for the Plac group.

Fig. 7.5a Plasma FFA concentration during both trials for the Cr group.
Fig. 7.5b Plasma FFA concentration during both trials for the Plac group.

Fig. 7.6a Plasma glycerol concentration during both trials for the Cr group.
Fig. 7.6b Plasma glycerol concentration during both trials for the Plac group.

Fig. 7.7 Plasma ammonia concentration during both trials for each group (*P<0.05 CrT1 vs. CrT2).
Table 7.1: Physical characteristics of the subjects (n=8).

<table>
<thead>
<tr>
<th>Variable</th>
<th>PLACEBO GROUP Mean (±SEM)</th>
<th>CREATINE GROUP Mean (±SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>21.5 ± 2.8</td>
<td>21.5 ± 2.5</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>177.3 ± 6.5</td>
<td>180.8 ± 4.8</td>
</tr>
<tr>
<td>Body Mass (kg)</td>
<td>77.3 ± 5.8</td>
<td>80.1 ± 10.1</td>
</tr>
<tr>
<td>%Fat*</td>
<td>16.1 ± 4.0</td>
<td>14.5 ± 5.6</td>
</tr>
<tr>
<td>VO$_2$ max (ml.kg$^{-1}$min$^{-1}$)</td>
<td>57.5 ± 4.4</td>
<td>59.0 ± 5.4</td>
</tr>
<tr>
<td>VO$_2$ max (l.min$^{-1}$)</td>
<td>4.4 ± 0.5</td>
<td>4.7 ± 0.3</td>
</tr>
</tbody>
</table>

*Durnin and Rahaman (1967)

Table 7.2: Mean sprint times (s) during trial 1 and trial 2 for Placebo and Creatine groups (n=8).

<table>
<thead>
<tr>
<th>SPRINT No.</th>
<th>Placebo Trial 1 Mean ± SEM</th>
<th>Placebo Trial 2 Mean ± SEM</th>
<th>Creatine Trial 1 Mean ± SEM</th>
<th>Creatine Trial 2 Mean ± SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2.38 ± 0.03</td>
<td>2.39 ± 0.04</td>
<td>2.44 ± 0.03</td>
<td>2.42 ± 0.04</td>
</tr>
<tr>
<td>2</td>
<td>2.41 ± 0.03</td>
<td>2.39 ± 0.04</td>
<td>2.45 ± 0.05</td>
<td>2.42 ± 0.04</td>
</tr>
<tr>
<td>3</td>
<td>2.43 ± 0.04</td>
<td>2.44 ± 0.04</td>
<td>2.47 ± 0.05</td>
<td>2.44 ± 0.05</td>
</tr>
<tr>
<td>4</td>
<td>2.45 ± 0.03</td>
<td>2.45 ± 0.04</td>
<td>2.47 ± 0.05</td>
<td>2.47 ± 0.05</td>
</tr>
<tr>
<td>5</td>
<td>2.45 ± 0.05</td>
<td>2.46 ± 0.03</td>
<td>2.47 ± 0.05</td>
<td>2.48 ± 0.05</td>
</tr>
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</table>
Table 7.3: Heart rate responses during each trial for both conditions (n=8).

<table>
<thead>
<tr>
<th></th>
<th>Heart Rate (b.min⁻¹)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>0-15min</td>
</tr>
<tr>
<td><strong>T1 Placebo</strong></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>166</td>
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<tr>
<td>± SEM</td>
<td>3</td>
</tr>
<tr>
<td><strong>T2 Placebo</strong></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>168</td>
</tr>
<tr>
<td>± SEM</td>
<td>3</td>
</tr>
<tr>
<td><strong>T1 Creatine</strong></td>
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</tr>
<tr>
<td>Mean</td>
<td>169</td>
</tr>
<tr>
<td>± SEM</td>
<td>3</td>
</tr>
<tr>
<td><strong>T2 Creatine</strong></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>162</td>
</tr>
<tr>
<td>± SEM</td>
<td>4</td>
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</tbody>
</table>

* a P<0.05 Part B vs. 15 min
* b P<0.05 Part B vs. 15 and 75 min
* c P<0.01 Part B vs. 15 min
* d P<0.05 Part B vs. 15 and 45 min

Table 7.4: Placebo and Creatine groups trials 1 and 2 body mass and relative body fat (n=8).

<table>
<thead>
<tr>
<th>GROUP/CONDITION</th>
<th>BODY MASS (kg)</th>
<th>BODY FAT (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo Trial 1</td>
<td>76.8 ± 2.1</td>
<td>16.1 ± 1.4</td>
</tr>
<tr>
<td>Placebo Trial 2</td>
<td>76.6 ± 2.0</td>
<td>16.5 ± 1.3</td>
</tr>
<tr>
<td>Creatine Trial 1</td>
<td>79.4 ± 3.8</td>
<td>14.5 ± 2.0</td>
</tr>
<tr>
<td>Creatine Trial 2</td>
<td>79.5 ± 3.5</td>
<td>14.5 ± 2.0</td>
</tr>
</tbody>
</table>
7.4 DISCUSSION

The purpose of the study was to investigate the effect of a Cr supplementation programme on sprint performance and endurance running capacity during intermittent high intensity exercise. The main finding was that Cr administration had no effect on 15 m sprint times or run time to exhaustion. Although the Cr group ran for 92 s longer following supplementation, this improvement was not statistically significant. Lower plasma ammonia concentrations were observed towards the end of intermittent exercise during trial 2 following Cr supplementation. There were no further differences observed for any of the measured physiological or performance variables in either the Cr or Plac group.

These results are in contrast to the findings of other studies which have reported significant performance improvements during repeated maximal exercise after Cr administration (Balsom et al., 1993a; Greenhaff et al., 1994b; Harris et al., 1993; Birch et al., 1994; Bogdanis et al., 1996). However, a few important points must be noted. During the PIHSRT, 15 m sprints were performed approximately every 90 s. Balsom et al. (1992) observed that 15 m sprints could be repeated every 30 s without decreases in performance. Creatine phosphate (PCr) and glycogen are the major sources of substrate which regenerate adenosine triphosphate (ATP) during brief maximal exercise (Boobis et al., 1987; Hultman et al., 1990). The half-time for PCr resynthesis in human mixed muscle fibres has been reported as 30-40 s (Harris et al., 1976), and therefore there was enough time for a significant resynthesis of PCr between sprints. Thus PCr concentration in the muscle would not be a limiting factor to repeated sprint performance in this protocol.

Dietary Cr supplementation has been shown to be a useful ergogenic aid only during exercise where PCr availability limits performance (Birch et al., 1994; Bogdanis et al., 1996). Subjects who performed six 10 s sprints on a non-motorised treadmill following 5 days of Cr supplementation generated a greater power output and ran further than the control group (Bogdanis et al., 1996). Therefore, the sprints during the PIHSRT should be
either repeated more frequently, or increased to a distance 40 m, in order to further stress the PCr energy system. This may also explain why run times to exhaustion were similar between groups. During Part B, subjects running speeds alternated between 55% and 95% VO$_2$ max, giving an average intensity of 75% VO$_2$ max. At this exercise intensity, glycogen and blood glucose are the predominant sources of energy substrate. Previous studies have reported that neither performance nor metabolism during submaximal exercise are altered following Cr ingestion (Green et al., 1994; Balsom et al., 1993b). However, in the protocol of this study, an enhanced muscle PCr content may nevertheless be beneficial during the acceleration phase every 20 m.

There were no changes in body mass or relative body fat following Cr supplementation in the present study. This is contrary to the observations of Balsom et al. (1993a) who reported a significant increase in body mass from 84.2 to 85.3 kg after the Cr administration period (5, 6 g doses of 5 g creatine monohydrate and 1 g of glucose per day for 6 days). This may be due to the different amounts of Cr supplement ingested by their subjects (9 g/day for 5 days in the present study vs 25 g/day for 6 days in the study of Balsom and colleagues). An increase in body mass from 73.4 to 74.3 kg was also reported following a supplementation regime of 75 mg kg bm$^{-1}$ of Cr and 1 g glucose, four times per day for 5 days (Bogdanis et al., 1996). It has been suggested the increase in body mass could be associated with a change in muscle fibre structure, possibly due to an enhanced rate of contractile protein resynthesis (Balsom et al., 1993a), but the gain in body mass over such a short time is mainly the consequence of water retention rather than protein synthesis.

Blood lactate concentrations were similar over the duration of each trial, averaging 6.8 and 4.6 mmol.l$^{-1}$ for the Plac and Cr groups, respectively. These results are similar to those previously observed following repeated 2.5 s bouts of sprinting 15 m (Balsom et al., 1992a), and illustrate that anaerobic glycolysis plays an important role as an energy source during prolonged intermittent high intensity exercise. Although there was a trend
for higher blood lactate values to be observed during trial 2 in the Plac group, yet lower in the Cr group, these differences were not statistically significant. However, considering the evidence of lower plasma ammonia concentrations, and a tendency for better performance times and lower blood lactate concentrations following dietary Cr administration, it may be suggested that Cr supplementation enhanced ATP resynthesis during exercise and reduced skeletal muscle adenine nucleotide loss. The increased total Cr content may regulate the rate of anaerobic glycolysis during exercise (Balsom et al., 1994), as one would assume that the rate of muscle lactate efflux was not altered. Thus, a lower blood lactate concentration would be indicative of a reduced reliance on anaerobic glycolysis for ATP resynthesis. If the rate of muscle glycogen degradation was reduced in the Cr group, one may anticipate an increased running time to exhaustion during Part B of the present study. Indeed, the Cr group ran for 92 s (27%) longer during trial 2 compared to trial 1, and 7 of the 8 subjects improved their trial 1 performance. However, although the Plac group ran for 15 s less during trial 2, there were no statistically significant differences between groups or trials.

