Strategies for Optimal Hydration and Energy Provision for Soccer-Specific Exercise

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A thesis submitted in partial fulfilment of the requirements for the degree of Doctor of Philosophy (Liverpool John Moores University) following work undertaken at The Research Institute for Sport and exercise Sciences.

May 2006

Abstract

One of the probable causes of fatigue in soccer match-play is depletion of muscle and liver glycogen. Other likely causes include dehydration and hyperthermia, especially when performed in the heat. The aim of the thesis was to investigate the effect of manipulating carbohydrate ingestion and the combined effect of pre-cooling and carbohydrate ingestion on the performance of soccer-specific exercise.

Study 1 was an investigation into the impact on metabolism of altering the timing and volume of ingested carbohydrate during soccer-specific exercise. It was demonstrated that ingesting a carbohydrate-electrolyte solution significantly increased blood glucose, insulin and carbohydrate oxidation, whilst suppressing NEFA, glycerol and fat oxidation. In addition, when the total volume of fluid consumed was equal, manipulating the timing and volume of carbohydrate ingestion elicited the same metabolic responses. However, consuming fluid in small volumes, frequently, reduced the sensation of gut fullness.

The effect of manipulating the timing and volume of carbohydrate ingestion on power output during the sprint portions of soccer-specific exercise was investigated in study 2. The experimental trials did not significantly affect power output when sprinting. It was also demonstrated that ingesting a carbohydrate solution, compared with placebo, significantly increased plasma glucose and carbohydrate oxidation. It was concluded that ingesting carbohydrate compared with a placebo during a soccer-specific protocol had no impact on peak sprint power output although it significantly altered metabolism.

In study 3 the effect of ingesting multi-carbohydrate solutions on the metabolic responses to soccer-specific exercise in the heat and the subsequent impact on high-intensity exercise capacity was examined. The ingestion of a multi-carbohydrate solution did not have a significant influence on muscle glycogen utilization, metabolism or exercise capacity compared with the ingestion of a glucose solution. This observation suggests that intestinal absorption does not limit the oxidation of exogenous carbohydrate during exercise of this nature in the heat. In addition, there was not a significant difference in muscle glycogen utilization or exercise capacity between treatments. It was concluded that an elevated core temperature, and not substrate availability limits exercise capacity during soccer-specific exercise in the heat.

In study 4 it was demonstrated that following 90 min of soccer-specific exercise in the heat high-intensity exercise capacity and performance as determined by a psychophysical test were significantly improved with the combination of pre-cooling and carbohydrate ingestion as was mental concentration during the protocol. Pre-cooling did not influence metabolism during exercise. These results suggest that carbohydrate ingestion can improve exercise capacity following soccer-specific exercise performed in the heat. Also, pre-cooling and the ingestion of carbohydrate can further enhance exercise capacity.

From these studies it was concluded that volume and timing of carbohydrate ingestion do not significantly influence metabolism or performance, providing the total volume is equal. In addition, core temperature, and not substrate availability appears to be the overall limiting factor to performing soccer-specific exercise in the heat.

Acknowledgements

I must start by thanking my supervisors Professor Tom Reilly, Professor Don MacLaren and Doctor Barry Drust, all of whom provided me with continuous encouragement, support and guidance throughout the development and completion of this thesis.

Thanks are also extended to GlaxoSmithKline, especially Manuela Finke, who financed the series of studies and provided support and copious amounts of Lucozade

I am grateful to the subjects, who volunteered their time and energy and provided blood, sweat and tears, well blood and sweat anyway. These studies would not have been possible without their help.

I would also like to thank the laboratory technicians, Dave Stokes, Pip Kennit, Andrew Hulton, Steve Wilson and Neil Chester, who kept me in supplies and always seemed keen to fix any problem when they arose. Also I must thank the medical staff, Louise Evans and Iain Campbell, who always made themselves available when required.

An added benefit of completing this PhD is the number of friends I've made; they have provided me with social support as well as helping me out during the more hectic periods.

I am very lucky to have a wonderful family and without them, I'm not sure I would have even got to University, let alone to this stage. Thank you for believing in me and the support, both financial and emotional.

Finally, I wish to thank (or should that be apologise to) Cheryl, who offered support, especially when things were not going as well as I wanted. Your support, patience and diplomacy have been greatly appreciated.

Declaration

I declare that the work presented in this thesis is entirely my own, with the exception of:

Some of the work reported in this thesis has already been presented as conference communications or published in international journals (see Appendix A).

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List of abbreviations

5-HIAA	5-Hydroxyindole acetic acid
5-HT	5-Hydroxytryptamine
ACTH	Adrenocorticotropic hormone
ADP	Adenosine diphosphate
ATP	Adenosine triphosphate
BCAA	Branched-chain amino acid
Ca^{2+}	Calcium
CHO	Carbohydrate
CHOf	Carbohydrate solution ingested frequently during the soccer-specific protocol
CHOv	Carbohydrate ingested prior to and at half-time of the soccer-specific protocol
CNS	Central nervous system
CoA	Coenzyme A
Cr	Creatine
f-TRP	free tryptophan
G-6-P	Glucose-6-phosphate
G-6-PDH	Glucose-6-phosphate dehydrogenase
GLU	Glucose ingestion
GLUc	Glucose ingestion with pre-cooling
GLUT2	Glucose transporter type 2 (glucose and fructose transporter)
GLUT5	Glucose transporter type 5 (fructose transporter)
HCl	Hydrochloric acid
IL-6	Interleukin-6
LIST	Loughborough intermittent shuttle test
MIX	Multi-carbohydrate solution
Mg^{2+}	Magnesium
NAD	Nicotinamide adenine dinucleotide
NADH	The reduced form of Nicotinamide adenine dinucleotide
NADP	Nicotinamide adenine dinucleotide phosphate
NADPH	The reduced form of Nicotinamide adenine dinucleotide phosphate
NEFA	Non-esterifed fatty acids
NMR	Nuclear magnetic resonance
PCr	Creatine phosphate
Pi	Inorganic phosphate
PLA	Placebo
PLAc	Placebo ingestion with pre-cooling
RER	Respiratory exchange ratio
rh	Relative humidity
SGLT1	Sodium glucose cotransporter
T_c	Core temperature
TRP	Tryptophan
VAS	Visual analogue scale
$\dot{V}CO_2$	Carbon dioxide production
$\dot{V}O_2$	Oxygen consumption
$\dot{V}E$	Minute ventilation
$\dot{V}O_{2max}$	Maximal oxygen uptake
Wmax	Maximum power output

Chapter 1

Introduction to the thesis

1.1. Introduction to the thesis

During a soccer match players perform a wide variety of activities ranging from walking to sprinting, and so the intensity of effort changes frequently. The energy cost of playing in a competitive soccer match has been estimated to be approximately 6700 kJ (Bangsbo, 1994b). Throughout a match, energy is provided predominantly by aerobic metabolism (Reilly *et al.*, 2000), with a rise in circulating free fatty acids as the game progresses (Bangsbo, 1994b). However, crucial components of activity e.g. tackling, jumping and sprinting, rely on anaerobic energy production and carbohydrate metabolism. Soccer does not just require physical effort, it also requires concentration and cognitive awareness in order to make the right decisions e.g. when to "make a tackle" or where to pass the ball and when and where to run.

The intensity of exercise associated with a competitive match is high enough to induce appreciable heat load, causing players to lose up to 3 litres of sweat in a game (Ekblom, 1986). Dehydration during exercise has been shown to raise core temperature and increase cardiovascular strain, and lead to a decrease in sweat loss (Sawka *et al.*, 1985). The elevation in core temperature has been demonstrated to be greater during intermittent exercise when compared with continuous exercise at the same average intensity (Ekblom *et al.*, 1971). An elevated core temperature (Gonzalez-Alonso *et al.*, 1999c; Nybo *et al.*, 2001) and a moderate level of dehydration (Walsh *et al.*, 1994) have been shown to limit exercise performance. As a consequence it is important that athletes consume fluid during prolonged exercise to reduce these adverse effects. It has been demonstrated that the addition of carbohydrate to the fluid can improve exercise performance (Wright *et al.*, 1991), possibly due to the sparing of muscle glycogen and delaying the onset of fatigue (Tsintzas *et al.*, 1995).

Many authors have investigated the impact of carbohydrate ingestion on the performance of exercise simulating the work-rate of soccer (Nicholas *et al.*, 1995; Walton and Rhodes, 1997; Nicholas *et al.*, 1999) and actual match-play (Kirkendall *et al.*, 1988; Leatt and

Jacobs, 1989; Zeederberg *et al.*, 1996) though the results have been somewhat equivocal. The different measurement tools used to assess performance i.e. run to exhaustion (Nicholas *et al.*, 1995) or high-intensity sprints and skills (Zeederberg *et al.*, 1996) may account for the differences between studies. These studies have focused on the ingestion of carbohydrate, and not the hydration strategy i.e. timing and volume of fluid. Consequently, there may be opportunities for enhancing performance during a game by adopting optimal refuelling and rehydration regimes.

The position stand of the American College of Sports Medicine (Convertino *et al.*, 1996) is that during exercise, athletes should start drinking early and at regular intervals in an attempt to consume fluids at a rate sufficient to replace the water lost through sweating, or consume the maximal amount that can be tolerated. However, most advice regarding rehydration during exercise has been based on continuous exercise (such as cycling or road-running) where fluid can be ingested during the activity or in sports such as American football or basketball where there are opportunities for breaks when fluid can be consumed. The rules of soccer coupled with gastric tolerance, and the perception of gut fullness do not allow for complete rehydration of players. Due to the continuous nature of play, with infrequent, unscheduled brief stoppages, the only two occasions that a player is guaranteed to be able to consume fluid is before the game kicks off and at half-time.

Gastric emptying is deemed to be a limiting factor in fluid replacement (Shi and Gisolfi, 1998) and is an important aspect in determining the rate at which nutrients enter the duodenum where glucose and water can be absorbed into the bloodstream (Brouns *et al.*, 1987). The exponential nature of gastric emptying (Rehrer *et al.*, 1989) highlights the importance of the volume of fluid in the stomach in controlling the rate of emptying. As fluid empties from the stomach, the volume decreases, as does the rate of gastric emptying. Maintaining a large fluid volume in the stomach, by repeated drinking maximises the rate of fluid and nutrient delivery to the small intestine (Mitchell and Voss, 1991; Noakes *et al.*, 1991). In addition, gastric emptying is also influenced by exercise

intensity; Leiper *et al.* (2005) demonstrated that the intensity associated with a soccer match is sufficient to slow gastric emptying.

Soccer matches at major tournaments are often played in temperatures exceeding 30°C (FIFA World Cup 2002 and UEFA Euro 2004). An elevated body temperature, along with reduced carbohydrate availability, has been demonstrated to be one of the factors that can limit endurance performance (Nybo and Nielsen, 2001). Consequently there has been an increase in the amount of attention paid to rehydration and energy provision during exercise, especially when performed in the heat. Fink *et al.* (1975) were the first to demonstrate that environmental temperature affects intramuscular substrate utilization when they observed that 60 min of intermittent exercise at 41°C increased muscle glycogen utilization, compared with exercise at 9°C. When the rise in body temperature is attenuated by preventing dehydration muscle glycogenolytic rate and carbohydrate oxidation are reduced. It has been demonstrated that sports drinks can provide the carbohydrate necessary to prevent hypoglycaemia (Coyle *et al.*, 1986) and improve exercise performance (Mitchell *et al.*, 1989). However, ingesting too much fluid during exercise can lead to hyponatraemia (Speedy *et al.*, 1999; Speedy *et al.*, 2001)

Jentjens *et al.* (2002) reported that the oxidation of ingested carbohydrate is reduced in the heat compared with a cool environment, and may be due to a reduced absorptive capacity of the intestine. Glucose is transported across the intestinal membrane by sodium/ glucose cotransporters (SGLT1), which is thought to become saturated at glucose ingestion rates exceeding 1 $g \cdot min^{-1}$ (Jeukendrup and Jentjens, 2000), which may explain why there is not a linear relationship between glucose ingestion rates and oxidation rates. This view is supported by the fact that exogenous carbohydrate oxidation rates have not exceeded approximately 1 $g \cdot min^{-1}$ despite carbohydrate in the form of glucose or glucose polymer being ingested at rates of up to 3 $g \cdot min^{-1}$ (Pirnay *et al.*, 1982; Wagenmakers *et al.*, 1993; Jeukendrup *et al.*, 1996). However, fructose is absorbed from the intestine by facilitative fructose transporters (GLUT5). Therefore, because glucose and fructose are absorbed by means of separate intestinal transport mechanisms, when a solution containing a mixture of glucose and fructose is ingested, there is less competition

for absorption compared with an isoenergetic amount of glucose. As a consequence there may be an increase in the amount of carbohydrate entering the bloodstream and subsequent availability for oxidation. An increase in exogenous carbohydrate could spare muscle glycogen, delay the onset of fatigue and improve performance.

An elevated core temperature limits exercise performance (Galloway and Maughan, 1997) and increases carbohydrate utilization (Febbraio, 2001). A number of strategies have been shown to be effective in reducing thermoregulatory strain and improving performance when exercising in the heat (Kay and Marino, 2000). Pre-cooling before prolonged exercise has been previously studied (Lee and Haymes, 1995; Booth *et al.*, 1997; Booth *et al.*, 2001), and may result in improved performance in terms of endurance time (Cotter *et al.*, 2001) and increased work-rate (Hessemer *et al.*, 1984). However, relatively little information has been published concerning the effects of pre-cooling prior to high-intensity intermittent exercise (Drust *et al.*, 2000a). There is some evidence that glycogen depletion is accelerated in the heat as a consequence of an elevated core temperature (Hargreaves *et al.*, 1996a), but the impact of pre-cooling on substrate utilization and performance of intermittent exercise (i.e. all-out sprints) has not been investigated. Also pre-cooling has not been examined in conjunction with hydration strategies. This is of interest because a pre-cooling strategy is easily implemented and could be used by soccer players (and other athletes) prior to matches to reduce thermoregulatory strain and potentially improve performance by sparing muscle glycogen.

In this thesis the potential responses to soccer-specific exercise after altering hydration strategies and implementing regimes to cope with performing in the heat are explored in a series of experiments.

1.2. Aims of the thesis

The overall aim of the thesis is to investigate the effect of altering the timing of carbohydrate ingestion, the composition of the ingested carbohydrate and the combined

effect of pre-cooling and carbohydrate ingestion on the performance of soccer-specific exercise.

All of the experiments conducted consisted of investigations into the metabolic and performance responses to intermittent exercise. The exercise protocol was designed to simulate the work-rate observed in competitive soccer match-play. The basis of the first two experiments was to manipulate the provision of carbohydrate to subjects so that the consequences could be examined:

1. For its effects on the metabolic responses to fixed exercise on a motorised treadmill.

2. With respect to its influence on performance (reflected in the sprint portion) during a soccer-specific protocol of the simulated work-rate on a non-motorised treadmill.

The basis of studies three and four was to examine the effect of strategies for performing soccer-specific exercise in the heat by means of:

3. Investigating the effect of carbohydrate formulation on the performance of soccer-specific exercise in the heat.

4. Investigating the effect of pre-cooling in conjunction with carbohydrate ingestion on the performance of soccer-specific exercise in the heat.

Chapter 2

Review of the literature

2.1. Introduction

This chapter outlines the energy sources required during exercise and the causes of fatigue. Furthermore, the benefits of fluid replacement and carbohydrate supplementation, and the factors that influence the effectiveness of these strategies are examined. The implications of exercising in the heat will be discussed, as well as methods to reduce thermal stress. Finally, these aspects will be discussed in relation to the performance of soccer.

2.2. Energy sources for exercise

During exercise energy is required for muscle action. The four metabolic pathways, phosphagen, anaerobic glycolytic, carbohydrate and lipid aerobic systems for the production of adenosine triphosphate (ATP) are summarized schematically in Figure 2.1.

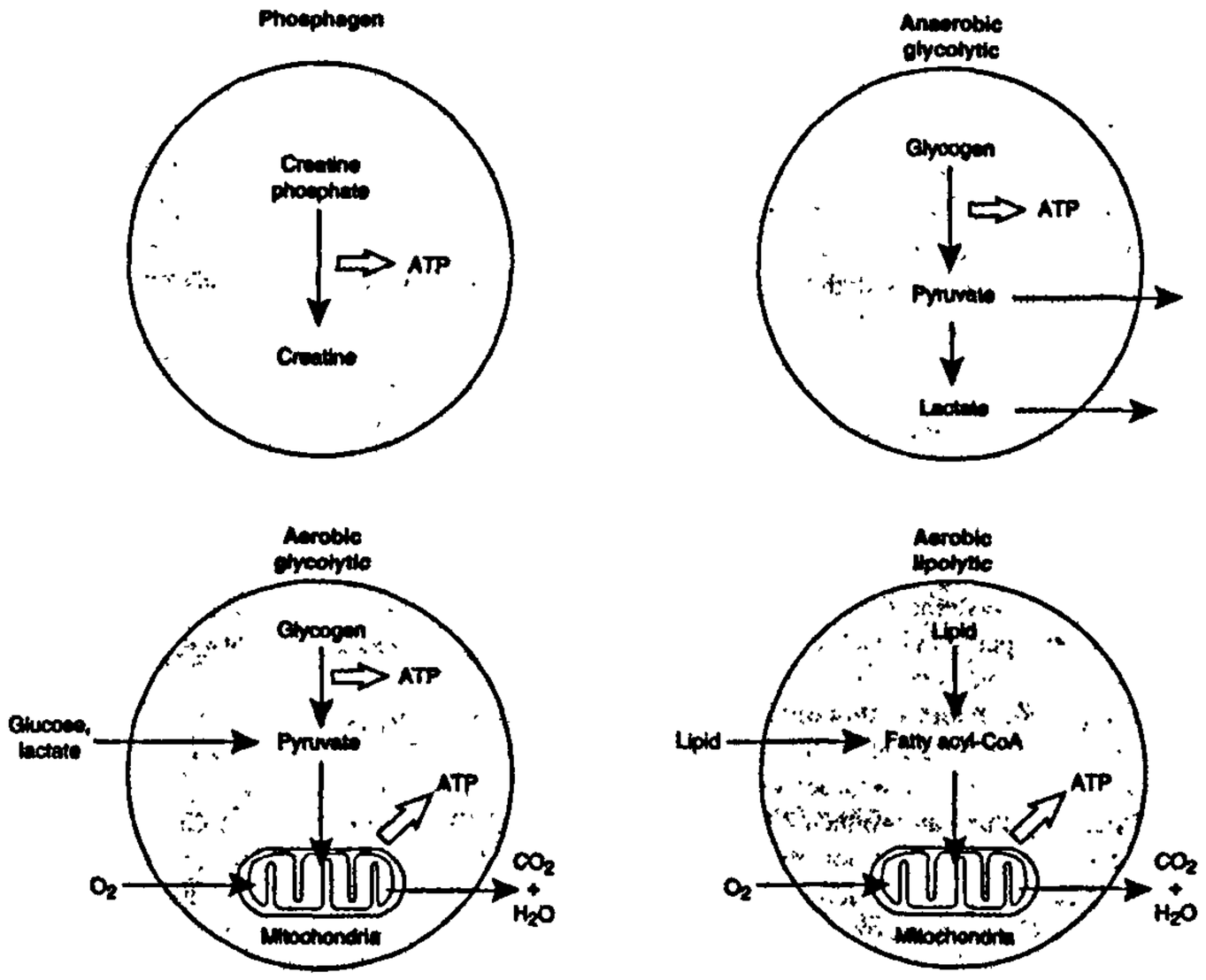

Figure 2.1: The four metabolic pathways for the production of ATP in muscle cells.

Abbreviation: CoA = coenzyme A (from Hawley and Hopkins, 1995).

2.2.1. The phosphagen system

In muscle the chemical energy liberated during the breakdown of ATP activates specific sites along the contractile element, causing the muscle fibre to contract. The store of ATP in the human body is only sufficient to supply energy for 1 or 2 seconds (Hawley and Hopkins, 1995), and since it cannot be transported in the blood or from other tissues, needs to be resynthesized at the rate of usage. The major sources of energy for ATP resynthesis are lipids and carbohydrates, but for rapid resynthesis the energy comes from a high-energy compound, phosphocreatine (PCr). Phosphocreatine acts as a temporary ATP buffer catalysed by the enzyme creatine kinase in which the phosphate group is transferred to adenosine diphosphate (ADP) to yield ATP and creatine (Cr) (Grassi, 2005). The reaction is also freely reversible and PCr can be rapidly resynthesized during periods of rest or low-intensity exercise.

2.2.2. The anaerobic glycolytic system

Carbohydrate, especially muscle glycogen, is utilized in anaerobic glycolysis at a rapid rate during high-intensity exercise. This is due to the energy demand exceeding the oxygen supply or its rate of utilization. Muscle glycogen and blood glucose utilization increases with increasing exercising intensity (Romijn *et al.*, 1993). As a consequence, muscle glycogen is the major source at intensities of greater than 65 to 70% $\dot{V}O_{2max}$. Cheetham *et al.* (1986) reported a significant decrease in muscle glycogen content (25%) following a 30-s sprint, with 62.9% of ATP produced being derived from glycolysis. In addition, Wootton and Williams (1983) proposed that during intermittent maximal exercise of brief duration (6 s) with short rest intervals (30 s) there is an increased demand on anaerobic glycolysis to maintain the rate of energy production because of incomplete resynthesis of PCr stores. As the duration of exercise increases the contribution of energy production from muscle glycogen decreases while that from blood glucose increases (Romijn *et al.*, 1993).

Lactate is produced when the regeneration of NADH to $NADH^+$ is insufficient to maintain the conversion of glyceraldehydes-3-phosphate to 1,3, biphosphoglycerate. This reaction typically occurs during periods of intense exercise or when oxidative phosphorylation is unable to meet energy production requirements (Stainsby, 1986). Once lactic acid is formed it diffuses into the blood, where it is buffered, forming hydrogen ions (protons) and lactate. It is then transported in the blood away from the muscle, allowing glycolysis to proceed and supply additional anaerobic energy for ATP resynthesis. Lactate should not be viewed merely as a "metabolic waste product". Brooks (1991) stated that lactate, derived from the anaerobic breakdown of muscle glycogen and blood glucose could be an important metabolic intermediate, a gluconeogenic precursor and a substrate for oxidative metabolism in both cardiac and skeletal muscle.

2.2.3. Aerobic energy production from carbohydrates

The relative amount of carbohydrate and lipid metabolised during exercise is dependent primarily on the intensity and duration (Romijn *et al.*, 1993). The preceding diet, training status and environmental conditions (especially temperature) can also influence fuel selection (Hargreaves, 1991).

Liver glycogen is predominantly used to maintain homeostasis of blood glucose to ensure that there is an adequate supply to the organs that are dependent on glucose for their energy needs e.g. brain, central nervous system (CNS), blood cells and kidney. During rest these tissues can account for as much as 75% of peripheral glucose utilization (Hargreaves, 1991), with resting skeletal muscle using 15 to 20%. Glycogenolysis represents 75% of hepatic glucose output, with gluconeogenesis accounting for the remaining 25%, although this fraction increases during prolonged exercise and fasting (Romijn *et al.*, 1993).

During exercise muscle glycogen is degraded to glucose-1-phosphate using the enzyme phosphorylase, and then to glucose-6-phosphate. This "site" of entry into glycolysis is the same for glucose entering the muscle, which is immediately phosphorylated by hexokinase. Glycolysis results in the production of pyruvate and reduced NAD, which can then be oxidized in the Tricarboxylic acid (TCA) cycle and electron transport chain, provided there is sufficient oxygen.

2.2.4. Aerobic energy production from lipids

For submaximal endurance activities, the utilization of lipids relative to carbohydrate in the aerobic pathways increases as the intensity of exercise decreases. This phenomenon has been termed the "crossover concept" (Brooks and Mercier, 1994). The major sources of lipids are plasma non-esterifed fatty acids (NEFA) mobilized from adipose tissue, and intramuscular triglycerides, with a minor contribution from plasma triglycerides. Triglycerides can be stored as droplets within the muscle fibres, which places the energy source within a short distance of the site of oxidation within the muscle mitochondria. Intramuscular triglycerides account for between 8400 and 12600 kJ of stored energy and are therefore a larger source of potential energy than muscle glycogen, which accounts for only approximately 6300 kJ (Coyle, 1997).

During prolonged exercise, lipolysis in fat depots is increased after 15-20 min of exercise, by the stimulation of the beta-receptors in adipocytes by adrenaline (Arner *et al.*, 1990). Hormone sensitive lipase is activated, which hydrolyses the triglyceride molecule into three NEFA and one glycerol molecule. However, in adipose tissue at rest, as much as 70% of the NEFA released is reattached to glycerol, forming new triglycerides within the adipocytes. During the performance of low-intensity exercise the rate of lipolysis increases and as a result NEFA in the plasma increases fivefold (Romijn *et al.*, 1993; Klein *et al.*, 1994).

Muscle is also capable of hydrolysing triglycerides, although the contribution to energy production from this source is limited (Kiens *et al.*, 1993). Plasma NEFA is utilized most during prolonged and low to moderate intensity exercise whereas intramuscular lipolysis is stimulated at higher intensities and at the beginning of prolonged exercise when plasma NEFA availability may be limited. Romijn *et al.* (1993) found that the oxidation of intramuscular triglycerides was low during exercise at 25% $\dot{V}O_{2max}$ compared with at 65% $\dot{V}O_{2max}$, where intramuscular triglyceride represented around half of the total fat oxidation (Figure 2.2). It should be noted that fat oxidation increases between 25% and 65% $\dot{V}O_{2max}$ even though the contribution from plasma NEFA decreases. When individuals exercise at 85% $\dot{V}O_{2max}$, muscle glycogen becomes the predominant energy source due to the higher exercise intensity requiring a greater demand for energy that cannot be supplied at a high enough rate by the oxidation of lipids.

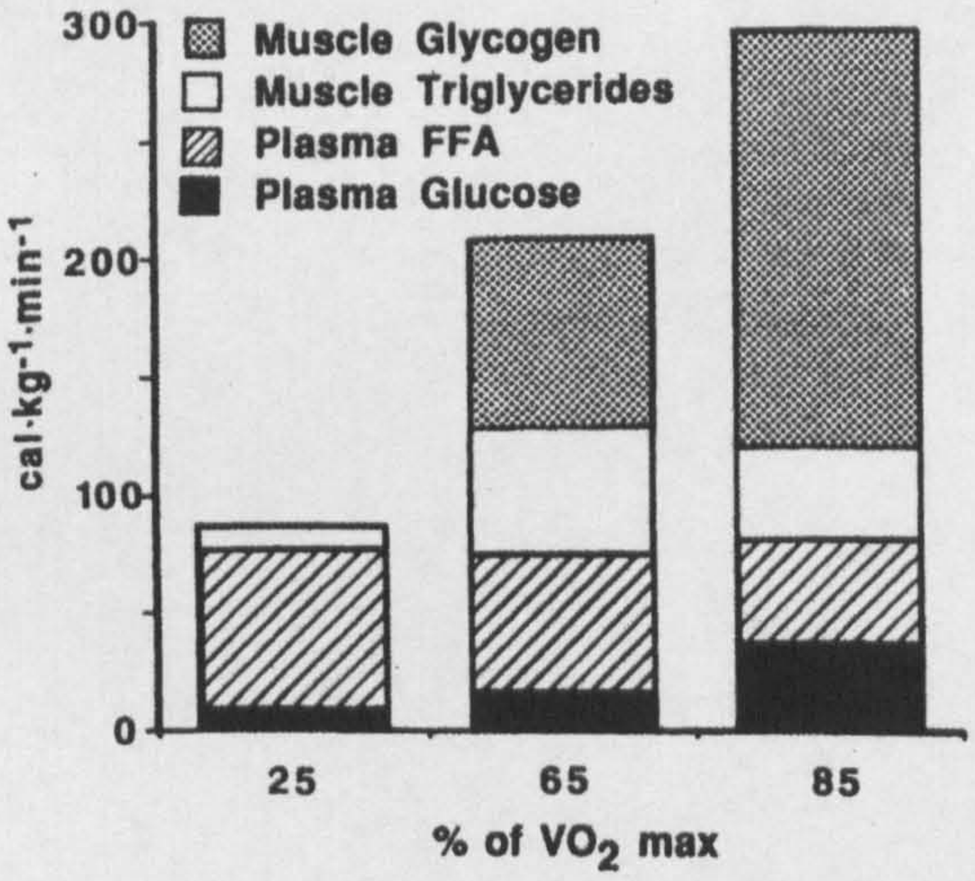

Figure 2.2: Contribution to energy expenditure from glucose, NEFA, muscle triglycerides and muscle glycogen after 30 min of exercise of different intensities (from Romijn *et al.*, 1993).

2.3. Fatigue during exercise

Fatigue can be described as the inability to maintain the required force or power output. It can also be viewed as a process that changes the functional state, possibly resulting in exhaustion and the termination of exercise or work (Kay and Marino, 2000). The causes of fatigue are based upon substrate depletion (Gollnick *et al.*, 1974), neurological impairment (Davis, 1995) and hyperthermia (MacDougall *et al.*, 1974).

2.3.1. Peripheral fatigue

Peripheral fatigue is typically thought to occur as a result of impaired skeletal muscle function. A limitation in energy supply is the classic hypothesis for the causes of muscle fatigue. This view is supported by the findings that fatigue coincides with the depletion of intra-muscular carbohydrate stores during prolonged exercise (Berstrom *et al.*, 1967) and depletion of PCr during high-intensity exercise (Hirvonen *et al.*, 1987).

Ammonia (NH_3) is produced by skeletal muscle as a byproduct of the breakdown of either ATP or amino acids (Graham *et al.*, 1995). During exercise, there is an increase in the release of NH_3 from contracting skeletal muscle, increasing circulating NH_3 levels. In addition, performing intermittent exercise in the heat has been shown to significantly elevate plasma ammonia levels (Snow *et al.*, 1993) and reduce sprinting capacity (Mohr *et al.*, 2006). As a consequence of NH_3 being able to cross the blood-brain barrier, a rise in plasma NH_3 increases cerebral NH_3 uptake, and this has the potential to influence brain neurotransmitters and cause central fatigue (Nybo and Secher, 2004).

Another potential cause of fatigue is the accumulation of potassium in the muscle interstitium (Nordsborg *et al.*, 2003). Renaud and Light (1992) demonstrated that extracellular potassium levels above 8 $mmol.l^{-1}$ reduces contractility and during high-intensity exercise, at the point of exhaustion, the interstitial potassium concentration is elevated to around 12 $mmol.l^{-1}$ (Nordsborg *et al.*, 2003), which may be high enough to

depolarize the muscle membrane potential and force development (Cairns and Dulhunty, 1995).

During high-intensity exercise a high rate of glycolysis is required to maintain power output, resulting in the formation of lactate. The consequence is a decrease in intramuscular pH, which is associated with a reduction in the release of Ca^{2+} and a decline in muscle contractility. Green (1995) discussed the implications of these mechanisms in a comprehensive review.

2.3.2. Central fatigue

Muscle fatigue has been attributed to the depletion of substrates, accumulation of metabolites, ionic changes and inadequate oxygen delivery. Analogously it has been suggested that metabolic, circulatory, neurotransmitter, thermodynamic changes or other disturbances of cerebral homeostasis could result in central fatigue (Nybo and Secher, 2004).

During exercise the generation of metabolic heat and depletion of carbohydrate have an adverse impact on exercise performance and capacity. As discussed earlier, carbohydrate depletion is classified as a peripheral cause of fatigue, but an increase in IL-6 release, as a consequence of a high rate of glycogenolysis, which acts to increase lipolysis has been linked with central fatigue (Nybo *et al.*, 2002a). Furthermore, the release of IL-6 from skeletal muscle could be involved in a feedback system, which ultimately decreases the central drive to continue exercise (Gleeson, 2000).

There is also evidence supporting the role of the neurotransmitters serotonin (5-HT or 5-hydroxtryptamine) and dopamine in central fatigue (Davis, 1995). During prolonged exercise NEFA are released from the adipose tissue, increasing the plasma concentration of NEFA and free tryptophan (f-TRP), as NEFA displaces some of the albumin-bound tryptophan (TRP) (Curzon *et al.*, 1973). Tryptophan is the amino acid precursor to

serotonin, and increased TRP availability is thought to elevate the cerebral serotonin level, because the enzyme that converts TRP to serotonin is not saturated under normal physiological conditions (Nybo and Secher, 2004). In this way, the cerebral level of serotonin increases in rats during prolonged exercise (Bequet *et al.*, 2001). In addition Watson *et al.* (2005) suggested that blood-brain barrier permeability may be altered during prolonged exercise in a warm environment, which may disturb normal brain function and contribute to the development of central fatigue. Studies have shown that the concentrations of 5-HT and its major metabolite, 5-HIAA, increase in several brain regions during prolonged exercise, peaking at fatigue (Bailey *et al.*, 1993b). The increase in brain 5-HT synthesis and turnover probably results from an increase in plasma f-TRP and f-TRP/ branched-chain amino acids (BCAA) ratio (Blomstrand *et al.*, 1989). The administration of 5-HT agonist and antagonist drugs can decrease and increase respective run times to fatigue in the absence of any peripheral markers of fatigue (Bailey *et al.*, 1993a).

The TRP mediated serotonergic activity has been shown to inhibit dopamine release, increase prolactin release (De Meirleir *et al.*, 1985), and consequently serum prolactin concentration and reduce central drive (Chaouloff, 1997). Carbohydrate supplementation has been shown to reduce f-TRP and f-TRP/ BCAA ratio, and fatigue is delayed using this strategy (Davis *et al.*, 1992), although it would be difficult to isolate the effects on central fatigue from the established benefits on skeletal muscle. However, in humans, it appears that the "serotonin-fatigue" hypothesis does not become relevant unless the exercise intensity and duration is sufficient to result in marked elevations of the circulating levels of NEFA and f-TRP (Nybo and Secher, 2004).

2.3.3. Hyperthermic fatigue

During exercise in the heat, or in situations where there is a large level of net heat storage, fatigue is probably caused by hyperthermia rather than substrate depletion (Febbraio *et al.*, 1996). It has been reported that high core temperature *per se* can cause

fatigue (Gonzalez-Alonso *et al.*, 1999c). Gonzalez-Alonso *et al.* (1999c) manipulated the initial core temperature, and the rate that it increased during exercise (cycling at 60% $\dot{V}O_{2max}$). Despite the different initial core temperatures (35.9°C – 37.4°C), all subjects fatigued at an identical level of hyperthermia (40.1°C – 40.2°C), and time to exhaustion was inversely related to initial core temperature and directly related to the rate of heat storage. These findings would suggest that exercise intensity is controlled by a central mechanism that forces a reduction in exercise intensity when core temperature reaches a critical level (Nielsen and Nybo, 2003). Inadequate hydration strategies have also been shown to accentuate thermal stress (Armstrong and Maresh, 1998).

2.4. Dehydration, fluid replacement and exercise performance

Water is the main component of the human body, accounting for approximately 60% of body mass and 72% of lean body mass in a healthy adult (Sawka and Pandolf, 1990). Dehydration during exercise increases core temperature and decreases blood volume, venous return, stroke volume, skin blood flow and sweat rate (Nadel *et al.*, 1980). Studies on the effects of dehydration on exercise performance have demonstrated increased cardiovascular strain, indicated by a disproportionate increase in heart rate during exercise, and a reduced ability of the body to transfer heat from the active muscles to the skin surface where it can be dissipated to the environment (Convertino *et al.*, 1996).

Dehydration that results in a loss of body mass of 1-2% contributes to an increase in core temperature and cardiovascular strain (Hoffman *et al.*, 1994). The magnitude of increase in core temperature and heart rate and the decline in stroke volume are graded in proportion to the amount of dehydration accrued during exercise (Montain and Coyle, 1992). The loss of body water can adversely affect exercise performance, although the severity depends upon the environmental conditions (Sawka and Pandolf, 1990) and the type of exercise. Although it appears that during exercise in temperate environments lasting less than 90 min, dehydration by 1-2% of body weight does not significantly influence performance (McConell *et al.*, 1999; Bachle *et al.*, 2001). However, exercise performance over 60 min has been shown to be impaired by dehydration amounting to

1.8% (Walsh *et al.*, 1994). Therefore, dehydration by 2% body weight during exercise in a hot environment (31-32°C) impairs performance, but when exercise is performed in a temperate environment (20-21°C), dehydration by 2% has a lesser effect on endurance performance (Coyle, 2004), possibly due to reduced cardiovascular strain. However, Dehydration does not alter isometric strength and endurance (Greiwe *et al.*, 1998) or anaerobic performance (Walsh *et al.*, 1994), but does reduce endurance (Barr *et al.*, 1991; Montain *et al.*, 1998) and maximal aerobic power (Sawka and Pandolf, 1990).

The ingestion of fluid during prolonged exercise can attenuate the detrimental effects of dehydration on body temperature and exercise performance (Montain and Coyle, 1992). It is important therefore, that athletes consume fluid during prolonged exercise, especially in the heat. However, there can be negative aspects associated with fluid consumption, including, gastrointestinal discomfort, reduced pace during competition due to the time spent drinking large volumes of fluid (Coyle and Montain, 1992) and hyponatraemia (Noakes *et al.*, 2004).

2.4.1. Carbohydrate ingestion during exercise

Originally it was considered that fluid replacement alone was of primary importance for optimising performance. It was not until commercially-funded scientific research into the value of carbohydrate ingestion during exercise did the use of carbohydrate solutions during exercise became common practice. Many authors (Table 2.1) have investigated the effect of carbohydrate ingestion using a number of modes of exercise and its effect on performance, with the majority demonstrating a positive impact on exercise capacity or performance. The effect of carbohydrate ingestion on soccer performance will be discussed at a later point.

Table 2.1: Effect of carbohydrate ingestion on exercise.

Authors	Mode of exercise	Intensity	Duration	Performance test	Improved
Ball *et al.* (1995)	Cycling	Time-trial	50 min	Wingate test	Yes
Coyle *et al.* (1986)	Cycling	70% $\dot{V}O_{2max}$		Cycle to exhaustion	Yes
Davis *et al.* (1988)	Cycling	Time-trials	120 min then another 30 min after 30 min rest		Improved 30 min but not 120 min
Sugiura and Kobayashi (1998)	Cycling	Continuous at 75% $\dot{V}O_{2max}$, or intermittent 65% and 100% $\dot{V}O_{2max}$ (5:1 ratio)	90 min (15 min rest after 45 min)	40-s Wingate test	Yes
Millard-Stafford *et al.* (1992)	Running	40 km (35 km self-paced, last 5 km at race pace)		Last 5 km time	Yes
Vergauwen *et al.* (1997)	Tennis	Strenuous training session	120 min	Leuven tennis performance test and shuttle run	Yes
Yaspelki *et al.* (1993a)	Cycling	Continuous at 45% or 75% $\dot{V}O_{2max}$	190 min	80% $\dot{V}O_{2max}$ ride to exhaustion	Yes

Coggan and Coyle (1989) demonstrated that even a single feeding of a high-carbohydrate solution during a prolonged bout of exercise had a beneficial effect on exercise capacity if the consumption occurred approximately 30 min prior to the onset of fatigue. Cyclists consumed either a placebo or a 50% solution of carbohydrate at a rate of 3 $g \cdot kg^{-1}$after 135 min of cycling at just below the individual's blood lactate threshold (mean 70% $\dot{V}O_{2max}$). The decline in blood glucose over the first 135 min was reversed and the time to fatigue was 21% longer for the carbohydrate trial. The authors concluded that a single carbohydrate feeding late in exercise can supply sufficient carbohydrate to restore euglycemia and increase carbohydrate oxidation, thereby delaying fatigue (Coggan and Coyle, 1989).

The main benefit of carbohydrate ingestion during exercise is thought to be due to the increased availability of blood glucose to replace that utilized by the muscle during exercise (Coyle *et al.*, 1986). Some studies have demonstrated a muscle glycogen sparing effect during intermittent cycling (Yasplekis *et al.*, 1993a) and prolonged submaximal running , but not prolonged submaximal cycling (Coyle *et al.*, 1986). It has also been demonstrated that carbohydrate ingestion allows for a higher rate of carbohydrate oxidation at a time when muscle glycogen levels are low (Coggan and Coyle, 1987). Another potential mechanism for the increased performance associated with carbohydrate ingestion is a change in brain neurotransmitter production (Walberg-Rankin, 1995). As discussed earlier, the "central fatigue hypothesis" is based on the fact that the production of serotonin in the brain is related to the amount of the precursor, tryptophan, in the blood. This is because tryptophan and branched-chain amino acids compete for entrance to the brain and the exercise-associated reduction in branched-chain can theoretically result in a greater production of serotonin, possibly resulting in early fatigue (Blomstrand *et al.*, 1991). Therefore the ingestion of carbohydrate may reduce the decline in branched-chain amino acids during exercise and delay centrally mediated fatigue.

As discussed earlier, the majority of studies have shown that consuming carbohydrate during exercise can improve capacity and performance. In contrast, Felig *et al.* (1982) reported that ingesting either 40 or 80g·h^{-1} of glucose did not significantly affect times to exhaustion when subjects cycled at 60 – 65% $\dot{V}O_{2max}$. This was despite the ingestion of glucose preventing the hypoglycaemia that was found in 37% of the subjects at exhaustion after consuming just water. Times to fatigue in subjects who consumed only water and had become hypoglycaemic during exercise were not significantly different from those who did not develop hypoglycaemia. These results led the authors to conclude that exercise can be continued in the presence of hypoglycaemia, which does not support the notion that glucose ingestion can improve performance during prolonged exercise (Felig *et al.*, 1982) and suggests that carbohydrate ingestion during exercise is only beneficial when carbohydrate stores are likely to be depleted due to the exercise.

2.5. Gastric emptying

One factor which affects the effectiveness of fluid replacement strategies is the rate of gastric emptying. Gastric emptying, or the rate at which fluid passes from the stomach into the small intestine, is considered a limiting factor in fluid replacement and is an important aspect in determining the rate at which nutrients enter the duodenum where they can be absorbed into the bloodstream (Brouns *et al.*, 1987). Various factors have been demonstrated to influence gastric emptying, including the volume of the fluid ingested, the carbohydrate content, the osmolality of the fluid and the temperature of the ingested fluid. Gastric emptying is also affected by solute acidity, exercise intensity and heat stress. Shi and Gisolfi (1998) considered that volume, carbohydrate content and osmolality were the most likely regulators of the rate of fluid delivery to the intestine.

2.5.1. Fluid volume

The mechanism by which fluid volume influences gastric emptying is related to the distension and pressure exerted on the stomach wall, which stimulate receptors in the gastric musculature altering the rate of gastric emptying (Costill and Saltin, 1974). Increasing the volume within the stomach stimulates the activity of the stretch receptors in the gastric mucosa, which increases the intragastric pressure, facilitating a faster rate of gastric emptying (Noakes *et al.*, 1991). Contractions move from the proximal to distal regions of the stomach, increasing the pressure within the antral region of the stomach. Increasing the pressure within the antral region increases the rate of gastric emptying (Struntz and Grossman, 1978). It is the total volume in the stomach that is important, which includes the volume of the fluid ingested, as well as the volume of gastric secretions and swallowed saliva (Leiper, 2001).

Studies using a single large ingestion (Costill and Saltin, 1974) or repetitive smaller ingestions (Duchman *et al.*, 1997) demonstrate that the maximum rate at which water and carbohydrate can be delivered from an ingested solution is influenced by the average

volume of fluid in the stomach, which in turn is determined by the volume ingested and the drinking pattern. The greater the initial volume of ingested fluid, or gastric volume, the greater the initial rate of gastric emptying. As the volume decreases, the absolute rate of gastric emptying decreases in proportion. Rehrer *et al.*(1989) demonstrated that the rate of gastric emptying falls exponentially with time. They also found that following a single ingestion, the rate of gastric emptying for any solution falls as a logarithmic function of the volume of fluid contained in the stomach. This observation means that during any equal period of time, a constant percentage of the fluid present in the stomach at the start of that period would have been emptied (Noakes *et al.*, 1991). Rehrer *et al.* (1989) reported that approximately 65% of water, 50% of an isotonic 7% carbohydrate solution and 25% of a 15% or 18% carbohydrate solution emptied during each successive 10-min period of exercise at 70% $\dot{V}O_{2max}$.

2.5.2. Carbohydrate content

The rate of gastric emptying decreases as the carbohydrate content of the ingested fluid increases (Costill and Saltin, 1974; Murray, 1987; Rehrer *et al.*, 1989). Studies have demonstrated an inverse relationship between the glucose concentration of the ingested fluid and the rate of gastric emptying (Brouns, 1998). Murray *et al.* (1999) found that the increase in carbohydrate content that decreases gastric emptying can be as little as 2%, when the carbohydrate concentration exceeds 6%. Even glucose concentrations of 4 to 5% produce a small but significant slowing of emptying (Costill and Saltin, 1974; Vist and Maughan, 1995). However, it is worth noting that when gastric emptying is expressed as the rate at which the energy content is emptied from the stomach carbohydrate content has no impact (Brener *et al.*, 1983). In fact, some studies (Hunt *et al.*, 1985) demonstrated an increase in substrate availability with increasing carbohydrate content.

2.5.3. Osmolality

The effect of osmolality on the rate of gastric emptying is debatable (Shi and Gisolfi, 1998). It would appear that carbohydrate concentration is more important than osmolality for influencing gastric emptying rate. The majority of studies have demonstrated little or no difference in the rates of gastric emptying of glucose or maltodextrin solutions, despite large differences in osmolality (Brouns *et al.*, 1995; Maughan, 1997). The lack of a difference may be as a consequence of the maltodextrins being hydrolysed before reaching the small intestinal osmoreceptors (Leiper, 2001). Therefore the osmolality of isoenergetic solutions are equal at the point of contact with the regulating osmoreceptors.

2.5.4. Exercise intensity

The volume ingested and the formulation of the drink are known to be the major factors on the rate of gastric emptying and intestinal absorption. It has been assumed that exercise intensity plays a relatively minor role in determining the absorption of ingested fluid in most sports (Maughan and Leiper, 1994). Maughan *et al.* (1990) demonstrated, using an isotopic water tracer method, that cycling at an intensity in excess of 40% $\dot{V}O_{2max}$ was sufficient to reduce the availability of the ingested fluid, and that this effect was proportional to the exercise intensity. However, general opinion appears to be that for euhydrated individuals exercising in a temperate environment power output at a constant level has to be more than 80% $\dot{V}O_{2max}$ before gastric emptying is slowed (Costill and Saltin, 1974). It has been assumed that during intermittent exercise, such as soccer, there is sufficient time at low-intensity exercise to allow for appropriate amounts of any ingested fluid to be emptied form the stomach and absorbed, and that the relatively short amount of time spent in high-intensity levels would not have an appreciable inhibitory effect on gastric emptying (Leiper *et al.*, 2005). However, the average intensity of an elite competitive soccer match has been reported to be around 70% $\dot{V}O_{2max}$ (Reilly *et al.*, 2000). Leiper *et al.* (2001) demonstrated that the pattern and intensity of exercise performed during an indoor 5-a-side competitive soccer match were sufficient to cause a

significant reduction in the rate of gastric emptying when compared with an equal duration of low-intensity walking. The authors concluded that it was the intermittent high-intensity sprinting that produced the slowing of gastric emptying.

