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**THE INFLUENCE OF FLUID INGESTION ON METABOLISM AND
SOCCER SKILLS FOLLOWING INTERMITTENT HIGH INTENSITY
SHUTTLE RUNNING**

by

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A Doctoral Thesis

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ABSTRACT

The impact of fatigue on the intermittent high intensity exercise undertaken during participation in team sports has not been extensively studied. Team sports are characterised not only by intermittent exercise, but also by the contribution of a wide range of skills. This thesis describes a series of studies conducted in a controlled environment to assess the influence of fluid ingestion and fatigue on selected soccer skills.

The aim of the first study was to examine the effect of 90-min of high intensity shuttle running with and without water ingestion on a soccer-dribbling test. The subjects were allocated to two randomly assigned trials either ingesting or abstaining from fluid intake during a 90 min intermittent exercise protocol (Loughborough Intermittent Shuttle Test: LIST). In the absence of water ingestion soccer skill deteriorated ($p < 0.05$) by 5% but was maintained when fluid was ingested.

The principal aim of the second study was to understand further the mechanisms contributing to the deterioration observed during the LIST. Subjects completed the LIST ingesting a 6.4% carbohydrate electrolyte solution (CHO), placebo (CON) or no fluid (NON). Free fatty acids, cortisol and aldosterone responses were lower ($p < 0.01$) at the end of exercise during both CHO and CON in comparison to NON. There was no difference in respiratory exchange ratio between trials. Fluid ingestion did not appear to cause a shift in substrate metabolism even though there were differences in plasma FFA concentrations.

The consumption of carbohydrate during exercise has been shown to increase physical performance, capacity and cognitive function. The aim of the third study was to assess the influence of a 6.4 % carbohydrate-electrolyte (CHO) placebo (CON) or no fluid (NON) on passing and dribbling soccer skills following the LIST. During the NON trial performance of the dribbling test followed a similar pattern to that in the first study and performance of the passing test decreased ($p < 0.05$). This reduction in performance was prevented during the CHO and CON trials.

The purpose of the final study was to identify whether a rehydration strategy following the LIST would result in a recovery of skill performance. Subjects were allocated to two randomly assigned trials either ingesting a volume of fluid equivalent to 150% (L) or 9% (S) of body mass loss during the LIST, over a 2 h recovery period. During the recovery period serum sodium and osmolality returned to resting concentrations in the L trial but remained elevated in the S trial ($p < 0.05$). Despite body mass returning to resting values following the rehydration period, performance of the skills tests remained impaired.

Deterioration in skill test performance may have been related to a reduction in neuromuscular control either by a reduction in muscle glycogen or by an increase in muscle damage during the no fluid trials. The mechanism responsible for the deterioration in skill performance remains to be elucidated.

Key words:

Intermittent exercise, fatigue, fluid ingestion, carbohydrate, soccer, skill

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 is in press and has been presented at the following conferences:

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DEDICATION

Words such as loving, caring, understanding and supportive are often used by sons and daughters to describe their parents. Those who are familiar with such terms may be interested to know of my other area of research. Over the past 25 years I have studied the area of “what it takes to make a great parent” and identified that John and Jackie M^cGregor are the most understanding, loving, caring and supportive parents anyone could ever have ($P < 0.00$). Although my dad thought it might be best to study law, I dedicate this thesis to them.

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CHAPTER 1

INTRODUCTION

1.1 Introduction

In many individual and team sports fluid ingestion during exercise is common practice and has been for many years. Traditionally soccer players have chosen a fluid replacement strategy of a cup of sweet tea and a slice of orange, ingested at the half-time interval. Indeed, until the 1994 World Cup finals fluid ingestion during soccer matches was prohibited. Most of the studies reporting fluid loss during soccer have been made under temperate conditions, during which fluid losses have ranged between 1-2.5 kg of body weight (Smaros, 1980; Leatt, 1986). Therefore a link between dehydration and performance is plausible. Many major soccer tournaments are played in hot climates, increasing the need for education about fluid ingestion.

The effect of dehydration on prolonged, continuous moderate - intensity (40 - 65% maximal oxygen uptake) exercise has been extensively investigated (see Coyle and Montain, 1992). Without fluid replacement there is a progressive decrease in systemic arterial, pulmonary arterial, right ventricular end-diastolic pressures and stroke volume (Ekelund, 1967; Rowell, 1986) and a progressive increase in rectal temperature and heart rate (Hamilton *et al.*, 1991). Cardiac output also declines during prolonged exercise when the reduction in stroke volume is relatively greater than the concomitant increase in heart rate (Sawka *et al.*, 1979). In addition, dehydration equivalent to the loss of only 2% body mass is sufficient to impair endurance performance significantly (Armstrong *et al.*, 1985). In contrast, fluid ingestion reduces the increase in heart rate and core temperature associated with dehydration (Hargreaves *et al.*, 1996). Water ingestion during exercise also improves endurance performance and capacity (Armstrong *et al.*, 1985; Fallowfield *et al.*, 1996).

The impact of dehydration on the intermittent high intensity exercise undertaken during participation in team sports has not been extensively studied. One of the main reasons for the dearth of information about the physiological and metabolic responses of players in intermittent sports is the practical difficulties associated with conducting field studies. Therefore the Loughborough Intermittent Shuttle Test (LIST) was designed to simulate the performance demands of intermittent sports such as soccer (Nicholas *et al.*, 1995). The influence of fluid intake, diet (Nicholas *et al.*, 1994, 1995, 1997), and environmental conditions (Morris *et al.*, 1996) on the performance of prolonged, intermittent exercise has been investigated using the LIST.

Team sports are characterised not only by intermittent high intensity exercise, but also by the contribution of a wide range of skills. One of the few studies examining the effects of dehydration on performance of a team sport showed that fluid deficits of 2 % of body mass had no significant effect on anaerobic power, vertical jumping height or goal shooting ability of players during a simulated basketball game (Hoffman *et al.*, 1995). However, the playing time in basketball is forty minutes, whereas in soccer it is more than twice as long.

During a soccer match there is an increase in the number of goals scored as the game reaches its end (Njorai, 1995). This phenomenon may occur because of a reduction in both physical and mental fatigue, leading to lapses in concentration and deterioration in skill (Reilly, 1996). The amount of work performed by soccer players, expressed as distance covered, decreases during the second half of a game (Karlsson, 1969) along with muscle glycogen concentration (Saltin, 1973). Therefore, the circumstantial evidence suggests an association between low work rate and low muscle glycogen stores.

Numerous authors have reported the beneficial effect of ingesting carbohydrate solutions during soccer (see Kirkendall, 1993). Carbohydrate ingestion decreases glycogen utilisation during a game and may delay fatigue (Jacobs, 1989). However, the influence of fluid intake per se on soccer skill remains to be determined.

The aim of the studies presented in this thesis was to examine the effect of fluid ingestion on selected soccer skills following the performance of prolonged intermittent, high intensity shuttle running.

This thesis is presented in eight main chapters. The review of literature deals with the most relevant literature on soccer metabolism, nutrition, physiology and skill with particular reference to the factors associated with fatigue and skill performance.

The General methods chapter (Chapter 3) describes the equipment and the testing procedures used during the administration of the experimental tests and the procedures associated with the collection of blood samples.

The main aims of the first study (Chapter 4) were to (i) investigate the effect of intermittent, high intensity shuttle running with and without water ingestion on soccer dribbling, and (ii) to describe some of the physiological and metabolic changes occurring during prolonged intermittent exercise.

The principal aim of the second study (Chapter 5) was to understand further the mechanisms underlying the deterioration observed in a soccer skill (Chapter 4).

The main aims of the third study (Chapter 6) were to (i) assess the influence of no fluid, a 6.4 % carbohydrate-electrolyte and a carbohydrate free solution on passing and dribbling soccer skill following prolonged intermittent, high intensity shuttle running, and (ii) to investigate further the possible role of central fatigue.

The purpose of the final study (Chapter 7) was to identify whether a rehydration strategy following the 90-min intermittent high intensity shuttle running would result in a recovery of skill performance.

The last chapter of this thesis (Chapter 8) summarises the findings of all studies and addresses some of the questions on which future research should focus.

CHAPTER 2

REVIEW OF LITERATURE

2.1 Introduction

This chapter draws together some of the findings from studies on the relationship between fluid ingestion, fatigue during prolonged high intensity exercise and the ability to perform selected soccer skills.

This review of literature has been divided into 6 main sections. The first section (section 2.2) examines the pertinent literature on the demands of soccer. Section 2.3 deals with the energy requirements of soccer. The potential causes of fatigue during soccer are reviewed in section 2.4 and the influence of carbohydrate and fluid ingestion during exercise in 2.5. Finally, section 2.6 reviews some of the relevant literature relating to skill performance.

2.2 Match Demands in Soccer

Various observation and recording techniques have been used to indirectly assess the sport specific requirements of soccer match play. Methods have included measurements of distances covered, activity patterns and running speeds (for a review of methods see Hughes, 1996).

2.2.1 Distance covered

The distance covered during a game of soccer is a function of positional role, with mid-fielders covering the greatest distances (Reilly and Thomas 1976; Ekblom, 1986). These findings, together with others taken from Tumilty (1993), are shown in Table 2.1.

Table 2.1. Distance (m) covered by players in a game (from Tumilty, 1993)

Reference	Players	Positions			
		Unspecified	Defence	Midfield	Attack
Agnevik (1970)	Swedish Division 1 team, 1968	10 200			
Whitehead (1975)	English players				
	Division 1		11 472	13 827	
	Division 2		10 826	11 184	
	Top amateur		9 679	9 084	
	College		6 609	8 754	
Reilly&Thomas (1976)	English Division 1		cb.7 759 fb. 8 245	9 805	8 397
Withers <i>et al.</i> (1982)	Australian National League team		cb.10 169 fb. 11 980	12 194	11766
Ekblom (1986)	4 Swedish teams from Divisions 1-4		9 600	10 600	10 100
	German Division 2		9 800		
Van Gool <i>et al.</i> (1988)	Belgian university team		9 903	10 710	9 820
Ohashi <i>et al.</i> (1988)	Japanese international players	9 303			
		10 387			
	Japanese league players	11 601			
		10 046			
Bangsbo <i>et al.</i> (1991a)	Danish Division 1 and 2 players		10 100	11 400	10 500

Abbreviations: cb = centre backs; fb = full backs

Table 2.1. indicates that the average distance covered by soccer players is around 10 km. The oxygen cost of completing 10 km in 90 min is approximately 35 ml. kg⁻¹ min⁻¹ or approximately 60% of the average maximum oxygen uptake ($\dot{V}O_2$ max) for soccer players (Tumilty, 1993). However, the average percentage of $\dot{V}O_2$ max sustained during a soccer game is closer to 80% (Ekblom, 1986; Bangsbo, 1994b). Higher energy requiring activities such as dribbling, running sideways and backwards, (Reilly and Ball, 1984, Reilly and Bowen, 1984) have been reasoned to be the cause

of this difference. Reilly and Thomas (1976) recorded a mean of 843 separate movement patterns during a game. Observations included that soccer players on average jumped 15.5 times, shot at goal 1.4 times and were getting up from the ground 5.3 times during a game. The distance covered in possession of the ball was found to be 1.7% of the overall distance.

2.2.2 Exercise pattern during a game

Not only have the observation studies identified the distances covered and the types of activities that are involved during a soccer match, various patterns of activity have also emerged.

2.2.3 Intensity

From a study performed on players in the North American Soccer League, Mayhew and Wenger (1985) observed that the high intensity (running) to low intensity activity (all other matchplay activities) ratio during a game was approximately 1:7. This consisted of standing (2.3% of the total time), walking (46.4%), jogging (38%), running (11.3%) and utility actions (backwards running, sideways shuffling and jumping, 2%).

In their study on English first Division players, Reilly and Thomas (1976) suggested that the ratio of high to low intensity exercise was closer to 1:11 consisting of sprints (11.2%), cruises (20.5%), jogs (36.8%), walking (24.8%) and other unspecified activities (6.7%). Observing players in the Australian Soccer League, Withers *et al.*, (1982) reported an overall time ratio for high to low intensity activity of 1:14. Such discrepancy has arisen due to the different methods used for data collection, the variances in the classification of the activity types and the level of competition. Nevertheless, these observations collectively show that the ratio is about 1:12.

2.2.4 First and second half differences

In a study performed on Swedish soccer players, Saltin (1973), identified that the total distance covered by the players in the second half was significantly lower than in the first, a finding which was also supported by Karlsson (1969).

Reilly and Thomas (1976) found that the total distance covered in the first half compared with the second half of a game was greater in more than 70% of the matches studied. Van Gool *et al.*, (1988) observed that not only did overall distance decrease by almost 9% between halves, the percentage of high and moderate intensity exercise also decreased, with an increase in lower intensity activities.

There are a number of possible reasons for the observed differences during the second half of soccer games. Gerisch *et al.* (1988) suggested that because of the prolonged nature of the game, players with lower aerobic fitness tend to compensate by more economical and 'clever play'. Bangsbo (1994a) explained that the shorter distances covered during the second half of a game may be due to a mental and physical fatigue where the players save their efforts for critical situations during the remaining part of the match.

Whether the difference between halves is due to fatigue is an area of debate. During a study of 14 top level Danish players, it was shown that although there were differences between halves in distance covered, there was no difference in the amount of high intensity running throughout the match. Although distances travelled decreased, performance potential had seemingly not (Bangsbo *et al.*, 1991a). Bangsbo (1994a) emphasised that in a separate study by Balsom, Ekblom and Bangsbo (unpublished findings) it was observed that sprinting speed over 20 m was reduced from 8.3 to 8.1 m.s⁻¹ as a result of match play. Therefore, it is not unreasonable to conclude that players probably do experience fatigue towards the end of a game.

2.2.5 Positional differences

Davis *et al.* (1992) performed a study on 135 soccer players prior to the commencement of the English league season. Mid-fielders had the highest predicted $\dot{V}O_2$ max values (61.4 ml. kg⁻¹ min.⁻¹), this being significantly greater than for centre backs (59.5 ml. kg⁻¹ min.⁻¹). The forwards were seen to be the fastest group, being significantly quicker than the goalkeepers, centre backs and full backs. Anaerobic power, knee extensor torque, extensor-flexor ratios and estimated body fat were similar between outfield players.

They concluded that while a distinction can be made between goalkeepers and outfield players in terms of physiological characteristics and role requirements, the difference between outfield players is less obvious. This is supported by Bangsbo *et al.* (1991a) who reported differing distances covered by positional role; however the percentages of the varying activity movement patterns such as walk, jog, sprint etc. were the same.

2.2.6 Level of play and tactics employed

Ekblom (1986) observed that players in the Swedish first division teams performed more periods of high intensity exercise than players from lower divisions. Through match observations of Danish first and second division players no difference in the total distance covered during a match was identified. However, the first division players performed more moderate speed (6.1% vs. 4.1% of total playing time), high speed (2.5% vs. 1.6%) and sprint (0.8% vs. 0.5%) running (Bangsbo, 1992). Thus confirming the observations of Ekblom (1986) that first division players have a higher work rate than second division players.

The tactics chosen by a team will also influence the type of demands to which a player is subjected. Gerisch *et al.* (1988) reported that the tactics of man-to-man marking produced higher blood lactate concentrations during match play than zone

defence. It was suggested that man-to-man marking was more stressful physiologically, than defending particular areas.

2.3 Energy requirements

Soccer makes greater overall physiological demands on players than many other sports. Therefore, there are a number of factors that influence the fatigue process during a soccer match. An important area of study is the metabolic processes that occur during match play. These factors include the amount and rate of energy provided by the aerobic and anaerobic reactions and their capacity to delay the onset of fatigue during soccer match play.

2.3.1 Aerobic energy production

There have been a number of attempts to directly assess the aerobic contribution to metabolism during soccer by measuring oxygen uptake ($\dot{V}O_2$) during match play (Durnin and Passmore, 1967; Seliger, 1968; Ogushi *et al.*, 1993). The values recorded during such trials are not representative of $\dot{V}O_2$ during games since the gas collection apparatus inevitably impedes the players' normal actions. Measurements have been made during practice games rather than actual competition, which further decrease the validity of such trials. The former problem has been minimised by using a lightweight (800 g) portable telemetry system (K2, Cosmed, Italy) to measure $\dot{V}O_2$. With this system $\dot{V}O_2$ has been measured during various soccer related activities, with values between 2 and 4 l.min⁻¹ being recorded for drills such as 1 vs. 1 and 3 vs. 1 games (Kawakami *et al.*, 1992).

One of the more common methods used to estimate the oxygen consumption during soccer, is the recording of heart rate during a match. Based on individual relationships between heart rate and oxygen uptake while running at a range of submaximal speeds,

the heart rate determinations for a player during match play can be transformed into oxygen uptake values. Using this approach, mean values for work rates of around 75% of $\dot{V}O_2$ max have been reported (Reilly and Thomas 1979). Similar studies by Ekblom (1986) suggest that aerobic energy production is very high and that many players exercise close to their maximal heart rate (HR_{max}) for long periods during the game. Rhode and Esperson (1988) found that heart rates during a match were below 73% of HR_{max} for only 10 min (11% of the playing time) but between 73% and 92% of HR_{max} for 57 min (63%), and higher than 92% HR_{max} for 23 min (26%). These observations confirm that soccer players do spend a significant amount of the 90-min playing time under high physiological stress.

Rectal temperatures have also been used as an indicator of the average energy production during soccer (Ekblom, 1986). This work was based on the findings that rectal temperature is related to relative energy consumption during high intensity exercise in temperate climates (Åstrand, 1960; Saltin and Hermansen 1966). Ekblom (1986) reported that players in the first division of the Swedish national league had a higher average rectal temperature during match play than players from the lower division. In several of the games studied (performed in a moderate climate; 20-22°C air temperature), rectal temperatures were over 40°C in some of the players. From such information and the known relationship between rectal temperature and relative oxygen uptake, Ekblom (1986) suggested that during national top level soccer, players were performing at about 80% of their $\dot{V}O_2$ max.

Ekblom *et al.* (1971) demonstrated that high intensity intermittent exercise gave rise to higher average rectal temperature when compared with continuous exercise of the same oxygen consumption. It was suggested that the differences were due to a higher rate of anaerobic metabolism during intermittent exercise. Caution should be used when extrapolating from measurements of oxygen uptake ($\dot{V}O_2$). Speeds may differ, but the total energy expenditure required to complete a set distance is the same. The $\dot{V}O_2$ during high intensity exercise will not reflect the total energy expenditure;

therefore it is important to monitor $\dot{V}O_2$ during recovery to give a direct comparison of energy expenditure.

The fact that intermittent sports such as soccer are more demanding than a corresponding bout of continuous exercise is supported by the observations of higher blood lactate concentrations during intermittent exercise. Blood lactate concentrations of 9.3 mmol.l^{-1} at treadmill speeds alternating between 8 km.h^{-1} (for 10 s) and 22 km.h^{-1} (for 15 s) were higher than the value (7.7 mmol.l^{-1}) obtained during continuous running at the same mean running speed (16.4 km.h^{-1}) (Bangsbo, 1994a).

From the general findings within the literature it appears that the mean relative work rate in soccer is around $70\% \dot{V}O_2 \text{ max}$, corresponding to an energy production of 1360 kcal for a player weighing 75 kg with a $\dot{V}O_2 \text{ max}$ of $60 \text{ ml kg}^{-1} \text{ min.}^{-1}$ (Bangsbo 1994b).

2.3.2 Anaerobic energy production

A significant amount of time during soccer is spent sprinting, jumping, tackling and heading which primarily stress the anaerobic energy system. Mayhew and Wenger (1985) suggested that approximately 12% of the total energy expenditure during a game is provided by the anaerobic system.

Although the anaerobic system is considered to play an important role in providing adenosine triphosphate (ATP) during soccer, the relative contribution from the phosphagen system or anaerobic glycolysis is unknown. Of the two anaerobic energy systems, the phosphagen rather than the glycolytic system, has been held to be of most importance (Tumilty, 1993). It is suggested this is due to the shortness and comparative infrequency of sprints in the game and so the more rapid provision of ATP by the hydrolysis of phosphocreatine (PCr) is more important than glycolytic provision of ATP.

2.3.3 The role of ATP and PCr

Total reserves of ATP and PCr may be small, $\sim 20 \text{ mmol.kg}^{-1}\text{w.w.}$, but they provide a considerable amount of energy during periods of high intensity exercise in general and soccer in particular (Shephard, 1992). Levels of PCr are almost totally depleted during an exhaustive sprint, resynthesis of which begins immediately, drawing upon a combination of glycolysis and oxidative metabolism. As PCr is synthesised during the periods of rest and low intensity exercise, the PCr concentration will rise and fall due to the intermittent demands for high rates of energy production during a game (Bangsbo, 1994b). Although the total utilisation of PCr is relatively small, it plays an important role as an energy buffer, providing phosphate for adenosine diphosphate (ADP) in the resynthesis of ATP. If PCr concentrations are not regenerated during the game, the rate of ATP resynthesis will not match its rate of utilisation and so fatigue will occur sooner rather than later.

2.3.4 Anaerobic glycolysis

The importance of anaerobic glycolysis for soccer performance has been examined by the analysis of blood lactate concentration. Blood lactate concentrations between $4\text{-}6 \text{ mmol.l}^{-1}$ have been recorded following the first and second halves of soccer match play (Gerisch *et al.*, 1988; Rhode and Esperson, 1988). From such observations it was concluded that because a relatively low concentration of lactate was produced, the anaerobic glycolysis system played a minor role in soccer performance. This view has been challenged by Bangsbo (1994b) and Tumilty (1993) who questioned the relevance of blood lactate as an indicator of lactate production.

Although high lactate concentrations are linked with the onset of muscular fatigue, the ability to perform high intensity, short-term exercise is dependent on the muscles' ability to derive energy from the glycogen to lactate pathway (Jacobs, 1981). The fate of the accumulated lactate in active skeletal muscle following intense, exhaustive exercise has not been resolved. It has a number of possible fates, for example, it can be metabolised within the muscle or lactate can be used as a substrate for

intramuscular glycogenolysis, plus gluconeogenesis in the liver. Lactate is also a ready substrate for oxidative metabolism in cardiac muscle.

Blood lactate concentrations reflect the balance between the release of lactate from muscle and its removal from the blood, and so it has been suggested that blood lactate concentrations underestimate lactate production, as not all of the lactate produced will appear in the blood. Also the rate of lactate production may be high, but the duration of activity too short to result in large blood lactate concentrations (Bangsbo, 1994a). Based on findings of high blood lactate concentrations approaching 10 mmol.l⁻¹, (Agnevik, 1970), it is reasonable to assume that lactate production is probably very high at certain times during a game. Although it is difficult to quantify the exact energy production from anaerobic glycolysis, it clearly plays an essential and significant role in energy provision during soccer.

2.3.5 Substrate utilisation during soccer

During prolonged strenuous exercise, carbohydrates and lipids are the main substrates for oxidative metabolism which fuels the contraction process in skeletal muscle. As soccer involves periods of high intensity exercise carbohydrate, in particular muscle glycogen, is utilised at an increased rate (Hargreaves, 1994). The role of protein metabolism in soccer is unclear, but studies with continuous exercise at a mean work rate and duration similar to soccer have shown that oxidation of proteins may contribute less than 10% to the total energy production (Wagenmakers *et al.*, 1989).

Although factors such as initial glycogen concentration, training status and environmental conditions may influence substrate selection, the relative contribution of carbohydrate and lipid to metabolism is primarily determined by the intensity and duration of exercise (Coggan and Coyle, 1991). Utilisation of muscle glycogen and blood-borne glucose by active muscle increases with exercise intensity. However, muscle glycogen is the major carbohydrate fuel at exercise intensities above 65-70% $\dot{V}O_2$ max. As soccer involves frequent periods of high intensity, intermittent exercise

with an average intensity between 60-80% $\dot{V}O_2$ max, soccer players rely heavily on their carbohydrate stores.

2.4 Causes of fatigue during soccer

Edwards (1983) defined muscle fatigue as the failure to maintain the required or expected power output. A number of sites and mechanisms may be involved in the fatigue process. Any explanation of its cause will be dependent upon the nature of the task, with factors such as duration, intensity, training status, recruited fibre types and environmental conditions all combining to influence the development of muscle tension. In his explanation of fatigue, Edwards (1983) produced a chain of command for muscular contraction and the possible mechanisms underlying fatigue. He suggested that the cause of fatigue might be due to a failure at any one of the links of the chain (Figure 2.1.).

The main factor that will cause a break in the chain and ultimately result in fatigue is undoubtedly the intensity of the exercise in relation to the capacity of the individual, (Maughan and Noakes, 1991). From match analysis it has been shown that soccer involves players performing at a high intensity for a prolonged period of time. As a result the deterioration in performance observed towards the end of a game will be a result of the fatigue processes that occur during both short intense and long-term exercise. Knowledge of the fatigue processes and metabolic changes associated with both intense and more prolonged continuous exercise, may then provide an understanding of the type of fatigue that is involved in soccer performance.

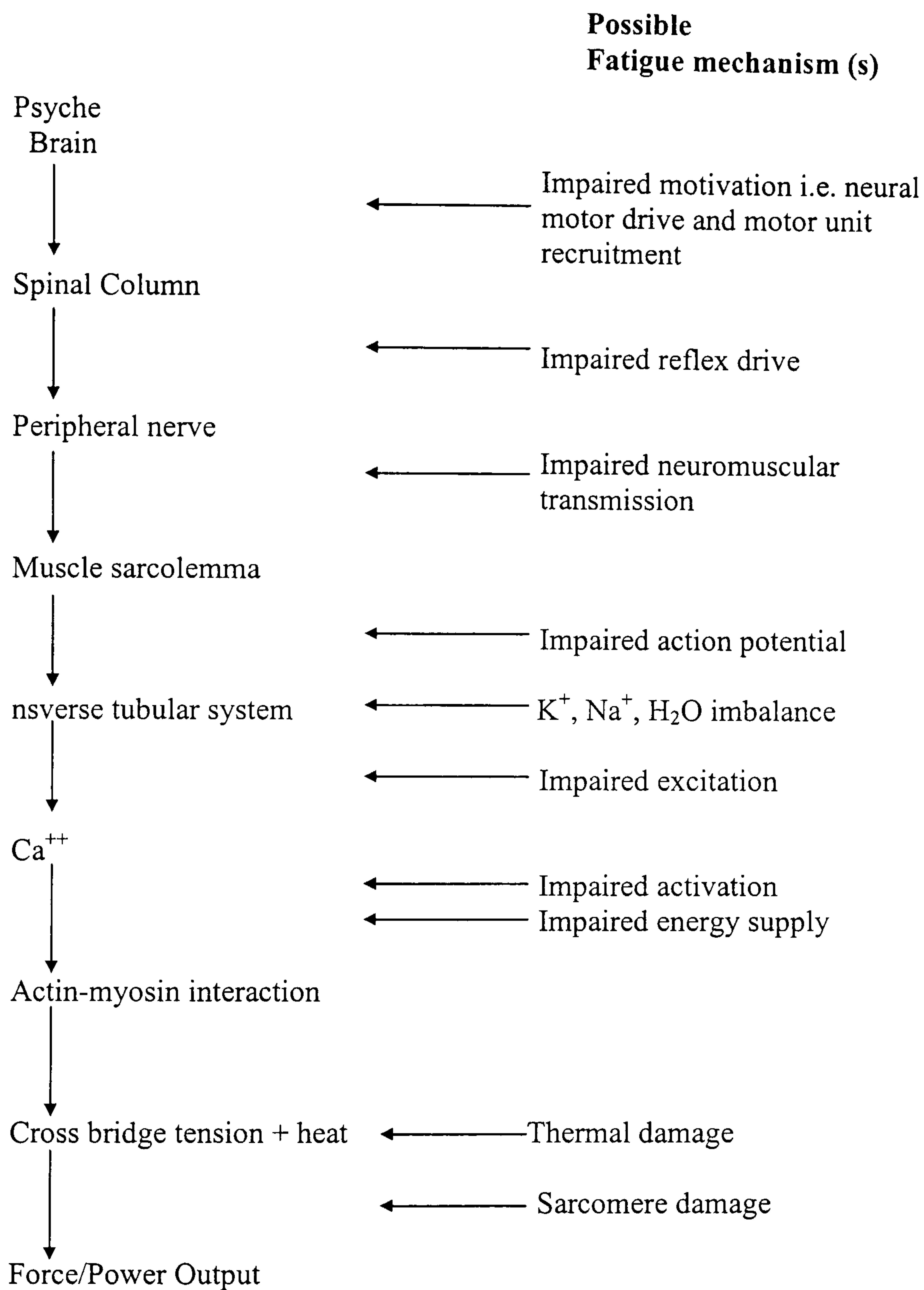


Figure 2.1. Chain of command for muscular contraction and possible mechanisms underlying fatigue. Modified from Edwards (1983)

2.4.1 Fatigue due to high intensity activity

As field tests of team sports are influenced by factors such as team tactics, position played and standard of play, researchers have attempted to gain a greater understanding of the aetiology of fatigue during high intensity intermittent exercise, through laboratory studies using cycle ergometry (Wootton and Williams, 1983; McCartney *et al.*, 1986; Gaitanos, 1993; Bogdanis *et al.*, 1996) and non motorised treadmill running (Holymard *et al.*, 1988). From such studies, estimations of the relative contribution of the energy systems and greater insights into the fatigue processes during high intensity intermittent activity have been achieved (see review by Boobis, 1987).

High intensity intermittent exercise such as soccer places a unique combination of demands on the metabolic processes in the muscle, where energy supply oscillates between fuelling contractile activity and restoring homeostasis (Balsom *et al.*, 1992). While energy is produced primarily via anaerobic glycogenolysis during a short bout of maximal exercise (Boobis, 1987), energy for recovery and subsequent bouts may have a significant aerobic contribution (Bogdanis *et al.*, 1996). In studies using high intensity intermittent exercise protocols, performance has been shown to gradually decrease during the latter stages of exercise. The rate of muscle lactate accumulation is reduced during the later stages of exercise and aerobic metabolism makes an ever increasing contribution to energy production (Gaitanos *et al.*, 1993; McCartney *et al.*, 1986).

An important function of the recovery process between repeated sprints is to replenish PCr stores and restore muscle pH (Balsom *et al.*, 1992). Balsom *et al.* (1992) studied the effect of recovery time on performance of 40 m sprints with rest intervals of 30 (R₃₀), 60 (R₆₀) or 120 s (R₁₂₀). Running speed in the R₁₂₀ trial was maintained for all 15 sprints whereas sprint times decreased over the testing period in the other two trials (R₃₀ and R₆₀). Acceleration from 0-15 m was only affected with the shortest 30 s rest period, findings similar to those reported by Wootton and Williams (1983).

The observed decrease in performance was suggested to be due to insufficient restoration of PCr and although not measured, a decline in muscle pH in the short rest periods (Balsom *et al.*, 1992). It would seem that the fatiguing effect of high intensity activities on subsequent performance is dependent upon the duration, length and frequency of the exercise bout. Match observations suggest that periods of maximal exertion in soccer are short (~5 s) and recovery periods relatively long (>30 s) (Reilly and Ball, 1984).

High intensity exercise is associated with a large production of lactate and an increase in acidity. While it is accepted that blood lactate concentrations have been recorded over 10 mmol.l⁻¹ during certain phases of a game (Bangsbo, 1994a), rest periods between these bouts are sufficiently long to cause a restoration of blood pH. Only in rare cases does muscle lactate concentrations reach values above 25 mmol. kg⁻¹ w.w. and muscle pH below 6.8. As a result Bangsbo (1994a) suggested that as lactate and pH are not always causally linked to a decrease in force development, the associated by-products of high intensity exercise may not be the main cause of fatigue in soccer.

2.4.2 Fatigue associated with glycogen depletion

The use of glycogen, as an energy substrate, by skeletal muscle is directly related to the duration and intensity of exercise (Saltin and Karlsson, 1971). During prolonged, strenuous exercise, muscle glycogen and blood borne glucose are essential substrates for contracting muscle and fatigue coincides with the depletion of muscle glycogen and a reduction in blood glucose concentration (Coggan and Coyle, 1987).

A reduction in substrate availability has been shown to impair both high intensity (Jacobs, 1981; Jacobs *et al.*, 1982b) and endurance performance (Bergstrom *et al.* 1967; Hultman, 1971), effects which might influence soccer performance during match play. Although the body draws upon carbohydrate, fat and to a limited extent protein to meet the energy demands of soccer, only carbohydrate reserves are sufficiently small to be threatened during a soccer match (Shephard, 1992).

During a competitive soccer game the blood glucose concentration is generally above the value recorded at rest with average values being above 4 mmol.l⁻¹ (Bangsbo, 1994b; Ekblom, 1986; Smaros, 1980). It appears that the liver releases enough glucose to maintain the blood glucose concentration during a match with the result that hypoglycaemia occurs in only very rare cases.

Depending on the level of competition and the extent of initial reserves, authors have reported 20-90% depletion of intramuscular glycogen during high level competitive play (Agnevik, 1970; Saltin, 1973; Smaros, 1980). As well as observing a rapid decrease in muscle glycogen during a soccer game, Karlsson (1969) using film analysis, observed that the players with the lowest glycogen content at half time had a slower average speed and covered less ground in the second half of the game.

These findings of low muscle glycogen concentrations at the end of a soccer match and a more pronounced use of glycogen in the first compared with the second half indicate that the level of muscle glycogen prior to a match may influence work rate towards the end of a game. In order to evaluate under standardised conditions, the effect of different pre-exercise muscle glycogen concentrations on endurance capacity, both Bangsbo *et al.* (1992) and Nicholas *et al.* (1997) developed an intermittent exercise protocol to simulate the physiological demands of soccer. Bangsbo *et al.* (1992) identified an improvement in high speed running following the ingestion of a carbohydrate-enriched diet for 2 days. Increasing the carbohydrate intake of a normal diet to 10 g.kg⁻¹bm.day⁻¹ improved intermittent high-intensity shuttle running after 22 h of recovery (Nicholas *et al.*, 1997). These studies confirm the need for increased carbohydrate intake to allow players to compete and train optimally on a daily basis.

The mechanisms responsible for fatigue when muscle glycogen is low remain unclear. Costill and Hargreaves (1992) suggested that carbohydrate depletion is associated with the accumulation of ATP breakdown products, namely inosine monophosphate, hypoxanthine, ammonia and ammonium. In glycogen depleted muscle, fatigue results

because of a failure of ATP resynthesis. It is suggested that this is due to a relative deficiency in pyruvate, which results in reduced substrate for anapleurotic reactions that supply the tricarboxylic acid cycle. Mitochondrial intermediates which are necessary for the continued oxidation of acetyl units are derived from other sources such as free fatty acids and amino acids. However, they cannot be metabolised as quickly as glycogen, hence the need for adequate glycogen stores before exercise.

2.4.3 Temperature regulation and dehydration

Increased muscular activity during exercise causes an increase in heat production in the body due to the inefficiency of the metabolic reactions involved in providing energy for muscle force development (Gleeson, 1998). Only a small proportion of the heat produced in active skeletal muscle is lost from the overlying skin. Most of the heat is passed to the body core via the convective flow of venous blood returning to the heart. Increases in body core temperature are sensed by thermoreceptors located in the hypothalamus. The hypothalamus also receives sensory input from skin thermoreceptors and integrates this information to produce appropriate reflex effector responses. These responses may include increasing blood flow to the skin and initiating sweating to limit the rise in body temperature. The hypothalamus also receives nonthermal sensory inputs that are capable of modulating the homeostatic regulation of body temperature. These other inputs include nervous signals from osmoreceptors and pressure receptors, so that changes in plasma osmolality and blood volume are capable of affecting sweating and cutaneous vasodilation (Gleeson, 1998).

Evaporation of 1 litre of sweat from the skin will remove 2.4 MJ (587 kcal) of heat from the body (Maughan and Shirreffs, 1998). Even in cool conditions, sweat losses may be high when the exercise duration is long or the intensity high. In a marathon held in conditions of 12°C, mean sweat losses were estimated to be 3.5 litres (Maughan, 1985). Most of the studies reporting fluid loss during soccer have been made under temperate conditions, during which fluid losses have ranged between 1-2.5 kg of body weight (Smaros, 1980; Leatt, 1986). In a study carried out under

varying temperatures (range 13.2°C - 33°C) Mustafa and Mahmoud (1979) reported evaporative water losses of 5 kg during soccer. It is not unusual that major soccer tournaments are played in demanding environmental conditions e.g. during the World Cup finals in Argentina (1978), Spain (1982), Italy (1990) and the United States of America (1994), ambient temperatures were often above 25°C.

2.4.4 Dehydration and performance

Although fluid loss presents a potential threat to exercise, relatively few studies have investigated the role of water ingestion on performance. Most emphasis in the literature has been placed on the influence of the addition of carbohydrate to a solution.

As little as a 2% reduction in body weight has been shown to impair endurance capacity (Saltin and Costill, 1988) and dehydration has also been shown to adversely affect exercise performance (Armstrong *et al.*, 1985; Craig and Cummings, 1966; Dengel *et al.*, 1992). Associated with the losses in exercise performance and capacity have been increases in core temperature (Hamilton *et al.*, 1991), stroke volume (Sawka, 1992; Below *et al.*, 1995) and increased heart rate (Hargreaves, 1996). These alterations in physical function are attenuated, if not prevented, by fluid ingestion (Hargreaves, 1996).

In a study by Walsh *et al.* (1994), subjects performed 2 bouts of cycling for 60 minutes at 70% $\dot{V}O_2$ peak and then exercised to exhaustion at 90 % of $\dot{V}O_2$ peak with or without ingesting 20 mmol.l⁻¹ of NaCl solution. Maintaining a euhydrated state by ingesting fluid at regular intervals throughout the 60-min enabled the subjects to cycle for 4 min longer during the exercise to exhaustion phase compared to the dehydration trial. Below *et al.* (1995) compared the effects of small (200 ml) and large (1330 ml) volumes of electrolyte and carbohydrate-electrolyte solutions on exercise performance during 1 h of intense exercise. The initial 50-min of exercise consisted of cycling at a work rate that elicited a $\dot{V}O_2$ which was 5% above the

subjects 'lactate threshold'. Following this the subjects immediately began a cycling performance test which required the completion of a set amount of work in the shortest time possible. Performance times were 6.5% faster during large fluid replacement than small fluid replacement and performance times were 6.3% better with carbohydrate ingestion than without carbohydrate ingestion. The effects of fluid and carbohydrate ingestion on performance were additive (Below *et al.*, 1995).

Montain and Coyle (1992) have also shown the physiological advantages associated with larger volumes of ingested fluid. Subjects exercised in the heat for 2 hours under the conditions of either no fluid, 20% of sweat loss replaced, 50% of sweat loss replaced or 80% of sweat loss replaced. The larger the volume of fluid ingested the better the control of physiological function in terms of heart rate, stroke volume, cardiac output and core temperature. Additional water intake in heat acclimated players has been shown to increase body water reserves and improve temperature regulation during soccer (Rico Sanz *et al.*, 1996).

Together with the attenuation of these responses to fluid loss, the ingestion of water has been suggested to contribute to an improvement in exercise capacity by sparing muscle glycogen. Fallowfield *et al.* (1996) and Hargreaves *et al.* (1996) observed an increase in endurance capacity with water ingestion. Respiratory exchange ratio data during both investigations indicated an increase in carbohydrate metabolism and a suppression of fat metabolism when subjects completed the trials without fluid ingestion. Hargreaves *et al.* (1996) identified that net muscle glycogen utilisation during exercise was reduced by 16% when water was ingested. In conclusion it was suggested that the reduction in glycogenolysis observed with water ingestion may account, in part, for the improved performance previously described with fluid ingestion.

2.4.5 Central factors

There is no doubt that changes within the active muscle contribute to fatigue (Edwards, 1981; Newsholme, 1981) however, changes within the central nervous system which are responsible for motor control of muscle may also contribute to the phenomenon of fatigue.

It is possible that fatigue during soccer may arise due to impairment of the central nervous system. Reilly and Lewis (1985) suggested that vigorous physical work, which causes thermoregulatory strain and induces appreciable sweat losses, leads to levels of dehydration where mental functioning may be adversely affected. In studies of prolonged high intensity intermittent exercise, endurance was increased (Reynolds *et al.*, 1985) and the ability to perform prescribed “neuromental” functions such as thought processing and accuracy tasks were improved when compared with performance in the absence of water consumption (Reynolds and Ekblom 1985).

Reilly and Lewis (1985) also suggested that a decrease in blood glucose concentration causes a drop in the supply of energy substrate to the nervous system, which may result in impaired mental functioning. In support of the importance of a normal blood glucose concentration for effective cognitive functioning, Niinimaa *et al.* (1977) noted a positive relationship between blood glucose concentrations and the mental ability of dinghy sailors.

It has been suggested that a reduction in performance may occur due to an increase in the activity of the brain neurotransmitter 5-hydroxytryptamine (serotonin, 5-HT) (Newsholme *et al.*, 1987; Blomstrand *et al.*, 1988, 1989). Serotonin is known to play a role in pain, arousal, sleepiness and mood (Young, 1986). It is also known that prolonged exercise is associated with increased brain 5-HT (Chaouloff *et al.*, 1986, 1987). Support for the theory that changes in 5-HT concentration play a role in fatigue has been presented in studies involving pharmacological manipulation of the 5-HT system. In rats administration of a 5-HT agonist was reported to impair running

performance in a dose-related manner (Bailey *et al.*, 1992) and administration of a 5-HT antagonist improved running performance (Bailey *et al.*, 1993).

The mechanism behind the increased level of 5-HT is thought to be an increase in the ratio of plasma free tryptophan (fTrp) to other large neutral amino acids (LNAA), which are released from muscle during prolonged exercise (Blomstrand *et al.*, 1988, Davis *et al.*, 1992). This concentration ratio is considered to be important because the LNAA (including tryptophan and the branched-chain amino acids (BCAAs; valine, leucine and isoleucine) are transported into the brain by the same carrier mechanism and compete for preferential translocation across the blood-brain barrier (Pardridge, 1977).

It is proposed that an increase in the concentration of 5-HT in the brain can result from two established peripheral effects of endurance exercise. These are, an increase in the rate of branched-chain amino acid (BCAA) utilisation by muscle and an increase in the rate of lipolysis in adipose tissue (Blomstrand *et al.*, 1991). The later results in an increase in 5-HT due to the interaction between the concentration of plasma free fatty acids (FFA) and tryptophan. Tryptophan and fatty acids bind to the same sites on albumin. If there is an increase in FFA there will be a lower number of binding sites available for albumin (Curzon *et al.*, 1973) and the fTrp concentration will increase (Blomstrand *et al.*, 1988). Consequently there is an increase in the concentration ratio of fTrp/BCAA in the plasma. Since BCAA compete with fTrp for entry into the brain (Pardridge and Oldendorf, 1975; Pardridge, 1977), an increase in this ratio should favour the entry of tryptophan across the blood-brain barrier. The increased concentration of tryptophan should lead to an increase in the concentration of 5-HT (Blomstrand *et al.*, 1991). This is due to the fact that tryptophan hydroxylase, the rate-limiting enzyme for 5HT synthesis, is normally unsaturated with tryptophan. Therefore any mechanism by which brain tryptophan concentration can be altered may conceivably be able to influence brain function (Curzon *et al.*, 1973).

2.4.6 Effect of branched-chain amino acids during exercise

A further prediction from the central fatigue hypothesis is that fatigue during exhaustive exercise should be delayed if the plasma concentration of BCAA is elevated during exercise to a level that would maintain the normal fTrp/BCAA ratio (Blomstrand *et al.*, 1991). Blomstrand *et al.*, (1991) provided volunteers with drinks containing BCAA during prolonged exercise and investigated the effects on physical and mental performance. Branched-chain amino acids were ingested during a 30-km cross-country race and a marathon. Mental performance was improved after the 30 km cross country-race when a BCAA supplement was given. The running performance in the marathon was improved for the “slower” runners (marathon completed in 3.05 h-3.30 h) when BCAA was taken during the race; however, there was no significant effect on the performance in the “faster” runners (<3.05 h). Blomstrand *et al.* (1991) suggested the findings indicated that both mental and physical performance could be improved by ingestion of BCAA during heavy sustained exercise. The reason for the lack of effect on performance in the faster runners was unknown. It was suggested that the better-trained (faster) runners were more resistant to fatigue and therefore less sensitive to additional BCAA. Alternatively, the slower runners may have depleted their glycogen stores at an earlier stage of the race, so that they would have increased the plasma concentrations of FFA and decreased that of BCAA earlier than the faster runners (Blomstrand *et al.*, 1991). It was also suggested that the ingestion of BCAA may have suppressed the increased rate of protein degradation that occurs during sustained exercise and that this effect of BCAA might have contributed to the improvement in physical performance found in the marathon runners.