Similar to previous observations (Greenhaff et al., 1993; Birch et al., 1994; Bogdanis et al., 1996), plasma ammonia concentrations were lower following repeated maximal exercise after Cr supplementation. These findings are also in agreement with the reduced plasma hypoxanthine accumulation reported by Balsom et al. (1993) following Cr ingestion. Ammonia and hypoxanthine are accepted markers of the loss of skeletal muscle adenine nucleotides (Harris et al., 1991), and a reduction in accumulation of IMP and ammonia reflects a higher muscle ATP turnover (Birch et al., 1994).

Exercise stimulates the catabolism of amino acids (predominantly BCAA) in muscle (Lemon and Nagle, 1981), and the extent of the resultant increases in blood ammonia will be determined by exercise intensity and duration and relative muscle fibre composition (Bannister and Cameron, 1990). An increase in the concentration of ammonia has also been associated with glycogen depletion due to the concomitant increase in amino acid
oxidation (Wagenmakers et al., 1991). Exhaustive exercise may induce a condition of ammonia toxicity which has been linked to central fatigue and possibly affect the rate of energy production and subsequent ATP availability. However, if ammonia toxicity was the mechanism for the onset of fatigue then the concentrations in plasma would be the same at the end of exercise, which was not the case.

Concentrations of FFA declined from initial resting values and then steadily increased over the duration of intermittent exercise and peaked 10 min after the cessation of exercise. Plasma FFA were higher after 75 min of the PIHSRT in the Cr supplemented group (P<0.05), which may suggest that the rate of glycolysis was reduced following Cr ingestion, thereby increasing the relative contribution of FFA to exercise metabolism.

Despite a possible enhanced rate of ATP turnover, indicated by the reduced ammonia concentrations following Cr supplementation, this metabolic alteration did not manifest itself in any performance improvements. There were only trends towards faster sprint times and increased run times to volitional fatigue. This may be associated with the dosage of Cr administered in the present study (3 g 3x/day for 5 days). Dietary Cr at a rate of 20 g/day for 3 days had no effect on either performance during a single 30 s cycling bout, or on the muscle PCr content (Odland et al., 1994). Indeed, low dose supplementation (3g/day) over a 1 month period was not as effective at raising tissue levels as the high dose 5 day administration of 20g/day (Greenhaff, 1995). Thus it may be postulated that a higher dosage of Cr at a rate of 20 g/day for 5 days may have resulted in an improvement in performance during the PIHSRT.

In summary, dietary Cr supplementation at a rate of 9 g/day for 5 days had no effect on sprint performance or endurance running capacity during a prolonged intermittent high intensity shuttle run test, although a reduction in plasma ammonia accumulation was observed after 1 h of exercise. Further study is required in order to establish whether a higher Cr dosage would influence performance during the existing PIHSRT protocol, or
whether a repeated sprint protocol which challenged muscle PCr availability both during exercise and recovery between sprints should be employed.
CHAPTER 8

GENERAL SUMMARY

The purpose of the studies in this thesis was to investigate the effect of nutritional intervention on performance of, and the physiological and metabolic responses to, prolonged, intermittent high intensity running. This was undertaken in a series of experiments in which the protocol developed was designed to replicate the combination of activities seen in the 'multiple sprint' sports such as soccer and rugby without the contact. Previous studies have attempted to simulate multiple sprint activities using cycle ergometers or treadmills, but the development of a controlled free-running intermittent high intensity test described in this thesis is the first of its kind.

The distances covered in soccer matches have been reported to range between 7.1 km and 12.9 km (Reilly and Thomas, 1976; Withers et al., 1982; Mayhew and Wenger, 1985; Ekblom, 1986; Bangsbo et al., 1991), which are similar to the distances covered by the subjects during the PIHSRT. In terms of total time, the activities of elite soccer players can be divided as follows; standing still (17%), walking (40%), low intensity running (35%) and high intensity running (8%) (Bangsbo et al., 1991) with sprints averaging 15 m every 90 s (Reilly and Thomas, 1976). These patterns of activity were performed in similar proportions in the PIHSRT, with the exception that a greater proportion of time spent performing high intensity exercise. The PIHSRT was designed to be more demanding than an actual soccer or rugby game and the increased physiological load was confirmed by the subjective assessment of the subjects. This perception was enhanced because during a game players slow down when they get tired, but in the PIHSRT, this is not possible as the speed of running is dictated by an audio signal. This would be equivalent to playing against very fit opponents who set the tempo of the game.
The total distance covered, heart rate responses, blood lactate concentrations measured and the amount of muscle glycogen utilised during the PIHSRT were similar to those recorded during a soccer match. These results, together with subjective assessments of the subjects (not documented) indicate that the PIHSRT closely resembles the activity pattern characteristic of soccer. The total amount of glycogen utilised during 90 min of the PIHSRT was 245.3 mmol (kg DM)$^{-1}$ and at the end of the test, muscle glycogen concentration was 39% of its initial value. This reduction in muscle glycogen concentration is similar to that of 30% and 43% observed during a professional soccer match (Karlsson, 1969 cited in Ekblom, 1986 and Jacobs et al., 1982b, respectively).

Although the physiological and metabolic responses measured represent the combination of activities of different intensities and not each activity in isolation, the results obtained are, nevertheless, an improvement on measurements at half-time and post-game observed in previous studies. Consequently, the observations made in this thesis have practical implications for games players.

In each of the studies the subjects were games players of similar training status. The experimental procedures were similar in each study and the physiological and metabolic responses to the exercise protocol during the control trials for all studies were consistent with each other. The main observations made during each study were integrated in order to facilitate a greater understanding of the physiological and metabolic responses to prolonged intermittent high intensity exercise. The mechanisms responsible for the improved performance and delayed fatigue observed during this type of exercise following dietary intervention were also examined. The main findings of these studies are summarised as follows:

1. Increasing the CHO availability during intermittent high intensity exercise, either by increasing CHO intake to the equivalent of 10 g kg$^{-1}$bm in the recovery period from exercise (PIHSRT)(Chapter 4), or through the ingestion of a CHO-E drink at regular
intervals during exercise (PIHSRT)(Chapter 5), increases the capacity for high intensity exercise at the end of a fixed bout of prolonged high intensity intermittent shuttle running. Subjects demonstrated improvements in exercise time to fatigue of 43% and 33%, respectively.

2. After 90 min of intermittent high intensity exercise, the amount of glycogen utilised in mixed muscle fibres was less in the trial when subjects ingested a CHO-E drink (192.8 mmol (kg DM)\(^{-1}\)) throughout compared with the control trial when a non-CHO placebo was administered (245.3 mmol (kg DM)\(^{-1}\)) (Chapter 6, Part A).

3. During the control trial, glycogen degradation during the PIHSRT was greater in the type II fibres compared with type I fibres [287.4 vs. 182.2 mmol glucosyl units (kg DM)\(^{-1}\), respectively]. The pattern of glycogen depletion in the individual fibre types varied between individual subjects for both trials (Chapter 6, Part A).

4. Following the 90 min PIHSRT, isokinetic muscle strength as measured by peak torque was significantly reduced at the angular velocity of 60°/s. No such reduction in peak torque was observed after subjects drank a CHO-E drink at regular intervals throughout the PIHSRT. However, total work and average power at the angular velocity of 60°/s were lower post-exercise than pre-exercise values in both trials (Chapter 6, Part B).

5. Dietary creatine (Cr) supplementation at a rate of 9g/day for 5 days had no effect on sprint performance or high intensity endurance capacity during the prolonged intermittent high intensity shuttle run test. However, plasma ammonia concentration was lower after 60 and 75 min of exercise and during the recovery measurements for 10 min after exercise following Cr supplementation. In addition, blood lactate concentration had a tendency to be lower when the PIHSRT was repeated after Cr supplementation.
The discussion which follows attempts to explain the above findings and propose possible mechanisms for the ergogenic effect of nutritional intervention on the fatiguing process.

8.1 Recovery of Functional Fitness Following Increased CHO Intake

Previous research on the recovery of functional capacity following exhaustive high intensity exercise has shown that physical work capacity was still approximately 7% lower than on the previous day even though muscle glycogen concentration was replaced after 22 h recovery (Keizer et al., 1986). Likewise, in the study of Nevill et al. (1993), a high CHO diet didn't allow the games players to maintain the same peak power output following a 24 h recovery. In contrast, Fallowfield and Williams (1993) showed that a high CHO recovery diet allowed runners to repeat their previous day's performance. However, this latter study didn't demand high intensity exercise from the subjects. Therefore, an examination of the influence of a high CHO diet on recovery of performance in the PIHSRT was relevant because the test requires the subjects to combine endurance with high intensity exercise. High intensity activity included sprinting 15 m approximately every 80 s, acceleration, deceleration, turns and running at 95% $\text{VO}_2\text{max}$ for 60 m out of a total of 200 m, beginning approximately every 80 s.

When the CHO intake of subjects was increased to the equivalent of 10 g kg$^{-1}$bm during the 22 h recovery period between two exhaustive bouts of intermittent high intensity shuttle running, there was a 43% difference in endurance capacity compared with an isocaloric diet, with additional energy provided from fat and protein (Chapter 4). This finding that an increased CHO intake during the recovery from prolonged intermittent high intensity exhaustive exercise is important for optimal performance during subsequent exercise is consistent with the findings of Fallowfield and Williams (1993) and advocates the importance of a high CHO diet during recovery from this type of exercise. The amount of muscle glycogen utilised during this type of exercise can be
estimated from the muscle glycogen concentration reported in Chapter 6 (Part A). For 80 min of high intensity intermittent exercise, the total glycogen utilised is 5.7 mmol (kg WW)⁻¹, [218 mmol (kg DM)⁻¹], assuming an active muscle mass of 25% (18 kg). This is the equivalent of 166 g CHO, assuming an equal rate of utilisation from each muscle fibre. The additional CHO in the recovery diet (705 g CHO in the CHO trial and 381 g CHO in the CON trial) clearly covered the CHO utilised during the PIHSRT. However, the 381 g of CHO provided in the CON trial would appear, at first glance, to also be sufficient to replenish the reduced muscle glycogen stores. However, it was probably not sufficient to sustain prolonged intermittent high intensity exercise performed twice in approximately 24 h. When subjects consumed their normal amount of CHO they did not perform as well compared with a recovery CHO intake the equivalent of 10 g kg⁻¹ bm d⁻¹. If the test had been repeated on the third day, it is likely that there would have been a further decrement in the performance during the CON trial due to cumulative glycogen depletion. Thus it is crucial for glycogen stores to be normalised within 24 h, by consuming the appropriate amount and type of CHO during recovery, particularly if exercise is performed on consecutive days, such as during a soccer or rugby tournament.