2.5.5. Other factors

Other potential factors influencing the rate of gastric emptying, include fluid acidity, fluid temperature and heat stress. It has been demonstrated that when a meal contains a high concentration of acid, gastric emptying slows. This observation is considered to be as a consequence of the stimulated duodenal receptors. However, the type and concentration of the acids, such as citric acid, found in the majority of sports drinks are unlikely to affect the rate of gastric emptying (Leiper, 2001).

Gastric emptying may also be affected by the temperature of the fluid. Costill and Saltin (1974) found that the rate of gastric emptying decreased slightly as the temperature of the ingested fluid increased. In contrast, Sun *et al.* (1988) reported that hot (50°C) and cold (4°C) isosmotic solutions appeared to empty from the stomach at a slower rate than the control solution (37°C). This observation suggests there may be an optimal temperature of the ingested fluid to promote gastric emptying. More studies would be required to substantiate these findings.

Heat stress has also been shown to affect the rate of gastric emptying. A number of authors (Owen *et al.*, 1986; Neufer *et al.*, 1989) have demonstrated that in a hot environment, the rate of gastric emptying slows. Owen *et al.* (1986) measured gastric residue following 2 hours of running (65% $\dot{V}O_{2max}$) in the heat (35°C) and in moderate environment (25°C) after consuming 200 ml of water every 20 min. The gastric residue was significantly less after exercise in the cooler condition. The authors suggested that the differences may have been due to the reduced splanchnic blood flow or increased plasma β-endorphin levels.

2.6. Intestinal transport of carbohydrate

The rate at which carbohydrate is absorbed from the intestine into the blood also influences the effectiveness of carbohydrate supplementation. The time course for the appearance of sugars in the blood and subsequent oxidation is dependent on the rate at which they are absorbed from the intestinal region. Intestinal sugar transporters are responsible for transporting the monosaccharides glucose, galactose and fructose from the intestinal lumen to the blood. The intestinal transport of glucose and galactose occurs via a sodium-dependent glucose transporter (SGLT1), located in the brush-border or apical membrane (Ferraris and Diamond, 1997). Fructose is transported from the lumen to the cytosol via GLUT5, a sodium-independent facilitative fructose transporter. GLUT2 is basolateral and transports all three monosaccharides from the cytosol to the blood (Figure 2.3).

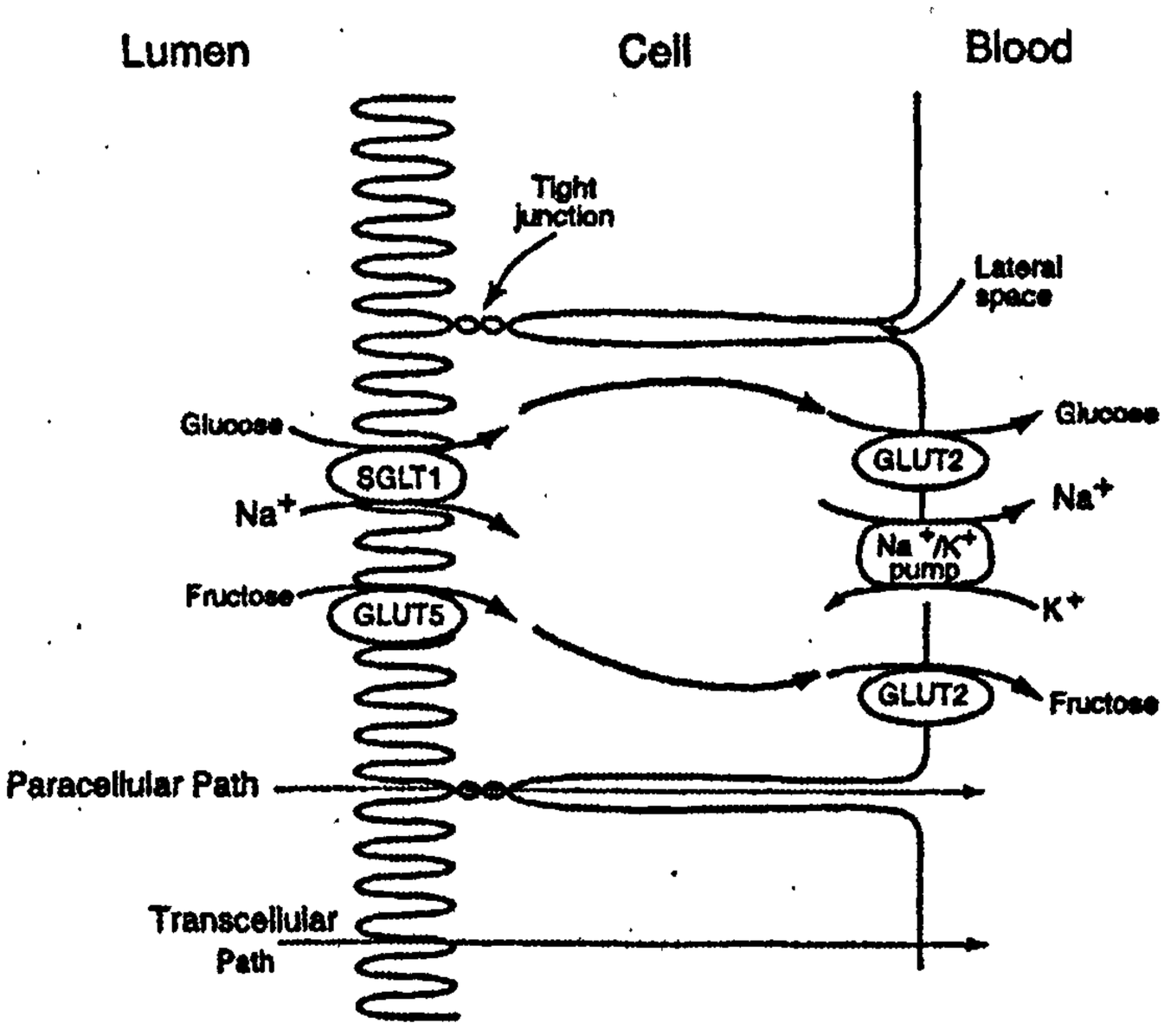

Figure 2.3: Intestinal absorption of carbohydrates (from Ferraris and Diamond, 1997).

The cotransporter SGLT1 is a high-affinity glucose transporter that is found in both the small intestine and kidney. Although transport and ligand-binding experiments suggest more than one type of sodium dependent glucose transport system, so far only SGLT1

has been identified (Ferraris, 2001). The facilitated transporter GLUT5 is a transporter which is highly stereospecific for fructose. It has also been suggested that GLUT2 is present at the brush border of normal rat intestine (Kellett and Helliwell, 2000), and therefore could mediate glucose and fructose transport.

A potential limiting factor for oxidation of exogenous carbohydrate is the rate of intestinal carbohydrate absorption (Jeukendrup *et al.*, 1999; Jentjens *et al.*, 2004b). When large amounts of glucose (>1 $g \cdot min^{-1}$) are ingested during exercise, intestinal absorption and/ or disposal by the liver may limit the rate of exogenous carbohydrate oxidation (Jeukendrup and Jentjens, 2000). It is thought that SGLT1 transporters are saturated at glucose ingestion rates exceeding 1 $g \cdot min^{-1}$, which may explain why there is not a linear relationship between glucose ingestion rates and oxidation rates. This view is supported by the fact that carbohydrate oxidation rates have not have been found to exceed approximately 1 $g \cdot min^{-1}$ despite carbohydrate in the form of glucose or glucose polymer being ingested at rates of up to 3 $g \cdot min^{-1}$ (Pirnay *et al.*, 1982; Wagenmakers *et al.*, 1993; Jeukendrup *et al.*, 1996).

As fructose is absorbed from the intestine by a GLUT5, glucose and fructose are absorbed via separate intestinal transport mechanisms. When a solution containing a mixture of glucose and fructose is ingested, there is less competition for absorption compared with an isoenergetic amount of glucose, which may increase the amount of carbohydrate entering the bloodstream and subsequent availability for oxidation. Furthermore, it has been demonstrated that fructose absorption is stimulated by the presence of glucose in a dose dependent fashion (Rumessen and Gudmandhoyer, 1986), which may contribute to the faster intestinal carbohydrate absorption rates seen when glucose and fructose are ingested simultaneously.

Jentjens *et al.* (2004b) demonstrated that during cycling at 50% maximum power output (Wmax), ingesting either an 8.7% (1.2 $g \cdot min^{-1}$) glucose drink, a 13.1% (1.8 $g \cdot min^{-1}$) glucose drink, an isoenergetic fructose plus glucose (0.6 $g \cdot min^{-1}$ fructose and 1.2 $g \cdot min^{-1}$ glucose) drink or water resulted in peak exogenous carbohydrate oxidation rates

approximately 55% higher during the fructose and glucose trial (1.26 g·min^{-1}) compared with the medium (0.80 g·min^{-1}) and high glucose (0.83 g·min^{-1}) drinks. This study also failed to demonstrate a significant difference in exogenous oxidation rates between the two glucose drinks, levelling off at 1.0-1.1 g·min^{-1}. Jentjens *et al.* (2004) suggested that the faster rate of carbohydrate absorption could have increased the availability of exogenous carbohydrate in the bloodstream, which may account for the higher exogenous carbohydrate oxidation rates when glucose and fructose were ingested, compared with a solution containing only glucose. Similar results have been reported using other combinations, such as, glucose, fructose and sucrose (Jentjens *et al.*, 2004a) and glucose and sucrose (Jentjens *et al.*, 2004c). The higher rate of exogenous carbohydrate oxidation was accompanied by a decrease in endogenous carbohydrate oxidation, suggesting that muscle glycogen utilization was reduced. A reduction in muscle glycogen utilization could delay its depletion, and possibly the onset of fatigue with an associated improvement in performance.

2.7. Exercise and the hot environment

The performance of prolonged continuous cycling (Galloway and Maughan, 1997) Parkin *et al.* (1999) and intermittent, high-intensity running (Morris *et al.*, 1998) has been shown to impaired as a consequence of high ambient temperatures (30-40°C). Parkin *et al.* (1999) reported that reduced exercise time to exhaustion during submaximal cycling in the heat (40°C) was associated with increased core and muscle temperature and a higher concentration of muscle glycogen at fatigue, suggesting that carbohydrate availability was not the cause of fatigue. Furthermore, Galloway and Maughan (1997) have shown that that the differences in the performance that occur in higher ambient temperatures (31°C compared with environment temperatures between 4°C and 21°C) are consistent with an influence of hyperthermia on the development of fatigue. Morris *et al.* (1998) demonstrated a significant reduction in the distance covered during prolonged, intermittent, high-intensity shuttle running in the heat (30°C) was as a consequence of significantly higher core temperature and heart rate in the heat compared with the trial performed in the moderate conditions. The decrement in performance was evident despite

blood glucose, lactate, NEFA and ammonia concentrations and RPE being similar between both trials.

2.7.1. Heat stress and metabolism

In general, the literature on the metabolic responses to exercise in the heat has demonstrated a shift towards increased carbohydrate and decreased fat utilization (Febbraio, 2001). Fink *et al.* (1975) were the first to demonstrate that environmental temperature affects intramuscular substrate utilization. They found that 60 min of intermittent exercise at 41°C increased muscle glycogen utilization, compared with exercise at 9°C, and this finding has since been replicated (Febbraio *et al.*, 1994b). Fink *et al.* (1975) also reported a shift in respiratory exchange ratio and a decrease in intramuscular triglyceride utilization, although not all studies have shown this change in substrate utilization (Nielsen *et al.*, 1990). It appears that during submaximal exercise in the heat, if there is a marked (>0.5°C) increase in core body temperature, intramuscular carbohydrate utilization is augmented (Febbraio, 2001). If the combined effect of exercise and heat stress does not markedly increase core temperature then it is unlikely that differences in metabolism will be observed.

There appears to be two mechanisms responsible for these metabolic changes, adrenaline concentration and muscle temperature. Exercise in the heat causes an approximately twofold increase in the level of circulating adrenaline (Hargreaves *et al.*, 1996a). Febbraio (1998) infused adrenaline into trained men exercising at 71% $\dot{V}O_{2peak}$ at 20°C to simulate the sympathoadrenal response at 40°C. The authors concluded that the increase in glycogen utilization and lactate accumulation was similar to that observed during exercise in the heat.

Muscle temperature *per se* appears to play a role in altering substrate metabolism by affecting key enzymes (Kozlowski *et al.*, 1985). It is thought that since muscle temperature can be approximately 2°C higher when exercising in the heat compared with

a cooler environment, enzyme reaction rates could be increased by as much as 30 to 40% (Febbraio, 2001). Relatively few authors have attempted to increase muscle temperature and investigate the effect on metabolism. Starkie *et al.* (1999) heated one leg whilst cooling the other for 40 min before and 20 min during exercise at 70% $\dot{V}O_{2peak}$ using water-perfused cuffs. Both regimes significantly increased muscle temperature. It was found that in addition to a higher muscle temperature in the heated leg, there was an increased rate of glycogen utilization. The authors concluded that muscle temperature *per se* was involved in the regulation of intramuscular carbohydrate utilization and was responsible, in part, for the increase in muscle glycogen utilization frequently observed during exercise in the heat.

An increase in environmental temperature increases core and muscle temperature, augmenting the exercise-induced increase. The higher core temperature and perceived effort results in a "feed-forward" increase in adrenaline secretion. This elevation in adrenaline, in addition to the increase in muscle temperature *per se*, augments muscle glycogen utilization in exercising skeletal muscle in the heat (Febbraio, 2000) (Figure 2.4).

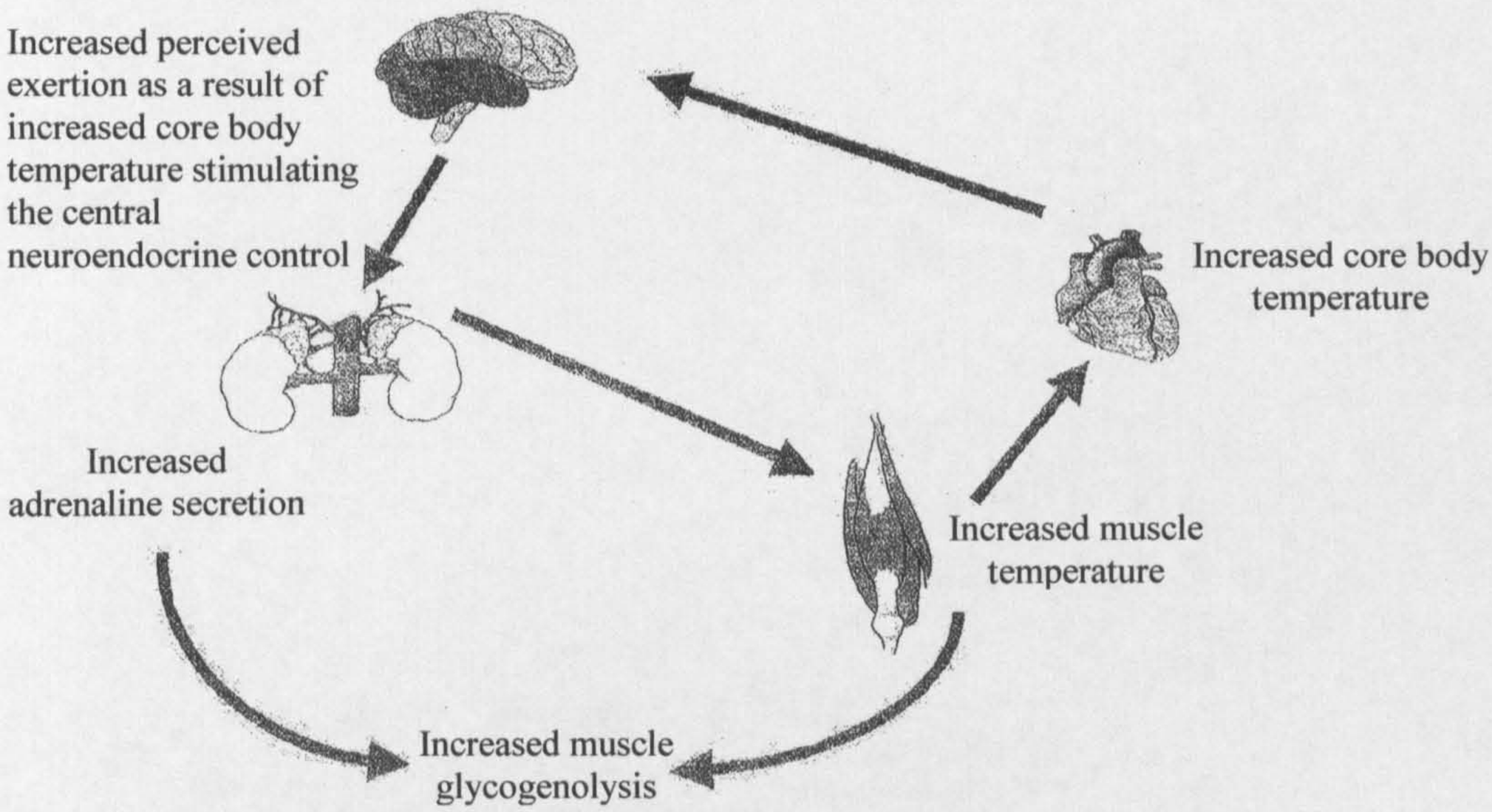

Figure 2.4: Proposed effect of increased environmental temperature on muscle glycogen utilization during exercise (from Febbraio, 2000).

Various other mechanisms have been proposed to explain the increase in carbohydrate utilization during exercise in the heat. These include reduced muscle blood flow (Gonzalez-Alonso *et al.*, 1999a), alterations in neuromuscular recruitment patterns (Sawka *et al.*, 1984) and increased adrenaline levels (Yasplekis *et al.*, 1993b). A decrease in muscle blood flow, as a consequence of increased skin blood flow, could alter metabolism due to a decrease in oxygen and substrate delivery. However, Gonzalez-Alonso *et al.* (1999b) demonstrated that even when muscle blood flow is reduced during exercise in the heat, arteriovenous oxygen difference is increased accordingly so that leg oxygen availability is not compromised. It has been hypothesised that exercise in the heat leads to a greater recruitment of fast glycolytic muscle fibres, which are more sensitive to changes in temperature than slow oxidative fibres (Sawka *et al.*, 1984; Young *et al.*, 1985). However, Febbraio *et al.* (1994a) demonstrated that during 40 min of exercise at 40°C or 20°C, there was no correlation between lactate accumulation and muscle fibre

type. It was suggested that slow oxidative fibres were preferentially recruited irrespective of environmental temperature.

2.7.2. Cardiovascular responses to exercise in the heat

The most notable effect of performing exercise in a hot environment is the increase in the amount of fluid lost (Burke, 2001). This increase in fluid loss is due to the evaporation of sweat being the main method of dissipating the heat produced by the exercising muscles and absorbed from the environment. If the fluid lost through sweating is not replaced, then dehydration occurs. Dehydration during exercise generally occurs because of the lack of sufficient fluid intake or there is a mismatch between thirst and body water requirements (Sawka *et al.*, 2001).

Compared with cooler ambient temperatures fatigue is accelerated in the presence of heat stress (Kay and Marino, 2000). The reduction in maximal aerobic power is caused by the redistribution of blood flow. In the heat, the superficial skin veins reflexively dilate to increase skin blood flow, reducing the percentage of cardiac output that perfuses exercising muscle and decreases the effective central blood volume and central venous pressure, thereby reducing venous return and cardiac output (Sawka and Montain, 2000).

Dehydration during prolonged exercise causes a reduction in total blood volume and stroke volume with a compensatory increase in heart rate (Gonzalez-Alonso *et al.*, 2000). This effect has been termed "dehydration induced cardiovascular drift" (Coyle, 1998) and due to a greater degree of dehydration, this is more pronounced when exercising in the heat. During exercise in hot ambient temperatures the rate of heat production and heat gain from the environment is disproportionate to the rate of heat dissipation, consequently leading to the development of hyperthermia. The increased requirement to dissipate heat, via evaporation, results in a loss of body water and electrolytes (Sawka, 1992). Hypovolemia has been associated with impaired cardiovascular function (Sawka, 1992),

dehydration (Gonzalez-Alonso, 1998) and further elevations in core temperature (Montain and Coyle, 1992).

The increase in skin temperature and blood flow compounds cardiovascular drift and a subsequent decrease in cardiac output and stroke volume occur (Montain and Coyle, 1992). Gonzalez-Alonso *et al.* (1997) reported that hyperthermia and dehydration independently reduced stoke volume and increased heart rate and the combined effect of dehydration hyperthermia during exercise in the heat the decline in stroke volume was greater and cardiac output declined synergistically. As a consequence there was a decrease in blood pressure impairing the dehydrated athlete's ability to cope with hyperthermia. Furthermore, the lowering of stoke volume observed with dehydration appears to be related to the increase in heart rate and decrease in blood volume (Gonzalez-Alonso *et al.*, 2000).

2.7.3. Fatigue in the heat

Another potential cause of the reduced aerobic capacity in the heat is that core temperature reaches a "critical temperature". Nielsen *et al.* (1993) observed that when exercising at 60% $\dot{V}O_{2max}$ in either 20°C or 40°C, exhaustion occurred when core temperature reached 39.7°C. The authors concluded that core temperature rather than circulatory failure is the critical factor limiting exercise capacity in the heat (Nielsen *et al.*, 1993). Gonzalez-Alonso *et al.* (1999c) reported consistent final core and thigh temperature (40.1– 40.3°C and 40.7– 40.9°C, respectively) at voluntary exhaustion in 40°C heat in trained humans, despite differences in starting core temperature, rate of heat storage, and final skin temperature. Galloway and Maughan (1997) demonstrated that during exercise in the heat (31°C), exercise capacity at 70% $\dot{V}O_{2max}$ was significantly reduced compared with lower ambient temperatures (4°C, 11°C and 21°C). Also $\dot{V}O_2$ was decreased and heart rate elevated in the hot condition. Core temperature at exhaustion in this condition was 40.1°C, suggesting that exercise capacity may have been limited by hyperthermia and not the depletion of endogenous carbohydrate stores.

In addition, it has been suggested that an elevated brain temperature may limit exercise duration (Fuller *et al.*, 1998). There is evidence that an elevated brain temperature may impair central arousal. Brain activity has been investigated in hot and cool environments, with a reduction in the β brain waves during exercise in the hot environment, increasing the ratio of α to β waves (Nielsen *et al.*, 2001). This observation is similar to what happens during sleep, so may reflect a reduced state of arousal in the hyperthermic individual (Cheung and Sleivert, 2004). An increase in core temperature has also been shown to increase resting metabolic activity in areas of the brain such as the cerebellum and hypothalamus (Nunneley *et al.*, 2002), which may lead to a reduced availability of cerebral glycogen.

Low *et al.* (2005b) reported that an elevated core temperature was the key stimulus for prolactin, which may be a marker of central serotonergic activity. An increase in serotonin (5-HT) decreases arousal, mood and motivation and increases RPE and in turn reduces voluntary activation causing fatigue and impaired exercise capacity (Cheung and Sleivert, 2004). Dopamine is another neurotransmitter that is a candidate for modulating hyperthermic fatigue, because it plays a role in the control and initiation of movement and may also reduce 5-HT production. Levels of dopamine also have been shown to increase during exercise, and a decrease in dopamine levels coincide with early fatigue (Davis and Bailey, 1997).

2.7.4. Strategies for improving exercise performance in the heat

Different strategies have been shown to be effective in reducing thermoregulatory strain and improving performance when exercising in the heat (Kay and Marino, 2000). Such interventions include acclimatization, part and whole-body pre-cooling using cold air, ice jackets or water immersion and fluid ingestion. Some of these strategies have negative aspects; heat acclimatization requires between 8 and 13 days before most adaptations are

seen (Nielsen *et al.*, 1993), and whole-body pre-cooling can be restrictive in terms of the equipment and time required to undertake the procedure (Marino and Booth, 1998). In contrast, fluid replacement is an easily implemented for improving the safety, health and performance of individuals undertaking exercise in the heat (Kay and Marino, 2000).

2.7.5. Fluid replacement strategies

Fluid intake has been shown to attenuate or prevent many of the metabolic, thermoregulatory and performance disturbances associated with exercise by reducing the degree of dehydration (Walsh *et al.*, 1994; Below *et al.*, 1995; Hargreaves *et al.*, 1996b). A number of authors (Below *et al.*, 1995; Hargreaves *et al.*, 1996b; Galloway and Maughan, 2000) have investigated the effect of fluid ingestion strategies on the thermoregulatory, cardiovascular and metabolic responses to exercise in a hot environment and the subsequent effect on performance. The majority of studies demonstrating that carbohydrate supplementation or water ingestion can delay the onset of fatigue have been conducted in neutral environments (~20°C). Ingesting water has been shown to attenuate the increase in core temperature and prevent the decline in stroke volume and cardiac output during prolonged exercise in moderate ambient temperatures (20-22°C) (Hamilton *et al.*, 1991b; Montain and Coyle, 1992). Also, when fluid is ingested in volumes such that body mass losses are replaced, muscle glycogen utilization is attenuated (Hargreaves *et al.*, 1996b). Coyle and Hamilton (1990) attributed the delay in the onset of fatigue in hot environments to the cardiovascular and thermoregulatory benefits of water provision during exercise. In addition, relatively few research groups have examined the effect of fluid provision in hot ambient temperatures (~30°C).

Below *et al.* (1995) examined the effect of ingesting water, carbohydrate or a combination on the cardiovascular and thermoregulatory responses to 50 min of exercise at 80% $\dot{V}O_{2max}$, followed by a time-trial in the heat (31.2°C). The ingestion of water resulted in a 6% improvement in time-trial performance; carbohydrate ingestion also

improved cycling performance by 6% and the combination of the two strategies enhanced performance by 12% (Figure 2.5). Therefore, the effects were independent and additive (Below *et al.*, 1995). This study also demonstrated that ingesting a larger volume (1.3 l, 79% of fluid losses) improved performance more effectively than a smaller volume (0.2 l, 13% of fluid losses).

Placebo 11.34±0.32 min	**Fluid** 10.51±0.27 min	**No carbohydrate** 10.92±0.32 min
Carbohydrate 10.55±0.29 min	**Fluid + Carbohydrate** 9.93±0.28 min	**Carbohydrate†** 10.23±0.28 min
Small fluid replacement 10.93±0.32 min	**Large fluid replacement*** 10.22±0.27 min	

Figure 2.5: Time trial performance in the heat (adapted from Below *et al.*, 1995). *Large volume quicker than small volume, †carbohydrate quicker than no carbohydrate ($P<0.05$).

Despite the differences in exercise performance, no difference in heart rate or core temperature was observed between the two carbohydrate trials when compared with the 'no carbohydrate' trial. However, core temperature was 0.33±0.04°C lower and heart rate 4±1 beats.min^{-1} lower during the 'large volume' trial compared with the 'small volume' trial. These results suggest that fluid volume, and not carbohydrate content is more important for reducing core temperature and heart rate during exercise when carbohydrate is consumed.

Galloway and Maughan (2000) investigated the effect of ingesting fluid containing different concentrations of carbohydrate on exercise capacity performed in a hot environment (30°C). The ingestion of either a dilute (2%) carbohydrate-electrolyte solution that replaced 150% of fluid losses or a solution with a high carbohydrate content (15%) that replaced 100% of fluid losses was compared with no fluid ingestion. Ingesting the carbohydrate-electrolyte solution significantly increased time to exhaustion when compared with no fluid (70.9 min). However, time to exhaustion during the trial with 2% carbohydrate solution (118.0 min) was significantly longer than during the 15% carbohydrate trial (84.0 min). No differences were observed in the thermoregulatory and cardiorespiratory responses between trials, although there was a tendency for a lower heart rate during exercise after ingesting the 2% carbohydrate solution. The study also demonstrated no differences in substrate oxidation rates, suggesting that carbohydrate or fluid provision had no impact on substrate oxidation and, in particular muscle glycogen utilization (Galloway and Maughan, 2000). These results show that fluid replacement with a large volume of dilute carbohydrate solution is effective during exercise in the heat, supporting the findings of Below *et al.* (1995), but the precise mechanisms for the improved exercise capacity are unclear.

There is clear evidence that both water and carbohydrate can improve exercise performance in the heat when compared with no fluid ingestion. Furthermore, the ingestion of carbohydrate solutions may further improve performance. It appears that ingesting a large volume of a dilute carbohydrate solution, which is primarily aimed at rapid fluid replacement, is more effective at delaying the onset of fatigue than a smaller volume of a more concentrated carbohydrate solution.

2.7.6. Pre-cooling

It is well documented that heat stress, as a consequence of an elevated core body temperature, is a major cause of reduced exercise performance and heat-related illnesses

(Gonzalez-Alonso *et al.*, 1999c). The question is whether a reduction in body temperature to delay reaching a critical value would benefit exercise performance.

The principle of the pre-cooling strategy is to reduce core body temperature prior to performing exercise. This creates a " heat sink", thereby increasing the margin for metabolic heat production, and so offsetting the time before a critical limiting temperature is attained when a given exercise intensity can no longer be maintained is reached (Nielsen *et al.*, 1993).

2.7.7. Pre-cooling and exercise performance

A major problem in evaluating the effectiveness of pre-cooling on exercise performance is the type of exercise and pre-cooling strategy protocol used. Various pre-cooling methods, such as cold air, water immersion, water perfused suits and ice jackets have been employed prior to performing exercise protocols in different environmental conditions (Table 2.2). These variations, including different protocols may explain the inconclusive results regarding the effects of pre-cooling on exercise performance.

Table 2.2: Summary of pre-cooling studies, method and results.

Author	Pre-cooling method	Exercise protocol	Ambient Conditions	Result
Arngrimsson *et al.* (2004)	Cooling vest	5 km run	32°C 50 *rh*	Significantly quicker (13 s)
Bergh and Ekblom (1979)	Water immersion (13-15°C)	Arm and leg exercise to exhaustion	20-22°C	Reduced physical performance
Booth *et al.* (1997)	Water immersion (23-24°C)	30 min self-paced treadmill running	31.6°C, 60% *rh*	Distance increased by 4% (304 m)
Booth *et al.* (2001)	Water immersion (24°C)	35 min cycling (65% $\dot{V}O_{2\,peak}$)	34.9°C 46.4 *rh*	Limited effect on muscle metabolism
Cotter *et al.* (2001)	Ice vest, with/ without thigh cooling and 3°C air	20 min cycling (65% $\dot{V}O_{2\,peak}$) and 15 min performance	33°C	Reduced physiological and psychophysical strain and increased endurance performance
Drust *et al.* (2000)	60 min cold shower (26°C)	90 min soccer-specific protocol	20.5°C 71.6 *rh*	No benefit on the physiological responses
Duffield *et al.* (2003)	Ice cooling jacket (5 min before, during rest periods, 2 * 5i, 1 * 10 min)	80 min intermittent, repeated sprint cycling exercise	30°C, 60% *rh*	No significant difference in work done
Gonzalez-Alonso *et al.* (1999)	30 min water immersion	Cycling to exhaustion (60% $\dot{V}O_{2\,max}$)	40°C 19% *rh*	Increased performance time and termination temperature identical
Hessemer *et al.* (1984)	Double cold air exposure (0°C)	60 min work-rate test	18°C	6.8% increase in work-rate
Kay *et al.* (1999)	Water immersion (24°C)	Cycle time trial (30 min)	31.4°C 60.2 *rh*	Decreased distance (-0.9 km) and increased heat storage
Kruk *et al.* (1990)	Cold air (5°C)	Cycling at 50% $\dot{V}O_{2\,max}$ (30 min)	5°C	Reduced exercise capacity
Marsh and Sleivert (1999)	30 min water immersion	70 second cycling power test	29°C 80 *rh*	Mean power output increased by 2.7%

Previous research has indicated that reducing core temperature before exercise can be beneficial for the performance of endurance exercise (Lee and Haymes, 1995; Booth *et al.*, 1997; Cotter *et al.*, 2001; White *et al.*, 2003; Arngrimsson *et al.*, 2004), although

others have demonstrated a negative effect (Kruk *et al.*, 1990; Kay *et al.*, 1999). However, limited research has been conducted using high-intensity exercise (Marsh and Sleivert, 1999; Cotter *et al.*, 2001; Sleivert *et al.*, 2001) or intermittent exercise (Drust *et al.*, 2000a; Duffield *et al.*, 2003).

Lee and Haymes (1995) used a protocol where subjects ran to exhaustion at 82% $\dot{V}O_{2max}$. After cold air pre-cooling, exercise duration increased from 22 to 26 min, a 16% increase. The effect of pre-cooling on the performance of endurance exercise in the heat (32°C) has also been assessed by Booth *et al.* (1997). Subjects performed a 30-min time-trial on a treadmill. After pre-cooling, the distance covered increased from 7250 m to 7550 m, an improvement of 4%. When the running speeds were analysed, after pre-cooling the subjects were capable of increasing their speed towards the end of the trial, whereas in the control condition, at best, speed could only be maintained. These results signify that the benefit of pre-cooling may be that the athlete is able to draw on reserves later in the performance, rather than just being able to maintain a given speed or intensity (Marino, 2002).

The effect of pre-cooling on the performance of intense exercise is equivocal. Bergh and Ekblom (1979) used a combined leg and arm exercise protocol designed to exhaust subjects within 8 minutes. After pre-cooling, work time was significantly reduced from 6.24 min to 4.36 and 3.06 min when core temperature was reduced to 35.8°C and 34.9°C respectively. The authors speculated that the cause of reduced exercise duration was that the lowered muscle temperature impaired anaerobic power as a consequence of depressed enzyme activity. Although this study demonstrated that pre-cooling may impair the performance of high-intensity exercise, the exercise protocol has limited practical applications to actual exercise performance. In contrast, Marsh and Sleivert (1999) demonstrated that in warm conditions (29°C), pre-cooling could improve the performance of high-intensity cycling over 70 s. It was shown that up to 30 minutes of pre-cooling could improve performance by approximately 3.3%. The authors suggested that the increase in performance was due to the cold-induced vasoconstriction of the skin increasing central blood volume, possibly increasing the flow of blood to the muscle, and

metabolite removal allowing for a higher exercise intensity to be maintained. This hypothesis relies on muscle blood flow being a limiting factor during exercise, which may not be the case. Nielsen *et al.* (1993) demonstrated that fatigue occurs, even when muscle blood flow is not reduced. Given these contradictory findings it is difficult to ascertain by what mechanism pre-cooling could enhance short-duration, high-intensity exercise (Marnio, 2002).

Pre-cooling has also been used as a technique before soccer-specific exercise. Drust *et al.* (2000) decreased core temperature by 0.6°C using a cold shower. There were no significant differences in the measured physiological variables. The authors concluded that there are no significant beneficial effects of pre-cooling on the physiological responses to soccer-specific exercise performed under normal environmental conditions. However, this protocol was performed in warm environmental conditions (26°C), so there may be benefits when performing in the heat.

The results from previous studies appear to suggest that pre-cooling is only beneficial for endurance exercise of a duration between 30 and 40 minutes, rather than intermittent or short duration exercise (Marino, 2002). Further research is required using more ecologically valid performance protocols before firm conclusions can be made regarding the benefits of pre-cooling on exercise performance.

2.7.8. Pre-cooling and metabolism

It has been established that pre-cooling can improve endurance exercise (60-80% $\dot{V}O_{2max}$) as a result of reduced thermoregulatory and cardiovascular strain (Booth *et al.*, 1997; Gonzalez-Alonso *et al.*, 1999c). There is also a link between hyperthermia and muscle metabolism. Metabolic disturbances are thought to be involved in the fatigue process. For example, exercise in the heat has been demonstrated to increase the rate of muscle glycogen utilization (Febbraio *et al.*, 1994b) and an associated reduction in lipid oxidation (Fink *et al.*, 1975). Other areas include the acceleration of muscle glycolysis,

PCr hydrolysis and ATP degradation (Kozlowski *et al.*, 1985). It has also been demonstrated that reducing the increase in core temperature the metabolic changes appear less pronounced (Fink *et al.*, 1975; Febbraio *et al.*, 1994b). However, to date, the metabolic responses to exercise following pre-cooling have not been widely investigated.

Booth *et al.* (2001) observed that whole-body pre-cooling did not significantly affect muscle metabolism during sub-maximal exercise performed in the heat. Muscle glycogen, triglyceride, ATP, PCr or lactate concentration at rest, or following 35 min of cycling at 60% $\dot{V}O_{2peak}$ at 35°C were unaffected by pre-cooling. The authors concluded that cardiovascular, rather than metabolic factors were the most likely mechanism for performance enhancement following whole-body pre-cooling. They also suggested that muscle temperature may need to exceed a critical level before muscle energy metabolism is altered significantly to impact on exercise performance (Booth *et al.*, 2001). In this study, the resting muscle glycogen concentrations before exercise were not similar, making the findings difficult to interpret.

2.7.9. The placebo effect of pre-cooling

There is the potential for pre-cooling to produce a placebo effect, caused by the subject's expectation that pre-cooling will improve performance, as it is impossible to blind the subject to temperature. This possibly may partially explain the improvements observed in the performance of short duration exercise, because core body temperature does not increase sufficiently to become a limiting factor. In one study (Yates *et al.*, 1996), subjects ingested a coloured water and were informed that it may offer a benefit in performance. Another possible method would be to inform the subjects that the experiment was designed to test if the lower core temperature improved performance, or the lower muscle temperature resulted in decreased performance.

2.7.10. Pre-cooling and hydration strategies

Pre-cooling has not been reported in conjunction with hydration strategies. This combination is of interest because a pre-cooling strategy, using a cooling vest is easily implemented and could be used by soccer players (and other athletes) prior to matches to reduce thermoregulatory strain and carbohydrate utilization, and potentially improve performance.

2.8. Soccer activity

Soccer is a team sport that that has been physiologically described as intermittent high-intensity exercise (Coyle, 1993). As stated previously, soccer players perform a wide range of different activities ranging from walking to maximal running, and so the intensity of effort alternates frequently. For a player to be successful there is a need to be able to perform prolonged intermittent exercise (endurance), high-intensity exercise and have the ability to sprint and develop high power output (force) for elements such as kicking and tackling. In a study of Danish elite soccer players, Bangsbo *et al.* (1991) found that standing represented 17.1% of the total playing time, 40.4% of the time was spent walking, and low-intensity activities (jogging, low-speed running and backwards running) accounted for 35.1%, moderate-speed running for 5.3%, high-speed running for 2.1% and sprinting for 0.7% (Figure 2.6). However, less than 2% of the total distance covered is in possession of the ball (Reilly, 1997), the majority of the activity is "off-the-ball".

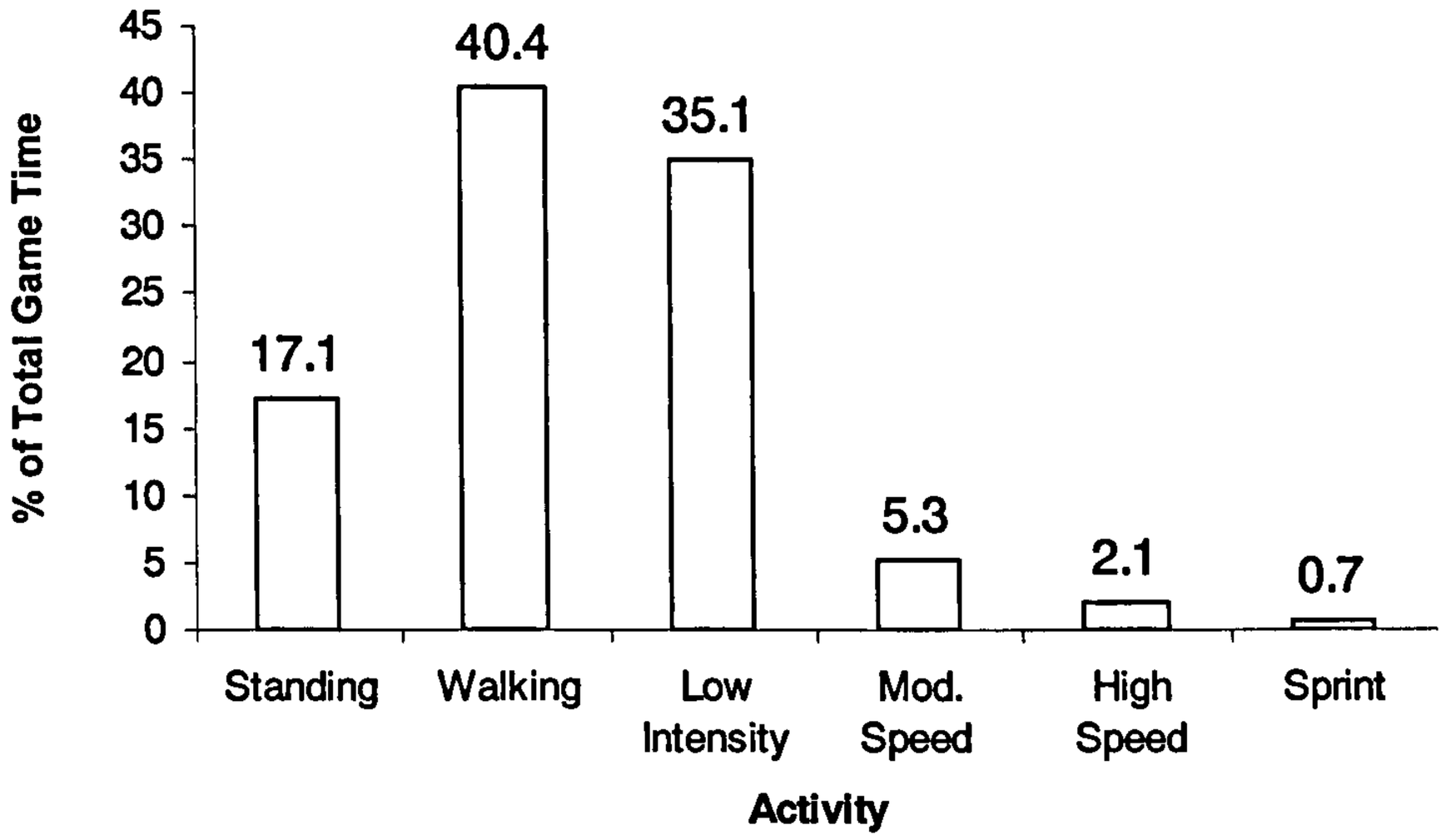

Figure 2.6: Activity profile for male elite players during Danish League soccer matches, expressed in relation to total playing time (from Bangsbo *et al.*, 1991).

Bangsbo *et al.* (1991) reported that Danish elite players had 1179 changes in playing activities during a match with each activity lasting for a mean duration of 4.5 seconds. These values are similar to the findings of Reilly and Thomas (1976) for English First Division players, where there were approximately 1000 changes per match, with each activity lasting for between 5 and 6 seconds. The greater number of changes in the Danish study was partly due to a larger number of exercise categories in the data analysis (Bangsbo, 1994b).

During a competitive soccer match, players typically cover between 10 and 12 km, with midfielders covering slightly more distance than the other playing positions (Withers *et al.*, 1982; Van Gool *et al.*, 1988; Bangsbo *et al.*, 1991; Reilly, 1994a). Within this value, the distance covered at high-intensity is relatively constant between a number of studies, Reilly and Thomas (1976) found that English professional players covered a distance of 2.8 km of high-intensity running, which is similar to the 2.2 km found by Withers *et al.* (1982) analysing Australian soccer players, and the 2.1 km found in elite Danish players

(Bangsbo *et al.*, 1991). In contrast, in a study of Swedish players (Ekblom, 1986) the total distance of high-intensity activity was only 0.8 km, possibly due to only two categories being recorded (Bangsbo, 1994b). With the exception of Ekblom (1986), these values are considerably higher than those found in an earlier study by Winterbottom (1960), who estimated that players covered a distance of 3361m, which comprised of 2347 m walking and jogging and 1015 m speed running. There is a number of possible explanations for these differences, the first being the development of soccer through changes in tactics and systems of play, and improvements in the physical capacities of the players. Also recording methods and player position would have affected the results.

The distance that a player covers is not the only activity that is performed during a soccer match. Other activities that use energy but do not require any significant distance moved include heading, jumping, tackling, getting up, shooting and passing. Ekblom (1986) found that the mean number of tackles per game for Swedish soccer players was 13.1 with 9.9 headers. In addition to these observations, English soccer players jumped on average 15.5 times (Reilly and Thomas, 1976). Activities such as accelerating and changing pace and direction also involve a large amount of energy utilization. Players may accelerate from a stationary position between 40 and 62 times a game (Smodlaka, 1978) and perform about 1000 changes in movement during a match, for example, change direction, with a change of activity occurring approximately 6 s (Yamanaka *et al.*, 1988). A more recent study has indicated about 1500 changes in movement occur, with a change of activity occurring every 4 s (Rienzi *et al.*, 2000). These differences may be due to a change in tactics and the nature of soccer. In addition, it has been shown that dribbling a soccer ball significantly increases energy expenditure (Reilly and Ball, 1984).

2.9. Energy sources during a soccer match

Due to the intermittent nature of activity in soccer, players need to perform prolonged intermittent exercise (endurance), where energy is primarily derived aerobically from carbohydrates and lipids. In addition a player needs to be able to perform high-intensity

exercise such as sprinting and develop high power output for activities such as kicking and tackling. Total energy expenditure during a match is thought to be 5-6 MJ (Shephard, 1992). Soccer is predominantly an aerobic sport with the majority of energy being derived from muscle glycogen (Figure 2.7).