In a more controlled laboratory environment no effect of BCAAs could be detected during exercise duration of approximately 30 min (Wagenmakers, 1992; Varnier *et al.*, 1994). Also when BCAAs were supplied together with carbohydrates during exercise of longer duration; 80 min and 1-3 h of exhaustive exercise (Blomstrand *et al.*, 1995; Van Hall *et al.*, 1995) no beneficial effect was observed.

With the limitations associated with field studies, Blomstrand *et al.* (1997) studied the effect of BCAA under more controlled conditions. The purpose of the study was to investigate the ingestion of BCAA on the amount of work performed, perceived exertion and on mental fatigue during cycling in the laboratory. Ingestion of BCAA decreased the perceived exertion and mental fatigue during 60 min of cycling at 70% VO_2 max. Following the 60-min exercise period, subjects exercised at their maximum for a further 20-min, no significant effect of BCAA could be detected. The concentration of fTrp increased during the later part of the exercise under both conditions. However, when the BCAA doses were ingested, the plasma concentration ratio of fTrp/BCAA was maintained or reduced below pre-exercise levels, which was expected to prevent an increase in the brain 5-HT level.

The conflicting results of field and laboratory studies may be due to the limitations of experimental design. Davis (1995) suggested that while field studies are designed to mimic athletes' actual situations, such studies are often limited in scientific value. Subjects are often not appropriately matched prior to their assignment to control and experimental groups and there is poor control over important variables such as exercise intensity and food and water intake across treatments.

2.4.7 Influence of carbohydrate ingestion on central fatigue

Carbohydrate ingestion has also been used as a strategy for delaying central fatigue. The ingestion of carbohydrate leads to a decreased mobilisation of fatty acids, so providing a greater number of binding sites on plasma albumin for tryptophan. As a result of this suppressive effect it was predicted that carbohydrate feeding would reduce concentrations of fTrp and free fTrp /BCAA, which would likely suppress the production of 5-HT in the brain and thereby minimise central fatigue (Davis *et al.*, 1992). Davis *et al.* (1992) observed that fTrp/BCAAs and FFA increased progressively during prolonged exercise until fatigue, when they were approximately 6 fold higher than pre-exercise values. These changes were attenuated in a dose-

related fashion and fatigue was delayed when subjects consumed drinks containing 6% or 12% carbohydrate. A lack of assessment of several variables, including muscle glycogen and specific measures of central fatigue, meant it was not possible to distinguish between the two treatments on central versus peripheral mechanisms of fatigue. Nevertheless, markers of peripheral fatigue involving cardiovascular, thermoregulatory and metabolic functions could not explain the delay in fatigue. Davis *et al.* (1992) therefore suggested that the results supported the possibility that central factors may have played a role in fatigue. The extent to which alterations in fTrp and the fTrp/BCAA are involved in the mechanisms underlying the onset of fatigue during prolonged exercise remains to be elucidated.

2.5 Carbohydrate and fluid replacement

It has been identified that glycogen depletion and dehydration contribute to the fatigue process in intermittent activities such as soccer (for a review see Maughan and Leiper, 1994). Prevention of glycogen depletion and dehydration can therefore delay fatigue and maintain performance potential. Numerous studies have been performed to identify the optimal strategy for carbohydrate and fluid ingestion prior to, during and post exercise to prevent or delay the fatigue process.

2.5.1 Carbohydrate ingestion prior to performance

A close correlation has been established between the time to exhaustion at a given intensity of exercise and pre-exercise muscle glycogen concentration (Bergstrom *et al.*, 1967; Hermansen *et al.*, 1967; Saltin and Karlsson, 1971). Bergstrom *et al.* (1967) advised athletes to undertake a bout of exhausting exercise followed by three days of low carbohydrate intake and three days of a high carbohydrate diet. Adherence to this regimen boosted muscle glycogen reserves from an average of 101 to 223 mmol. kg⁻¹ w.w.. More recently Sherman *et al.* (1981) have suggested that the low carbohydrate phase can be omitted without loss of supercompensation and will reduce the risk of symptoms such as headaches and irritability which have been found as a result of the

low carbohydrate phase. It is less clear that attempts at supercompensation would be helpful to a soccer player as normal training sessions would be interrupted and players may be involved in more than one game a week.

Jacobs *et al.* (1982a) studied the dietary repletion of muscle glycogen of soccer players by sampling the vastus lateralis of Swedish players following a game and for two subsequent days. Diet records based on recall showed that 14% of the players' intake was protein, 29% was fat and 47% was carbohydrate. This diet increased muscle glycogen by 59% over two days to a mean value of 73 mmol.kg⁻¹ w.w. which was below that usually observed for sedentary subjects (76 mmol.kg⁻¹ w.w.)(Felig and Wahren, 1975). Such a diet was deemed to be insufficient to replenish muscle glycogen stores during the reported weekly routine of the players which consisted of training, one day off and one to two games per week. During the simulation study of Bangsbo (1992), similar diets were reported to those of the players studied by Jacobs *et al.* (1982a). Bangsbo (1992) observed that the players' usual diet consisted of only 46% carbohydrate (426 g per day) and five of the players had an intake below 400 g per day.

If these diets are typical of professional soccer players then they probably influence the players' capacity to run throughout the game. Saltin (1973) reported observations on nine players: before the game 5 had a normal glycogen concentration and 4 players had concentrations that were 50% lower. At the end of the game, members of the normal group had less than 10% of their initial muscle glycogen concentration and the low-glycogen concentration group had totally depleted their muscle glycogen stores. The total distance covered was less and the fraction of the total distance as walking was greater in the low-glycogen group.

Jacobs (1988) stated that although it may not be possible for soccer players to repeatedly follow the extreme carbohydrate loading techniques used by endurance athletes, a high carbohydrate diet (65 to 70% carbohydrate in diet of 600 g per day) should be sufficient to ensure adequate replenishment of glycogen stores between

games. It is clear that a nutritional strategy needs to be adopted by players if they want to be adequately prepared for competition.

2.5.2 Pre-game carbohydrate ingestion

Ingestion of a concentrated carbohydrate solution in the hour before prolonged exercise has been found to provoke a large increase in plasma insulin concentration, with a resultant hypoglycaemia (Costill *et al.*, 1977) and deterioration in performance (Foster *et al.*, 1979; Koivisto *et al.*, 1981). As a result athletes were advised to avoid consuming carbohydrate shortly before exercise (Costill and Miller, 1980). Other studies have since reported no hypoglycaemia or increased rate of glycogenolysis and fatigue with similar feedings (Fielding *et al.*, 1987; Chryssanthopoulos *et al.*, 1994). In some cases the ingestion of carbohydrate solutions shortly before exercise has been seen to improve performance (Hargreaves *et al.*, 1984; Mitchell *et al.*, 1989; Below *et al.*, 1995).

2.5.3 Practical issues concerning carbohydrate ingestion during exercise

The ingestion of a carbohydrate solution during exercise can be effective in the prevention of the exercise induced changes to body homeostasis such as a decrease in plasma volume, increase in plasma osmolality and core temperature, decrease in available muscle fuels and maintenance of blood glucose concentration (Burke and Read, 1993). The process of rehydration and energy provision initially depends on both the rate of gastric emptying and subsequent intestinal absorption of the ingested fluid (Hawley *et al.*, 1994). The composition of the fluid will therefore be influenced by the relative importance of the need to supply carbohydrate and water. This will depend upon the intensity and duration of the exercise task, the environmental conditions and the physiological and biochemical characteristics of the individual.

As the rate of gastric emptying (Costill and Saltin, 1974; Fordtran and Saltin, 1967; Rehrer *et al.*, 1989) and intestinal absorption (Fordtran and Saltin, 1967; Rehrer *et al.*,

1989; Gisolfi *et al.*, 1991) are not influenced by exercise intensity up to 80% VO_2 max, the existing data on continuous exercise are applicable to most intermittent forms of exercise (Shi and Gisolfi, 1998).

2.5.4 Volume of fluid

A major factor in the regulation of gastric emptying is the volume of fluid consumed. Studies using single (Costill and Saltin, 1974) or repetitive ingestions (Duchman *et al.*, 1997; Rehrer *et al.*, 1990; Ryan *et al.*, 1989) show that gastric emptying rate is strongly influenced by gastric volume. The greater the ingested volume, the greater the rate of gastric emptying. The mechanism determining volume dependent gastric emptying rate is the distension and pressure created by the increased volume in the stomach (Shi and Gisolfi, 1998). This in turn stimulates receptors in the gastric musculature to increase the rate of gastric emptying (Costill and Saltin, 1974; Minami and McCallum, 1984).

During exercise, the normal rate of gastric emptying is in the range of 600-1000 ml h^{-1} (Shephard, 1990). If the fluid intake exceeds this range, it merely accumulates in the stomach and may lead to abdominal discomfort. Thirst is a poor guide to fluid needs when a person is involved in physical exercise and often athletes find it difficult to drink an adequate volume of fluid. The recommendations for fluid intake during exercise are no longer given as absolute values. Instead it is stated that athletes should attempt to match their fluid losses during exercise, or, if this is not possible, they should consume the maximum amounts that can be tolerated (Montain and Coyle, 1992; Maughan and Shirreffs, 1998).

2.5.5 Energy density and osmolality

It was previously believed that osmolality was one of the most important factors in the control of gastric emptying. Brouns *et al.* (1995) reported the results of a study which compared the effect of six drinks with osmolalities ranging from 240 - 390 mOsmol.kg⁻¹, but all with a carbohydrate content of 60 g.l⁻¹. It was observed that all drinks emptied at the same rate (Brouns *et al.*, 1995). Therefore, although high osmolalities may reduce gastric emptying rate (Vist and Maughan 1995), it is not as an important factor in relation to the consumption of most sports drinks (range of 200-400 mOsmol.kg⁻¹) (Brouns, 1998).

The rate of glucose uptake is dependent on the luminal concentrations of glucose and sodium. Costill and Saltin (1974) found a slowing of emptying with glucose concentrations higher than 5%, and Barnes *et al.* (1984) observed a slowing of gastric emptying with glucose concentrations above 2.5%. Glucose polymers (maltodextrins formed by the controlled hydrolysis of starch) have the theoretical advantage of providing equal amounts of glucose at a lower osmolality than glucose or sucrose solutions. Similar effects are seen with short-chain (3-10 glucosyl units) glucose polymers (Coggan and Coyle, 1988) sucrose, (Sasaki *et al.*, 1987) or mixtures of sugars (Carter and Gisolfi, 1989). Noakes *et al.* (1991) suggested that the failure to find a clear advantage with low osmolality solutions, may be due to a rapid near-total hydrolysis of polymers and starch occurring in the intestine before the solutions reach the osmoreceptors controlling gastric emptying.

Some investigators have attempted to provide more carbohydrate without boosting the osmotic pressure and insulin levels by providing fructose as the ingested substrate (Hargreaves *et al.*, 1985; Koivisto *et al.*, 1981). However, intestinal distress is sometimes provoked by high concentrations of fructose intake (Maughan *et al.*, 1989). In conclusion, it appears that 5 to 10% solutions of sucrose, glucose, glucose polymers or combinations of these sugars all have suitable gastric emptying characteristics for the delivery of fluid and moderate amounts of carbohydrate

substrate (Burke and Read, 1993). However it should be noted that other than the recent study by Shi and Gisolfi (1998), there is little information on the gastric emptying and intestinal absorption of carbohydrate-electrolyte solutions during intermittent high intensity exercise of brief duration. Therefore the successful translation to recommend to soccer players is based on circumstance rather than direct evidence.

2.5.6 Rate of carbohydrate ingestion

The rate of carbohydrate intake necessary to achieve enhanced performance during endurance exercise is greater than $20 \text{ g}\cdot\text{h}^{-1}$, with the typical rate being approximately 40 to $60 \text{ g}\cdot\text{h}^{-1}$ (Murray, 1987; Tsintzas and Williams, 1998). The maximum carbohydrate oxidation rate following the ingestion of glucose solutions is of the order $0.5\text{-}1.0 \text{ g}\cdot\text{min}^{-1}$ (Hawley *et al.*, 1992).

2.5.7 Temperature of fluid

In a study by Costill and Saltin (1974), subjects were given 400 ml of a dilute glucose solution at temperatures ranging from 5 to 35°C . The volume emptied in the first 15-min after ingestion was approximately twice as great for the solution at 5°C as for the solution at 35°C . Sun *et al.* (1988) reported that warm (50°C) and cold (4°C) drinks appeared to empty from the stomach more slowly than a control drink of 37°C . Maughan and Noakes (1991) suggested that there is no difference between the gastric emptying rates of hot and cold beverages; however, there may be an advantage of an increase in palatability if the solution provided it is at a lower temperature.

2.5.8 Factors influencing intestinal absorption

Water and many electrolytes move in both directions relatively easily across the mucosa of the small intestine. This movement occurs under the influence of the prevailing relative osmotic, concentration and electrochemical gradients, with the net movement of water and solute representing the difference between their individual bi-directional mucosal fluxes (Guandalini, 1988). Water absorption in the intestine is mainly a passive process caused by the creation of local osmotic gradients that promote greater net movement of water out of the intestinal lumen (Leiper and Maughan, 1988). Hypotonicity of the luminal contents *per se* will also establish osmotic gradients that can promote water absorption (Maughan, 1991).

The brush border membrane of the enterocytes covering the intestinal villi and the intercellular junctions between these cells, provide the major barrier for absorption of carbohydrate. Within the membrane there is a variety of transport carriers of differing specificity that unidirectionally translocate carbohydrate monomers, amino acids, peptides and other organic solutes into the cytoplasm of the cell (Silk and Dawson, 1979). The energy dependent active carriers usually require the presence of sodium ions that are cotransported with the nutrient molecule (Leiper, 1998) and are therefore dependent upon the activity of the sodium-potassium-ATPase pumps (Silk and Dawson, 1979). Diffusion facilitating carriers are driven by a concentration gradient favouring transport of the specific solute; however, the carrier mechanism increases the rate of transport compared with simple diffusion (Guandalini, 1988).

2.5.8.1 Osmolality

Studies using the technique of segmental perfusion to measure intestinal absorption (Schedl *et al.*, 1994) indicate that water absorption is inversely related to the osmolality of the perfusate (Cunha Ferreira *et al.*, 1992; Hunt *et al.*, 1991). Hypotonic solutions produce greater water absorption as compared with iso- or hypertonic

solutions. Most studies have shown that solutions, even those containing carbohydrate and sodium, with an osmolality of less than 200 mOsmol.kg⁻¹ promote slower rates of water absorption than those with an osmolality between 200 and 260 mOsmol.kg⁻¹ (Leiper, 1998). The most effective osmolality range for rehydration solutions appears to be relatively narrow and controversy still exists regarding the optimal osmolality of an ingested solution to promote optimal absorption (Shi and Gisolfi, 1998).

2.5.8.2 Carbohydrate and intestinal absorption

Solutions with a very high glucose concentration will not necessarily promote an increased glucose uptake relative to more dilute solutions, but, because of their osmolality, will cause a net movement of fluid into the intestinal lumen (Maughan and Noakes, 1991). Although in perfusion studies glucose absorption continues to increase up to a concentration of at least 200 mmol.l⁻¹, water absorption tends to be faster from carbohydrate solutions containing between 50 to 100 mmol.l⁻¹ (Leiper, 1998). As this effect is mainly due to an increase in osmolality, more complex carbohydrates have been used to increase the carbohydrate content without markedly increasing the osmolality. Glucose polymers or maltose have been used to try and activate the maximum number of glucose transporters in the intestinal segment. Perfusion of a segment of jejunum with hypotonic solutions with similar osmolality but with different types of carbohydrate failed to show any increase in the rate of water absorption (Leiper *et al.*, 1996). There was, however, a tendency for water uptake to be slower from the solution containing mainly sucrose. Many authors have found that water uptake from sucrose solutions is slower than from glucose solutions; this finding has been attributed to an increase in osmolality caused by an accumulation of fructose hydrolysed from sucrose. Absorption of fructose is slower than an equivalent amount of glucose due to the difference in the brush border carrier mechanisms that are used by the two monosaccharides (Leiper, 1998).

2.5.9 Value of carbohydrate during exercise

The basic hypothesis underlying carbohydrate ingestion is it will boost blood glucose, providing additional substrate for the working muscle and thus spare local glycogen reserves (Shephard and Leatt, 1987). Whereas increased blood glucose concentration following glucose feeding before exercise has been confirmed (Hargreaves *et al.*, 1984; Ivy *et al.*, 1979), the value of the added glucose is yet to be conclusively established.

Tsintzas *et al.* (1995a) reported that glycogen utilisation was 28% less after a 60 min treadmill run when subjects had consumed a 5.5% carbohydrate solution during the run, compared with the utilisation rate when they drank water. In more prolonged (4 hour) exercise consisting of low intensity cycle exercise interspersed with high intensity sprints, Hargreaves *et al.* (1984) found that muscle glycogen utilisation during the last 3 hours of exercise was 37% less in subjects who had consumed drinks containing 43 g of sucrose per hour compared to a placebo solution. Following 90 min of the Loughborough Intermittent Shuttle Test (LIST), Nicholas *et al.* (1994) identified that ingesting a 6.9% carbohydrate electrolyte solution resulted in a 22% reduction in muscle glycogen utilisation compared with ingesting an equal amount of a non-carbohydrate electrolyte solution.

Other studies which have employed a variety of exercise models, types and amounts of carbohydrate solutions during exercise, have shown no effect of carbohydrate feeding on muscle glycogen utilisation (Coyle *et al.*, 1986, Hargreaves and Briggs, 1988). These and other authors (Coggan and Coyle, 1991) suggested that the beneficial effect of ingesting carbohydrate lies in the maintenance of an adequate total carbohydrate availability during the final stages of prolonged exercise rather than the sparing of muscle glycogen. At present the reason for these different results is not clear. Tsintzas *et al.* (1995) showed glycogen sparing during the first hour of prolonged exercise rather than at the end of exercise (see Tsintzas and Williams, 1998).

2.5.10 Rehydration and post-exercise recovery

Even when fluids are available, the volume ingested is seldom sufficient to match the rate of sweat loss, and some degree of fluid deficit normally accompanies exercise. Replacement of these losses, as well as replenishment of energy substrate stores, must be achieved in the recovery period, before the next training period or competition (Maughan, 1998).

The major electrolytes lost in sweat are sodium and chloride. Due to the large variability in the electrolyte loss that occurs in different situations, it has been difficult to formulate a general recommendation for electrolyte replacement (Maughan, 1998). Costill and Sparks (1973) first highlighted the importance of electrolytes in promoting post-exercise rehydration. The ingestion of a glucose-electrolyte solution after a reduction in body mass of 4%, resulted in a greater restoration of plasma volume than plain water. González-Alonso *et al.* (1992) confirmed that a dilute carbohydrate-electrolyte solution (60 g.l⁻¹ carbohydrate) is more effective in promoting post-exercise rehydration than either plain water or a low-electrolyte diet cola. The mechanism of this action was not identified in either study but the authors did establish, that because of the high urine flow that ensued, even drinking large volumes of electrolyte free drinks did not allow subjects to remain in positive fluid balance for more than a short time.

Nose *et al.*, (1988a) showed that ingestion of plain water in the post-exercise period results in a rapid fall in plasma sodium and plasma osmolality. Both of these factors are important in determining fluid balance and have the effect of reducing the stimulus to drink and of stimulating urine output. A reduced fluid intake and an increased urine production will delay or prevent the rehydration process. When plain water was ingested together with capsules containing sucrose, plasma volume was not restored until after 60 min. In contrast, when sodium chloride capsules were ingested with the water to give a saline solution with an effective concentration of 0.45% (77 mmol.l⁻¹), plasma volume was restored within 20 min. In the sodium chloride

trial, voluntary intake was higher and urine output was less; 71% of the water loss was retained within 3 h compared with 51% in the plain water trial (Nose *et al.*, 1988a). It was later established that the delayed rehydration in the water trial was due to a reduction in the stimulus for plasma renin activity and aldosterone release, thus allowing a greater urinary loss of sodium and water (Nose *et al.*, 1988b).

Maughan and Leiper (1995) evaluated the importance of the addition of sodium to rehydration fluids. Subjects were initially dehydrated by the equivalent of 1.9% of body mass by intermittent exercise on four occasions. Beginning 30 min after the exercise period, subjects ingested a volume of fluid equal to 150% of the body mass lost. One of the test drinks was consumed over a 60 min period and the subjects' recovery was followed for a further 6 h. The test drinks contained 2, 26, 52, or 100 mmol.l⁻¹ sodium as well as flavouring intended to mask, the taste differences. Urine output over the subsequent few hours was inversely proportional to the sodium content of the ingested fluid. Only when the sodium content exceeded 50 mmol.l⁻¹ were the subjects in positive fluid balance throughout the recovery period. These observations were confirmed in a further study that examined the interaction between the volume of fluid ingested and the sodium content of rehydration drinks (Shirreffs *et al.*, 1996). Drinking large volumes of fluid (twice the sweat loss) did not allow subjects to remain in positive fluid balance for more than 2 h when the sodium content of the drinks was low (23 mmol.l⁻¹). Increasing the sodium content to 61 mmol.l⁻¹ allowed subjects to remain in positive fluid balance when volumes equal to 1.5 times or twice the sweat loss were ingested. When smaller volumes of fluid, equal to the sweat loss, were consumed, effective rehydration was not achieved due to ongoing urine losses. It is clear that rehydration after exercise can only be achieved if the sodium concentration and volume of the rehydration solution is greater than that lost during exercise (Maughan, 1998).

2.5.11 Fluid intake and soccer performance

Foster *et al.* (1986) found that the administration of a 300 ml, 25% glucose polymer solution in the hour interval between two indoor soccer matches increased the total cruise and walk-jog distance covered by the players. Following glucose ingestion of a similar concentration, taken before and at the half-time interval of a game, Kirkendall (1988) observed that players covered greater distances (25%) and achieved those distances at greater intensities than players who had consumed a placebo solution. These improvements were attributed to two possible mechanisms, the elevation of blood glucose to spare muscle glycogen or by offering an alternative fuel source once glycogen had been depleted.

Jacobs (1989) studied the influence of drinking a glucose polymer solution on muscle glycogen utilisation. Although blood glucose concentrations remained similar between those players who had and had not taken a glucose drink, muscle glycogen was 31% greater in the players who ingested the glucose solution (500 ml of 7% glucose; 10 min before and at half time). As blood glucose concentrations were similar, Jacobs (1989) concluded that the beneficial effect of carbohydrate feeding was to decrease the utilisation of glycogen rather than prevent hypoglycaemia. Furthermore Jacobs (1989) suggested that avoiding or delaying the time of onset of glycogen depletion may commensurately delay or avoid the performance impairments found by authors such as Saltin (1973).

2.6 Human performance, control and skill

In order for performance to be successful the performer must be in control of the situation. The notion of control involves the processing of information and the use of this information to ensure that the output matches the input (Glencross, 1980). When considering skilled performance, the principal components of the control system include input and perceptual processes, central and translatory processes and output and motor processes. Input and perceptual processes are concerned with discriminating and giving meaning to incoming data. Central and translatory processes relate to perception and memory and initiating decisions about the appropriate actions and responses. Finally, output and motor processes specify the serial organisation of the actual movement patterns (Glencross, 1980). Failure to complete a skill successfully may be due to a failure of any one of these processes.

Acquisition of skills which involves complex information processing and in which there is high uncertainty in input and output takes place over extended periods of time. The development of skill and hence control, may be related to adopting the most suitable strategy or plan of action. In most situations, there are usually a number of alternative methods or strategies for meeting the task demands. “ Some strategies are more efficient than others and what is termed skill seems to lie in choosing the most efficient out of any range available” (Welford, 1976).

2.6.1 Exercise and cognitive function

Studies that have assessed the effects of exercise on cognitive function have used a variety of procedures and produced conflicting findings. In order to highlight some of these differences the studies have been classified by: exercise mode, the test or task of cognitive function employed, the duration of the task and the outcome of the particular study (Table 2).

Table 2.2: Studies of exercise and cognitive functioning

Study	Exercise mode	Cognitive task	Time of task	Findings
Bills (1927)	Hand dynamometer	Addition	During exercise	Facilitation
Davey (1973)	Cycle ergometer (constant resistance; 15s, 30s, 2, 5 and 10 min)	Short term memory	After exercise	Facilitation after 30 s and 2 min; impairment after 10 min
Flynn (1972)	Cycle ergometer (5 resistive loads; 6 min)	Simple arithmetic	After exercise	No effect
Gutin & DiGennaro (1968)	Treadmill run to exhaustion	Simple addition	After exercise	Impairment
McGlynn, Laughlin, & Bender (1977)	Treadmill run (12 min)	Discrimination task	During and after exercise	Facilitation during exercise
Sjöberg (1980)	Cycle ergometer (3 resistive loads; 12 min)	Short term memory; simple arithmetic	During and after exercise	Impairment of low fitness subjects on arithmetic after exercise
Tomporowski, Ellis & Stephens (1985)	Treadmill run (80%VO ₂ max to exhaustion)	Free-recall test of memory	After exercise	No effect
Marriott, Reilly & Miles (1991)	90 min treadmill simulated soccer test	Decision making	After exercise	No effect
McMorris & Keen (1994)	cycle ergometer (70% and 100% max power output)	Reaction time	During exercise	No effect during 70% Impairment at 100%
McMorris & Graydon (1998)	cycle ergometer (70% and 100% max power output)	Speed of visual search	During exercise	Improvement of speed of search with increasing intensity

Tomporowski and Ellis (1986) proposed two possible explanations for the discrepancies in the literature. The first explanation assumes that exercise does alter cognitive functioning and the disparate results are due to two states brought about by exercise; arousal of the central nervous system and physical fatigue of the skeletal-motor system. It is suggested that exercise may initially facilitate attentional processes by directly affecting the central nervous system, but as exercise intensity or duration increases, the debilitating effects of muscular fatigue may cancel the facilitative effects of exercise (Tomporowski and Ellis, 1986). It is believed that the dominant state determines the ability of subjects to perform tests of cognition. Thus it may be possible for exercise to either facilitate or impair performance on the same cognitive test depending on the level of physical fitness of the subject and the point at which the subject is tested. The second explanation for the variety of findings relating to exercise and cognitive functioning assumes that exercise *per se* does not alter cognitive functioning, but rather that motivational variables affect test performance. This is, that people who do not exercise on a regular basis tend to view exercise as both physically and psychologically stressful, and may expect that exercise will produce a decrement in their abilities. Thus, motivational variables may play an important role in all studies assessing the effects of exercise on mental performance (Tomporowski and Ellis, 1986).

2.6.2 Fatigue and motor learning

Motor skill learning and performance has received a considerable amount of attention. To explore this area investigators have typically employed a simple transfer paradigm in which some of the subjects are fatigued during day 1 (acquisition trials) and are then switched to practice without fatigue (control conditions) on day 2 (Schmidt, 1969). If fatigue depresses the performance of experimental subjects only on the first day, it is considered a performance variable. Should decrements persist after transfer to practice on the subsequent day, then fatigue is concluded to be a learning variable. That is, practice under fatigue conditions is assumed to distort mechanisms that govern learning as well as performance (Wrisberg and Herbert, 1976). Research in this area has indicated that fatigue is principally a performance variable. Practice in a

state of physical fatigue results in immediate motor performance impairment, but motor learning is largely unaffected (Alderman, 1965; Carron, 1969; Cotton *et al.*, 1974; Pack *et al.*, 1974). There are some exceptions to this; some studies have indicated that learning is also affected by fatigue (Barnett *et al.*, 1973; Carron and Ferchuk, 1971)

As with the studies investigating exercise and cognitive function, the diversity of findings seems to have resulted from the use of many different motor tasks under a variety of experimental conditions. The major cause of these diverse findings appears to be exercise intensity. For example, three different investigations of the effects of exercise on a stabilometer, indicated that either exercise facilitated learning and performance (Cochran, 1975), produced null effects (Bartz & Smithy, 1970), or impaired both (Carron and Ferchuk, 1971).

Comparisons among studies using different motor tasks indicate that while light exercise can have a facilitative effect (Benson, 1968; Cochran, 1975; Richards, 1968), moderate levels of exercise usually impair performance without affecting learning (Alderman, 1965; Carron, 1969; Whitley, 1973). High levels of exercise generally cause a decrement in both performance and learning (Williams *et al.*, 1977). The changes in performance under conditions of heavy physical exertion have been related to the accumulation of acid metabolites (Carron, 1972). Williams *et al.* (1977) suggested that under such conditions a motor task may change enough to require different neuromotor coordination patterns for control. This is a theory shared by Berger and Smith-Hale (1991). In their study, practising while fatigued not only reduced the quality of performance but also impeded the learning of a gross motor task. They suggested that the physiological event associated with high levels of neuromuscular fatigue influenced performance in a relatively permanent way and thus adversely affected motor learning of relatively complex total body movements. The reasoning behind this was that neural integration with muscle fatigue or force generation might not involve the usual ordering of motor unit recruitment. This idea is based on evidence that the usual patterns of motor activity that minimise metabolic cost of work, are sacrificed in ballistic type movements in favour of mechanical force

(Burke, 1984). Berger and Smith-Hale (1991) suggested that if this correct, then the usual patterns of motor activity under conditions of minimal fatigue would activate more type II motor units in attempts to achieve the necessary forces to accomplish the task.

2.6.3 Skill and lowered blood glucose

Based on circumstantial evidence that a modest reduction in blood glucose during exercise reduced control of movement in healthy adults, Brooke *et al.* (1982) investigated the impact of exercise-induced low blood glucose and skill under controlled conditions. Their work originated from findings of carbohydrate insufficiency being implicated in imprecision of actions of forge workers (Brooke *et al.*, 1980), pilots (Fraser *et al.*, 1974), motorway drivers (Christian, 1972) and people missing breakfast (Tuttle *et al.*, 1949). Due to the problems of poor experimental control with such studies, Brooke *et al.* (1982) investigated the control of movement of muscle and joint complexes and exogenous or endogenous interventions under standardised conditions. To study such a complex movement, Brooke *et al.* (1981) developed an on-line measurement of the muscle forces applied in two-legged cycling on an ergometer. With a computerised system, calculations were made of the variability of force applied at 26 points in the revolution of each pedal. Increases in the variation are suggested to indicate decreased neural control. Subjects exercised for 20 min on 6 occasions. Exercise involved loads that elicited approximately 60% (3 trials) and 40% (3 trials) $\dot{V} O_2$ max on the modified cycle ergometer. For each workload, trials for three different dietary conditions were made: a) 3.5 h postprandial (called fasting), b) the fast terminated by ingestion of 100 g of glucose dissolved in 150 ml distilled water and 10 ml of lemon juice at the start of exercise and c) as in b) but fed 30 min before exercise. Blood glucose concentrations fell modestly below basal values with exercise under conditions a) and c) but not b). These two treatments i.e. a) and c) were more variable in force applied and with altered pattern of average forces. Brooke *et al.* (1982) suggested that such deterioration of movement control was due to nutritional and exercise changes that disturb the neural control of a habituated adapted task.

2.6.4 Impact of raising blood glucose

Early studies relating to the effects of breakfast on performance were carried out in the 1940's and 50's and were collectively known as the Iowa Breakfast Studies. Tuttle *et al.* (1949, 1950, 1952, and 1954) compared the effects of breakfast vs. no-breakfast on mental and physical performance. It was these studies that were influential in promoting the common belief that avoiding breakfast is detrimental to performance. The actual findings, were inconsistent and have been criticised (Dickie and Bender, 1982) for their small subject numbers, the use of only simple reaction time as a performance measure and the subjective nature of some of the other assessments.

Under more controlled conditions Lloyd *et al.* (1996) demonstrated significant acute effects of the manipulation of the fat to carbohydrate ratio of breakfast on morning mood in habitual breakfast eaters. In agreement with a previous study on the effects of lunch (Lloyd *et al.*, 1994), consumption of a breakfast low in fat (27%) and high in carbohydrate (62%), resulted in a more positive mood state than after a iso-caloric meal higher in fat (38.5%) and lower in carbohydrate (34%). The mechanisms underlying such behavioural effects are less clear. Modulation of serotonergic function by high carbohydrate low protein meals has been suggested as one route by which the post absorptive effects of food could alter mood (Rogers, 1995). Meals have also been suggested to influence brain functioning through effects on gut hormones released in response to eating (Young, 1993). Stacher *et al.* (1979) demonstrated substantial dose-related effects of intravenously administered cholecystikinin (CCK) on mood and performance (with subjects feeling more relaxed, drowsy, sluggish and inert with increasing doses of the hormone). Lloyd *et al.* (1996) suggested that this latter result is consistent with the fact that fat and protein are more potent stimulants for CCK release than carbohydrate (Liddle *et al.*, 1983). Therefore CCK concentrations may be involved in the mechanism responsible for the different mood states following meals.

It has been proposed that improvement in memory, associated with consumption of breakfast, is linked to the availability of glucose for the brain. A series of studies reported an enhancement of memory following glucose drinks in the elderly (Goner-Frederick *et al.*, 1987; Hall *et al.*, 1989; Manning *et al.*, 1990) and younger adults (Lapp, 1981; Benton and Owens, 1993). Benton and Sargent (1992) reported a significant correlation between the concentration of blood glucose and performance in a test of spatial memory in a group of university students. The higher the blood glucose concentration the better the performance, a finding supported by Smith *et al.* (1992).

There is not complete agreement about the influence of changes in blood glucose concentrations on cognitive performance. Smith *et al.* (1994) found no differences in reaction time performance or sustained attention between subjects given breakfast or fasted and Pollitt *et al.* (1981) observed an improvement in memory with fasting. Given this evidence, Owens and Benton (1994) investigated the impact of raising blood glucose on other measures of human ability including inspection and reaction time. Under a double-blind procedure, 96 subjects were allocated to one of two conditions, receiving either a drink containing 50 g of glucose or a placebo drink. Inspection and reaction times were tested 15 min after the ingestion of the drink. Inspection time was unaffected by glucose ingestion and blood glucose concentration. However, increasing blood glucose concentration resulted in faster decision times when reaction time was measured. Benefits of glucose ingestion were observed despite the fact blood glucose concentrations did not fall to hypoglycaemic values (2.2 mmol.l^{-1}). It appears that the change in blood glucose rather than the absolute value was the critical factor (Owens and Benton, 1994). Given that blood glucose concentration was well within the normal range, it was suggested that glucose could influence information processing under frequently controlling physiological conditions. As the study found lower intra-individual variability when blood glucose concentrations were increasing, the authors suggested that an increased blood supply of glucose results in more consistent functioning near the physiological limit.

The improved decision times and reduced variability with the increasing glucose concentration were suggested to be due to an increased production and release of acetylcholine. This was based on the fact that stimulation of acetylcholine synthesis is a possible mechanism that could mediate the impact of glucose on memory (Durkin *et al.*, 1992) and reaction times. Durkin *et al.* (1992) produced the first experimental evidence that raised concentrations of glucose facilitate acetylcholine synthesis when they measured acetylcholine from the rat hippocampus during conditions of increased neural activity. In mice, raising glucose concentration attenuates the amnesia induced by the anticholinergic drug scopolamine. As short-term memory can limit information processing, Owens and Benton (1994) suggested that greater supplies of acetylcholine may have improved the memory capacity of their subjects. As a result this allowed them to process information faster when the decision making task approached the limits of short term memory (Owens and Benton, 1994). They went on to suggest that taken together these studies provide evidence that under periods of increased neural activity, raising the glucose supply is associated with a beneficial increased synthesis of acetylcholine. However, other explanations such as enhanced neuronal efficiency or attention could also account for the findings.

2.6.5 Carbohydrate ingestion and skill performance

The maintenance of plasma glucose concentration with a carbohydrate drink has been suggested to reduce error and maintain skill performance in tennis (Burke and Ekblom, 1982; Keul, 1995; McCarthy, 1997). Following a 2 h tennis simulation test, Burke and Ekblom (1982) showed a reduction in the amount of hitting error and an 11.6% improvement in explosive power (vertical jump) with carbohydrate ingestion. It was suggested that carbohydrate availability in the form of a drink not only maintains plasma glucose for muscle metabolism, but may also maintain the metabolism of nerve cells and thus the integration controlling the contraction of skeletal muscle over long periods (Burke and Ekblom, 1992). In a study of tennis hitting to exhaustion, McCarthy (1997) showed that the ingestion of a 6.9%

carbohydrate-electrolyte solution maintained tennis hitting accuracy in comparison to a drop in performance during a placebo trial.

Carbohydrate ingestion also appears to improve motor racing skill. This is based upon work performed using a driving simulator, in which all driving and reaction errors were registered (Keul *et al.*, 1982). The error rate in the last third of a 110 km driving test was significantly lower following glucose administration. Concentration exercises performed prior to and after the driving simulation test improved following glucose administration. Keul (1995) suggested that nutrition should not only be planned for sports requiring a high energy level, but also for those which are limited by mental and neuromuscular parameters.

2.6.6 Soccer skill

Playing soccer involves the use of cognitive, perceptual and motor skills (Bate, 1996). Skills are generally expressed as being either open or closed and gross or motor. If the environment is stable and predictable then skill is classified as closed, whereas if the skill involves an ever changing, unpredictable environment, then the skill is classified as being open (Magill, 1989). Soccer has been defined as an open-skilled game requiring rapid responses to unpredictable situations but with some closed-skill elements such as free kicks, corner and penalty kicks (Bate, 1996).

Gross motor skills are characterised as involving large musculature where the precision of movement is not as important to the successful execution of the skill as it is for fine motor skills. Fine motor skills require control of the small muscles of the body to achieve the aim of the skill (Magill, 1989). Examples of gross motor skills include running, jumping and throwing whereas fine skills are those such as writing, drawing or playing the piano. Soccer skill falls on the continuum between gross and fine skills.

Although there have been numerous studies into the game of soccer there are surprisingly few studies that have investigated fatigue and soccer skill. Those that have attempted to investigate this topic (Muckle, 1973; Zeederberg *et al.*, 1996) have studied skill performance during match play and therefore the results reported may be questioned because of other factors influencing the outcome. This may be the reason for the contrasting findings of these authors, with Muckle (1973) suggesting an increase in soccer skill with carbohydrate ingestion and Zeederberg *et al.* (1996) finding no increase in playing performance with carbohydrate ingestion.

CHAPTER 3

GENERAL METHODS

3.1. Introduction

The specific procedures followed in each study are described in the methods section of each experimental chapter. Methods common to all the studies in this thesis are described in this chapter.

All procedures were approved by the Ethical Advisory Committee of Loughborough University and were carried out in accordance with the 'Code of Practice for Workers having Contact with Body Fluids'. All individuals who expressed an interest in the studies were fully informed about the aims, procedures and the demands that the study would place upon them. In addition any possible risks and discomforts were explained before written consent was obtained. Subjects were also required to complete a health history questionnaire and were informed that they could withdraw from the study at any time, even after giving their written consent. Subjects with diabetes mellitus, asthma or any other medical condition were excluded (Appendix A).

This chapter consists of two sub-sections. The first section outlines the apparatus and procedures employed during the administration of the preliminary and main experimental tests. The second describes the procedures concerned with the collection, treatment, storage and analysis of blood samples.

3.2 Experimental testing procedures

3.2.1. Preliminary measurement - estimation of maximal oxygen uptake

Maximal oxygen uptake ($\dot{V}O_2 \text{ max}$) was estimated by means of the progressive multistage fitness test, following speeds dictated by a pre-recorded tape (Ramsbottom *et al.*, 1988), according to the original protocol (Leger and Lambert, 1982). No more than 5, and no less than 3 subjects performed the test on any one occasion, for reasons of space confinements and motivation. In addition, subjects were verbally encouraged throughout the test. A 10 min warm up consisting of running and stretching, ad libitum, was allowed before the start of the test. During this time the subjects were reminded that the test was progressive and maximal and that they should continue to run at the required pace for as long as possible.

During the multistage test, subjects were required to complete the 20-m shuttles at a progressively increasing speed. The test began at a shuttle speed of $2.22 \text{ m}\cdot\text{s}^{-1}$, which increased by approximately $0.14 \text{ m}\cdot\text{s}^{-1}$ every minute. Auditory signals, or bleeps were generated from a cassette player (Sanyo, model MCD-231L) to indicate the pace at which each 20 m should be covered. At the end of each shuttle, subjects were required to place one foot on the taped line marking 20-m. Individuals ran until they were unable to maintain the required pace and withdrew themselves, or were withdrawn by one of the investigators. The level attained and the number of shuttles reached, when the subjects retired, were recorded and maximal oxygen uptake was estimated using a table of predicted values (Ramsbottom *et al.* 1988). Speeds (shuttle levels) equivalent to 55% and 95% of each individual's estimated $\dot{V}O_2 \text{ max}$ were then calculated.

Following completion of preliminary measurements, subjects were familiarised with test procedures. They were obliged to become accustomed to the pace required during the different exercise intensities within one exercise cycle, the ingestion of fluid between exercise bouts, heart rate telemetry and the Borg Scale of perceived exertion.

Familiarisation with the skill tests was also included which required the subjects to perform the tests until they achieved a consistent score. A full description of the skill tests used can be found later in this chapter.

3.2.2 Apparatus and instrumentation

The experimental trials were performed in the Loughborough University Sports Hall on a flat, non-slippery wooden surface and in an adjacent sport science laboratory. Two striped lines were marked (Rabonne Chesterman 30-m steel tape) on the floor of the Sports Hall to indicate the 20-m distance. A 10-m line was also measured and marked to indicate the halfway mark for the 20-m shuttle run.

3.2.3 The Loughborough Intermittent Shuttle Test (LIST)

Due to the lack of control of the pattern and intensity of exercise in a game of soccer, it is difficult to assess the benefits of intervention, such as fluid ingestion. Therefore, to investigate the influence of free running on selected soccer skills a protocol was required which closely resembles the activity pattern of a soccer match. The exercise protocol used during the main trials of this thesis was based on the LIST developed by Nicholas *et al.* (1999). Some alterations were made, most notably the elimination of the run to exhaustion. The LIST in the present studies consisted of the main body of the original design, which consisted of 6 cycles of a standard set of exercise. Each set consisted of the following :

	PACE	DISTANCE	INTENSITY
1	Walking	3 x 20 metres	1.54 m.s. ⁻¹
2	Maximum sprint	1 x 15 metres	Maximum Speed
3	Recovery/Walk	~ 3 metres	4 second duration
4	Running (Cruise)	3 x 20 metres	~ 95% $\dot{V}O_2$ max
5	Jogging	3 x 20 metres	~ 55% $\dot{V}O_2$ max

Two auditory signals, or bleeps were generated from a micro-computer (BBC Master Series) to indicate the pace at which each 20 m should be covered. These signals were amplified via a computer programme (H.K.A. Lakomy, Loughborough University unpublished) for the purpose of the development of the LIST. The first bleep indicated when subjects should be at the start or end of each run and the second, a higher pitched tone, signified when they should be in the middle. Sprint times were determined in one direction over the first 15 m by 2 infrared photoelectric cells (RS Components Ltd), interfaced with the microcomputer.

Ten completed cycles plus 3 walks and a sprint were performed per set of activity. Each set lasted approximately 15-min and was separated by 3-min rest. The six 15-min sets and five 3-min rest periods during each trial resulted in a total exercise time of 90 min and a rest interval of 15 min.

3.2.4 Environmental conditions

Dry and wet bulb temperatures were measured at 15-min intervals during exercise using a whirling hygrometer (Brannan Thermometers Ltd). From these values, relative humidity was calculated. All experimental tests were conducted in an ambient temperature ranging between 13-20 °C. Barometric pressure was measured using a wall-mounted barometer (Griffin and George Ltd.).

3.2.5 Heart rate monitoring and subjective ratings of perceived exertion

In all preliminary and experimental tests, heart rate was monitored throughout exercise by short range radio telemetry (Polar Sport Tester™, Polar Electro, Kempele, Finland) and stored in memory mode. Subjective ratings of perceived exertion (Borg, 1973) were obtained during the last few minutes of each 15-min block of exercise.