8.2 Improvement of High Intensity Exercise Capacity with CHO-E Ingestion

The majority of previous research studies in this area have shown that ingesting a CHO-E solution has a positive effect on performance during constant load cycling, treadmill running and road running (Coggan and Coyle, 1991; Tsintzas et al., 1993a; Tsintzas et al., 1995a; Tsintzas et al., 1995b). Therefore it was logical to examine the influence of ingesting a CHO-E solution on performance of intermittent high intensity exercise.
The ingestion of a 6.9% CHO-E solution resulted in a 33% improvement in endurance capacity compared with placebo ingestion (Chapter 5). One possible explanation for this improvement was glycogen sparing in the active muscle fibres, as demonstrated in the follow-up study (Chapter 6, Part A). The reduced rate of decline in muscle glycogen during the CHO trial was most likely due to the elevated serum insulin levels, significant after 30 min of exercise, and the tendency for both blood glucose and serum insulin concentrations to be elevated throughout the CHO trial. Such a metabolic environment would favour the uptake of glucose into the muscle. In addition, it is possible that muscle glycogen synthesis occurred in the type II fibres during the low intensity exercise bouts in the CHO trial as has been previously reported (Kuipers et al., 1987). This would account for the higher concentration of muscle glycogen post-exercise in type II fibres following CHO ingestion throughout exercise [CHO trial = 165.6 mmol (kg DM)\(^{-1}\) and CON trial = 137.3 mmol (kg DM)\(^{-1}\)].

The evidence of a glycogen sparing effect during intermittent exercise is in agreement with previous studies which have used both intermittent exercise (Yaspelkis et al., 1993; Hargreaves et al., 1984) and continuous constant paced exercise (Tsintzas et al., 1993b; Tsintzas et al., 1995a), but in contrast to those which have reported that CHO supplementation had no influence over muscle glycogen utilisation during exercise at 70% \(\text{VO}_2\) max (Coyle et al., 1986; Hargreaves and Briggs., 1988).

During the 90 min of shuttle running without CHO ingestion, glycogen utilisation in type II muscle fibres [3.2 mmol kg (DM)\(^{-1}\)min\(^{-1}\)] was nearly double that in type I fibres [2.0 mmol (kg DM)\(^{-1}\)min\(^{-1}\)]. This is in contrast to the utilisation rates in type I fibres (3.3 mmol (kg DM)\(^{-1}\)min\(^{-1}\)) and type II fibres (1.2 mmol (kg DM)\(^{-1}\)min\(^{-1}\)) during 60 min submaximal constant paced running (Tsintzas et al., 1993b). Both fibre type populations were recruited during the intermittent exercise, as would be expected due to the nature of the test - a mixture of endurance and high intensity exercise. The average exercise intensity was approximately 50% of \(\text{VO}_2\) max, but the greater glycogen
utilisation in the type II fibres reflected the importance of recruiting this fibre population during the brief periods of high intensity exercise and the acceleration, deceleration and turning phases. However glycogen sparing was more pronounced in the type I fibre population compared with type II fibres in two out of three subjects analysed. Whether the rates of glycogen utilisation were representative of the whole duration of exercise is uncertain. Further biopsies would be required after 45 or 60 min of exercise in order to answer this question.

8.3 Impairment of Muscle Function Following the PIHSRT

The reduction in isokinetic muscle function following prolonged intermittent high intensity shuttle running was attenuated when a CHO-E beverage was ingested at regular intervals throughout exercise. The loss in muscular strength and function following prolonged sub-maximal and intermittent exercise has also been observed by other investigators (Forsberg et al., 1978; Symons and Jacobs, 1989; Keizer et al., 1986), the loss of force being more pronounced under low CHO than high CHO conditions (Young and Davies, 1984). Results suggest that performance of maximal exercise is determined by factors other than substrate availability, that is the restoration of glycogen stores, although the ingestion of CHO attenuates the observed changes in muscle function. The greater glycogen depletion observed following the CON trial may result in a more pronounced detrimental effect on the electromechanical coupling capacity of the contracting fibres (Edwards, 1981). It is also possible that individual type I and type II fibres were glycogen depleted and were not recruited during the isokinetic measurements. This reduction in muscle mass activated would explain the reduction in peak torque, total work and average power observed, as studies have shown that a high proportion of type II fibres is a prerequisite for maximal performance (Thorstensson et al., 1976; Jacobs et al., 1981). It is difficult to determine whether the changes in muscle function are due to the effects of the prolonged intermittent high intensity exercise or to the reduction of muscle glycogen per se. Further research is
needed to elucidate the exact mechanisms by which glycogen depletion can affect the force generation of human muscle fibres. Thus CHO ingestion exerts its ergogenic effect not only on the ability to perform high intensity exercise late in exercise, but may also enhance performance during activities which require explosive contractions, such as kicking the ball in soccer.

8.4 No Performance Improvements with Creatine Supplementation

Carbohydrate ingestion during the PIHSRT had no effect on sprint performance. Previous studies which have shown that performance during repeated maximal exercise is enhanced with Cr supplementation (Harris et al., 1993; Balsom et al., 1993a; Birch et al., 1994) prompted the investigation in this thesis (Chapter 7). However, supplementation with Cr for the five days prior to performance of the PIHSRT did not improve sprint times when 15 m sprints were performed approximately every 80 s. Therefore, PCr content was not a limiting factor to performance under these conditions. This result is in agreement with an earlier study which reported that 15 m sprints could be repeated 40 times every 30 s without decreases in performance (Balsom et al., 1992a).

Creatine supplementation showed a tendency to increase endurance capacity, although this improvement was not statistically significant and therefore cannot be considered as an important observation (Chapter 7). Plasma ammonia concentrations were reduced following Cr supplementation, an observation that is indicative of the loss of muscle tissue adenine nucleotides (Harris et al., 1991). Thus, lower plasma ammonia concentrations following Cr supplementation may be related to better ATP buffering and more efficient ADP rephosphorylation associated with higher intracellular PCr levels. This may result in a decreased contribution from anaerobic glycolysis during the sprints, thus sparing muscle glycogen for use at the end of exercise. Indeed, in agreement with
this was the observation that blood lactate concentrations had a tendency to be lower following Cr supplementation.

The increases in the plasma ammonia concentration observed over the course of the PIHSRT may also be due to an increase in amino acid oxidation with increasing intensity and duration of exercise (Lemon et al., 1982; Lemon, 1994). The lower ammonia concentrations during exercise following Cr supplementation may also be explained by a reduced amino oxidation due to differences in muscle glycogen concentration. It is known that amino acid oxidation is inversely related to glycogen availability (Lemon and Mullin, 1980). Although muscle glycogen concentrations were not measured, it is possible that Cr supplementation resulted in a higher initial PCr content and rate of resynthesis, reducing the contribution of anaerobic glycolysis to energy production during the sprints and continual accelerations and decelerations in the process of turning. Thus the rate of glycogen utilisation would be lower and the contribution of amino acids to total energy supply less. In support of a reduced rate of anaerobic glycolysis, there was a tendency for blood lactate concentrations to be reduced following Cr supplementation. Some (Balsom et al., 1993a), but not all (Greenhaff et al., 1994b; Birch et al., 1994) studies have found that anaerobic glycolysis was altered by Cr supplementation. This change in glycogenolysis has been suggested as the consequence of a feedback mechanism regulated by ADP and AMP accumulation in the muscle (Ren and Hultman, 1990).

8.5 Mechanisms for Enhanced Performance and Delay in the Onset of Fatigue

The physiological and metabolic responses to prolonged intermittent high intensity shuttle running with the different nutritional strategies described in the studies reported in this thesis are summarised in Figure 8.1, along with the possible causes of fatigue during this type of exercise.
[Blood glucose] maintained at euglycaemic levels throughout exercise

Both type I and type II fibres recruited during PIHSRT

Type II glycogen utilized greater than type I

↑ CHO during recovery from PIHSRT

No differences in [blood lactate] or [blood glucose]

↑ [Blood glucose] and [Serum insulin] early in exercise

↑ PCr utilisation

↑ Glycogen utilisation

↑ [Plasma ammonia]

↑ Oxidation of BCAA

↑ Glycogen sparing in both type I and II fibres

Subjects ran for 43% longer compared with CON trial

Subjects ran 33% longer compared with CON trial

Subjects ran 13% longer compared with CON trial (ns)

↑ Glycogen depletion in type II fibres

↓ ATP resynthesis in type II fibres

FATIGUE

Failure to maintain running speed at the required intensity

Fig. 8.1 Physiological and metabolic responses to prolonged intermittent high intensity shuttle running with different nutritional strategies.
Prolonged, intermittent high intensity shuttle running over a set distance or to exhaustion without CHO ingestion did not result in low blood glucose concentrations or the development of hypoglycaemic symptoms at the end of exercise. This is consistent with the observations made at the end of exhaustive constant paced running and in contrast to the those findings at the end of prolonged cycling to exhaustion. Therefore, although CHO oxidation rates were not measured, it is unlikely that a severe reduction in the total CHO availability was associated with the onset of fatigue during intermittent high intensity shuttle running.