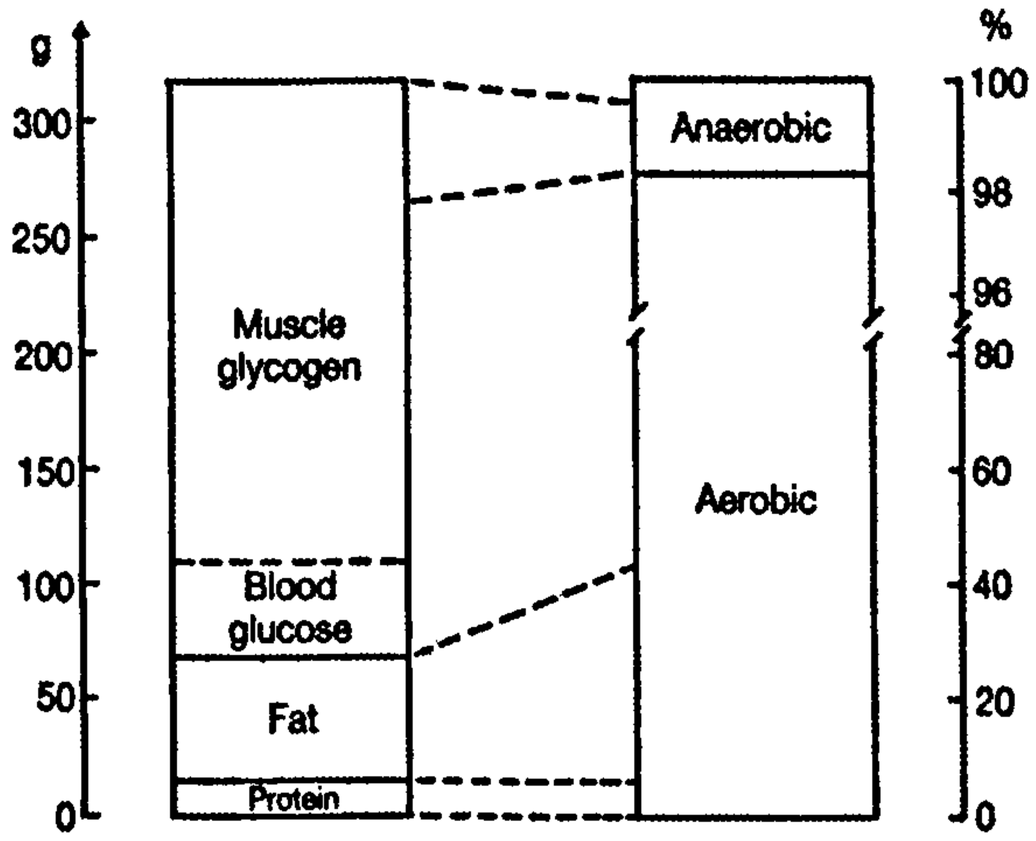

Figure 2.7: Energy provision during soccer (from Bangsbo, 1994a).

2.9.1. Anaerobic energy production

Bangsbo *et al.* (1991) reported that during a soccer match an elite male player performs around 7 minutes of high-intensity exercise, which includes about 19 sprints lasting on average 2.0 seconds. To meet the energy demands of these activities, anaerobic energy production is required. The degradation of stored ATP and PCr provides a considerable amount of the energy required during periods of high-intensity exercise during a match (Bangsbo, 1994b). Phosphocreatine is used for rapid resynthesis of ATP and as this reaction is freely reversible and PCr is rapidly resynthesized during periods of rest or low-intensity exercise. Therefore, due to the intermittent nature of a soccer match the PCr concentration varies during the course of the game when the net utilization of PCr is quantitatively small. Figure 2.8 shows that during periods of high-intensity exercise the concentration of PCr as determined by nuclear magnetic resonance (NMR) during isometric contractions with the calf muscles, decreases (Bangsbo, 1994a).

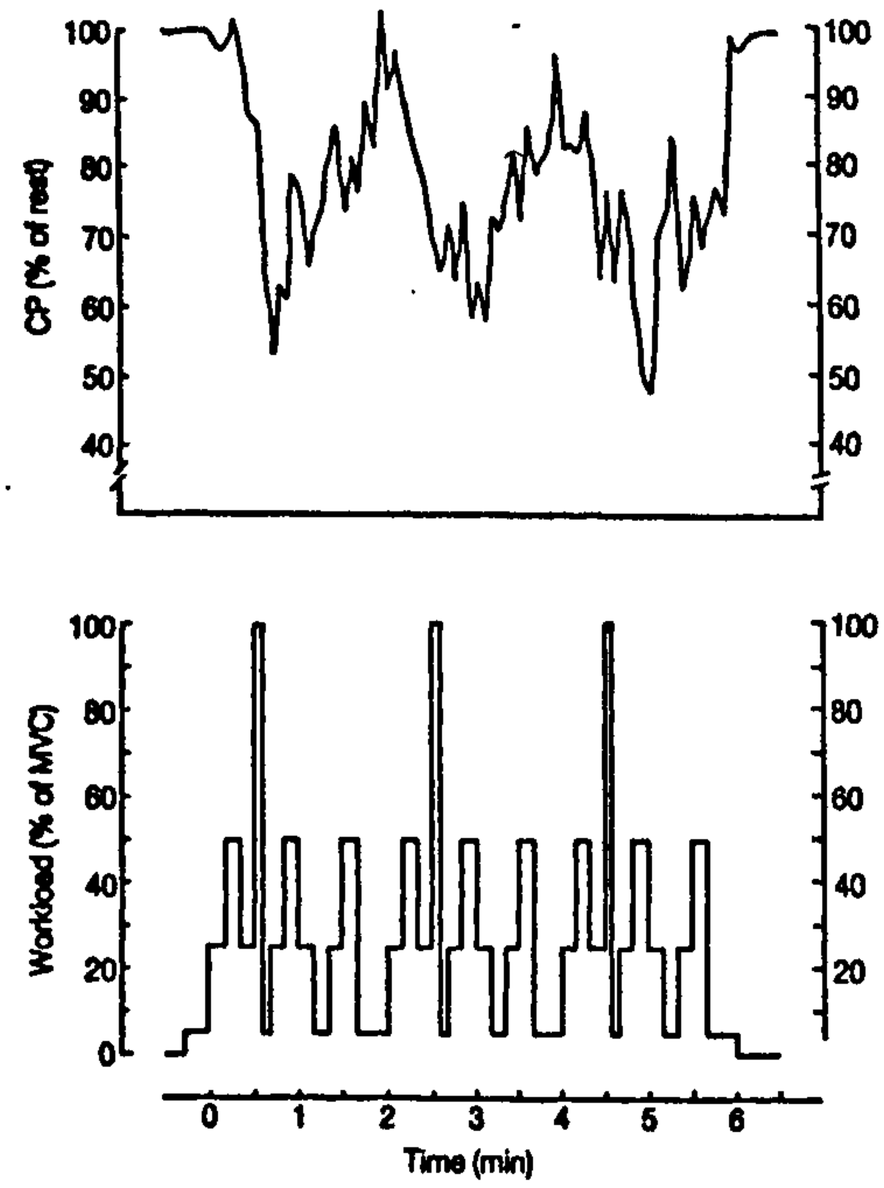

Figure 2.8: Phosphocreatine concentration in the gastrocnemius (upper panel) at alternating workloads (lower panel) (from Bangsbo, 1994a).

Bangsbo (1994b) stated that the lactate producing system probably contributed less than 10% to the total energy production. Nevertheless, anaerobic energy production is important during a soccer match as it necessary to be able to produce energy at a high rate to meet the demands of the periods of intense activity associated with soccer. The contribution of anaerobic energy production is often determined through measurements of blood lactate during match-play. Values have been reported to range from 2.4 $mmol \cdot l^{-1}$ post-match (Carli *et al.*, 1986) to values greater than 12 $mmol \cdot l^{-1}$ Ekblom (1986).

The concentration of lactate tends to be lower in the second half compared with the first. This is probably due to a reduction in high-intensity running and total distance covered in the second half with a shift from carbohydrate to fat utilization (Bangsbo, 1994b). It is also worth noting that blood lactate measurements may underestimate lactate production

since not all the lactate produced in the muscle appears in the blood as it is taken up by other tissues (Hermansen and Stensvold, 1972). Therefore, the anaerobic energy turnover during an entire soccer match cannot be established from single blood lactate measurements taken before, during and after the match (Bangsbo, 1994a).

2.9.2. Aerobic energy production

As discussed earlier, soccer is predominantly an aerobic sport with the proportion of time spent in aerobic and anaerobic activities being approximately 88% and 12%, respectively (Mayhew and Wenger, 1985). Therefore, the majority of a match is spent in lower-intensity activities, such as jogging and walking. Direct measures of muscle glycogen utilization suggest that glycogen usage amounts to about 155-160 g, providing approximately 2.5 MJ of energy (Shephard, 1999). Plasma glucose also provides a source of energy, suggesting that a combination of gluconeogenesis and the release of glucose from the liver could provide 900 kJ of energy (Shephard, 1999).

It had been observed that NEFA concentration increases throughout a soccer match, especially the second half (Bangsbo, 1994b). This occurrence is possibly a consequence of the more frequent low-intensity exercise observed during the second half of soccer matches. In contrast there is only a minor increase in glycerol levels (Bangsbo, 1994b), suggesting a high uptake of glycerol in tissues such as the liver. Based on the heart rates observed during laboratory-based studies, as much as 40% of the total energy needs can be met from the oxidation of NEFA (1.9 – 2.3 MJ) (Bangsbo, 1994a). This finding would suggest that there is likely to be utilization of fatty acids derived from adipose tissue and intramuscular triglyceride reserves. However, the degradation of carbohydrates, especially muscle glycogen, provides the majority of energy during a soccer match (Hargreaves, 1994).

Protein metabolism may also provide a small proportion of the energy required, Wagenmakers *et al.* (1989) demonstrated that during continuous exercise at a mean work-rate and duration equivalent to soccer less than 10% of the energy required was derived from the oxidation of protein.

2.10. Fatigue in soccer

The causes and impact of fatigue in soccer performance has been studied extensively. The distance covered in the second half of a match tends to be less than that during the first half (Reilly and Thomas, 1976; Bangsbo *et al.*, 1991; Bangsbo, 1994b) and is a manifestation of fatigue (Reilly, 1997). Van Gool *et al.* (1988) reported that Belgium university players, on average, covered 444 m less in the second half compared with the first. Similar findings have been replicated with professional players, Bangsbo *et al.* (1991) found that a 5% greater distance was covered in the first half. It has also been shown that the amount of high-intensity exercise declines towards the end of a match (Reilly and Thomas, 1976; Bangsbo *et al.*, 1991). Rahnama *et al.* (2003) demonstrated after exercise simulating the work-rate of competitive soccer the capacity of the knee extensor and flexor muscles to develop force was reduced. These findings suggest that performance in the second half is impaired and fatigue occurs towards the end of a match.

One of the most probable causes for the decline in work-rate is reduced muscle glycogen content. Saltin (1973) filmed Swedish club players and found that players with low glycogen content in the *vastus laterlis* muscle covered 25% less distance than the other players. A greater effect was observed in the running speed, those players with low pre-match glycogen stores covered 50% of the total distance walking and 15% sprinting, in contrast the players with high concentrations covered 27% walking and 24% sprinting. These findings suggest that the pre-match glycogen content has an important protective function against fatigue. Other potential causes include physiological changes in the muscle cell such as accumulation of hydrogen ions, lactate and ammonia and potassium imbalance (Bangsbo, 1994a), dehydration and hyperthermia as players can lose more than 3 litres of fluid during a match (Bangsbo, 1994b) and core temperature often exceeds

39.5°C (Ekblom, 1986; Mohr *et al.*, 2004). However, it has proved difficult to identify any single factor (hydrogen ions, lactate, potassium imbalance, ammonia or glycogen depletion) or precise combination of factors that would completely explain the causes of fatigue (Reilly, 1997), although it appears that hypoglycaemia is not a cause as blood glucose concentration does not reach critical values during a match (Bangsbo, 1994b).

During a match a player's physical performance appears to be reduced after a period of the match with a large amount of high-intensity exercise (Mohr *et al.*, 2003), suggesting that fatigue occurs both temporarily during a match following intense periods of activity and towards the end of the match. Mohr *et al.* (2003) found that in the 5-min period immediately after the most intense 5-min period recorded during the game, the amount of high-intensity running was significantly lower than the average of the entire game. It is possible that this was caused by the natural fluctuations in the intensity of the match as a consequence of tactical or psychological factors (Mohr *et al.*, 2005). However, Krustrup *et al.* (2003) demonstrated that after periods of intense exercise during the first half, sprint performance was reduced, although at half-time the ability to perform repeated sprints was unaffected. These results together indicate that players can experience periods of temporary fatigue during a match.

A potential cause for the temporary fatigue may be a disruption in muscle ion homeostasis (Mohr *et al.*, 2005) and not an accumulation of muscle lactate, high muscle acidosis or low PCr concentration in the muscle. It has been suggested that fatigue during high-intensity exercise may be due to an accumulation of potassium in the muscle interstitium (Nordsborg *et al.*, 2003). This view is based on the observations that during high-intensity exercise, at the point of exhaustion, the interstitial potassium concentration is elevated to around 12 $mmol.l^{-1}$ (Nordsborg *et al.*, 2003), which may be high enough to depolarize the muscle membrane potential, reducing force development (Cairns and Dulhunty, 1995). However, little is known about the turnover of potassium in the muscle during intermittent activity such as soccer, and so further research is required to substantiate these suggestions.

2.11. Soccer and environmental temperature

The environmental conditions can also influence the work-rate profile of players during a match. When matches are played in cool conditions with low ambient temperatures, muscle performance can be suboptimal (Shephard, 1999) and the risk of injury increased (Reilly, 1994b). Countermeasures include performing an extended warm-up and wearing extra layers of clothing, although this could reduce mobility.

In contrast, many major soccer tournaments are held during the summer months where ambient temperatures can reach 30-35°C (FIFA World Cup 2002 and UEFA Euro 2004), possibly causing hyperthermia. For example, 34 cases of heat exhaustion occurred in a 2-day youth soccer tournament played in ambient temperatures >30°C in the USA (Kirkendall, 1993). A high ambient temperature in combination with high humidity will affect work-rate due to an increase in core temperature, dehydration and an inability to dissipate heat through sweat production (Reilly, 1994b). Core temperatures in excess of 40°C have been reported during matches played in hot conditions (Ekblom, 1986). Playing in hot environment (30-38°C), compared with cool conditions (10-15°C) has been shown to increase sweat loss from 1.5-2.0 litres to 3.5-4.0 litres (Bangsbo, 1994b).

As a consequence playing in a hot environment physical performance during match-play may be impaired, possibly due to more rapid muscle glycogen depletion and an earlier onset of fatigue. Rico-Sanz *et al.* (1996) demonstrated that the performance of a soccer-specific test was significantly reduced following a match in warm environment (25.3°C). It has also been reported that performing in the heat, combined with high humidity can result in sprinting distance being reduced by approximately 50% when compared to performance at 20°C, 500 m and 900 m respectively (Ohashi *et al.*, 1988) and that this reduction is especially evident in the second half (Ekblom, 1986). The impaired performance is probably due to factors such as elevated core temperature, dehydration and ineffective sweat production.

In order to combat the effects of a high temperature on physical performance, adequate hydration strategies before, during and after a match are essential (Rico-Sanz *et al.*, 1996). Other strategies include allowing sufficient time for acclimatisation to the hotter environmental temperatures, especially when playing in major tournaments and reducing the length of the warm-up. In these conditions, pre-cooling the body prior to exercise has not been explored.

2.12. Soccer simulations

Due to the acyclic and unpredictable nature of activity in soccer combined with the lack of control of pattern and exercise intensity during a game, it is difficult to asses the benefits of interventions in a "real match" situation. As a consequence of this problem, sports physiologists have been reluctant to examine soccer due to the relative lack of experimental models to simulate soccer matches in a laboratory. However, a number of laboratory tests have been developed, which simulate the activity pattern and work-load associated with competitive match-play (Drust *et al.*, 2000a; Drust *et al.*, 2000b; Nicholas *et al.*, 2000). Laboratory tests are performed in a controlled environment to reduce the impact of external variables (MacDougall and Wenger, 1991). This type of protocol has many research applications, such as evaluating the impact of nutritional interventions and has be used to assess the effect of ingesting carbohydrate-electrolyte solutions on endurance running (Nicholas *et al.*, 1995), muscle glycogen utilization (Nicholas *et al.*, 1999), recovery of intermittent endurance running capacity (Nicholas *et al.*, 1997), soccer skill (McGregor *et al.*, 1999), muscle function (Gleeson *et al.*, 1998), muscle fatigue (Rahnama *et al.*, 2003) and the impact of pre-cooling on physiological responses (Drust *et al.*, 2000a).

2.13. Carbohydrate solution ingestion and soccer

As discussed earlier, two of the probable causes of fatigue during a soccer match are energy depletion in the form of muscle glycogen, and dehydration. The ingestion of

carbohydrate-electrolyte solutions, or sports drinks, has been shown to spare muscle glycogen during exercise and delay the onset of fatigue, with some studies showing performance improvements. Studies of the effect of carbohydrate ingestion and soccer performance have been done during actual match-play (Leatt and Jacobs, 1989; Zeederberg *et al.*, 1996; Guerra *et al.*, 2003) or soccer-specific exercise simulating the work load of a competitive match in a laboratory in a variety of conditions (Nicholas *et al.*, 1995; McGregor *et al.*, 1999; Welsh *et al.*, 2002; Morris *et al.*, 2003) (Table 2.3).

Table 2.3: Effect of carbohydrate ingestion on soccer-specific situations.

Authors	Solution	Situation	Effect of carbohydrate
Guerra *et al.* (2003)	6% carbohydrate-electrolyte at 15 min intervals or no fluid	Match-play	Greater amount of time running and number of sprints
Leatt and Jacobs (1989)	7% glucose-polymer or flavoured water before match and at half-time	Match-play	Not measured
McGregor *et al.* (1999)	Flavoured water before and at 15 min intervals or no fluid	90 min intermittent exercise protocol (LIST)	Quicker 15 m sprint times towards the end and maintained skill performance
Morris *et al.* (2003)	6.5% carbohydrate-electrolyte, flavoured water or no fluid before and during every exercise set and rest period (19 min)	30°C, 75 min intermittent exercise protocol (LIST) followed by 60 s run/ 60 s rest until exhaustion	No impact on distance covered or 15 m sprint times
Nicholas *et al.* (1995)	6.9% carbohydrate-electrolyte or flavoured water before and every 15 min	75 min intermittent exercise protocol (LIST) followed by run to exhaustion	No effect on 15 m sprint times. Significant longer run to exhaustion
Welsh *et al.* (2002)	Carbohydrate or flavoured water before, the end of each quarter and at half-time	60 min intermittent high-intensity shuttle running with physical and mental function tests	Longer time to fatigue, faster sprint times, improved motor skill test performance
Zeederberg *et al.* (1996)	6.9% glucose-polymer or flavoured water before match and at half-time	Match-play	No measurable benefits on motor skill proficiency

Note: LIST – Loughborough intermittent shuttle test.

Zeederberg *et al.* (1996) investigated the effect of ingesting a glucose-polymer solution before a match and at half-time. There were no measurable benefits of glucose-polymer

ingestion for the motor skill (such as tackling, controlling, passing, dribbling, heading and shooting) proficiencies of soccer players during games played in a cool environment. In contrast Guerra *et al.* (2003) reported that ingesting a carbohydrate-electrolyte at regular intervals compared with no fluid ingestion resulted in a greater amount of time running and performed more sprints during a soccer match. This led to the conclusion that ingesting carbohydrate was beneficial by preventing the deterioration in performance, possibly as a consequence of reducing the level of dehydration and energy depletion. Leatt and Jacobs (1989) also investigated the effect of ingesting glucose-polymer solution before a game and at half-time and whilst actual performance was not measured, a 31% higher concentration of muscle glycogen was found compared with the control group at the end of the match. The authors concluded that carbohydrate ingestion may delay fatigue. Whilst field studies provide a more realistic representation, an important consideration to make when interpreting the results from field studies is the lack of control over work-rate. Also there is probably a significant inter-player variation in the amount of work performed during a soccer match (Leatt and Jacobs, 1989). These results suggest that ingesting carbohydrate during a soccer match may aid the performance in terms of distance covered or sprinting ability. In contrast, individual skills such as passing are unaffected.

High-intensity intermittent protocols have been widely used to examine the effect of carbohydrate ingestion due to the greater control over work-rate and environmental factors. There appears to be some inconsistencies in the findings regarding carbohydrate supplementation during soccer-specific exercise, which is possibly due to the different measurement tools employed, whether exercise performance or capacity was measured. One of the most commonly used protocols is the Loughborough Intermittent Shuttle Test (LIST). Whilst McGregor *et al.* (1999) did not investigate the effect of carbohydrate ingestion, they did demonstrate that water ingestion significantly improved sprinting ability towards the end of the protocol and prevented the drop in skill performance observed during the no fluid trial. The implication of this study is that by ingesting fluid during soccer, dehydration can be reduced or prevented, skill performance can be maintained and the onset of fatigue can be delayed due to reduced thermal strain.

Nicholas *et al.* (1995) demonstrated that ingesting carbohydrate did not improve sprinting ability but did significantly enhance the time to exhaustion after 75 min of the intermittent protocol. These findings led the authors to conclude that the improvement in exercise capacity was due to the muscle glycogen sparing effect of the ingested carbohydrate (Nicholas *et al.*, 1995). Welsh *et al.* (2002) employed a similar protocol, although the duration was only 60 min organized into quarters with a 20-min rest between the second and third quarters to represent half-time, a schedule which is not representative of soccer. Nevertheless, ingesting carbohydrate significantly improved exercise capacity by a similar margin as reported by Nicholas *et al.* (1995). In contrast to the findings of Nicholas *et al.*(1995), sprint performance was significantly enhanced with the ingestion of carbohydrate, as well as improvements in the performance of a motor skill test and self-reported perceptions of fatigue. These authors (Welsh *et al.*, 2002) also investigated the effects of carbohydrate ingestion on mental parameters, and although not significant there was a trend for improvements in the Stroop Color-Word Test with carbohydrate ingestion, suggesting that carbohydrate could maintain mental performance. This observation is supported by the findings of Reilly and Lewis (1985) who demonstrated that carbohydrate ingestion could be beneficial to cognitive performance in terms of the number of tasks attempted and fewer resultant errors during 120-min of cycling at 60% $\dot{V}O_{2max}$ when compared with water or no fluid ingestion. Whilst the protocol used by Welsh *et al.* (2002) was not soccer-specific, the combined results from this, and other studies suggest that carbohydrate ingestion can improve certain aspects of soccer performance.

Morris *et al.* (2003) examined the effect of carbohydrate ingestion on high-intensity intermittent shuttle running performance in the heat. This study failed to show any benefit of carbohydrate ingestion, possibly due to hyperthermia being the main fatiguing factor and not dehydration or energy. However, all but one subject failed to complete the first part of the protocol. It would have also been interesting to have investigated the effect of carbohydrate ingestion on heat acclimatised subjects, possibly reducing the impact of hyperthermia.

The results from these studies suggest that fluid replacement and carbohydrate ingestion have an important function during soccer match-play by reducing the level of dehydration, sparing muscle glycogen and delaying the onset of fatigue. It is also clear that further research is required into the effect of manipulating the timing, volume and composition of the fluid ingested, as well as the environmental conditions.

2.14. Summary

Carbohydrate depletion, dehydration and hyperthermia are considered to be the some of the major causes of fatigue during exercise. It has also been demonstrated that exercising in the heat accelerates these processes. A number of strategies aimed at alleviating the effects of fatigue have been investigated, including fluid replacement with and without carbohydrate ingestion, and pre-cooling. Soccer is considered an intermittent high-intensity activity which is played in a variety of environmental conditions. Energy production during soccer match-play is provided by a combination of aerobic and anaerobic systems, during low and high-intensity contributing exercise periods respectively. Fatigue, defined as a reduction in work-rate, has been shown to occur both temporarily during the match and towards the end of the match. The ingestion of carbohydrate improves performance during match-play and soccer-specific exercise, simulating match-play in controlled conditions by delaying the onset of fatigue.

Chapter 3

General methods

3.1. General methods

3.1.1. Location of Testing and Ethical Approval

All procedures and methods contained within this work were conducted in the physiology laboratories of the Research Institute for Sport and Exercise Sciences at Liverpool John Moores University. All the studies conducted were approved by the University's Human Ethics Committee at Liverpool John Moores University.

3.1.2. Subjects

All participants were in good health and regularly exercised (at least three times a week). Written and verbal information regarding the nature and risks of the experimental procedures was provided to all participants (Appendix B). Participation was entirely voluntary and subjects signed an appropriate consent form and were free to withdraw from the experiments at any time.

Prior to all experimental sessions subjects were asked to refrain from strenuous exercise such as a competitive match and the consumption of alcohol for the previous 24 h and caffeine for 12 h. Subjects were also asked to record food intake and physical activity for the preceding 3 days prior to their first experimental session in order to replicate their dietary and activity behaviour for subsequent sessions to avoid differences in diet and physical condition. Fours hours prior to subjects consumed a snack (65% CHO; 20% Fat; 15% Protein; 117g CHO). Each experimental session was separated by 5-7 days and was conducted at the same time of day (approximately 15:00 hours) to avoid circadian variation in any of the measured variables (Reilly and Brooks, 1986).

3.1.3. Anthropometry

Subjects' heights were measured whilst standing in the Frankfurt plane using a stadiometer (Seca, Birmingham, U.K). Prior to the commencement of each experimental session, and upon completion, subjects weighed themselves nude using precision calibrated weighing scales (Seca, Birmingham, U.K) for the determination of body weight loss. Changes in body mass loss were calculated from the difference in dry body mass between pre- and post-exercise. Values were corrected for the volume of fluid ingested, urine excreted and respiratory and metabolic losses (Mitchell *et al.*, 1972) for calculating sweat loss.

3.1.4. Soccer-specific protocol

The soccer-specific protocol devised for the experimental work in chapters 4, 6 and 7 was performed on a motorised treadmill (H/P/Cosmos Pulsar 4.0, H/P/Cosmos Sports & Medical GmbH, Nussdorf-Traunstein, Germany) (Figure 3.1) and was a modified version of the one designed by Drust *et al.* (2000b). The protocol consisted of the various exercise intensities that are regularly observed during competitive soccer matches (i.e. walking, jogging, cruising and sprinting). The proportions of these activities were based on the observations of Reilly and Thomas (1976), although utility movements (e.g. backwards and sideward movements) were not included. The proportions of these activities were divided between walking and jogging. Therefore the proportion of time for each activity was as follows: static 3.8% walking 27.9%; jogging 38.9%; cruising 19.9%; sprinting 9.5%. The duration of each activity was determined by matching the proportions observed by Reilly and Thomas (1976) to the total time of the block, after the deduction of the total time for the treadmill speed changes had been made (Figure 3.2 and Table 3.1). The duration of each discrete bout was as follows: static 8.0 s; walking 27.8 s; jogging 38.7 s; cruising 34.8 s; sprinting 9.4 s. Subjects undertook two familiarisation sessions, consisting of two blocks of the soccer-specific protocol (i.e. 30 minutes) before the first trial.

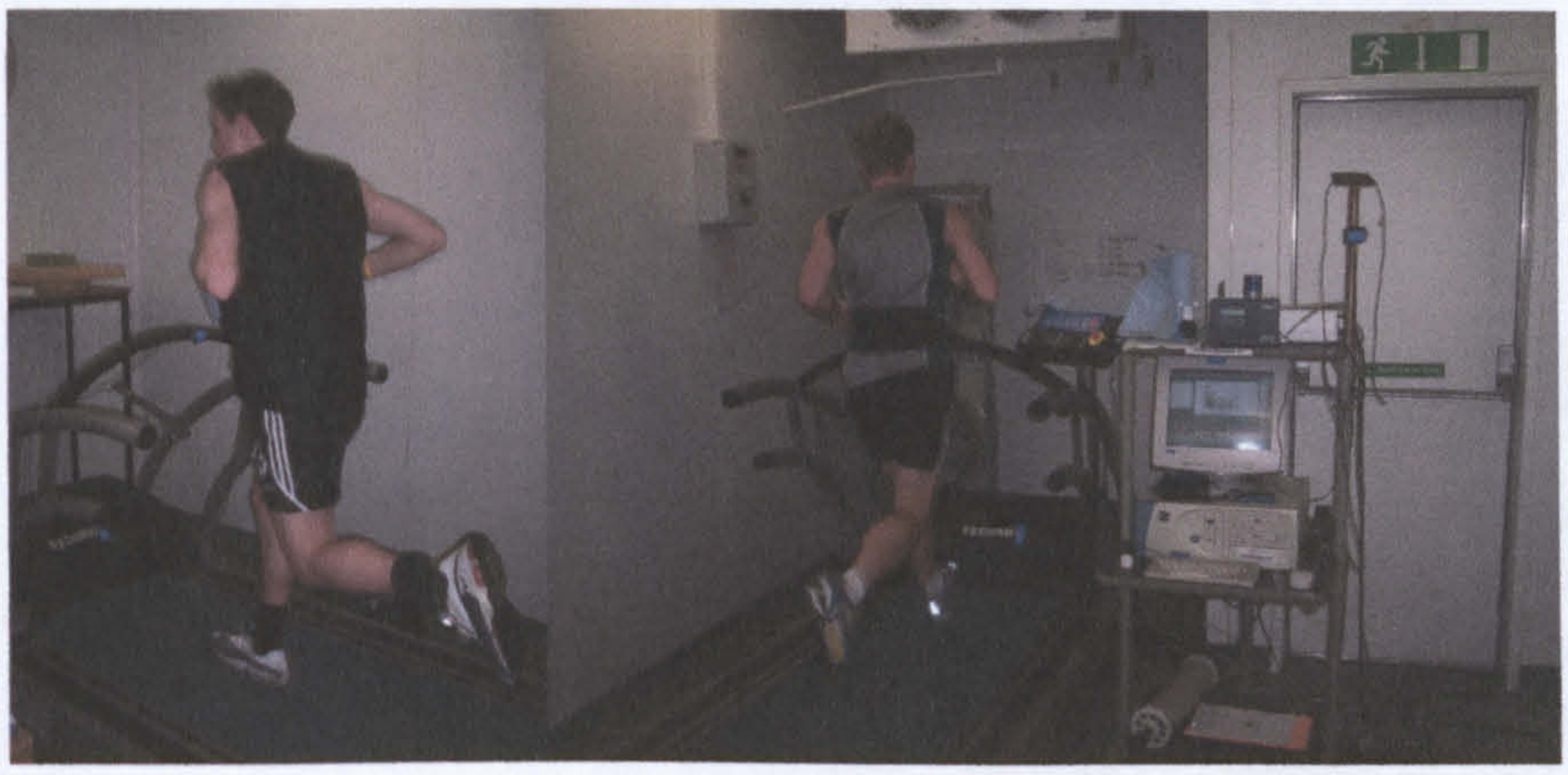

Figure 3.1: Subject performing the soccer-specific protocol on a motorised treadmill.

Table 3.1: Soccer-specific protocol, with corresponding speeds, acceleration time and total duration.

Activity	Speed ($km \cdot h^{-1}$)	Acceleration time (s)	Activity duration (s)	Total (s)
Cruise	13	9.75	34.75	44.50
Jog	10	2.25	38.72	40.97
Sprint	19	6.75	9.40	16.15
Stop	0	14.00	8.00	22.00
Jog	10	7.50	38.72	46.22
Walk	4	4.50	27.8	32.30
Sprint	19	11.25	9.70	20.95
Walk	4	11.25	27.80	39.05
Cruise	13	6.75	34.75	41.50
Jog	10	2.25	38.72	40.97
Sprint	19	6.75	9.40	16.15
Walk	4	11.25	27.80	39.05
Jog	10	4.50	38.72	43.22
Sprint	19	6.75	9.40	16.15
Stop	0	14.00	8.00	22.00
Walk	4	3.00	27.80	30.80
Cruise	13	6.75	34.75	41.50
Jog	10	2.25	38.72	40.97
Sprint	19	6.75	9.40	16.15
Walk	4	11.25	27.80	39.05
Jog	10	4.50	38.72	43.22
Sprint	19	6.75	9.40	16.15
Walk	4	11.25	27.80	39.05
Cruise	13	6.75	34.75	41.50
Jog	10	2.25	38.72	40.97
Sprint	19	6.75	9.40	16.15
Walk	4	11.25	27.80	39.05
Stop	0	3.00	11.00	14.00

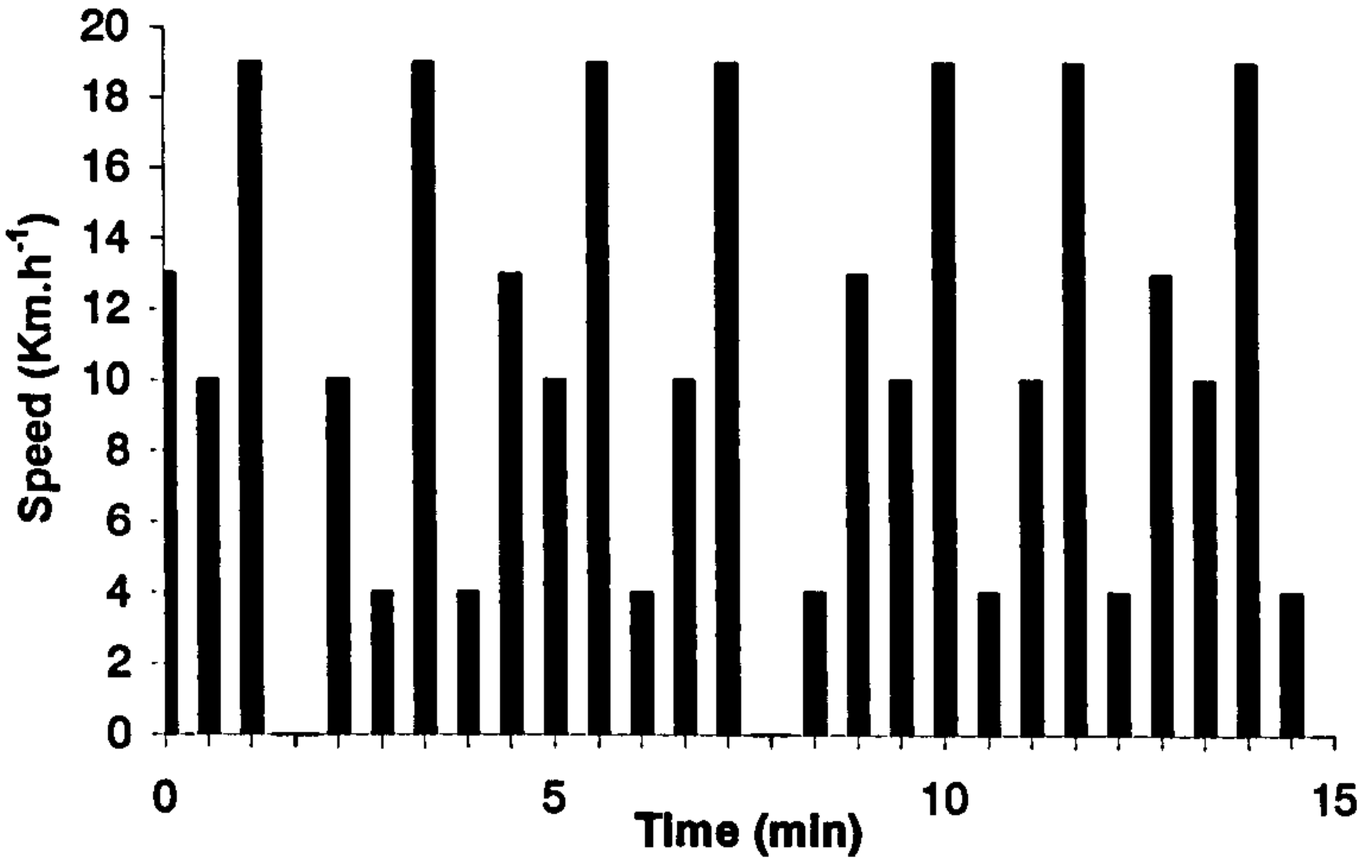

Figure 3.2: Activity profile of one block of the soccer-specific protocol.

3.2. Cardio-respiratory measures

3.2.1. Heart rate

Heart rate was measured continuously at 5 s intervals by means of a short–range radio telemetry system (Polar S610i, Polar Electro, Kempele, Finland) during all exercise.

3.2.2. Assessment of respiratory gases during exercise

Oxygen consumption ($\dot{V}O_2$), carbon dioxide production ($\dot{V}CO_2$), respiratory exchange ratio (RER) and minute ventilation ($\dot{V}E$) were recorded using an on-line automated gas analyser (Metalyzer3B, Cortex Biophysic GmbH, Leipzig, Germany) after calibration with known reference gases. This system has previously been reported to be a reliable measurement tool for assessment of respiratory gases during exercise (Meyer *et al.*, 2001b).

3.2.3. Assessment of maximal oxygen uptake ($\dot{V}O_{2max}$)

Prior to each study, all subjects were assessed for aerobic power by determining their $\dot{V}O_{2max}$ on a motorised treadmill (H/P/Cosmos Pulsar 4.0, H/P/Cosmos Sports & Medical GmbH, Nussdorf-Traunstein, Germany). All subjects started running at 10 $km \cdot h^{-1}$ and increased by 2 $km \cdot h^{-1}$ every 2 min up to 16 $km \cdot h^{-1}$. Thereafter, the treadmill was inclined by 2% every 2 min until volitional exhaustion. The $\dot{V}O_{2max}$ was taken as the highest $\dot{V}O_2$ value obtained in any 10-s period, and was stated as being achieved by the following end point criteria: 1. Failure of heart rate to increase with further increases in exercise intensity; 2. RER >1.15; 3. Plateau of oxygen consumption (<150 $ml.min^{-1}$) despite increased work-load (American College of Sports Medicine, 2000).

3.2.3. Substrate oxidation rates

Total carbohydrate and fat oxidation rates ($g \cdot min^{-1}$) were calculated by using stoichiometric equations of Frayn (1983) with the assumption that protein oxidation during exercise was negligible:

$$\text{Carbohydrate oxidation } (g \cdot min^{-1}) = 4.55\,\dot{V}CO_2 - 3.21\,\dot{V}O_2 \quad (1)$$

$$\text{Fat oxidation } (g \cdot min^{-1}) = 1.67(\dot{V}O_2 - \dot{V}CO_2) \quad (2)$$

where $\dot{V}O_2$ and $\dot{V}CO_2$ represent oxygen consumption and carbon dioxide production, respectively, in litres per minute.

3.3. Measurement of core body temperature

Core body temperature (T_c) was monitored by means of an ingestible temperature sensor pill and external data logger (HQ inc., Florida, USA). Approximately 3-4 hours prior to testing, subjects swallowed a heat-sensitive telemetry pill to ensure that the sensor had passed into the small intestine so that it was unaffected by any exothermic reactions that

may have been occurring in the stomach or the ingestion of cold fluid. While inside the gastrointestinal tract, the internal crystal sensor vibrated at a frequency relative to the temperature of the substance surrounding it, producing a magnetic flux. The sensor wirelessly transmitted the core body temperature signal to the ambulatory data logger worn on the outside of the body. Each sensor was factory calibrated and supplied with a calibration reference correction value, which was programmed into the data logger prior to ingestion to ensure that it was operating correctly. The data were subsequently downloaded at the end of each trial. This method of T_c measurement has been shown to be an accurate measure of T_c and is comparable to other measures such as rectal and oesophageal temperature (Sparling *et al.*, 1993; O'Brien *et al.*, 1998; Lee *et al.*, 2000; Edwards *et al.*, 2002) and has recently been used to monitor core temperature during actual match-play (Edwards and Clark, 2006).

3.4. Assessment of psychological variables

3.4.1. Rating of Perceived Exertion (RPE)

Rating of perceived exertion (RPE) was measured using a 6-20 scale (Borg, 1970). The category ratio scale that was used is displayed in Table 3.2.

Table 3.2: Borg scale used for subjects' RPE during exercise.

Rating	Description
6	No exertion at all
7	Extremely light
8	
9	Very light
10	
11	Light
12	
13	Somewhat hard
14	
15	Hard
16	
17	Very hard
18	
19	Extremely hard
20	Maximal exertion

3.4.2. Rating of Thermal sensation

Subjects reported rating of thermal sensation during exercise according to a 17-point thermal sensation scale (Toner *et al.*, 1986). The category ratio scale that was used is displayed in Table 3.3.

Table 3.3: Thermal sensation scale of Toner *et al.* (1986).

Rating	Description
0.0	Unbearably cold
0.5	
1.0	Very cold
1.5	
2.0	Cold
2.5	
3.0	Cool
3.5	
4.0	Neutral (comfortable)
4.5	
5.0	Warm
5.5	
6.0	Hot
6.5	
7.0	Very hot
7.5	
8.0	Unbearably hot

3.4.3. Assessment of subjective feelings of gut fullness and thirst

Gut fullness and thirst were measured using 100-mm visual analogue scales (VAS) (Figure 3.3). Unlike a Graphic Rating Scale that contains descriptors placed at equal intervals, the VAS allows the individual to rate the feeling of fullness or thirst without having to invoke their own descriptive terms and have been shown to exhibit a high level of within subject reliability and validity (Stubbs *et al.*, 2000).

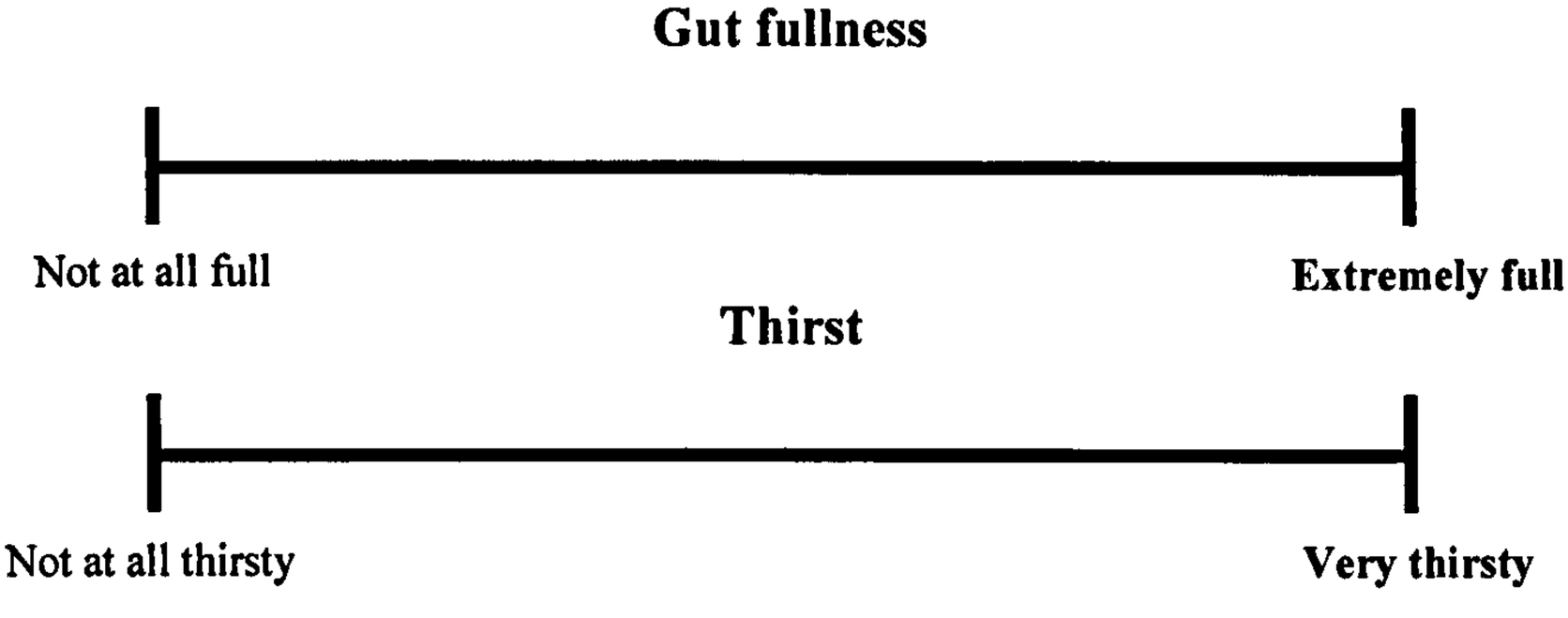

Figure 3.3: Visual Analogue Scale (VAS) used to assess subjective feelings of gut fullness and thirst.

3.5. Urine colour and osmolality

To ensure hydration status was constant for each trial, a urine sample was obtained pre-exercise. The sample was tested for colour (Armstrong *et al.*, 1994) and osmolality (Advanced Micro-osmometer Model 3300, Advanced Instruments inc, Massachusetts, USA). The principle of the test is based on freezing point osmometry, where the solute concentration of a solvent raises the osmotic pressure and boiling point, and reduces the freezing point of the sample. These properties change in proportion to the number of particles in the sample, which allows the concentration to be calculated. The micro-osmometer utilises the change in freezing point to calculate osmolality, by supercooling the sample then allowing the temperature to rise to the freezing point. Intra-sample coefficient of variations were 0.4 % at 278.5 $mOsm \cdot kg^{-1}$ and 2.6 % at 925.5 $mOsm \cdot kg^{-1}$.

3.6. Blood procurement and storage

Venous blood samples were drawn from a superficial vein in the antecubital crease of the forearm using standard venepuncture techniques (Vacutainer Systems, Becton, Dickinson, Europe) by a qualified phlebotomist (the author was trained in phlebotomy prior to the first study, see Appendix C) whilst the subject was standing. Serum was obtained by collecting samples in to serum separation tubes. The blood was stored at

room temperature for 60 min before being centrifuged at 4°C for 15 min. Plasma was obtained by collecting the samples into tubes that had been pre-treated with the anti-coagulant lithium heparin or EDTA. These samples were then gently mixed and immediately centrifuged at 4°C for 15 min. Once centrifugation was completed the plasma or serum was aliquoted into storage tubes (Eppendorf, Hamburg, Germany) and stored at -80°C for later analysis.

3.7. Blood analyses

All metabolite analysis was conducted using a bench top clinical chemistry analyser (ILab 300 plus, Instrumentation Laboratories, Warrington, UK). The analyser was calibrated prior to use with the relevant standard solutions supplied with the assay kits and samples were tested in duplicate.

3.7.1. Plasma glucose analysis

Glucose concentrations were determined in plasma using a commercially available kit (IL Test™ Glucose Oxidase kit, Instrumentation Laboratory, Warrington, UK). A summary of the enzymatic reactions involved in the determination of plasma glucose concentration can be seen below:

$$\beta\text{-D-Glucose} + O_2 + H_2O \xrightarrow{\text{Glucose Oxidase}} \text{Gluconic acid} + H_2O_2$$

$$2\,H_2O_2 + \text{phenol} + \text{4-aminoantipyrine} \xrightarrow{\text{Glucose Peroxidase}} \text{red quinoneimine} + 4H_2O$$

The red quinoneimine dye generated an increased absorbance and is proportional to the glucose concentration in the sample. Primary measurements were recoreded at a wavelength of 510 nm and CV was 1.3% at 4.2 $mmol \cdot l^{-1}$.

3.7.2. Plasma lactate analysis

Plasma lactate concentrations were determined using a commercially available kit (Randox Lactate PAP, Randox Laboratories Ltd, Co. Antrim, UK) using enzymatic methods. The principle of the test was:

$$\text{Lactate} + O_2 \xrightarrow{\text{Lactate Oxidase}} \text{pyruvate} + H_2O_2$$

$$H_2O_2 + \text{4-aminoantipyrine} + \text{TOOS} \xrightarrow{\text{Peroxidase}} \text{purple product} + 4H_2O$$

(TOOS = N-ethyl-N-(2 hydroxy-3-sulphopropyl) m-toluidine)

The purple product generated an increased absorbance and is proportional to the lactate concentration in the sample. Measurements were recorded at a wavelength of 550 nm. The test is linear up to lactate concentrations of 12.21 $mmol \cdot l^{-1}$. The coefficient of variations were 5.7% at 1.1 $mmol \cdot l^{-1}$ and 4.6% at 4.5 $mmol \cdot l^{-1}$.