3.2.6 Expired air collection and analysis

Expired air was collected using either modified Douglas bag equipment or a portable gas analyser (Aerosport[®] KB1 - C) (Chapter 5).

3.2.5.1 The modified Douglas bag technique

To collect expired air samples during the LIST a modified Douglas bag collection technique was used. A 150 l capacity Douglas bag (Harvard Equipment Ltd.) was attached to the frame of a rucksack for conveyance during the protocol. The rucksack was placed on the subject during the walk phase of the cycle prior to collection. Subjects were handed a noseclip (Harvard Equipment Ltd.) and mouthpiece (Harvard Equipment Ltd.) to ensure evacuation of 'dead space' with expiratory air. The mouthpiece was connected to the Douglas bag via a lightweight two-way valve (Jakeman and Davies 1979), a 0.5 m length of wide-bore (30 mm) lightweight tubing (Fulconia; Baxter, Woodhouse and Taylor Ltd.) and a two-way tap (Harvard Equipment Ltd.). Thus, a closed circuit was formed when the nose-clip and mouthpiece were correctly worn, allowing expired air to be collected over the measured time interval of one cycle of the LIST.

A paramagnetic oxygen analyser (Sybron - Taylor, model 570A) was used to measure the percentage oxygen content of expired air. This operates on the basis of the susceptibility of oxygen to a paramagnetic gas. The analyser was accurate to $\pm 0.01\%$ and provided oxygen concentration as a digital readout. The percentage carbon dioxide of expired air was measured by an infrared carbon dioxide analyser (Lira, model 303; Mines Safety Appliances Ltd). This has an analogue scale from which carbon dioxide concentrations were calculated with reference to a calibration curve. Both analysers were calibrated against nitrogen, a calibration gas, and room air immediately prior to each series of gas analyses.

A digital dry gas meter (Harvard Equipment Ltd.) was used to determine gas volumes. This had previously been calibrated using a 600 l Tissot Spirometer (Collins Ltd,

USA). The temperature of expired air was monitored as each bag was evacuated by a thermistor probe (Edale type 2984, Model C). This was fitted on the inner surface of the air inlet pipe connecting the Douglas bag to the gas meter. The analogue scale of the thermistor was calibrated prior to each set of analyses against two internal standard settings of 25°C and 50°C.

Using the temperature of the expired air and the barometric pressure, all the gas volumes obtained were corrected to Standard Temperature and Pressure of a Dry gas (STPD). Thus the values for ventilation rate, oxygen and carbon dioxide concentration were used in the Haldane transformation to calculate the oxygen uptake ($\dot{V}O_2$) and carbon dioxide production ($\dot{V}CO_2$). From these values of $\dot{V}O_2$ and $\dot{V}CO_2$ the respiratory exchange ratio (RER) was also calculated using software designed for this purpose (D.G. Kerwin).

3.2.5.2 Estimation of energy expenditure

Energy expenditure was estimated by indirect calorimetry, using open circuit spirometry. The proportions of energy derived from carbohydrate and fat were estimated from the non-protein respiratory exchange ratio (RER) value. This assumes that the contribution of protein to energy metabolism is relatively small (Consolazio, Johnson and Pecora, 1963).

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

The oxidation of 1 g of carbohydrate uses 0.828 l of oxygen, and produces 0.828 l of carbon dioxide and 17 kJ of energy. The oxidation of 1 g of fat uses 1.989 l of oxygen and produces 1.419 l of carbon dioxide and 39 kJ of energy. Whole body oxygen consumption ($\dot{V}O_2$) and carbon dioxide ($\dot{V}CO_2$) production is calculated from expired air analyses. Rates of carbohydrate ($x \text{ g}\cdot\text{min}^{-1}$) and fat ($y \text{ g}\cdot\text{min}^{-1}$) oxidation can be determined using simultaneous equations:

$$\dot{V}O_2 = 0.828 x + 1.989 y \dots (i)$$

$$\dot{V}CO_2 = 0.828 x + 1.419 y \dots (ii)$$

Solving for 'x' and 'y' by subtracting (ii) from (i), gives rise to the following equations of energy metabolism:

$$x = \frac{\dot{V}O_2 - (y * 1.989)}{0.828} \text{ where, } y = \frac{\dot{V}O_2 - \dot{V}CO_2}{0.570}$$

Thus total energy expenditure is given by:

$$\text{Energy Expenditure} = (x * 17) + (y * 39) \text{ kJ min}^{-1}$$

3.2.5.3 Aerosport[®] KB1 - C Portable Gas Analyser (PGA)

The PGA measures 22.9 by 12.1 by 7.6 cm (width by depth by height) and weighs approximately 1 kg. The respiratory gas is exhaled across a pneumotacheometer, where a micro-sample proportional to the expired flow is drawn off through the centre line of the pneumotacheometer. A sample is drawn into the mixing system at a fixed rate. The ventilatory volume is measured with a variable selector flatplate orifice pneumotacheometer. The flow of gas, which passes over the pneumotacheometer, produces a pressure drop proportional to the square root of the flow. For each sample drawn off for calculation of ventilatory volume, an identical volume of sample is emitted to the O₂ and CO₂ analysers. Oxygen uptake, carbon dioxide production and respiratory exchange ratio were calculated according to the principles described when using the adapted Douglas bag technique.

The PGA was set to record expired air every 60 seconds, on a medium flow rate setting of 10-120 l.min⁻¹ (STPD). Subjects were connected to the analyser via a mouthpiece and nose clip. The battery pack and main control panel rested both comfortably and securely on a velcro belt behind the subject. The subject inserted the

mouthpiece and data collection commenced; the data output from the PGA was stored and subsequently down-loaded into a lap top computer (Ezbook MegaPro 486D).

3.2.6 Body mass and height

In all studies nude body mass was determined to the nearest 0.1 kg using beam balance scales (Avery Ltd., Model 3306ABV). Body mass was recorded before exercise, after the subject had voided and immediately post-exercise, after sweat had been removed from the skin. From these measurements and after correcting for fluid intake, sweat rates were estimated for the exercise period. Height was evaluated to the nearest 0.1 cm using a stadiometre (Holtain Ltd). Gentle traction was applied on the mastoid process of the subject, in order to try and compensate for any shrinkage in the intervertebral discs. The experimenter ensured that the subject maintained heel contact with the heel plate and the floor during these measurements.

3.2.7 Nutritional control

Subjects reported for each experimental trial following an overnight fast of between 10 and 12 h. This was to ensure that subjects began each trial with an empty stomach, thus eliminating any negative effect a previous meal might have, both on exercise metabolism and gastric emptying. For the 2 days prior to each experimental 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 weighed and recorded their food and drink in a food record diary during the 2 days before the first trial. This diet was then replicated during the corresponding period prior to any further trials. From this information, an analysis of the nutritional content of their normal diets was made by a registered dietician. All energy values are reported as kcal rather than as kJoules because the former units are widely used by sports people. Subjects maintained the same training schedule which they had performed prior to the first trial for the corresponding time periods leading up to any

further main trials. These restrictions were also made to standardise as far as possible the initial state of hydration for each trial.

In Chapter 4 subjects were required to ingest a commercially available "No Added Sugar Concentrated Lemon Drink", diluted 1:4 with tap water; 52 mOsmol.kg⁻¹ (J Sainsbury plc, London, UK). During the studies reported in Chapters 5 and 6 subjects ingested a 6.4% carbohydrate electrolyte solution, which is a commercially available sports drink (Lucozade Sport, SmithKline Beecham) and an artificially sweetened placebo solution (SmithKline Beecham). Following exercise in the study reported in Chapter 7, subjects ingested a non-carbohydrate experimental sports drink (SmithKline Beecham). The administration of drinks in each study is described in the relevant chapters. The prescribed volume of fluid was measured using a measuring cylinder and stored in separate plastic drinking bottles, in order to avoid spillage and to ensure that the correct amount was consumed. During the studies reported in Chapters 4, 5 and 6 the subjects drank the prescribed fluids prior to the commencement of the LIST and during the 3-min recovery periods between exercise bouts.

3.3 The soccer skill tests

The skill tests (Chapters 4, 6 and 7) were devised specifically to fit within the dimensions of the sports hall used during the main trials. The tests had to be simple to set up, measure and most importantly be valid and reliable tests of selected soccer skill.

3.3.1 An examination of the validity and reliability of the soccer skill tests

Various statistical techniques have been adopted to assess whether a new method of measuring a variable is either repeatable or equivalent to an established method (Nevill and Atkinson, 1997). These techniques include Pearson's correlation coefficient, the correlated *t* test and repeated measures analysis of variance. Recently, such techniques have been criticised in particular the use of correlation coefficients because they are measures of relation rather than agreement (for a review see Atkinson, 1995). An alternative approach for assessing the reliability of a particular method has been proposed by Bland and Altman (1986) which involves reporting a simple interval known as the "limits of agreement". Assuming the differences between measurements are normally distributed, the limits should contain 95% of such differences. Bland and Altman (1986) argued that the scientist can then use their own judgement to assess the agreement associated with this interval and hence the measurement errors. In order to determine the reliability of the two tests of soccer skills to be employed within this thesis the level of agreement was calculated using the methods described by Bland and Altman (1986).

3.3.2 Subjects

Sixty seven male university level soccer players, aged 18 - 24 years, volunteered to participate in the study. Playing experience and ability ranged from county representative to semi-professional. Thirty players were randomly assigned to the

Loughborough Soccer Passing Test (LSPT) and thirty seven to the Loughborough Soccer Dribbling Test (LSDT).

3.3.3 Protocol

To complete the LSDT the players dribbled a ball between a line of 6 cones, 3 m apart, as fast as possible. An additional cone was used to indicate the starting line of the test. The players started with feet behind the starting line as illustrated in Figure 3.1. The examiner timed the player from the moment the ball was touched in a forward direction after the signal “go” was given. The time for the test finished when the player stopped the ball on the finishing line. The test was timed manually using a stopwatch (Accusplit 725 XP). The player had to finish with the ball in his control. Ten such attempts were made with a 1-min break between each and the total of all ten times was added to give a final score.

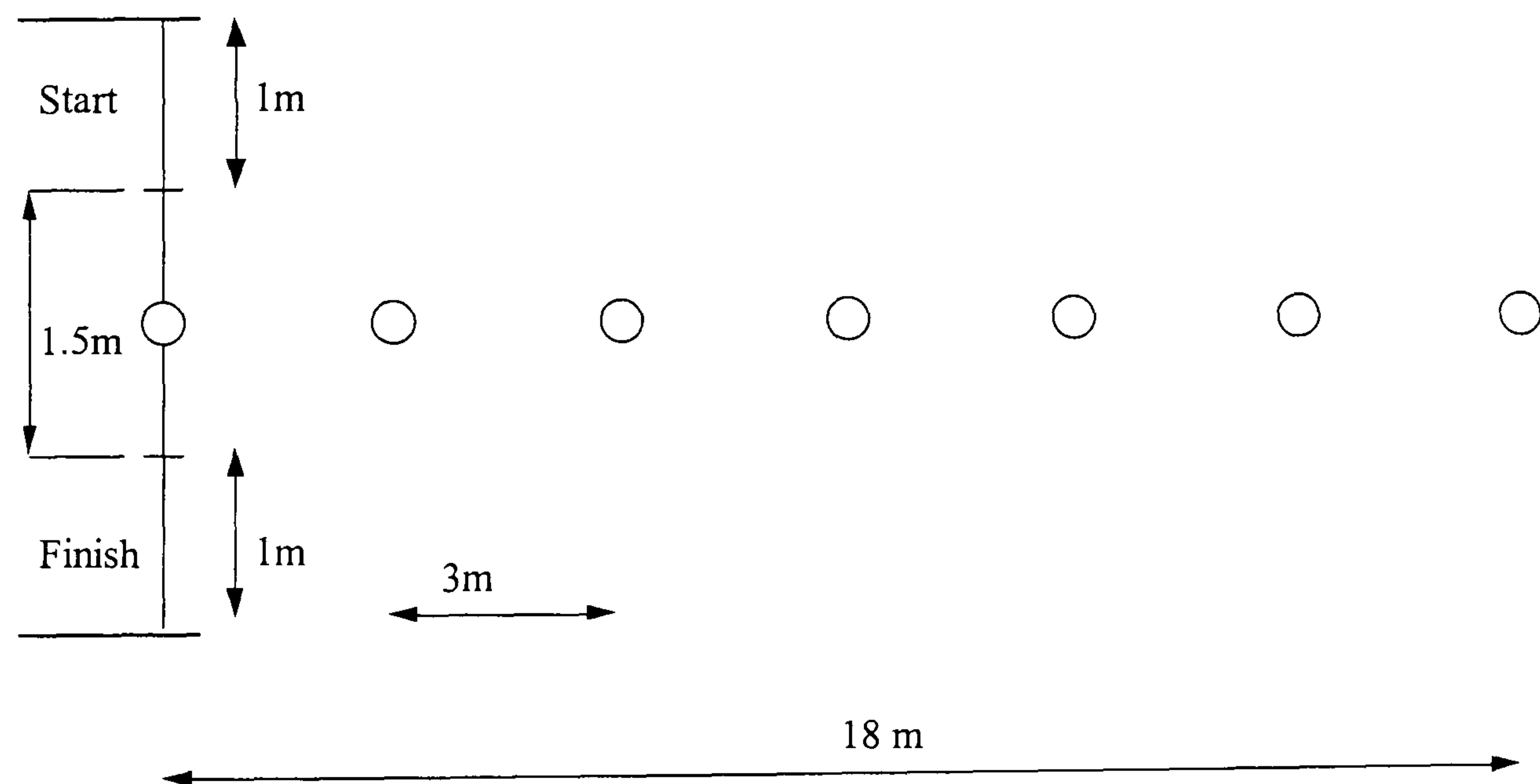


Figure 3.1: Diagrammatic representation of the Loughborough Soccer Dribbling Test (LSPT)

The LSPT involved the performance of 16 passes made from a central zone to four 0.5-m colour coded targets that were marked on to benches. The benches were used to allow the ball to rebound and the targets colour coded for identification. Small disc cones were placed around the central zone. The passes were randomly selected and called out to the player by the test examiner. The time to complete the test was recorded using a stop watch (Accusplit 725 XP). If the marked areas were missed, or one of the cones was hit during the test, additional 'penalty seconds' were added. If the player ventured out of the outer zone, missed the 0.5 m target or a cone was hit by the player, 2 s was added. If the bench was missed then a 5 s penalty was added. The LSPT is shown in Figure 3.2.

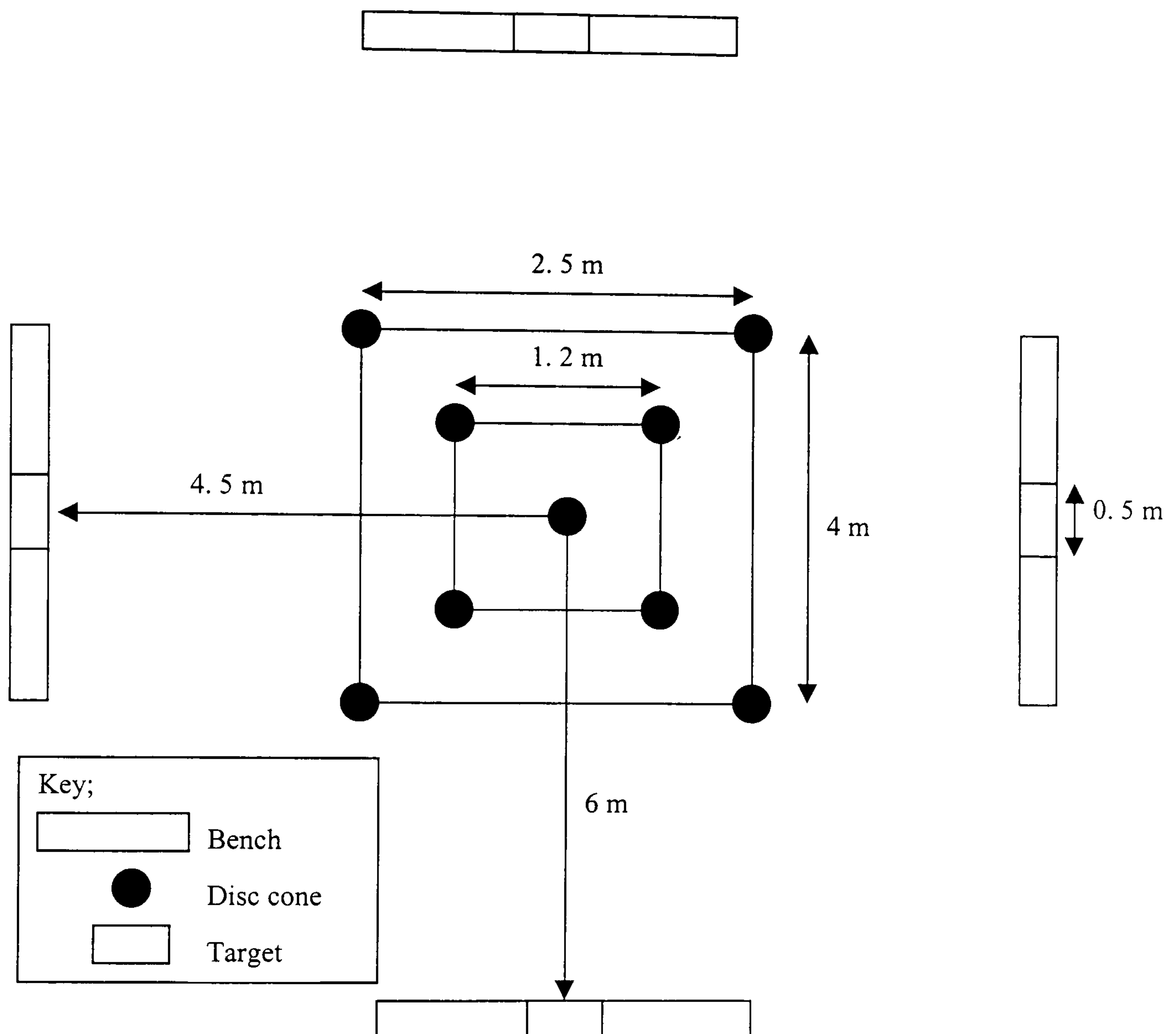


Figure 3.2: Diagrammatic representation of the Loughborough Soccer Passing Test (LSPT)

3.3.4 Statistical analysis

Following familiarisation, subjects performed the skill tests twice so that the level of agreement between the two trials could be calculated (Bland and Altman, 1986) and the reliability of each test determined. The score generated from each test was

correlated (Spearman's rank order) against the group's skill ability ranking, as assessed by the players' coach to determine the validity of the tests.

3.3.5 Results

The differences (errors) between the two scores generated for each skill test were plotted against the measurement means to assess heteroscedastic error. It was identified that there was no association between the errors and measurement means, indicating the absence of heteroscedastic error. The mean (SD) skill test scores for the LSDT and the LSPT were 149.0 ± 18.4 s and 55.0 ± 4.5 s respectively. The mean (SD) difference between trial 1 and trial 2 for both tests are presented in Table 3.1, together with some more commonly used statistical tests. The validity coefficient was significant for both tests, LSDT $r = 0.78$ ($p < 0.01$); LSPT $r = 0.64$ ($p < 0.05$).

Table 3.1 Test - retest reliability using different statistical techniques

Method	LSDT	LSPT
Mean (SD) difference in score between test 1 and test 2 (s)	0.08 ± 3.28	-0.03 ± 3.13
95% agreement limits (s)	-6.35 to 6.51	-6.17 to 6.11
t-test	$t = 0.89$	$t = 0.95$
Pearson's correlation	$r = 0.99$	$r = 0.83$

Following the completion of the preliminary study a decision was required as to whether the skill tests were reliable and valid tests of selected soccer skills. It was concluded that the agreement limits were at a value that could be accepted for reliability and their purpose of determining the influence of intermittent exercise on selected soccer skills. In addition to reliability the validity correlation coefficients were at a value that were deemed sufficient to represent valid tests of soccer skills. In summary, the results of this study suggest that the LSDT and LSPT are reliable indicators of dribbling and passing skill for the group of players chosen. Additional research is required to identify if these tests can be applied to other groups of the population.

3.4 Collection, treatment, storage and analysis of blood samples

3.4.1 Sample collection

An indwelling cannula (Venflon, 16-18G) was inserted into an antecubital vein and kept patent by periodic flushing with a sterile saline (10 U.ml⁻¹ heparin). Subjects rested for 15-20 min before local anaesthetic was administered (0.5 ml of 1% lignocaine) prior to inserting the cannula. Subjects then stood up for 15-20 min before a 10 ml resting venous blood sample was drawn. Further venous blood samples were obtained throughout exercise at pre-determined times (Chapters 4,5,6 and 7) and during recovery (Chapter 7).

3.4.2 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 stood for 15-20 min before a 10 ml resting blood sample was drawn.

3.4.3. Treatment, storage and analysis of venous blood samples

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

- 5 ml of whole blood was dispensed into a lithium-heparinised tube and 5 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 analyses on the 20 µl aliquots of perchloric acid extract using the G.O.D period method (Boehringer Mannheim GmbH Diagnostica, Mannheim,

Germany). Blood lactate concentration was determined by fluorimetric analyses (Locarte, Model 8-9) on 20 μl aliquots of perchloric acid extract using a method adapted from Maughan (1982). A coefficient of variability of less than 3.0% performed on a single blood sample and a regression equation of correlation 0.999 on the standards was achieved before commencing sample analyses. The lactate standards ranged between 0 and 10 mmol.l^{-1} , which accommodated the sample values measured in the studies reported in this thesis. Haemoglobin concentration was determined in duplicate (2 x 20 μl) by the cyanomethaemoglobin method (Boeringher Mannheim GmbH Diagnostica, Mannheim, Germany). Triplicate 50 μl samples of whole blood were collected using heparinised pipettes and then micro-centrifuged (Hawksley Ltd, Lancing, UK) for 15 min at 11000 rev.min^{-1} . Packed cell volume was measured using a sliding haematocrit reader (Hawksley Ltd, Lancing, UK). 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 analyses of FFA, using a commercially available kit (Wako chemicals GmbH kit, UK), and glycerol (Laurell and Tibbling, 1966).
- Serum was obtained by centrifuging 5 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 (Coat-A-Count Insulin, Diagnostica Products Corporation (DPC) kit, Caernarfon, UK), cortisol (Coat-A-Count Cortisol, DPC kit, Caernarfon, UK), prolactin (Coat-A-Count Prolactin, DPC kit, Caernarfon, UK) and aldosterone (Coat-A-Count Aldosterone, DPC kit, Caernarfon, UK), using commercially available kits. The tubes for insulin, cortisol, prolactin and aldosterone assays were counted using an automated gamma counter (Packard, Cobra 5000, Pangbourne, UK). Serum sodium and potassium concentrations were determined by flame photometry (Ciba Corning 480) and serum osmolality by

freezing-point depression (automatic cryoscopic osmometer, Gonotec Osmotat 030).

- An additional 1 ml of whole blood was collected during the studies reported in Chapters 6 and 7. This sample 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 GmbH Diagnostica, Mannheim, Germany).

The coefficient of variation [(Standard deviation / mean) * 100] of the blood, plasma and serum assays is shown in Table 3.1.

Table 3.2: Coefficient of variation of blood, plasma and serum metabolite assays (n = 15)

Constituents/Metabolite	Coefficient of variation (%)
Haemoglobin	1.1
Heamatocrit	0.6
Blood glucose	1.4
Blood lactate	1.2
Plasma ammonia	2.0
Plasma FFA	0.7
Plasma glycerol	0.5
Serum aldosterone	5.8
Serum cortisol	6.5
Serum insulin	5.5
Serum osmolality	0.2
Serum potassium	0.2
Serum prolactin	1.6
Serum sodium	0.4

3.5 Statistical Analyses

A two-way analysis of variance for repeated measures on both factors (trials-by-time) was used to establish if any significant differences existed between subject response. A one-way analysis of variance for repeated measures was used to establish if any significant differences existed between environmental conditions during each trial and energy intake prior to each trial. When differences were found a *post-hoc* Newman-Kuels test was used to identify them. The level of significance was accepted at $p < 0.05$. Results are represented as mean \pm SEM.

CHAPTER 4

INFLUENCE OF INTERMITTENT, HIGH-INTENSITY SHUTTLE RUNNING AND FLUID INGESTION ON THE PERFORMANCE OF A SOCCER SKILL

4.1 Introduction

The effect of dehydration on prolonged, continuous moderate - intensity (40 - 65% maximal oxygen uptake) exercise has been extensively investigated (see Coyle and Montain, 1992). Dehydration equivalent to the loss of only 2% body mass is sufficient to impair endurance performance significantly (Armstrong *et al.*, 1985). In contrast, fluid ingestion reduces the physiological strain associated with dehydration (Hargreaves *et al.*, 1996) and improves endurance performance and capacity (Armstrong *et al.*, 1985; Fallowfield *et al.*, 1996). The impact of dehydration on the intermittent high intensity exercise undertaken during participation in team sports has not been extensively studied.

In addition, team sports are characterised not only by intermittent high intensity exercise, but also by the contribution of a wide range of skills. Yet there does not appear to have been studies examining the effect of water ingestion alone on physical performance during soccer or the effect that this may have on soccer skills.

The aim of this study was to examine the influence of fluid intake on a test of soccer skill following the completion of 90 min of intermittent, high intensity shuttle running (LIST). Soccer demands a range of skills, one of which is dribbling with the ball. Therefore it is reasonable to include a soccer-dribbling test an example of one of the many skills required by soccer players.

4.2 Methods

4.2.1 Subjects

Nine male university and semi-professional soccer players volunteered to participate in this study. The mean \pm SEM age, body mass (BM) and estimated maximal oxygen uptake ($\dot{V}O_2$ max) of the group were 20.2 ± 0.4 years, 73.2 ± 1.8 kg and 59.1 ± 1.3 ml.kg⁻¹. min⁻¹ respectively.

4.2.2 Preliminary tests

Subjects performed preliminary tests to: (i) predict $\dot{V}O_2$ max in order to calculate the relative exercise intensities as previously described (Chapter 3) and (ii) to familiarise themselves with the experimental procedures. Following completion of preliminary measurements, subjects were familiarised with the soccer skill and mental concentration tests.

4.2.3 Experimental design

Subjects acted as their own controls in a repeated measures cross-over design. They were allocated to two randomly assigned trials either ingesting or not ingesting fluid. To increase the palatability of the fluid ingested during the fluid trials, subjects ingested a commercially available "No Added Sugar Concentrated Lemon Drink", diluted 1:4 with tap water; 52 mOsmol.kg⁻¹, (J Sainsbury plc, London, UK) immediately prior to the LIST (5 ml.kg⁻¹BM) and every 15 min thereafter (2 ml.kg⁻¹BM).

In the two days prior to the main trials subjects did not participate in any prolonged, heavy exercise. They reported to the laboratory after an overnight fast of between 10 and 12 h. They then emptied their bladders prior to the preliminary measurement of nude body mass, which was recorded before and immediately following each trial. Nude body mass was determined to the nearest 0.1 kg using a beam balance (Avery

Ltd., Model 3306ABV). These measurements before and after exercise along with fluid intake during exercise were used to calculate sweat losses during exercise. An indwelling cannula (Venflon, 16-18G, BOC Ohmeda, Sweden) was inserted into an antecubital vein before exercise and kept patent by periodic flushing with sterile saline (10 U.ml⁻¹ heparin). The subjects stood for 15-20 min before a resting blood sample was obtained.

A standardised 15-min warm-up was performed by each subject prior to each trial, and consisted of jogging, stretching and striding. Immediately after the warm up the subjects were required to perform the Loughborough Soccer Dribbling Test (LSDT, as described in Chapter 3) and a mental concentration test (Hardy and Fazey, 1990). The mental concentration test involved the identification of numbers ascending from 1 to 100 from a randomised 10 x 10 grid. Subjects had to identify as many numbers as possible within one minute. There then followed a 2-min period during which subjects in the fluid trial ingested the prescribed solution and prepared to begin the LIST. During the no fluid trial the subjects had a similar 2-min break before the start of the LIST.

Once the LIST began the subjects were required to run or walk at 4 different speeds. The speeds were dictated by a single bleep and based on the % $\dot{V}O_2$ max calculated for each subject. To help the subjects maintain the appropriate pace, one of the investigators would continually provide information about the nature of each forthcoming 20 m i.e. “walk”, “sprint”, “cruise”, “jog” or “rest”.

During the LIST investigators made sure that the subjects placed at least one foot on the line delimiting the 20-m distance at each end of the Sports Hall. The subjects were also told to adjust their pace if they ran at the wrong speeds at any time during the test. As the subjects approached the sprints and during each sprint they were verbally encouraged to perform maximally.

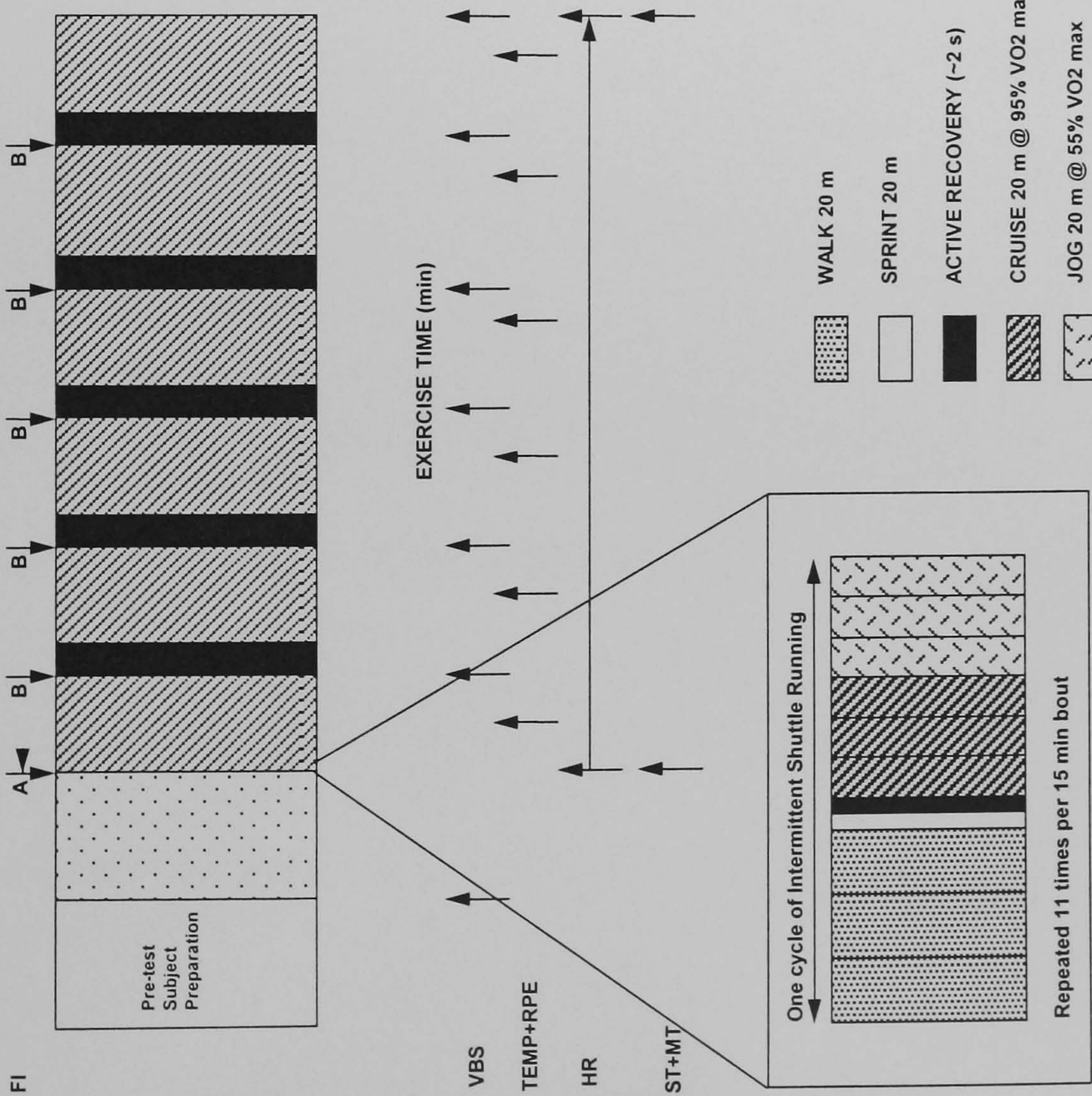
Heart rate was recorded every 15 s during the trials by short - range radio telemetry (Polar Sport Tester™, Kempele, Finland). The temperature of the Sports Hall was

recorded every 20 min and maintained over the study between 13 - 20°C by opening windows and doors. Dry-bulb and wet-bulb temperatures were measured using a whirling hygrometer (Brannan Thermometers Ltd).

On completion of the LIST the subjects had a 5 min break before they performed the soccer dribbling skill test and the mental concentration test for a second time. The overall design of the study is illustrated in Figure 4.1.

LOUGHBOROUGH INTERMITTENT SHUTTLE TEST (LIST)

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



- Warm-up
- 15 min. Intermittent Exercise
- 3 min. Rest Period

VBS = Venous Blood Sample

HR = Heart Rate (every 15 s)

ST+MT = Skill+Mental Tests

FI = Fluid Intake

A = 5 ml/kg body weight

B = 2 ml/kg body weight

TEMP = Environmental Temperature

RPE = Rating of Perceived Exertion

Figure 4.1: Schematic illustration of the LIST and experimental design

4.2.4 Blood sampling and analysis

Ten ml of blood was withdrawn at rest, after 15, 30, 45, 60, 75 and 90 min of exercise. The blood was dispensed, treated and stored as previously described (Chapter 3). Serum was analysed for insulin, cortisol, aldosterone, osmolality and electrolyte concentrations and plasma was analysed for FFA and glycerol concentrations. Whole blood was assayed for lactate and glucose concentrations and plasma volume changes, using methods that have been previously described (Chapter 3).

4.2.5 Statistical analysis

Results were analysed as described under statistical analysis (Chapter 3). Results are represented as mean \pm SEM.

4.3 Results

4.3.1 Skill and mental tests

There were no differences between trials for the pre-LIST skill test scores (fluid: 131.0 ± 4.1 s, no fluid: 129.5 ± 4.6 s). Post-LIST skill test performance times were longer in the no fluid trial (135.7 ± 4.9 s) (main effect of time x trial: $F_{1,8} = 13.4$, $p < 0.05$) while no difference was observed at completion of the fluid trial (129.1 ± 4.7 s). There was no difference between pre-exercise and post-LIST scores for the mental concentration test (pre-fluid: 13 ± 1 , pre-no fluid: 12 ± 1 , post-fluid: 13 ± 1 , post-no fluid: 13 ± 2).

4.3.2 Changes in plasma volume and body mass

There were no differences between trials in the changes in plasma volume from rest to the end of exercise (no fluid $2.0 \pm 1.1\%$; fluid $3.5 \pm 1.1\%$). During the no fluid trial body mass loss following the LIST was 1.75 ± 0.16 kg. This was greater than the body mass loss in the fluid trial, 1.01 ± 0.07 kg (main effect of time x trial: $F_{1,8} = 29.4$, $p < 0.05$). Mean sweat rate (calculated from net body mass loss and uncorrected for respiratory water loss or metabolic water gain) was higher during the fluid trial (1.4 ± 0.1 l.h⁻¹) compared with the no fluid trial (1.2 ± 0.1 l.h⁻¹) ($p < 0.05$).

4.3.3 Heart rate and rating of perceived exertion

Mean heart rate for the 90 min of exercise was higher during the no fluid trial (170 ± 4 beats.min⁻¹) than during the fluid trial (164 ± 3 beats.min⁻¹) (main effect of trial: $F_{1,8} = 6.2$, $p < 0.05$). The rating of perceived exertion (RPE) described by the subjects during each trial was found to be similar at completion of the fourth block of exercise. Thereafter, RPE continued to increase during the no fluid trial, while remaining unchanged during the fluid trial. This resulted in significant differences

during the fifth and sixth block of the LIST (main effect of time x trial: $F_{1,8} = 7.7$, $p < 0.01$), a period corresponding to the final 30 minutes of exercise (Figure 4.2).

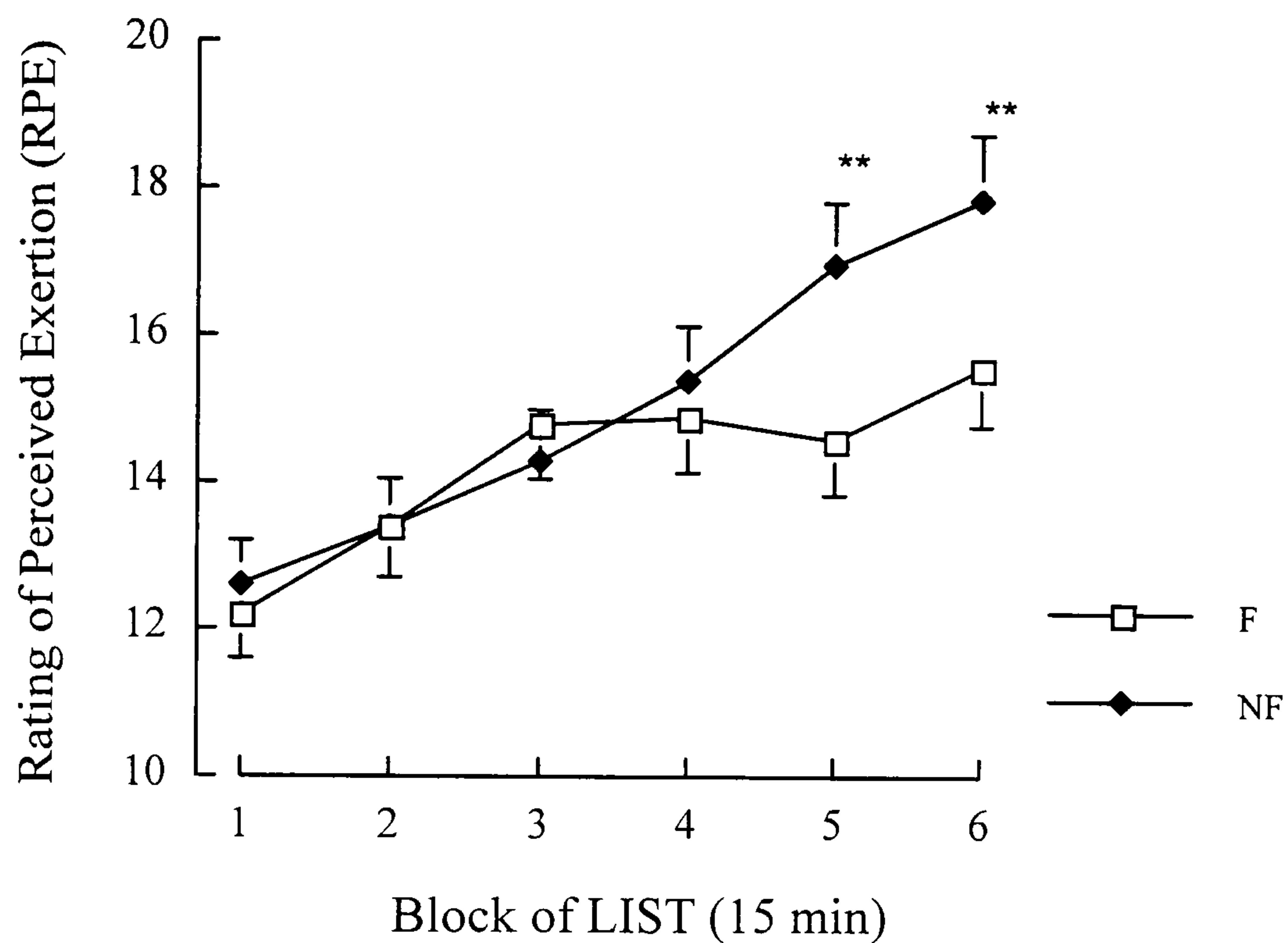


Figure 4.2. Rating of perceived exertion (Borg Scale) during each 15-min block of the LIST. F = fluid trial, NF = no fluid. ** *post-hoc* difference, $p < 0.01$ NF vs F

4.3.4 Environmental conditions

There was no difference between trials in the dry and wet bulb temperature and relative humidity during the study (Table 4.1).

Table 4.1: Mean dry and wet bulb temperature ($^{\circ}\text{C}$) and relative humidity (%) (Values are mean \pm SEM).

	Dry bulb temperature ($^{\circ}\text{C}$)	Wet bulb temperature ($^{\circ}\text{C}$)	Relative humidity (%)
Fluid	13.4 \pm 0.3	9.2 \pm 0.3	57.1 \pm 1.6
No Fluid	12.8 \pm 0.5	8.7 \pm 0.3	58.0 \pm 2.9

4.3.5 Serum cortisol and aldosterone

Serum cortisol concentrations were similar in both trials up to the completion of the fourth block of the LIST, (i.e. up to 60 min). Thereafter, cortisol concentration continued to rise in the no fluid trial at an increased rate, leading to a difference between trials at 75 (main effect of time x trial: $F_{6,42} = 5.09$, $p < 0.05$) and at 90 min (main effect of time x trial: $F_{6,42} = 5.09$, $p < 0.01$) (Figure 4.3a). The rise in serum aldosterone concentrations from resting values to completion of the fourth block of the LIST was similar in both trials. During the no fluid trial aldosterone concentrations continued to increase in the final 30 min of exercise and a difference between trials was observed at 90 min (main effect of time x trial: $F_{6,42} = 2.63$, $p < 0.05$) (Figure 4.3b).

4.3.6 Serum osmolality and electrolytes

Serum osmolality followed a similar response in both trials up to the completion of the fourth block of the LIST, (i.e. up to 60 min). Following this time osmolality continued to rise in the no fluid trial but was reduced during the fluid trial. This resulted in a difference between trials at 90 min (main effect of time x trial: $F_{6,42} = 3.45$, $p < 0.01$) (Figure 4.3c). There was no significant difference between trials for potassium concentration but serum sodium concentration followed a similar pattern to osmolality, with differences between trials at 45, 75 and 90 min (main effect of time x trial: $F_{6,42} = 8.7$, $p < 0.01$) (Figure 4.3d).

4.3.7 Fifteen metre sprint times

There was no difference between trials for the 15-m sprint times up to completion of the 5th block of the LIST. During the final block of the LIST the mean sprint time in the no fluid trial was longer than during the fluid trial (main effect of time x trial: $F_{5,40} = 2.4$, $p < 0.05$). Within the no fluid trial the mean sprint time during the sixth

block of exercise was longer than during the first three blocks (main effect of time x trial: $F_{5,40} = 2.4$, $p < 0.05$) (Figure 4.4).

4.3.8 Energy Intake

There was no difference between trials in the total energy, carbohydrate, fat and protein consumed in the 48 h prior to the main trials. (Table 4.2).