It is well documented that fatigue during maximal single and intermittent exercise bouts is associated with intracellular accumulation of metabolic end products of glycogen metabolism, including lactate, ionic disturbances over the cell membrane and decreased sarcoplasmatic reticulum function (Sahlin, 1986; Gaitanos et al., 1993). However, in all the studies in this thesis, blood lactate concentration at the end of exercise was no higher than 9 mmol.l⁻¹ and thus did not increase to levels which would reflect muscle acidosis prior to fatigue. It is unlikely, therefore, that lactate accumulation in the muscle was a contributory factor to the reduction in speed, i.e., fatigue, observed in these studies.

At the end of 90 min of exercise (Chapter 6, Part A), there was still sufficient muscle glycogen left in both type I and II fibres to continue exercising for possibly another 30 min if the rate of glycogen utilisation remained the same. This is due to the nature of the test, which requires recruitment of both type I and type II muscle fibres. However, the exercise intensity was increased after the fixed bout of intermittent exercise in Chapters 4, 5 and 7, placing additional demands on the type II fibres. As these fibres became glycogen depleted, tension development would decrease and type I fibre recruitment could not compensate sufficiently for this loss of muscle tension. Consequently, impaired type II muscle function would adversely affect high intensity exercise performance. When this critical point was reached, the exercise intensity could not be sustained and fatigue, or a reduction in running speed, occurred. Thus fatigue was
possibly due to glycogen reduction to a critical level in the type II fibres. This is in agreement with other studies who have found that energy production from glycogen is impaired when muscle glycogen concentration falls below 20-30 mmol.(kg WW)-1 [86-129 mmol. (kg DM)-1] (Costill, 1988).

The mechanism responsible for the glycogen sparing observed with CHO ingestion may possibly be due to differences in serum insulin response. The hyperinsulinaemia after 30 min of intermittent exercise was of a sufficient magnitude to reduce muscle glycogenolysis. Hyperglycaemia alone does not appear to be a sufficient stimulus to reduce muscle glycogenolysis during exercise (Coyle et al., 1991) and may account for the discrepancies between studies with regard to the effect of CHO supplementation on muscle glycogen utilisation. The increased glucose uptake by muscle in response to higher concentrations of circulating insulin thus spares the muscles glycogen stores; however, the exact mechanism by which the uptake of glucose by the active muscles alters the rate of glycogen utilisation is at present unknown.

In addition, it has been reported that blood glucose oxidation cannot support exercise at an intensity greater than 75% \(\text{VO}_2\max\), so it is unlikely that exercise could be sustained during Part B at the end of the PIHSRT without adequate muscle glycogen stores. Therefore, fatigue during this type of exercise was probably associated with the depletion of glycogen in the type II muscle fibres. The decreased mixed muscle PCr concentration observed in Chapter 6, Part A immediately post-exercise following placebo ingestion would support such an hypothesis. Moreover, oral Cr supplementation did not improve high intensity endurance performance at the end of the PIHSRT, as noted in Chapter 7.

However, other factors which may have contributed to fatigue during the PIHSRT cannot be dismissed. These include a change in muscle function (contractility)(Chapter 6, Part B), the reduction of ATP turnover rate as a consequence of adenine nucleotide
loss (Chapter 7), and central fatigue, possibly induced by ammonia toxicity, or an increase in the brain uptake of aromatic amino acids and brain serotonin concentration (Bannister and Cameron, 1990). The lower plasma ammonia concentrations observed during exercise after Cr supplementation in Chapter 7 may thus be indicative of a more efficient rephosphorylation of ADP (Greenhaff et al., 1993) or to a reduced catabolism of amino acids (predominantly BCAA) in muscle (Lemon and Nagle, 1981), or both. Thus, there are a variety of possible causes of fatigue during prolonged intermittent high intensity running and more work is required in order to understand the exact mechanisms in the fatiguing process during this type of exercise.

The failure to observe glycogen sparing with CHO ingestion in some previous studies may be due to the pre-exercise nutritional status of the subjects. In the studies in this thesis the subjects consumed their normal food intake for two to three days prior to exercising after an overnight fast. This would result in normal pre-exercise glycogen stores. The initial glycogen concentration in the exercising muscles has been observed to affect its subsequent use in some (Galbo et al., 1979; Sherman et al., 1981) but not all (Vandenberghe et al., 1995), depending on the intensity and duration of exercise. Therefore it is not surprising that in some of the studies which have not observed a glycogen sparing effect, the pre-exercise glycogen concentration was almost twice the values reported in Chapter 6 (Part A) of this thesis. The physiological response would be an increased glycogen utilisation which would increase the concentration of G-6-P resulting in the inhibition of hexokinase in the working muscle. In this metabolic environment, glucose phosphorylation to G-6-P would be the limiting step to glycolysis, and glycogenolysis would continue at the same rate and would not be influenced by CHO ingestion. Thus, there would be no additional benefit of CHO ingestion during exercise when pre-exercise muscle glycogen concentration is elevated above normal values.
8.6 Muscle Soreness, Damage and Oxygen Free Radical Production

After participating in the PIHSRT, the majority of subjects reported muscle soreness lasting for several days. In many instances this soreness is caused by structural damage to the tissue induced by mechanical stress; the type of contraction undergone by the muscle being the major influencing factor (Newham et al., 1986; Armstrong, 1991). However, it has been suggested that free radicals are involved, in some way, in this damaging process (Davies et al., 1982).

However, evidence for a fundamental role of free radicals in the muscle damage and the protective effect of antioxidants is inconclusive. Thus, in addition to the four studies reported in this thesis, an additional study was carried out in collaboration with the Muscle Research Centre, University of Liverpool to investigate the effects of a 90 min bout of intermittent high intensity shuttle running on metabolism during the 72 h recovery following exercise. The possibility that the reported muscle soreness may be due to muscle damage as a consequence of increased production of free radicals was examined. Detailed descriptions of the methods and results of this study appear elsewhere (Woodger, unpublished MPhil thesis). The study showed that there were no significant changes in the accepted markers of muscle damage and free radical activity (serum creatine kinase, TBars, red blood cell catalase, glutathione peroxidase, total and oxidised glutathiones). Serum creatine kinase (CK) activity peaked at 116.6 U.l⁻¹ after 24 h post-exercise. However, 3 individuals had peak values ranging between 189.8 and 306 U.l⁻¹. These CK concentrations are well below those observed 24 h following marathon running (Tsintzas et al., 1995b) and greater than those recorded following walking for 37 km at an average speed of 5.4 km.h⁻¹ on each of 4 consecutive days (Clarkson et al., 1988). In general, these CK values are not exceptionally high and appear out of proportion to the extent of the post-exercise soreness experienced by the subjects.
Thus there was no evidence to suggest that the production of free radicals increased during the PIHSRT. However, there was a decrease in Vitamin E concentration 48 h after the cessation of the PIHSRT, which in addition to the observation of an initial mobilisation in Vitamin C concentration in the immediate post-exercise period, followed by a progressive decrease 24 h, 48 h and 72 h after exercise may indicate that free radicals were produced as a consequence of participation in the PIHSRT and consumption of antioxidants had occurred. Whether these concentrations would be further reduced if the exercise protocol was repeated within 3 days, or whether Vitamin E and Vitamin C supplementation would attenuate the post-exercise reductions observed requires investigation.

Several studies have reported that antioxidant supplements benefit performance in humans (Jakeman and Maxwell, 1993; Krotkiewski et al., 1994; for review see Dekkers et al., 1996). Jakeman and Maxwell (1993) showed that Vitamin C supplementation may exert a protective effect against eccentric exercise-induced muscle damage. In their study, recovery of maximal voluntary contraction (MVC) in the first 24 h post-exercise was greater in the group supplemented with Vitamin C for 21 days prior to and for 7 days after performing 60 min of box-stepping exercise. Supplementation with Vitamin E had no effect on the recovery of MVC.

In the study by Krotkiewski et al. (1994), pre-treatment of subjects with a free radical-scavenging preparation (pollen extract) for 4 weeks was shown to increase post-exercise muscle glycogen concentration and decrease both post-exercise creatine kinase concentration and eliminated the subjective symptoms of muscle soreness. However, more research is necessary on the role of free radicals in muscle damage and the protective effect of antioxidants before any recommendations for supplementation programmes can be made.
8.7 Conclusions

Increasing the CHO content of the recovery diet to 10 g.kg\(^{-1}\)bm.day\(^{-1}\) enhances high intensity exercise at the end of a fixed bout of prolonged intermittent high intensity shuttle running possibly due to an increased glycogen availability at the end of exercise. It is also evident that the ingestion of a CHO-E beverage during exercise improves the capacity to perform high intensity exercise and enhances isokinetic muscle function at the end of a fixed bout of prolonged intermittent high intensity shuttle running by sparing the limited stores of glycogen in the exercising muscle. It is likely that fatigue during prolonged intermittent high intensity exercise is caused by glycogen depletion in the type II muscle fibres to a critically low level, resulting in the inability to maintain ATP resynthesis at the required rate. Thus an adequate CHO intake before, during and after prolonged intermittent high intensity shuttle running is essential in order to ensure optimal performance during this type of exercise. However, whether some other mechanism is influential in the fatiguing process in this metabolic environment cannot be ruled out.
REFERENCES


APPENDIX A

MEDICAL HISTORY QUESTIONNAIRE

NAME __________________ AGE _______ DATE OF BIRTH ______

OCCUPATION _______________ DOCTOR _______________ (TEL. _____)

If you are happy to answer the questions posed below, please proceed. Your answers will be treated in the strictest confidence.