3.7.3. Plasma NEFA analysis

Non-esterified fatty acid was analysed in plasma and determined using a commercially available kit (NEFA-C, Wacko Chemicals GmbH, Neuss, Germany). The principle of the NEFA measurement was:

$$\text{RCOOH} + \text{ATP} + \text{CoA-SH} \xrightarrow{\text{Acyl-CoA synthase}} \text{Acyl-CoA} + \text{AMP} + \text{Ppi}$$

$$\text{Acyl-CoA} + O_2 \xrightarrow{\text{Acyl-CoA oxidase}} \text{2,3-trans-Enoyl-CoA} + H_2O_2$$

$$2H_2O_2 + \text{4-Aminoantipyrine} + \text{3-methyl-N-ethyl-N-(}\beta\text{-hydroxyethyl)-alanine}$$

$$\xrightarrow{\text{Peroxidase}} \text{Coloured reaction product} + 4H_2O$$

The concentration of NEFA is proportional to the formation of the coloured product. Primary measurements were taken at a wavelength of 500 nm and coefficient of variations were 2.3% at 0.2 mmol·l^{-1} and 3.1% at 1.1 mmol·l^{-1}.

3.7.4. Plasma glycerol analysis

Glycerol concentration was determined in plasma using a direct calorimetric method using a commercially available kit (Randox Laboratories Ltd, Co. Antrim, UK). The intra-assay CV was 1.9% at 248 μmol·l^{-1}. The principle of the NEFA measurement was:

$$\text{Glycerol} + \text{ATP} \xrightarrow{\text{Glycerol kinase}} \text{Glycerol-3-phosphate} + \text{ADP}$$

$$\text{Glycerol-3-phosphate} + O_2 \xrightarrow{\text{Acyl-CoA oxidase}} H_2O_2 + \text{Dihydroxyacetone-phosphate}$$

$$2H_2O_2 + \text{3,5-dichloro-2-hydroxybenzene sulphonic acid} + \text{4-aminophenazone}$$

$$\xrightarrow{\text{Peroxidase}} \text{n-(4-antipyryl)-3-chloro-5-sulphonate-p-benzoquinoneimine}$$

3.7.5. Hormone analysis: Enzyme-linked immunosorbent assay (ELISA)

Catecholamines (Catcombi ELISA, IBL GmbH, Hamberg, Germany), insulin (Insulin ELISA, DRG Instruments GmbH, Germany), cortisol (Cortisol ELISA, DRG Instruments GmbH, Germany), prolactin (Prolactin ELISA, DRG Instruments GmbH, Germany) and IL-6 (IL-6 ELISA, BLK diagnostics, Spain) concentrations were assessed using a Microplate reader (Anthos Labtech Instruments, Austria) for the experimental work in chapters 4 and 5. A fully-automated Immunoassay system (Triturus, Grifols, Cambridge, U.K) was used for the experimental work in chapters 6 and 7. All samples were analysed in duplicate in one batch.

The ELISA technique utilised an antibody-labelled reaction to determine the concentration of a specific antigen in the sample. A monoclonal antibody was attached inside a series of plastic wells, forming a microplate, which constituted the solid phase of the reaction. After the sample had been added to the well, any antigen present was recognised by the antibody and bound to it. After a period of incubation, the wells were washed to remove any unbound molecules. This procedure was followed by a second period of incubation, where another antibody, which had an enzyme linked to it, facilitated a colourless reaction to produce a colour change, which was dependent of the antigen concentration.

3.7.6. Plasma osmolality

Plasma osmolality was assessed using the same osmometer (Advanced Micro-osmometer Model 3300, Advanced Instruments inc, Massachusetts, USA) as described in section 3.5.

3.7.7. Measurement of haematocrit, haemoglobin and change in plasma volume

Venous blood was drawn into duplicate haematocrit tubes containing lithium heparin (Micro haematocrit tubes, L.I.P Equipment, Yorkshire, U.K) and two β-haemoglobin microcuvettes (Hemocue AB, Angelholm, Sweden). The haematocrit tubes were sealed at one end using Critoseal (Gelman-Hawksley Ltd, Sussex, UK) and centrifuged at 13000 rpm for 5 min (Mikro 12-24 Zentrifugen, Hettich, Tuttlingen, Germany) and the amount of haemotocrict was measured (Gelman-Hawksley Ltd, Sussex, UK). The concentration of haemoglobin in whole blood was determined using a Hemocue met-Hb Photometer system (Hemocue, Angelholm, Sweden). The accuracy of the Hemocue system was determined before each measurement using the standard reference cuvette. The measurement of haemoglobin (red blood cell volume) and haematocrit (packed cell volume as a percentage of the total blood volume) allowed the calculation of changes in plasma volume relative to the baseline sample according to Dill and Costill (1974).

3.8. Statistics

All statistical analyses were performed using Statistical Package for Social Science (SPSS) for Windows (version 11) (SPSS inc, Chicago, USA). Results were reported as the mean ± the standard error of the mean (SEM). When Mauchley's test of sphericity indicated a minimal level of violation (>0.75) the degrees of freedom was corrected using the Huynh-Feldt adjustment and when the sphericity was <0.75, the Greenhouse-Geiser correction was used (Field, 2000). Where differences were noted, pairwise comparisons (Bonferroni adjusted) were employed to identify where the significant differences occurred. A level of $P<0.05$ was considered statistically significant.

Chapter 4
Study 1

The present study is the first of two investigations into the metabolic and performance responses to soccer-specific exercise. The exercise protocol was designed to simulate the work-rate observed in competitive soccer match-play. The basis of the experiments was to manipulate the provision of energy drinks to subjects so that their effects could be examined.

4.1. Introduction

A significant reduction in the glycogen content of the thigh muscles of players has been observed by the end of a match (Jacobs *et al.*, 1982). This decline in glycogen stores is reflected in lower running speeds and shorter distances covered during the second half (Saltin, 1973). There is evidence supporting the consumption of carbohydrate during exercise simulating the work-rate of competitive soccer, but this is somewhat inconclusive and may depend upon the measurement tool, such as run to exhaustion or high-intensity sprints. Nicholas *et al.* (1995) established that by ingesting a 6.9% carbohydrate solution, exercise capacity in a simulation of exercise equivalent to soccer could be improved, whereas sprint performance was not. Zeederberg *et al.* (1996) investigated the effect of ingesting a 6.9% glucose-polymer solution before a match and at half-time and found that there were no measurable benefits of glucose-polymer ingestion on motor skills of soccer players during games played in a cool environment. Their study involved measurement of discrete skills, whereas Nicholas *et al.* (1995) assessed time to exhaustion after a 75-min intermittent exercise protocol. Leatt and Jacobs (1989) also investigated the effect of ingesting glucose-polymer solution before a game and at half-time. Whilst performance aspects were not measured, a higher muscle glycogen concentration was found post-game compared with the control group, which led to the conclusion that carbohydrate ingestion does not hinder performance and may delay fatigue.

Another factor that has been linked with fatigue during a soccer match is dehydration. The intensity of exercise associated with a competitive match is high enough to induce appreciable thermal stress, causing players to lose up to 3 litres of sweat (Ekblom, 1986).

In general there are not sufficient opportunities, i.e. breaks in play, during a match for players to ingest enough fluid to replace what is lost through sweating. Another problem with fluid ingestion is that gastric discomfort may result from attempting to ingest a large volume of fluid at half-time (Reilly and Ekblom, 2005). As a consequence there are opportunities for enhancing performance during a game by adopting optimal refuelling and rehydration regimes.

Gastric emptying is considered a limiting factor in fluid replacement (Shi and Gisolfi, 1998). Studies using a single large ingestion (Costill and Saltin, 1974) or repeated smaller ingestions (Duchman *et al.*, 1997) have demonstrated that gastric emptying is strongly affected by gastric volume. Gastric emptying is also influenced by exercise intensity, and Leiper *et al.* (2001) demonstrated that the intensity associated with a soccer match is sufficient to slow gastric emptying. The drinking strategy employed in the majority of studies related to soccer has been to ingest a large volume before the activity and again at half-time, despite frequent administration of carbohydrate being shown to be necessary to improve performance (Fielding *et al.*, 1985). Nevertheless, the effect of frequent fluid ingestion during soccer-specific exercise has not been previously investigated.

This study was designed to investigate the metabolic and performance responses to intermittent exercise. The exercise protocol was designed to simulate the work-rate in competitive soccer match-play. The aims of the experiment were to manipulate the provision of energy drinks for subjects to:

1) establish energy provision and hydration strategies for soccer-specific exercise;
2) investigate the effect of consuming a carbohydrate-electrolyte drink in a single bolus or frequent ingestion on the metabolic responses to soccer-specific exercise.

4.2. Methods

4.2.1. Subjects

Twelve male university soccer players of age: 24±1 years; height: 1.80±0.1 m; body mass: 76.5±3 kg; $\dot{V}O_{2max}$: 61.1±1 $ml \cdot kg^{-1} \cdot min^{-1}$ participated in this study. All subjects provided written informed consent to participate, in accordance with Liverpool John Moores University's ethical procedures.

4.2.2. Experimental Protocol

Each subject attended the laboratory on six separate occasions. During the first visit the subject's $\dot{V}O_{2max}$ was assessed. The subjects also undertook two familiarisation sessions, consisting of two blocks of the soccer-specific protocol (i.e. 30 min).

Subjects completed the full soccer-specific protocol, outlined in section 3.1.4, on three occasions in "normal" laboratory conditions (mean temperature 18.4±0.3 °C, relative humidity 58.6±2 %, wind speed 0 $m.s^{-1}$). During one session 7 $ml \cdot kg^{-1}$ body mass of carbohydrate electrolyte solution (Lucozade Sport, GlaxoSmithKline, Gloucestershire, UK) was consumed before (mean 538±19 ml) and at half-time (mean 538±19 ml, i.e. mean total 1075±38 ml). The emphasis was on volume and hence the treatment was designated CHOv. On another occasion a placebo (a similarly coloured, flavoured and textured electrolyte solution) (GlaxoSmithKline, Gloucestershire, UK) was consumed at the same time points (PLA). During the other session the same total volume of carbohydrate electrolyte was consumed but drinking occurred more frequently and in smaller volumes (i.e. 179±6 ml) at 0, 15, 30, 45, 60, 75 min of exercise, during the walking phase of the block (CHOf). During the carbohydrate trials the total amount of carbohydrate ingested at a rate of 45±0.9 $g.h^{-1}$. The trials were performed in a counter-balanced fashion and where possible were double-blind.

4.2.3. Heart rate and RPE

Heart rate was measured continuously by means of a short–range radio telemetry system (Polar S610i, Polar Electro, Kempele, Finland) outlined in section 3.2.1. Data were presented as the mean value for each 15-min block. At the completion of each 15-min block RPE was measured using a 6-20 scale (Borg, 1970) outlined in section 3.4.1.

4.2.4. Assessment of respiratory gases during exercise

Oxygen consumption ($\dot{V}O_2$), carbon dioxide production ($\dot{V}CO_2$), respiratory exchange ratio (RER) and minute ventilation ($\dot{V}E$) were recorded for 2 min using an on-line automated gas analyser (Metalyzer3B, Cortex Biophysic GmbH, Leipzig, Germany) after 10 min of each block. Carbohydrate and fat oxidation rates were calculated using the stoichiometric equations of Frayn (1983) outlined in section 3.2.2.

4.2.5. Measurement of core body temperature

Core body temperature (outlined in section 3.3) was monitored continuously by means of an ingestible temperature sensor pill and external data logger (HQ inc., Florida, USA). Data were presented as the mean value for each 15-min block.

4.2.6. Blood procurement and analysis

Prior to exercise, at half-time and at the completion of exercise venous blood samples were drawn from an antecubital vein using standard venepuncture techniques (Vacutainer Systems, Becton, Dickinson, Europe). The blood samples were later analysed for glucose, NEFA, glycerol, lactate, adrenaline, insulin and cortisol outlined in (described in section 3.7).

4.2.7. Statistics

All variables were analysed using two-way ANOVAs with repeated measures except for sweat loss, which was analysed using a one-way ANOVA with repeated measures. All results are reported as the mean ± the standard error of the mean (SEM) and a level of $P<0.05$ was considered statistically significant.

4.3. Results

4.3.1. Plasma metabolites

Pre-exercise plasma glucose concentration was similar for all three trials. There was a significant effect of trial of the concentration on plasma glucose ($F_{2,22}$=12.326; P<0.05; Figure 4.1). The plasma glucose concentration was significantly higher at 45 and 90 min during CHOf than during PLA. There was also a significant effect of time ($F_{2,22}$=6.175; P<0.05). During trials CHOv and CHOf, plasma glucose concentration was elevated significantly above resting levels at half-time and at completion of the soccer-specific protocol (P<0.05). The repeated measures ANOVA identified a significant time and trial interaction ($F_{4,44}$=3.114; P<0.05); plasma glucose remained relatively constant during the first half of placebo trial, in contrast to during the carbohydrate trials when plasma glucose increased markedly. In all trials plasma glucose decreased during the second half although no subjects were found to be hypoglycaemic.

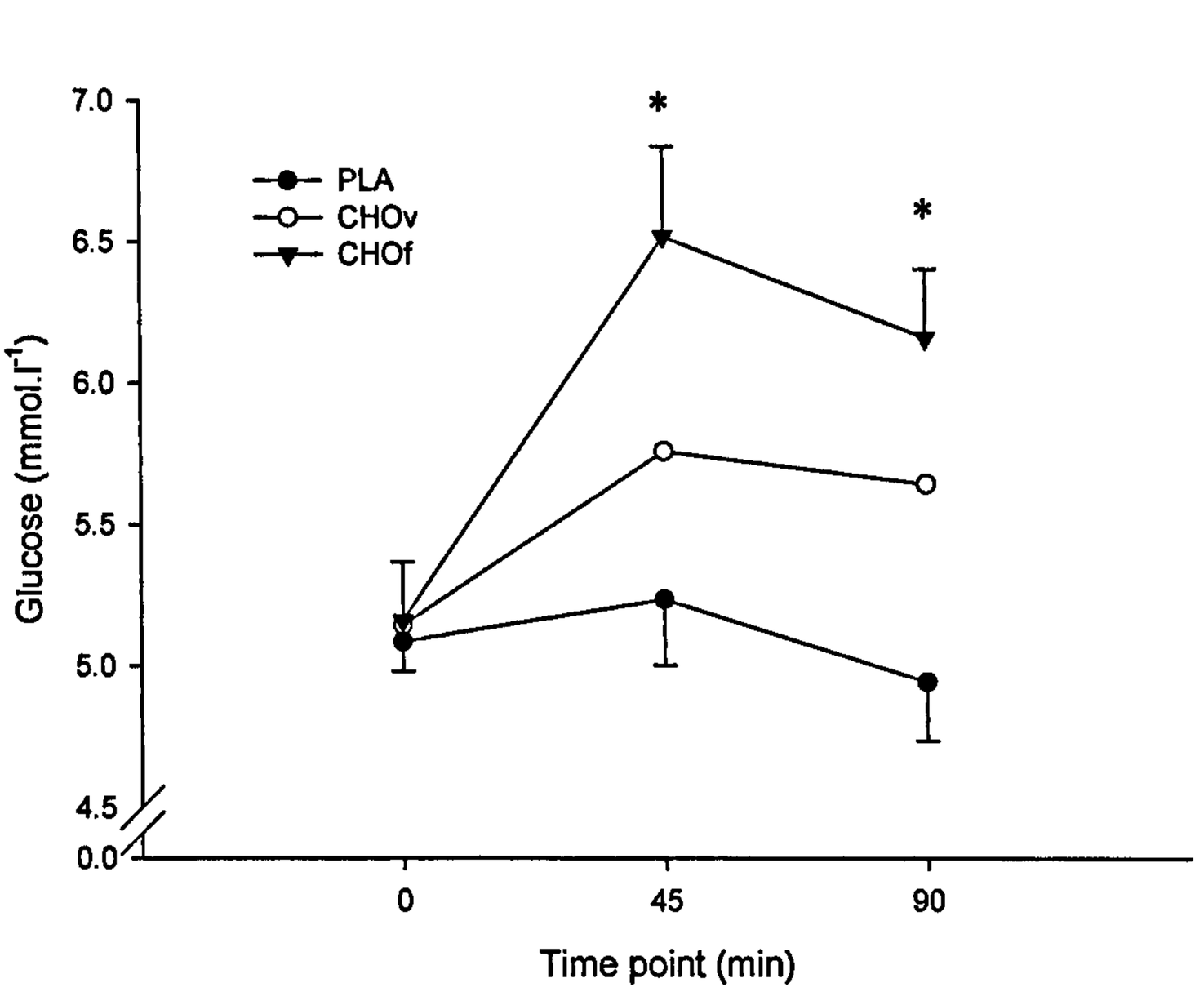

Figure 4.1: Plasma glucose concentration during the soccer-specific protocol. *CHOf significantly greater than PLA.

The repeated measures ANOVA revealed that there was a significant trial effect on the plasma concentration of NEFA ($F_{2,22}$=2.691; P<0.05; Figure 4.2). There was a significant effect of time on the concentration of plasma NEFA ($F_{2,22}$=34.679; P<0.05), which increased significantly between each time point as exercise progressed. There was also a significant ($F_{4,44}$=3.579; P<0.05) trial and time interaction; after half-time NEFA concentration increased markedly more during PLA compared with CHOv and CHOf, in which it increased at a steady rate.

The plasma concentration of glycerol was significantly affected by the trial ($F_{2,22}$=4.828; P>0.05), and the concentration was significantly (P<0.05) higher during the PLA trial compared with CHOv (Figure 4.3). Plasma glycerol concentration increased significantly between each time point ($F_{2,22}$=49.141; P<0.05). There was also a significant ($F_{4,44}$=3.067; P<0.05) trial and time interaction; after half-time glycerol concentration increased markedly more during PLA compared with CHOv and CHOf.

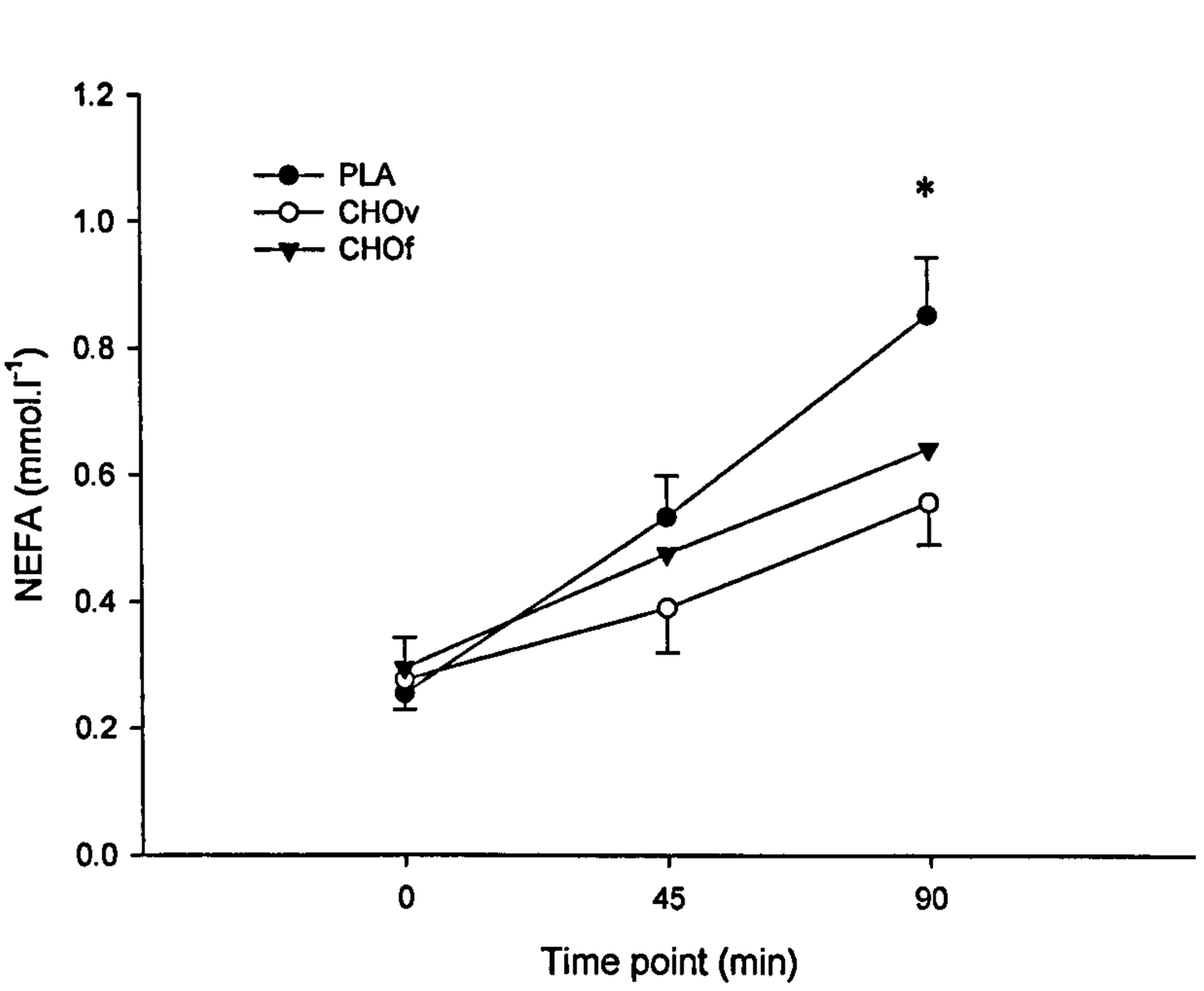

Figure 4.2: Plasma NEFA concentration during the soccer-specific protocol. *PLA significantly greater than CHOv.

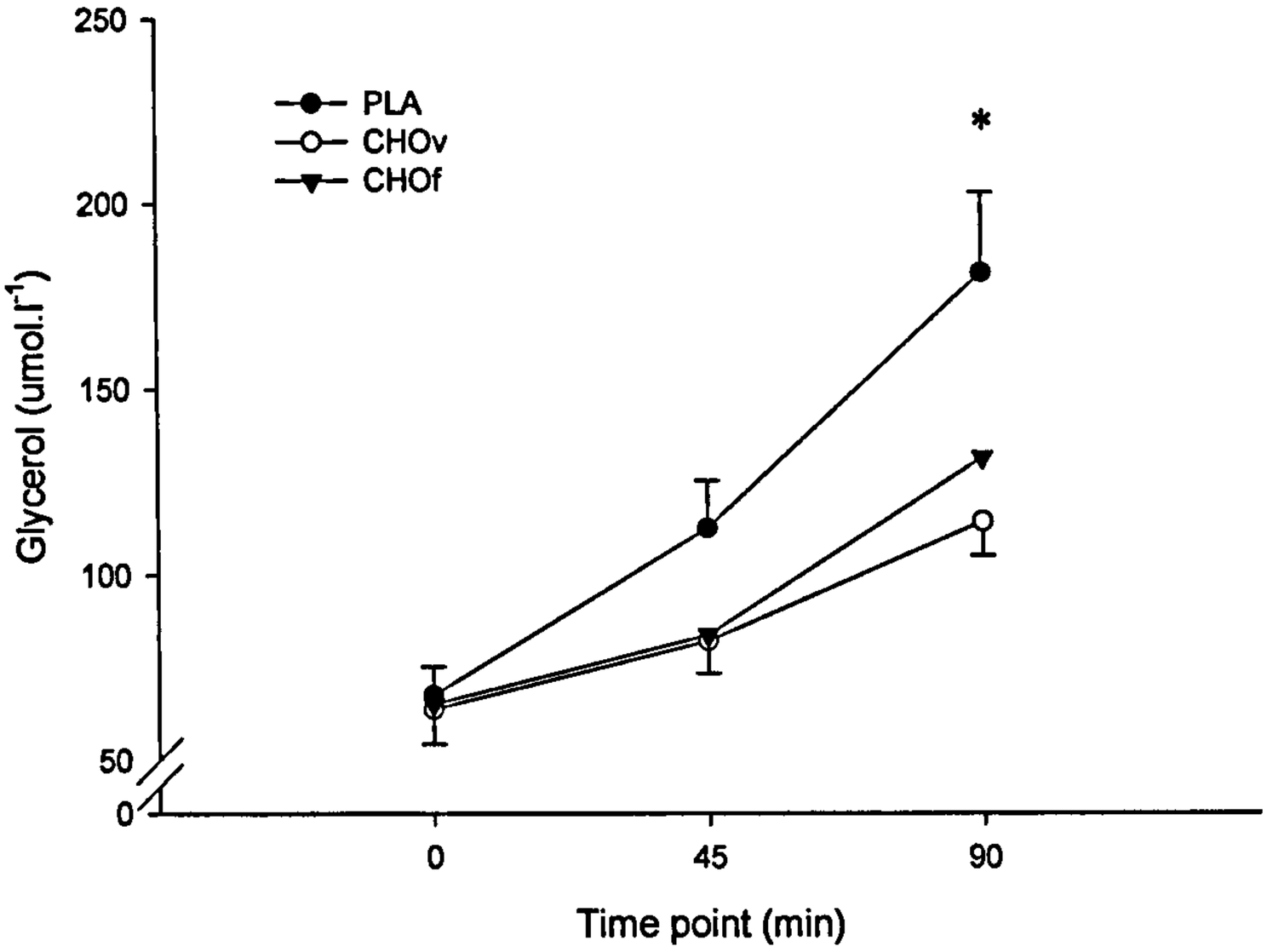

Figure 4.3: Plasma glycerol concentration during the soccer-specific protocol.

*PLA significantly greater than CHOv.

The repeated measures ANOVA revealed that there was no significant trial effect on the plasma concentration of lactate ($F_{2,22}$=0.109; P>0.05) (PLA: 3.38±0.45 mmol·l^{-1}, 3.9±0.4 mmol·l^{-1}; CHOv: 3.5±0.3 mmol·l^{-1}, 3.9±0.5 mmol·l^{-1}; CHOf: 3.2±0.4 mmol·l^{-1}, 3.8±0.4 mmol·l^{-1}, 45 and 90 min respectively). There was a significant effect of time on the concentration of plasma lactate ($F_{2,22}$=74.209; P<0.05), which was significantly higher at half-time and post-exercise compared with pre-exercise levels. There was no significant difference in plasma osmolality between the three trials ($F_{2,22}$=2.962; P>0.05) nor by completing the soccer-specific protocol ($F_{2,22}$=1.270; P>005).

4.3.2. Hormones

The concentration of adrenaline was found to be similar during all trials ($F_{2,22}$=0.946; P>0.05, Figure 4.4), and increased significantly ($F_{2,22}$=23.821; P<0.05;) between each time point. The repeated measures ANOVA revealed a significant interaction

($F_{2,26}$=5.808; P<0.05). Between half-time and the completion of the soccer-specific protocol adrenaline concentration increased at a greater rate during PLA (1.78±0.12 nmol·l^{-1}) compared with CHOv (1.59±0.11 nmol·l^{-1}) and CHOf (1.61±0.11 nmol·l^{-1}).

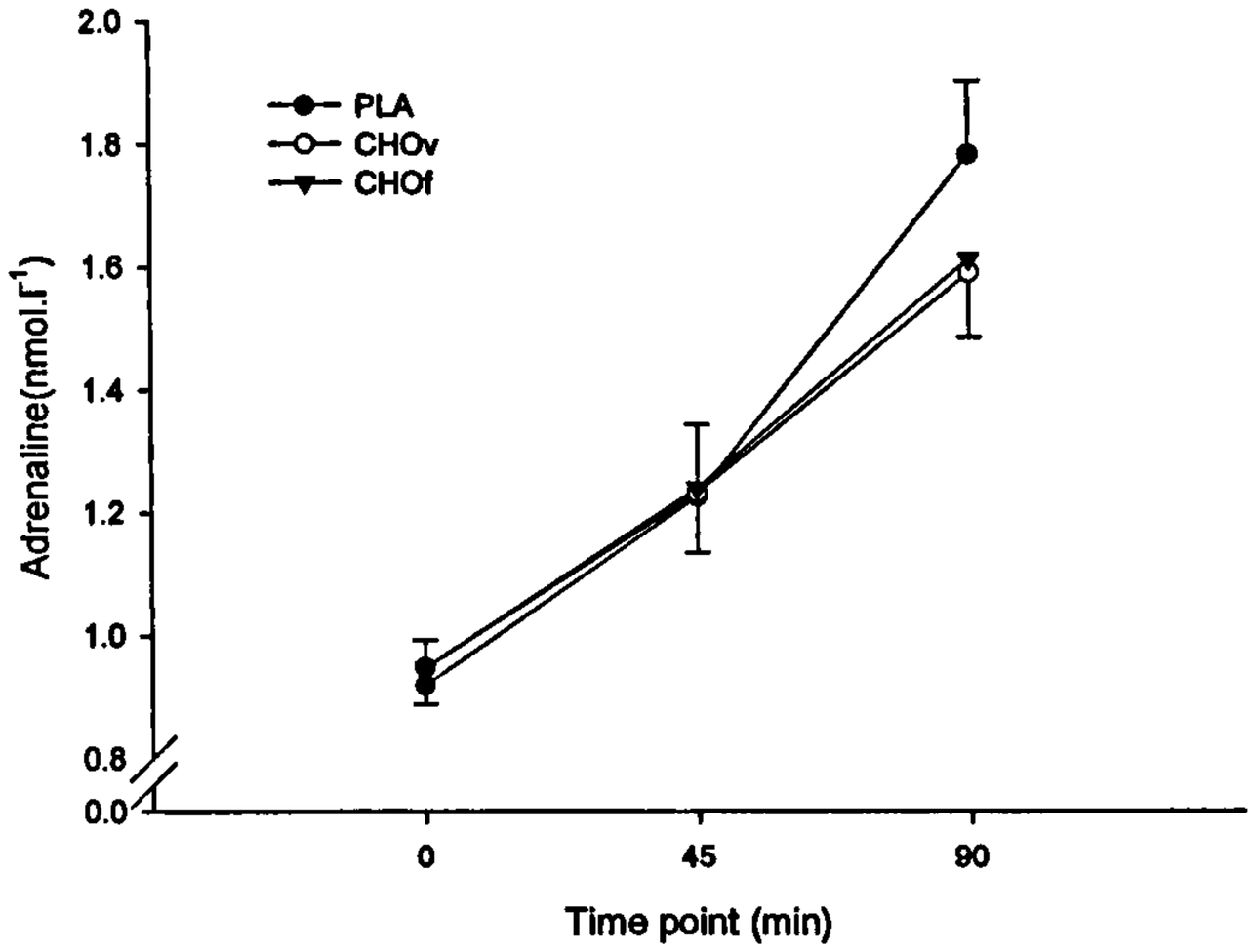

Figure 4.4: Plasma adrenaline concentration during the soccer-specific protocol.

There was no significant trial effect of the concentration of plasma cortisol ($F_{2,22}$=0.263; P>0.05; Figure 4.5). The repeated measures ANOVA revealed that there was not a significant effect of time ($F_{2,22}$=0.421; P>0.05) or interaction ($F_{2,22}$=0.696; P>0.05).

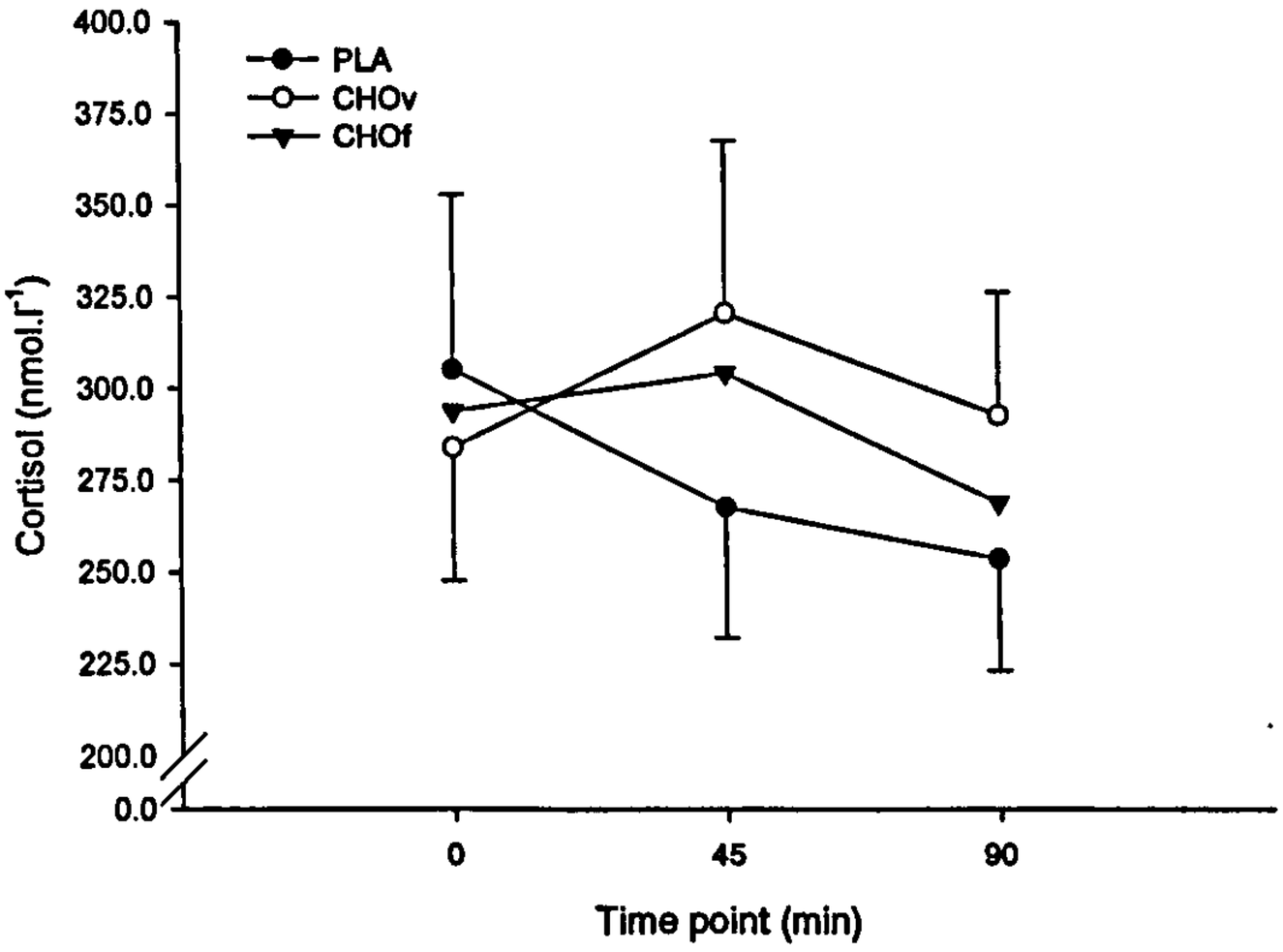

Figure 4.5: Serum cortisol concentration during the soccer-specific protocol.

There was a significant trial effect on the concentration of serum insulin ($F_{2,22}$=5.416; P<0.05; Figure 4.6). The serum insulin concentration was significantly higher during CHOf than during the placebo trial. The repeated measures ANOVA identified a significant time and trial interaction ($F_{4,44}$=3.194; P<0.05) whereby, serum insulin concentration increased during the first half of CHOf, whereas it decreased during CHOv and more markedly during PLA. All trials demonstrated a decreased insulin response during the second half.

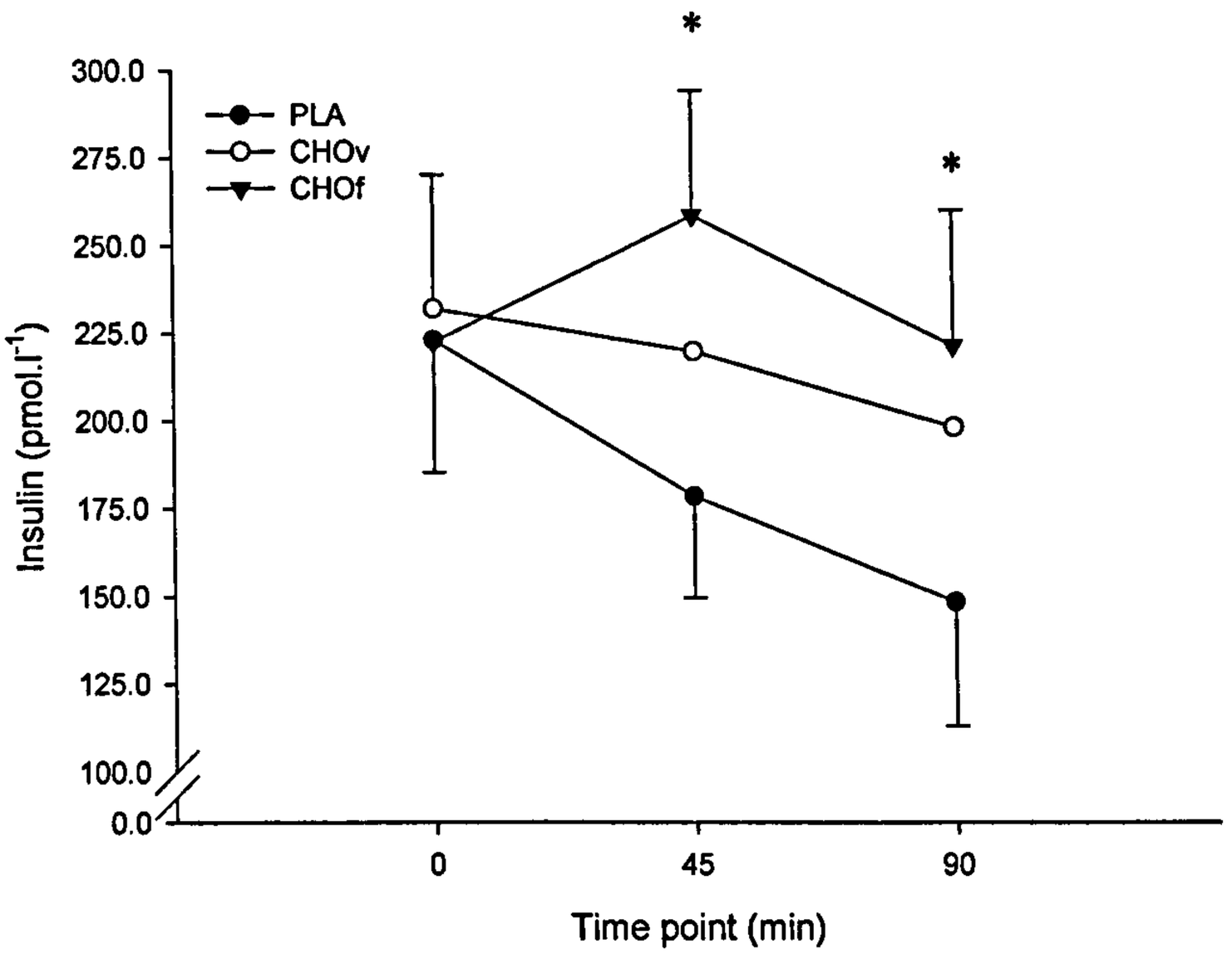

Figure 4.6: Serum insulin concentration during the soccer-specific protocol.
*CHOf significantly greater than PLA.

4.3.3. Indirect calorimetry

There was a significant difference in RER between the three trials, ($F_{2,22}$=5.194; P<0.05, Figure 4.7). During PLA, RER was significantly lower than during CHOv or CHOf, indicating a greater proportion of fat oxidation. There was a significant effect of time on RER ($F_{5,55}$=4.560; P<0.05). Block 6 showed significantly (P<0.05) lower values than the previous 75 min, indicating that a greater proportion of fat was oxidized during this 15-

min period. There was a significant interaction effect of time and treatment ($F_{3,34}$=2.717; P<0.05). During the carbohydrate trials RER remained relatively constant throughout the soccer-specific protocol, whereas during PLA, RER decreased throughout both halves of the soccer-specific protocol.

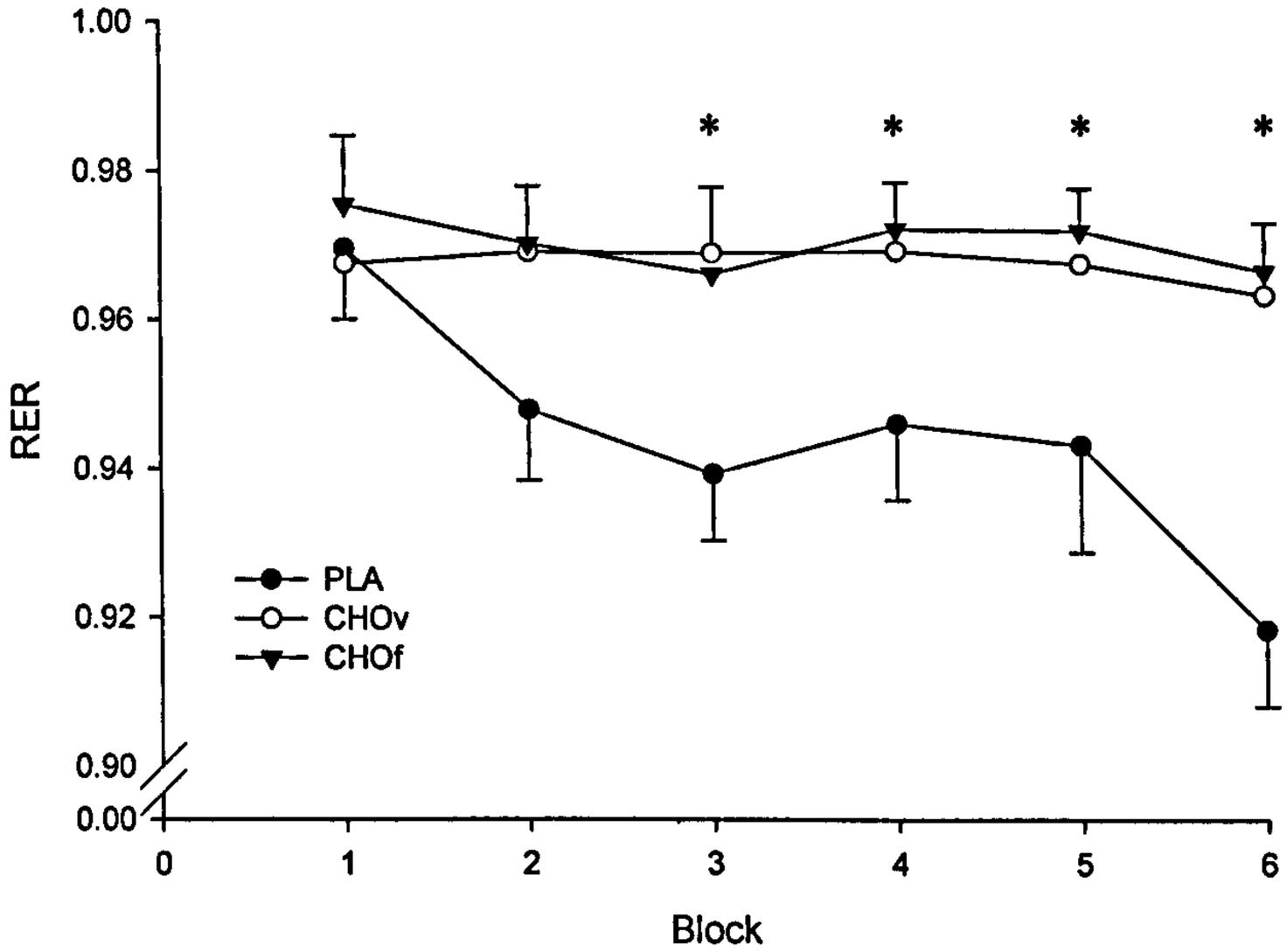

Figure 4.7: Respiratory exchange ratio during the soccer-specific protocol.
*CHOf and CHOv significantly greater than PLA.

The repeated measures ANOVA showed carbohydrate oxidation was significantly ($F_{2,22}$=4.555; P<0.05) affected by the experimental treatments (Figure 4.8). Carbohydrate oxidation was greater during CHOf compared to PLA (P<0.05). There were no significant differences between CHOf and CHOv (P>0.05). Carbohydrate oxidation was significantly ($F_{23,29}$=2.421; P<0.05) lower during block 6 compared with blocks 1 and 5. No significant interaction was observed ($F_{4,44}$=1.625; P>0.05).

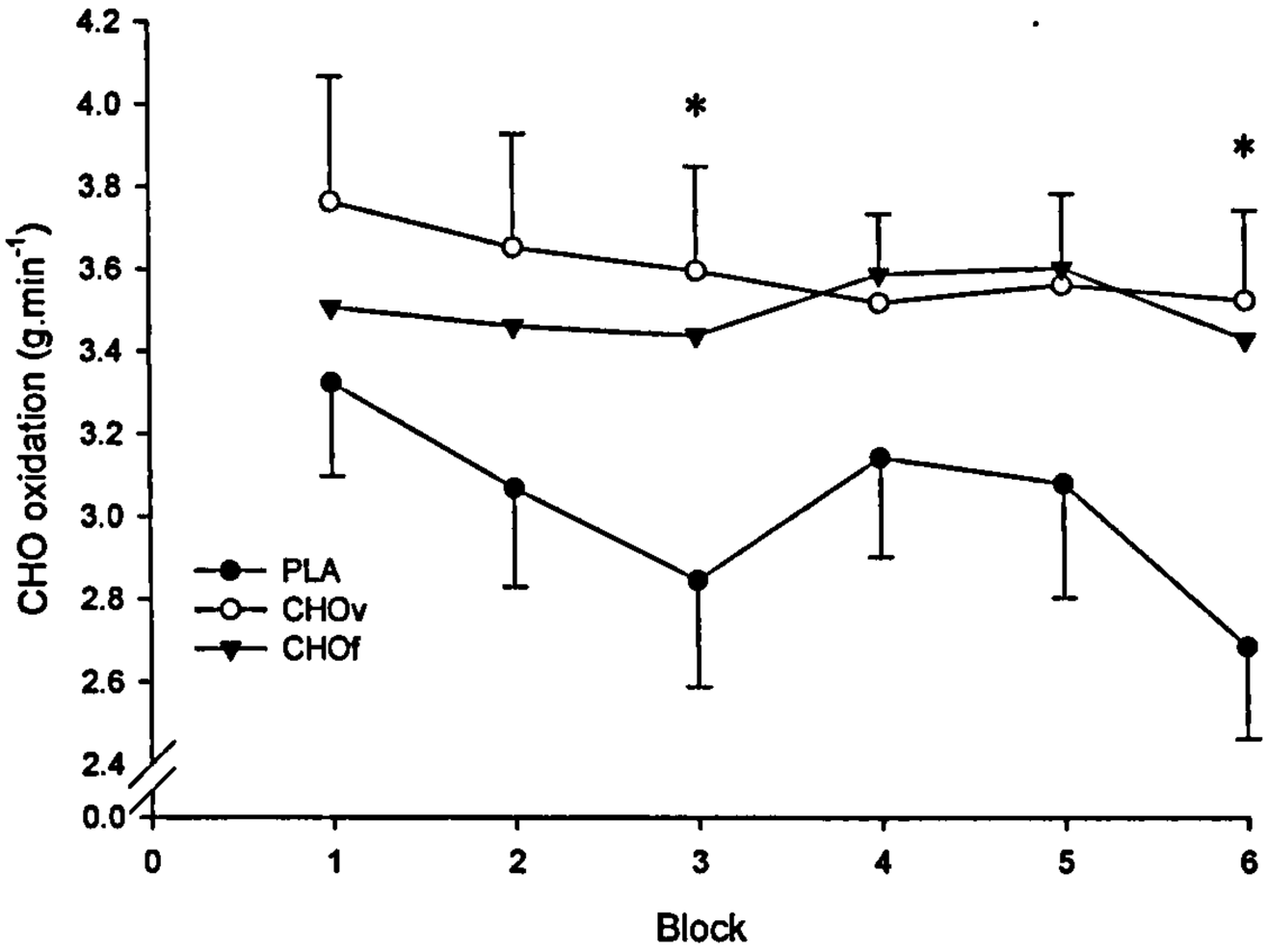

Figure 4.8: CHO oxidation during the soccer-specific protocol.