Table 4.2: Daily total energy (kcal), carbohydrate (%), fat (%) and protein (%) in the 48 h prior to the main trials (Values are mean \pm SEM)

Trial	Total energy (kcal)	Carbohydrate (%)	Fat (%)	Protein (%)
Fluid	2360 \pm 237	52.6 \pm 2.4	32.2 \pm 2.6	15.2 \pm 1.2
No Fluid	2334 \pm 297	53.6 \pm 2.6	31.5 \pm 3.0	14.9 \pm 1.4

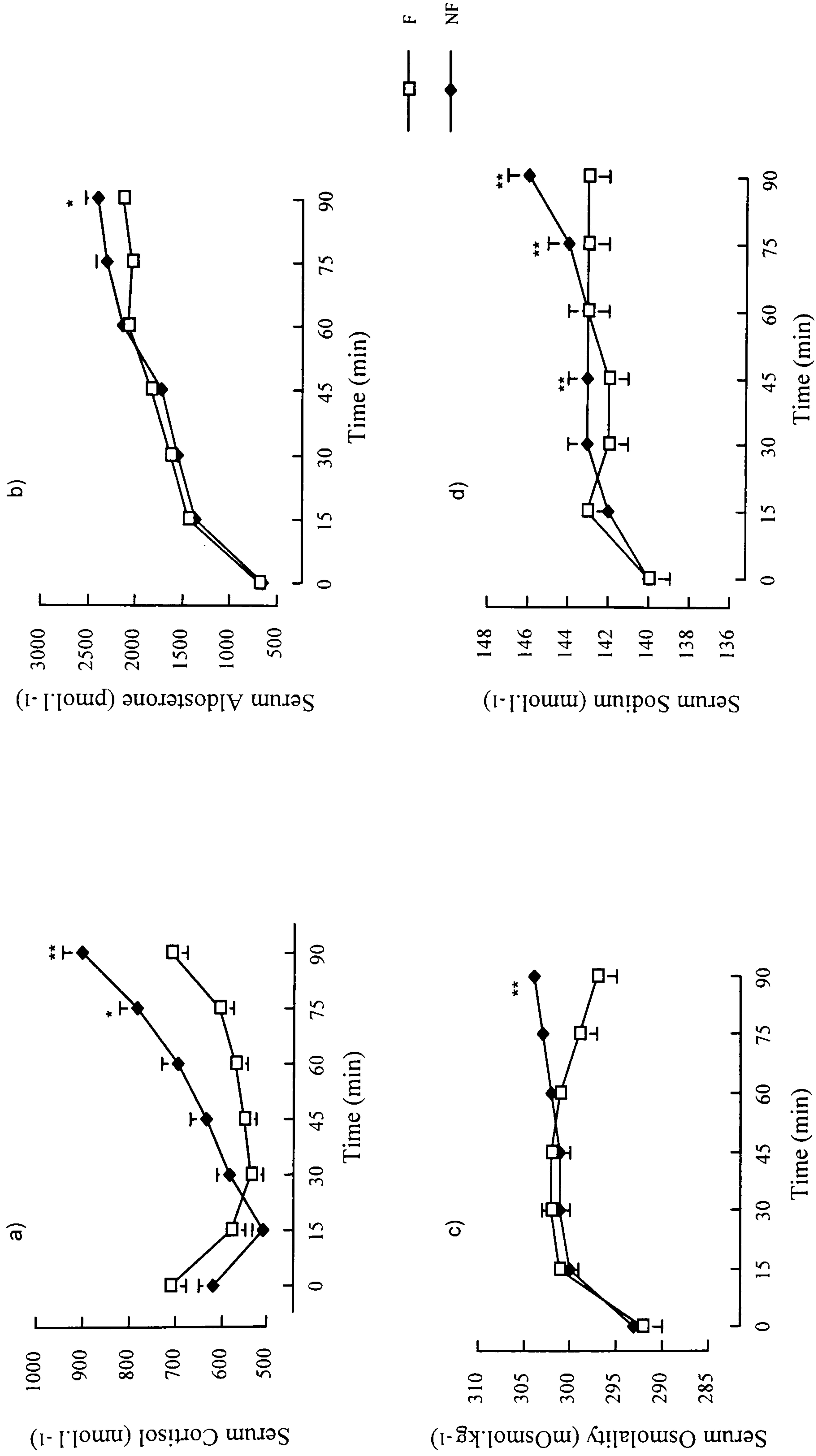


Figure 4.3: F = fluid trial, NF = no fluid. a) Serum cortisol concentration (nmol.l⁻¹) during the LIST: * p<0.05 NF vs F, ** p<0.01 NF vs F; b) Serum aldosterone concentration (pmol.l⁻¹) during the LIST: * p<0.05 NF vs F; c) Serum osmolality concentration (mOsmol.kg⁻¹) during the LIST: **p<0.01 NF vs F; d) Serum sodium concentration (mmol.l⁻¹) during the LIST: **p<0.01 NF vs F

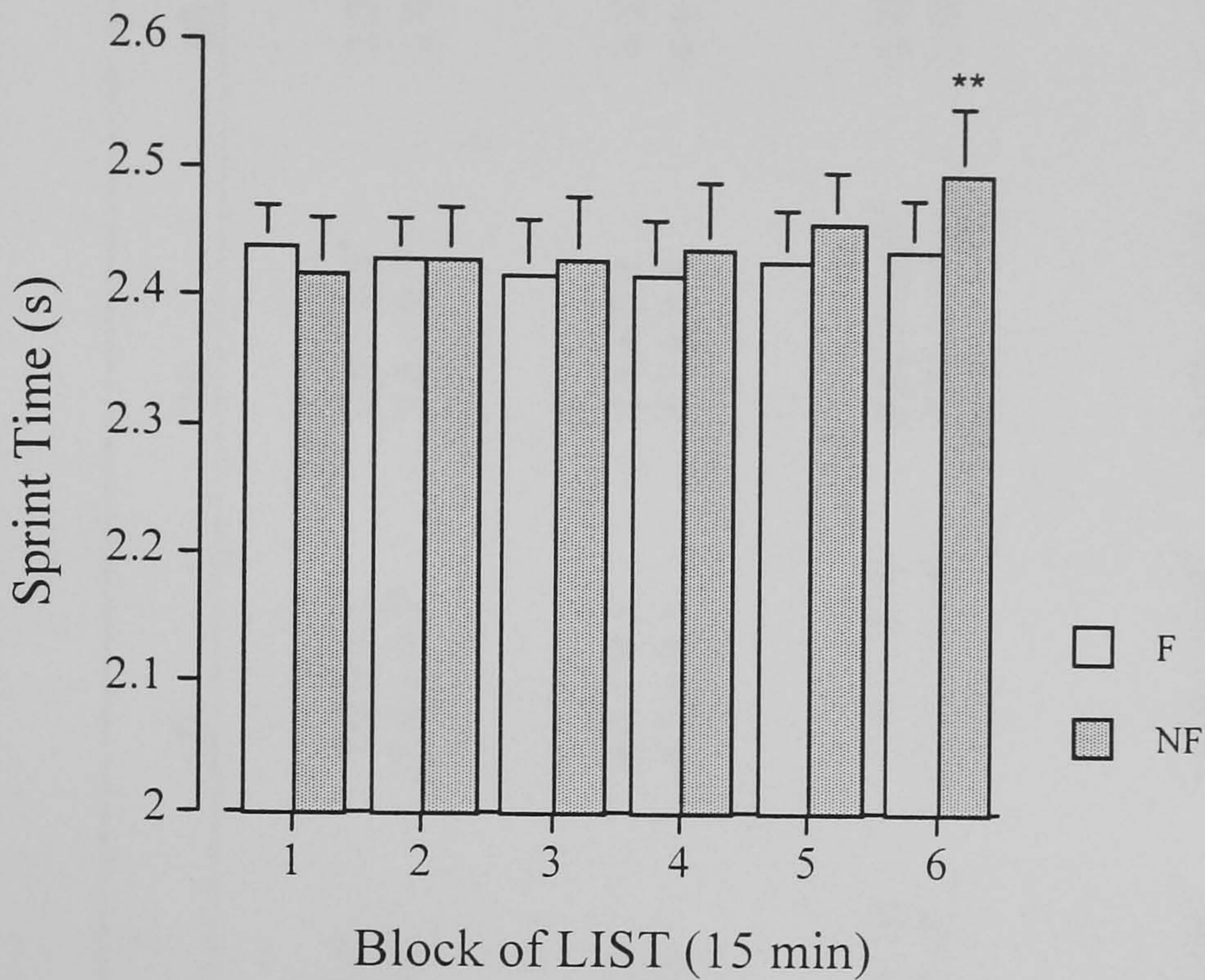


Figure 4.4: Mean sprint time (s) during each 15-min block of the LIST. F = fluid trial, NF = no fluid. ***post-hoc* difference $p < 0.05$ NF vs F

4.3.9 Other metabolites measured

Blood lactate, blood glucose, plasma FFA, plasma glycerol and serum insulin concentrations increased during exercise (main effect of time $p < 0.01$). There was no difference between trials (Table 4.3).

Table 4.3: Blood lactate (mmol.l⁻¹), blood glucose (mmol.l⁻¹), plasma FFA (mmol.l⁻¹), plasma glycerol (mmol.l⁻¹) and serum insulin (mU.l⁻¹) concentrations during the LJUST (Values are mean ± SEM)

	Exercise time (min)						
	0	15	30	45	60	75	90
Blood lactate (mmol.l⁻¹)							
FLU	0.7 ± 0.1	3.6 ± 0.7 ^a	3.2 ± 0.5 ^a	3.2 ± 0.5 ^a	3.0 ± 0.5 ^a	2.6 ± 0.5 ^a	2.3 ± 0.4 ^a
NON	0.7 ± 0.1	2.8 ± 0.4 ^a	2.6 ± 0.4 ^a	2.6 ± 0.2 ^a	2.6 ± 0.3 ^a	2.6 ± 0.2 ^a	2.4 ± 0.1 ^a
Blood glucose (mmol.l⁻¹)							
FLU	4.5 ± 0.2	5.1 ± 0.4 ^b	5.0 ± 0.3 ^b	4.7 ± 0.3	4.6 ± 0.3	4.4 ± 0.4	4.3 ± 0.3
NON	4.4 ± 0.2	5.0 ± 0.4 ^b	5.1 ± 0.5 ^b	4.8 ± 0.5	4.5 ± 0.3	4.1 ± 0.3	4.0 ± 0.3
Plasma FFA (mmol.l⁻¹)							
FLU	0.43 ± 0.09	0.32 ± 0.08	0.34 ± 0.09	0.41 ± 0.10	0.46 ± 0.11	0.68 ± 0.08 ^c	0.88 ± 0.17 ^c
NON	0.49 ± 0.10	0.44 ± 0.08	0.46 ± 0.10	0.45 ± 0.07	0.65 ± 0.06	0.71 ± 0.08 ^c	0.90 ± 0.13 ^c
Plasma glycerol (mmol.l⁻¹)							
FLU	0.06 ± 0.01	0.17 ± 0.03 ^d	0.22 ± 0.03 ^d	0.26 ± 0.04 ^d	0.30 ± 0.05 ^d	0.35 ± 0.05 ^d	0.41 ± 0.06 ^d
NON	0.07 ± 0.01	0.20 ± 0.03 ^d	0.25 ± 0.04 ^d	0.31 ± 0.03 ^d	0.36 ± 0.04 ^d	0.41 ± 0.03 ^d	0.43 ± 0.05 ^d

a: *post-hoc* difference, $p < 0.01$ vs 0 min b: *post-hoc* difference, $p < 0.01$ vs 90-min, *post-hoc* difference, $p < 0.01$ vs 0-min c: *post-hoc* difference, $p < 0.01$ vs all other time points d: *post-hoc* difference, $p < 0.01$ vs 0-min

4.4 Discussion

The main finding of the present study was that prolonged intermittent, high intensity shuttle running without water ingestion resulted in a 5% deterioration in performance of a soccer skill. The decline did not occur when the soccer players drank a carbohydrate free solution throughout the trial.

Because of the lack of information relating to dehydration during intermittent sports it is difficult to compare the findings of this study with others in the literature. As little as 2% of body mass has been shown to impair mental functioning (Gopinathan *et al.*, 1988) and endurance performance (Armstrong *et al.*, 1985). During the no fluid trial, body mass decreased by 2.4%, which was greater than in the fluid trial (1.38%, $p < 0.05$). The increased fluid loss during the no fluid trial may have led to an increased thermal strain with a resulting negative impact on skill maintenance.

The fact that an increased body mass loss may have caused an increased thermal load during the no fluid trial is supported by the evidence of an increased heart rate during the no fluid trial. In general heat dissipation is increased by an increased peripheral blood flow. Blood may then accumulate in the compliant veins and cause a reduction in central blood volume. In turn, a reduction in venous return and stroke volume may result, causing a decrease in cardiac output. Initial compensation for this decreased cardiac output normally occurs by means of a small increase in heart rate (Montain and Coyle, 1992). Therefore, the differences in the heart rate response to the two trials in the present study, may have been due to a greater reduction in stroke volume and an increased core temperature in the no fluid trial compared with the fluid trial.

Montain and Coyle (1992) reported that during 2 h of exercise of moderate intensity, the magnitude of the increase in core temperature and heart rate was directly related to the degree of dehydration. Such an effect of dehydration on heart rate is also supported by the findings of Hamilton *et al.* (1991) and

Hargreaves (1996) who reported that heart rates were higher during exercise when no fluid was ingested.

Of interest in the present study was the absence of a difference in changes in plasma volume between trials. Aldosterone is centrally involved in the regulation of fluid homeostasis, especially in the homeostasis of plasma volume (Altenkirch *et al.*, 1990). The increased aldosterone response during the no fluid trial is likely to have been a key regulatory response in maintaining plasma volume. During the fluid trial the estimated sweat rate ($1.4 \pm 0.1 \text{ l.h}^{-1}$) was higher than in the trial in which fluid was prohibited ($1.2 \pm 0.1 \text{ l.h}^{-1}$) ($p < 0.05$). Aldosterone concentration is linearly related to hypohydration and a reduced total body sodium concentration (Montain *et al.*, 1997); therefore the increased aldosterone concentration during the no fluid trial may have acted to reduce sweat rate.

A reduction in sweat rate will result in an increased core temperature, an event which is strongly associated with fatigue. A rise in core temperature can be reduced by ingesting fluid (Costill *et al.*, 1970). Montain and Coyle (1992) observed that fluid ingestion during prolonged cycling maintained skin blood flow and reduced hyperthermia during the later stages of exercise. They suggested that fluid ingestion maintains thermoregulation by preventing an increase in serum osmolality, a response observed during the present study. An increased cortisol response during the no fluid trial reflects the greater strain on the subjects when they performed the 90-min LIST without fluid ingestion. This is supported by the higher RPE during the last 30 min of the LIST during the 'no fluid' trial.

The deterioration in skill found in the 'no fluid' trial may also be linked to depletion of muscle glycogen. Low muscle glycogen stores have been associated with changes in work rate and performance during soccer matches (Karlsson, 1969; Saltin, 1973). Jacobs (1981) demonstrated that short term (<1 min) maximal intensity exercise is also impaired when muscle glycogen is depleted, and suggested that this impairment may be a function of a reduced glycogenolysis. Since both endurance and explosive power are integral components of

performance in soccer, a low intramuscular glycogen concentration may directly impair playing performance (Jacobs *et al.*, 1982b).

Jacobs (1981) suggested that the critical level of muscle glycogen concentration below which impairment of anaerobic ATP resynthesis occurs, is about 40 mmol.kg w.w.⁻¹. During the performance of the LIST, muscle glycogen concentrations were recorded below 40 mmol. kg w.w.⁻¹ in the vastus lateralis for subjects ingesting a non-carbohydrate placebo solution (Nicholas *et al.*, 1994). Although muscle biopsy samples were not obtained in the present study, it is reasonable to assume that at the end of the LIST the muscle glycogen concentrations of the subjects in the present study were as low as those reported by Nicholas *et al.* (1994).

The ingestion of water during prolonged running improves endurance capacity beyond that achieved while running without fluid intake. Without fluid ingestion there is increased carbohydrate oxidation and suppressed fat oxidation (Fallowfield *et al.*, 1996). In a study using muscle biopsies, Hargreaves *et al.* (1996) showed that glycogen sparing occurs as a result of water ingestion with a consequential shift towards increased fat oxidation. They also found that heart rates and ratings of perceived exertion were lower during the fluid trial which were confirmed in the present study. Hargreaves *et al.* (1996) suggested that water ingestion attenuates the increased plasma adrenaline concentration which normally occurs during exercise without fluid, which in turn may explain the reduction in glycogen utilisation. In contrast, an increased contribution of carbohydrate to energy metabolism when no fluid is ingested would lead to an increased rate of glycogenolysis and earlier onset of fatigue. Although the mean duration of each sprint was only 2.45 s there was some evidence to suggest a negative influence of running without fluid ingestion. The last sprint in the no fluid trial was significantly slower than the first. This was not the case in the fluid trial. Of course the duration of the study was such that endurance capacity was not tested since all subjects completed 90 min of the LIST.

Nicholas *et al.* (1994) reported that the muscle glycogen concentrations at the end of the LIST, when a placebo solution had been ingested were 31 and 27 mmol. kg w.w.⁻¹ for Type I and Type II fibres, respectively. If muscle glycogen concentrations are further lowered in the absence of fluid ingestion (Hargreaves *et al.*, 1996), then a deterioration in skill performance due to a low muscle glycogen concentration in the no fluid trial is plausible. Whether a shift in metabolism causing a sparing of muscle glycogen during the fluid trial has an impact on skill performance remains to be elucidated.

Blood glucose is the main substrate for energy metabolism within the central nervous system. Therefore, it is not surprising that hypoglycaemia has been suggested as a possible reason for the deterioration in performance observed in sports, such as soccer, which require both tactical thought and co-operative interaction between players (Shephard and Leatt, 1987). As there was no difference in blood glucose concentration between trials or between rest and exercise in each trial, it could be concluded that lowered blood glucose concentrations were not the cause of the decrement in performance of the skill test in the no fluid trial.

In summary, performance of a soccer skill test decreased following prolonged intermittent shuttle running when no fluid was ingested. The ingestion of fluid throughout the LIST reduced the increase in heart rate, RPE, serum osmolality, sodium and cortisol concentrations and also prevented the loss in skill performance.

CHAPTER 5

THE INFLUENCE OF CARBOHYDRATE INGESTION ON EXERCISE METABOLISM DURING INTERMITTENT HIGH INTENSITY SHUTTLE RUNNING

5.1 Introduction

The physical demands of soccer have mainly been described using notational analysis techniques, (Reilly and Thomas, 1976; Mayhew and Wenger 1985). These indirect methods have been used because of the difficulty of monitoring players during real match play. The development of the LIST as a method of simulating the demands of intermittent sports, such as soccer has provided the opportunity to investigate the influence of environmental temperature (Morris *et al.* 1996) and dietary interventions (Nicholas *et al.*, 1995, 1997) on endurance capacity and performance of a soccer skill test (Chapter 4).

Prolonged exercise undertaken without fluid ingestion appeared to increase the demands on the body's carbohydrate stores (Fallowfield *et al.*, 1996; Hargreaves *et al.*, 1996) and so accelerate glycogen depletion and the onset of fatigue. This shift in substrate metabolism during exercise without fluid ingestion may also contribute to the deterioration in skill performance (Chapter 4). Therefore, the aim of the present study, was to assess the influence of no fluid (NON), a 6.4 % carbohydrate-electrolyte (CHO) and a carbohydrate free solution (CON) on exercise metabolism during prolonged intermittent, high intensity shuttle running.

5.2. Methods

5.2.1 Subjects

Ten male amateur club level games players (soccer $n = 5$; hockey $n = 2$; rugby union $n = 2$, squash $n = 1$) volunteered to participate in the study. The mean \pm SEM age, body mass (BM) and estimated maximal oxygen uptake of the group were 24 ± 0.7 years, 74.3 ± 2.8 kg and 58.7 ± 1.6 ml.kg⁻¹ min⁻¹ respectively.

5.2.2 Preliminary tests

Subjects performed preliminary tests to: (i) estimate $\dot{V}O_2$ max in order to calculate the relative exercise intensities as previously described (Chapter 3) and (ii) to familiarise themselves with the experimental procedures.

5.2.3 Experimental design

Subjects completed 3 main trials separated by at least 7 days. The order of the trials was randomised to offset any training or order effects. In each trial, subjects were assigned to one of the following treatments: (i) ingestion of a 6.4 % carbohydrate - electrolyte (CHO) solution (Lucozade Sport, SmithKline Beecham, UK), (ii) ingestion of an identical solution to (i) with the same concentration of electrolytes, artificially sweetened and flavoured but excluding carbohydrate (CON) (SmithKline Beecham, UK) and (iii) no fluid intake (NON).

The solutions ingested were of a similar colour, texture and taste and were administered in a double blind design. The subjects consumed the solutions immediately prior to the LIST (5ml.kg⁻¹BM) and every 15-min thereafter (2ml.kg⁻¹BM) (Figure 5.1).

For the two days prior to the main trials subjects did not participate in any prolonged, heavy exercise. They reported to the laboratory after an overnight fast of between 10 and 12 h. They then emptied their bladders prior to the preliminary measurement of nude body mass, which was recorded before and immediately following each trial. Nude body mass was determined to the nearest 0.1 kg using a beam balance (Avery Ltd., Model 3306ABV). These measurements before and after exercise along with fluid intake during exercise were used to calculate sweat losses during exercise. An indwelling cannula (Venflon, 16-18G, BOC Ohmeda, Sweden) was inserted into an antecubital vein before exercise and kept patent by periodic flushing with sterile saline (10 U.ml⁻¹ heparin). The subjects stood for 15-20 min before a resting blood sample was obtained. A resting expired air sample was then collected. To collect expired air 5 subjects were randomly assigned to the Douglas bag method and 5 to the portable gas analyser (Chapter 3). Further expired air samples were collected during each block of the LIST (Figure 5.1)

The games players performed a standardised 15-min warm-up prior to each trial, consisting of jogging, stretching and striding. Once the LIST began the subjects were required to run or walk at 4 different speeds dictated by a single bleep corresponding to relative values of each subjects' individual $\dot{V}O_2$ max. To help the subjects maintain the appropriate pace, one of the investigators continually provided information about the nature of each forthcoming 20 m i.e. walk, sprint, cruise, jog or rest.

Heart rate was recorded every 15 s during the trials by short - range radio telemetry (Polar Sport Tester™, Kempele, Finland). The temperature of the Sports Hall was recorded every 20 min and maintained over the study between 13 - 20°C by opening windows and doors. Dry-bulb and wet-bulb temperatures were measured using a whirling hygrometer (Brannan Thermometers Ltd).

5.2.4 Blood Sampling and Analysis

Ten millilitres of blood were withdrawn at rest, after 15, 30, 60 and 90 min of exercise. The blood was dispensed, treated and stored as previously described (Chapter 3).

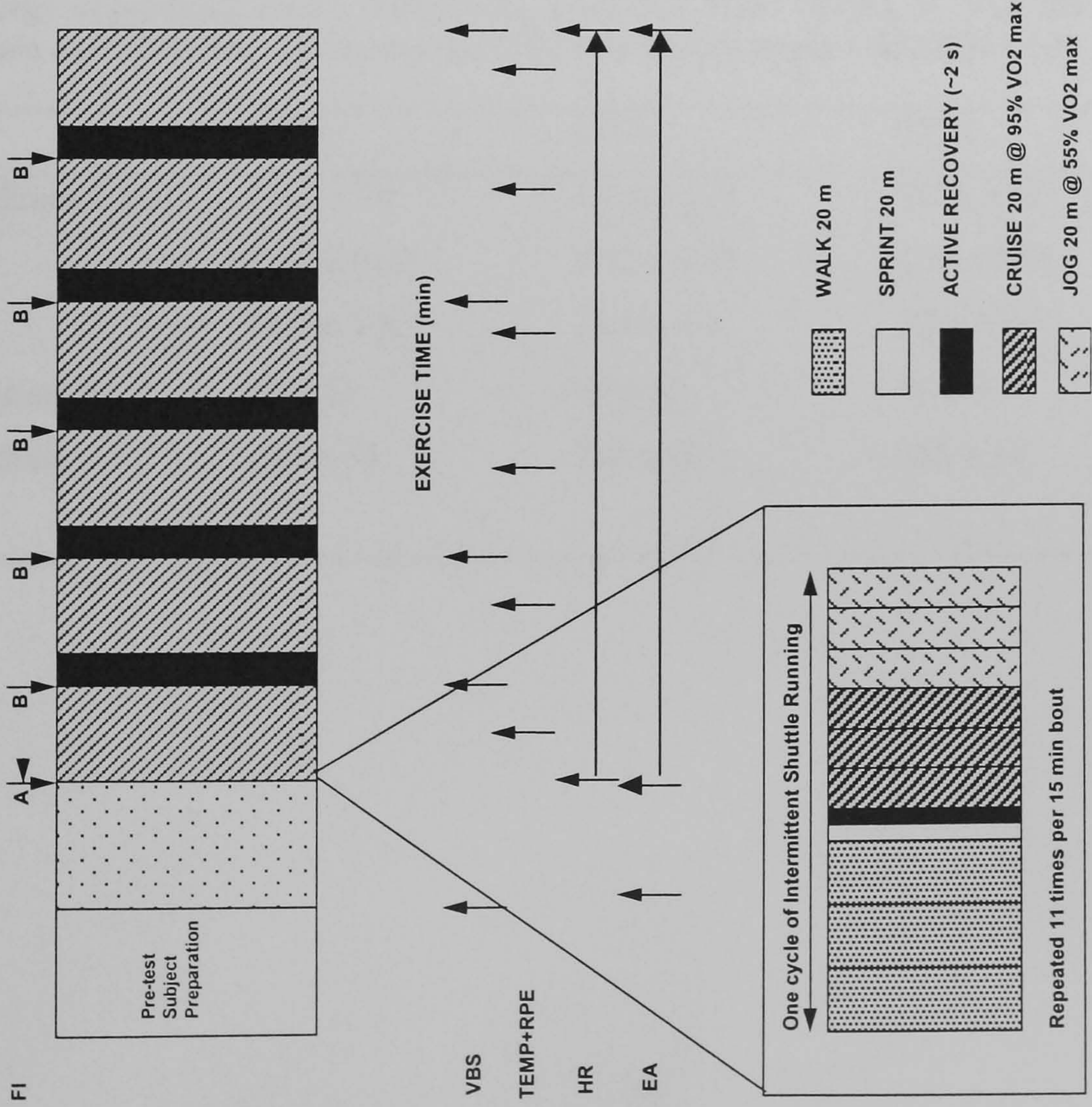
Serum was analysed for insulin, cortisol, aldosterone and electrolyte concentrations and plasma was analysed for FFA concentrations. Whole blood was assayed for lactate and glucose concentrations and plasma volume changes, using methods that have been previously described (Chapter 3).

5.2.5 Statistical Analysis

Results were analysed as described under statistical analysis (Chapter 3). Results are represented as mean \pm SEM. During one of the trials no blood samples were collected for one of the subjects, therefore, statistics are based on $n = 10$ for expired air analysis and $n = 9$ for blood analysis.

LOUGHBOROUGH INTERMITTENT SHUTTLE TEST (LIST)

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



- Warm-up
- 15 min. Intermittent Exercise
- 3 min. Rest Period

- VBS = Venous Blood Sample
- HR = Heart Rate (every 15 s)
- EA = Expired Air collection
- FI = Fluid Intake
- A = 5 ml/kg body weight
- B = 2 ml/kg body weight
- TEMP = Environmental Temperature
- RPE = Rating of Perceived Exertion

Figure 5.1: Schematic illustration of the LIST and experimental protocol

5.3 Results

5.3.1 Expired air analysis

Analysis of expired air was firstly performed for both methods of collection independently ($n = 5$) and is referred to in Appendix 3 and also when grouped together ($n = 10$). There were no differences in the respiratory exchange ratios (RER) between trials at any time during the LIST. Although the total rate of carbohydrate and fat oxidation appeared different between the CHO and the other two trials, no statistically significant difference was observed; both when analysed independently ($n = 5$) or collectively ($n = 10$). Mean $\% \dot{V}O_2$ max and total energy expenditure were also similar in all three trials (Table 5.1).

Table 5.1: Energy expenditure (kcal), Respiratory Exchange Ratio (RER), $\% \dot{V}O_2$ max, fat and carbohydrate oxidation rates (g) during the LIST (Values are mean \pm SEM)($n = 10$)

	CHO	CON	NON
Energy expenditure (kcal)	1392 \pm 79	1375 \pm 107	1320 \pm 92
RER value	0.93 \pm 0.02	0.93 \pm 0.02	0.92 \pm 0.01
$\% \dot{V}O_2$ max	73.7 \pm 1.6	73.0 \pm 3.0	70.0 \pm 2.0
Total fat oxidation (g)	42 \pm 11	52 \pm 13	49 \pm 11
Total carbohydrate oxidation (g)	247 \pm 14	221 \pm 16	215 \pm 13

5.3.2 Environmental conditions

There was no difference between trials in the dry and wet bulb temperature and relative humidity during the study (Table 5.2).

Table 5.2: Mean dry and wet bulb temperature (°C) and relative humidity (%) (Values are mean \pm SEM).

	Dry bulb temperature (°C)	Wet bulb temperature (°C)	Relative humidity (%)
CHO	19.2 \pm 0.7	15.4 \pm 0.5	67.5 \pm 2.5
CON	18.0 \pm 0.7	14.9 \pm 0.5	72.8 \pm 2.0
NON	19.6 \pm 0.8	15.6 \pm 0.7	67.5 \pm 2.5

5.3.3 Plasma Free Fatty Acids (FFA)

Plasma FFA concentration increased over time in all 3 trials (main effect of time x trial: $F_{8,56} = 4.8$, $p < 0.05$). During the CHO trial plasma FFA concentrations were lower than in both the CON and NON trials at the end of exercise (90 min)(main effect of time x trial: $F_{8,56} = 4.8$, $p < 0.05$). There was also a difference in plasma FFA concentrations, at 90 min, between the NON and CON trial (main effect of time x trial: $F_{8,56} = 4.8$, $p < 0.05$) (Figure 5.2)

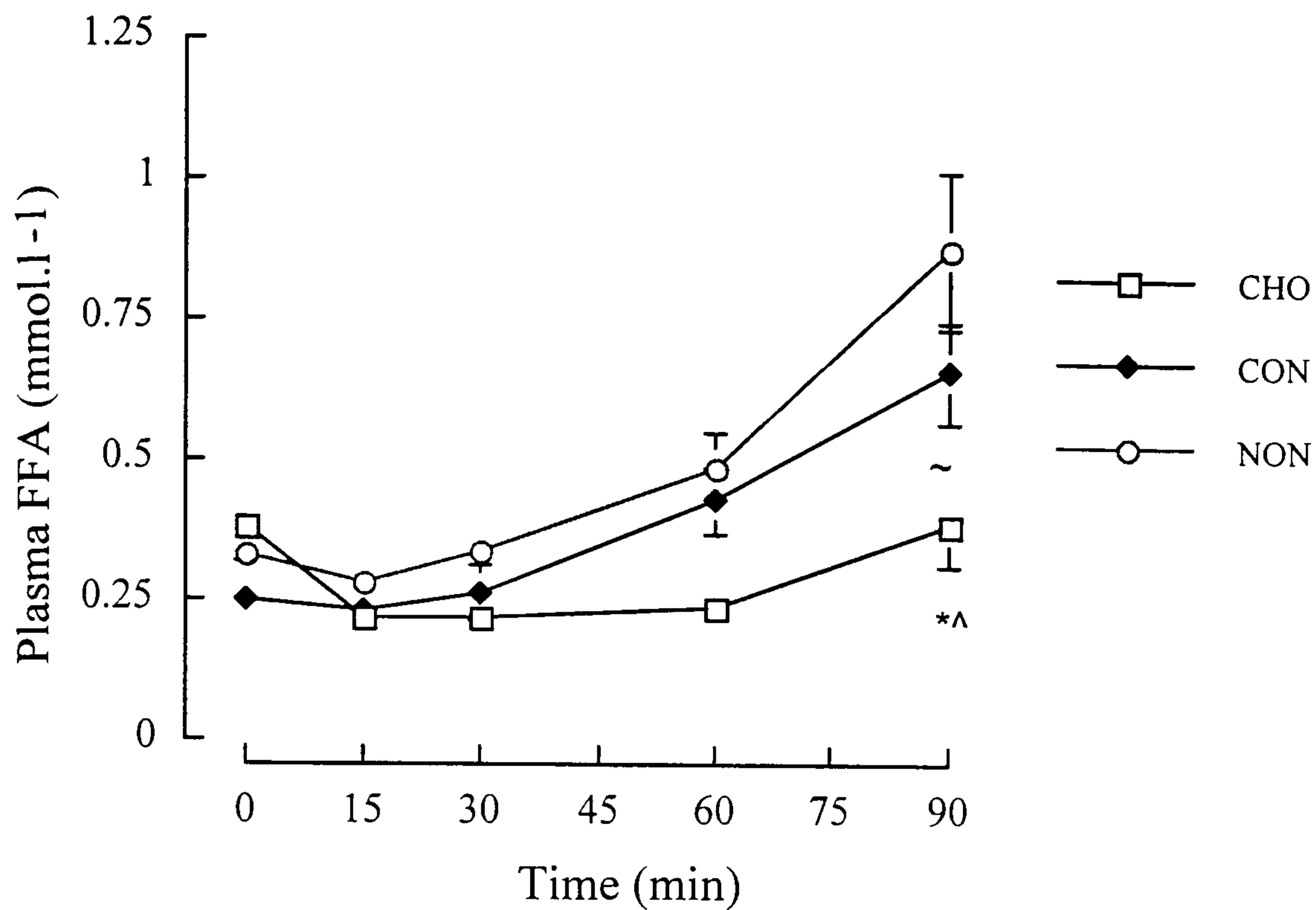


Figure 5.2: Plasma FFA concentration (mmol.l⁻¹) during the LIST

*^ *post-hoc* difference $p < 0.01$ CHO vs NON and CON, ~ *post-hoc* difference $p < 0.01$ CON vs NON

5.3.4 Serum insulin

Serum insulin concentrations were similar in the CON and NON trials. There was an initial increase in serum insulin concentration and a peak value was recorded after 15-min of exercise in the CHO trial, following which the values declined towards resting concentrations (main effect of time x trial: $F_{8,40} = 5.7, p < 0.01$). Both at 15 and 30-min of exercise, serum insulin concentrations were greater in the CHO trial (Figure 5.3).

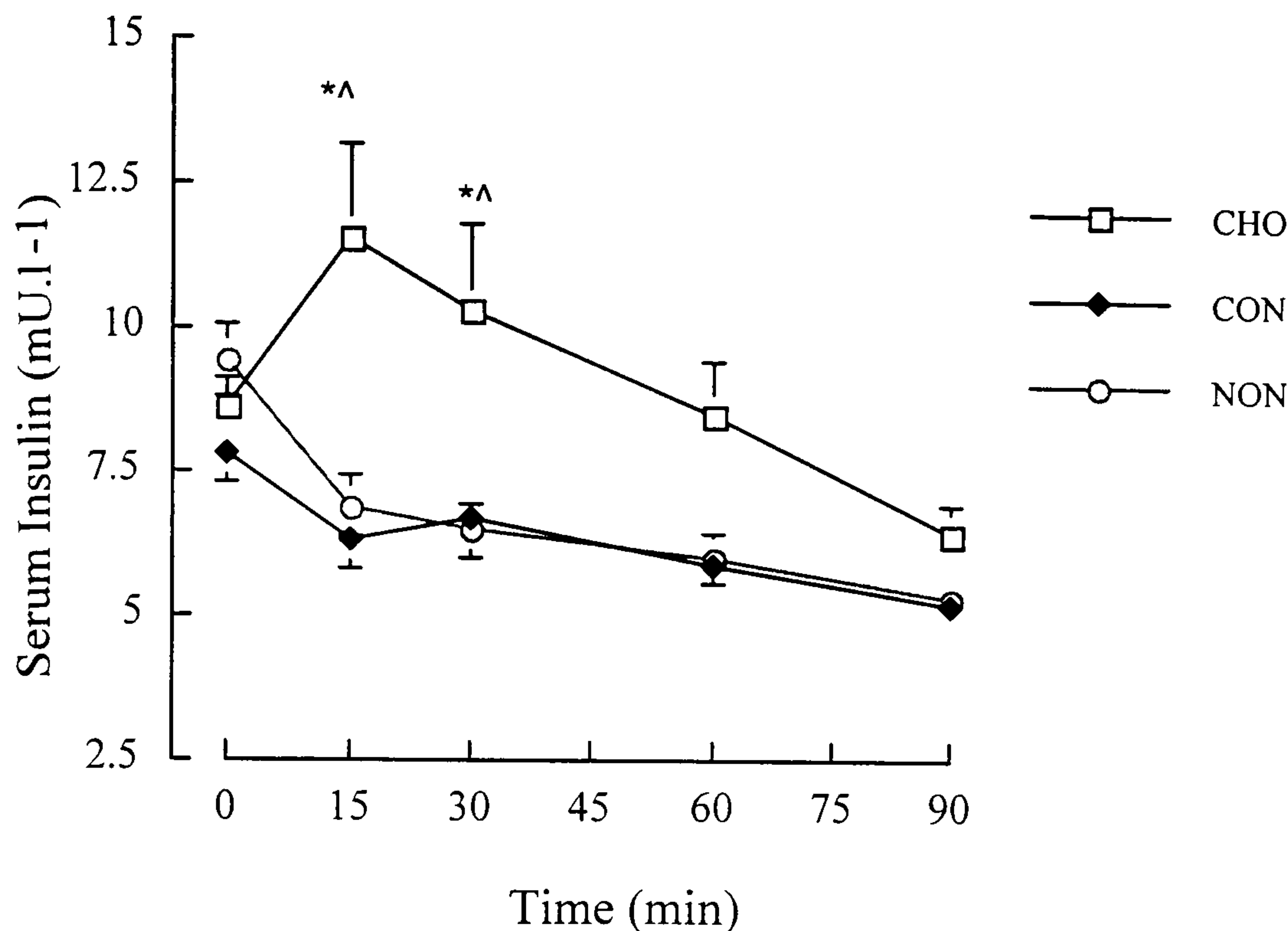


Figure 5.3. Serum insulin concentration (mU.l⁻¹) during the LIST.
 *^ *post-hoc* difference $p < 0.01$ CHO vs CON and NON

5.3.5 Changes in plasma volume and body mass

There were no differences between trials in the changes in plasma volume from rest to the end of exercise (CHO $-5.3 \pm 1.4\%$; CON $-3.4 \pm 2.1\%$; NON -3.6 ± 1.8). During the NON trial body mass loss following the LIST was 2.31 ± 0.2 kg. This was greater than the body mass loss in the CHO (1.22 ± 0.18 kg) and CON trials (1.30 ± 0.19 kg) (main effect of time x trial: $F_{2,18} = 40.5$, $p < 0.01$) but there was no difference between CHO and CON.

5.3.6 Heart rate and rating of perceived exertion

There was no difference between trials for mean heart rate during the LIST (CHO 163 ± 1 ; CON 164 ± 1 ; NON 168 ± 1). The rating of perceived exertion (RPE) described by the subjects increased in all 3 trials (main effect of time: $F_{5,45} = 16.7$, $p < 0.01$). The increase in RPE during the NON trial was greater than both the CHO and CON trials (main effect of trial: $F_{2,18} = 9.2$, $p < 0.01$) (Figure 5.4).

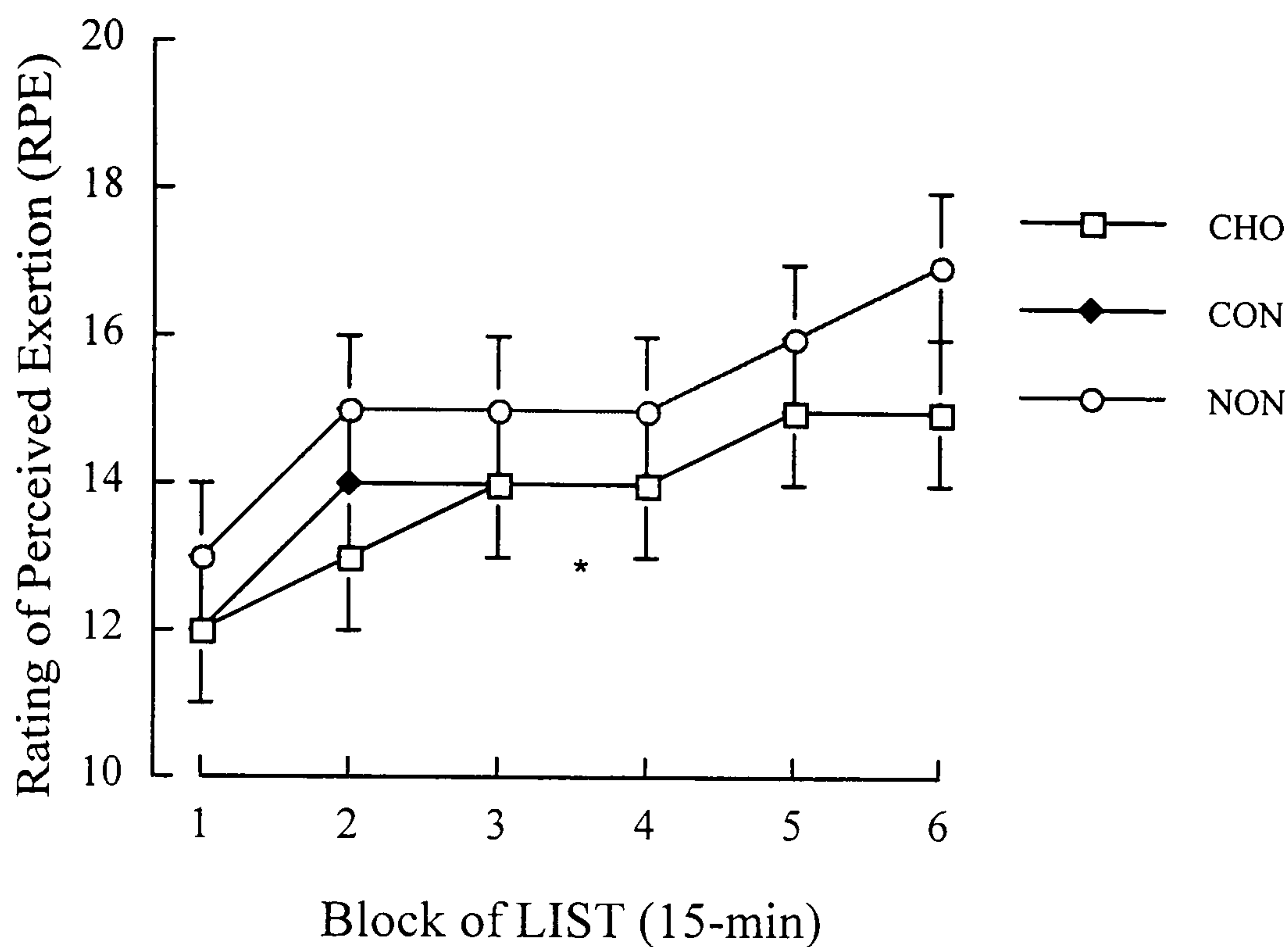


Figure 5.4: Rating of perceived exertion (RPE) during the LIST.
 * Main effect of trial, $p < 0.01$ NON vs CON and CHO.