1. Are you at present under medical treatment of any kind? YES/NO*
   If YES, please state the condition ________________________________

2. Are you suffering from or have suffered from or received medical treatment for any of the following conditions?
   a. Heart or circulation condition? YES/NO*
   b. Stomach or duodenal ulcers? YES/NO*
   c. Low back pain? YES/NO*
   d. Asthma or bronchial complaints? YES/NO*
   e. Diabetes Mellitus YES/NO*

   Have any of your immediate family or grandparents had any of the above? YES/NO*
   If YES, please state ________________________________

3. Do you smoke at all now? YES/NO*
   or, have you in the past been a regular smoker? YES/NO*

4. Do you wish your own doctor to be asked for an opinion as to your fitness to take part as a subject in this experiment, involving prolonged, intermittent, high intensity exercise until volitional fatigue? YES/NO*

ACTIVITY LEVEL EVALUATION

1. Do you engage in regular physical activity? YES/NO*
   If so, what type? ________________________________
   How many days per week? ______
   How much time per day (please tick one)
   Less than 15 minutes 15 to 30 minutes
   30 to 60 minutes More than 60 minutes

2. Do you play competitive sport? YES/NO*
   What sport? ____________________________ Current playing level? ______

3. Do you ever experience shortness of breath during exercise? YES/NO*

4. Do you ever experience chest discomfort during exercise? YES/NO*

5. If so, does it go away with rest? YES/NO*

6. How would you describe your state of well-being at this time?
   Very, very good = Poor =
   Very good = Very poor =
   Good = Very, very poor =
   Neither good nor poor =

*delete as necessary
## APPENDIX B

**PREDICTED VO₂ MAX VALUES AND RELATIVE EXERCISE INTENSITIES**

<table>
<thead>
<tr>
<th>Level (ml.kg⁻¹.min⁻¹)</th>
<th>VO₂ max (ms⁻¹)</th>
<th>Time for 20m (sec)</th>
<th>VO₂ (95%) (sec)</th>
<th>VO₂ (55%) (sec)</th>
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## APPENDIX C

### COMPOSITION OF 6.9% CHO SOLUTION
(Lucozade Sport; SmithKline Beecham)

<table>
<thead>
<tr>
<th>Component</th>
<th>Value</th>
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<tbody>
<tr>
<td>DEXTROSE (g)</td>
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<td>FRUCTOSE (g)</td>
<td>0.5</td>
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<td>MALTOSE (g)</td>
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<tr>
<td>HIGHER SACCHARIDES</td>
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<tr>
<td>SODIUM (mg.100⁻¹ ml)</td>
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<tr>
<td>POTASSIUM (mg.100⁻¹ ml)</td>
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<tr>
<td>OSMOLALITY (mOsm.kg⁻¹)</td>
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<tr>
<td>ENERGY VALUE (KJ.100⁻¹ ml)</td>
<td>110</td>
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</table>
APPENDIX D

MIXED MUSCLE METABOLITE ASSAYS

1/ Adenosine triphosphate and Phosphocreatine

**Principle:**

\[
\text{PCr} + \text{ADP} \xrightarrow{\text{CPK}} \text{Creatine} + \text{ATP}
\]

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

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

**Reagents:**

- **Buffer:** TRIS-HCl 50 mmol.l\(^{-1}\), pH 8.1 with 0.02% BSA
- **Cofactor:** NADP 5 mmol.l\(^{-1}\)
- **Enzymes:** CK 1260 U.ml\(^{-1}\); HK 28 U.ml\(^{-1}\); G6P-DH 14 U.ml\(^{-1}\)
- **Reagents:** ADP 10 mmol.l\(^{-1}\); Glucose 10 mmol.l\(^{-1}\); Dithiothreitol 50 mmol.l\(^{-1}\); MgCl\(_2\) 0.1 mol.l\(^{-1}\)
- **Standard:** ATP 2 mmol.l\(^{-1}\) (stock solution); PCr 2 mmol.l\(^{-1}\)
- **Additional reagents:** Carbonate buffer 20 mmol.l\(^{-1}\), pH 10.0; TRIS-HCl 20 mmol.l\(^{-1}\), BSA 0.02%, pH 8.1 (diluent for enzyme solutions)

Working standards were prepared daily from the stock solution as follows:

<table>
<thead>
<tr>
<th>stock solution (µl)</th>
<th>0</th>
<th>25</th>
<th>50</th>
<th>100</th>
<th>200</th>
</tr>
</thead>
<tbody>
<tr>
<td>water (µl)</td>
<td>2000</td>
<td>1975</td>
<td>1950</td>
<td>1900</td>
<td>1800</td>
</tr>
<tr>
<td>ATP concentration (µmol/l)</td>
<td>0</td>
<td>25</td>
<td>50</td>
<td>100</td>
<td>200</td>
</tr>
<tr>
<td>PCr concentration (µmol/l)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>200</td>
</tr>
</tbody>
</table>

Immediately prior to analysis sufficient reaction mixture for 3 sets of duplicate samples and quadruplicate double distilled water blanks, and a set of quadruplicate standards were prepared as follows:
Reaction Mixture - per ml of buffer (final concentrations):
NADP 10 μl (0.046 mmol.l⁻¹); ADP 10 μl (0.091 mmol.l⁻¹); Glucose 10 μl (0.091 mmol.l⁻¹); Dithiothreitol 10 μl (0.457 mmol.l⁻¹); MgCl₂ 50 μl (4.566 mmol.l⁻¹); G6P-DH 5 μl (0.064 mmol.l⁻¹)

Procedure:

1/ Twenty μl of extract was pipetted into a fluorimeter tube and diluted with 100 μl of double-distilled water. Following this, 10 μl of the diluted extract was transferred into 3 pair of tubes (3 sets of duplicate samples). Three sets of water blanks and 1 set of standards were also prepared using 10 μl volumes.
2/ Two hundred μl of reaction mixture was added to one pair of tubes and blanks (G-6-P determination; 1st step)
3/ Five μl of HK (0.127 U.ml⁻¹) was added per ml of remaining reaction mixture. Two hundred μl of this reaction mixture was then added to the second pair of tubes, blanks and standards (ATP + G-6-P determination; 2nd step).
4/ Ten μl of CK (11.351 U.ml⁻¹) was added per ml of remaining reaction mixture. 200 μl of this reaction mixture was then added to the third pair of tubes and blanks (ATP + G-6-P + PCr determination; 3rd step).
5/ A further 1 ml of this reaction mixture was added to 50 μl of 0.2 mmol.l⁻¹ PCr standard, and the reaction followed in the fluorimeter to confirm it was running, and to check the time course of incubation. After incubating for 30-40 min at room temperature, 1 ml of carbonate buffer was added to each tube, and after thorough mixing fluorescence was measured. The PCr reagent gives the sum of PCr, ATP and G-6-P, the ATP reagent the sum of ATP and G-6-P, and the G-6-P reagent G-6-P on its own. The concentrations of ATP and PCr were determined from the standard curve following the subtraction of G-6-P from (ATP+G-6-P), and (ATP+G-6-P) from (PCr+ATP+G-6-P), respectively. Values in mmol.Kg dry muscle⁻¹ were obtained after multiplying each value by 0.75 to account for the initial extraction dilution (factor 0.125) and the dilution of the extract (factor 6). Before any calculation was made, the corresponding blanks were subtracted from each sample and standard in every step.
2. Creatine

Principle:

Creatine + ATP $\xrightarrow{CK} PCr + ADP$

ADP + PEP $\xrightarrow{PK} ATP + Pyruvate$

Pyruvate + NADH + H⁺ $\xrightarrow{LDH} Lactate + NAD⁺$

Reagents:

Buffer: Low fluorescence imidazole, 50 mmol.l⁻¹, pH 7.5
Cofactor: NADH 1 mmol.l⁻¹
Enzymes: CK 1260 V.ml⁻¹; PK 75 U.ml⁻¹; LDH 240 U.ml⁻¹
Reagents: ATP 10 mmol.l⁻¹; PEP 2 mmol.l⁻¹; MgCl₂ 0.1 mol.l⁻¹; KCl 3 mol.l⁻¹; EDTA 0.1 mol.l⁻¹
Standard: Creatine 2 mmol.l⁻¹ (stock solution)
Additional reagents: Carbonate buffer 20 mmol.l⁻¹, pH 10.0; TRIS-HCl 20 mmol.l⁻¹, pH 8.1 with 0.02% BSA (diluent for enzyme solutions)

Working standards were prepared daily from the stock solution as follows:

<table>
<thead>
<tr>
<th>stock solution (µl)</th>
<th>0</th>
<th>50</th>
<th>100</th>
<th>150</th>
</tr>
</thead>
<tbody>
<tr>
<td>water (µl)</td>
<td>2000</td>
<td>1950</td>
<td>1900</td>
<td>1850</td>
</tr>
<tr>
<td>creatine concentration (µmol/l)</td>
<td>0</td>
<td>50</td>
<td>100</td>
<td>150</td>
</tr>
</tbody>
</table>

Immediately prior to the analysis sufficient reaction mixture for 2 sets of duplicate samples and quadruplicate double distilled water blanks, and a quadruplicate set of standards were prepared as follows:

Reaction mixture- per ml of buffer (final concentrations):

MgCl₂ 50 µl (4.413 mmol.l⁻¹); KCl 10 µl (26.478 mmol.l⁻¹); ATP 20 µl (0.177 mmol.l⁻¹); PEP 25 µl (44 µmol/l); NADH 15 µl (13 µmol/l); EDTA 1 µl (88 µmol.l⁻¹); LDH 2 µl (0.424 U.ml⁻¹); PK 10 µl (0.662 U.ml⁻¹)
Procedure:

1/ Twenty µl of extract was pipetted into a fluorimeter tube and diluted with 100 µl of double distilled water. Following this, 10 µl of the diluted extract was transferred into 2 pair of tubes (2 sets of duplicate samples). Two sets of water blanks and 1 set of standards were also prepared using 10 µl volumes.

2/ Two hundred µl of reaction mixture was added to one pair of tubes and blanks (ADP + Pyruvate determination; 1st step)

3/ Ten µl of CK (11.024 U.ml⁻¹) was added per ml of remaining reaction mixture. Two hundred µl of this reaction mixture was then added to the second pair of tubes, blanks and standards (Creatine + ADP + Pyruvate determination; 2nd step).