*CHOf and CHOv significantly greater than PLA.

Repeated measures ANOVA showed that the rate of fat oxidation during PLA was significantly higher ($F_{2,22}$=11.295; P<0.05, Figure 4.9) compared with the CHOf and CHOv trials but did not identify a significant effect of time ($F_{2,24}$=1.328; P>0.05) or interaction ($F_{4,40}$=1.328; P>0.05).

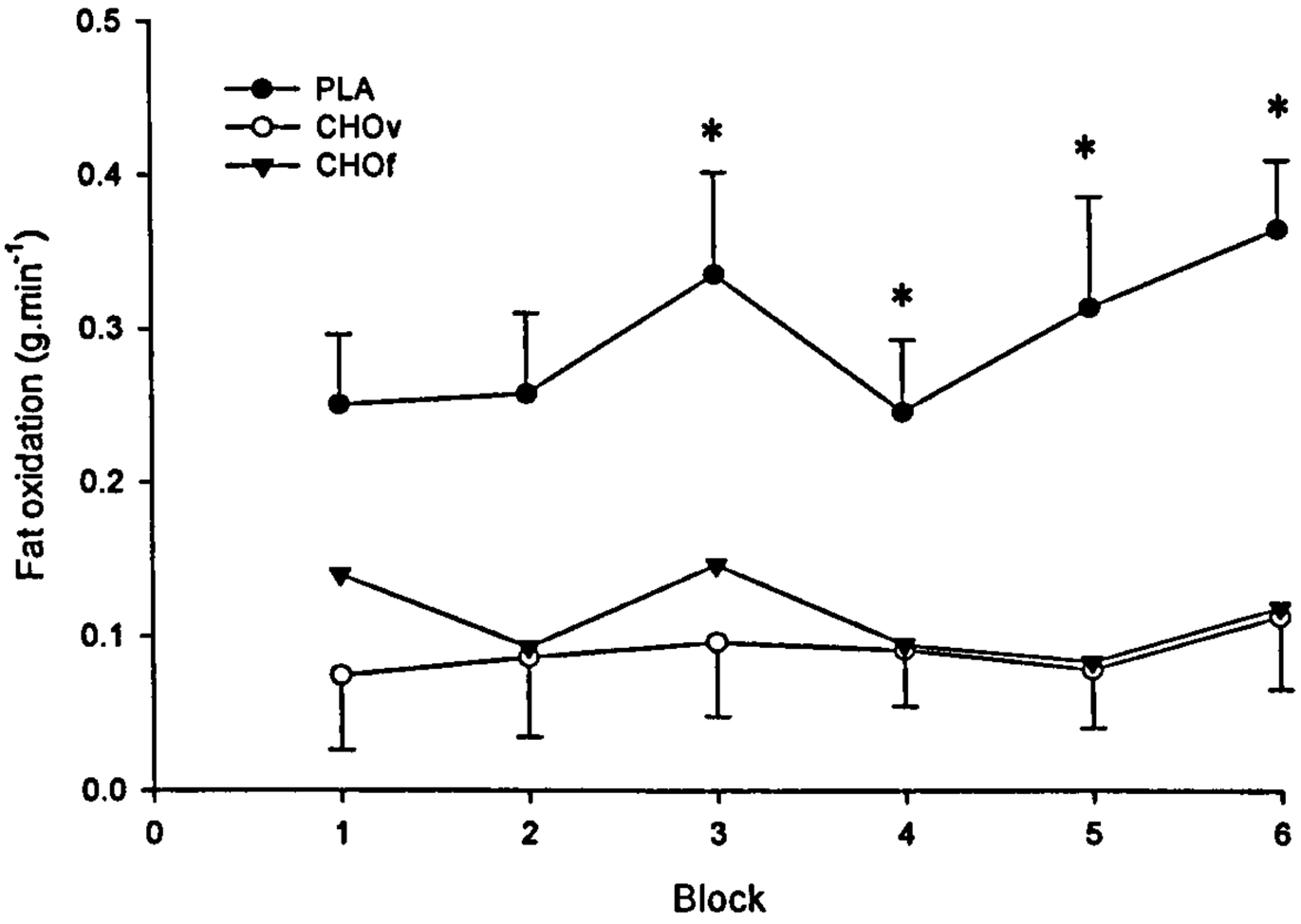

Figure 4.9: Fat oxidation during the soccer-specific protocol.

* PLA significantly greater than CHOf and CHOv.

4.3.4. Gut Fullness and thirst

Gut fullness was significantly ($F_{2,22}$=6.608; P<0.05) less during CHOf compared with PLA and CHOv (Figure 4.10). There was also a significant effect of time, ($F_{3,16}$=13.555; P<0.05); pairwise comparisons showed that gut fullness increased significantly after fluid ingestion and was significantly higher after fluid ingestion pre-exercise and at half-time compared with blocks 3 and 6. There was a significant interaction ($F_{5,51}$=3.805; P<0.05). Gut fullness was relatively constant throughout CHOf; in comparison during PLA and CHOv it increased markedly after fluid ingestion and decreased throughout each half of the soccer-specific protocol.

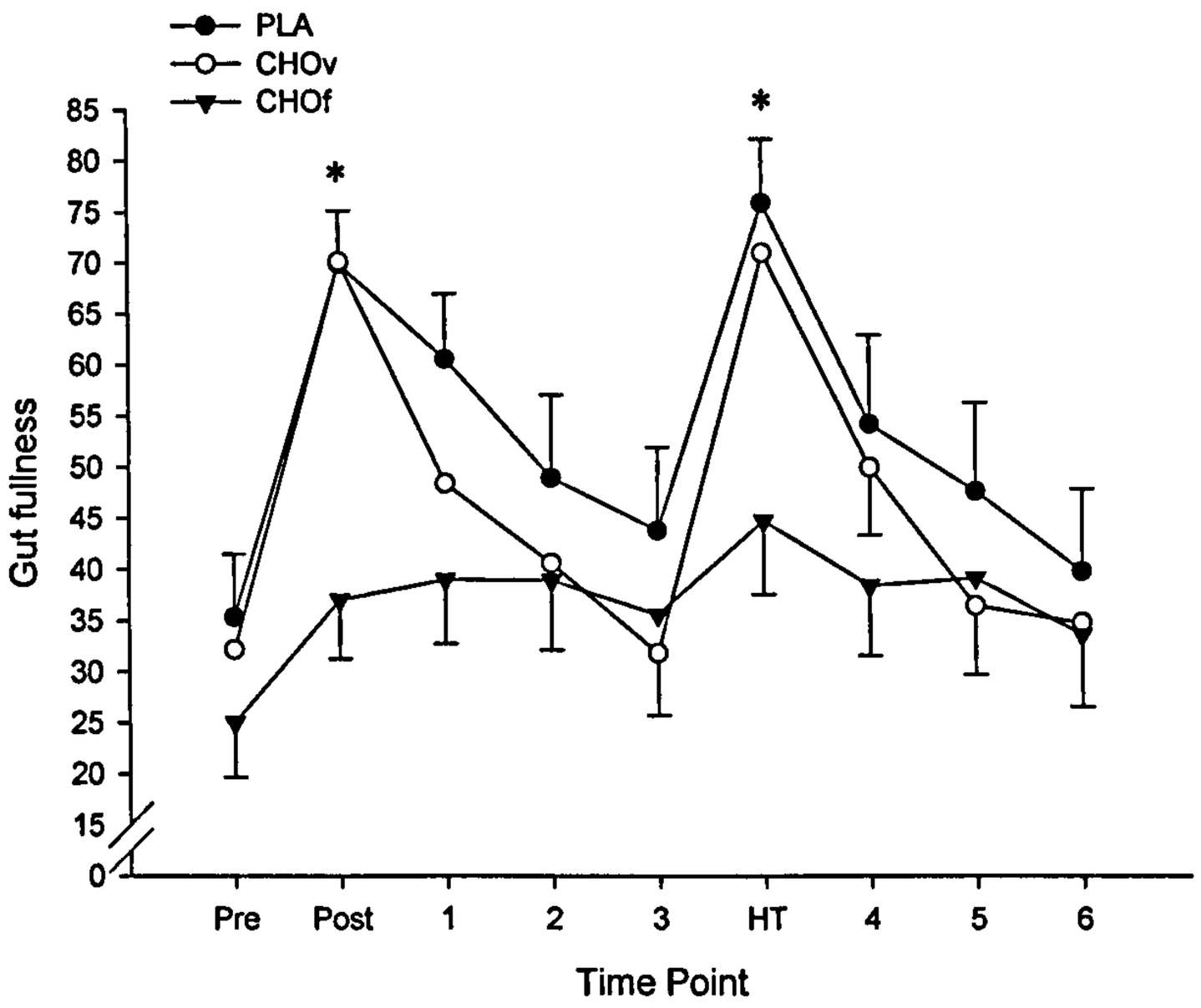

Figure 4.10: Gut Fullness during the soccer-specific protocol.

Pre – pre-fluid, Post – post-fluid, HT – half-time, 1-6 soccer-specific protocol block.
*PLAand CHOv significantly greater than CHOf.

There was no significant difference in thirst between the trials ($F_{2,22}$=2.910; P>0.05, Figure 4.11). There was a significant difference between time points, ($F_{3,29}$=15.361; P<0.05), and pairwise comparisons showed these differences occurred between post-fluid ingestion and the end of the first half. The subjective feeling of thirst was significantly

higher (P<0.05) throughout the second half, compared with half-time. A significant interaction between time and condition was also identified ($F_{16,176}$=2.776; P<0.05). The feeling of thirst was relatively consistent throughout CHOf, which was in contrast during PLA and CHOv, the feeling of thirst decreased markedly after fluid ingestion before increasing steadily throughout the subsequent 45 min.

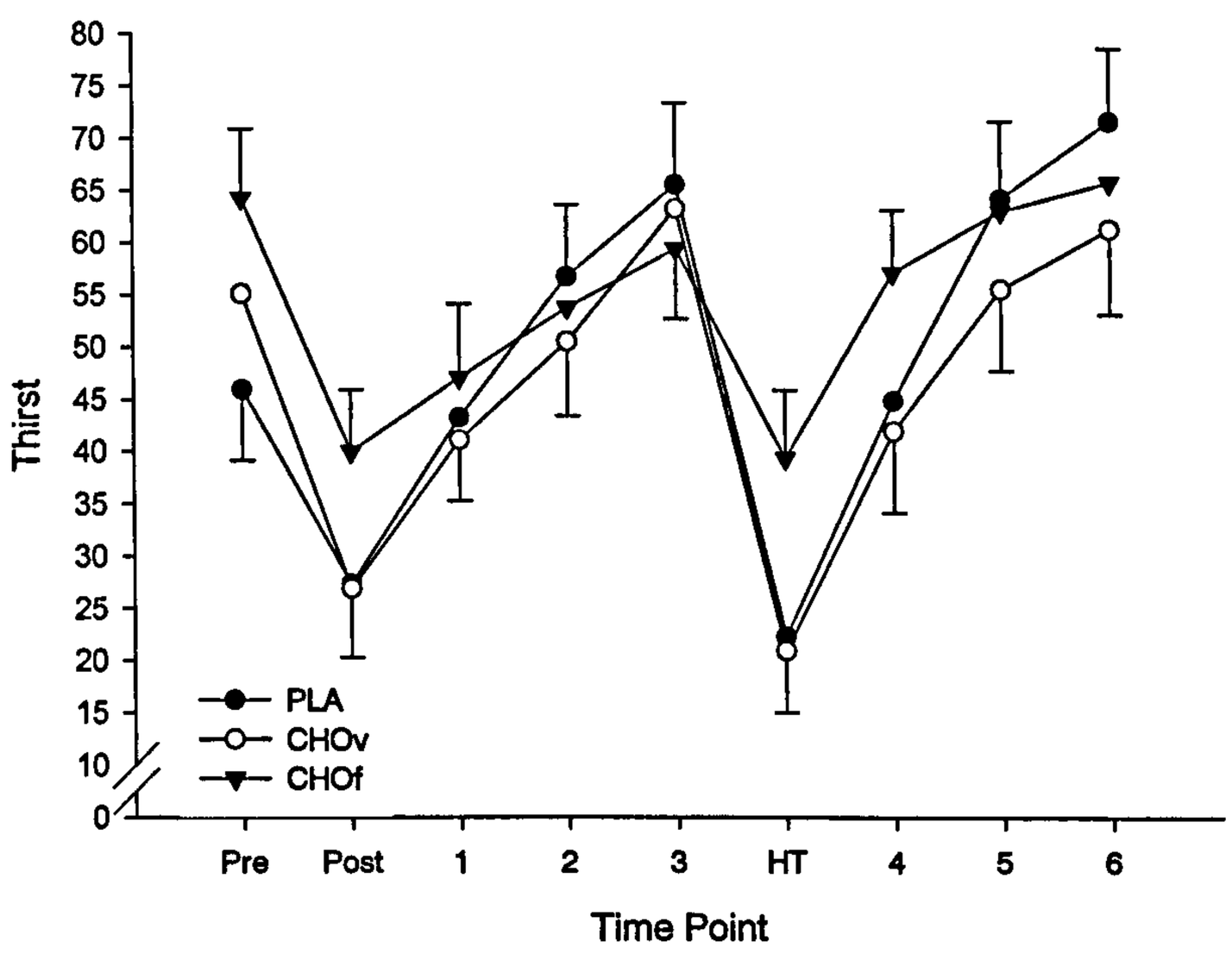

Figure 4.11: Subjective feeling of thirst during the soccer-specific protocol.
Pre – pre-fluid, Post – post-fluid, HT – half-time, 1-6 soccer-specific protocol block.

4.3.6. Core temperature

There was no significant trial effect on core temperature ($F_{2,22}$=1.993; P>0.05, Figure 4.12). A significant effect of time was observed ($F_{5,55}$=44.950; P<0.05); pairwise comparisons demonstrated these differences occurred between the first block and the following five blocks. Core temperature increased significantly (P<0.05) during each half of the soccer-specific protocol, but there was no significant difference (P>0.05) between the end of the first half and the start of the second, i.e. the half-time fall in core

temperature did not reach statistical significance. No significant interaction was identified ($F_{5,51}$=0.716; P>0.05).

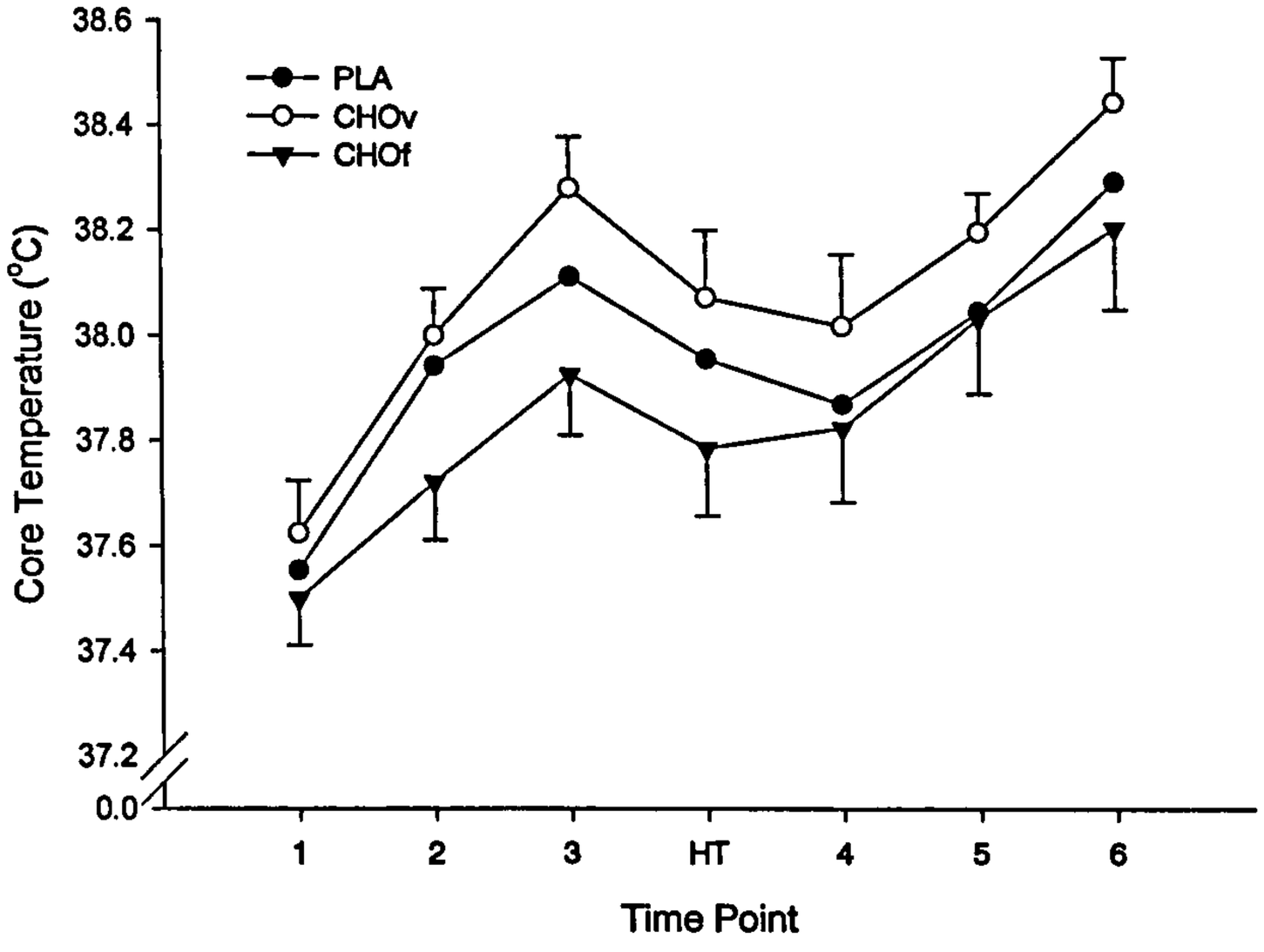

Figure 4.12: Core temperature during the soccer-specific protocol.

4.3.7. Heart rate and RPE

There was no significant trial effect on heart rate ($F_{2,22}$=0.259; P>0.05, Figure 4.13). Heart rate increased significantly ($F_{2,23}$=41.127; P<0.05) throughout each half of the soccer-specific protocol. There was also a significant interaction ($F_{3,37}$=3.210; P<0.05); heart rate increased by a greater margin during the second half of the PLA trial than during CHOv and CHOf.

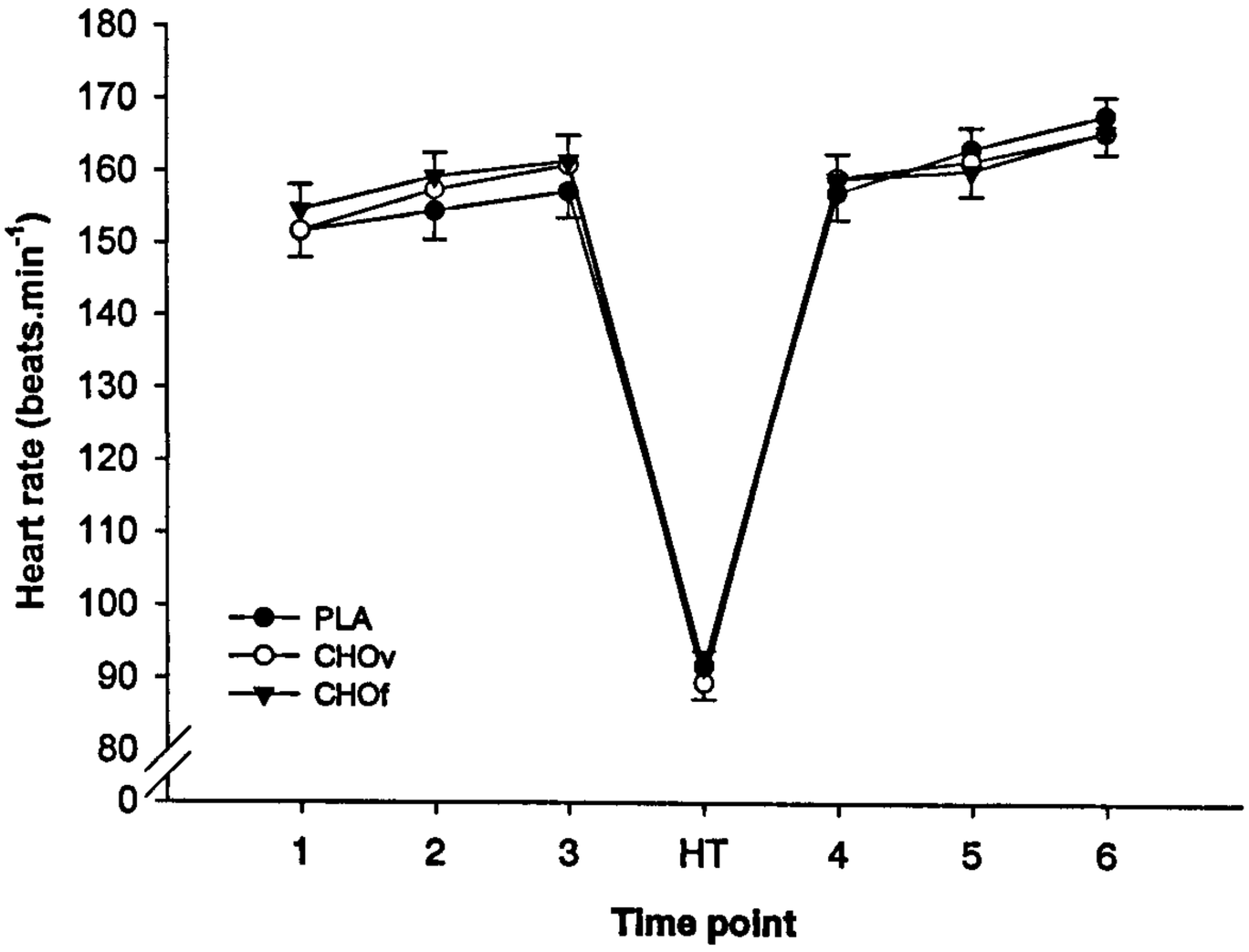

Figure 4.13: Heart rate during the soccer-specific protocol.

There was no significant ($F_{2,22}$=1.470; P>0.05) difference in RPE between trials (Figure 4.14). A significant effect of time was detected ($F_{2,19}$=57.122; P<0.05), with RPE increasing significantly throughout each half of the soccer-specific protocol although no interaction was observed ($F_{5,49}$=1.202; P>0.05).

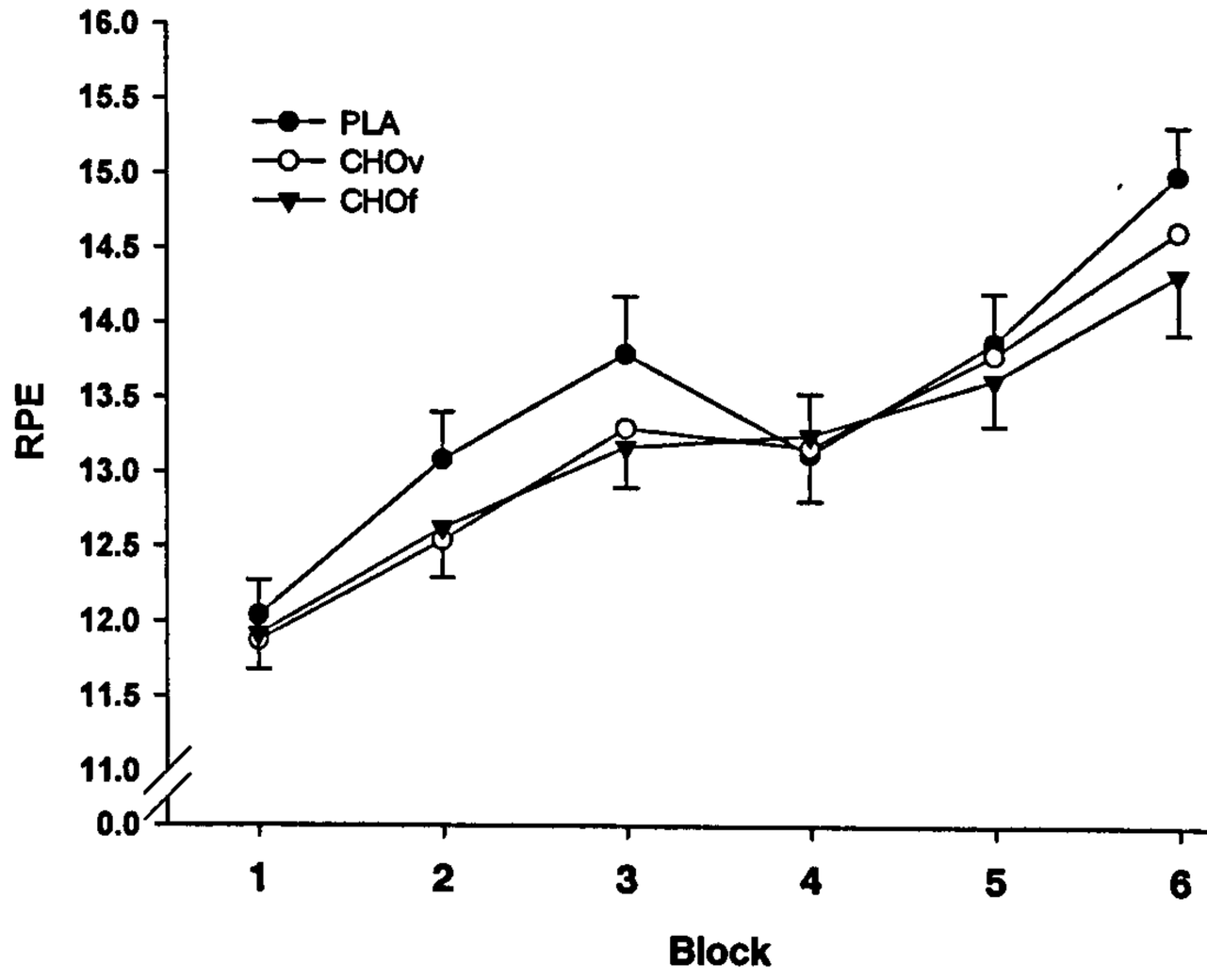

Figure 4.14: RPE during the soccer-specific protocol.

4.3.8. Sweat Loss

There was no significant difference ($F_{2,22}$=1.536; P>0.05) in sweat loss between the three trials. The mean losses were: PLA (1.43±0.10 kg), CHOv (1.22±0.08 kg) and CHOf (1.43±0.12 kg). The absolute weight loss (uncorrected for fluid ingestion) was not significantly different between trials ($F_{2,18}$=1.024; P>0.05); PLA: 0.62±0.1 kg, CHOv: 0.48±0.1 kg and CHOf: 0.67±0.1 kg.

4.4. Discussion

The main finding of the present study was that altering the timing and ingested volume of a carbohydrate-electrolyte solution did not significantly affect metabolism. In addition, consuming a carbohydrate-electrolyte solution compared with PLA significantly increased plasma glucose concentration and carbohydrate oxidation, whilst NEFA, glycerol and fat oxidation were reduced.

Two of the most probable causes of fatigue during a soccer match are dehydration and muscle glycogen depletion (Reilly, 1997). In the present study the ingestion of a carbohydrate solution, irrespective of timing and volume, during soccer-specific exercise significantly elevated plasma glucose levels. The lack of a difference between the two carbohydrate trials may have been as a consequence of the same total volume of carbohydrate being ingested. The elevated blood glucose levels associated with carbohydrate ingestion also supports previous findings (Coyle *et al.*, 1983; Wright *et al.*, 1991; Nicholas *et al.*, 1995), that the ingestion a carbohydrate solution during exercise can maintain or increase the concentration of blood glucose during exercise. Consuming a carbohydrate solution during exercise has also been demonstrated to attenuate the exercise induced decrease in plasma insulin (Coyle *et al.*, 1983; Coyle *et al.*, 1986), as was observed in the present study.

Plasma NEFA and glycerol concentrations increased during the soccer-specific protocol, with the greater increase occurring during the second half of the PLA trial. This observation suggests that consuming carbohydrate solution during exercise suppressed the release of NEFA and glycerol during CHOv and CHOf, possibly as an effect of an elevated insulin concentration. Coyle *et al.* (1991) reported that the concentration of plasma NEFA is depressed following the ingestion of carbohydrate during cycling, which is a consequence of the elevated insulin levels, as was observed in the present study. The increase in NEFA supports the findings of Bangsbo (1994b), that the concentration of NEFA in the plasma increases during a soccer match, and more so during the second half.

Plasma lactate concentrations were similar for all conditions. Previous studies (Coyle *et al.*, 1983; Nicholas *et al.*, 1995) have demonstrated that carbohydrate ingestion can alter the concentration of blood substrates, but lactate concentration is unaffected. In matches, blood lactate tends to be higher at the end of the first half (Ekblom, 1986; Stolen *et al.*, 2005). This observation may be as a consequence of a reduction in work-rate that occurs during the second half. In the present study the work-rate was not reduced in the second half and may explain why lactate values continued to rise during this period.

The adrenaline concentration increased significantly during the soccer-specific protocol, but was not significantly different between the trials. However, there was a trend for adrenaline levels to be lower at the completion of the carbohydrate trials similar to the findings of Coyle *et al.* (1983). A number of authors (Felig *et al.*, 1982; Fritzsche *et al.*, 2000) have reported that when carbohydrate is ingested the adrenaline response is blunted. This observation may be related to the large increase in insulin concentration associated with carbohydrate ingestion (Fritzsche *et al.*, 2000). However, when carbohydrate is ingested during exercise and it does not affect insulin concentration, i.e. when the carbohydrate dose is low (~ 13g $CHO \cdot h^{-1}$), the adrenaline response does not seem to be affected (Mitchell *et al.*, 1989; Burgess *et al.*, 1991).

High-intensity exercise has been associated with elevated plasma cortisol concentrations in order to maintain blood glucose concentration, and this effect can be attenuated by the consumption of carbohydrate drinks (Henson *et al.*, 1998). This was not the case in the present study, as the concentration of cortisol was highest in the two carbohydrate trials. A possible explanation for the lack of an increase in cortisol is that glucose concentration did not fall below resting levels. However, Bishop *et al.* (1999) concluded that during soccer-specific exercise the change in stress hormones was minimal and carbohydrate supplementation had a negligible effect. Carli *et al.* (1986) reported a significant increase in plasma cortisol concentration compared with pre-match levels in semi-professional players. Although this increase may have been partially due to the increased psychological tension associated with a competitive match, a similar trend was observed in the present study.

Similar patterns of RER, carbohydrate oxidation and fat oxidation were observed when carbohydrate was consumed during exercise, irrespective of timing and volume. The RER gradually declined during the soccer-specific protocol but was significantly lower than in the other conditions during PLA. This observation indicates an increased fat oxidation, and consequently decreased carbohydrate oxidation, during the soccer-specific protocol, and is reflected in the carbohydrate oxidation rate being significantly lower and fat oxidation significantly higher during PLA. This result is similar to the findings of Wright *et al.* (1991), where in the trials in which carbohydrate was consumed, either during exercise or as a pre-exercise meal, both RER and carbohydrate oxidation were significantly elevated compared to the placebo trial. The higher RER and carbohydrate oxidation after carbohydrate feedings were attributed to either greater muscle glycogenolysis or glucose uptake and oxidation (Wright *et al.*, 1991), reflected in the significantly higher rate of carbohydrate oxidation during CHOf and CHOv compared to PLA in the present study. Since it appears that carbohydrate feeding reduces muscle glycogen degradation during prolonged exercise, especially in type 1 muscle fibres (Tsintzas *et al.*, 1995) and intermittent exercise (Nicholas *et al.*, 1994; Nicholas *et al.*, 1999), the higher carbohydrate oxidation was most likely caused by increased blood glucose uptake and oxidation.

Plasma osmolality did not increase significantly during the soccer-specific protocol, remaining within normal values. This observation indicates that the subjects did not suffer from severe dehydration, a conclusion supported by a mean weight loss (uncorrected for fluid consumption) of 0.77%. These results suggest that 7 $ml \cdot kg^{-1}$ ingested at the start of each half is an adequate volume of fluid to consume to prevent dehydration during soccer-specific exercise, when performed in a moderate ambient temperature. The similar sweat loss values for the trials suggest that it is the total volume of fluid that is important in preventing dehydration, more so than the timing and volume.

Despite the different timings and volume of fluid consumed there were no significant differences between trials in the subjective feeling of thirst. This observation is in contrast to Ferguson *et al.* (2005), who reported that the thirst response was higher when

a small volume of fluid was consumed at 15 min intervals during exercise compared with a single bolus pre-exercise. Although towards the end of exercise, when similar volumes had been ingested for both conditions, thirst was similar. However, this study was performed in the heat, which is likely to increase the sensation of thirst due to increased sweat loss. In the present study, irrespective of volume, thirst decreased significantly following the consumption of fluid at rest, prior to the soccer-specific intermittent protocol and at half-time. Gut fullness was significantly lower and relatively constant during CHOf compared with PLA and CHOv. In contrast during PLA and CHOv gut fullness increased sharply after fluid ingestion and decreased throughout the half. Consuming large volumes of fluid increases gut fullness (perceived or actual), which may cause discomfort and adversely influence performance. Therefore, a small volume of fluid consumed regularly may be a preferable option. This option may not be practical during a soccer match, as there are not any scheduled breaks in play where fluid can be ingested.

There were no statistical differences in either heart rate or RPE between trials during the soccer-specific intermittent protocol, supporting the findings of Nicholas *et al.* (1995). Core temperature increased significantly during all of the trials, but there was no difference between trials. These findings indicate that the physiological stress imposed by the protocol was similar in all three conditions. In contrast, previous studies have reported lower heart rate (Melin *et al.*, 1994; Ferguson *et al.*, 2005) and core temperature (Melin *et al.*, 1994) following a single bolus compared with intermittent fluid intake, although these studies were low intensity (50% $\dot{V}O_{2max}$) and performed in the heat .

In conclusion, ingesting carbohydrate-electrolyte solution significantly affected plasma metabolites and increased carbohydrate oxidation. Also, when the total volume of fluid consumed was equal, manipulating the timing and volume of carbohydrate ingestion elicited the same metabolic responses. Furthermore, consuming a small volume of fluid at regular intervals, compared with a single large volume before and at half-time of the soccer-specific protocol, resulted in a reduced sensation of gut fullness. As there are no scheduled breaks in soccer matches the results suggest that ingesting carbohydrate in a

sports drink before a game and again at half-time is a practical strategy for fluid provision.

Chapter 5
Study 2

The previous study demonstrated that ingesting carbohydrate prior to, and during soccer-specific exercise significantly increased plasma glucose and carbohydrate oxidation. Increased blood glucose has been demonstrated to maintain carbohydrate oxidation during exercise which can improve performance. The effect of manipulating the timing and volume of carbohydrate ingestion on sprint power output during soccer-specific exercise was the subject of investigation in this study.

5.1. Introduction

Soccer players tend to cover less distance in the second half of a match compared with the first half (Reilly and Thomas, 1976; Bangsbo *et al.*, 1991; Bangsbo, 1994b), the reduction in work-rate being a sign of fatigue. One of the likely causes for the decline in distance covered is a reduction in muscle glycogen content. Saltin (1973) demonstrated that players with low glycogen content in the *vastus lateralis* muscle at the start of the game covered 25% less distance than the other players with "normal" levels. The lower muscle glycogen also altered running speed. Players with low pre-match glycogen stores covered 50% of the total distance walking and 15% sprinting, in contrast to the players with high concentrations who covered 27% walking and 24% sprinting.

Another potential cause of fatigue during a soccer match is dehydration and a mild level of dehydration (Walsh *et al.*, 1994) can limit exercise performance. It is, therefore important that athletes consume fluid during prolonged exercise. Consequently, much research has focused on rehydration during soccer matches and soccer-specific exercise (Kirkendall *et al.*, 1988; Nicholas *et al.*, 1995; McGregor *et al.*, 1999). The addition of carbohydrate to this fluid can further improve exercise capacity (Nicholas *et al.*, 1995), possibly due to the sparing of muscle glycogen and delaying the onset of fatigue (Leatt and Jacobs, 1989). Therefore, carbohydrate provision and rehydration may be key factors influencing performance during a game. Some authors have investigated the impact of carbohydrate ingestion on exercise capacity during exercise corresponding to a simulation of the intensity of a soccer match (Nicholas *et al.*, 1995; Walton and Rhodes, 1997) and actual match-play (Kirkendall *et al.*, 1988; Leatt and Jacobs, 1989; Zeederberg

et al., 1996) and have reported improvement in terms of endurance time and muscle glycogen content.

Gastric emptying is deemed to be a limiting factor in fluid replacement (Shi and Gisolfi, 1998) and is an important aspect in determining the rate at which nutrients enter the duodenum where glucose and water can be absorbed into the bloodstream (Brouns *et al.*, 1987). Studies using a single large ingestion (Costill and Saltin, 1974) or repetitive smaller ingestions (Rehrer *et al.*, 1992; Duchman *et al.*, 1997) have demonstrated that the maximum rate at which water and carbohydrate can be delivered from an ingested solution is influenced by the average volume of fluid in the stomach, which in turn is determined by the volume ingested and the drinking pattern. The drinking strategy employed in the majority of studies investigating fluid provision during soccer-specific exercise or competitive matches has been to ingest a large volume before the activity and again at half-time, or a large volume at the start with a small volume throughout the protocol. This is a strategy that is potentially uncomfortable, as ingesting a large volume of fluid during exercise, especially when running, is likely to lead to feelings of abdominal discomfort, possibly due to the accumulation of unabsorbed fluid in the small intestine or colon (Noakes, 1993). Despite the intensity associated with a soccer match being sufficient to slow gastric emptying (Leiper *et al.*, 2001; Leiper *et al.*, 2005), no previous study has focused on the effect of consuming small repetitive doses on performance of soccer-specific exercise.

As a consequence of the acyclic nature of activity in soccer, there are no scheduled breaks where fluid can be consumed; besides, gastric tolerance and the perception of gut fullness do not allow for adequate rehydration for soccer players. Due to play being continuous, with infrequent, unscheduled stoppages, the only two occasions that a player is guaranteed to be able to consume fluid are before the game and at half-time. In the American College of Sports Medicine's position stand on exercise and fluid replacement (Convertino *et al.*, 1996) it is stated that during exercise, athletes should start drinking early and at regular intervals in an attempt to consume fluids at a rate sufficient to replace

the water lost through sweating, or consume the maximal amount that can be tolerated. Most advice regarding rehydration during exercise has been based on continuous exercise e.g. cycling and road-running or sports where there are opportunities for breaks when fluid can be consumed e.g. American Football and basketball.

The aim of this study was to compare the effect ingesting a large volume of sports drink before and at half-time of a soccer-specific protocol with ingesting the same total volume but in frequent smaller doses during the protocol on exercise performance assessed by measuring power output during repetitive brief sprints.

5.2. Methods

5.2.1. Subjects

Twelve male university soccer players participated in this study. Mean (±SEM) age: 25±3 years; height: 1.77±0.1 m; body mass: 74.5±6 kg; $\dot{V}O_{2max}$: 59.4±6 $ml \cdot kg^{-1} \cdot min^{-1}$. All subjects provided written informed consent to participate, in accordance with Liverpool John Moores University's ethical procedures.

5.2.2. Experimental Protocol

The subjects undertook two familiarisation sessions, consisting of two cycles of the soccer-specific intermittent protocol (i.e. 30 minutes). Before the first familiarisation session, 500 ml of carbohydrate electrolyte solution (Still Lucozade Sport, (6.35±0.05 $g.100\ ml^{-1}$ CHO, 48±1 $mg.100\ ml^{-1}$ Na) GlaxoSmithKline, Gloucestershire, UK) was consumed whereas 500 ml of a similarly coloured, flavoured and textured placebo (GlaxoSmithKline, Gloucestershire, UK) was consumed before the start of the second familiarisation trial. These procedures ensured that there were no adverse gut reactions to the volume and composition of the fluid consumed and reliable sprint power outputs were obtained (CV = 6.9%) within this 30-min period. The second of the familiarisation sessions took place at least 5 days before the first trial.

During the subsequent sessions, subjects completed a soccer-specific intermittent exercise protocol (Drust *et al.*, 2000a) on a non-motorised treadmill (Woodway, Vordem, Auf Schrauben, Germany). The soccer-specific intermittent protocol consisted of 90 min activity divided into 2 x 45 min identical blocks, separated by a period of 15 min, representing half-time. Each 45-min block consisted of three 15-min cycles of different exercise intensities associated with competitive soccer (e.g. walking, jogging and sprinting). The movement categories incorporated in this protocol were walking, jogging,

cruising and sprinting. Static periods, where the subject remained stationary on the treadmill were also incorporated although due to the technical limitations of the equipment, utility movements (sideways and backwards) were not included within the protocol. The 15-min cycle of activity consisted of 33 discrete bouts, 9 walking bouts, 9 jogging bouts, 3 cruises (sub-maximal high-intensity), 3 sprints and 9 static pauses. The duration of each bout was as follows: walking 47.3 s, jogging 33 s, cruising 15.3 s, sprint 3.3 s and static 15.3 s. These durations were determined by matching the percentage of the total time observed during match-play to that during each sub-cycle (Drust, 1997). The order of these bouts was arranged so that periods of high-intensity activity were separated by periods of low-intensity recovery periods and static pauses to simulate the acyclical nature of the exercise pattern experienced during soccer (Drust *et al.*, 2000a).

The treadmill speeds for each activity were designated on the basis of Van Gool *et al.* (1988) with a correction for the intrinsic resistance associated with running on a non-motorised treadmill. It has been demonstrated that this resistance can reduce maximal velocity by approximately 20%, compared to normal running (Lakomy, 1987). Therefore the speeds selected for each activity were as follows: walking 4 $km.h^{-1}$, jogging 8 $km.h^{-1}$, cruising 10 $km \cdot h^{-1}$. There were not any speed restrictions placed on sprinting as the subjects were instructed to produce maximum effort and this response constituted as a performance measure.

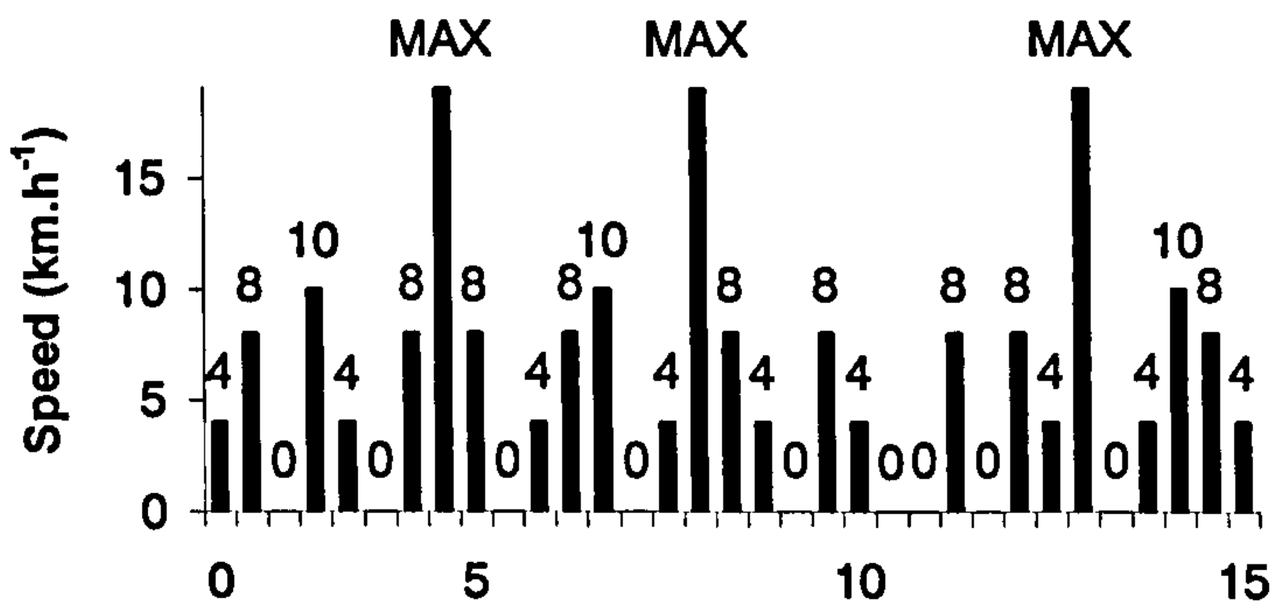

Figure 5.1: Graphical representation of soccer-specific intermittent protocol.

The full soccer-specific protocol was performed on three occasions, and consisted of 90 min of activity. The 90-min period was divided into two 45-min identical blocks, separated by a 15-min half-time break. On two occasions either 7 $ml \cdot kg^{-1}$ BM of carbohydrate-electrolyte (CHOv) or placebo (PLA) solution was ingested before and at half-time (mean 533±11 ml; i.e. mean total 1065±22 ml). On a third occasion the same volume of carbohydrate-electrolyte solution was consumed (CHOf) but in smaller volumes at 0, 15, 30, 45, 60 and 75 min (mean 177±4 ml) during the final walking phase of each block. During the carbohydrate trials the total amount of carbohydrate ingested was 67.71±1.40 g CHO. Subjects acted as their own controls in a double-blind repeated-measures crossover design with the order randomly assigned. The soccer-specific protocol was performed in "normal" laboratory conditions (mean temperature 18.9±0.4 °C, relative humidity 59.3±4 %). A standard 15-min warm up was performed, consisting of jogging, sprinting and stretching, before the subject began the 90 min of exercise.