5.3.7 Serum cortisol and aldosterone

Changes in serum cortisol concentrations were similar in all trials up to the completion of the fourth block of the LIST, (i.e. up to 60 min). Following this time serum cortisol concentration continued to rise in the NON trial at an increased rate, leading to a difference between the CHO and CON trials at 90 min (main effect of time x trial: $F_{8,64} = 2.2$, $p < 0.05$) (Figure 5.5). The rise in serum aldosterone concentration from resting values to completion of the third block of the LIST, (i.e. up to 45 min) was similar in all trials. During the NON trial serum aldosterone concentration continued to increase in the final 30 min of exercise and a difference between the NON and CHO trial was observed at 60 (main effect of time x trial: $F_{8,40} = 3.0$, $p < 0.05$) and 90 min (main effect of time x trial: $F_{8,40} = 3.0$, $p < 0.01$). Aldosterone concentration was also different between the NON and the CON trial at 90 min (main effect of time x trial $F_{8,40} = 3.0$, $p < 0.01$) (Figure 5.6)

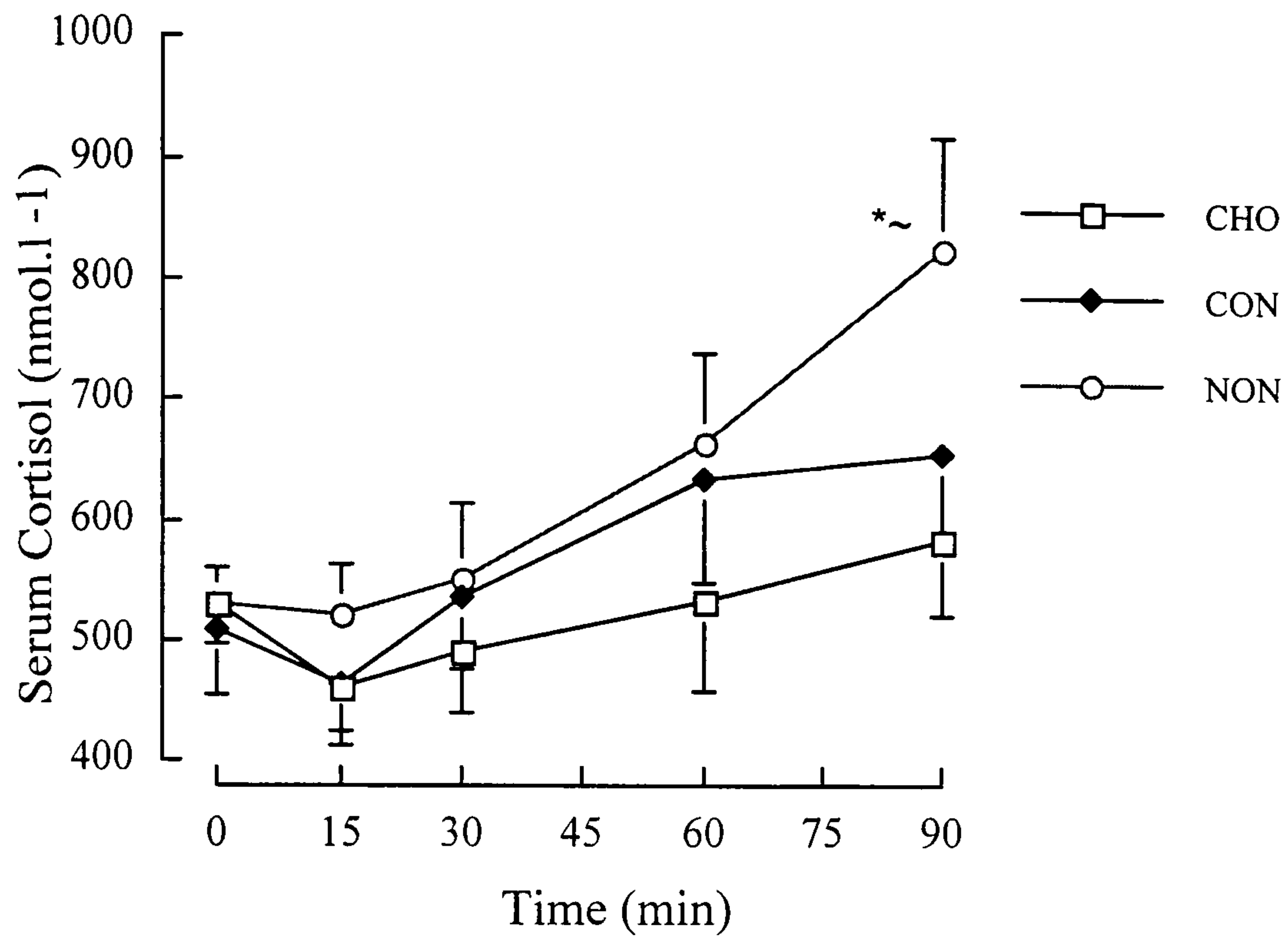


Figure 5.5: Serum cortisol concentration (nmol.l⁻¹) during the LIST.
 *~ *post-hoc* difference $p < 0.01$ NON vs CHO and CON

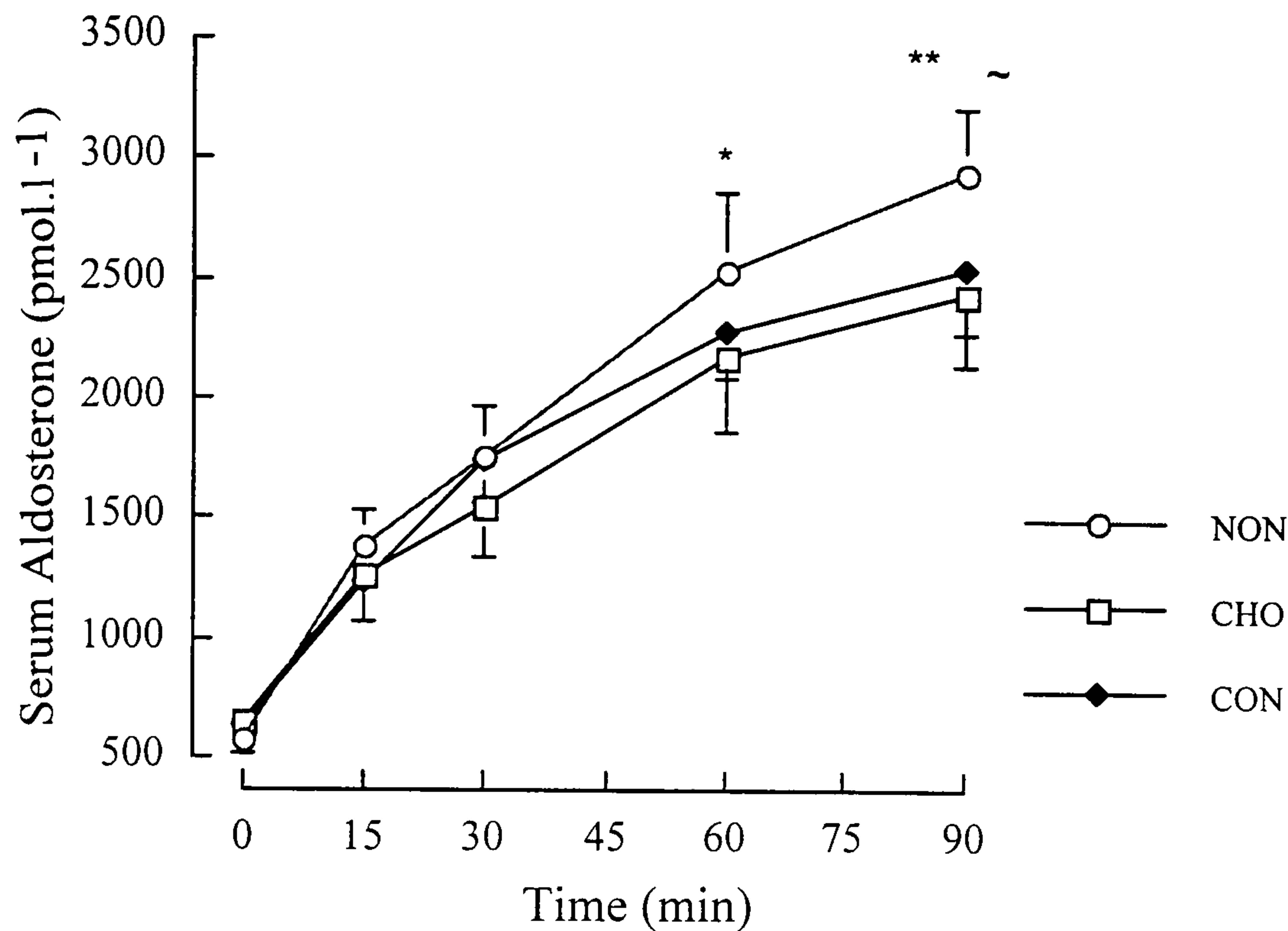


Figure 5.6: Serum aldosterone concentration (pmol.l^{-1}) during the LIST.
 * *post-hoc* difference $p < 0.05$ NON vs CHO, ** *post-hoc* difference $p < 0.01$ NON vs CHO,
 ~ *post-hoc* difference $p < 0.01$ NON vs CON

5.3.8 Serum osmolality and electrolytes

Although serum osmolality appeared to be higher during the NON trial there was no statistical significant difference between trials. There was no significant difference between trials for potassium concentration and serum sodium concentration followed a similar pattern to osmolality, with no statistically significant difference between trials (Table 5.3).

5.3.9 Blood glucose and lactate

There was no statistical difference between trials for blood glucose or lactate concentration over the 90 min period. Both metabolites increased during exercise (glucose: main effect of time: $F_{4,28} = 27.9, p < 0.01$; lactate: main effect of time: $F_{4,28} = 38.1, p < 0.01$) (Table 5.3).

Table 5.3: Serum osmolality (mOsmol.kg⁻¹), serum sodium (mmol.l⁻¹), serum potassium (mmol.l⁻¹), blood glucose and lactate concentrations during the LIST (Values are mean ± SEM)

Metabolites	Trial	0-min	15-min	30-min	60-min	90-min
Serum osmolality (mOsmol.kg ⁻¹)	CHO	295 ± 1	303 ± 2 ^a	303 ± 2 ^a	303 ± 2 ^a	304 ± 2 ^a
	CON	292 ± 1	302 ± 2 ^a	302 ± 2 ^a	302 ± 2 ^a	302 ± 2 ^a
	NON	292 ± 2	300 ± 1 ^a	304 ± 2 ^a	306 ± 2 ^a	307 ± 2 ^a
Serum sodium (mmol.l ⁻¹)	CHO	140 ± 1	142 ± 1 ^b	142 ± 1 ^b	143 ± 1 ^b	144 ± 1 ^b
	CON	139 ± 1	142 ± 1 ^b	142 ± 1 ^b	143 ± 1 ^b	144 ± 1 ^b
	NON	140 ± 1	142 ± 1 ^b	142 ± 1 ^b	144 ± 1 ^b	145 ± 1 ^b
Serum potassium (mmol.l ⁻¹)	CHO	4.1 ± 0.1	4.1 ± 0.1	4.1 ± 0.1	4.2 ± 0.1	4.4 ± 0.3
	CON	4.2 ± 0.1	4.2 ± 0.1	4.2 ± 0.1	4.2 ± 0.1	4.2 ± 0.1
	NON	4.0 ± 0.1	4.1 ± 0.1	4.1 ± 0.1	4.2 ± 0.1	4.2 ± 0.0
Blood glucose (mmol.l ⁻¹)	CHO	4.1 ± 0.1	6.0 ± 0.5 ^c	6.2 ± 0.5 ^c	5.2 ± 0.4 ^c	4.5 ± 0.3
	CON	4.2 ± 0.2	5.1 ± 0.2 ^c	5.4 ± 0.3 ^c	4.7 ± 0.2 ^c	4.3 ± 0.2
	NON	4.4 ± 0.1	5.4 ± 0.3 ^c	6.0 ± 0.3 ^c	5.2 ± 0.2 ^c	4.6 ± 0.3
Blood lactate (mmol.l ⁻¹)	CHO	0.9 ± 0.1	4.6 ± 0.5 ^d	5.3 ± 0.5 ^d	4.6 ± 0.5 ^d	4.6 ± 0.5 ^d
	CON	0.7 ± 0.1	4.6 ± 0.4 ^d	4.4 ± 0.4 ^d	3.7 ± 0.3 ^d	4.0 ± 0.4 ^d
	NON	0.7 ± 0.0	4.4 ± 0.3 ^d	4.5 ± 0.3 ^d	4.2 ± 0.6 ^d	4.0 ± 0.6 ^d

a: *post-hoc* difference, $p < 0.01$ vs 0-min b: *post-hoc* difference, $p < 0.01$ vs 0-min c: *post-hoc* difference, $p < 0.01$ vs 0-min d: *post-hoc* difference, $p < 0.01$ vs 0-min

5.3.10 Fifteen metre sprint times

Mean sprint time increased in all three trials during the 90-min LIST (main effect of time: $F_{5,45} = 5.5, p < 0.01$). Mean sprint times during the last block of exercise (CHO: 2.59 ± 0.06 s; CON: 2.58 ± 0.04 s; NON: 2.66 ± 0.05 s) were significantly slower than the first four blocks.

5.3.11 Fluid and carbohydrate ingested

The mean total volume of fluid ingested during the CHO and CON trials was 1.1 ± 0.03 l. During the CHO trial the subjects ingested a total of 70.6 ± 2.2 g of carbohydrate at a rate of 47.1 ± 1.5 g.h⁻¹.

5.3.12 Energy Intake

There was no difference between trials in the total energy, carbohydrate, fat and protein consumed in the 48 h prior to the main trials. (Table 5.5).

Table 5.4: Daily total energy (kcal), carbohydrate (%), fat (%) and protein (%) in the 48 h prior to the main trials (Values are mean \pm SEM)

Trial	Total energy (kcal)	Carbohydrate (%)	Fat (%)	Protein (%)
CHO	2955 ± 192	53.8 ± 2.2	29.7 ± 2.8	16.5 ± 1.3
CON	2937 ± 260	56.8 ± 2.9	26.0 ± 2.8	17.3 ± 1.4
NON	2858 ± 190	54.4 ± 2.9	29.6 ± 2.9	16.0 ± 1.4

5.4 Discussion

The main finding of the present study was that despite the ingestion of a 6.4% carbohydrate-electrolyte and an identical carbohydrate free solution there was no difference in estimated fat and carbohydrate oxidation rates compared to when no fluid was ingested.

During prolonged constant pace running Fallowfield *et al.* (1996) identified that without fluid ingestion there was an increased carbohydrate and decreased fat oxidation rate. Hargreaves *et al.* (1996) also identified small differences in substrate oxidation between fluid and no fluid trials. In the fluid trial they reported an increase in fat oxidation. This was confirmed from analysis of muscle biopsies which showed glycogen sparing. This shift in substrate metabolism was supported by their earlier work (Febbraio *et al.*, 1994) and attributed to increased activity of the sympathetic nervous system in response to heat stress. Hargreaves *et al.* (1996) suggested that water ingestion attenuates the increased plasma adrenaline concentration that normally occurs during exercise without fluid.

During exercise, an elevated adrenaline concentration has been found to increase glycogenolytic rate by stimulating the conversion of phosphorylase *b* to its active form phosphorylase *a* (Richter, 1982). Adrenaline infusion increases plasma concentrations and also results in an increase in glycogenolysis (Jansson *et al.*, 1986). Although catecholamines were not measured during the present study, serum cortisol concentration was greater during the NON trial than in the fluid trials. As catecholamines and cortisol increase during exercise with an increase in physiological strain, the higher cortisol concentration during the NON trial may be indicative of a similar catecholamine response. In addition to reducing the rise in cortisol concentration, the fluid ingested during the CHO and CON trials may have reduced the catecholamine response over the 90-min of exercise and subsequently reduced glycogenolysis. However, this was not reflected in whole body estimations of carbohydrate oxidation i.e. RER values.

During the present study there was no difference in the estimated fat oxidation rates between trials. This was despite higher plasma FFA concentrations during the NON trial than in the

fluid trials. These findings suggest that although there was a decreased availability of plasma FFA during the fluid trials, the exercising muscle was able to oxidise a similar amount of fat in all trials. Intramuscular triglyceride oxidation may have been increased during the CHO and CON trials to compensate for the reduced availability of plasma FFA (Romijn *et al.* 1993).

Recently, an increase in plasma FFA concentration during exercise has been linked with factors relating to central causes of fatigue (Davis *et al.*, 1992; Wilson, 1994). An increase in plasma FFA causes less tryptophan to be bound to albumin (Curzon *et al.*, 1973) and consequently increases the plasma free tryptophan (fTrp) concentration (Blomstrand *et al.*, 1988). An increase in plasma fTrp correlates with an increase in brain tryptophan concentration and subsequent synthesis of brain 5-hydroxytryptamine (5-HT)(Curzon and Knott 1974). Because 5-HT has been associated with pain and arousal (Jacobs and Azmitita, 1992), it is possible that an increase in 5-HT synthesis may affect the ability to sustain performance (Blomstrand *et al.*, 1991). Therefore, central fatigue may be a cause in the difference in the skill test performance observed between the no fluid and fluid trials of Chapter 4. However, there is not enough evidence to support such a theory in the present study.

If as proposed, fluid ingestion reduces muscle glycogenolysis, a difference in carbohydrate oxidation rate between trials may be expected. During the present study there was no statistically significant difference between trials in estimated carbohydrate oxidation rate. The estimated carbohydrate oxidation rate for the CHO ($2.7 \pm 0.1 \text{ g}\cdot\text{min}^{-1}$) and CON ($2.5 \pm 0.2 \text{ g}\cdot\text{min}^{-1}$) trials during the LIST are similar to the those reported following the ingestion of similar solutions and during exercise at a similar intensity (Tsintzas *et al.*, 1996). A number of authors have reported that carbohydrate ingestion during exercise reduces the rate of muscle glycogen utilisation (glycogen sparing) in comparison to water ingestion (Bjorkman *et al.* 1984; Hargreaves *et al.* 1984; Erikson *et al.* 1987; Nicholas *et al.*, 1994; Tsintzas *et al.*, 1995). Although muscle biopsy samples were not obtained in the present study, it is reasonable to assume that at the end of the LIST the muscle glycogen concentrations of the subjects were similar to those reported by Nicholas *et al.* (1994). Using similar conditions to

those in the present study, Nicholas *et al.* (1994) reported that the consumption of a carbohydrate-electrolyte solution during the LIST reduced the amount of muscle glycogen utilised in comparison to flavoured water. Unfortunately, indirect calorimetry was not performed during the study of Nicholas *et al.* (1994). However, during the studies of prolonged cycling (Erikson *et al.*, 1987) and running (Tsintzas *et al.*, 1995), carbohydrate ingestion resulted in glycogen sparing eventhough there were no differences in carbohydrate oxidation rates between trials. The contribution of carbohydrate may be from different sources. For example a reduction in muscle glycogenolysis may be compensated by an increase in blood glucose oxidation following the ingestion of carbohydrate (Tsintzas and Williams, 1998). Therefore, total carbohydrate oxidation will remain similar and reason for the absence of a difference between the two fluid trials in the present study.

Similarly an increase in blood glucose utilisation could explain the observation that there were no differences between the CHO and NON trials in glucose oxidation rates but not between the NON and CON trials. Muscle glycogenolysis may have been greater during the NON trial than in the CON trial and glucose oxidation remained similar through an enhanced rate of glucose release from sources other than muscle. King *et al.* (1985) identified a muscle glycogen sparing effect during exercise in the heat following 8 days of acclimatisation. They also found no difference in carbohydrate and fat oxidation rates pre and post acclimatisation yet showed a 42% reduction in muscle glycogen utilisation. The absence of a difference in carbohydrate oxidation rates was suggested to be due to an increased hepatic glucose production and subsequent use by active skeletal muscle in the pre-acclimatisation trial (King *et al.* 1985). As with fluid ingestion, they suggested that acclimatisation resulted in a reduced core temperature and muscle glycogen sparing, possibly as a result of a lowering of the catecholamine concentration post acclimatisation.

Another possible reason for a sparing of muscle glycogen without an associated increase in glucose oxidation rate could be a greater blood flow to the active tissues during the CON trial. This is based upon studies performed during thermal stress (Rowell, 1974; Fink *et al.*, 1975) and following acclimatisation (Rowell *et al.*, 1967; King *et al.*, 1985). It has been suggested that following heat acclimatisation, less of the cardiac output is required for heat

dissipation and hence a greater proportion may be available for perfusion of muscle (Rowell, 1974). This area is equivocal as many authors have found only small or no differences in muscle blood flow following exercise under similar conditions (Kirwan *et al.*, 1987; Savard *et al.*, 1988; Nielsen *et al.*, 1990; Nielsen *et al.*, 1993).

It is possible that during the NON trial muscle glucose uptake was reduced secondary to an accelerated muscle glycogenolysis (Jansson *et al.*, 1986). This would be a further reason why carbohydrate oxidation rates were similar in all trials despite a potentially greater muscle glycogenolysis in the NON trial.

It has been suggested that the use of pulmonary exchange data during high intensity exercise is not sensitive to shifts in metabolism. The RER calculations assume a steady state of exercise, and do not consider the influence of lactate production (Heigenhauser *et al.*, 1983) and the bicarbonate response to lactic acid buffering (Stringer *et al.*, 1995) following high intensity exercise. During exercise at intensities that cause hyperventilation, $\dot{V}CO_2$ may overestimate CO_2 production and potentially result in the overestimation of the rate of carbohydrate oxidation and, concomitantly, an underestimation of fat oxidation (Ferranini 1988). However, a study using stable isotope tracers and indirect calorimetry to evaluate the regulation of endogenous fat and glucose metabolism, identified that indirect calorimetry is valid up to and including exercise at 85% $\dot{V}O_2$ max (Romijn *et al.*, 1993). As the mean exercise intensity was 70% $\dot{V}O_2$ max during the present study, the use of indirect calorimetry to estimate energy expenditure during the LIST appears valid. As with any other investigation, the true validity of the estimation of substrate oxidation can only be quantified following direct measurements of glucose and FFA exchange across the working muscle (King *et al.*, 1985).

A major finding of the present study was that through indirect calorimetry the energy expenditure during the LIST was quantified for the first time. The LIST has been used in a number of studies (Nicholas *et al.*, 1994; Nicholas *et al.*, 1997; Morris *et al.*, 1996; Thompson *et al.*, 1999) and was designed specifically to simulate the activity pattern characteristic of the game of soccer (Nicholas *et al.*, 1995). The similarity between the LIST

and match analysis data in terms of distance covered and physiological parameters such as heart rate, glucose and lactate concentrations has been discussed previously (Nicholas *et al.*, 1999). The values recorded for heart rate and blood metabolites during this study and those in the previous chapter are also similar to those reported by Nicholas *et al.* (1999). Estimated total energy expenditure, % $\dot{V}O_2$ max, carbohydrate and fat oxidation rates during the present study are consistent with those estimated from match analysis (for a review see Bangsbo, 1998).

Plasma volume changes, RPE, body mass loss, heart rate response and electrolyte concentrations were similar to those reported in Chapter 4. In addition to the greater rise in cortisol and aldosterone concentrations during the NON trial, the data from this study add further evidence to there being a greater strain on the body in the absence of fluid ingestion.

In summary, during 90-min of intermittent high intensity shuttle running there appears to be an increase in perceived, circulatory and thermoregulatory strain in the absence of fluid ingestion. Data obtained from analysis of expired air did not identify a shift in metabolism during exercise without fluid.

CHAPTER 6

THE INFLUENCE OF A CARBOHYDRATE-ELECTROLYTE SOLUTION ON PASSING AND DRIBBLING SOCCER SKILL FOLLOWING PROLONGED INTERMITTENT RUNNING

6.1 Introduction

Prolonged intermittent high intensity shuttle running without fluid ingestion has been shown to reduce the performance of a soccer dribbling test (Chapter 4). Dribbling is only one of a number of skills performed during the game of soccer. It could be argued that dribbling with a ball relies more on physical attributes, such as speed and power, rather than successful completion of skilled elements such as decision-making and control. It is therefore of interest to identify if a similar bout of exercise influences a skill that is more cognitive than physically demanding.

The consumption of carbohydrate during running and cycling exercise increases exercise performance (Murray *et al.*, 1987; Mitchell *et al.*, 1988; Tsintzas *et al.*, 1995) capacity (Coyle *et al.*, 1983; Hargreaves *et al.*, 1984; Nicholas *et al.*, 1995) and cognitive function (Reilly and Lewis, 1985). There are relatively few studies that have investigated the influence of carbohydrate ingestion during exercise on soccer skills. Those studies that have been reported have not all found that carbohydrate ingestion helps skill retention. For example ingestion of carbohydrate immediately prior to and or during soccer has been shown to improve (Muckle, 1973) or have no effect (Zeederberg *et al.*, 1996) on components of soccer skill. The reason for the disparity in findings may be due to the lack of adequate control associated with these field studies.

Changes in metabolic concentration may have an influence on central neural control. For example, exhaustive bouts of exercise may increase plasma ammonia concentration to the

point where they could impair the function of the central nervous system (Mutch and Bannister 1983; Banister and Cameron, 1990). Central fatigue has also been related to an increase in the transport of plasma tryptophan across the blood-brain barrier and subsequent synthesis of brain 5-hydroxytryptamine (Blomstrand *et al.*, 1991; Davis *et al.*, 1992).

The aim of the present study, was to assess the influence of no fluid (NON), a 6.4 % carbohydrate-electrolyte (CHO) and a carbohydrate free solution (CON) on passing and dribbling soccer skills following prolonged intermittent, high intensity shuttle running. To investigate further the possible role of central fatigue, plasma ammonia and serum prolactin were measured in addition to the metabolites reported in previous chapters.

6.2. Methods

6.2.1 Subjects

Nine male university and semi-professional soccer players volunteered to participate in the study. The mean \pm SEM age, body mass (BM) and estimated maximal oxygen uptake of the group were 20.7 ± 0.4 years, 71.6 ± 1.4 kg and 59.3 ± 0.9 ml.kg⁻¹ min⁻¹ respectively.

6.2.2 Preliminary tests

Subjects performed preliminary tests to: (i) estimate $\dot{V}O_2$ max in order to calculate the relative exercise intensities as previously described (Chapter 3) and (ii) to familiarise themselves with the experimental procedures.

6.2.3 Experimental Procedures

Subjects completed 3 main trials separated by at least 7 days. The order of the trials was randomised to offset any training or order effects. In each trial, subjects were assigned to one of the following treatments: (i) a 6.4 % carbohydrate - electrolyte (CHO) solution (Lucozade Sport, SmithKline Beecham, UK), (ii) an identical solution to (i) with the same concentration of electrolytes, artificially sweetened and flavoured but excluding carbohydrate (CON) (SmithKline Beecham) and (iii) no fluid (NON).

The solutions ingested were of a similar colour, texture and taste and were administered in a double blind design. The subjects consumed the solutions immediately prior to the LIST ($5\text{ml.kg}^{-1}\text{BM}$) and every 15-min thereafter ($2\text{ml.kg}^{-1}\text{BM}$) (Figure 6.1).

In the two days prior to the main trials subjects did not participate in any prolonged, heavy exercise. They reported to the laboratory after an overnight fast of between 10 and 12 h. They then emptied their bladders prior to the preliminary measurement of nude body mass, which

was recorded before and immediately following each trial. Nude body mass was determined to the nearest 0.1 kg using a beam balance (Avery Ltd., Model 3306ABV). These measurements before and after exercise along with fluid intake during exercise were used to calculate sweat losses during exercise. An indwelling cannula (Venflon, 16-18G, BOC Ohmeda, Sweden) was inserted into an antecubital vein before exercise and kept patent by periodic flushing with sterile saline (10 U.ml⁻¹ heparin). The subjects stood for 15-20 min before a resting blood sample was obtained (Figure 6.1).

The subjects performed a standardised 15-min warm-up prior to each trial, consisting of jogging, stretching and striding. Immediately after the warm up the subjects performed the Loughborough Soccer Dribbling Test and the Loughborough Soccer Passing Test (LSPT and LSDT, as described in Chapter 3). There then followed a 2-min period during which subjects ingested the prescribed solution and prepared to begin the LIST. During the no fluid trial the subjects had a similar 2-min break during which they awaited the commencement of the LIST.

Heart rate was recorded every 15 s during the trials by short - range radio telemetry (Polar Sport Tester™, Kempele, Finland). Temperature of the Sports Hall was recorded every 20 min and maintained over the study between 14 - 20°C by opening windows and doors. Dry-bulb and wet-bulb temperatures were measured using a whirling hygrometer (Brannan Thermometers Ltd).

On completion of the LIST the subjects performed the soccer skill tests again. The overall design of the study is illustrated in Figure 6.1.

6.2.4 Blood Sampling and Analysis

Eleven millilitres of blood were withdrawn at rest, after 15, 30, 60 and 90 min of exercise. The blood was dispensed, treated and stored as previously described (Chapter 3). Serum was analysed for prolactin, insulin, cortisol, aldosterone and electrolyte concentrations and plasma was analysed for FFA concentrations. Whole blood was analysed for ammonia, lactate and

glucose concentrations and plasma volume changes, using methods that have been previously described (Chapter 3).

6.2.5 Statistical Analysis

Results were analysed as described under statistical analysis (Chapter 3). Results are represented as mean \pm SEM.

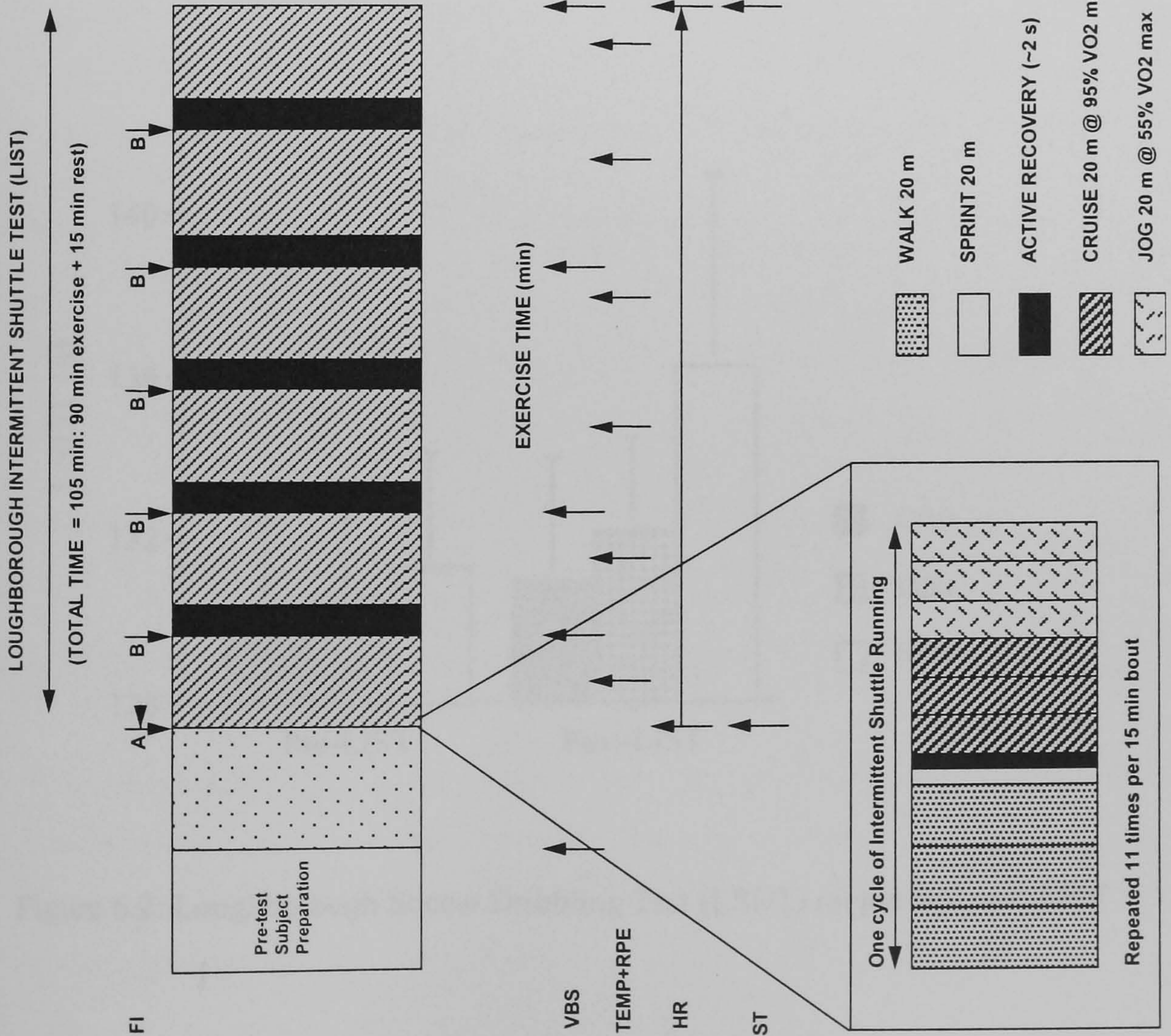


Figure 6.1: Schematic illustration of the LIST and experimental procedures

6.3 Results

6.3.1 Skill tests

There were no differences between the pre-LIST test scores between trials for the LSPT (CHO: 54.2 ± 2.2 s, CON: 53.5 ± 1.5 s, NON: 51.7 ± 1.7 s). There was also no difference between Pre- and Post-LIST skill test performance times in the CHO and CON trials; however, Post-LIST skill test times were longer than Pre-LIST during the NON trial (57.1 ± 1.8 s) (main effect of time x trial: $F_{2,16} = 4.4, p < 0.05$). Although there appeared to be a similar finding for the LSDT there was no statistically significant difference between pre-exercise and post-LIST scores (Figure 6.2).

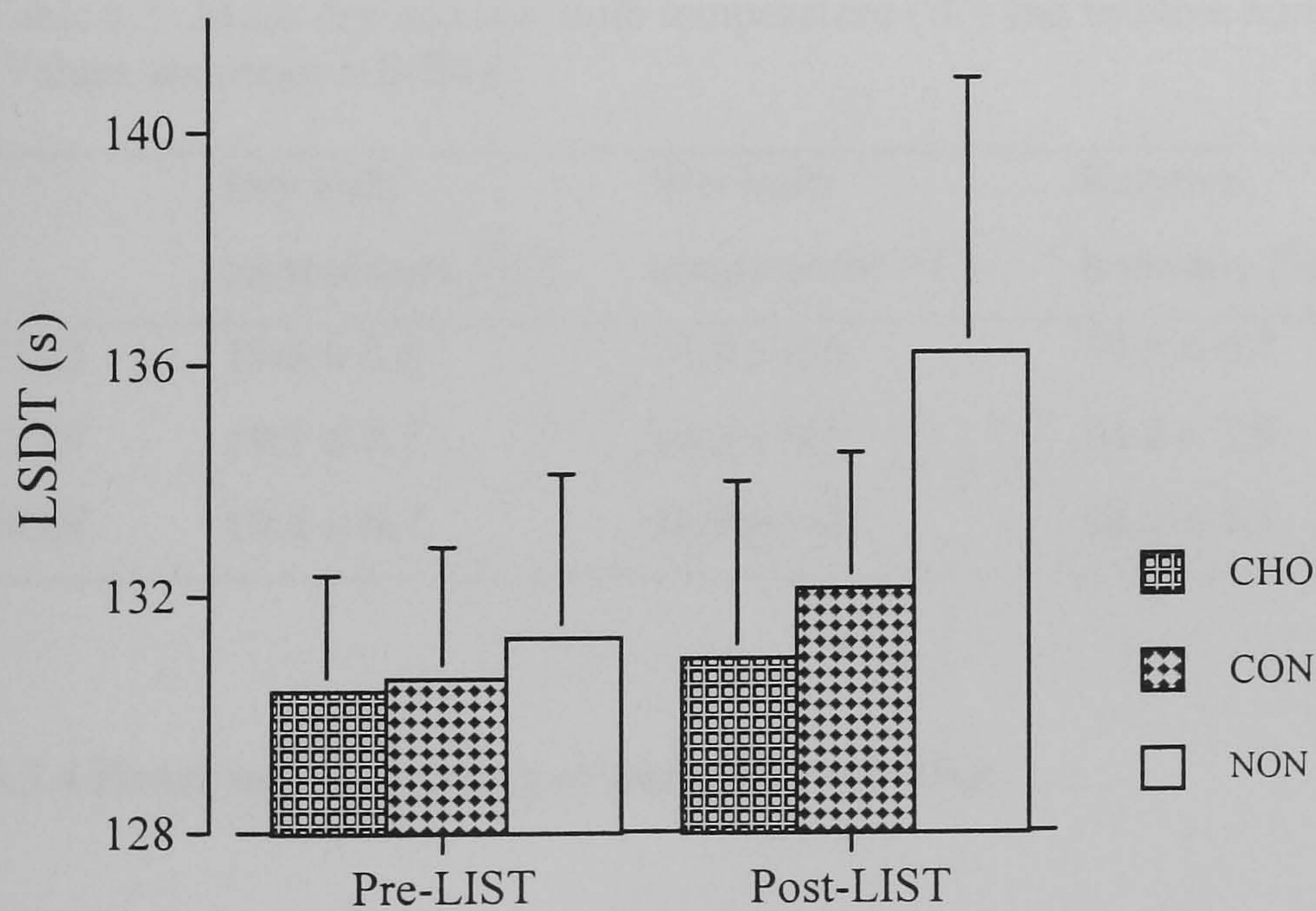


Figure 6.2: Loughborough Soccer Dribbling Test (LSDT) (s) pre and post-LIST

6.3.2 Changes in plasma volume and body mass

There were no differences between or within trials in the changes in plasma volume from rest to the end of exercise (CHO $-3.9 \pm 2.7\%$; CON $-2.5 \pm 1.5\%$; NON $-4.1 \pm 1.2\%$). During the NON trial body mass loss following the LIST was 2.35 ± 0.2 kg. This was greater than the body mass loss in the CHO trial (1.54 ± 0.09 kg) and CON trial (1.32 ± 0.24 kg) (main effect of time x trial: $F_{2,16} = 14.6, p < 0.01$). There was no difference in body mass changes between CHO and CON trials.

6.3.3 Environmental conditions

There was no difference between trials in the dry and wet bulb temperature and relative humidity during the study (Table 6.1).

Table 6.1: Mean dry and wet bulb temperature ($^{\circ}\text{C}$) and relative humidity (%) (Values are mean \pm SEM).

	Dry bulb temperature ($^{\circ}\text{C}$)	Wet bulb temperature ($^{\circ}\text{C}$)	Relative humidity (%)
CHO	19.8 ± 0.8	14.9 ± 0.6	59.8 ± 0.7
CON	19.1 ± 0.7	14.9 ± 0.5	64.4 ± 3.9
NON	19.3 ± 0.7	14.9 ± 0.4	64.2 ± 4.1

6.3.4 Heart rate and rating of perceived exertion

There was no difference in mean heart rate for the 90 min of exercise between trials ($n = 7$). The rating of perceived exertion (RPE) described by the subjects during the NON trial was greater than in the CHO and CON trials during the second and each subsequent block of the LIST (main effect of time x trial: $F_{10,80} = 2.8, p < 0.01$) (Figure 6.3).

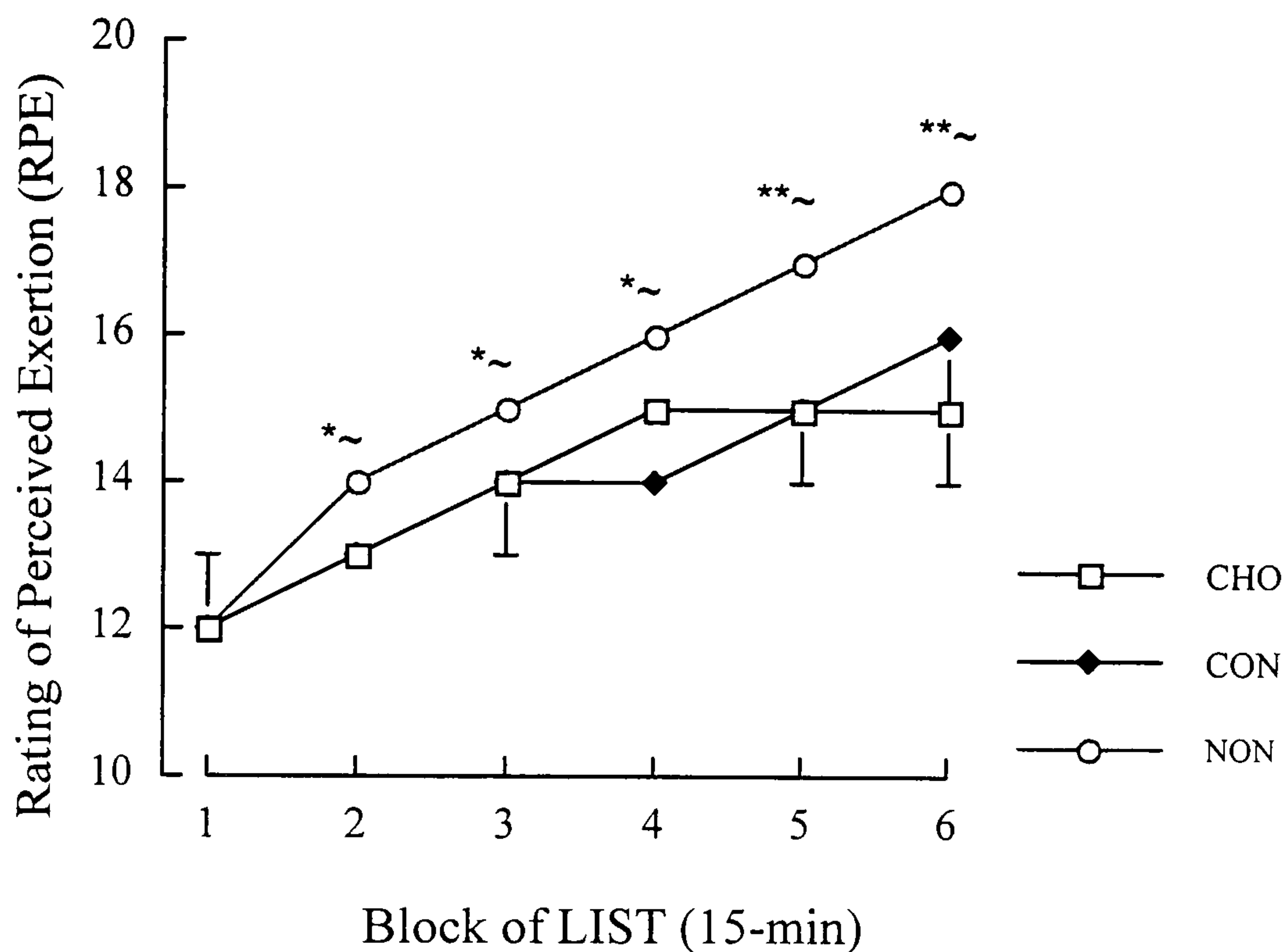


Figure 6.3: Rating of perceived exertion (Borg scale) during the LIST.
 ~ *post-hoc* difference $p < 0.01$ NON vs CON, * *post-hoc* difference $p < 0.05$ NON vs CHO, ** *post-hoc* difference $p < 0.01$ NON vs CHO

6.3.5 Metabolites measured

Prior to three of the main trials during this study there was difficulty with cannulation and no blood was collected for those particular trials. Therefore, there were only 6 and for some metabolites only 5 complete sets of subject data for blood analysis.

6.3.6 Blood glucose and lactate

Higher blood glucose concentration (main effect of time: $F_{4,12} = 3.8$, $p < 0.05$) was observed at 15-min than at 0 and 90-min during all trials. There was no statistically significant difference between trials (Figure 6.4) ($n = 6$). Blood lactate concentrations were also similar between trials. Resting blood lactate values were lower than all other time points (main effect of time: $F_{4,8} = 10.5$, $p < 0.01$) (Figure 6.5).