4/ A further 1 ml of the creatine reagent was added to 50 µl of the 150 µmol/l creatine standard and the reaction in this tube followed on the chart recorder. After incubating for 60-65 min at room temperature, 1 ml of carbonate buffer was added to each tube. Following thorough mixing fluorescence was measured. The Creatine reagent gives the sum of Cr, ADP and pyruvate and the ADP reagent the sum of ADP and pyruvate. Concentrations of Creatine were determined from the standard curve following subtraction of (ADP+Pyruvate) from (Cr+ADP+Pyruvate). Values in mmol.Kg dry muscle⁻¹ were obtained after multiplying each value by 0.75 to account for the initial extraction dilution (factor 0.125) and the dilution of the extract (factor 6). Before any calculation was made, the corresponding blanks were subtracted from each sample and standard in every step.
3) Lactate

**Principle:**

Lactate + NAD\(^+\) \overset{LDH}{\longrightarrow} \text{Pyruvate} + \text{NADH} + \text{H}^+

**Reagents:**

Buffer: Hydrazine 1.1 mol.l\(^{-1}\), pH 9.0 with 1 mmol.l\(^{-1}\) EDTA
Cofactor: NAD 50 mmol.l\(^{-1}\)
Enzymes: LDH 5500 U.ml\(^{-1}\) (undiluted)
Standard: Lactate 2 mmol.l\(^{-1}\) (stock solution)
Additional reagent: Carbonate buffer 20 mmol.l\(^{-1}\), pH 10.0

Working standards were prepared daily from the stock solution as follows:

<table>
<thead>
<tr>
<th>Stock solution (μl)</th>
<th>0</th>
<th>50</th>
<th>100</th>
<th>150</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water (μl)</td>
<td>200</td>
<td>1950</td>
<td>1900</td>
<td>1850</td>
</tr>
<tr>
<td>Lactate concentration (μmol/l)</td>
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<td>50</td>
<td>100</td>
<td>150</td>
</tr>
</tbody>
</table>

Immediately prior to the analysis sufficient reaction mixture for 1 set of duplicate samples, and quadruplicate double distilled water blanks, a set of quadruplicate standards, plus 1 ml to follow the reaction on the chart recorder were prepared as follows:

**Reaction mixture- per ml of buffer (final concentration):**

NAD 10 μl (0.491 mmol.l\(^{-1}\)); LDH 8 μl (43.22 U.ml\(^{-1}\))

**Procedure:**

1/ Ten μl of undiluted extract was added to 1 set of duplicate samples. A set of double distilled water blanks and 1 set of standards were also prepared using 10 μl volumes.

2/ Two hundred μl of reaction mixture was then added to each tube.
3/ A further 1 ml of reaction mixture was added to 50 µl of 0.15 mmol.l⁻¹ lactate standard and the reaction in this tube followed on the chart recorder.

4/ After 30 min incubation, 1 ml of carbonate buffer was added, the contents were mixed and fluorescence was read. Lactate concentration was calculated from the standard curve after subtracting blanks from samples and standards. Values in mmol.Kg dry muscle⁻¹ were obtained by multiplying each value by 0.125 to account for the initial extraction dilution.
4) Glycogen

The assay for glycogen was carried out on both the acid precipitated muscle pellet (acid insoluble glycogen) and the neutralised extract (acid soluble glycogen) by analysis of glucosyl units obtained after acid hydrolysis of glycogen by the method of Jansson (1981). The muscle glycogen values reported were the sum of soluble and insoluble glycogen, minus the free glucose and G-6-P present in the neutralised extract.

Acid Hydrolysis:

Reagents:

HCl 1 mol.l⁻¹; NaOH 6 mol.l⁻¹

Procedure:

For each muscle sample, one hundred µl of 1 mol.l⁻¹ HCl per 1 mg of muscle powder was added to the precipitated muscle pellet. One hundred µl of 1 mol.l⁻¹ HCl was also added to 20 µl of undiluted neutralised extract. Both samples were gently mixed and incubated for 2 hours in tightly sealed screw top micro-reaction (Eppendorf) tubes in a boiling water bath. The tubes were then centrifuged for 1 min and left at room temperature to cool down. The acid hydrolysed extract only was neutralised with 15 µl of 6 mol.l⁻¹ NaOH.

Acid insoluble glycogen was assayed spectrophotometrically using a Glucose Test Combination, (GOD/Perid method). Acid soluble glycogen was assayed fluorimetrically for glucose.

Acid insoluble glycogen

Principle:

\[
glucose + O_2 + H_2O \xrightarrow{GOD} \text{Gluconate} + H_2O_2 \\
H_2O_2 + ABTS \xrightarrow{POD} \text{coloured complex} + H_2O
\]

where:

GOD = glucose oxidase
POD = horseradish peroxidase
ABTS = di-ammonium 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonate)

The intensity of the colour change is directly proportional to the concentration of glucose. Therefore, the glucose concentration of a sample can be calculated by using a standard of known concentration.

**Reagents:**

Buffer/enzymes/chromogen: made up as directed in kit (phosphate buffer 0.1 mol.l\(^{-1}\), pH 7.0; POD 0.8 U.ml\(^{-1}\); GOD 10 U.ml\(^{-1}\); ABTS 1.0 mg.ml\(^{-1}\)). This was found to remain active for several months when stored protected from light at 0-4°C.

Standard: Glucose 0.505 mmol.l\(^{-1}\)

**Procedure:**

Five blanks, five standards and duplicate samples were prepared by adding 10 µl of double-distilled water, standard and the unneutralised hydrolysed extract of the precipitated muscle pellet, respectively, to 2 ml of the GOD/Perid reagent. The samples were incubated at room temperature for 30 mins and their absorbance (A) of samples and standards was then determined against the distilled water blanks on a spectrophotometer at 436 nm.

Acid insoluble glycogen concentration (glucosyl units.Kg dry muscle\(^{-1}\)) was calculated using the following equation:

\[
[Glycogen] = \frac{(A\ \text{sample} \div A\ \text{standard}) \times 0.505 \times 100}{100}
\]
Acid soluble glycogen

**Principle:**

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

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

**Reagents:**

**Buffer:** TRIS-HCl 0.1 mol.l\(^{-1}\), pH 8.1 with 0.02% BSA

**Cofactor:** NADP 5 mmol.l\(^{-1}\)

**Enzymes:** HK 28 U.ml\(^{-1}\); G6P-DH 7 U.ml\(^{-1}\)

**Reagents:** ATP 200 mmol.l\(^{-1}\); MgCl\(_2\) 0.1 mol.l\(^{-1}\); EDTA 0.1 mol.l\(^{-1}\); Dithiothreitol 50 mmol.l\(^{-1}\);

**Standard:** Glucose 0.505 mmol.l\(^{-1}\) (stock solution)

**Additional reagents:** Carbonate buffer 20 mmol.l\(^{-1}\), pH 10.0; TRIS-HCl 20 mmol.l\(^{-1}\), pH 8.1 with 0.02% BSA (diluent for enzyme solutions).

Working standards were prepared daily from the stock solution as follows:

<table>
<thead>
<tr>
<th>stock solution (µl)</th>
<th>0</th>
<th>20</th>
<th>50</th>
<th>100</th>
</tr>
</thead>
<tbody>
<tr>
<td>water (µl)</td>
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<td>485</td>
<td>455</td>
<td>405</td>
</tr>
<tr>
<td>glucose concentration (µmol/l)</td>
<td>0</td>
<td>20</td>
<td>50</td>
<td>100</td>
</tr>
</tbody>
</table>

Immediately prior to the analysis, sufficient reaction mixture for two sets of duplicate samples and quadruplicate double distilled water blanks, and a set of quadruplicate standards were prepared as follows:

**Reaction mixture- per ml of buffer (final concentrations):**

- NADP 6 µl (29 µmol.l\(^{-1}\)); ATP 1.5 µl (0.29 mmol.l\(^{-1}\)); MgCl\(_2\) 10 µl (0.966 mmol.l\(^{-1}\));
- EDTA 5 µl (0.483 mmol.l\(^{-1}\)); Dithiothreitol 10 µl (0.483 mmol.l\(^{-1}\)); G6P-DH 3 µl (0.02 U.ml\(^{-1}\))
Procedure:

1/ Two sets of 20 μl of water blanks and samples (neutralised hydrolysed extract) and 1 set of standards were pipetted into fluorometric tubes.

2/ Two hundred μl of reaction mixture was added to one set of samples and blanks (G-6-P determination; 1st step)

3/ Five μl of HK (0.135 U.ml⁻¹) was added per ml of remaining reaction mixture and 200 μl of this reaction mixture was then added to the second pair of samples, blanks and standards (Glucose + G-6-P determination; 2nd step).

4/ A further 1 ml of the Glucose reagent was added to 50 μl of the 100 μmol.l⁻¹ glucose standard and the reaction in this tube followed on the chart recorder. Following a 30 min incubation at room temperature, 1 ml of carbonate buffer was added to each tube, and after thorough mixing fluorescence was measured. Acid soluble glycogen concentration (as glucosyl units) was calculated from the standard curve after subtracting G-6-P values from values obtained in step 3 above. The resulting value was multiplied by (0.125*6.75) to correct for the dilution involved in the extraction and acid hydrolysis. Before any calculation was made the corresponding blanks were subtracted from each sample and standard in every step. Muscle free glucose was subtracted from the result to give the true acid-soluble glycogen concentration. This assay also enabled the concentration of G-6-P to be determined by using appropriate standards.
Free glucose assay

Principle:

Glucose + ATP $\xrightarrow{\text{HK}}$ G6P + ADP

G6P + NADP$^+$ $\xrightarrow{\text{G6P-DH}}$ 6-P-Gluconolactone + NADPH + H$^+$

Reagents:

Buffer: TRIS-HCl 0.1 mol.l$^{-1}$, pH 8.1 with 0.02% BSA
Cofactor: NADP 5 mmol.l$^{-1}$
Enzymes: HK 28 U.ml$^{-1}$; G6P-DH 7 U.ml$^{-1}$
Reagents: ATP 200 mmol.l$^{-1}$; MgCl$_2$ 0.1 mol.l$^{-1}$; EDTA 0.1 mol.l$^{-1}$; Dithiothreitol 50 mmol.l$^{-1}$
Standard: Glucose 0.505 mmol.l$^{-1}$ (stock solution)
Additional reagents: Carbonate buffer 20 mmol.l$^{-1}$, pH 10.0; TRIS-HCl 20 mmol.l$^{-1}$, pH 8.1 with 0.02% BSA (diluent for enzyme solutions)