For the three days prior to the first test session, subjects completed a diet and physical activity diary, which provided a dietary template prior to subsequent trials and was analysed using Microdiet for Windows Version 1.2 (Downlee Systems Limited, High Peak, UK).

5.2.3. Physiological Measurements

Ratings of perceived exertion (Borg, 1970), gut fullness and thirst (Wu *et al.*, 2003) were recorded during the double static period (10 min) of each 15-min block. Gut fullness and thirst were also recorded immediately before and after fluid ingestion prior to commencing exercise. Heart rate was measured continuously by means of a short–range radio telemetry system (Polar S610i, Polar Electro, Kempele, Finland) and was presented as the mean value for each 15-min block. During each sprint phase, the power output was recorded to monitor performance and indicate the occurrence of fatigue. Power output was calculated using the horizontal component of applied force (the restraining forced,

measured using a force transducer) and the treadmill belt speed (Figure 5.2) (Lakomy, 1987). Peak power output was defined as the maximum value obtained during each sprint.

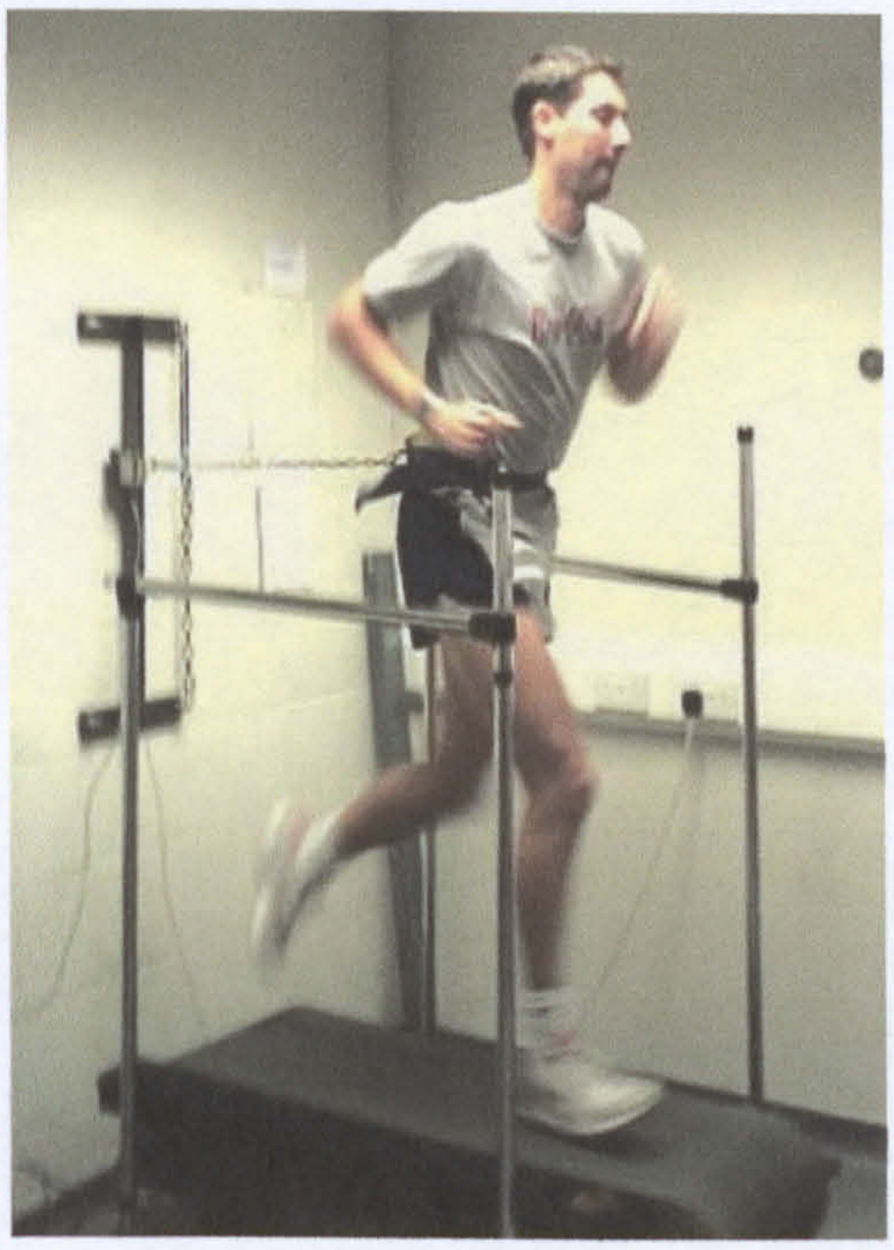

Figure 5.2: Subject performing the soccer-specific protocol on a non-motorised treadmill.

To assess the reliability of peak power output during sprinting, twelve university soccer players, all of whom were familiar with treadmill sprinting, performed six maximal 3.3 s sprints during 30 min of soccer-specific exercise, on two occasions 1 week apart. Both tests were performed on a non-motorized treadmill (Woodway, Vordem, Auf Schrauben, Germany), interfaced with a data acquisition system. Using a two-way ANOVA with repeated measures, there were no significant differences (P>0.05) between power output for repeated trials on the same day or for repeated trials on different days. The coefficient of variation for the measure of peak power output was 6.9%. Hence, the treadmill system and protocol provided a reliable measure of peak power output.

5.2.4. Assessment of respiratory gases during exercise

Oxygen consumption ($\dot{V}O_2$), $\dot{V}CO_2$, RER and $\dot{V}E$ were recorded for 2 min using an on-line automated gas analyser (Metalyzer3B, Cortex Biophysic GmbH, Leipzig, Germany) after 10 min of each block. Carbohydrate and fat oxidation rates were calculated using the stoichiometric equations of Frayn (1983) as described in section 3.2.3.

5.2.5. Measurement of core body temperature

Core body temperature was monitored continuously by means of an ingestible temperature sensor pill and external data logger (HQ inc., Florida, USA) as described in section 3.3 Data were presented as the mean value for each 15-min block.

5.2.6. Blood sampling and analysis

Venous blood samples (14 ml) were drawn prior to exercise (0-min), at half-time (immediately after the completion of the 45 min) and at completion of each trial (90-min). The blood samples were later analysed for glucose, NEFA, glycerol, lactate, catecholamines and cortisol as described in section 3.7.

5.2.7. Statistics

All variables were analysed using two-way ANOVAs with repeated measures except for sweat loss, which was analysed using a one-way ANOVA with repeated measures. All results are reported as the mean ± the standard error of the mean (SEM) and a level of $P<0.05$ was considered statistically significant.

5.3. Results

5.3.1. Pre-trial conditions

The pre-trial conditions were similar for all trials (Table 5.1). There were no significant differences in the carbohydrate ($F_{1,,11}$=1.920; P>0.05) or energy ($F_{1,1}$=0.122; P>0.05) content of the participant's pre-trial diet. Pre-trial hydration status was similar for all conditions, urine colour $F_{2,22}$=1.055; P>0.05) and osmolality ($F_{2,22}$=1.311; P>0.05) were not significantly different.

Table 5.1: Pre-trial dietary and hydration status.

	Diet		Urine	
Trial	**CHO (%)**	**Energy ($MJ \cdot d^{-1}$)**	**Colour**	**Osmolality ($mOsm \cdot kg^{-1}$)**
PLA	54.6±1.6	7.1±0.4	2.4±0.3	354.8±53.4
CHOv	53.5±1.7	7.1±0.5	2.1±0.3	299.6±47.2
CHOf	53.7±1.6	7.1±0.5	2.0±0.3	293.4±58.8

5.3.2. Power output

There was no significant effect of the treatments on peak power output during sprinting ($F_{2,22}$=0.133; P>0.05) (PLA: 1080±70 W; CHOv: 1104±66 W; CHOf: 1091±39W) (Figure 5.3). Peak power output remained constant throughout each trial ($F_{5,55}$=1.379; P>0.05).

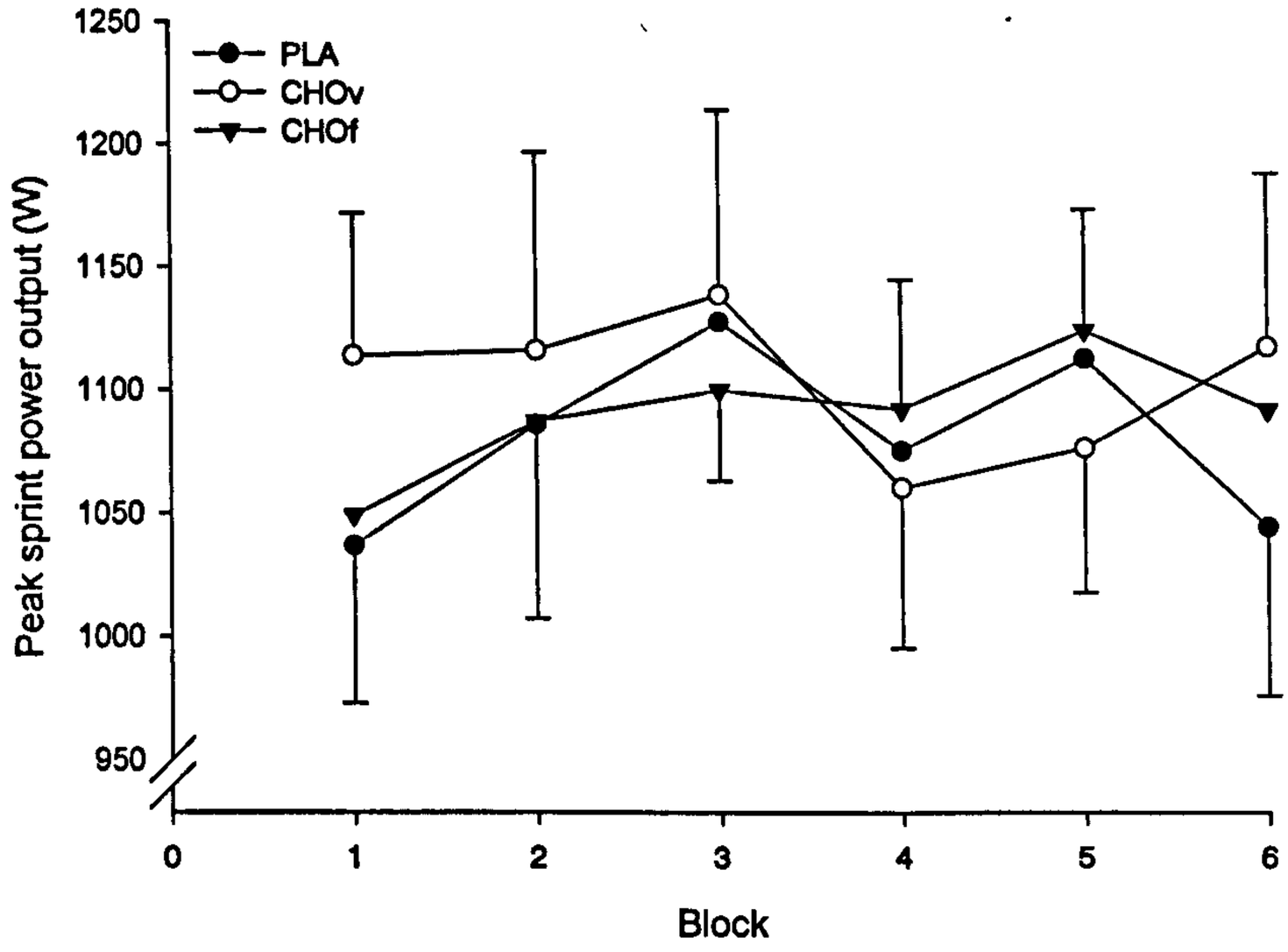

Figure 5.3: Peak sprint power output during the soccer-specific protocol.

5.3.3. Plasma metabolites

Plasma glucose concentration (Figure 5.4) was not significantly different between CHOv and CHOf pre-exercise, at half-time or post-exercise. However, plasma glucose was significantly higher during CHOf compared with PLA ($F_{2,22}$=4.909; P<0.05) at half-time and post-exercise. For all trials plasma glucose was significantly lower post-exercise than at half-time ($F_{2,22}$=21.197; P<0.05).

The concentration of NEFA was not significantly different between CHOv and CHOf (Figure 5.5), although it was significantly higher during PLA at half-time and post-exercise compared with CHOv and CHOf ($F_{2,22}$=22.802; P>0.05). Plasma NEFA concentration increased significantly between each time point ($F_{1,13}$=35.809; P<0.05). Glycerol concentration was significantly higher post-exercise following PLA compared to CHOv and CHOf (Figure 5.6), and increased significantly between each time point ($F_{1.1,13}$=61.592; P<0.05).

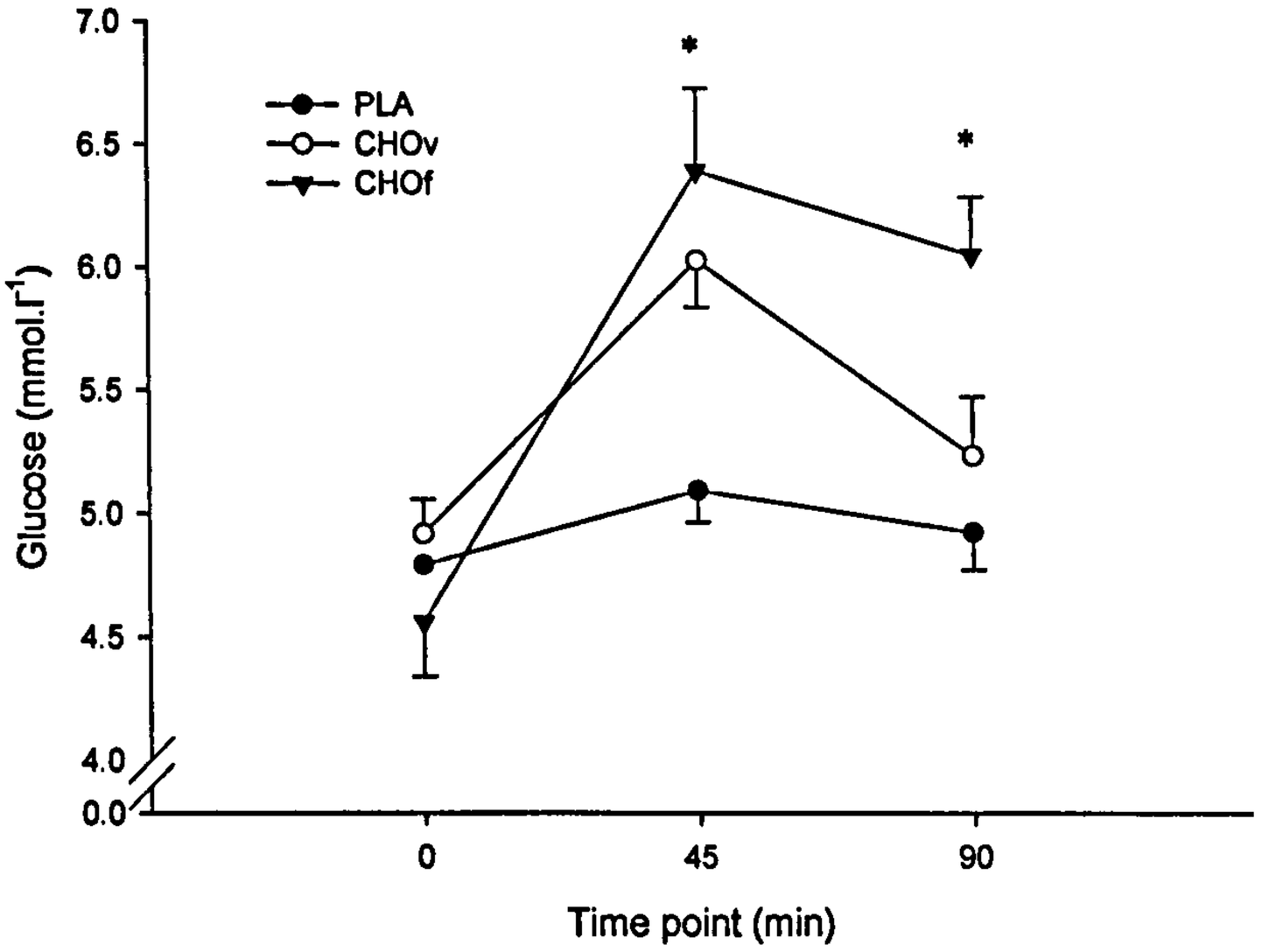

Figure 5.4: Plasma glucose concentration during the soccer-specific protocol.

*CHOf significantly greater than PLA.

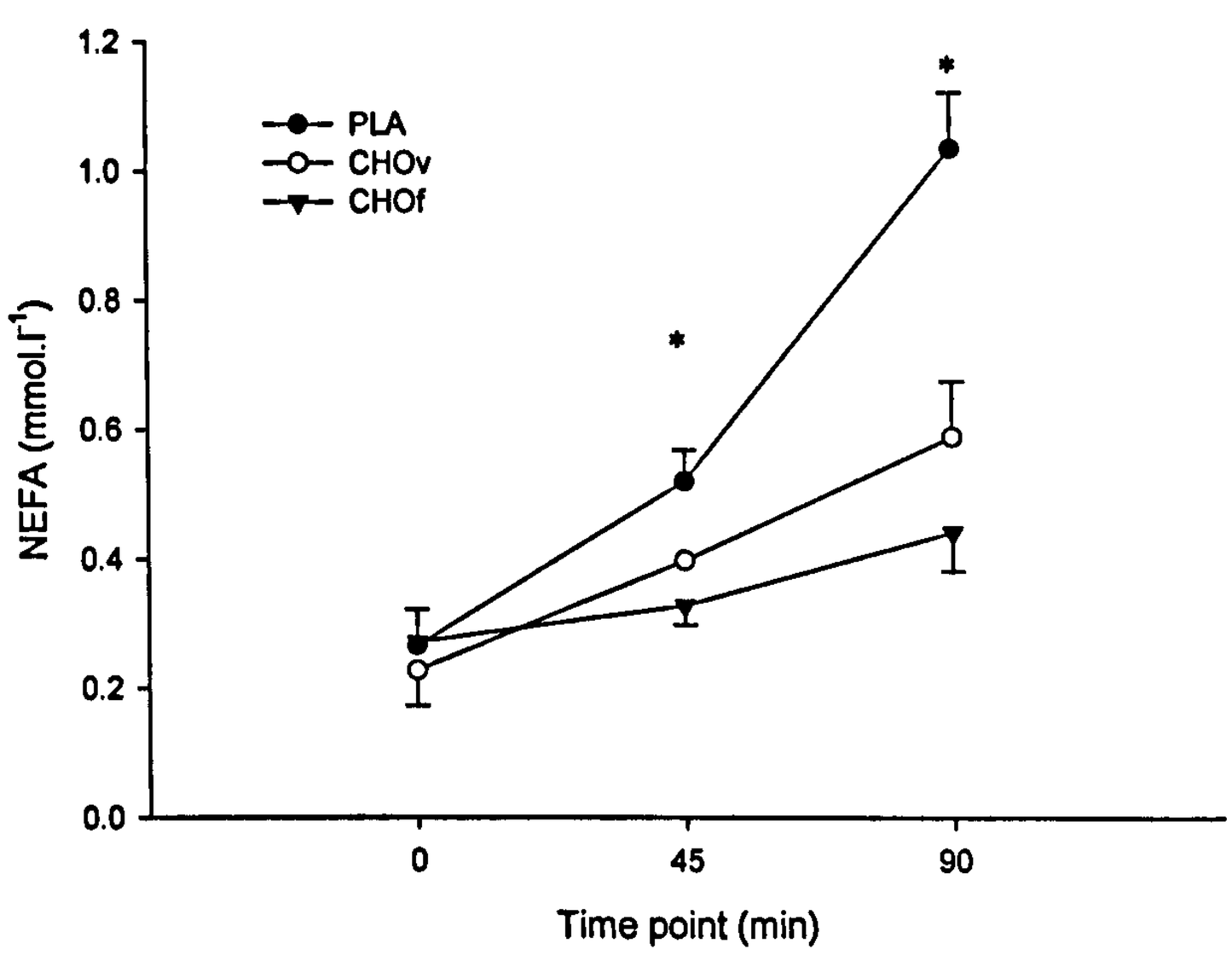

Figure 5.5: Plasma NEFA concentration during the soccer-specific protocol.

* PLA significantly greater than CHOf and CHOv.

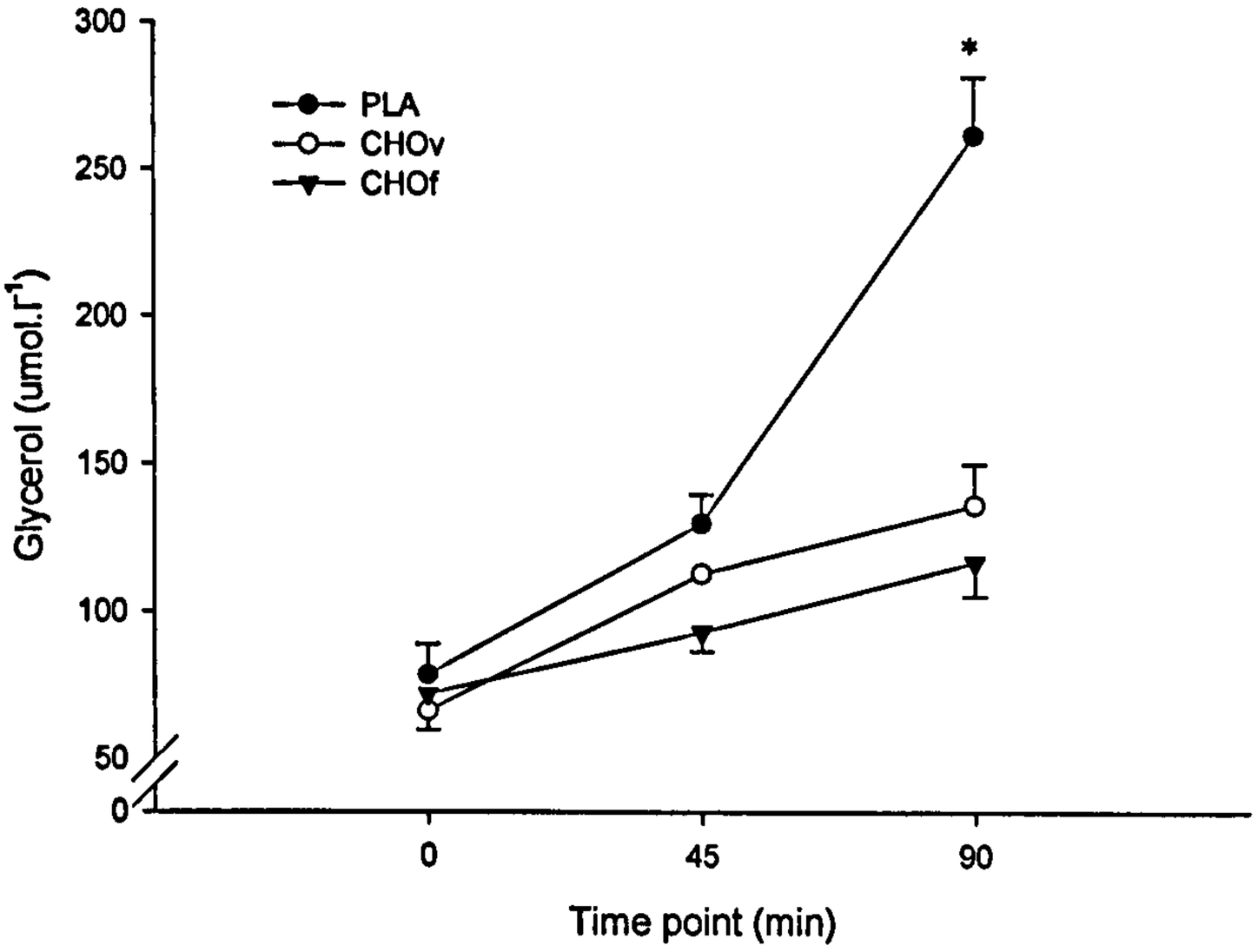

Figure 5.6: Plasma glycerol concentration during soccer-specific protocol.

* PLA significantly greater than. CHOf and CHOv.

Lactate concentration was not significantly different between any of the trials ($F_{2,22}$=0.583; P>0.05), although it increased significantly above resting levels after the onset of exercise, with peak values at half-time (PLA: 2.8±0.6 mmol.l^{-1}; CHOv: 3.3±0.4 mmol.l^{-1}; CHOf: 3.0±0.6 mmol.l^{-1}) ($F_{1,12}$=10.592; P<0.05). Mean plasma osmolality during the soccer-specific protocol was not significantly affected by the trials (PLA: 279.7±3 mOsm·kg^{-1}; CHOv: 278.3±3 mOsm·kg^{-1}; CHOf: 279.6±3 mOsm·kg^{-1}) ($F_{2,22}$=0.071; P>0.05). There were no significant differences between trials in plasma volume changes (PLA: -1.32±0.3%; CHOv: -1.71±0.2%; CHOf: -1.24±0.3%) ($F_{2,22}$=1.447; P>0.05) or sweat loss (PLA: 1.80±0.2 kg; CHOv: 1.62±0.1 kg; CHOf: 1.58±0.1 kg) ($F_{1,11}$=0.605; P>0.05). The absolute weight loss (uncorrected for fluid ingestion) was not significantly different between trials ($F_{1,11}$=0.710; P>0.05); PLA: 0.76±0.3 kg, CHOv: 0.53±0.1 kg and CHOf: 0.60±0.1 kg.

5.3.4. Hormones

Adrenaline levels (Figure 5.7) were found to be similar during all trials ($F_{2,22}$=0.609; P>0.05) with significant increases between each time point ($F_{1.,13}$=127.216; P<0.05). A similar pattern was observed for noradrenaline levels ($F_{2,22}$=185.51; P<0.05, Figure 5.8), with no significant effect of the treatments ($F_{2,22}$=0.091; P>0.05).

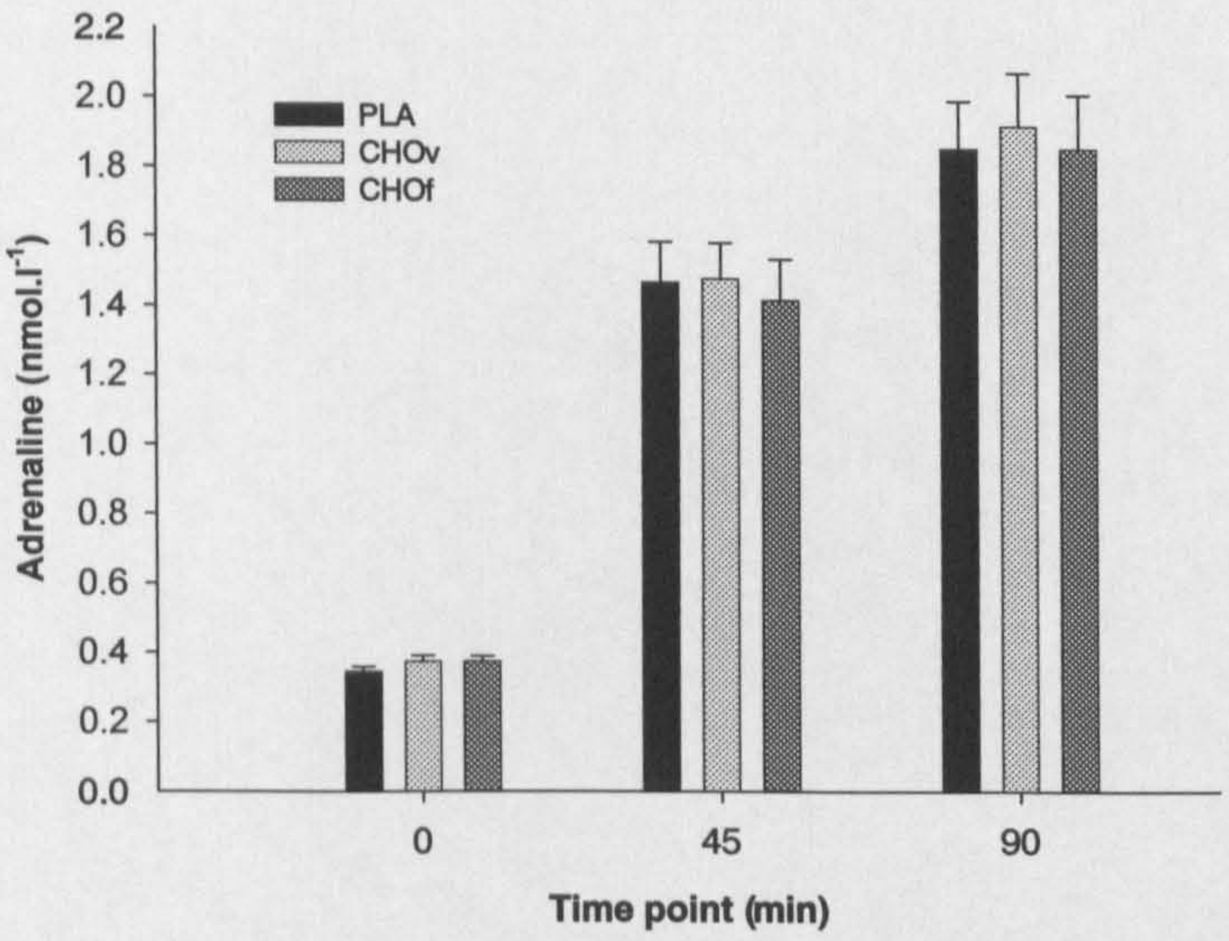

Figure 5.7: Adrenaline concentration during the soccer-specific protocol.

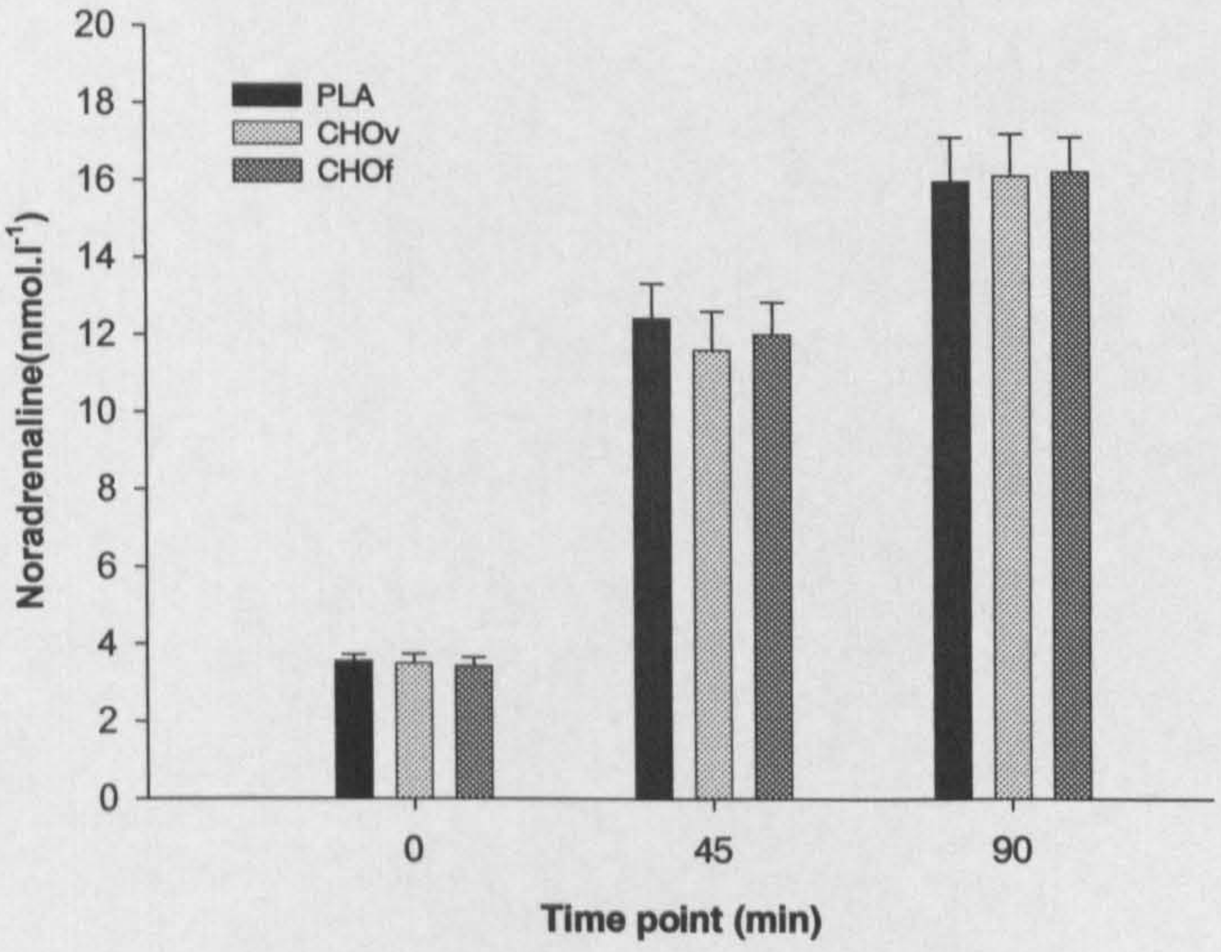

Figure 5.8: Noradrenaline concentration during the soccer-specific protocol.

Cortisol concentration was significantly elevated post-exercise for CHOf (257.9±27.4 nmol.l^{-1}) compared with PLA (186.4±24.4 nmol.l^{-1}) ($F_{2,22}$=4.053; P<0.05; Figure 5.9), although no significant difference was identified between CHOv and CHOf. A significant effect of time ($F_{2,22}$=4.937; P<0.05) was observed, values were significantly lower at full time, compared with pre-exercise and half-time.

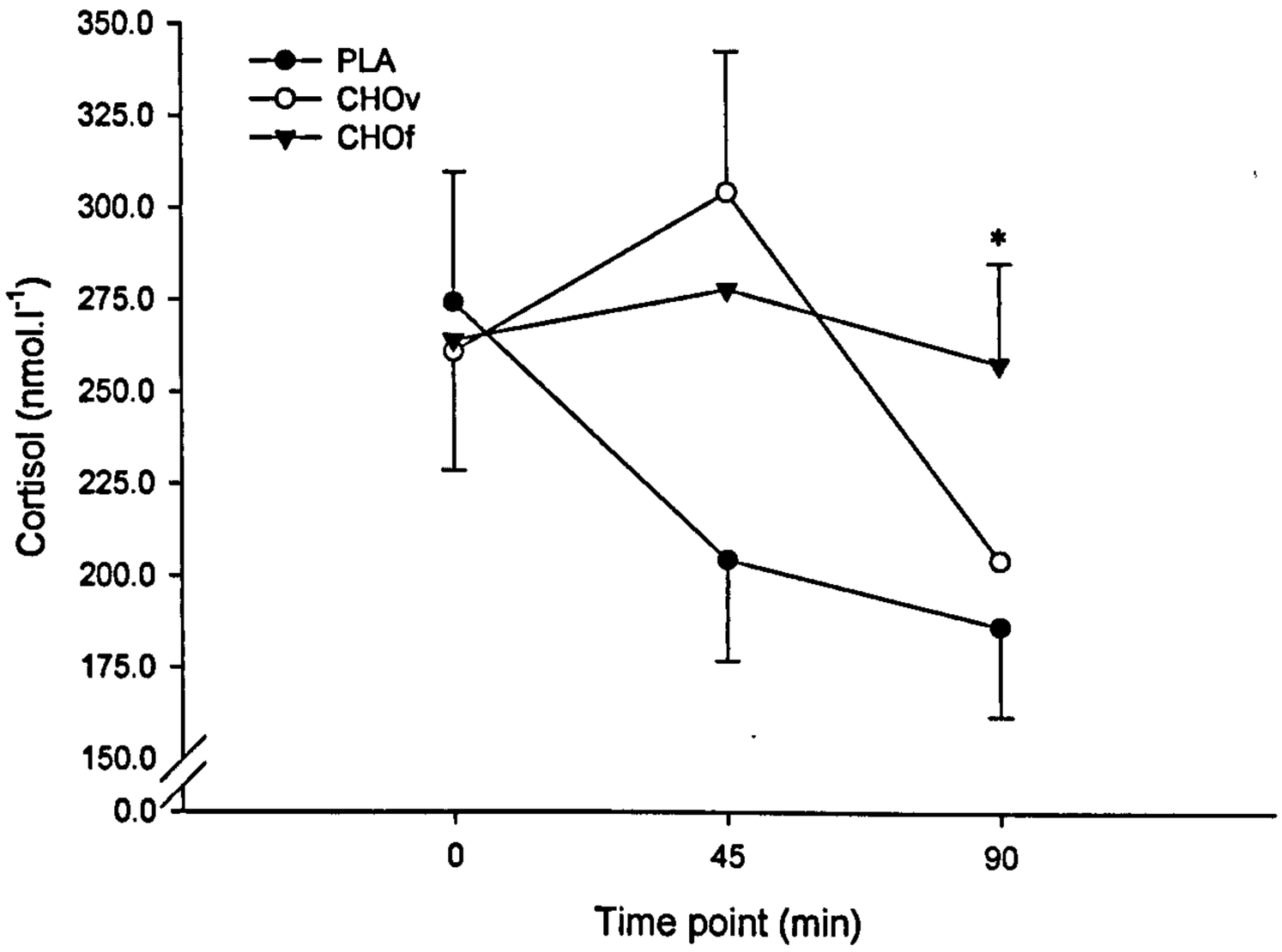

Figure 5.9: Plasma cortisol concentration during soccer-specific protocol.
* CHOf significantly greater than PLA.

5.3.5. Substrate oxidation rates

Carbohydrate oxidation (Figure 5.10) was not significantly different between CHOf and CHOv (P>0.05), although it was significantly ($F_{2,22}$=3.759; P<0.05) greater during CHOf and CHOv compared to PLA. In contrast fat oxidation was not significantly ($F_{2,22}$=2.428; P>0.05) different between trials (PLA: 0.51±0.03 g.min^{-1}; CHOv: 0.50±0.04 g.min^{-1}; CHOf: 0.42±0.04 g.min^{-1}).

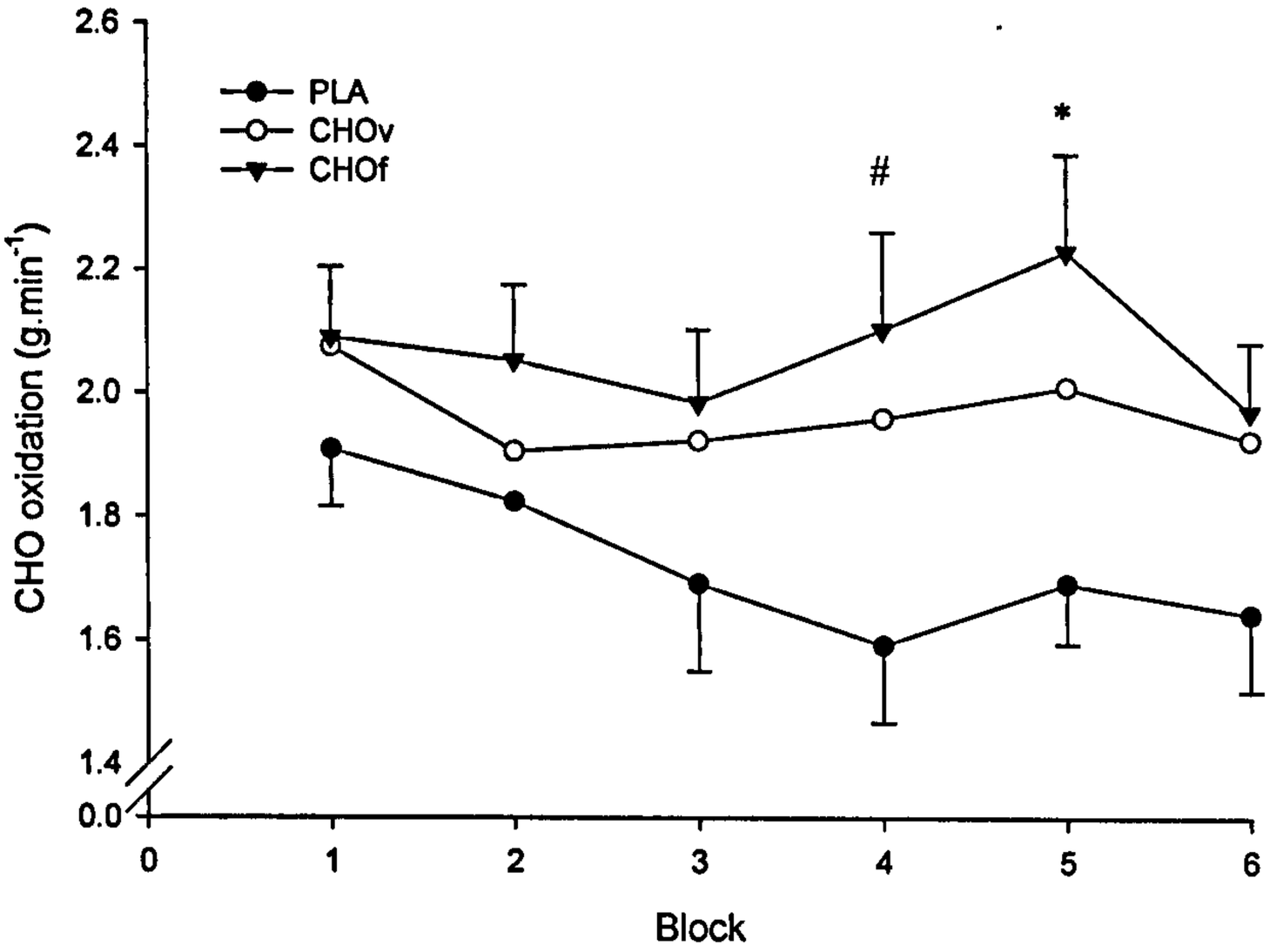

Figure 5.10: Carbohydrate oxidation during the soccer-specific protocol.

CHOv and CHOf significantly greater than PLA.

* CHOf significantly greater than PLA.

5.3.6. Perceived thirst and gut fullness

There was no significant trial effect on the rating of thirst ($F_{2,22}$=0.573; P>0.05; Table 5.2). There was a significant ($F_{3,32}$=25.425; P<0.05) effect of time on rating of thirst. Thirst decreased significantly (P<0.05) following the consumption of fluid pre-exercise but was significantly (P<0.05) higher during the first half of the soccer-specific protocol compared with immediately post-fluid ingestion. Subjective rating of thirst also increased steadily throughout the second half of the soccer-specific protocol and during block 6 (75-90 min) was significantly (P<0.05) higher than at half-time.

Table 5.2: Perceived thirst throughout the soccer-specific protocol.

Trial	Pre-fluid	Post-fluid	Block 1	Block 2	Block 3	Half-time	Block 4	Block 5	Block 6
PLA	12.7 ±0.4‡†	8.0 ±0.3	10.3 ±0.3‡†	11.0 ±0.4‡†	11.6 ±0.5‡†	8.3 ±0.6	9.3 ±0.5	9.7 ±0.3‡†	11.8 ±0.6‡†
CHOv	11.4 ±0.6‡†	7.8 ±0.2	9.6 ±0.5‡†	10.8 ±0.4‡†	11.1 ±0.6‡†	8.0 ±0.4	9.4 ±0.4	10.5 ±0.4‡†	11.3 ±0.5‡†
CHOf	12.2 ±0.9‡†	9.4 ±0.8	11.1 ±0.5‡†	10.5 ±0.6‡†	10.8 ±0.5‡†	8.9 ±0.4	10.0 ±0.4	10.3 ±0.5‡†	10.3 ±0.5‡†

‡ significantly higher than post-fluid,† significantly higher than half-time

Table 5.3: Perceived gut fullness throughout the soccer-specific protocol.

Trial	Pre-fluid	Post-fluid	Block 1	Block 2	Block 3	Half-time	Block 4	Block 5	Block 6
PLA	7.8 ±0.2	11.6 ±0.7‡	9.9 ±0.5‡†	9.8 ±0.4‡†	9.5 ±0.6‡†	11.7 ±0.8‡	10.3 ±0.8†	9.7 ±0.5†	8.9 ±0.5†
CHOv	8.0 ±0.3	12.3 ±1.0‡	10.6 ±0.7‡†	10.3 ±0.5‡†	9.8 ±0.5‡†	12.9 ±0.9‡	10.7 ±0.7†	10.7 ±0.7†	10.1 ±0.8†
CHOf	8.1 ±0.4	10.4 ±1.0‡	9.5 ±0.8‡†	9.5 ±0.5‡†	9.3 ±0.4‡†	11.0 ±1.0‡	10.3 ±0.9†	9.7 ±0.7†	9.8 ±0.7†

‡ significantly higher than pre-fluid, † significantly lower than half-time

There was a significant ($F_{2,29}$=16.445; P<0.05) effect of time on rating of gut fullness (Table 5.3), which was lower (P<0.05) immediately prior to fluid consumption pre-exercise compared with any stage during the first half or at half-time. Gut fullness increased significantly (P<0.05) following the consumption of fluid at the start of the first half and was significantly higher at half-time following fluid ingestion, compared with any stage of the second half (P<0.05). Subjective rating of gut fullness decreased steadily throughout each half of the soccer-specific protocol. There were no significant differences ($F_{2,22}$=1.061; P>0.05) in rating of gut fullness between the three trials.

5.3.7. Core temperature

There were no significant differences in core temperature between trials ($F_{2,10}$=2.210; P>0.05). Core temperature increased steadily throughout each half and decreased during

the half-time break (Figure 5.11). The repeated measures ANOVA revealed there was a significant ($F_{6,30}$=15.215; P>0.05) effect of time on core temperature. Core temperature was significantly higher during the fifth and sixth blocks, compared with the first 15-min period (P<0.05). There was also an increase at the start of the second half, with temperature being significantly higher in block 5 than block 4 (P<0.05).