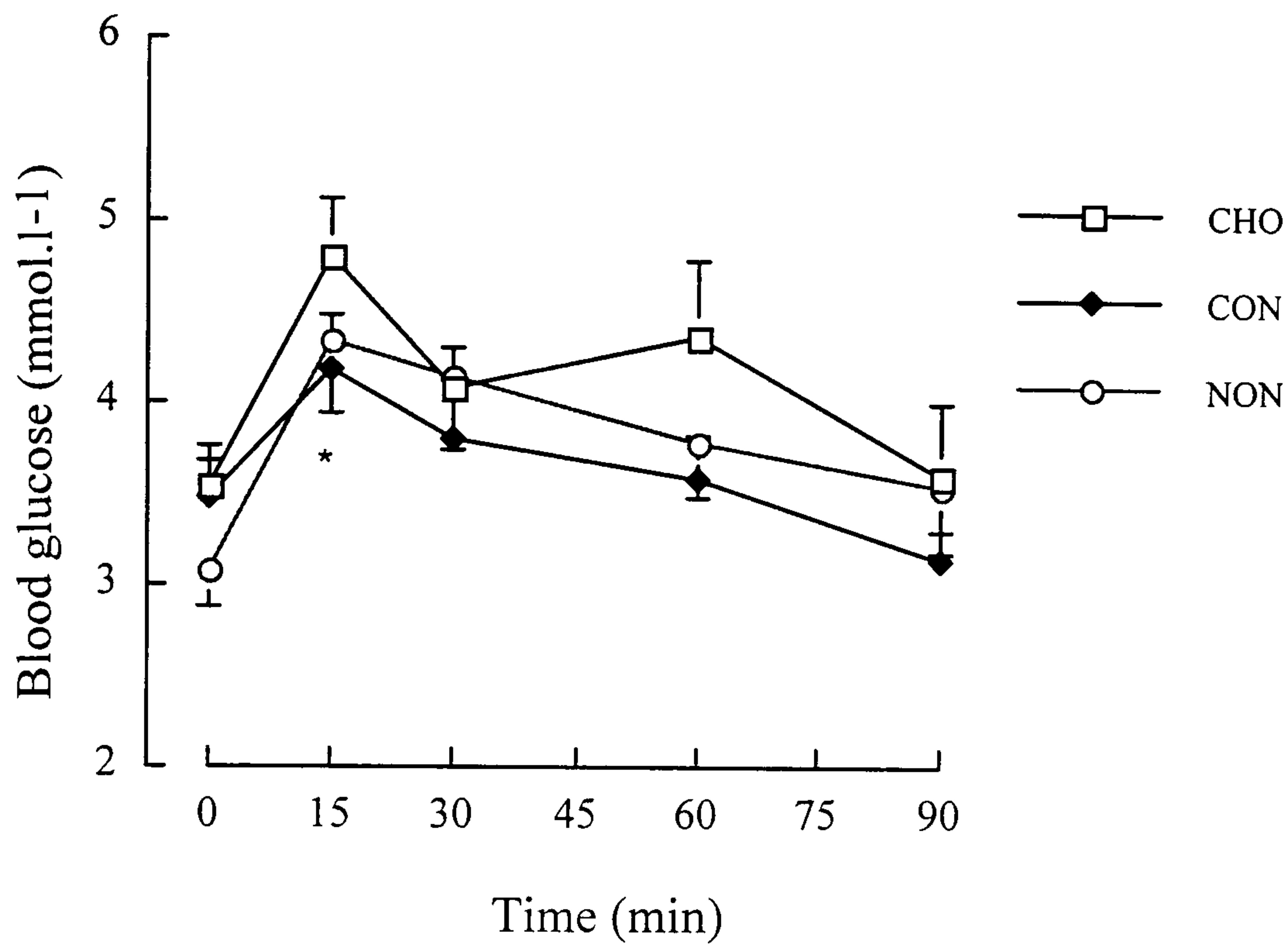


Figure 6.4 Blood glucose concentration (mmol.l⁻¹) during the LIST.
 * *post-hoc* difference $p < 0.05$ 15 vs 0 and 60-min

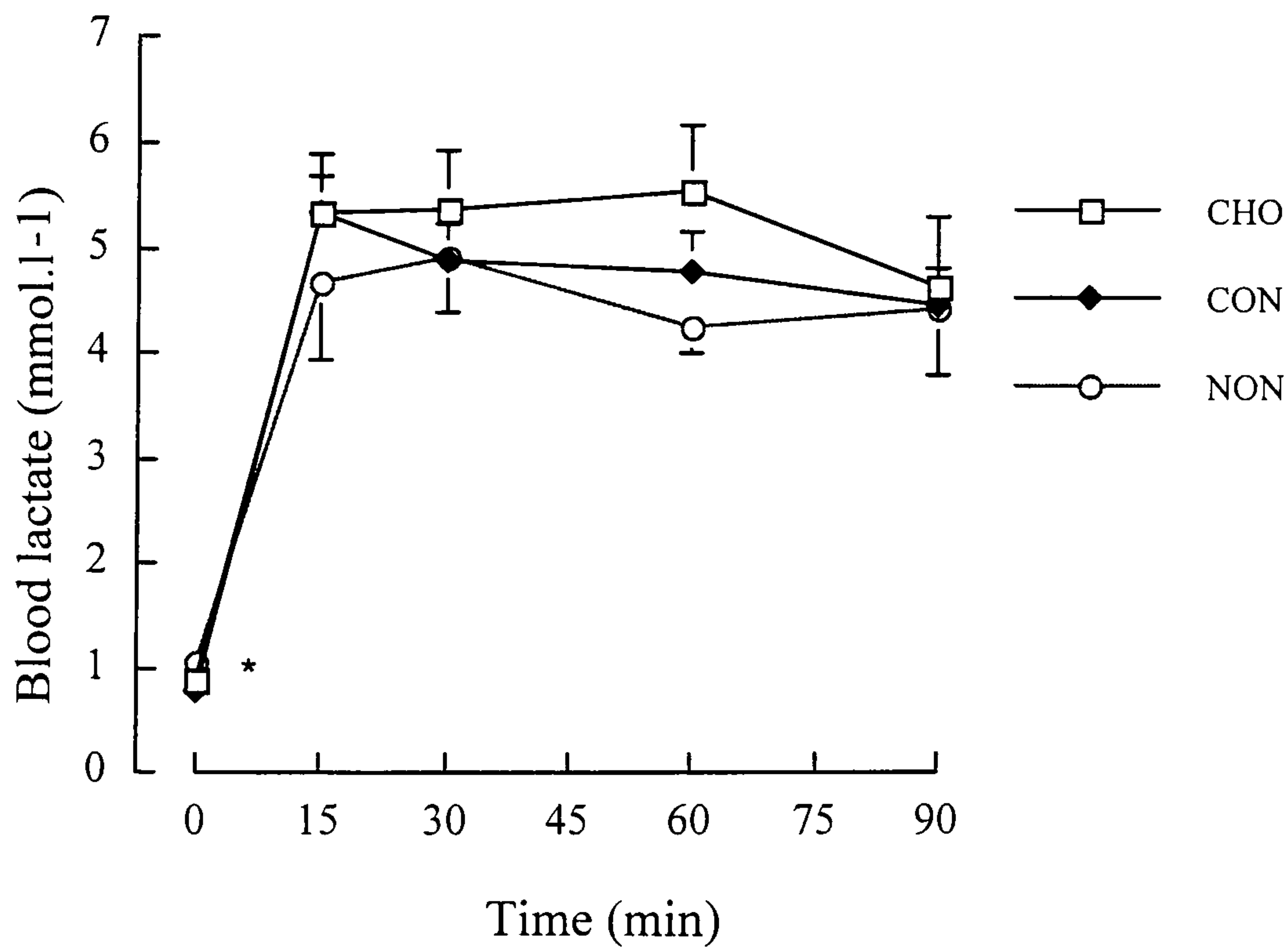


Figure 6.5 Blood lactate concentration (mmol.l⁻¹) during the LIST.
 * *post-hoc* difference $p < 0.01$ 0 vs all other time points

6.3.7 Plasma ammonia

Plasma ammonia increased in all trials over the 90-min LIST (main effect of time: $F_{4,12} = 14.9$, $p < 0.01$). Although plasma ammonia concentrations appeared to be lower during the CHO trial than the CON and NON, there was no statistically significant difference between trials (Figure 6.6).

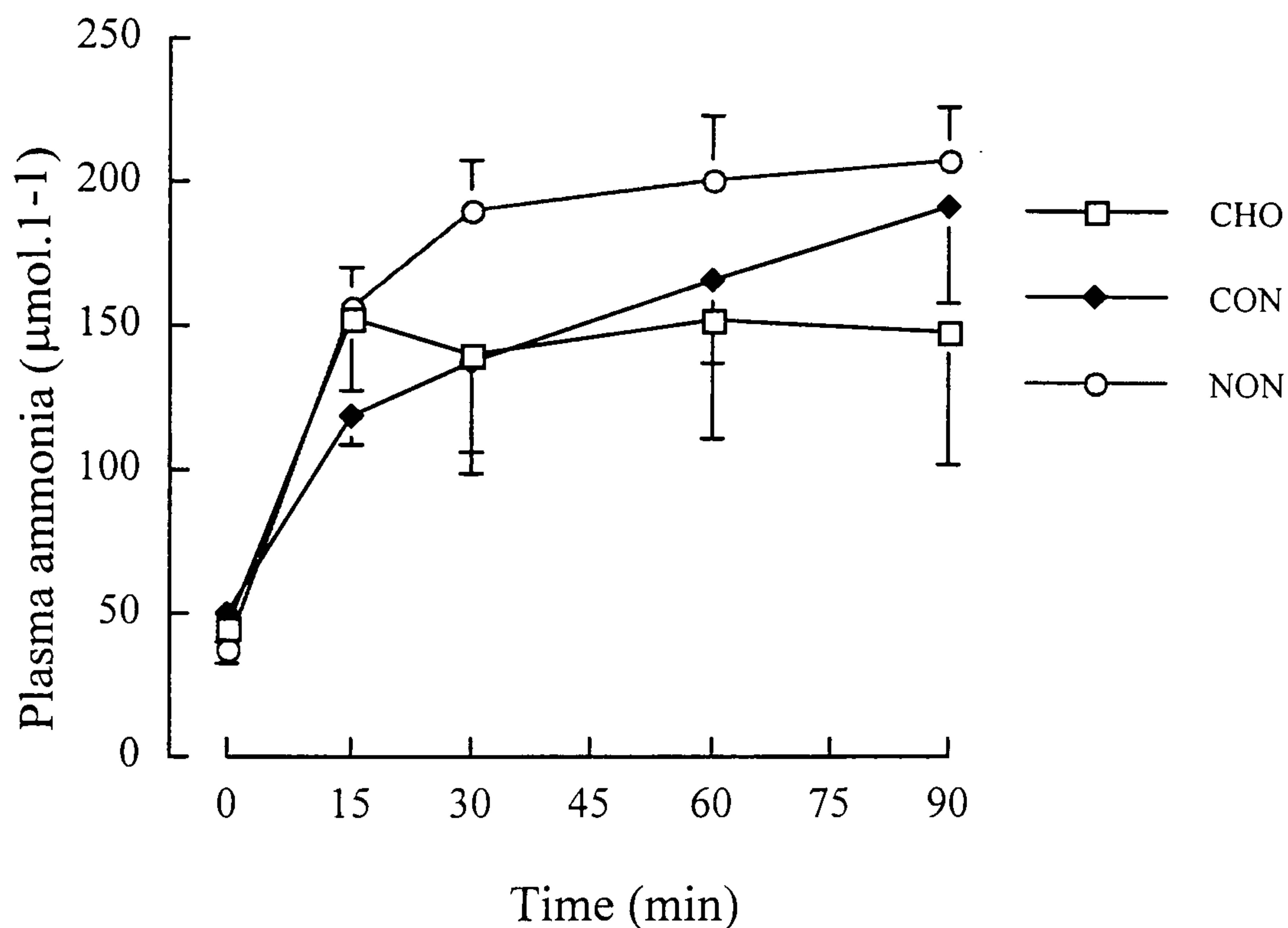


Figure 6.6 Plasma ammonia concentration ($\mu\text{mol.l}^{-1}$) during the LIST (n = 6).
**post-hoc* difference $p < 0.01$ 0 vs all other time points

6.3.8 Plasma Free Fatty Acids (FFA)

Plasma FFA concentrations increased over time in all 3 trials, with values being higher at 90-min than all other time points (main effect of time: $F_{4,12} = 10.1$, $p < 0.01$). The pattern of response was similar to that reported previously (Chapter 5), although during this study there was no statistically significant difference between trials (Figure 6.7).

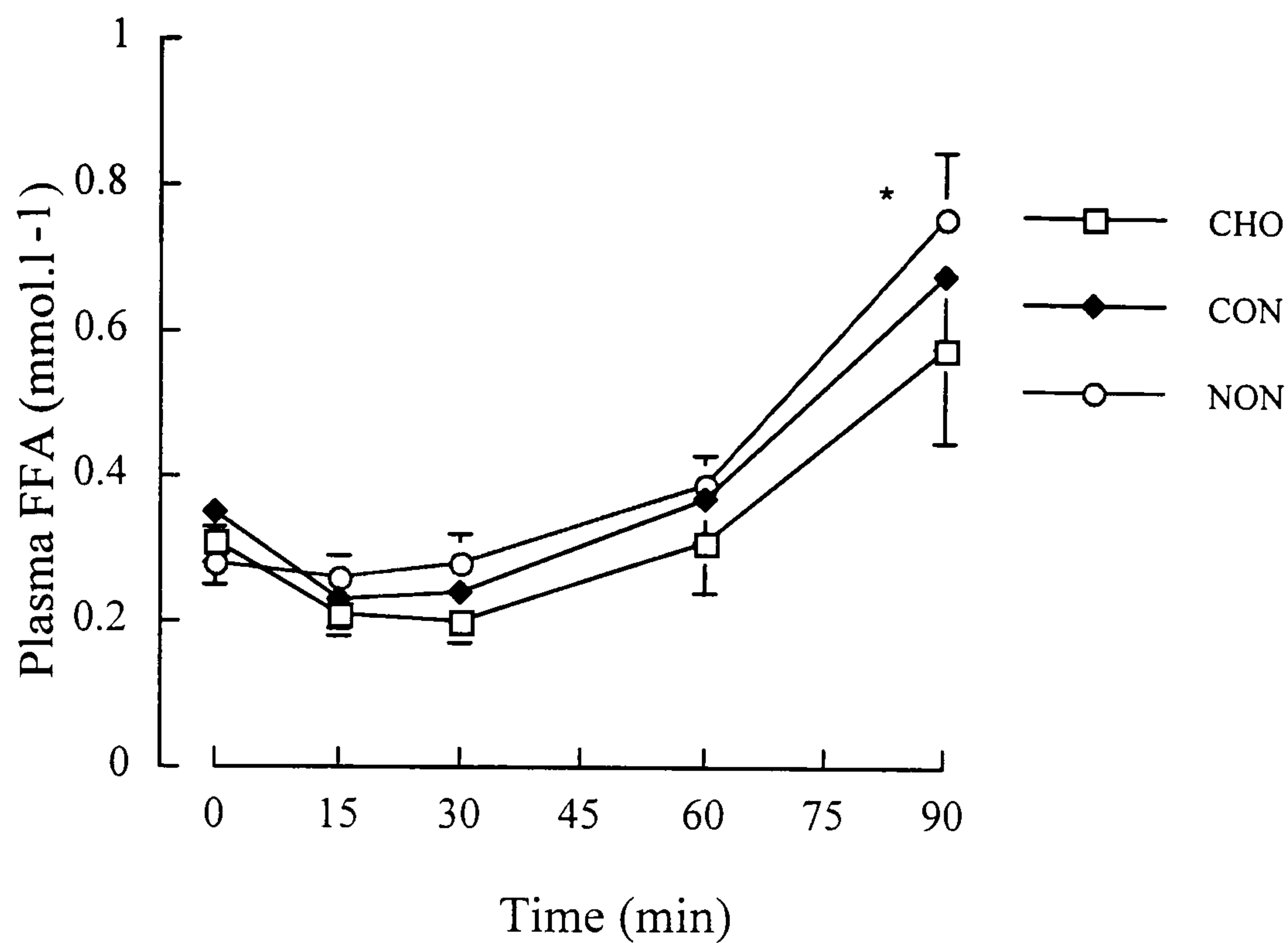


Figure 6.7 Plasma FFA concentration (mmol.l⁻¹) during the LIST.
 **post-hoc* difference $p < 0.01$ 90-min vs all other time points

6.3.9 Serum insulin

Serum insulin concentrations followed a similar pattern to that reported previously. Following an initial increase in concentration, the values progressively declined (main effect of time: $F_{4,12} = 7.1$, $p < 0.01$) (Figure 6.8). There was no statistically significant difference between trials.

6.3.10 Serum aldosterone

Serum aldosterone increased within all trials (main effect of time: $F_{4,4} = 6.2$, $p < 0.05$), with no statistically significant difference between trials (Figure 6.9).

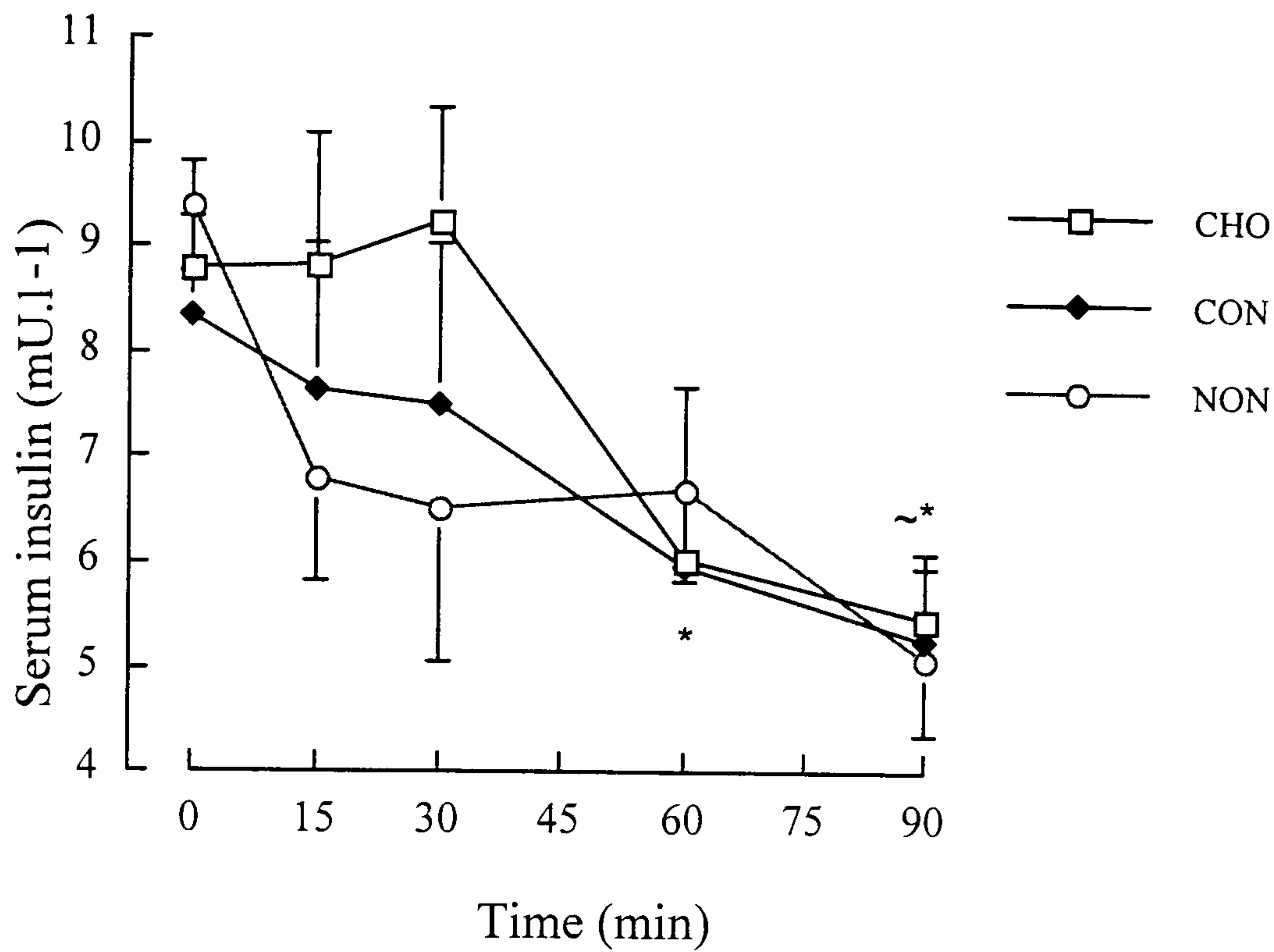


Figure 6.8 Serum insulin concentration (mU.l⁻¹) during the LIST (n = 5).
 * *post-hoc* difference $p < 0.05$, 0 vs 60 and 90-min, ~* *post-hoc* difference $p < 0.05$, 15 and 30 vs 90-min.

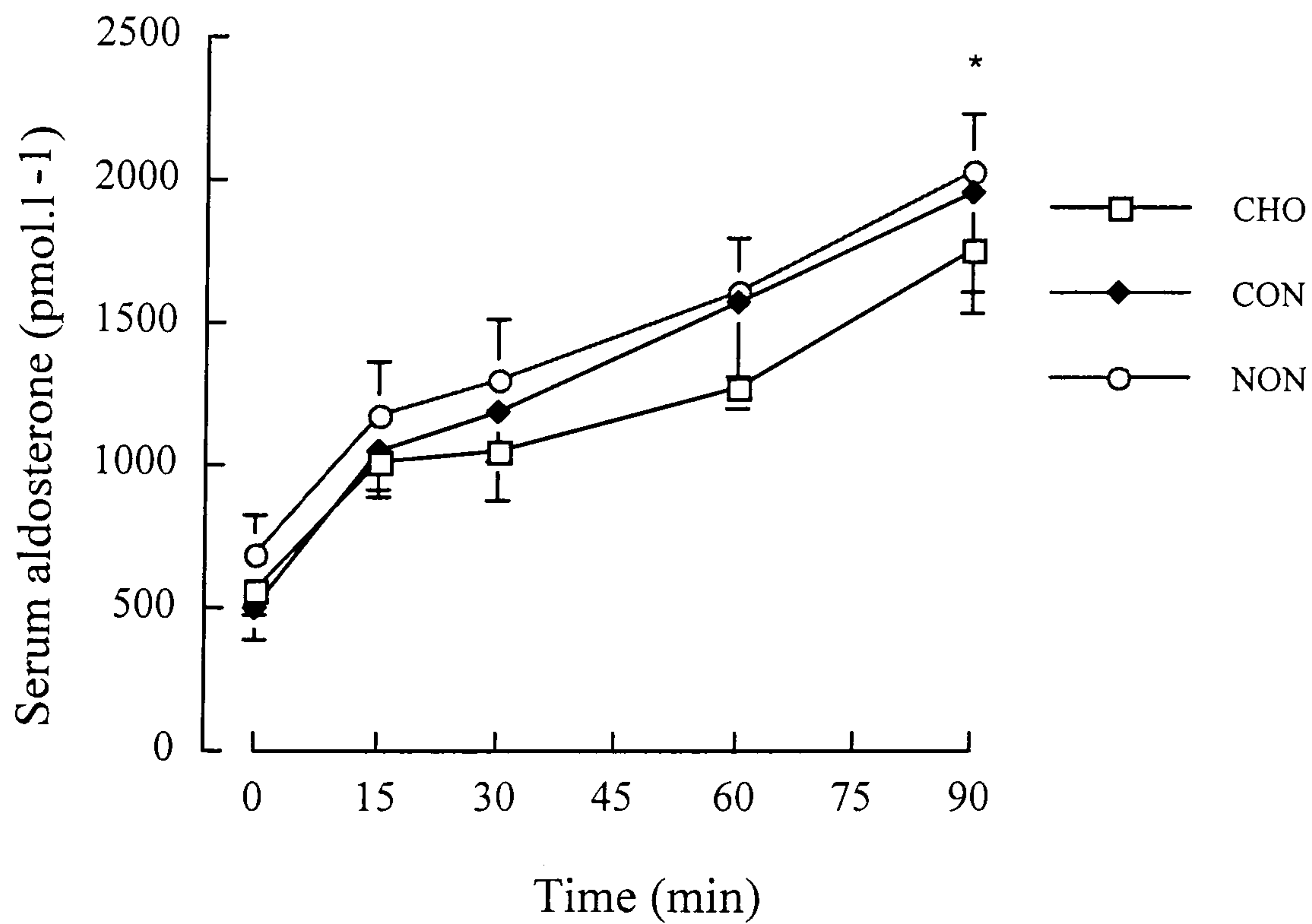


Figure 6.9: Serum aldosterone concentration (pmol.l⁻¹) during the LIST (n = 5).
 * *post-hoc* difference $p < 0.05$, 0 vs 90-min.

6.3.11 Serum prolactin

Serum prolactin concentration was measured at 0, 45 and 90 min. There was no statistically significant difference between or within trials (Figure 6.10).

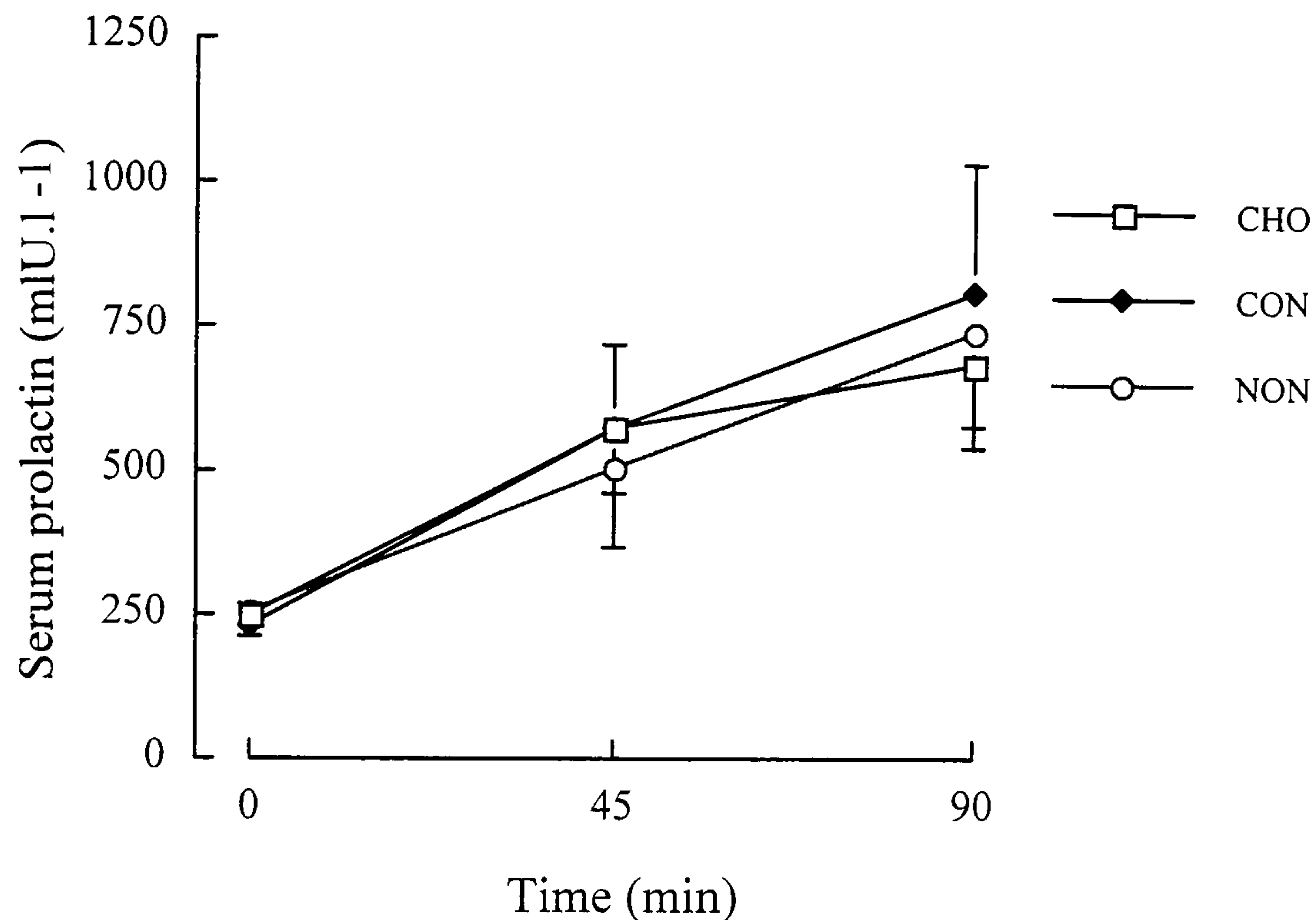


Figure 6.10: Serum prolactin concentration ($\mu\text{mol.l}^{-1}$) during the LIST ($n = 5$).

6.3.12 Serum cortisol

Serum cortisol increased in all trials during exercise (main effect of time: $F_{4,4} = 83.9, p < 0.01$). Although there appeared to be a greater serum concentration towards the end of exercise during the NON trial there was no statistically significant difference between trials (Figure 6.11).

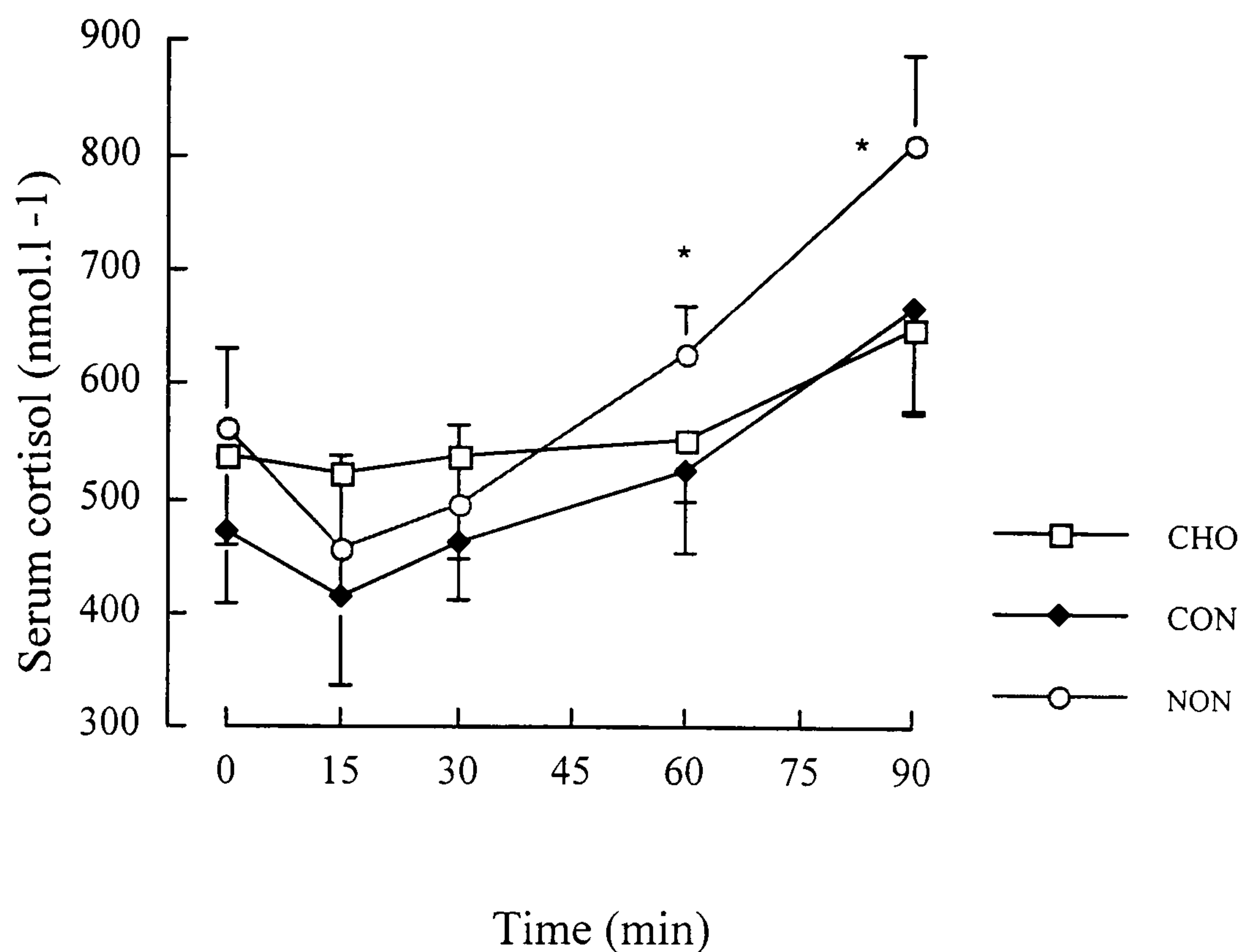


Figure 6.11: Serum cortisol concentration (nmol.l⁻¹) during the LIST.
 * *post-hoc* difference $p < 0.01$: 60 and 90-min vs all other time points.

6.3.13 Serum osmolality and electrolytes

Serum osmolality and electrolyte concentrations are shown in Table 6.2. There was no statistically significant difference within the 90-min or between trials for all concentrations ($n = 5$).

6.3.14 Fifteen metre sprint times

There was no statistically significant difference between trials for the 15-m sprint times ($n = 9$). There was a main effect of time ($F_{5,40} = 3.9$, $p < 0.01$), with the mean sprint time during the final block of the LIST being slower than blocks 1 ($p < 0.01$), and blocks 2-4 ($p < 0.05$).

Table 6.2: Serum osmolality (mOsmol.kg⁻¹), sodium (mmol.l⁻¹) and potassium (mmol.l⁻¹) concentrations during the LIST (n =5) (Values are mean ± SEM).

Metabolites	Trial	0-min	15-min	30-min	60-min	90-min
Serum osmolality (mOsmol.kg ⁻¹)	CHO	293 ± 1	301 ± 2	301 ± 3	301 ± 3	302 ± 2
	CON	292 ± 1	300 ± 2	300 ± 2	300 ± 2	301 ± 2
	NON	292 ± 2	299 ± 2	302 ± 4	305 ± 4	305 ± 3
Serum sodium (mmol.l ⁻¹)	CHO	138 ± 1	141 ± 1	141 ± 1	142 ± 1	142 ± 1
	CON	139 ± 1	141 ± 1	141 ± 1	142 ± 1	143 ± 1
	NON	138 ± 1	140 ± 1	141 ± 1	142 ± 1	143 ± 1
Serum potassium (mmol.l ⁻¹)	CHO	4.2 ± 0.1	4.0 ± 0.1	4.0 ± 0.1	4.1 ± 0.1	4.3 ± 0.3
	CON	4.2 ± 0.1	4.0 ± 0.1	4.1 ± 0.1	4.3 ± 0.1	4.2 ± 0.1
	NON	4.1 ± 0.1	4.1 ± 0.1	4.1 ± 0.1	4.2 ± 0.1	4.0 ± 0.1

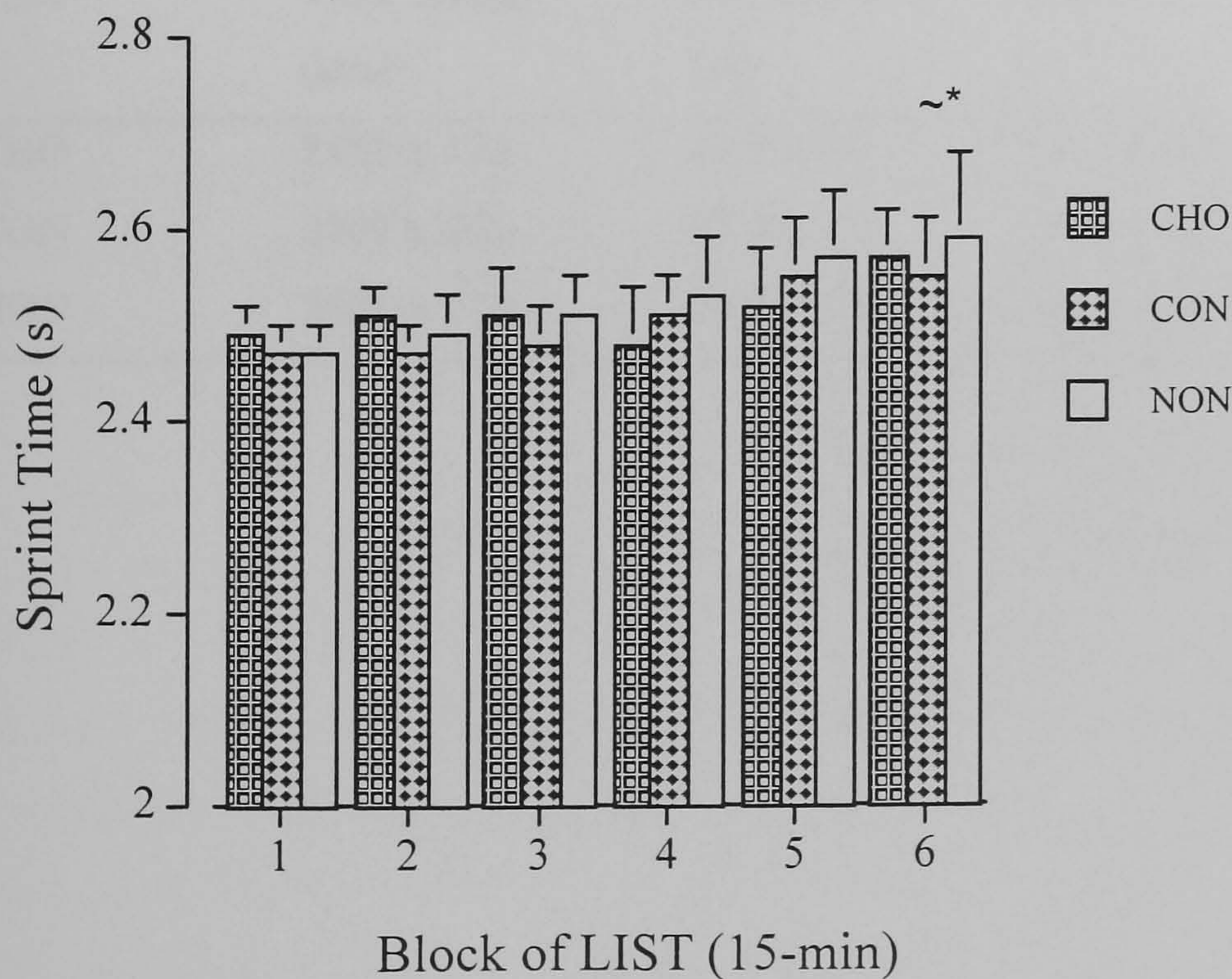


Figure 6.12 Mean sprint time (s) during the LIST.

~ *post-hoc* difference $p < 0.01$, block 6 vs block 1, * *post-hoc* difference $p < 0.05$ block 6 vs blocks 2-4.

6.3.15 Fluid and carbohydrate ingested

The mean total volume of fluid ingested during the CHO and CON trials was 1.07 ± 0.02 l. During the CHO trial the subjects ingested a total of 68.7 ± 1.4 g of carbohydrate at a rate of 45.8 ± 0.8 g.h⁻¹.

6.3.16 Energy Intake

There was no difference between trials in the total energy, carbohydrate, fat and protein consumed in the 48 h prior to the main trials. (Table 6.3).

Table 6.3: Daily total energy (kcal), carbohydrate (%), fat (%) and protein (%) in the 48 h prior to the main trials (Values are mean \pm SEM)

Trial	Total energy (kcal)	Carbohydrate (%)	Fat (%)	Protein (%)
CHO	2285 ± 192	51.9 ± 3.1	30.5 ± 3.8	17.4 ± 1.8
CON	2507 ± 276	53.2 ± 3.7	30.0 ± 3.8	16.8 ± 1.7
NON	2526 ± 270	53.7 ± 3.3	30.2 ± 3.5	16.0 ± 1.8

6.4 Discussion

The main finding of the present study was that prolonged intermittent, high intensity shuttle running without water ingestion resulted in a 9% deterioration in performance of a soccer passing test. A decline in the performance of a soccer-dribbling test was also observed, although the difference was not statistically significant. Ingestion of a 6.4 % carbohydrate-electrolyte and a carbohydrate free solution maintained the performance of the passing test with no difference between pre and post-LIST scores.

The reduction in the performance of the passing test is consistent with the results from the dribbling test reported in Chapter 4. The performance of the soccer-dribbling test post-LIST was not statistically slower during any of the trials. This was despite 7 out of the 9 subjects during this investigation being slower post-LIST during the NON trial. The overall results of this test were skewed by the performance of one particular subject. The mean difference in scores, pre and post-LIST, during the NON trial was a slower time of 5 s. Of the two subjects who did not perform slower post-LIST one subject remained within 1 s of his pre-score, however the other subject actually improved by 9s. The performance difference of one individual is difficult to explain but important to note, as the trend of results for the dribbling test was similar to that reported previously (Chapter 4).

The absence of a difference between the CON and CHO trials in the tests of skill performance is consistent with those of Zeederberg *et al.* (1996). Following the ingestion of a 6.9% carbohydrate-electrolyte solution during two soccer matches, no difference in heading, dribbling and shooting ability was observed (Zeederberg *et al.*, 1996). Hypoglycaemia has been suggested as a possible reason for the deterioration in performance observed in sports, such as soccer (Shephard and Leatt, 1987). Therefore, the ingestion of carbohydrate during exercise has the potential to delay the onset of hypoglycaemia and fatigue. As there was no difference in blood glucose concentration between or within any trial, the results from this study are consistent with findings reported in Chapter 4 that hypoglycaemia is not the cause of fatigue during the NON trial.

As hypoglycaemia was not a limiting factor, the potential beneficial effect of carbohydrate supplementation was not observed during this study.

Carbohydrate ingestion during exercise has been shown to spare muscle glycogen, delay the onset of fatigue and improve exercise capacity in comparison to a placebo solution (Tsintzas *et al.*, 1995; Nicholas *et al.*, 1994b, 1995). As there was no difference in skill performance between the CHO and CON trials muscle glycogen concentration does not appear to limit performance of a skill when fluid is ingested. However, a further reduction in muscle glycogen in the absence of fluid ingestion may be the cause of the decrement in skill (Chapter 4).

Body mass loss, heart rate response, sprint times and RPE were similar to those reported in the previous two chapters. Problems during blood sampling resulted in a small number of completed data sets for most of the metabolites measured. Therefore, although the concentrations of the metabolites measured during this study were similar to those reported in the previous two chapters, no statistically significant difference was identified between trials. As the results of this study were similar to those in Chapters 4 and 5, conclusions about the cause(s) of fatigue during the NON trial and the reduction in skill performance remain the same. In addition to an increased muscle glycogenolysis when fluid is not ingested (Hargreaves *et al.*, 1996) the increased fluid loss during the NON trial may have led to an increased thermal strain with a resulting negative impact on skill maintenance (Chapter 4).

In addition to the metabolites reported in Chapters 4 and 5, plasma ammonia (NH₃) and serum prolactin were measured. As with the other metabolites, there was no statistically significant difference between trials for NH₃ and prolactin. As the sample size was small, discussion of the pattern of the metabolite response in this particular study may be more significant than the use of statistical tests.

Plasma ammonia increased in all 3 trials during exercise and appeared to be greater during the NON trial. Numerous studies on prolonged exercise have reported that NH₃

concentration increases throughout exercise (Wagenmakers *et al.*, 1991; Maclean *et al.*, 1991; Maclean *et al.*, 1992; Tsintzas *et al.*, 1995; Nicholas *et al.* 1995). There are two potential sources of NH_3 in skeletal muscle during prolonged exercise. The first involves the deamination of AMP to inosine 5-monophosphate (IMP) and NH_3 as one of the reactions of the purine nucleotide cycle. The second involves the deamination and release of NH_3 from oxidation of branched chain amino acids (Maclean and Graham 1993). Ammonia metabolism is also altered by the availability of other energy substrates. For example, ammonia production increases during submaximal exercise when muscle glycogen stores are low (Maclean *et al.*, 1992). A large proportion of the ammonia produced when muscle glycogen concentration is low has been attributed to BCAA metabolism (Wagenmakers *et al.*, 1991; Maclean *et al.*, 1992). It is therefore difficult to determine whether low muscle glycogen, increased BCAA availability or both result in the increased NH_3 concentration observed during exercise.

A rise in ammonia concentration may be related to the development of local fatigue (Heald, 1975; Washio and Mashima, 1963; Wilkerson *et al.*, 1975; Mutch and Banister, 1983; Sahlin, 1990). In addition, the ammonia released from muscle during exercise may have direct access to brain tissue, via the circulation, where it could cause toxic effects in the central nervous system (Barnes *et al.*, 1964). Interestingly, ammonia can be produced in the brain by the deamination of brain catecholamines (Iverson and Simmonds, 1969; Schildkraut *et al.*, 1966). There have been numerous observations that in response to exercise there is an increase in plasma catecholamines (Banister, 1972; Chin *et al.*, 1971; Raven, 1970) and in the brain (Brown *et al.*, 1979). If the fate of circulating catecholamines is similar to brain catecholamines (i.e. deamination), then this could be contributory to an elevation of blood, and indirectly, of brain NH_3 concentrations. Thus, increased endogenous production of NH_3 from catecholamine deamination may contribute to the overall central and local effects of fatigue (Mutch and Banister 1983). Whether a central nervous system component is more important in the perception of fatigue or the actual decline of motor function has yet to be determined.

The association between an increase in catecholamines and NH_3 is of interest as it was suggested that an increased production of catecholamines during the LIST, may have led to an increased muscle glycogenolysis and a subsequent effect on skill performance when no fluid is ingested (Chapters 4 and 5). Therefore, in addition to an increased glycogenolysis (King *et al.*, 1985; Jansson *et al.*, 1986; Hargreaves *et al.*, 1996), catecholamine production when no fluid is ingested may increase NH_3 production and subsequently reduce skill performance. Consequently, both central and peripheral factors may contribute to the deterioration in skills performance observed when fluid is not ingested.

An increase in the concentration of the brain neurotransmitter 5-hydroxytryptamine (5-HT, serotonin) has also been associated with central fatigue (Blomstrand *et al.*, 1989, Blomstrand *et al.*, 1991; Bailey *et al.*, 1992; Wilson and Maughan, 1992). Due to the difficulties in assessing 5-HT, prolactin release has been used as a marker of central serotonergic activity (Marvin *et al.*, 1998). The prolactin response during the present study was similar in all 3 trials, with the response during the CHO trial being slightly but not statistically lower. Carbohydrate ingestion during exercise has been suggested to reduce central fatigue due to the suppression of plasma FFA's and therefore the rise in plasma fTrp (Davis *et al.*, 1992). In so doing, carbohydrate ingestion has been proposed to lower the concentration of fTrp crossing the blood brain barrier and thereby reduce the production of 5-HT (see Chapter 5). As the tendency for prolactin concentration was higher during the CON than the NON trial there is no evidence in the present study of 5-HT being a cause of the difference observed in skill performance between the NON and fluid trials.