Working standards were prepared daily from the stock solution as follows:

<table>
<thead>
<tr>
<th>Stock solution (μl)</th>
<th>0</th>
<th>20</th>
<th>50</th>
<th>100</th>
</tr>
</thead>
<tbody>
<tr>
<td>Double distilled water (μl)</td>
<td>505</td>
<td>485</td>
<td>455</td>
<td>405</td>
</tr>
<tr>
<td>Glucose concentration (μmol.l$^{-1}$)</td>
<td>0</td>
<td>20</td>
<td>50</td>
<td>100</td>
</tr>
</tbody>
</table>

Immediately prior to the analysis, sufficient reaction mixture for two sets of duplicate samples and quadruplicate double distilled water blanks, and a set of quadruplicate standards were prepared as follows:

Reaction mixture- per ml of buffer (final concentrations):

NADP 6 μl (29 umol.l$^{-1}$); ATP 1.5 μl (0.29 mmol.l$^{-1}$); MgCl$_2$ 10 μl (0.966 mmol.l$^{-1}$); EDTA 5 μl (0.483 mmol.l$^{-1}$); Dithiothreitol 10 μl (0.483 mmol.l$^{-1}$); G6P-DH 3 μl (0.02 U.ml$^{-1}$)
**Procedure:**

1/ Twenty μl of water blanks, samples (neutralised undiluted extract) and standards were pipetted into fluorometric tubes.

2/ Two hundred μl of reaction mixture was added to one set of samples and blanks (G-6-P determination; 1st step).

3/ Five μl of HK (0.135 U.ml⁻¹) were added per ml of remaining reaction mixture and 200 μl of this reaction mixture were then added to the second set of samples, blanks and glucose standards (Glucose + G-6-P determination; 2nd step). A further 1 ml of the reaction mixture was added to 50 μl of the 100 μmol.l⁻¹ glucose standard and the reaction in this tube followed on the chart recorder. Following a 30 min incubation at room temperature, 1 ml of carbonate buffer was added to each tube, and after thorough mixing fluorescence was measured. Glucose concentration was calculated from the standard curve. G-6-P was subtracted from the result to give the true glucose concentration. Values in mmol.Kg dry muscle⁻¹ were obtained after multiplying each value by 0.125 to account for the initial extraction dilution. Before any calculation was made the corresponding blanks were subtracted from each sample and standard in every step.
APPENDIX E

SPECTROPHOTOMETRIC ASSAYS FOR VALIDATION OF STANDARDS

All assays were carried out in a 1 ml semi-micro cuvette at 340 nm, reading against air, and were followed to completion on a chart recorder. The concentration of the standard was calculated using the following equation:

\[
[C] = \frac{V_c \times \Delta A}{6.22} \times V_s
\]  

(1)

where:

- \([C]\) = the concentration of the standard (mmol.l\(^{-1}\))
- \(V_c\) = the final cuvette volume (ml)
- \(V_s\) = the volume of standard added (ml)
- \(\Delta A\) = change in absorbance = \(A_f - A_i - A_E\)
  - \(A_f\) = final absorbance, \(A_i\) = the initial absorbance,
  - \(A_E\) = the absorbance of the enzyme
- 6.22 = the extinction coefficient for NADH/NADPH at 340 nm
1/ Adenosine triphosphate

**Principle:**

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

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

**Reagents:**

Buffer: TRIS-HCl 50 mmol.l\(^{-1}\), BSA 0.02%, pH 8.1
Cofactor: NADP 50 mmol.l\(^{-1}\)
Enzymes: HK 28 U.ml\(^{-1}\); G6P-DH 7 U.ml\(^{-1}\)
Other reagents: Glucose 0.1 mol.l\(^{-1}\); MgCl\(_2\) 0.1 mol.l\(^{-1}\); DTT 50 mmol.l\(^{-1}\); Tris-HCl 20 mmol.l\(^{-1}\), pH 8.1 with 0.02% BSA
Standard: ATP 2 mmol.l\(^{-1}\)

**Reaction mixture- per ml of buffer (final concentrations):**

NADP 10 µl (0.459 mmol.l\(^{-1}\)); MgCl\(_2\) 50 µl (5 mmol/l); Glucose 10 µl (0.917 mmol.l\(^{-1}\)); G6P-DH 10 µl (0.064 U.ml.l\(^{-1}\)); DTT 10 µl (0.459 mmol.l\(^{-1}\))

**Procedure:**

One ml of reaction mixture was added to 20 µl of 2 mmol.l\(^{-1}\) ATP standard. A\(_1\) was then read. Ten µl of HK (0.272 U.ml.l\(^{-1}\)) was then added and the reaction followed to completion (5 min). A\(_2\) was then read. A further 10 µl of HK was then added and absorbance read again (AF) to determine the absorbance of the enzyme (AE = |A\(_2\) - AF|). The concentration of standard was calculated using the formula (1).
2/ Creatine

Principle:

Creatine + ATP $\mathit{\text{CK}}$ $\rightarrow$ PCr + ADP

ADP + PEP $\mathit{\text{PK}}$ $\rightarrow$ ATP + Pyruvate

Pyruvate + NADH + H$^+$ $\mathit{\text{LDH}}$ $\rightarrow$ Lactate + NAD$^+$

Reagents:

Buffer: Low fluorescence imidazole, 50 mmol.l$^{-1}$, pH 7.5
Cofactor: NADH 1 mmol.l$^{-1}$
Enzymes: CK 1260 U.ml$^{-1}$; PK 75 U.ml$^{-1}$; LDH 50 U.ml$^{-1}$
Reagents: ATP 10 mmol.l$^{-1}$; PEP 30 mmol.l$^{-1}$; MgCl$_2$ 0.1 mol.l$^{-1}$; KCl 3 mol.l$^{-1}$; Tris-HCl 20 mmol.l$^{-1}$, pH 8.1 with 0.02% BSA
Standard: Creatine 2 mmol.l$^{-1}$

Reaction mixture- per ml of buffer (final concentrations):

MgCl$_2$ 50 $\mu$l (4.523 mmol.l$^{-1}$); KCl 10 $\mu$l (27.137 mmol.l$^{-1}$); NADH 75 $\mu$l (0.068 mmol.l$^{-1}$); ATP 100 $\mu$l (0.905 mmol.l$^{-1}$); PEP 6.5 $\mu$l (0.176 mmol.l$^{-1}$); PK 10 $\mu$l (0.678 U.ml$^{-1}$); LDH 4 $\mu$l (0.181 U.ml$^{-1}$)

Procedure:

One ml of reaction mixture was added to 20 $\mu$l of standard. A1 was then read. Ten $\mu$l of CK (12.233 U.ml$^{-1}$) was then added and the reaction followed to completion (60 min). A2 was then read. A further 10 $\mu$l of CK were then added and the absorbance was read again (AF). The concentration of standard was calculated using the formula (1).
3/ Glucose-6-phosphate

Principle:

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

Reagents:

Buffer: TRIS-HCl 0.1 mol.l\(^{-1}\), BSA 0.02%, pH 8.1
Cofactor: NADP 5 mmol.l\(^{-1}\)
Enzymes: G6P-DH 7 U.ml\(^{-1}\)
Reagent: EDTA 0.1 mol.l\(^{-1}\)
Standard: G-6-P 2 mmol.l\(^{-1}\)

Reaction mixture - per ml of buffer (final concentrations):

- NADP 10 µl (0.05 mmol.l\(^{-1}\))
- EDTA 5 µl (0.5 mmol.l\(^{-1}\))

Procedure:

One ml of reaction mixture was added to 20 µl of standard. A1 was then read. 10 µl of G6P-DH (0.07 U/ml) was then added and the reaction followed to completion (3-5 min). A2 was then read. A further 10 µl of G6P-DH (0.07 U/ml) were then added and the absorbance was read again (AF). The concentration of standard was calculated using the formula (1).
4) Lactate

**Principle:**

\[
\text{Lactate} + \text{NAD}^+ \xrightarrow{\text{LDH}} \text{Pyruvate} + \text{NADH} + H^+
\]

**Reagents:**

- **Buffer:** Hydrazine $1.1 \text{ mmol.l}^{-1}$, pH 9.0 with $1 \text{ mmol.l}^{-1}$ EDTA
- **Cofactor:** NAD $50 \text{ mmol.l}^{-1}$
- **Enzymes:** LDH $5500 \text{ U.ml}^{-1}$ (undiluted)
- **Standard:** Lactate $2 \text{ mmol.l}^{-1}$

**Reaction mixture- per ml of buffer (final concentrations):**

- NAD $40 \mu l (1.923 \text{ mmol.l}^{-1})$

**Procedure:**

One ml of reaction mixture was added to 20 $\mu l$ of standard. A1 was then read. Five $\mu l$ of LDH ($27.363 \text{ U.ml}^{-1}$) was then added and the reaction followed to completion (15-20 min). A2 was then read. A further 5 $\mu l$ of LDH were then added and absorbance was read again (AF?). The concentration of standard was calculated using the formula (1).
5/ Phosphocreatine

**Principle:**

\[ \text{PCr + ADP } \overset{\text{CK}}{\longrightarrow} \text{Creatine + ATP} \]

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

\[ \text{G6P + NADP } \overset{\text{G6P-DH}}{\longrightarrow} \text{6-P-Gluconolactone + NADPH + H}^+ \]