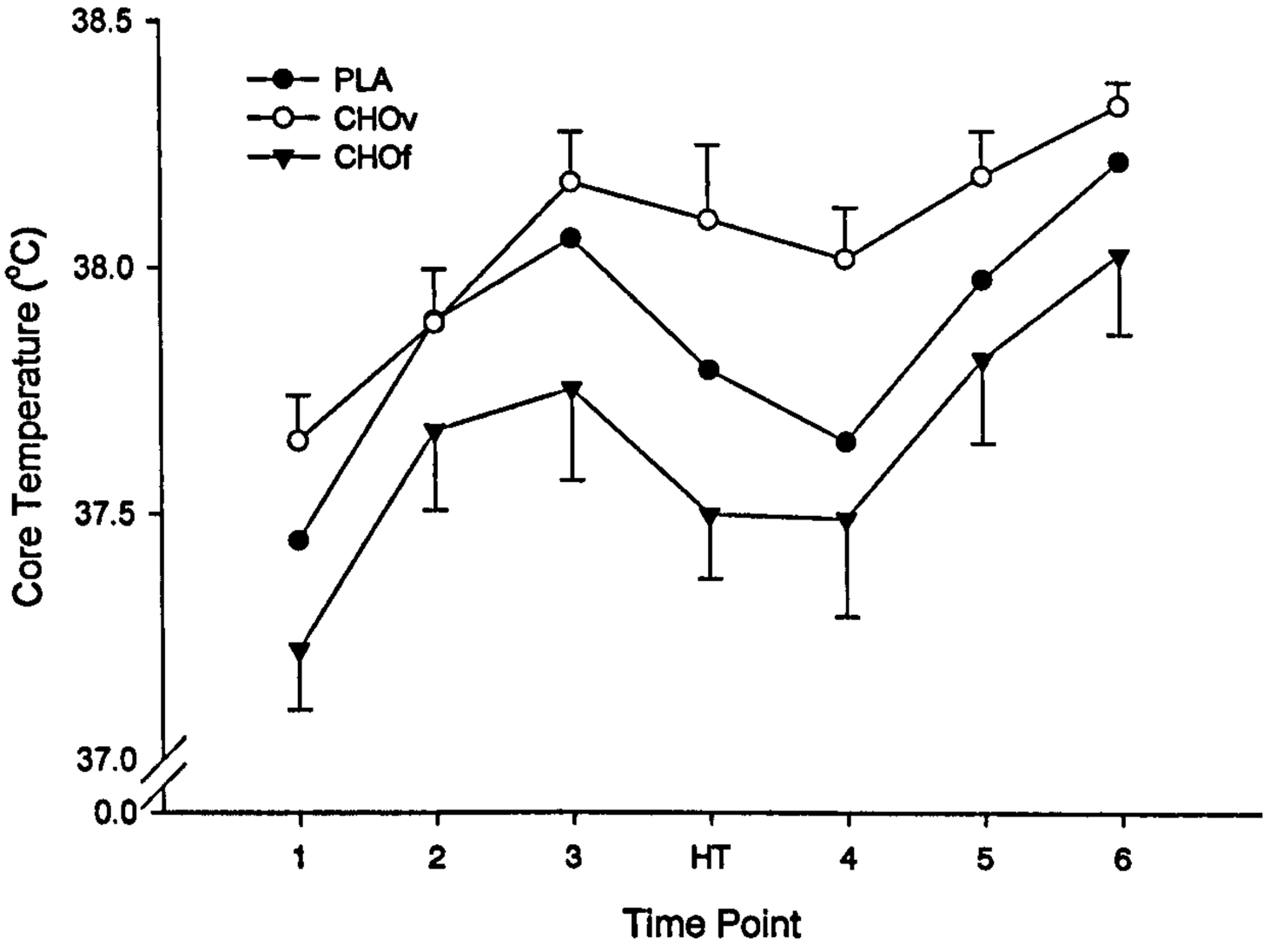

Figure 5.11: Changes in core temperature during the soccer-specific protocol.

5.3.8. Heart rate and RPE

The repeated measures ANOVA showed that there was a significant ($F_{3,30}$=611.048; P<0.05) effect of time on heart rate. Heart rate was significantly elevated (P<0.05) above pre-fluid and post-fluid ingestion resting values throughout the soccer-specific protocol (Figure 5.12). Over the half-time interval, heart rate was significantly higher than pre-exercise values and significantly below that measured during the soccer-specific exercise (both P<0.05). No significant differences in heart rate were observed between the three trials ($F_{2,22}$=0.165; P>0.05).

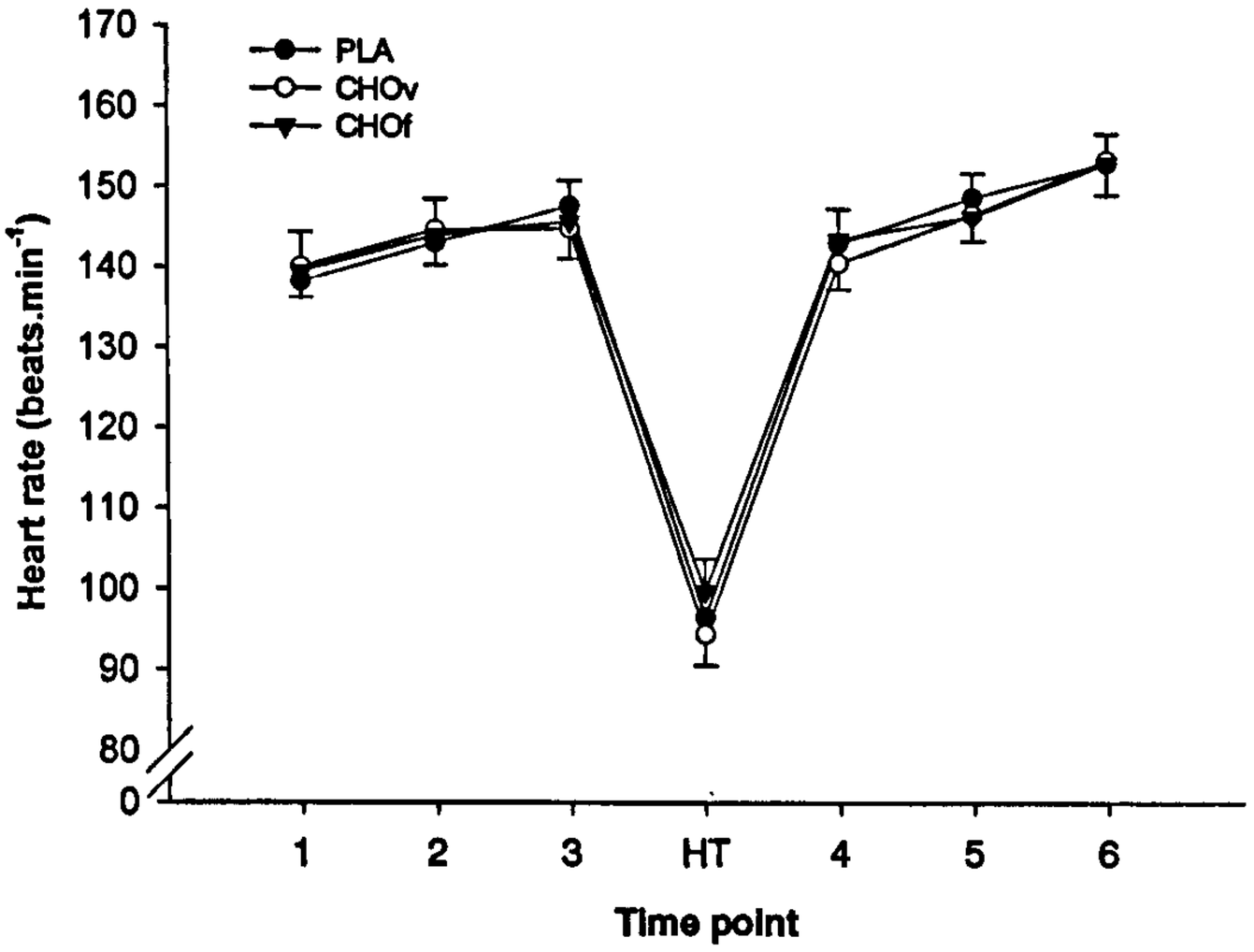

Figure 5.12: Heart rate during the soccer-specific protocol.

The RPE increased progressively throughout the exercise period for all of the trials (Figure 5.13). There was a significant effect of time ($F_{3,33}$=15.693; P<0.05) on RPE. Rating of perceived exertion was significantly (P>0.05) higher at the end of each half compared to the start of that half. The repeated measures ANOVA revealed that there were no significant differences in RPE between the three trials ($F_{2,22}$=1.090; P>0.05).

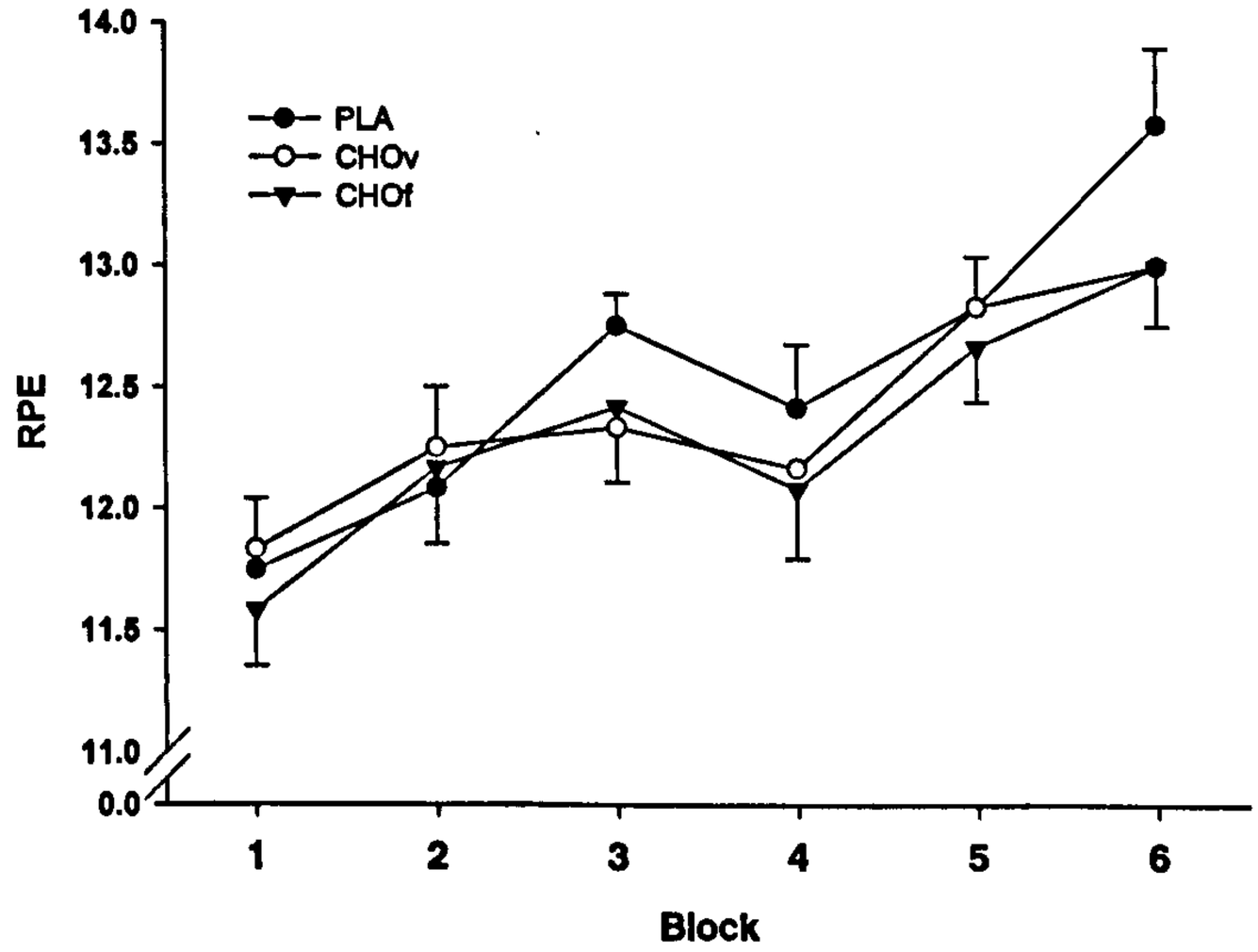

Figure 5.13: RPE during the soccer-specific protocol.

5.4. Discussion

The main findings of the present study were that i) consuming a carbohydrate-electrolyte solution significantly increased plasma glucose concentration and carbohydrate oxidation and suppressed NEFA and glycerol compared to placebo, but had no impact on sprint power output during sprinting and ii) altering the timing and volume of carbohydrate ingestion did not significantly affect metabolism or sprint power output during CHOv or CHOf.

One of the key factors in sustaining prolonged exercise at intensities ranging from 65 to 85% $\dot{V}O_{2max}$ is the concentration of muscle glycogen (Bergstrom *et al.*, 1967). Fatigue during exercise of this nature is associated with the depletion of the muscle's limited glycogen stores (Nicholas *et al.*, 1999) and a reduction in blood glucose (Coyle *et al.*, 1986). In the present study, plasma glucose concentration during CHOv and CHOf was not significantly affected by manipulating the timing and volume of ingesting a carbohydrate-electrolyte solution. This result may have been as a consequence of the same total volume being ingested, and the same amount of glucose being made available. This study also reaffirmed the finding that consuming carbohydrate during exercise increases plasma glucose concentration (Coyle *et al.*, 1983; Nicholas *et al.*, 1995).

Plasma NEFA and glycerol concentration increased during the soccer-specific protocol, with a greater increase occurring during the second half, supporting the findings of Bangsbo (1994b). The largest increase occurred during PLA, confirming that consuming a carbohydrate solution during exercise suppresses the release of NEFA and glycerol during CHOv and CHOf, possibly as an effect of an elevated insulin concentration, which has been shown to occur following carbohydrate ingestion during a simulation of the exercise intensity of soccer match-play (Nicholas *et al.*, 1995).

Plasma lactate concentrations were similar for all conditions. This finding is in agreement with the majority of studies (Coyle *et al.*, 1983; Nicholas *et al.*, 1995), which have

demonstrated that despite significant differences in the concentration of blood glucose, the concentration of lactate during exercise remains relatively constant.

A similar pattern of carbohydrate oxidation was observed when carbohydrate was consumed during exercise, irrespective of timing and volume, possibly due to the total amount of carbohydrate ingested being the same during both trials. However, carbohydrate oxidation was significantly higher during CHOf and CHOv compared with PLA. Previous studies have demonstrated that consuming carbohydrate during exercise maintains high rates of carbohydrate oxidation late in exercise (Coyle *et al.*, 1986). The higher carbohydrate oxidation after ingesting carbohydrate has been attributed to either increased muscle glycogenolysis or elevated glucose uptake and oxidation (Wright *et al.*, 1991).

High-intensity exercise has been associated with elevated plasma cortisol concentration in order to maintain blood glucose concentration, and this rise can be attenuated by the consumption of carbohydrate drinks (Henson *et al.*, 1998). In the present study plasma glucose concentration did not fall significantly below resting levels, which may explain why plasma cortisol concentration was maintained during the carbohydrate trials. The failure of cortisol to increase during PLA may have been due to the overall exercise intensity not being high enough (67±1 % $\dot{V}O_{2max}$) to elicit a cortisol response, although this intensity is close to values observed during a match [70% $\dot{V}O_{2max}$ (Reilly *et al.*, 2000)]. Nieman *et al.* (1994) found that high-intensity exercise (80% $\dot{V}O_{2max}$) produced a significantly greater cortisol response compared to moderate-intensity exercise (50% $\dot{V}O_{2max}$). It has been demonstrated (Mitchell *et al.*, 1989; Burgess *et al.*, 1991) that when carbohydrate ingested during exercise fails to affect insulin concentration, the adrenaline response does not seem to be affected, and so may explain the lack of a difference between the carbohydrate trials and PLA in the present study. Bishop *et al.* (1999) concluded that during soccer-specific exercise the change in stress hormones was minimal and carbohydrate supplementation had a negligible effect.

The change in plasma volume significantly increased during exercise, although there was not a significant difference between trials. Plasma osmolality also increased during all experimental trials of the soccer-specific protocol, but remained within normal values, indicating that the subjects did not suffer from severe dehydration. There were no significant differences between the trials, suggesting that the overall rate of gastric emptying was the same and it is the total volume of fluid ingested rather than the timing of ingestion that is important in preventing dehydration during soccer-specific exercise. A possible explanation for this occurrence is that the time-course for the volume of fluid ingested to be distributed throughout the body after gastric emptying, intestinal absorption and osmotic flow is 40-60 min (Noakes *et al.*, 1991; Schedl *et al.*, 1994) and within this time scale a similar volume of fluid would have emptied from the stomach. Total sweat loss was comparable with previous studies (Nicholas *et al.*, 1995; Bishop *et al.*, 1999; Nicholas *et al.*, 2000), in which the exercise intensity of soccer was simulated. The value was relatively low, indicating that the volume of fluid intake was appropriate for the environmental conditions and fitness of the subjects.

Despite the different timings and volume of fluid consumed, there were no significant differences in either thirst or gut fullness between trials. Irrespective of how the total volume was consumed, thirst decreased significantly following the consumption of fluid at rest, prior to the soccer-specific intermittent protocol and at half-time. In contrast gut fullness increased significantly at these time points. Gastric emptying is affected by the volume of fluid ingested, the larger the volume the faster the rate i.e. drinking a large volume prior to a match (CHOv). As the volume in the stomach declines the rate of gastric emptying decreases proportionally. However, if the volume is maintained by repeated ingestion of smaller volumes of fluid i.e. every 15 min (CHOf), the rate of gastric emptying remains relatively constant (Rehrer *et al.*, 1990). This may explain the small differences, although not significant, in plasma metabolites and carbohydrate oxidation between CHOf and CHOv. During the CHOf trial, carbohydrate may have passed from the stomach into the small intestine at a constant rate, enabling glucose absorption to occur at a constant rate and plasma glucose and carbohydrate oxidation to be maintained at higher levels. When fluid was consumed the subjective rating of gut

fullness increased significantly, especially during PLA and CHOv. This finding agrees with Mitchell and Voss (1991) who demonstrated that ingesting large volumes caused an increased frequency of complaints of gastric fullness.

The increases in blood glucose and carbohydrate oxidation following carbohydrate ingestion were not reflected in performance as peak sprint power output was relatively constant throughout all of the trials and not significantly different between trials. The values obtained for peak sprint power output were consistent with previous studies that have employed the non-motorised treadmill (Hamilton *et al.*, 1991a; Tong *et al.*, 2001). The finding that substrate availability did not affect sprint performance was similar to that of Nicholas *et al.* (1995) who demonstrated that the ingestion of a carbohydrate-electrolyte significantly extended a run to exhaustion following a period of intermittent exercise, but had no impact of the performance of high-intensity exercise, 15-m sprints. It has also been demonstrated that ingesting carbohydrate during a soccer match has no impact on the ability to perform high-intensity skills such as tackling and heading (Zeederberg *et al.*, 1996).

The mean sprint duration in a competitive match is 3.7 seconds (Drust *et al.*, 1998). In the present study the duration of each sprint was 3.3 seconds. At high-intensity exercise of these durations, PCr is the major energy source, and a reduction in carbohydrate availability might increase PCr degradation during exercise (Gaitanos *et al.*, 1993; Tsintzas *et al.*, 2001). However, Balsom *et al.* (1992) demonstrated that forty 15-m sprints could be performed at 30-s intervals unimpaired without carbohydrate supplementation. Although Balsom *et al.*, (1999b) reported that subjects who had consumed a high-carbohydrate diet (65% CHO) were able to perform 33% more high-intensity exercise than after a low-carbohydrate diet (30% CHO) and in a trial to fatigue, subjects who had consumed a high-carbohydrate diet were able to perform 265% more 6-s intervals (~200% $\dot{V}O_{2max}$) than during a low-carbohydrate diet (Balsom *et al.*, 1999a). In the present study 18 sprints were performed separated by approximately 200 s of lower-intensity exercise, suggesting there was sufficient time for PCr resynthesis between

sprints and that the PCr system was able to meet the energy demands during the sprints and may explain the relatively constant sprint power output. Also no signs were displayed of hypoglycaemia during PLA, this state being associated with fatigue and reduced performance (Coyle *et al.*, 1986), suggesting carbohydrate availability was not a factor limiting peak sprint power output in the present study. The maintenance of plasma glucose levels is possibly a consequence of the exercise intensity induced increase in circulating adrenaline promoting hepatic glucose production (Vranic *et al.*, 1984).

Core temperature and heart rate were similar for all trials, possibly due to the sweat loss and changes in plasma volume values being comparable between trials. Therefore, the cardiovascular strain appears to be unaffected by manipulating fluid ingestion patterns. In contrast, previous studies have reported lower heart rate (Melin *et al.*, 1994; Ferguson *et al.*, 2005) and core temperature (Melin *et al.*, 1994) following a single bolus compared with intermittent fluid intake.There were no statistical differences in RPE between the trials during the soccer-specific protocol. This observation is most likely a consequence of core temperature and heart rate being unaffected by the experimental conditions and supports the findings of Ferguson *et al.* (2005). These observations indicate that the physiological stress imposed by the protocol was similar in all three trials.

The general recommendation for fluid ingestion during exercise is that fluid should be consumed early, and at regular intervals in an attempt to replace the water lost through sweating, or to consume the maximal amount that can be tolerated (Convertino *et al.*, 1996). This study indicates that if sufficient carbohydrate-electrolyte solution is ingested before and at half-time, sprint performance and metabolism are not significantly affected when compared to consuming the same total volume ingested at the recommended 15-min intervals. The absence of scheduled breaks in soccer prevents players taking regular feedings of carbohydrate other than at half-time. These findings indicate that consuming a carbohydrate-electrolyte solution before a match and at half-time is a practical strategy for fluid provision during soccer at moderate ambient temperatures. When employing this strategy, extra fluid could be consumed during a match when the opportunities arise as a consequence of the natural breaks that typically occur e.g. during pauses for injuries,

as the rules require that players must go to the perimeter lines to avail of any drinks provided by support staff during the game.

In conclusion, ingesting a carbohydrate-electrolyte solution compared with a flavoured placebo during a soccer-specific protocol significantly altered metabolism, although it had no impact on peak sprint power output. Furthermore, providing that the total volume of fluid consumed is equal, manipulating the timing and volume of carbohydrate ingestion did not influence periodic all-out exercise performance and elicited the same metabolic responses.

Chapter 6

Study 3

The previous studies have demonstrated that blood glucose and carbohydrate oxidation can be maintained by ingesting carbohydrate during the performance of soccer-specific exercise in a thermo-neutral environment. However, international soccer matches and tournaments are often played in hot conditions. The aim of the present study was to investigate the effect of ingesting sports drinks on metabolism and exercise capacity during soccer-specific exercise performed in the heat.

6.1. Introduction

Soccer matches at major tournaments are regularly played in temperatures exceeding 30°C (FIFA World Cup 2002 and UEFA Euro 2004). During prolonged exercise in the heat large amounts of water may be lost as a result of sweating. Dehydration during exercise has been shown to raise core temperature and increase cardiovascular strain (Sawka *et al.*, 1985). An elevated body temperature has been demonstrated to limit exercise performance (Nybo and Nielsen, 2001). The ingestion of fluid containing carbohydrate has been shown to offset dehydration, minimize disturbances in cardiovascular function, improve thermoregulation (Coyle and Coggan, 1984), spare muscle glycogen (Nieman *et al.*, 2005), maintain blood glucose concentration and improve performance (Davis *et al.*, 1988).

Performing high-intensity intermittent exercise in the heat has been shown to increase muscle glycogen utilization (Morris *et al.*, 2005), fluid loss, cardiovascular stress and impair performance (Morris *et al.*, 1998; Morris *et al.*, 2005). In addition, the ingestion of a glucose solution during this type of exercise has been reported to be ineffective in attenuating the effects of dehydration and delaying the onset of fatigue (Morris *et al.*, 2003). Previous studies (Davis *et al.*, 1990; Jentjens *et al.*, 2006) have indicated that fluid availability during exercise in the heat is lower with a glucose drink compared with a combined glucose and fructose drink or water. Shi *et al.* (1995) also demonstrated that the ingestion of a glucose and fructose solution resulted in greater water absorption than a glucose solution. These findings suggest that a glucose drink may be less effective for fluid replacement during exercise in the heat. Therefore, ingesting a multi-carbohydrate

drink may increase the intestinal absorption fluid, thus minimising the impact of dehydration.

A potential limiting factor for the oxidation of exogenous carbohydrate is the rate of intestinal absorption of carbohydrate. It is thought that the intestinal glucose transporters (SGLT1) are saturated when the rate of glucose ingestion exceeds 1 $g \cdot min^{-1}$, which may explain why there is not a linear relationship between glucose ingestion rates and oxidation rates (Jeukendrup and Jentjens, 2000). However, Shi *et al.* (1995) suggested that the inclusion of 2 or 3 carbohydrates (glucose, fructose and sucrose) may increase water and carbohydrate absorption despite increased osmolality. This effect was attributed to the separate transport mechanisms across the intestinal wall for glucose, fructose and sucrose. Therefore, when a solution containing a mixture of glucose and fructose is ingested there is less competition for absorption compared with an isoenergetic amount of glucose. As a consequence there is the possibility of an increase in the amount of carbohydrate entering the bloodstream and its subsequent availability for oxidation, which in turn may spare muscle glycogen and delay the onset of fatigue, and improve performance. However, the majority of investigations into the effect of multi-carbohydrate solutions on carbohydrate oxidation have employed prolonged low-intensity exercise protocols (Jentjens *et al.*, 2004a; Jentjens *et al.*, 2004b; Jentjens *et al.*, 2004c; Jentjens *et al.*, 2006) and have not studied the impact on exercise capacity or performance. The impact of ingesting multi-carbohydrate solutions during high-intensity intermittent activity such as soccer-specific exercise and the subsequent effect on performance has not been previously examined.

The aim of this study was to investigate the effect of ingesting a multi-carbohydrate sports drink compared with a glucose only solution on metabolism and exercise capacity during soccer-specific exercise performed in the heat.

6.2. Methods

6.2.1. Subjects

Eleven male university soccer players of age: 27±2 years; height: 1.78±0.1 m; body mass: 76.1±2 kg; $\dot{V}O_{2max}$: 63.1±2 ml·kg^{-1}·min^{-1} participated in this study. All subjects provided written informed consent to participate, in accordance with Liverpool John Moores University's ethical procedures.

6.2.2. Experimental design

Subjects undertook two familiarisation sessions consisting of two blocks of the soccer-specific protocol (i.e. 30 minutes) outlined in section 3.1.4.

Subjects completed the full soccer-specific protocol on a motorised treadmill on three occasions in the environmental chamber (30.2±0.5°C and 45±4% relative humidity) (Figure 6.1). During one session 228±6 ml of carbohydrate-electrolyte solution [Still Lucozade Sport, (6.6 g·100 ml^{-1} CHO, 49 mg·100 ml^{-1} Na, 296±0.5 mOsm.kg^{-1}), GlaxoSmithKline, Gloucestershire, UK] was consumed at 0, 15, 30, 45, 60, 75 minutes of exercise (GLU). On another occasion 228±7 ml of a multi-carbohydrate (fructose, dextrose, maltodextrin) solution (6.6 g·100 ml^{-1} CHO, 50 mg·100 ml^{-1} Na, 296±0.5 313±0.6 mOsm.kg^{-}1, GlaxoSmithKline, Gloucestershire, UK) was consumed at the same time points (MIX). During the other session 228±6 ml of a placebo (a similarly coloured, flavoured and textured electrolyte solution) (<0.5 g·100 ml^{-1}, 49.5 mg·100 ml^{-1} Na, 65±0.3 mOsm.kg^{-1}; GlaxoSmithKline, Gloucestershire, UK) was consumed (PLA). During the carbohydrate trials the total amount of carbohydrate ingested at a rate of 60±0.5 g.h^{-1}. The trials were performed in a double-blind, counter-balanced fashion.

Figure 6.1: The environmental chamber where the trials were performed.

6.2.3. Heart rate

Heart rate was measured continuously by means of a short–range radio telemetry system (Polar S610i, Polar Electro, Kempele, Finland) during all exercise (outlined in section 3.2.1) and was presented as the mean value for each 15-min block.

6.2.4. Assessment of respiratory gases during exercise

Oxygen consumption ($\dot{V}O_2$), $\dot{V}CO_2$, RE and $\dot{V}E$ were recorded for 2 min using an on-line automated gas analyser (Metalyzer3B, Cortex Biophysic GmbH, Leipzig, Germany) after 10 min of each block. Carbohydrate and fat oxidation rates were calculated using the stoichiometric equations of Frayn (1983) as described in section 3.2.3.

6.2.5. Measurement of core body temperature

Core body temperature was monitored continuously by means of an ingestible temperature sensor pill and external data logger (HQ inc., Florida, USA). Data were presented as the mean value for each 15-min block. See section 3.3.

6.2.6. Ratings of Perceived Exertion (RPE) and thermal sensation

At the completion of each 15-min block perceived exertion was measured using a 6-20 scale (Borg, 1970). Subjects also rated their thermal sensation during exercise according to a 17-point thermal sensation scale (Toner *et al.*, 1986) as described in section 3.4.

6.2.7. Blood procurement and analysis

Prior to exercise, at half-time and at the completion of exercise venous blood samples were drawn from an antecubital vein using standard venepuncture techniques (Vacutainer Systems, Becton, Dickinson, Europe). The blood samples were later analysed for glucose, lactate, NEFA, glycerol, catecholamines, insulin and IL-6 (outlined in section 3.7).

6.2.8. Cunningham and Faulkner test

After completing the soccer-specific protocol, subjects performed the Cunningham and Faulkner test (Cunningham and Faulkner, 1969), which required the subject to run at a gradient of 20% and a speed of 12.8 $km \cdot h^{-1}$ until fatigue. The time began when the subject started running unsupported and stopped when he grabbed the handrails at the point of fatigue. This test was a measure of fatigue resistance to high-intensity exercise and has been shown to be both valid and reliable as a measurement tool (Thomas *et al.*, 2002).

To assess reliability following familiarisation, the subjects performed the Cunningham and Faulkner test on six occasions 1 week apart on a motorized treadmill (H/P/Cosmos Pulsar 4.0, H/P/Cosmos Sports and Medical GmbH, Nussdorf-Traunstein, Germany). There was no significant difference ($P>0.05$) in time to exhaustion between trials. The coefficient of variation for time to exhaustion was 5.2%. Hence, the Cunningham and

Faulkner test was deemed to provide a reliable measure of resistance to high-intensity exercise.

6.2.9. Muscle biopsies

A percutaneous needle biopsy of *vastus lateralis* has been shown to significantly reduce insulin-stimulated glucose uptake (Holck *et al.*, 1994), although the mechanism of this phenomenon is not known. To exclude the possibility that the biopsy procedure *per se* influenced glucose uptake, a basal biopsy was obtained approximately three weeks before the first trial. An additional biopsy was taken on completion of the soccer-specific protocol during each trial. After local anaesthesia [2 ml 0.5% Bupivacaine Hydrochloride (Marcain Polyamp, AstraZeneca, UK)] and incision of the skin and muscle fascia, percutaneous muscle samples (~30 mg) were taken from the lateral vastus of the quadriceps femoris muscle using an automated procedure (Pro-Mag 2.2 Automatic Biopsy System, Manan Medical Products, USA) with a 14 gauge needle (ACN Biopsy needles, InterV, Denmark) in the distal to proximal direction (Figure 6.2). The biopsy was immediately frozen in liquid nitrogen and stored at -80°C for subsequent glycogen analysis. To determine the concentration of muscle glycogen the tissue was acid hydrolyzed allowing the glucose residues to be measured enzymatically (Powerwave X340, BioTek Instruments Inc, USA) as described by Lowry and Passonneau (1972) (see Appendix D). Glycogen concentrations are expressed as "wet weight".

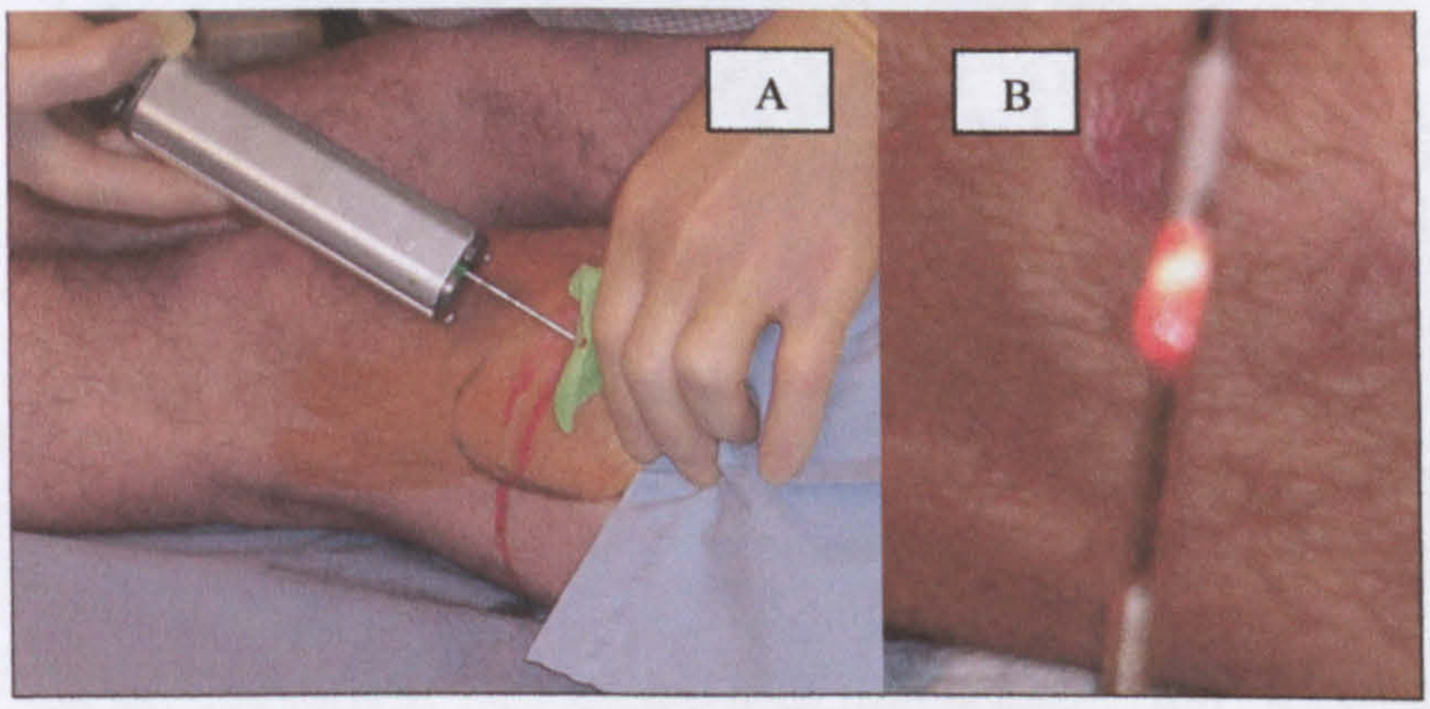

Figure 6.2: Muscle biopsy being performed (A) and a sample of muscle (B).

6.2.10. Statistics

All variables were analysed using two-way ANOVAs with repeated measures except for muscle glycogen concentration, sweat loss and the time to exhaustion during the Cunningham and Faulkner test and the reliability of the Cunningham and Faulkner test, which were analysed using a one-way ANOVA with repeated measures. Results are reported as the mean ± the standard error of the mean (SEM) and a level of $P<0.05$ was considered statistically significant.

6.3. Results

6.3.1. Pre-trial hydration status

Pre-trial hydration status was similar for all of the experimental conditions. Urine colour PLA (2.00±0.22), GLU (2.00±0.22) and MIX (2.09±0.24) ($F_{2,15}$=0.476; P>0.05) and osmolality PLA (509.86±106.16 mOsm·kg^{-1}), GLU (517.41±107.76 mOsm·kg^{-1}) and MIX (567.41±106.21 mOsm·kg^{-1}) ($F_{2,17}$=0.438; P>0.05) did not differ significantly between trials.

6.3.2. Muscle glycogen

Muscle glycogen concentration was significantly lower following the soccer-specific protocol compared with pre-exercise ($F_{1,7}$=7.141; P<0.05). The difference between trials was not significant (P>0.05):- Pre (127.23±17.36 mmol·kg wet weight^{-1}), PLA (62.99±8.39 mmol·kg wet weight^{-1}), GLU (68.62±2.70 mmol·kg wet weight^{-1}) and MIX (76.63±6.92 mmol·kg wet weight^{-1}).

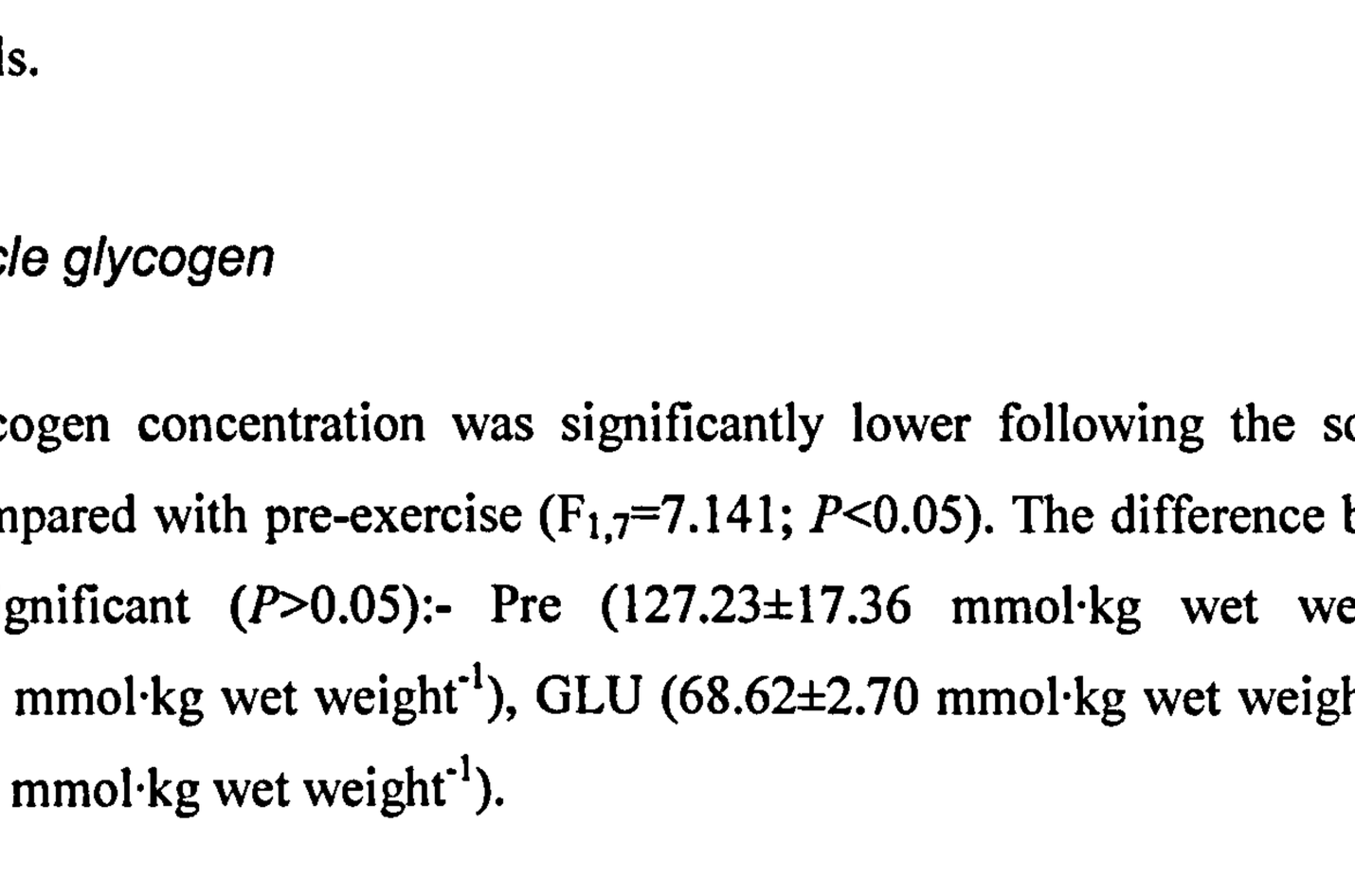

Figure 6.3: Muscle glycogen concentration at pre-exercise (PRE) and after the soccer-specific protocol. * significantly lower than pre-exercise.

6.3.3. Plasma metabolites

Pre-exercise plasma glucose concentration was similar for the three trials. There was a significant trial effect on the concentration of plasma glucose ($F_{2,16}$=7.055; P<0.05; Figure 6.4), whereby the concentration was significantly higher in GLU compared with PLA throughout the soccer-specific protocol. There was also a significant effect of time ($F_{2,17}$=13.703; P<0.05). In trials GLU and MIX, plasma glucose concentration was elevated significantly (P<0.05) above resting levels at half-time and on completion of the soccer-specific protocol. The repeated measures ANOVA identified a significant time and trial interaction effect ($F_{2,24}$=2.243; P<0.05); plasma glucose increased during the first half in all trials, whereas during the second half plasma glucose was relatively constant during GLU and MIX, but decreased during PLA.

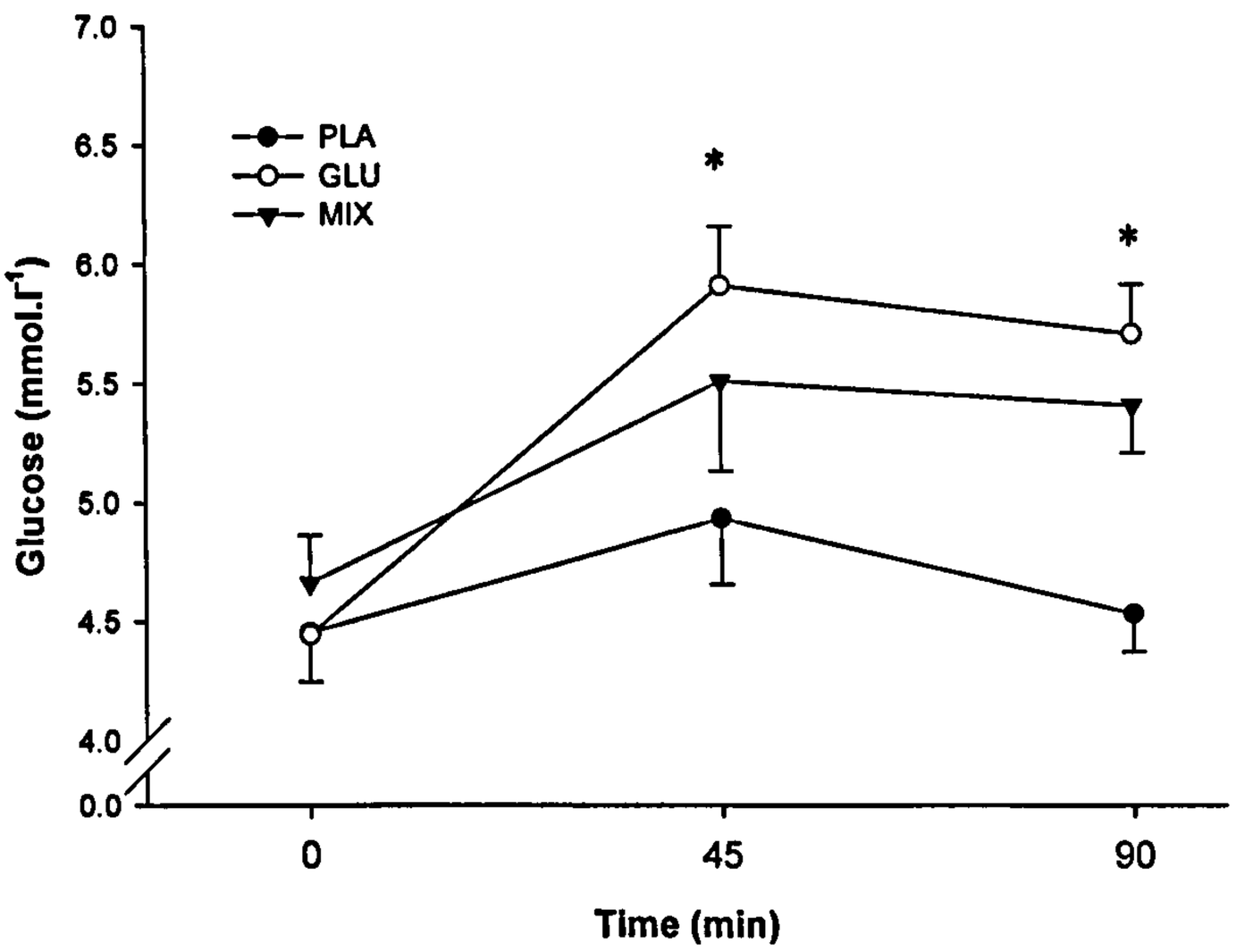

Figure 6.4: Plasma glucose concentration during the soccer-specific protocol.

* GLU significantly greater than PLA.

The repeated measures ANOVA revealed that there was a significant trial effect on the plasma concentration of NEFA ($F_{1,13}$=13.406; P<0.05). The concentration of NEFA was significantly (P<0.05) higher during PLA compared with GLU and MIX (Figure 6.5). There was a significant effect of time on the concentration of plasma NEFA

($F_{1,13}$=35.663; P<0.05) which increased significantly between each time point as exercise progressed. There was also a significant ($F_{2,18}$=7.759; P<0.05) trial and time interaction; after half-time NEFA concentration increased markedly more during PLA compared with GLU and MIX.