In summary, performance of a soccer-passing test decreased following prolonged intermittent shuttle running when no fluid was ingested. This reduction in performance was prevented with the ingestion of a carbohydrate-electrolyte and carbohydrate-free solution throughout the LIST. The cause of the decrement in passing and dribbling skill remains to be elucidated.

CHAPTER 7

THE INFLUENCE OF REHYDRATION ON SOCCER SKILL FOLLOWING INTERMITTENT SHUTTLE RUNNING

7.1 Introduction

During exercise without fluid replacement there is a progressive decrease in systemic arterial, pulmonary arterial, right ventricular end-diastolic pressures and stroke volume, (Ekelund, 1967; Rowell, 1986) and a progressive increase in rectal temperature and heart rate (Hamilton *et al.*, 1991). Cardiac output also declines during prolonged exercise when the reduction in stroke volume is relatively greater than the concomitant increase in heart rate (Sawka *et al.*, 1979). In addition, dehydration equivalent to the loss of only 2% body mass is sufficient to impair endurance performance (Armstrong *et al.*, 1985) and soccer dribbling and passing skill (Chapters 5 and 6). In contrast, fluid ingestion reduces the increase in heart rate and core temperature associated with dehydration (Hargreaves *et al.*, 1996). Water ingestion during exercise also improves endurance performance, capacity and skill (Armstrong *et al.*, 1985; Fallowfield *et al.*, 1996; Chapters 5 and 6).

Some of the potential mechanisms underlying the retention of skill may be that fluid ingestion reduces the circulatory and thermoregulatory strain on the body (Sawka *et al.*, 1979; Hamilton *et al.*, 1991) and/or reduces the catecholamine response during exercise, with a subsequent sparing of muscle glycogen (King *et al.*, 1985; Jansson *et al.*, 1986; Hargreaves 1996).

In an attempt to isolate the effects of muscle glycogen depletion and hypohydration, the aim of the present study was to establish whether a rehydration period following completion of the LIST without fluid would result in recovery of skill performance.

7.2 Methods

7.2.1 Subjects

Eight male university and semi-professional soccer players volunteered to participate in the study. The mean \pm SEM age, body mass (BM) and estimated maximal oxygen uptake ($\dot{V}O_2$ max) of the group were 21.9 ± 1.4 years, 73.4 ± 2.4 kg and 59.0 ± 0.6 ml.kg⁻¹.min⁻¹ respectively.

7.2.2 Preliminary Tests

Subjects performed preliminary tests to: (i) estimate $\dot{V}O_2$ max in order to calculate the relative exercise intensities as previously described (Chapter 3) and (ii) to familiarise themselves with the experimental procedures. Following completion of preliminary measurements, subjects were familiarised with the LSDT and LSPT.

7.2.3 Experimental design

Subjects acted as their own controls in a repeated measures cross-over design. They were allocated to two randomly assigned trials either ingesting a large (L) or small (S) volume of fluid during a 2-h recovery period following the LIST. During the L trial the prescribed volume ingested was divided into four equal parts, each of which was consumed over a 30-min period. The prescribed volume (in ml) was calculated as 150% of the weight loss (in g) during the 90-min LIST. During the S trial the subjects ingested 100 ml at the beginning of the recovery period and again 1 h later. The solution consumed during both trials was a flavoured non-carbohydrate experimental sport drink with a sodium concentration of 60mg.100ml⁻¹ (SmithKline Beecham, UK).

In the two days prior to the main trials subjects did not participate in any prolonged, heavy exercise. Subjects reported to the laboratory after an overnight fast of between 10

and 12 h. They then emptied their bladders prior to the preliminary measurement of nude body mass, which was recorded before, following the LIST and recovery period during each trial. Nude body mass was determined to the nearest 0.1 kg using beam balance (Avery Ltd., Model 3306ABV). Immediately following body mass measurements, core temperature was recorded using a rectal probe inserted to a depth of 10 cm and connected to a thermometer (Edale Instruments Ltd, model C). A cannula (Venflon, 16-18G, BOC Ohmeda, Sweden) was inserted into an antecubital vein and kept patent by periodic flushing with sterile saline (10 U.ml⁻¹ heparin). The subjects stood for 15-20 min before a resting blood sample was obtained.

A standardised 15-min warm-up was performed by each subject prior to each trial and consisted of jogging, stretching and striding. Immediately after the warm up the subjects performed the LSDT and LSPT. This was followed by a 2-min period during which subjects prepared to begin the LIST. An identical warm up period was performed prior to the final set of skill tests following the 2-h recovery period.

Heart rate was recorded every 15 s during the trials by short - range radio telemetry (Polar Sport Tester™, Kempele, Finland). Temperature of the Sports Hall was recorded every 20 min and maintained over the study between 14 - 20°C by opening windows and doors. Dry-bulb and wet-bulb temperatures were measured using a whirling hygrometer (Brannan Thermometers Ltd).

On completion of the LIST the subjects were required to perform the LSDT and LSPT for a second time. Immediately following the tests, the subjects core temperatures were recorded. Following exercise the subjects dried themselves and were weighed to determine the extent of body mass loss during the exercise period. This was all completed within 30-min of the completion of the LIST. Over the following 2-h the subjects ingested either the large volume or small volume of fluid. Additional blood samples were collected at 30-min intervals during recovery, the first corresponding to 1 h post-LIST. Throughout the recovery period subjects remained standing for the 15-20 min prior to blood sampling. If a subject required to urinate at any time, they were free to do so and

the urine voided was collected, so that the volume excreted over the entire period could be measured. Following the recovery period subjects were weighed and core temperature was measured before they completed the LSPT and LSDT for a final time. Whole body net fluid balance was calculated from the body mass loss, volume of fluid ingested and urinary volume excreted. The overall design of the study is illustrated in Figure 7.1.

7.2.4 Blood sampling and analysis

Eleven millilitres of blood were withdrawn at rest, after 45 and 90-min of the LIST and every 30-min during the 2 h recovery period. Thus the sampling times were at 0, 45, 90, 150, 180, 210 and 240-min. The blood was dispensed, treated and stored as previously described (Chapter 3). Serum was analysed for prolactin, insulin, cortisol, aldosterone, osmolality and electrolyte concentrations and plasma was analysed for FFA concentrations. Whole blood was assayed for ammonia, lactate and glucose concentrations and plasma volume changes, using methods that have been previously described (Chapter 3).

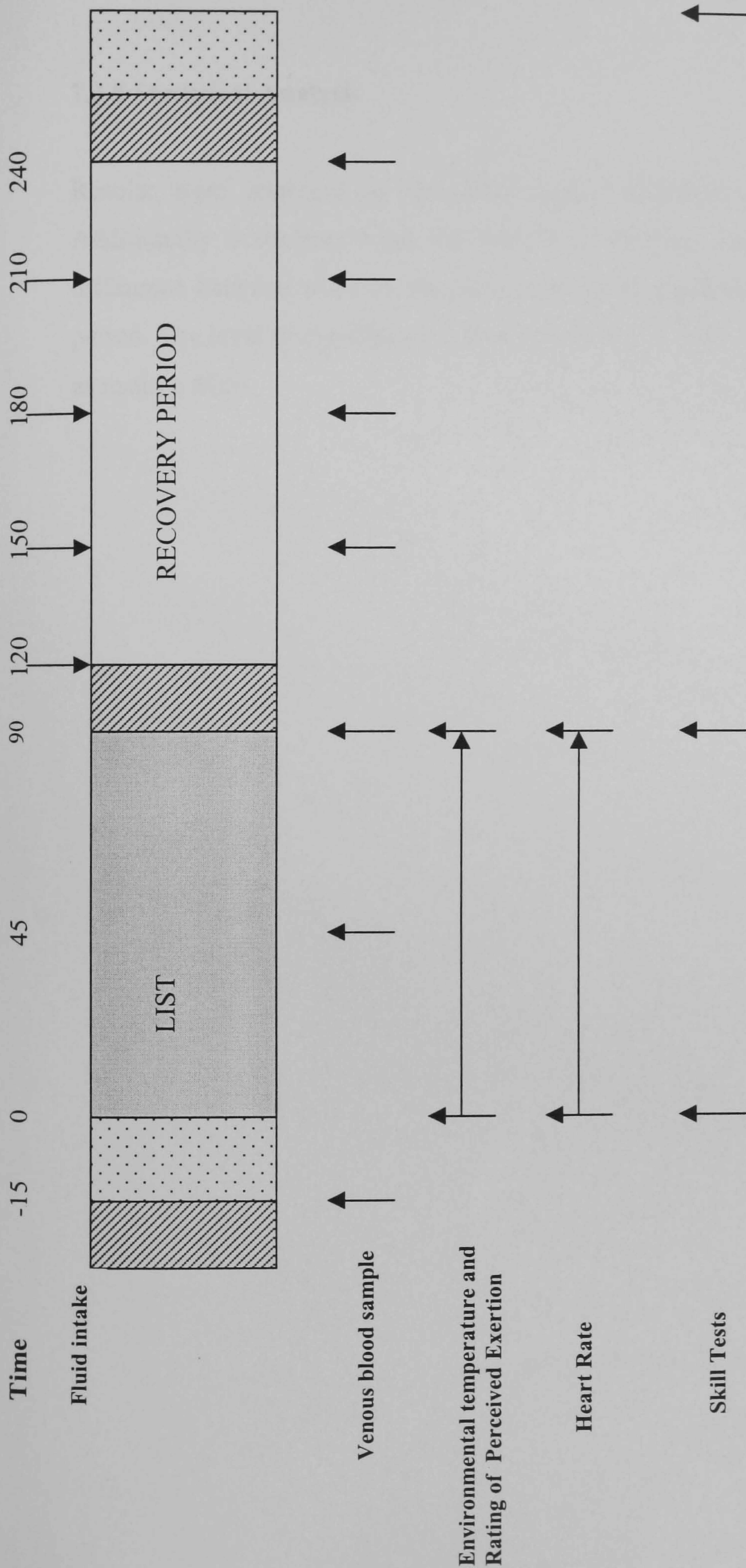


Figure 7.1: Schematic illustration of the study protocol

LIST = Loughborough Intermittent Shuttle Test

-  Body Mass + Core Temperature
-  Warm up
-  Body Mass + Core Temperature

7.2.5 Statistical Analysis

Results were analysed as described under statistical analysis (Chapter 3). Additionally a students t-test for correlated samples was used to analyse the difference between trials in the volume of urine produced during the recovery period. The level of significance was accepted at $p < 0.05$. Results are represented as mean \pm SEM.

7.3 Results

7.3.1 Skill tests

There was no difference between trials in the pre-LIST scores for the LSDT. Following exercise LSDT performance times were longer in both trials (main effect of time: $F_{2,14} = 5.7, p < 0.05$) and remained longer following the 2-h recovery period (main effect of time: $F_{2,14} = 5.7, p < 0.05$) (Figure 7.2). Performance of the LSPT followed a similar pattern to the LSDT, with post-LIST and post-recovery times being longer than pre-LIST scores (main effect of time: $F_{2,14} = 5.7, p < 0.05$) (Figure 7.3).

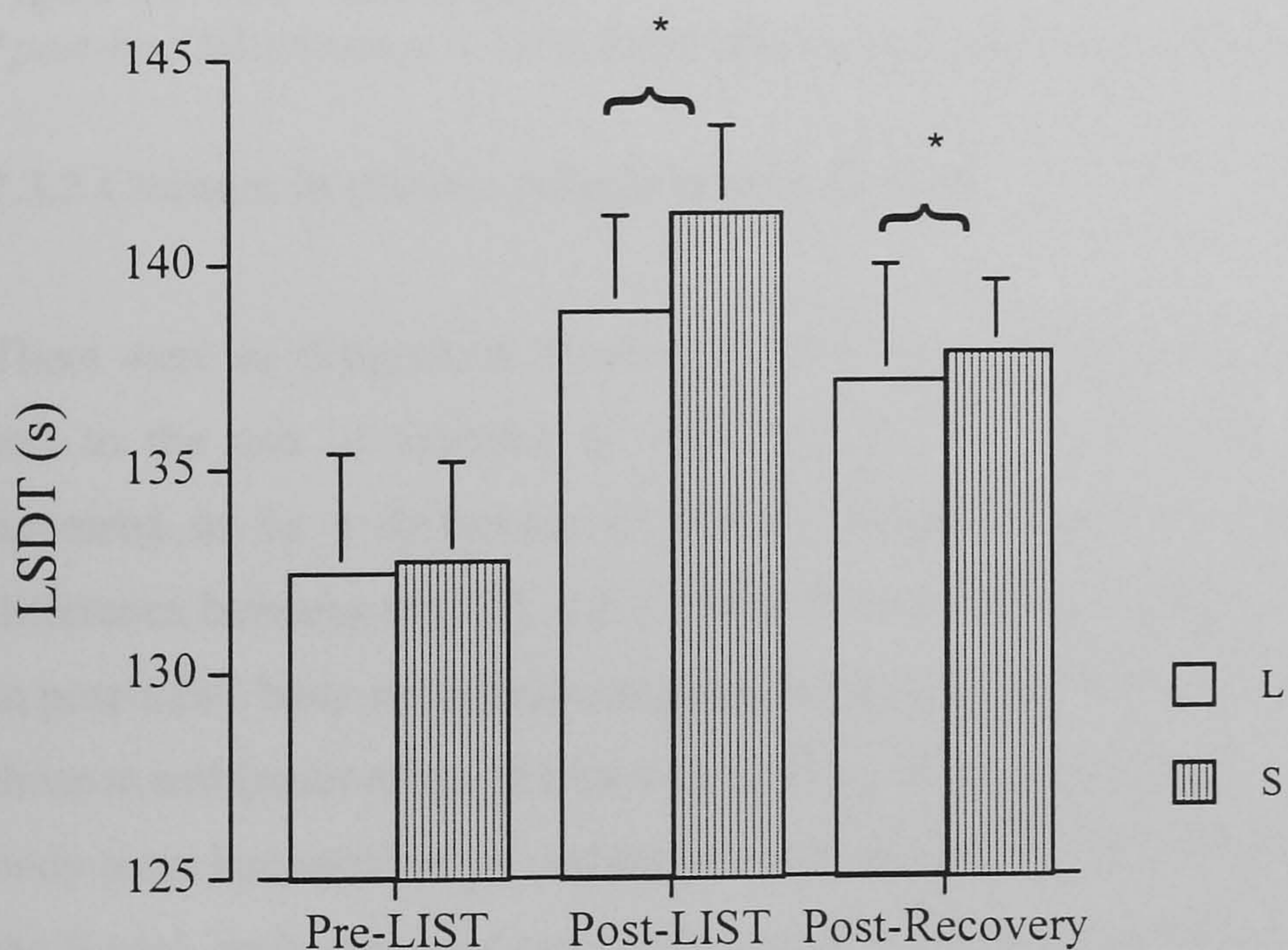


Figure 7.2: LSDT scores (s)

**post-hoc* difference $p < 0.05$ Pre-LIST vs Post-LIST and Recovery

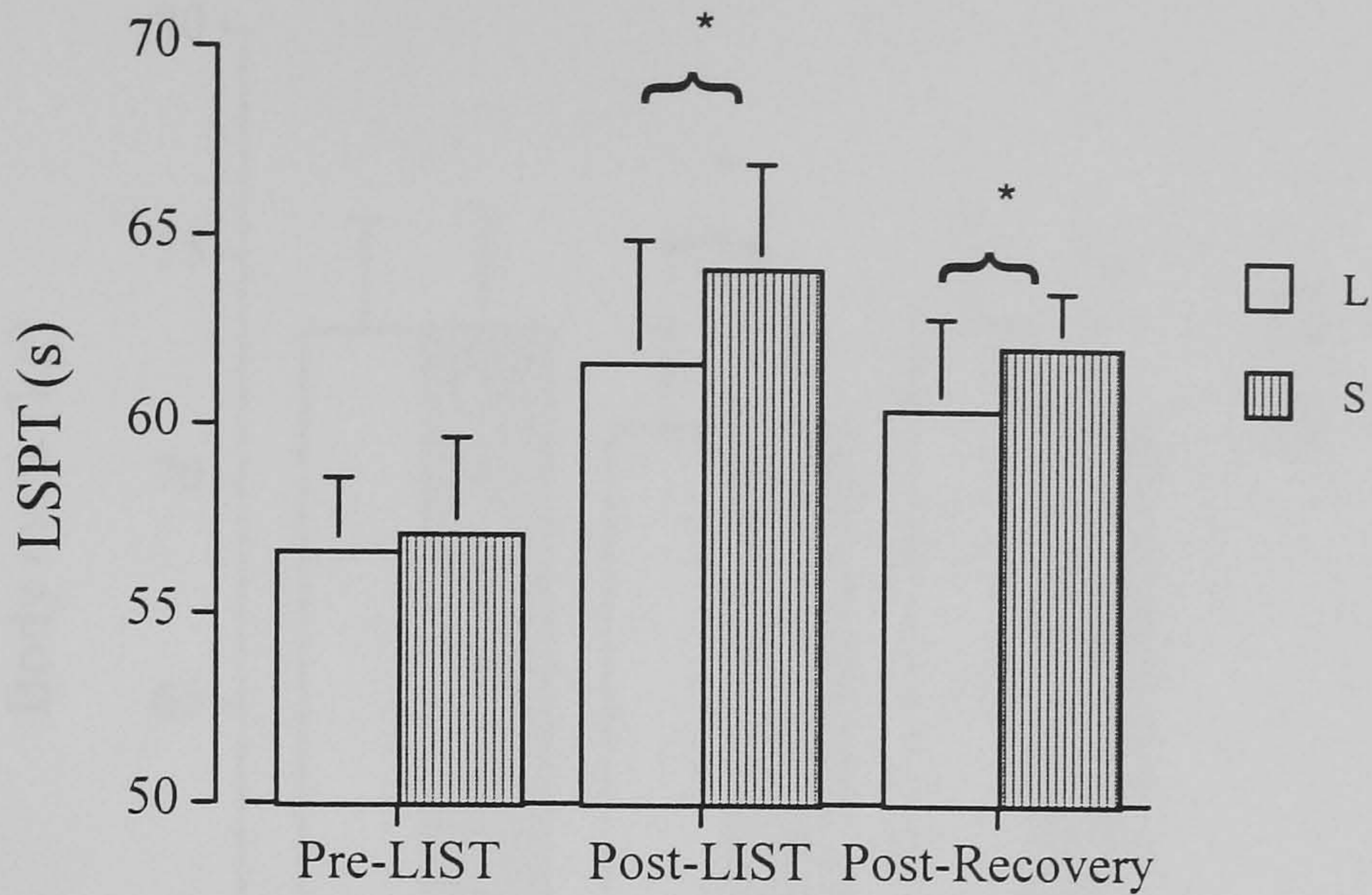


Figure 7.3: LSPT scores (s)

**post-hoc* difference $p < 0.05$ Pre-LIST vs Post-LIST and Recovery

7.3.2 Changes in plasma volume and body mass

There were no differences between trials in the changes in plasma volume from rest to the end of exercise (L $-2.6 \pm 1.7\%$; S $-3.3 \pm 1.3\%$). Although there appeared to be a difference in plasma volume post-Recovery, there was no difference between trials (L $1.8 \pm 1.0\%$; S $-0.1 \pm 1.4\%$). There was no difference in post-LIST body mass between trials, with values in both trials being lower than those at rest (main effect of trial x time: $F_{2,14} = 23.7$, $p < 0.01$). During the L trial body mass had returned to resting values at the end of the 2 h recovery, but during the S trial, body mass did not return to pre-exercise values (main effect of time x trial: $F_{2,14} = 5.7$, $p < 0.01$) (Figure 7.4).

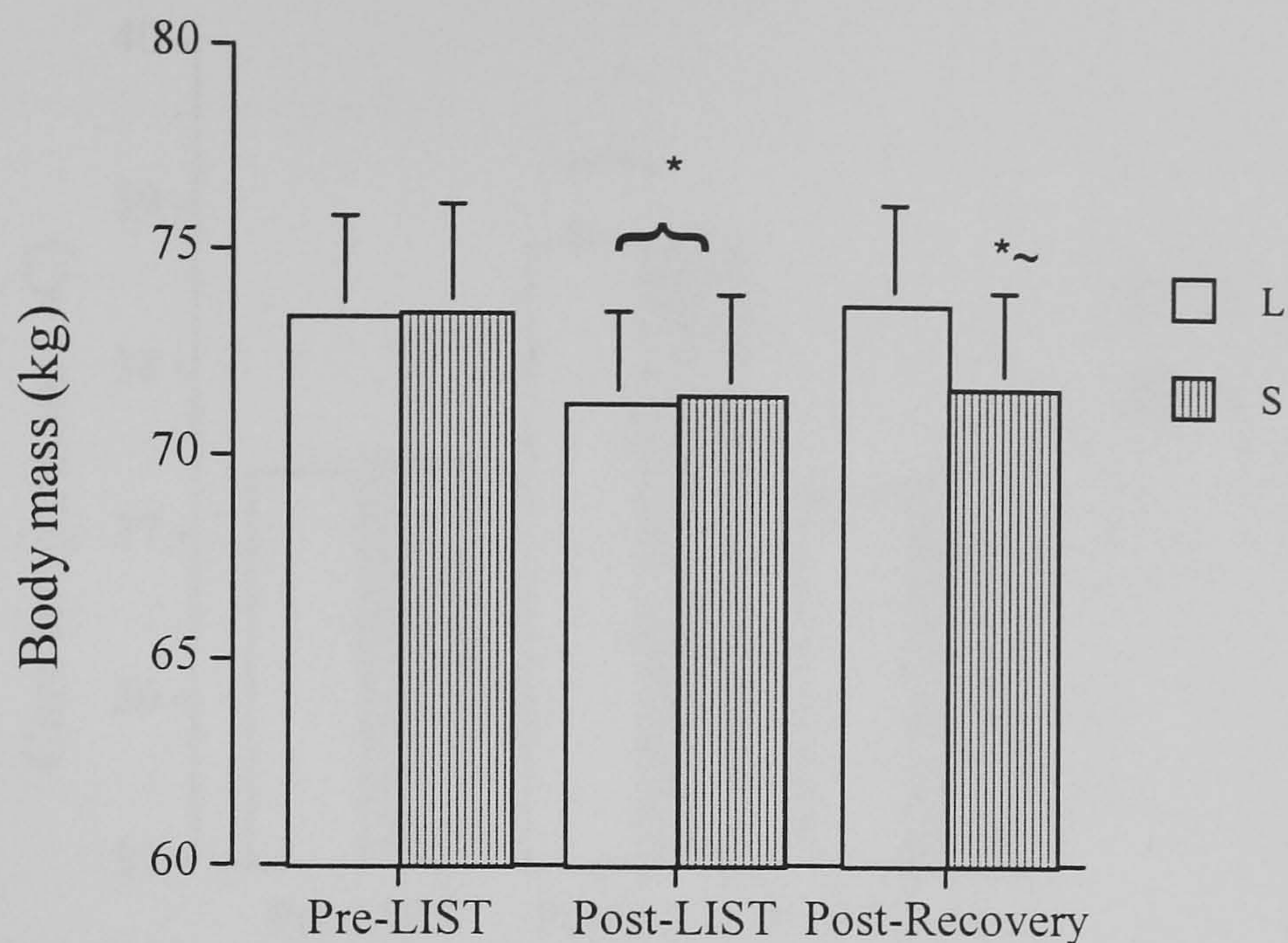


Figure 7.4: Body mass (kg)

* *post-hoc* difference $p < 0.01$ Pre-List vs Post-LIST both trials, Pre-List vs Post-Recovery S trial. ~ *post-hoc* difference $p < 0.01$ Post-List L vs Post-List S

7.3.3 Core temperature

Core temperature increased in both trials during exercise with values being greater post-LIST (main effect of time: $F_{2,14} = 5.7, p < 0.01$). Values returned to those recorded before the beginning of exercise after the 2 h recovery. There was no difference between trials at any time (Figure 7.5).

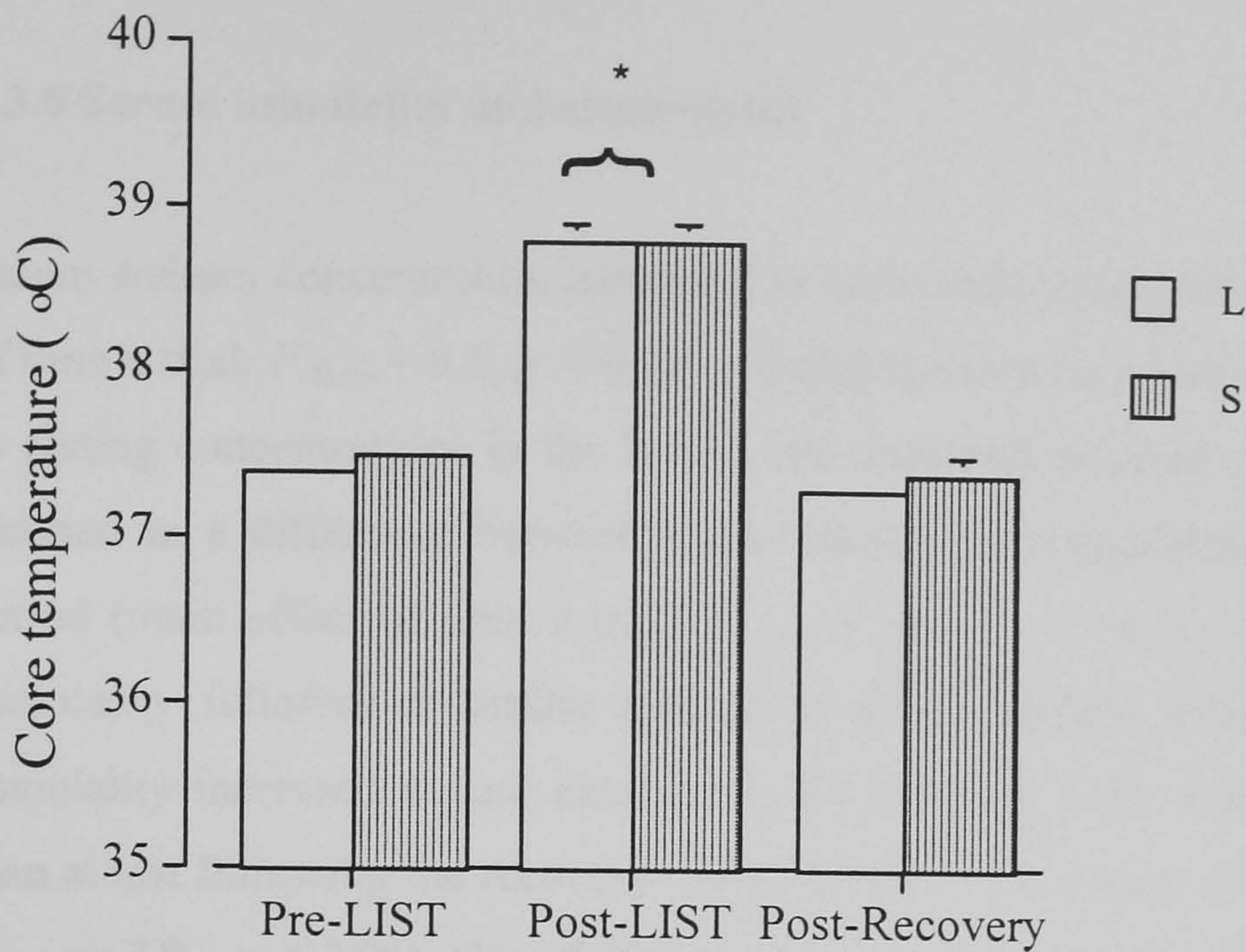


Figure 7.5. Core temperature (°C)

**post-hoc* difference $p < 0.01$ Post-LIST vs Pre-LIST and Post-Recovery

7.3.4 Environmental conditions

There was no difference between trials in the dry and wet bulb temperature and relative humidity during the study (Table 7.1).

Table 7.1: Mean dry and wet bulb temperature (°C) and relative humidity (%) (Values are mean \pm SEM).

	Dry bulb temperature (°C)	Wet bulb temperature (°C)	Relative humidity (%)
L	13.7 \pm 0.4	9.4 \pm 0.5	57.3 \pm 2.3
S	14.4 \pm 0.3	10.5 \pm 0.5	57.8 \pm 3.6

7.3.5 Heart rate, rating of perceived exertion and sprint times

There was no difference between trials during each 15-min block of the LIST for mean heart rate, rating of perceived exertion or sprint times (Table 7.2). All reported variables increased during exercise (main effect of time, $p < 0.01$).

7.3.6 Serum osmolality and electrolytes

Serum sodium concentration increased in both trials during exercise (main effect of time x trial: $F_{6,30} = 9.6, p < 0.01$). During the recovery period sodium returned to resting concentrations in the L trial but remained elevated in the S trial. This resulted in a difference between trials towards the completion of the recovery period (main effect of time x trial: $F_{6,30} = 9.6, p < 0.01$) (Figure 7.6). Serum osmolality followed a similar pattern to serum sodium concentration. Serum osmolality increased during exercise in the S and L trials and remained greater than at rest following the recovery period in the S trial (main effect of time x trial: $F_{6,30} = 7.9, p < 0.01$). Osmolality returned to resting values during the recovery period in the L trial, resulting a difference between trials (main effect of time x trial: $F_{6,30} = 9.6, p < 0.01$) (Figure 7.7). There was no difference between or within trials in serum potassium concentrations.

Table 7.2: Mean heart rate (beats.min⁻¹), Rating of Perceived Exertion (RPE) and sprint time (s) during each 15-min block of the LIST. (Values are mean ± SEM)

Variable	Trial	Block-1	Block-2	Block-3	Block-4	Block-5	Block-6
Heart Rate (beats.min ⁻¹)	L	164 ± 3	165 ± 3	167 ± 3 ^a	169 ± 2 ^a	170 ± 2 ^a	171 ± 2 ^a
	S	166 ± 3	166 ± 3	168 ± 2 ^a	169 ± 3 ^a	169 ± 3 ^a	172 ± 3 ^a
RPE	L	13 ± 0 ^b	13 ± 0 ^b	14 ± 1 ^b	15 ± 1 ^b	16 ± 0 ^b	17 ± 1 ^b
	S	12 ± 0 ^b	13 ± 0 ^b	14 ± 1 ^b	15 ± 1 ^b	16 ± 1 ^b	16 ± 1 ^b
Sprint Time (s)	L	2.42 ± 0.02	2.42 ± 0.02	2.44 ± 0.02	2.46 ± 0.02	2.47 ^c ± 0.02	2.48 ^c ± 0.02
	S	2.45 ± 0.01	2.44 ± 0.02	2.44 ± 0.02	2.47 ± 0.02	2.48 ^c ± 0.03	2.48 ^c ± 0.02

a: *post-hoc* difference, $p < 0.01$ vs block 1 b: *post-hoc* difference, $p < 0.01$ vs all other blocks c: *post-hoc* difference, $p < 0.01$ vs block 1

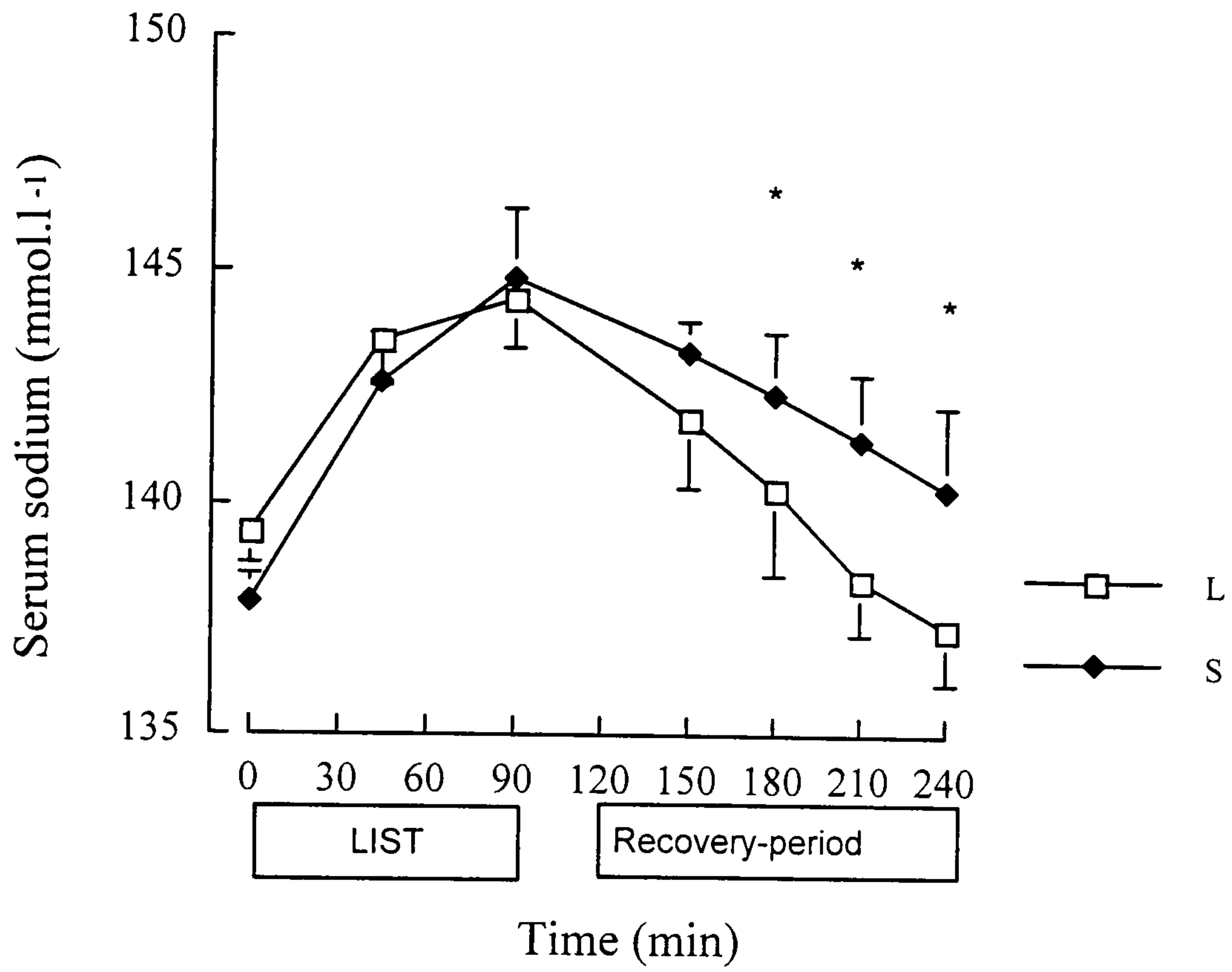


Figure 7.6: Serum sodium concentration (mmol.l⁻¹)
 **post-hoc* difference $p < 0.01$, L vs S

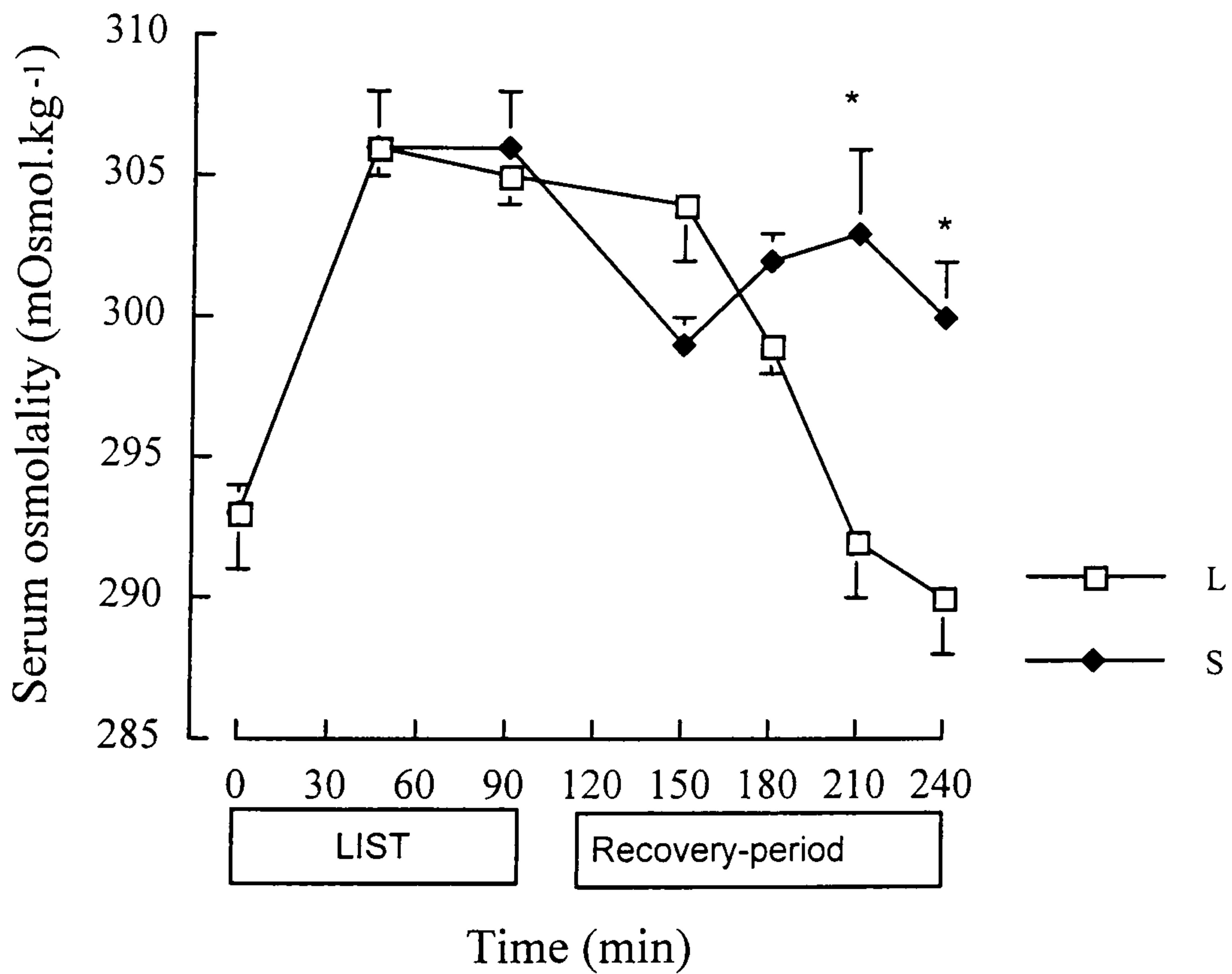


Figure 7.7: Serum osmolality concentration (mOsmol.kg⁻¹)
 **post-hoc* difference $p < 0.01$, L vs S

7.3.7 Plasma Free Fatty Acids (FFA)

Plasma FFA concentration increased during exercise in both trials (main effect of time: $F_{6,24} = 21.3$, $p < 0.01$). Plasma FFA also continued to increase during the recovery period in both trials, leading to a difference between the end of exercise and all time points during recovery (main effect of time: $F_{6,24} = 21.3$, $p < 0.01$)(Figure 7.8).

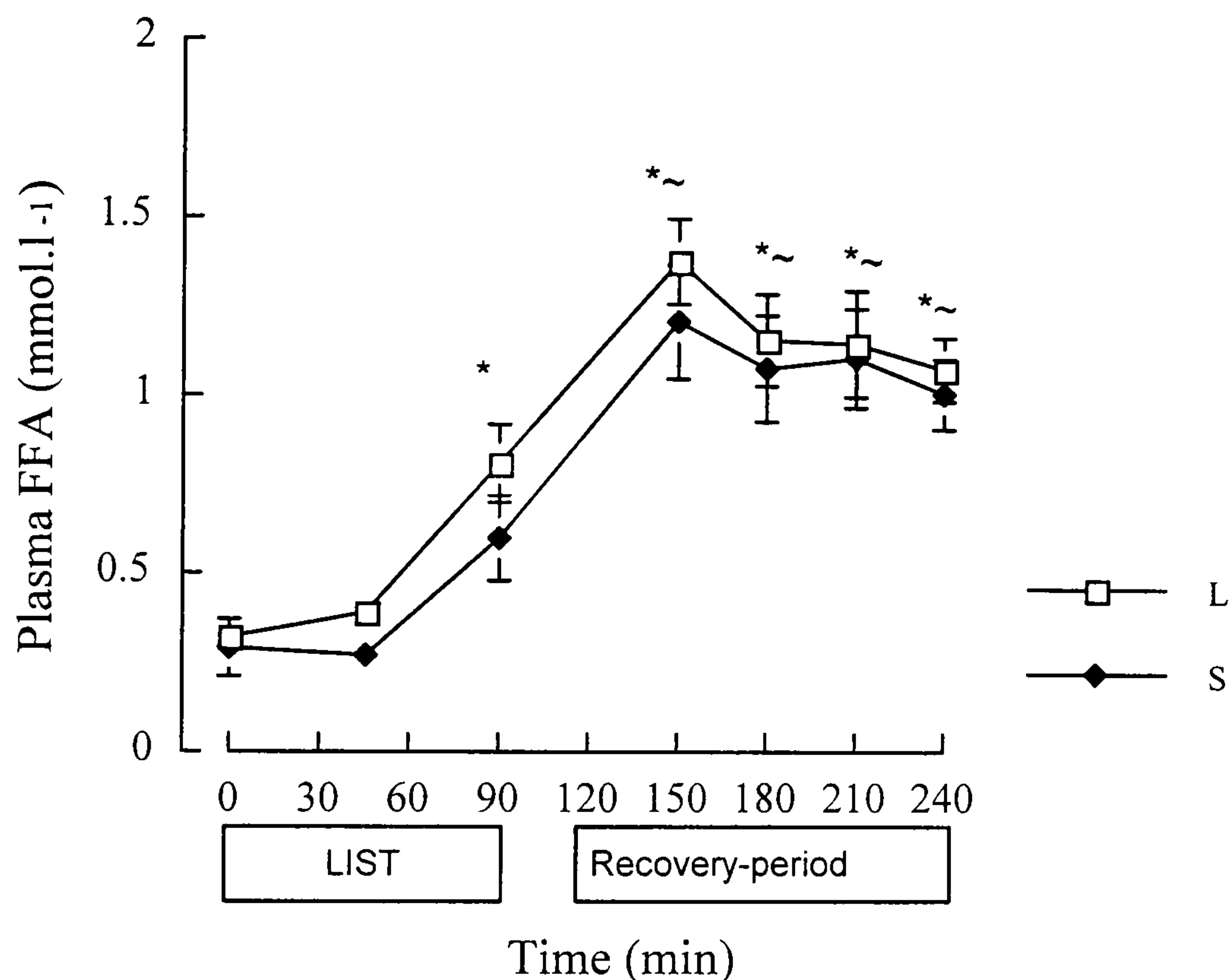


Figure 7.8: Plasma FFA concentration (mmol.l⁻¹)

**post-hoc* difference $p < 0.01$ 0 and 45-min vs all other time points. ~ *post-hoc* difference $p < 0.01$, recovery period vs exercise time points

7.3.8 Plasma ammonia

Plasma ammonia concentration increased during exercise (main effect of time: $F_{6,30} = 22.4$, $p < 0.01$) with a peak concentration at 90-min (L: 168.8 ± 14.4 , S: 158.0 ± 38.5 $\mu\text{mol.l}^{-1}$). Plasma ammonia concentration then returned to resting concentrations (L: 43.0 ± 6.5 , S: 47.2 ± 6.5 $\mu\text{mol.l}^{-1}$) during the recovery period.

7.3.9 Serum aldosterone and cortisol

Serum aldosterone concentration increased from rest (L: 486.3 ± 3 , S: 476.7 ± 45.8 pmol.l⁻¹) to a peak concentration immediately post-LIST (L: 1573.3 ± 226.3 , S: 1908.7 ± 270 pmol.l⁻¹) (main effect of time: $F_{6,30} = 24.9$, $p < 0.01$). Following this time point serum aldosterone concentration declined resulting in no difference between rest and post-recovery (240-min) concentration (L: 688 ± 93.1 , S: 669.1 ± 101 pmol.l⁻¹). There was no statistically significant difference between trials at any time. There was also no difference between trials for serum cortisol concentration. Serum cortisol concentration increased during exercise (main effect of time: $F_{6,30} = 7.9$, $p < 0.01$) with a peak concentration at 150-min. Following this time cortisol concentration declined towards resting concentration (Figure 7.9).