**Reagents:**

Buffer: TRIS-HCl 50 mmol.l\(^{-1}\), BSA 0.02%, pH 8.1
Cofactor: NADP 50 mmol.l\(^{-1}\)
Enzymes: CK 1290 U.ml\(^{-1}\); HK 28 U.ml\(^{-1}\); G6P-DH 7 U.ml\(^{-1}\)
Reagents: ADP 50 mmol.l\(^{-1}\); Glucose 100 mmol.l\(^{-1}\); DTT 50 mmol.l\(^{-1}\); MgCl\(_2\) 0.1 mol.l\(^{-1}\); Tris-HCl 20 mmol.l\(^{-1}\), pH 8.1 with 0.02% BSA
Standard: PCr 2 mmol.l\(^{-1}\)

**Reaction mixture - per ml of buffer (final concentrations):**

NADP 10 µl (0.450 mmol.l\(^{-1}\)); ADP 10 µl (0.450 mmol.l\(^{-1}\)); DTT 10 µl (0.450 mmol.l\(^{-1}\)); MgCl\(_2\) 50 µl (5 mmol.l\(^{-1}\)); Glucose 10 µl (0.901 mmol.l\(^{-1}\)); G6P-DH 10 µl (0.063 U.ml\(^{-1}\)); HK 10 µl (0.252 U.ml\(^{-1}\))

**Procedure:**

One ml of reaction mixture was added to 20 µl of 2 mmol.l\(^{-1}\) ATP standard. A1 was then read. Ten µl of CK was then added and the reaction followed to completion (10 min). A2 was then read. A further 10 µl of CK was then added and absorbance was read again (AF) to determine the absorbance of the enzyme. The concentration of standard was calculated using the formula (1).
APPENDIX F

SINGLE MUSCLE FIBRE ANALYSIS

Separation and identification of individual fibres: One part of the freeze-dried muscle kept at -70 °C was used to determine the glycogen content of single muscle fibres. This part was also washed twice with petroleum ether to remove the fat, and then left in the fume cupboard to dry.

Fragments of single muscle fibres were dissected under low power microscopy (magnification x 10). Three pieces from the end of each individual fibre fragment were then cut off, mounted on three different glass slides and stored at -70 °C. The remaining part of the individual fibre was then placed in a methanol-washed plastic tube (eppendorf 1.2 ml vol.) and stored at -70 °C.

At a later date, the mounted ends of the fibre fragments were stained in duplicate, and where necessary in triplicate, for myofibrillar ATPase to identify type I (slow-twitch) and type II (fast-twitch) fibres using a modification of the method of Brooke and Kaiser (1970). Following the identification of each dissected fibre, 5-10 fibres of each type were pooled for each sample and weighed on a Perkin-Elmer balance, the total weight ranging from 15 to 30 μg. The reproducibility of the weight of the pooled fibres was 1%. The pooled fibres were placed at the bottom of methanol-washed plastic tubes and stored at -70 °C until analysis.

Extraction procedure: On the day of the analysis, the tubes with the pooled fibres were removed from the freezer, left to thaw and centrifuged to ensure all fibres were gathered at the bottom of the tube. Glycogen was then extracted by adding 20 μl KOH (1 mol.l⁻¹) to each tube. Following this, samples were gently agitated on a vortex mixer and then warmed in a water bath at 50 °C for 15 min. Samples were then removed from the water bath, and 60 μl of double-distilled de-ionised water was added before being mixed. This procedure resulted in the complete digestion of the fibres. The volume of each muscle extract was 80 μl.

Glycogen degradation: Following the extraction procedure, the extract of each muscle sample was neutralized by adding 80 μl of HCl/Acetate buffer (0.25 mol.l⁻¹ HCl and 0.15 mol.l⁻¹ Acetate) to two tubes, each containing 40 μl. For every series of samples being degraded, one control sample (containing 40 μl of 0.25 mol.l⁻¹ KOH and 80 μl of HCl/Acetate buffer) was also used. All samples were then mixed, and 4 μl of the enzyme
Amyloglucosidase (6 units mg⁻¹) was added to the control sample (Zc) and to one tube of each pair of muscle extracts (Za), before being mixed once again. The other tube with the neutralised extract was analysed without prior glycogen degradation (Zb). Samples were left to stand for 60 min at room temperature, centrifuged for 2 min and the supernatant was then removed, placed into clean plastic tubes and put on ice.

**Glycogen assay:** The muscle extract was assayed fluorometrically for glucose using a modification of the method by Harris et al. (1974). Detailed procedures of this assay are presented later in Appendix F. The coefficient of variation of the assay was 4.5%. Glucose concentration in each cuvette was calculated using a NADH standard curve, since the emission light from the fluorimeter lamp was not stable. The slope of the NADH curve enabled the values determined by the fluorimeter to be related to the values accurately determined spectrophotometrically (in this Appendix). The glycogen concentration of each sample was calculated using the glucose concentration in cuvette, and taking into account double-distilled water blanks, control samples (Zc), dilution and extraction factors and the total weight of the pooled fibres (in this Appendix).

Single fibre analysis was performed on the biopsy samples of three subjects from both trials and additional biopsy samples from two subjects were also analysed from the CON trial. As with the mixed muscle metabolites, single muscle fibre glycogen concentration was expressed as mmol (kg DM)⁻¹.
APPENDIX F

SINGLE MUSCLE FIBRE GLYCOGEN ASSAY

Principle:

ATP + Glucose \[\text{HK} \rightarrow \text{G-6-P} + \text{ADP}\]

NADP + G-6-P \[\text{G6P-DH} \rightarrow 6\text{-P-Gluconolactone} + \text{NADPH} + \text{H}^+\]

Reagents:

Buffer: Tethanolamine-Hydrochloride (TEA-HCl), 400 mmol/l, pH 7.8
Cofactors: NADP 10 mmol/l; MgCl\textsubscript{2} 100 mmol/l; EDTA 20 mmol/l; DTT 50 mmol/l; ATP 32 mmol/l; BSA in NaHCO\textsubscript{3} (1% solution)
Enzymes: Hexokinase 11.2 U/ml; G6P-DH 350 U/ml
Standard: Glucose 20 mmol/l (stock solution)

The working standard (100 μmol/l) was prepared daily from the stock solution as follows: 50 μl of stock solution into 10 ml of double-distilled water.

Immediately prior to the analysis sufficient reaction mixture for blanks, standards, control and samples was prepared as follows:

Reaction mixture:

Buffer 1 ml; MgCl\textsubscript{2} 160 μl; EDTA 100 μl; DTT 40 μl; NADP 200 μl; ATP 144 μl; BSA, NaHCO\textsubscript{3} 160 μl; H\textsubscript{2}O 250 μl; G6P-DH 0.32 μl
Procedure:

i/ Reaction mixture (0.6 ml) was added to cuvettes containing 50 µl of
double-distilled water (blank), standard, control and samples.

ii/ The cuvettes were mixed and incubated for 15 min.

iii/ Fluorescence was then read.

iv/ Following this, 10 µl of hexokinase was added to each cuvette before they
were mixed and incubated for 15 min.

v/ Fluorescence was then read.

vi/ Where enough sample was left the above procedure was repeated
(duplicate analysis).
Determination of NADH standard curve

i/ A 5 mmol/l NADH stock solution was prepared

ii/ Six NADH standards were then prepared from the stock solution as follows:

<table>
<thead>
<tr>
<th>Stock solution (μl)</th>
<th>Water (ml)</th>
<th>[NADH] μmol/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>2.5</td>
<td>0</td>
</tr>
<tr>
<td>10</td>
<td>2.5</td>
<td>20</td>
</tr>
<tr>
<td>25</td>
<td>2.5</td>
<td>50</td>
</tr>
<tr>
<td>50</td>
<td>2.5</td>
<td>100</td>
</tr>
<tr>
<td>75</td>
<td>2.5</td>
<td>150</td>
</tr>
<tr>
<td>100</td>
<td>2.5</td>
<td>200</td>
</tr>
</tbody>
</table>

iii/ The absorbance of the NADH standards was then measured against the absorbance of water (blank) on a spectrophotometer at 340 nm.

iv/ The exact concentration of the NADH standards (mmol/l) was then calculated using the formula:

\[
\text{Absorbance of standard} - \frac{\text{Absorbance of blank}}{6.22}
\]

where: 6.22 is the coefficient of extinction of NADH at 340 nm.

v/ Following this, 50 μl of each NADH standard was pipetted into fluorometric cuvettes. Double-distilled water (0.6 ml) was then added to each cuvette. The cuvettes were mixed and their fluorescence was read.

vi/ The fluorescence of the standards was then plotted against the product of the concentrations of the NADH standards and the dilution factor (i.e. 50/650). The resulting regression equation enabled the calculation of the slope K, which related the values determined by the fluorimeter to the values accurately determined by the spectrophotometer.
Determination of glucose concentration in each cuvette

The concentration of glucose in each cuvette (Z) was calculated using the formula:

\[
Z = \frac{(V_2 \cdot Y_2 - V_1 \cdot Y_1) - (V_2 \cdot X_2 - V_1 \cdot X_1)}{V_2 \cdot K}
\]

where:
- \(V_1\) = volume in cuvette without hexokinase added
- \(V_2\) = volume in cuvette with hexokinase added
- \(X_1\) = Fluorescence of blank before hexokinase added
- \(X_2\) = Fluorescence of blank after hexokinase added
- \(Y_1\) = Fluorescence of standard, control or sample before hexokinase added
- \(Y_2\) = Fluorescence of standard, control or sample after hexokinase added
Determination of glycogen concentration (G) from glucose concentration in each cuvette

The glycogen concentration (G) of each sample was calculated using the formula:

\[ G = \frac{(Z_a - Z_b - Z_c) \times EF \times V}{W} \times D \]

where:

- \( Z_a \) = concentration of glucose in cuvette containing sample with the enzyme amyloglucosidase
- \( Z_b \) = concentration of glucose in cuvette containing the sample without the enzyme amyloglucosidase
- \( Z_c \) = concentration of glucose in cuvette with control sample
- \( EF \) = Dilution factor in extraction and degradation
- \( V \) = Volume of KOH in which pooled fibres were extracted
- \( W \) = weight of pooled fibres (μg)
- \( D \) = Dilution factor in glucose assay (13.2)