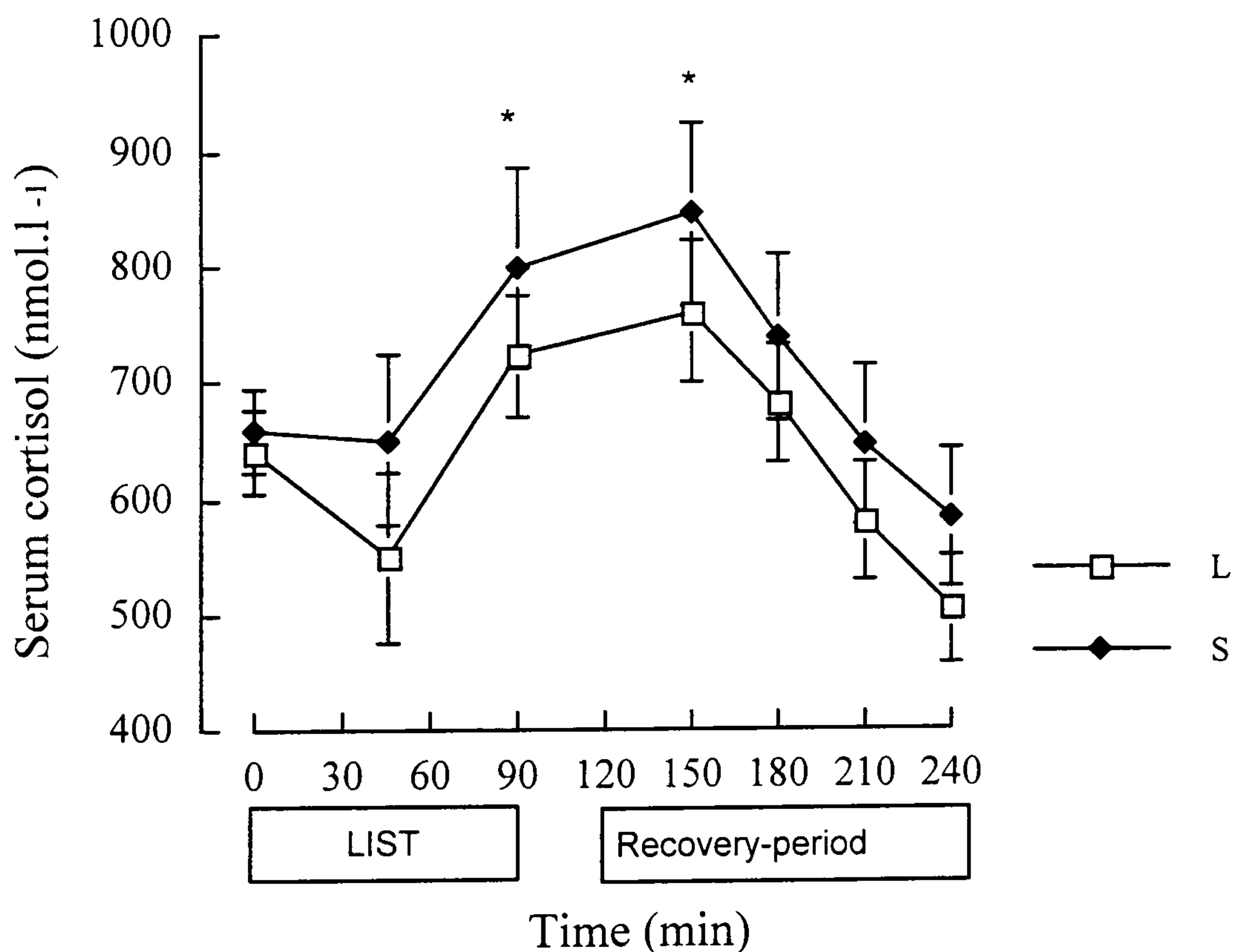


Figure 7.9: Serum cortisol concentration (nmol.l⁻¹)
*post-hoc difference $p < 0.01$, 90 and 150-min vs 0, 45, 210 and 240-min

7.3.10 Other metabolites measured

There was no statistically significant difference between trials for blood glucose and lactate concentration during exercise and recovery. Blood glucose and lactate concentration peaked during exercise and returned to resting concentration during the recovery period (main effect of time, $p < 0.01$). Serum prolactin concentration followed a similar pattern, increasing during exercise and then returning to resting concentrations (main effect of time x trial time: $F_{6,30} = 3$, $p < 0.05$). There was no statistically significant difference between trials (Table 7.3).

Table 7.3: Blood glucose (mmol.l^{-1}), lactate (mmol.l^{-1}) and serum prolactin (mIU.l^{-1}) concentration during the LIST. (Values are mean \pm SEM).

Metabolite	Trial	0	45	90	150	180	210	240
Blood glucose (mmol.l^{-1})	L	4.3	5.1 ^a	4.3	3.8	3.6	3.8	3.8
		± 0.1	± 0.4	± 0.2	± 0.3	± 0.2	± 0.2	± 0.2
	S	4.3	5.2 ^a	4.6	3.9	3.7	3.7	3.7
		± 0.1	± 0.4	± 0.3	± 0.2	± 0.2	± 0.1	± 0.2
Blood lactate (mmol.l^{-1})	L	0.8	4.1 ^b	3.5 ^b	1.1	1.0	0.8	0.7
		± 0.05	± 0.6	± 0.5	± 0.2	± 0.1	± 0.1	± 0.1
	S	0.8	4.7 ^b	4.4 ^b	1.2	1.0	0.9	0.9
		± 0.1	± 0.6	± 0.7	± 0.1	± 0.1	± 0.1	± 0.1
Serum prolactin (mIU.l^{-1})	L	260.0	232.2	271.8	331.4 ^c	303.0 ^c	271.7	257.3
		± 27.2	± 33.3	± 24.4	± 30.0	± 22.3	± 24.0	± 18.4
	S	281.7	232.9	303.0 ^d	284.7 ^e	231.4	194.7	179.3 ^f
		± 34.7	± 23.0	± 40.5	± 40.8	± 31.3	± 22.8	± 17.4

a: *post-hoc* difference, $p < 0.05$ vs all other time points b: *post-hoc* difference, $p < 0.01$ vs 0-min c: *post-hoc* difference, $p < 0.05$ vs 45-min d: *post-hoc* difference, $p < 0.01$ vs 210 and 240-min e: *post-hoc* difference, $p < 0.01$ vs 240-min f: *post-hoc* difference, $p < 0.05$ vs 0-min

7.3.11 Fluid ingested, urine produced and net fluid balance

The mean volume of fluid consumed during the L and S trials was 3.1 ± 0.3 and 0.2 ± 0.0 l respectively. The cumulative volume of urine excreted over the rehydration period was proportional to the volume of drink consumed. Urine production during the L trial (0.4 ± 0.1 l) was greater than during the S trial (0.1 ± 0.1 l) ($p < 0.01$). Exercise resulted in a fluid loss of 2.1 ± 0.2 l in both the S and L trial. Net fluid balance at the end of the rehydration period was significantly lower in the S trial (-2.0 ± 0.2 l) than in the L trial ($+0.6 \pm 0.1$ l) ($p < 0.01$).

7.3.12 Energy Intake

There was no difference between trials in the total energy, carbohydrate, fat and protein consumed in the 48 h prior to the main trials. (Table 7.4).

Table 7.4: Daily total energy (kcal), carbohydrate (%), fat (%) and protein (%) in the 48 h prior to the main trials (Values are mean \pm SEM)

Trial	Total energy (kcal)	Carbohydrate (%)	Fat (%)	Protein (%)
L	3459 ± 362	51.9 ± 3.0	34.7 ± 3.1	13.5 ± 1.3
S	3317 ± 256	55.9 ± 3.9	31.4 ± 3.4	12.8 ± 0.8

7.4 Discussion

The main finding of the present study was that in the absence of fluid ingestion performance of both a soccer dribbling and passing skill test deteriorated following the LIST. Fluid ingestion during a 2-h recovery period failed to return performance of these tests to pre-exercise values.

During the present study body mass decreased by 2.8% following exercise, which is similar to the findings in the studies reported in Chapters 4, 5 and 6. Hypohydration reduces physical work capacity (Saltin, 1964; Caldwell *et al.*, 1984), physical performance (Armstrong *et al.*, 1985), and cognitive performance (Adolph, 1947; Ladell, 1955; Gopinathan *et al.*, 1988). Although it may be clear that performance is adversely affected by hypohydration, the mechanism(s) responsible is not. As the performance of the skill tests employed in the present study require mental and physical processes, the decrement immediately following exercise could be attributed to hypohydration. However, as skill performance remained impaired following fluid replenishment the mechanism responsible for the decrement in skill performance appears to be more complex.

Hypertonicity and hypovolaemia adversely affect temperature regulation and exercise performance following hypohydration (for a review see Sawka, 1992). In the present study hypertonicity was observed following exercise in both trials, however, following the L trial a return of osmolality was not accompanied by a recovery in skill. Although important in thermoregulation, hypovolaemia *per se* does also not appear to limit skill performance, as there was no difference at any point pre- or post-exercise in plasma volumes.

The maintenance of plasma volume despite a large fluid deficit has been observed in a number of studies (Sawka *et al.*, 1980; Houston *et al.*, 1981; Gass *et al.*, 1983). Reasons for the stable plasma volume during intense exercise, despite progressive dehydration, might include the release of water from glycogen breakdown (Sawka, 1992), metabolic water (Pivarnik *et al.*, 1984) and the redistribution of water from inactive skeletal muscle (Sawka, 1988). Plasma

volume has been shown to be defended by the mobilisation of fluid from the intracellular to the extracellular compartments in response to plasma hypertonicity (Nose *et al.*, 1988a). It has been speculated that the endocrine system contributes to the redistribution of water into the intravascular space during intense exercise (Convertino, 1987). Therefore, in combination with other fluid conservation hormones such as angiotensin and vasopressin, the increase in aldosterone concentration during exercise is likely to have been a key regulatory response in maintaining plasma volume.

At the end of exercise core temperature had increased to 38.8°C. A high core temperature is closely associated with the onset of fatigue (Costill *et al.*, 1970; Montain and Coyle, 1992) and was proposed as a potential mechanism for the decrement in skill performance observed in the studies reported in Chapters 4 and 6. Fatigue under hyperthermic conditions may be due to an effect of heat stress on brain function (Brück and Olschewski, 1987; Nielsen *et al.*, 1990; Nielsen *et al.*, 1993). Nielsen *et al.* (1990) suggested that core temperatures >39°C may reduce the function of motor centres and the ability to recruit motor units required for activity, perhaps via an effect on the motivation for motor performance. Sawka *et al.* (1992) developed heat stress curves to estimate exhaustion for a given level of physiological strain. Exhaustion from heat strain was rarely associated with a core temperature below 38°C, and exhaustion always occurred before a temperature of 40°C. The core temperatures at the end of exercise during this study were within this predicted range and therefore post-LIST skill may have been affected by a loss in cognitive function resulting from an increase in core temperature. However, core temperature returned to resting values in both trials following the recovery period and yet skill performance was still less than pre-exercise performance. Therefore, the extent to which core temperature affects skill performance is unclear.

Increased serotonergic activity has been associated with a central mechanism of fatigue during heat stress (Marvin *et al.*, 1998). Head cooling resulted in a 51% improvement in exercise time to fatigue in a hot environment. A reduction in prolactin response during the head cooling trial was suggested to indicate that

central serotonergic activity was suppressed and played a key role in the mechanism of central fatigue (Marvin *et al.*, 1998). Serum prolactin concentrations increased during exercise but returned to resting values during recovery. Therefore, an increase in serotonergic activity being the cause of the decrement observed following exercise, particularly following the recovery period is disputable.

In support of this, plasma FFA continued to rise after exercise and yet no difference was observed in skill test performance post-exercise and recovery. Plasma FFA and fTrp have been shown to be positively correlated (Davis *et al.*, 1992; Wilson 1994) and associated with central fatigue. The correlation between FFA and fTrp has been explained by the action of FFA in displacing Trp from albumin (Curzon *et al.*, 1973). As FFA increased in the recovery period, a similar rise in fTrp can therefore be expected. As the increase in FFA during the present study was not associated with a further decrement in skill performance, 5-HT synthesis was probably not a factor relating to fatigue in the present study. Central fatigue associated with an increase in ammonia concentration (Chapter 6) is also doubtful as plasma ammonia concentration returned to resting values following recovery yet performance of the skill tests remained impaired.

The cause of the reduction in the skills tests observed immediately post-LIST may be due to a number of circulatory, thermoregulatory, hormonal or metabolic changes within the body. However, independent of the fluid consumed during the recovery period, skill test performance remained below pre-exercise values. The mechanism involved in the reduction in skills performance appears to be one that occurs during the exercise period and can not be altered through the ingestion of a carbohydrate free solution or within the time of the recovery period.

Exercise induced muscle fibre injury is one such mechanism and has been discussed in several reviews (Armstrong, 1984; Ebbeling and Clarkson, 1989; Armstrong *et al.*, 1991). Muscle damage as a consequence of eccentric actions (Armstrong *et al.*, 1983; Lieber and Fridén, 1988; McCully and Faulkner, 1985) has been associated with a loss of contractile force (Davies and White, 1981;

Friden *et al.*, 1983; OgiLie *et al.*, 1985; Warren *et al.*, 1990), impaired muscle function (Friden *et al.*, 1983; Newham and Jones, 1983) and the sensation of soreness (Armstrong, 1984; Ebbeling and Clarkson, 1989). Indeed, muscle function has been shown to be impaired immediately following the LIST (Nicholas *et al.*, 1996) and in addition to severe muscle soreness, impaired for a few days following the test (Thompson; unpublished data).

The shuttle running during the performance of the LIST involves considerable acceleration and deceleration, with 624 changes of direction. During these parts of the test, the extent and magnitude of eccentric work is considerable (Thompson *et al.*, 1999). In addition to maximum voluntary force production, some studies have examined the effect of muscle damage and soreness on other aspects of neuromuscular control. Miles *et al.* (1993) showed a disruption of the pattern of agonist and antagonist activity following eccentric exercise of the elbow flexors, whilst Saxton *et al.* (1995) demonstrated an increase in muscle tremor and impaired proprioception of joint position following eccentric exercise of the forearm flexors. Pearce *et al.* (1998) studied motor skill of the elbow flexor muscles following a bout of exercise-induced muscle damage. Isometric muscle strength and tracking ability both declined immediately following exercise. A number of factors were proposed for the losses observed in neuromuscular control and motor skill. The impairment may have been related to a loss in force generating capacity or an alteration in proprioceptive feedback from the moving limb (Saxton *et al.*, 1995; Pearce *et al.*, 1998).

In the absence of carbohydrate ingestion, muscle glycogen resynthesis will have been slow (Bergstrom *et al.*, 1967; Hultman *et al.*, 1971; Costill *et al.*, 1971; Ivy *et al.*, 1988a, 1988b) and therefore not dramatically changed during the 2-h recovery. A reduction in muscle glycogen following exercise has been associated with a reduction in muscle force (Jacobs *et al.*, 1982b; Young and Davies, 1984; Sherman *et al.*, 1984; Nicholas, 1996), exercise capacity (Bergstrom *et al.*, 1967; Hermansen *et al.*, 1967) and work rate during soccer (Karlsson, 1969; Saltin, 1973). Therefore, the level of stored glycogen within the musculature may directly affect its ability to produce force and possibly influence playing performance in

soccer. Although muscle biopsy samples were not obtained in the present study, it is reasonable to assume that at the end of the LIST the muscle glycogen concentrations of the subjects in the present study were as low as those reported by Nicholas *et al.* (1994). If muscle glycogen concentrations are further lowered in the absence of fluid ingestion then a deterioration in skill performance due to a low muscle glycogen concentration is plausible (Chapter 4).

In summary, following the dehydration induced by exercise, rehydration resulted in a return of fluid balance following a 2 h recovery period. Despite a negative fluid balance being observed during the S trial there was no difference between trials in skill test performance post-exercise. As it has been identified that fluid ingestion attenuates the decrement in skill observed without fluid (Chapters 4 and 6), the beneficial effect of fluid ingestion seems only to occur when fluid is ingested during exercise. The decrement in skill performance may occur due to an increase in muscle damage or an increased glycogenolysis as a result of hypohydration.

CHAPTER 8

GENERAL SUMMARY

The impact of fatigue on the intermittent high intensity exercise undertaken during participation in team sports has not been extensively studied. Team sports are characterised not only by intermittent exercise, but also by the contribution of a wide range of skills. The aim of the studies presented in this thesis was to examine the effect of intermittent, high intensity shuttle running and fluid ingestion on the performance of selected soccer skills. This was undertaken in a series of experiments in which the Loughborough Intermittent Shuttle Test (LIST) was employed to simulate the minimal physical demands of soccer. As the activity pattern and the physiological and metabolic responses during the LIST closely simulate the match demands of soccer (Nicholas *et al.*, 1999), the observations made in this thesis have practical implications for soccer players.

In all studies the subjects were of comparable training status, the experimental procedures were similar as were most of the changes associated with and without water ingestion. The main findings from these studies were drawn together to facilitate a greater understanding of the physiological and metabolic responses to prolonged intermittent high intensity exercise and its impact on soccer skill. The mechanisms responsible for the maintenance of skill performance observed during this type of activity, following fluid ingestion, were also considered.

The main findings of these studies are summarised as follows:

- Performance of a soccer dribbling and passing skill tests deteriorated following 90-min of prolonged intermittent shuttle running when no fluid was ingested.
- Retention of skill was achieved when fluid was ingested throughout exercise.
- The ingestion of a 6.4% carbohydrate-electrolyte solution had no additional effect on skill performance than a carbohydrate free solution.

- Rehydration following exercise without fluid ingestion did not return skill performance to pre-exercise levels.

The discussion which follows attempts to use the results in a collective way and propose mechanisms for the beneficial effect of fluid ingestion during exercise on soccer skill.

Body water deficit (hypohydration) reduces physical work capacity (Saltin, 1964; Caldwell *et al.*, 1984), physical (Armstrong *et al.*, 1985) and cognitive performance (Adolph, 1947; Reilly and Lewis, 1985; Gopinathan *et al.*, 1988). The extent of the decrement in performance is related to how the fluid is lost, the size of the fluid deficit, the fluid-containing space that predominantly sustains the loss, the type of exercise, and the thermal environment to which the individual is exposed (Sawka and Pandolf, 1990). In an attempt to understand the possible causes for the reduction in skill performance, the focus of the discussion is on how the size of the fluid deficit influences performance because in the studies undertaken all other variables were controlled.

Although hypohydration has been shown to adversely affect exercise performance, the mechanisms underlying fatigue are unclear. Sawka and Pandolf (1990) suggested that the electrolyte imbalance and elevated body temperature accompanying hypohydration are the physiological mechanisms responsible for the onset of fatigue during high intensity exercise. Supporting evidence for this theory was provided by the work of Nielsen *et al.* (1981). They reported that ability to sustain high intensity cycling was inversely related to potassium concentration and muscle temperature during hypohydration (Nielsen *et al.*, 1981). There was no difference in potassium concentration between fluid and no fluid trials during the experiments reported in this thesis. Sodium concentration was higher when no fluid was consumed; however a return to pre-exercise concentrations of both sodium and potassium following exercise was not associated with a return of skill performance (Chapter 7). Therefore, acute electrolyte imbalance does not appear to be the cause of a reduction in skill.

In addition to brief high intensity exercise, increases in muscle and core temperature have been associated with a reduction in walking capacity (Craig and Cummings 1966), 5000 and 10000 m race times (Armstrong *et al.*, 1985) and running capacity (Morris *et al.*, 1996). Similar increases in muscle and core temperature may have a debilitating effect on the performance of soccer skills.

In comparison to euhydration, hypohydration has been shown to increase core temperature during exercise even in temperate conditions (Sawka *et al.*, 1980; Neuffer *et al.*, 1989; Hamilton *et al.*, 1991). As the magnitude of water deficit increases, there is a concomitant graded elevation in core temperature during exercise (Greenleaf and Castle, 1971; Gisolfi and Coppling 1974; Sawka *et al.*, 1985; Montain and Coyle 1992). In the present studies the water deficit was greatest in all trials when fluid was not ingested (Chapters 4, 5 and 6). When hypohydrated, the increased heat storage results from either a disproportionate increase in metabolic heat production or a disproportionate decrease in metabolic heat loss (Sawka, 1992). As the estimated total energy expenditure was similar in all trials (Chapter 5), the hypohydration-mediated increase in heat storage during exercise is likely to have been due to a decrease in heat dissipation, rather than differences in metabolic rate.

Mean sweat rate was lower during the no fluid trial than when fluid was ingested (Chapter 4). However, in subsequent studies a lower sweat rate was not found even though conditions were similar (Chapters 5 and 6). Hypohydration has been associated with both reduced (Senay, 1968; Moroff and Bass, 1965; Strydom and Holdsworth, 1968) and unchanged (Strydom *et al.*, 1966; Claremont *et al.*, 1976; Swamy *et al.*, 1981) sweat rate. Although sweat rate was unchanged during some of these previously published studies, an elevated core temperature was still present during hypohydration (Strydom *et al.*, 1966; Swamy *et al.*, 1981; Sawka *et al.*, 1984). Therefore, sweat rate is lower for a given core temperature, and the potential for heat dissipation via evaporation is reduced when subjects are hypohydrated during exercise (Sawka, 1992).

The mechanism by which fluid replacement reduces heat storage during exercise is equivocal. Plasma hypertonicity (Harrison *et al.*, 1978; Senay, 1979; Candas *et al.*, 1986; Liebert *et al.*, 1988), hypovolaemia (Hertzman and Ferguson, 1960; Fortney *et al.*, 1981,1988; Morimoto, 1990) or a combination of both have been suggested as the cause of the reduced sweating response during exercise-induced heat stress. In agreement with previous studies, serum osmolality and sodium concentration were higher in the no fluid trials reported in Chapters 4, 5 and 6. Although hypertonicity was greater in the absence of fluid ingestion, a return of osmolality was not associated with a recovery of skill (Chapter 7). There may, of course, be a lag time between the appearance of measures of successful rehydration and the return of motor skills.

There was no difference at any time in plasma volume during all trials reported in this thesis, however, this does not imply that blood volume was unaffected. The maintenance of plasma volume despite a large fluid deficit has been observed in a number of previously published studies (Sawka *et al.*, 1980; Houston *et al.*, 1981; Gass *et al.*, 1983). Reasons for the maintenance of plasma volume during intense exercise, despite progressive dehydration, might include the release of water from glycogen breakdown (Sawka, 1992), metabolic water (Pivarnik *et al.*, 1984) and the redistribution of water from inactive skeletal muscle (Sawka, 1988). Plasma volume has been shown to be defended by the mobilisation of fluid from the intracellular to the extracellular compartments in response to plasma hypertonicity (Nose *et al.*, 1988). Although plasma volume is maintained, the cost of these alterations may be a reduction in performance. Therefore, despite a return of total body fluid following rehydration, a delayed return of intracellular fluid may delay the return of skill performance (Chapter 7).

In addition to thermoregulatory function, the cardiovascular response to exercise is altered by hypohydration (Nadel, 1980; Nadel *et al.*, 1981; Sawka *et al.*, 1979; Morimoto, 1990). During prolonged submaximal exercise without fluid replacement there is a progressive decrease in systemic arterial, pulmonary arterial, right ventricular end-diastolic pressures and stroke volume, as well as a gradual increase in heart rate (Ekelund, 1967; Rowell, 1986). When the reduction

in stroke volume is relatively greater than the concomitant increase in heart rate cardiac output declines (Sawka *et al.*, 1979). As a consequence, competition arises between the central and peripheral circulation for a limited blood volume (Nadel *et al.*, 1980; Rowell, 1986).

Several investigators (Nadel *et al.*, 1980; Fortney *et al.*, 1981, 1985; Kenney *et al.*, 1990) have reported that these conditions reduce skin blood flow for a given core temperature and therefore decrease the potential heat loss. It has also been suggested that the redistribution of cardiac output can reduce the blood supply to the working muscles and thereby limit performance (Rowell, 1974). This area is indefinite as many authors have found only small or no differences in muscle blood flow following exercise under similar conditions (Kirwan *et al.*, 1987; Savard *et al.*, 1988; Nielsen *et al.*, 1990; Nielsen *et al.*, 1993).

Fatigue under hyperthermic conditions may be due to an affect of heat stress on brain function (Brück and Olschewski 1987; Nielsen *et al.*, 1990; Nielsen *et al.*, 1993). Neilsen *et al.* (1990) suggested an elevated core temperature may reduce the function of motor centres and the ability to recruit motor units required for activity, perhaps via an effect on the motivation for motor performance. The elevated core temperatures, when fluid was not consumed, may therefore have affected skill performance immediately post-exercise by a loss in cognitive function. However, as core temperature returned to resting values and skill performance remained impaired (Chapter 7), the extent to which heat stress affects skill performance requires further investigation.

Increased serotonergic activity has been associated with a central mechanism of fatigue during heat stress (Marvin *et al.*, 1998). Due to the difficulties in assessing 5-HT, prolactin release has been used as a marker of central serotonergic activity. The prolactin response to exercise is potentiated at higher temperatures and this is accompanied by a reduction in endurance capacity (Galloway and Maughan 1995). Cooling the head appears to increase endurance capacity and attenuate the prolactin response to exercise (Brisson *et al.*, 1989, Marvin *et al.*, 1998). In the present series of studies serum prolactin concentrations increased during exercise

(Chapters 6 and 7) but returned to resting values during recovery (Chapter 7). A return of prolactin and core temperature during recovery suggests that an increase in serotonergic activity being the cause of the decrement in skill is doubtful.

Another physical mechanism by which hypohydration might limit submaximal exercise performance is by altering skeletal muscle metabolism. During prolonged exercise without fluid ingestion there is increased carbohydrate oxidation and decreased fat oxidation (Fallowfield *et al.*, 1996). Hargreaves *et al.* (1996) also identified small differences in substrate oxidation between fluid and no fluid trials. In the fluid trial they reported an increase in fat oxidation. This was confirmed from analysis of muscle biopsies which showed glycogen sparing. This shift in substrate metabolism was supported by their earlier work (Febbraio *et al.*, 1994) and attributed to increased activity of the sympathetic nervous system in response to heat stress.

Hargreaves *et al.* (1996) suggested that water ingestion attenuates the increased plasma adrenaline concentration that normally occurs during exercise without fluid, which in turn may explain the reduction in glycogen utilisation. In contrast, an increased rate of glycogenolysis when no fluid is ingested would lead to an earlier onset of fatigue. Although the mean duration of each sprint during the LIST was approximately 2.5 s there was some evidence to suggest a negative influence on running performance in the absence of fluid ingestion. Sprint times in the no fluid trial tended to be slower than in the fluid trials towards the end of exercise (Chapters 4, 5 and 6).

Nicholas *et al.* (1994) reported that the muscle glycogen concentrations at the end of the LIST, when a placebo solution had been ingested, were 31 and 27 mmol. kg w.w.⁻¹ for Type I and Type II fibres, respectively. Although muscle biopsy samples were not obtained, it is reasonable to assume that at the end of the LIST the muscle glycogen concentrations of the subjects who took part in the studies reported in this thesis, were as low as those reported by Nicholas *et al.* (1994). If muscle glycogen concentrations are lowered even further in the absence of fluid

ingestion (Hargreaves *et al.*, 1996), then a deterioration in skill performance due to a low muscle glycogen concentration is plausible.

A reduction in the force-producing capability of muscle has been associated with a reduction in visuomotor tracking skill (Pearce *et al.*, 1998). A close correlation was reported between tracking error and strength loss. It was suggested that the pronounced weakness may have impaired judgement, whereby subjects judged the isometric force of the exercise arm to be a similar percentage of maximum force despite a reduced force generating capacity (Pearce *et al.*, 1998). It is important to note that the tracking test used by Pearce *et al.* (1998) required the subjects to generate very low forces, that were little more than that necessary to flex the arm. When combined with prolonged dynamic exercise, exercise-induced muscle weakness is greater under conditions of low muscle glycogen (Young and Davies, 1984). Judgement also plays a significant role in the successful completion of the LSPT and LSDT in the present studies, therefore similar alterations in judgement following the LIST may have affected skill performance.

This is supported by the work of Nicholas, (1996) who showed that after completing the LIST muscle function was reduced as were muscle glycogen concentrations in both Type I and Type II fibres. It was not clear whether the reduction in muscle function was due to reduced glycogen availability in the muscle fibres, or as a consequence of the influence of exercise on muscle *per se*, or both. As the decrease in muscle function and muscle glycogenolysis was less when subjects ingested a carbohydrate-electrolyte solution, muscle glycogen concentration would appear to be important in maintaining force development.

Successful completion of the soccer skill tests reported in this thesis requires a certain pattern of fibre type recruitment. Due to the failure to maintain the required energy input in the fibres recruited to perform the skill tests, an altered recruitment pattern may have led to an inefficient and therefore slower skill test time. An alteration in neurone recruitment pattern has also been suggested as a possible fatigue mechanism in the performance of motor skill tasks following muscle damage. Saxton *et al.* (1995) assessed physiological tremor following

eccentric exercise. They found an increase in muscle tremor and impaired proprioception of joint position following muscle damage. One of the mechanisms that were attributed to the increased tremor amplitude was a need to increase the number of large motor neurones recruited following eccentric exercise. Large motor neurones have less fine control and so need to be recruited in greater numbers following eccentric work to compensate for the prolonged loss of force production at low frequencies of stimulation (Davies and White, 1981; Newham *et al.*, 1987).

Similar to the idea of an alteration in recruitment pattern following glycogen depletion, an alteration in recruitment pattern due to damage of muscle fibres in the motor unit may influence skill performance. In addition to a reduction in muscle function, performance of the LIST has been associated with severe muscle soreness after exercise and for a few days following the test (Thompson; unpublished Ph.D.). Independent of glycogen depletion, an increase in muscle temperature may lead to an increase in muscle damage (Armstrong *et al.*, 1991). Therefore, a greater heat stress during the no fluid trials may have led to a greater muscle damage and a reduction in motor unit recruitment potential. Interestingly even a modest rise in muscle temperature has been shown to increase the amplitude of physiological tremor (Smith, 1991). Recent evidence suggests that low frequency fatigue (i.e. the disproportionate loss of force production at low frequencies of stimulation) results from structural changes to the proteins involved in excitation-contraction coupling, resulting in impaired calcium release from the sarcoplasmic reticulum (Westerblad, 1993).

Many of the associated consequences of exercise without fluid ingestion are related and therefore difficult to isolate as a single mechanism responsible for the reduction in skill performance. As skill performance remained impaired following rehydration and core temperature returned to normal, these may not be the most influential factors governing skill performance under these conditions. The circumstantial evidence appears to point to a combination of a greater reduction in muscle glycogen and exercise-induced damage in muscle fibres as a consequence of an increased fluid deficit. However, whether some other mechanisms are

influential in skill performance under these conditions can not be excluded and requires further investigation.

On a practical note, irrespective of the mechanisms responsible for the deterioration in skill following the LIST, it is clear from the series of studies reported in this thesis that performers of prolonged high intensity exercise such as soccer will benefit from fluid ingestion. However, traditionally players have not been encouraged to drink water during a soccer match and until recently were prohibited from doing so. Therefore, fluid consumption during games should be encouraged.

Recommendations for future research:

- Examine further the role of muscle glycogen and skill performance. Possibly achieved through dietary manipulation prior to performance of skills tests.
- Biopsy study to identify the influence of water ingestion on muscle glycogen concentration during intermittent exercise.
- Assess the influence of a 'no fluid' trial on muscle function and markers of muscle damage during intermittent exercise.
- Divide the LIST into two 45-min periods. This will increase the specificity of the test and allow for the examination of skill performance prior to, during and post exercise.

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HEALTH SCREEN FOR STUDY VOLUNTEERS Name

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

Please complete this brief questionnaire to confirm fitness to participate:

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

2. **In the past two years**, have you had any illness which require you to:
 - (a) consult your GPYes No
 - (b) attend a hospital outpatient departmentYes No
 - (c) be admitted to hospitalYes No

3. **Have you ever** had any of the following:
 - (a) Convulsions/epilepsy.....Yes No
 - (b) Asthma.....Yes No
 - (c) Eczema.....Yes No
 - (d) DiabetesYes No
 - (e) A blood disorderYes No
 - (f) Head injuryYes No
 - (g) Digestive problemsYes No
 - (h) Heart problemsYes No
 - (i) Problems with bones or jointsYes No
 - (j) Disturbance of balance/co-ordination.....Yes No
 - (k) Numbness in hands or feet.....Yes No
 - (l) Disturbance of vision.....Yes No
 - (m) Ear / hearing problems.....Yes No
 - (n) Thyroid problemsYes No

(o) Kidney or liver problems.....Yes No

(p) An allergic reaction, eg., swelling or breathing difficulties
Yes No

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

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

Additional questions for female participants

(a) are your periods normal/regular?.....Yes No

(b) are you on “the pill”?.....Yes No

(c) could you be pregnant?Yes No

(d) are you taking hormone replacement therapy (HRT)?
Yes No

Thank you for your cooperation!

Introduction

A pilot study was performed to: (i) identify the reliability of the 2 methods of indirect calorimetry used within the thesis and (ii) identify the level of agreement between the two methods (iii) to measure the oxygen uptake during the completion of the Progressive Multistage Fitness Shuttle Run Test (Ramsbottom *et al.*, 1988) using the Aerosport KB1-C portable gas analyser and compare the results with those from a table of predicted values (Ramsbottom *et al.*, 1988).

Methods**Subjects**

Eleven male recreational games players volunteered to participate in this study. The mean \pm SEM age, body mass (BM) and height of the group were 21.2 ± 0.6 years, 80.1 ± 2.0 kg and 1.78 ± 1.01 m respectively.

Preliminary tests

Subjects performed preliminary tests to: (a) estimate $\dot{V} O_2$ max in order to calculate the relative exercise intensities as previously described (Chapter 3) (b) to familiarise themselves with the experimental procedures (c) compare the Aerosport KB1-C portable gas analyser with estimated values.

Experimental design

Subjects acted as their own controls in a repeated measures cross-over design. They completed two 15-min blocks of the LIST on four occasions, the order of which was randomised. Each trial was separated by at least 7 days. Subjects completed the test on 2 occasions with the Aerosport KB1-C portable gas analyser (PGA) and on 2 further occasions with the adapted Douglas bag method (DB).

In the two days prior to the main trials subjects did not participate in any prolonged, heavy exercise. They reported to the laboratory after an overnight fast of between 10 and 12 h. They then emptied their bladders prior to the measurement of nude body

mass which was determined to the nearest 0.1 kg using a beam balance (Avery Ltd., Model 3306ABV).

A standardised 15-min warm-up was performed by each subject prior to each trial, which consisted of jogging, stretching and striding.

Once the LIST began the subjects were required to run or walk at 4 different speeds. The speeds were dictated by a single bleep and based on the % $\dot{V} O_2$ max calculated for each subject. To help the subjects maintain the appropriate pace, one of the investigators would continually provide information about the nature of each upcoming 20 m i.e. “walk”, “sprint”, “cruise”, “jog” or “rest”.

During the LIST investigators made sure that the subjects placed at least one foot on the lines delimiting the 20-m distance at each end of the Sports Hall. The subjects were also told to adjust their pace if they ran at the wrong speeds at any time during the test. As the subjects approached the sprints and during each sprint they were verbally encouraged to perform maximally.

Heart rate was recorded every 15 s during the trials by short - range radio telemetry (Polar Sport Tester™, Kempele, Finland). Temperature of the Sports Hall was recorded every 20 min and maintained over the study between 14 - 20°C by opening windows and doors. Dry-bulb and wet-bulb temperatures were measured using a whirling hygrometer (Brannan Thermometers Ltd).

Results

Over the time of the investigation (7 weeks) a number of the subjects failed to complete all 4 trials; because of injury and other commitments. As all trials were randomised the number of completed data sets for a particular trial varied. Therefore, it was not appropriate to perform statistical tests on the data. Table 1 summarises the results:

Table 1: Summary of results (mean \pm SEM)

	DB 1 Block 1	DB1 Block 1	DB2 Block 2	DB 2 Block 2	PGA 1 Block 1	PGA 1 Block 1	PGA 2 Block 2	PGA 2 Block 2
N	10	9	7	6	10	8	5	5
$\dot{V}O_2$ (l.min ⁻¹)	3.47 \pm 0.11	3.53 \pm 0.15	3.37 \pm 0.14	3.52 \pm 0.14	3.77 \pm 0.25	3.82 \pm 0.27	3.21 \pm 0.39	3.22 \pm 0.43
$\dot{V}CO_2$ (l.min ⁻¹)	3.35 \pm 0.13	3.37 \pm 0.15	3.22 \pm 0.12	3.33 \pm 0.11	3.38 \pm 0.19	3.44 \pm 0.27	2.8 \pm 0.29	2.49 \pm 0.35
$\dot{V}E$ (l.min ⁻¹)	86.67 \pm 2.63	88.34 \pm 3.23	85.81 \pm 3.59	87.39 \pm 4.12	85.83 \pm 4.18	87.79 \pm 5.57	70.8 \pm 6.07	74.0 \pm 7.59
FEO ₂ (%)	16.94 \pm 0.11	16.97 \pm 0.12	17.03 \pm 0.13	16.93 \pm 0.13	16.65 \pm 0.12	16.71 \pm 0.1	16.58 \pm 0.18	16.74 \pm 0.2
FECO ₂ (%)	3.9 \pm 0.11	3.85 \pm 0.12	3.8 \pm 0.12	3.86 \pm 0.13	3.96 \pm 0.12	3.85 \pm 0.09	3.97 \pm 0.17	3.88 \pm 0.20
RER	0.97 \pm 0.01	0.95 0.01	0.96 0.01	0.95 0.02	0.87 0.02	0.88 0.02	0.89 0.04	0.9 0.03
Heart Rate (beats.min ⁻¹)	169 \pm 5	170 \pm 5	162 \pm 4	162 \pm 4	175 \pm 6	171 \pm 5	162 \pm 8	171 \pm 9
Sprint times (s)	2.52 \pm 0.04	2.54 \pm 0.05	2.54 \pm 0.06	2.55 0.06	2.57 0.05	2.57 0.05	2.58 0.06	2.57 0.06
Temperature (Dry Bulb °C)	15.3 \pm 0.3	15.3 \pm 0.3	16.5 \pm 0.4	16.5 \pm 0.4	16.2 \pm 0.4	16.2 \pm 0.4	16.9 \pm 0.8	16.9 \pm 0.8
Temperature (Wet Bulb °C)	11.2 \pm 0.3	11.2 \pm 0.3	10.9 \pm 0.4	10.9 \pm 0.4	11.0 \pm 0.4	11.0 \pm 0.4	10.3 \pm 0.3	10.3 \pm 0.3

DB 1 = Completion of LIST with Douglas bag (1st trial)

DB 2 = Completion of LIST with Douglas bag (2nd trial)

PGA 1 = Completion of LIST with the Aerosport KB1-C portable gas analyser (1st trial)

PGA 2 = Completion of LIST with Aerosport KB1-C portable gas analyser (2nd trial)

The estimated $\dot{V}O_{2max}$ for the group was 52.6 ± 2.5 ml.kg⁻¹BM (Ramsbottom *et al.*, 1988) and measured as 57.2 ± 2.1 ml.kg⁻¹BM.

Discussion

Although the pilot study was not completed, it identified that both measures of indirect calorimetry were reliable methods of estimated energy expenditure during the LIST.

Appendix 3 Expired air raw data

The energy expenditure results reported in Chapter 5 are a combination of results from 5 subjects whose energy expenditure was estimated from the adapted Douglas bag method and 5 estimated from the Aerosport KB1-C portable gas analyser. The results from each independent method are shown in the tables below.

$\dot{V} E$ (l.min⁻¹)

Method	Trial	Rest	Block 1	Block 2	Block 3	Block 4	Block 5	Block 6
DB	CHO	9.7	78.1	78.5	75.7	75.2	76.5	75.6
		± 1.3	± 2.7	± 2.3	± 2.9	± 2.3	± 3.7	± 2.0
	CON	9.8	79.2	76.5	79.2	74.9	74.8	75.8
		± 0.3	± 6.4	± 1.9	± 8.2	± 4.5	± 1.8	± 4.3
	NON	9.9	74.0	75.3	73.0	68.1	68.7	71.2
		± 1.3	± 2.3	± 5.8	± 5.5	± 4.5	± 4.5	± 5.1
PGA	CHO	11.2	80.6	83.0	76.8	79.9	81.2	80.7
		± 1.5	± 2.0	± 2.3	± 2.4	± 2.4	± 2.7	± 2.6
	CON	10.7	79.9	81.2	80.4	78.4	78.8	80.3
		± 0.6	± 2.2	± 2.6	± 3.0	± 3.3	± 3.0	± 2.7
	NON	11.3	76.2	77.4	78.8	73.2	74.7	78.7
		± 0.8	± 2.2	± 3.9	± 3.4	± 3.2	± 3.2	± 3.7

$\dot{V} O_2$ (l.min⁻¹)

Method	Trial	Rest	Block 1	Block 2	Block 3	Block 4	Block 5	Block 6
DB	CHO	0.3	3.1	3.0	3.0	2.9	2.9	2.9
		± 0.03	± 0.2	± 0.1	± 0.2	± 0.2	± 0.2	± 0.2
	CON	0.3	3.1	3.0	3.1	3.0	2.9	3.1
		± 0.04	± 0.3	± 0.3	± 0.3	± 0.3	± 0.2	± 0.2
	NON	0.3	3.0	2.9	2.9	2.7	2.8	2.8
		± 0.07	± 0.2	± 0.2	± 0.2	± 0.2	± 0.1	± 0.1
PGA	CHO	0.5	3.7	3.6	3.4	3.4	3.5	3.6
		± 0.06	± 0.2	± 0.2	± 0.2	± 0.2	± 0.2	± 0.2
	CON	0.5	3.7	3.6	3.5	3.5	3.4	3.4
		± 0.05	± 0.2	± 0.3	± 0.3	± 0.4	± 0.3	± 0.3
	NON	0.5	3.6	3.4	3.5	3.2	3.4	3.4
		± 0.03	± 0.2	± 0.3	± 0.2	± 0.2	± 0.3	± 0.3

$\dot{V} \text{CO}_2$ (l.min⁻¹)

Method	Trial	Rest	Block 1	Block 2	Block 3	Block 4	Block 5	Block 6
DB	CHO	0.3	2.9	2.8	2.8	2.7	2.7	2.7
		± 0.03	± 0.1	± 0.1	± 0.1	± 0.1	± 0.1	± 0.1
	CON	0.2	2.9	2.7	2.8	2.7	2.7	2.7
		± 0.02	± 0.1	± 0.2	± 0.2	± 0.2	± 0.1	± 0.1
	NON	0.2	2.7	2.7	2.5	2.4	2.4	2.4
		± 0.03	± 0.1	± 0.2	± 0.2	± 0.2	± 0.2	± 0.2
PGA	CHO	0.4	3.3	3.2	2.9	3.1	3.2	3.2
		± 0.05	± 0.1	± 0.1	± 0.1	± 0.1	± 0.1	± 0.1
	CON	0.3	2.5	2.6	2.6	2.8	2.9	3.1
		± 0.05	± 0.4	± 0.4	± 0.4	± 0.4	± 0.4	± 0.6
	NON	0.4	3.1	3.0	3.1	2.8	2.9	3.0
		± 0.02	± 0.2	± 0.2	± 0.1	± 0.2	± 0.2	± 0.1

Key:

DB = Douglas bag group (n = 5): The mean ± SEM age, body mass (BM) and estimated maximal oxygen uptake ($\dot{V}O_2 \text{max}$) of the DB group were 24.6 ± 1.0 years, 68.3 ± 3.4 kg and 58.7 ± 2.5 ml.kg⁻¹. min⁻¹ respectively.

PGA = Aerosport KB1-C portable gas analyser group (n = 5): The mean ± SEM age, body mass (BM) and estimated maximal oxygen uptake ($\dot{V}O_2 \text{max}$) of the PGA group were 23.4 ± 1.0 years, 80.4 ± 2.4 kg and 58.7 ± 2.4 ml.kg⁻¹. min⁻¹ respectively.