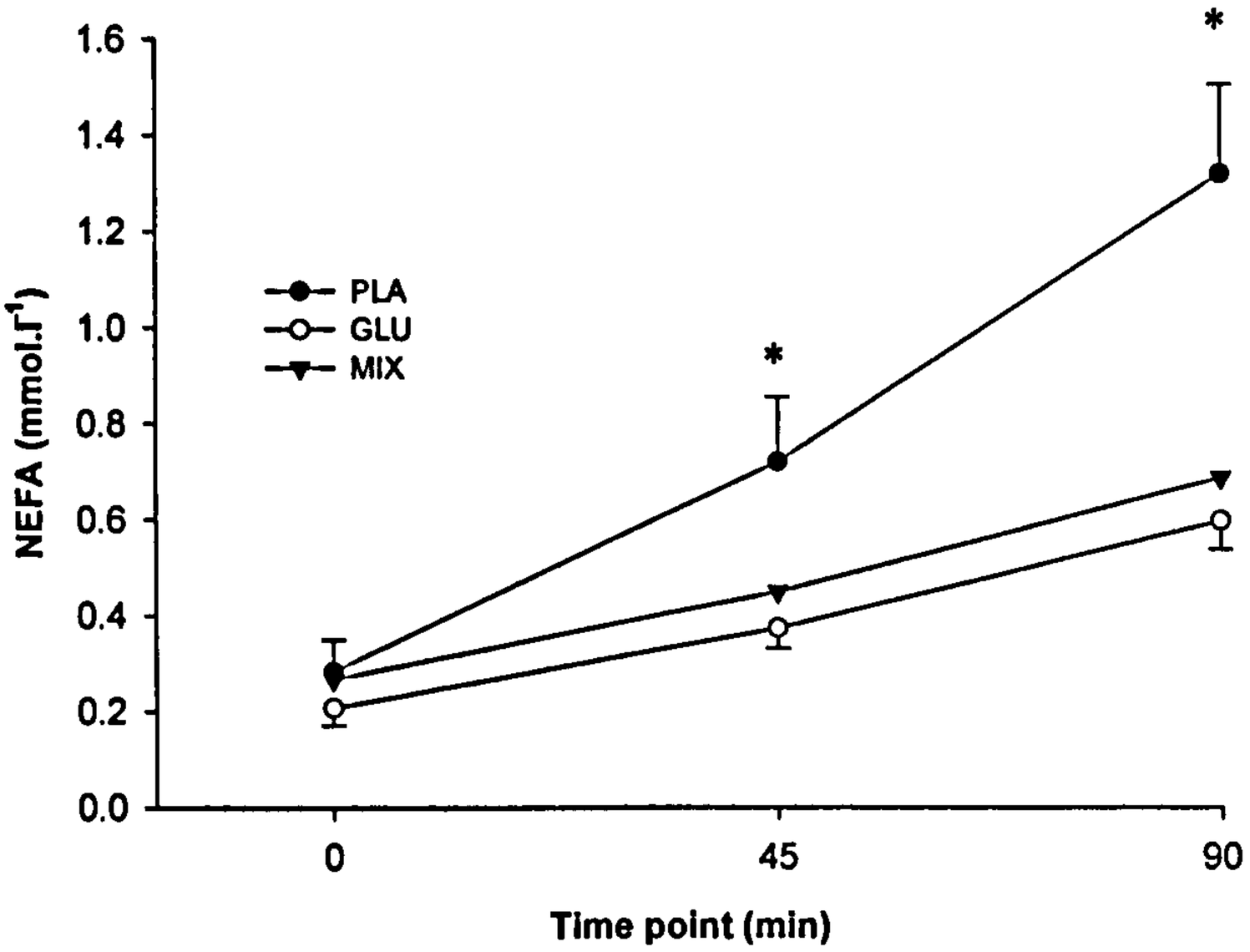

Figure 6.5: Plasma NEFA concentration during the soccer-specific protocol.
* GLU and MIX significantly lower than PLA.

The plasma concentration of glycerol was significantly affected by the trial ($F_{2,17}$=12.472; P<0.05), and the concentration was significantly (P<0.05) higher during the PLA trial compared with GLU throughout exercise and significantly higher than MIX at 90 min (Figure 6.6). Plasma glycerol concentration increased significantly between each time point ($F_{1,12}$=61.745; P<0.05). There was also a significant trial and time interaction ($F_{2,22}$=9.530; P<0.05); after half-time glycerol concentration increased markedly more during PLA compared with GLU and MIX.

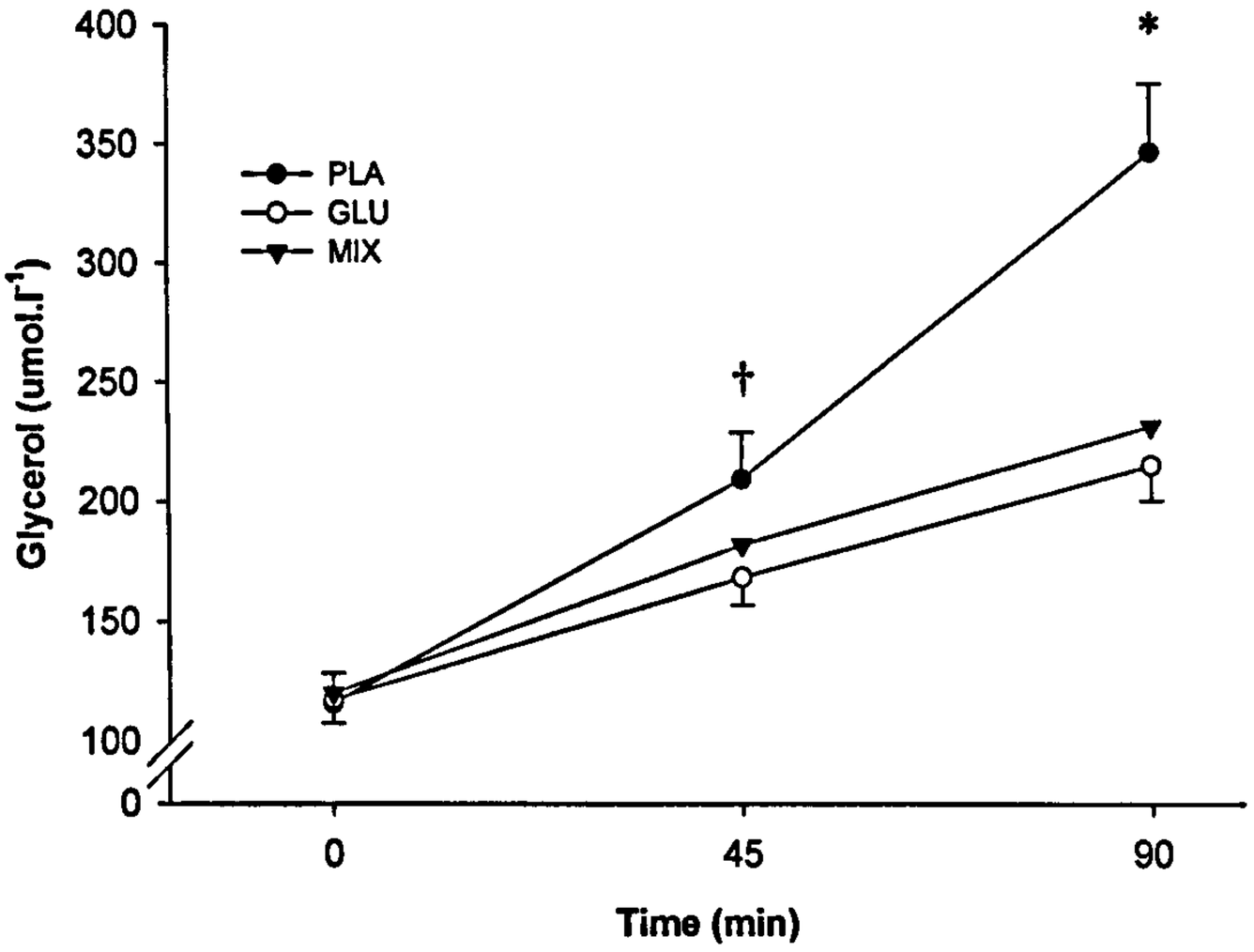

Figure 6.6: Plasma glycerol concentration during the soccer-specific protocol.

* PLA significantly greater than GLU and MIX; † PLA significantly greater than GLU.

No significant trial effect on the plasma concentration of lactate was observed ($F_{1,10}$=1.051; P>0.05). Plasma lactate concentration increased significantly above resting levels after the onset of exercise, with peak values at half-time ($F_{2,16}$=15.527; P<0.05) (Figure 6.7).

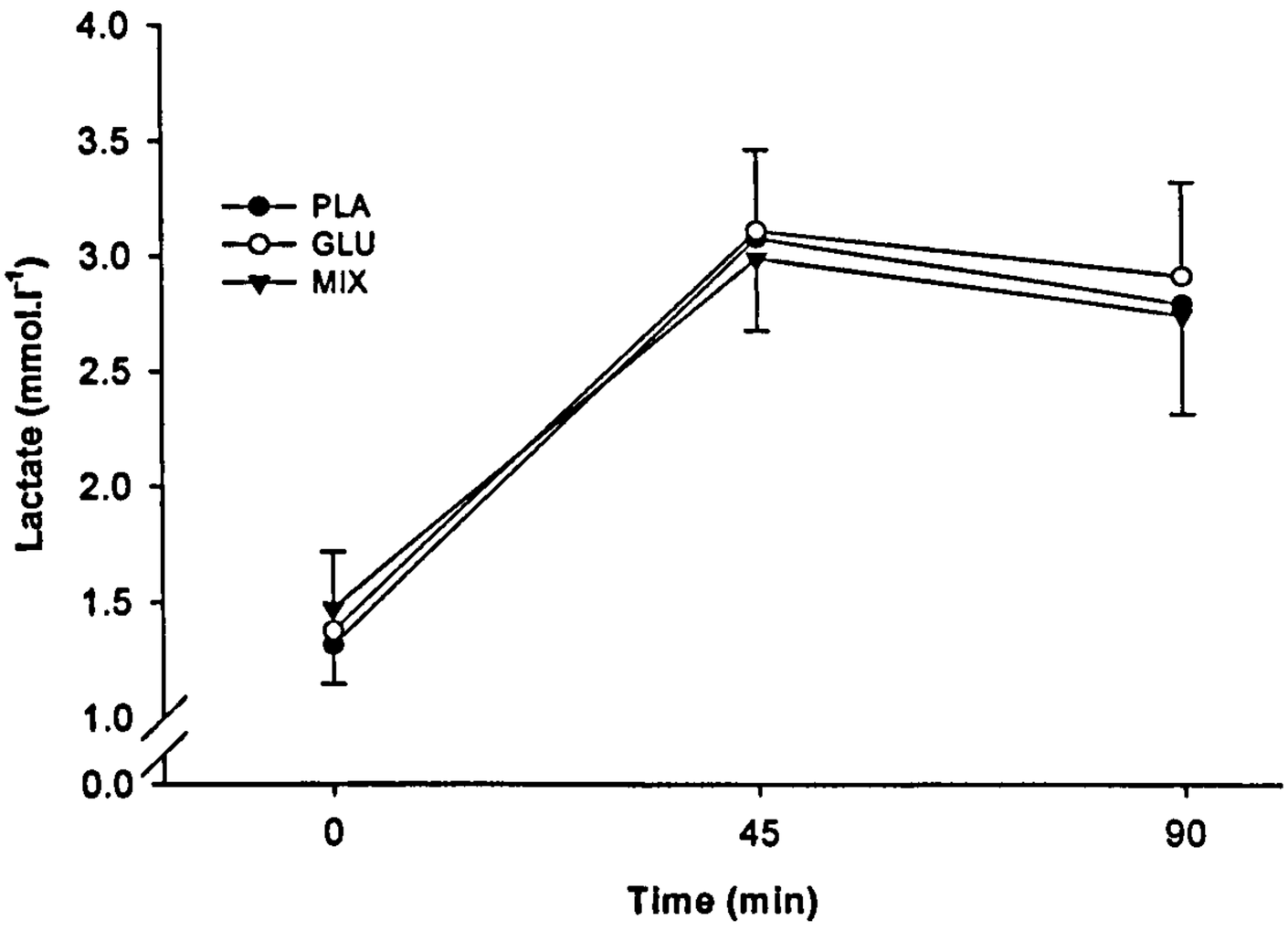

Figure 6.7: Plasma lactate concentration during the soccer-specific protocol.

6.3.4. Hormones

The concentration of adrenaline was found to be similar when all trials were compared ($F_{2,19}$=0.840; P>0.05, Figure 6.8). The adrenaline values increased significantly ($F_{1,11}$=12.971; P<0.05) between each time point.

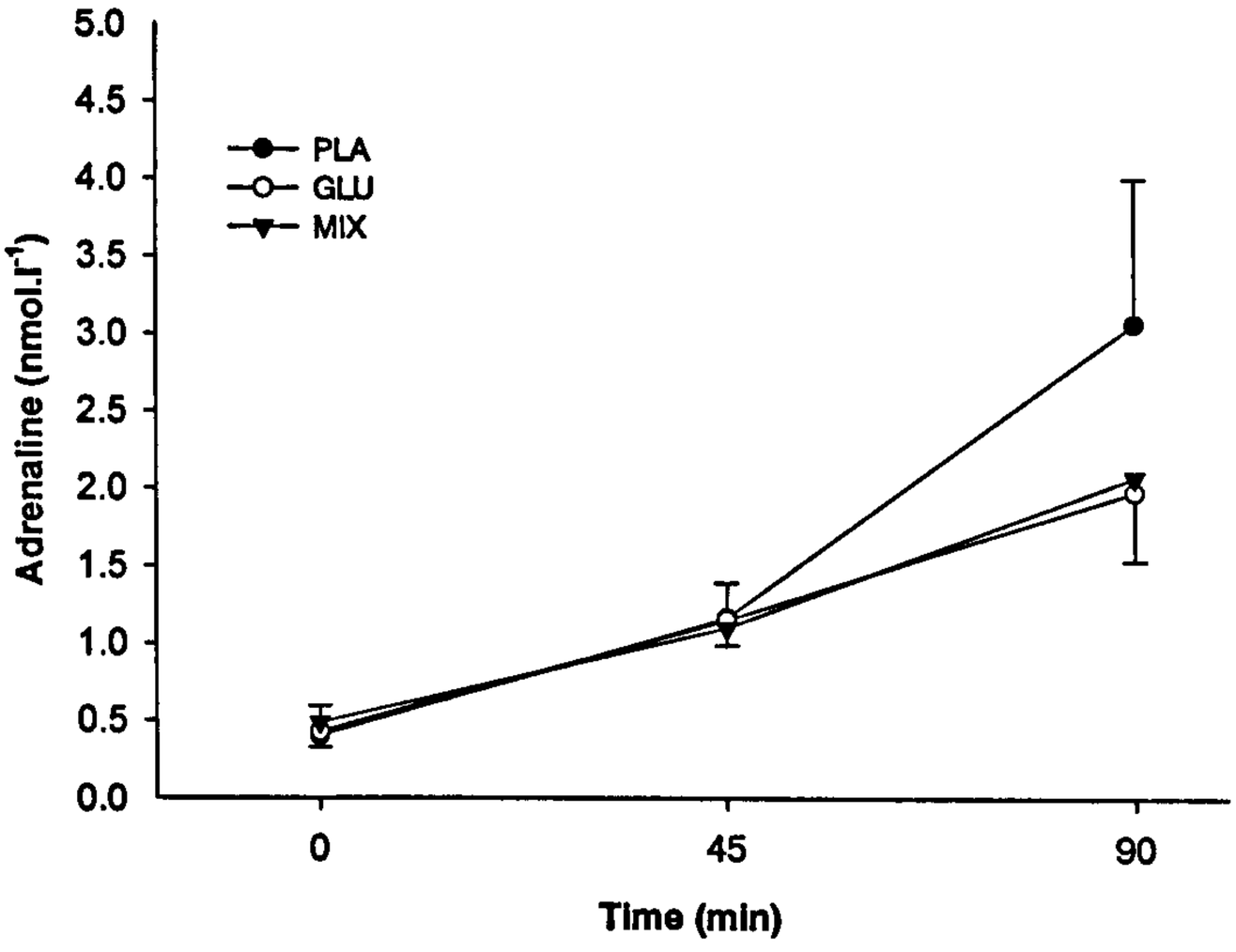

Figure 6.8: Plasma adrenaline concentration during the soccer-specific protocol.

A similar pattern was observed for noradrenaline levels (Figure 6.9); there were no significant differences between any of the trials ($F_{2,15}$=0.284; P>0.05). The concentration of noradrenaline increased significantly ($F_{2,15}$=73.117; P<0.05) between each time point.

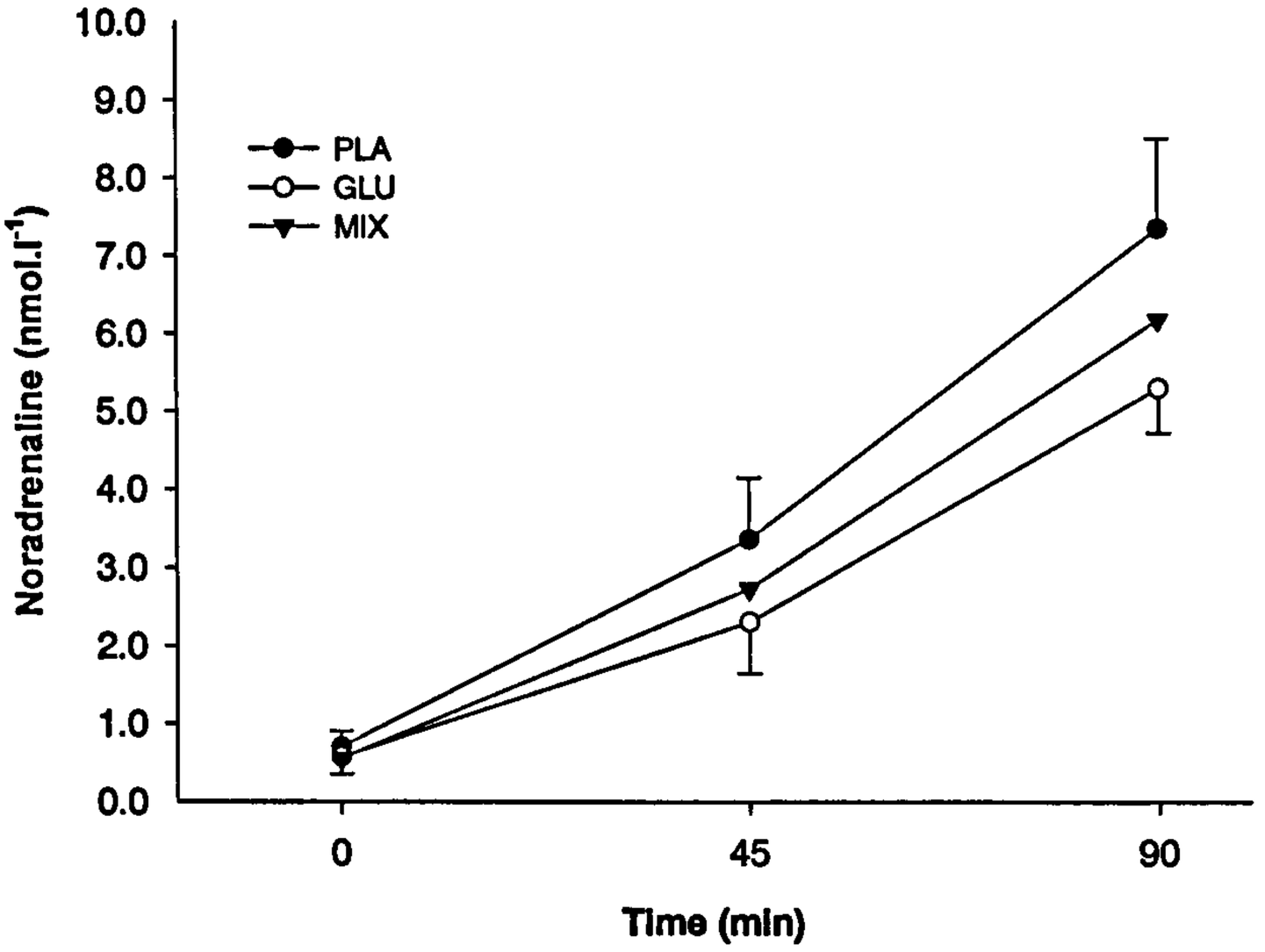

Figure 6.9: Plasma noradrenaline concentration during the soccer-specific protocol.

The concentration of IL-6 was significantly affected by the experimental trials ($F_{2,17}$=16.311; P<0.05), and was significantly (P<0.05) higher during the PLA trial compared with GLU and MIX throughout the protocol (Figure 6.10). The values for IL-6 increased significantly between each time point ($F_{2,20}$=157.194; P<0.05). There was also a significant trial and time interaction ($F_{3,28}$=8.802; P<0.05); during the first 45 min the IL-6 concentration increased markedly more during PLA compared with GLU and MIX, although the increase was less pronounced during the second 45 min.

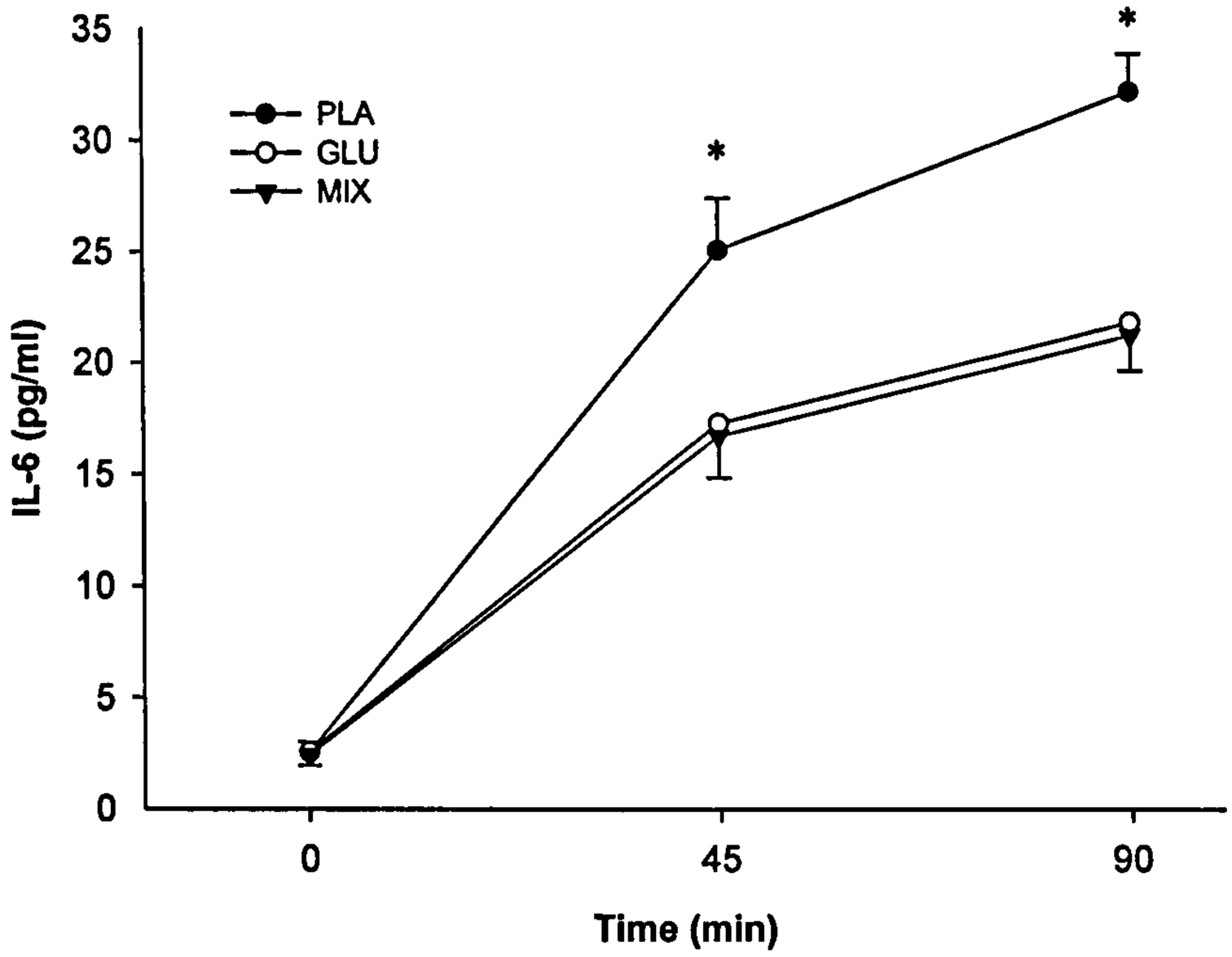

Figure 6.10: Interleukin-6 concentration during the soccer-specific protocol.

* PLA significantly greater than GLU and MIX

There was a significant trial effect of the concentration of serum insulin ($F_{2,16}$=12.251; P<0.05; Figure 6.11). The serum insulin concentration was significantly higher during GLU and MIX than during the PLA condition (P<0.05). Serum insulin concentration increased significantly between pre-exercise and half-time ($F_{1,15}$=4.327; P<0.05). The repeated measures ANOVA identified a significant time and trial interaction ($F_{2,24}$=4.083; P<0.05). Serum insulin concentration increased during the first half of GLU and MIX, whereas in contrast it decreased during PLA. All trials demonstrated decreased insulin response during the second half, markedly so during GLU.

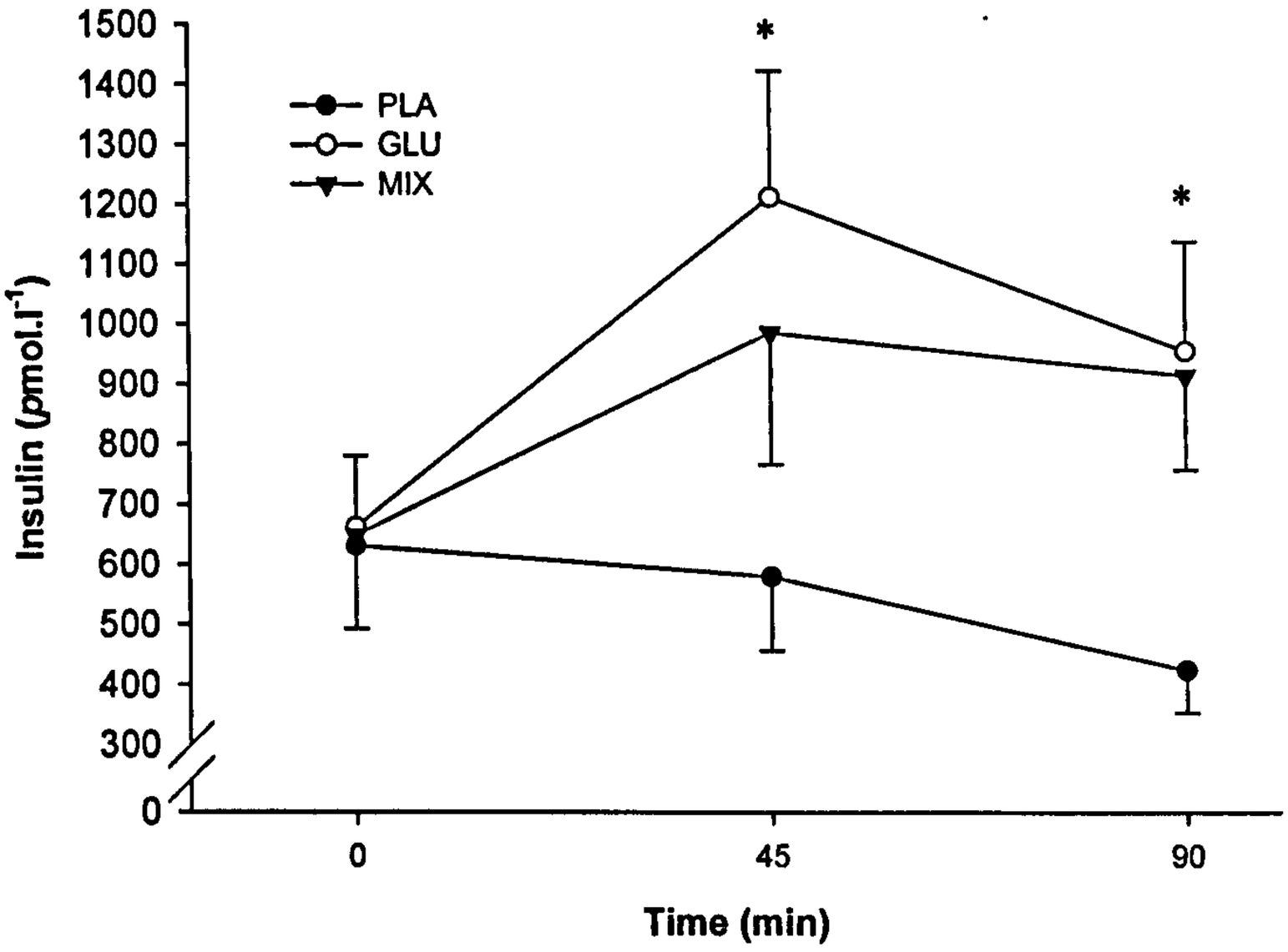

Figure 6.11: Serum insulin concentration during the soccer-specific protocol.

* GLU and MIX significantly higher than PLA.

6.3.5. Plasma osmolality

There was no significant difference in plasma osmolality (Figure 6.12) between the three experimental conditions ($F_{1,14}$=0.011; P>0.05). Plasma osmolality was significantly higher ($F_{2,17}$=5.404; P<0.05) at the completion of the soccer-specific protocol (302.73±1.93 mOsm.kg^{-1}), compared with pre-exercise values (295.61±1.08 mOsm.kg^{-1}).

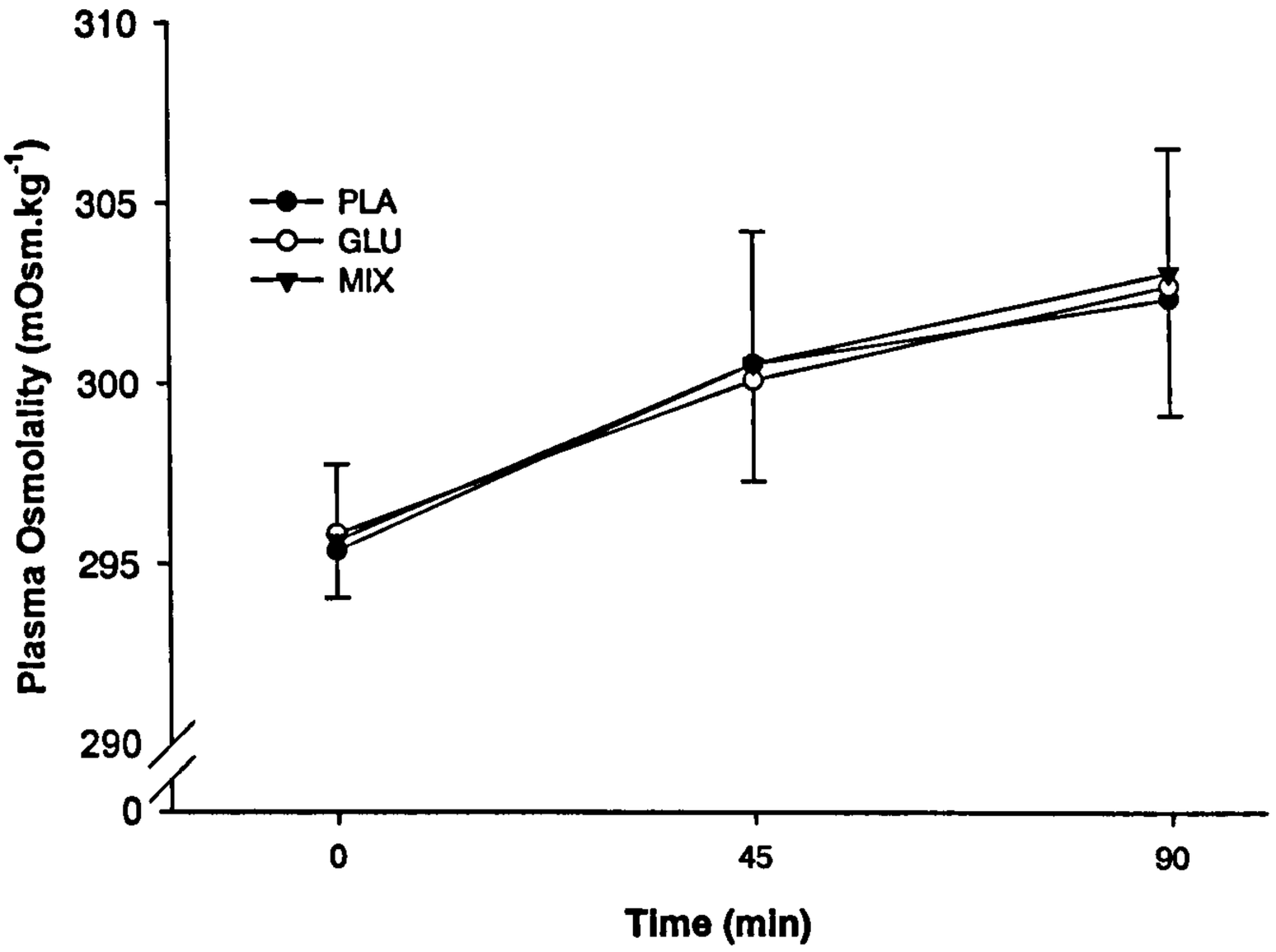

Figure 6.12: Changes in plasma osmolality during the soccer-specific protocol.

6.3.6. Indirect calorimetry

There was no significant difference in RER between the three trials, ($F_{2,17}$=3.138; P>0.05, Figure 6.13). During PLA, RER was lower than during GLU or MIX, indicating a greater proportion of fat oxidation; however, this difference was not significant. There was a significant effect of time on RER ($F_{2,21}$=12.838; P<0.05). Block 1 showed significantly (P<0.05) higher values than the subsequent 75 min, indicating that a greater proportion of carbohydrate was oxidised. There was a significant interaction ($F_{4,38}$=4.264; P<0.05); RER during MIX remained relatively constant throughout the soccer-specific protocol, whereas during GLU it increased sharply after half-time, before steadily decreasing during the second half. In contrast, RER decreased throughout both halves of the soccer-specific protocol during PLA.

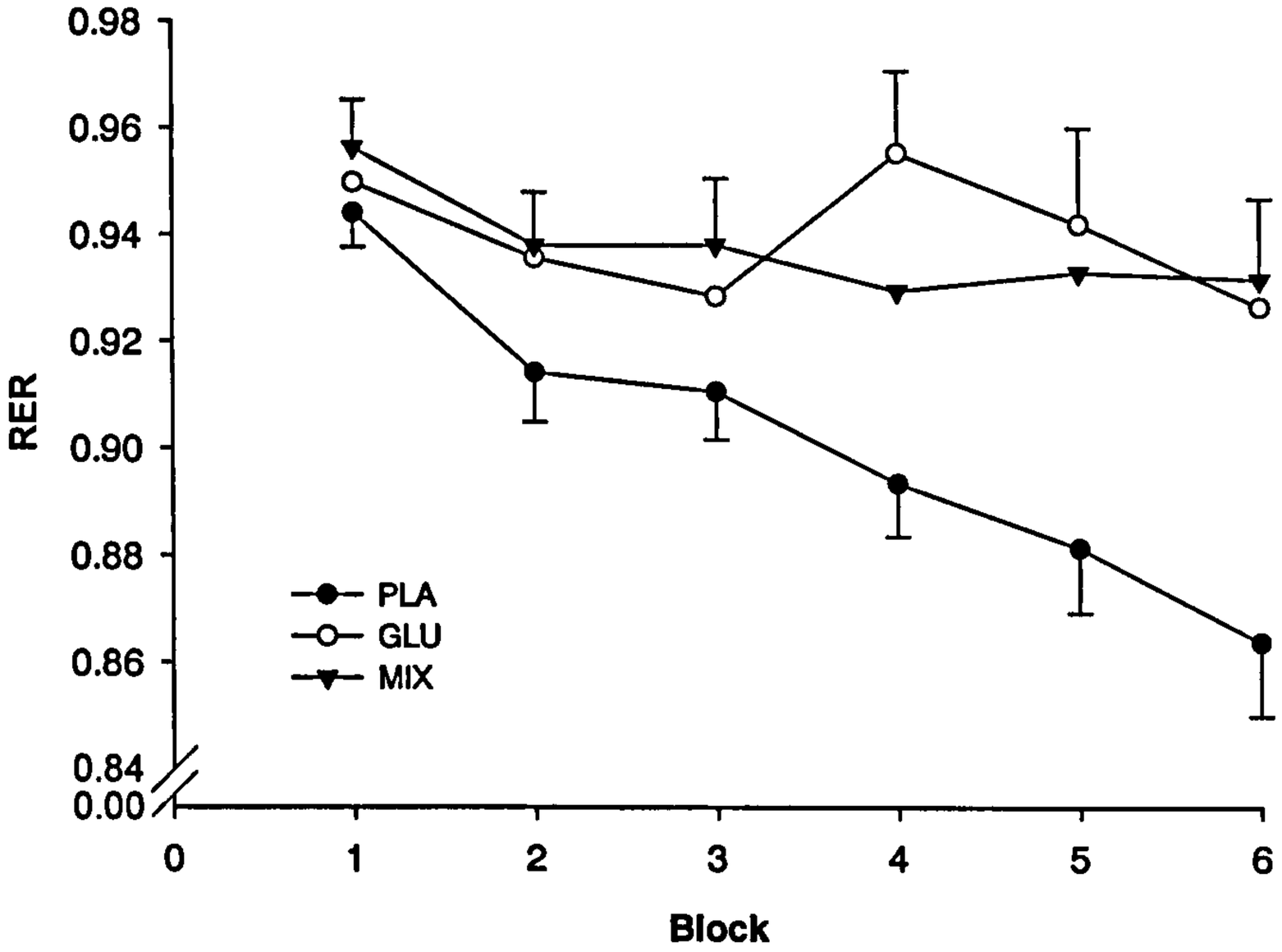

Figure 6.13: Respiratory exchange ratio during the soccer-specific protocol.

Total carbohydrate oxidation (Figure 6.14) was significantly ($F_{2,20}$=3.556; P<0.05) affected by the trials. Carbohydrate oxidation was greater during MIX compared to PLA (P<0.05). There were no significant differences between MIX and GLU (P>0.05) or GLU and PLA (P>0.05). Carbohydrate oxidation was significantly ($F_{3,28}$=11.883; P<0.05) higher during block 1 compared with the rest of the protocol. There was a significant interaction between trial and time ($F_{4,37}$=3.802; P<0.05); carbohydrate oxidation was stable during GLU and MIX, in contrast it declined steadily during PLA.

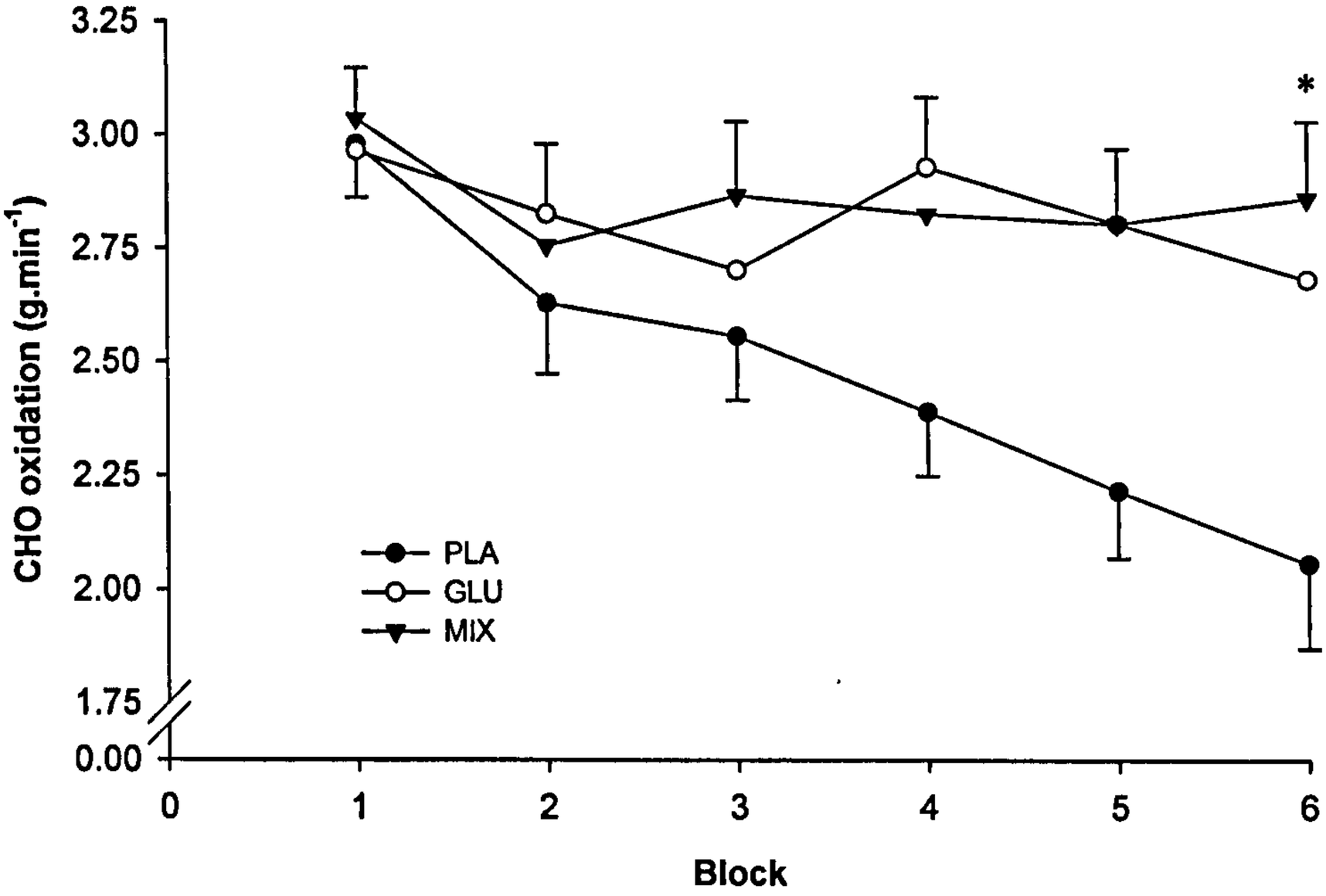

Figure 6.14: Carbohydrate oxidation during the soccer-specific protocol.

* MIX significantly higher than PLA.

Repeated measures ANOVA showed that the rate of fat oxidation during PLA was significantly higher ($F_{2,19}$=4.112; P<0.05, Figure 6.15) than MIX, and there was no significant difference between GLU and MIX (P>0.05) or PLA and GLU (P>0.05). The repeated measures ANOVA identified a significant effect of time ($F_{2,22}$=10.701; P<0.05). The Bonferroni corrected pairwise comparisons revealed that fat oxidation was significantly lower (P<0.05) during block 1 compared with the remainder of the protocol. There was a significant interaction ($F_{3,31}$=4.440; P<0.05), fat oxidation increased steadily during PLA, in contrast with the carbohydrate trials, in particular MIX, during which after an initial increase, fat oxidation was relatively constant.

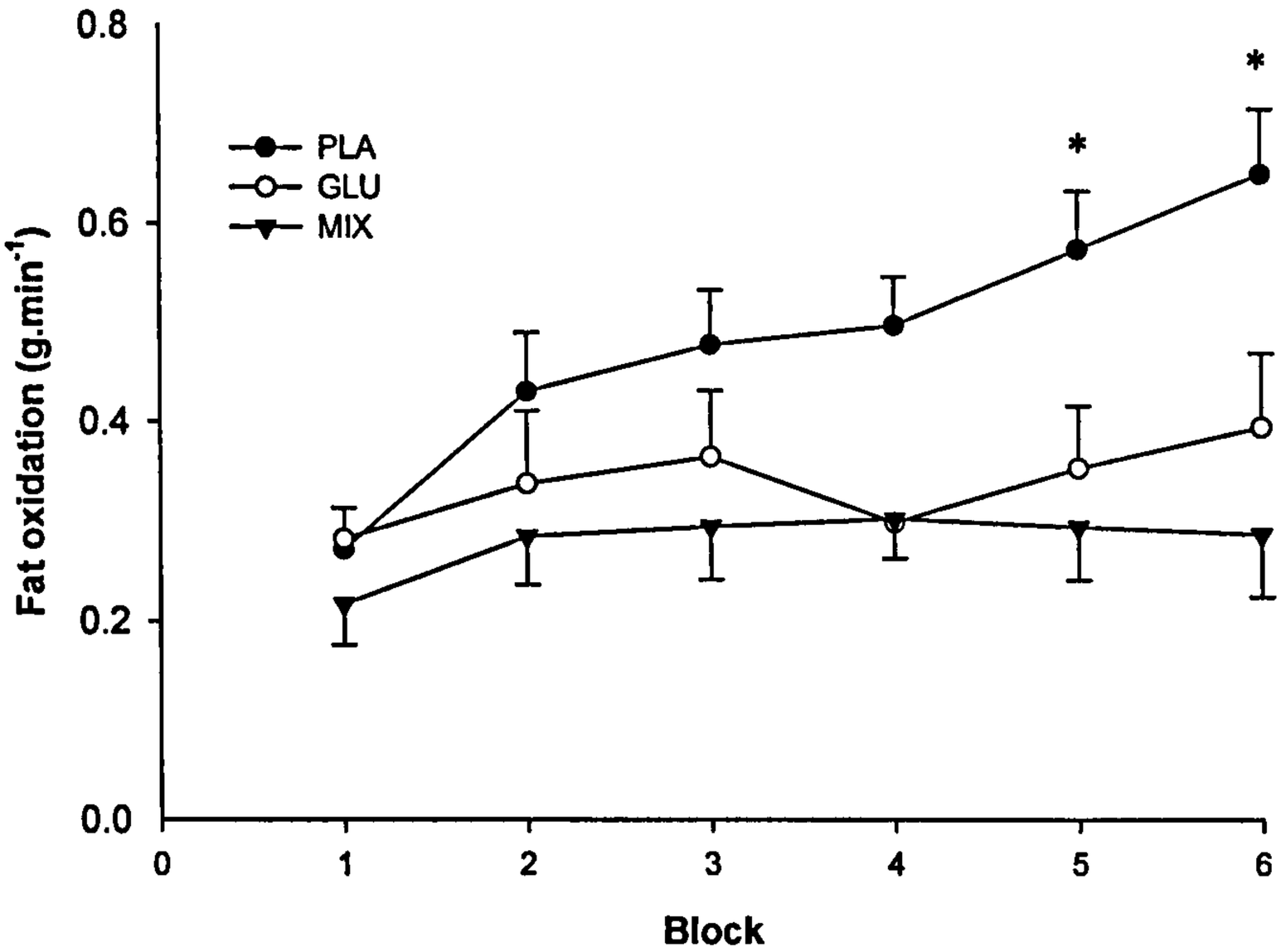

Figure 6.15: Fat oxidation during the soccer-specific protocol.

* PLA significantly higher than MIX.

6.3.7. Gut Fullness and thirst

Gut fullness was not significantly ($F_{2,18}$=0.132; P>0.05) affected by the trials (Table 6.1). There was a significant effect of time ($F_{3,31}$=9.491; P<0.05), pairwise comparisons revealed that gut fullness increased significantly post-fluid ingestion prior to completing the protocol, and remained significantly (P<0.05) elevated throughout.

Table 6.1: Subjective sensation of gut fullness during soccer-specific protocol.

	Time point								
	Pre-	Post-	1	2	3	HT	4	5	6
PLA	23.0±4.3	41.3±3.3	42.7±5.4	43.1±3.7	55.5±5.4	53.5±7.6	57.0±5.8	57.5±6.7	50.9±5.6
GLU	32.0±8.3	49.5±7.0	45.0±6.1	48.5±8.0	55.3±7.6	54.6±7.6	49.8±7.8	50.3±7.1	56.5±7.1
MIX	24.5±4.6	41.0±4.7	37.8±6.6	42.6±7.6	51.0±6.5	60.8±6.5	52.1±7.3	56.5±7.2	55.0±7.3

Note: Pre – Pre-fluid ingestion; Post – Post-fluid ingestion; 1-6 – Blocks; 1-6, HT – Half-time.

There was a significant difference in thirst between the trials ($F_{2,19}$=4.580; P<0.05, Figure 6.16); the subjective sensation of thirst was significantly (P>0.05) higher during MIX compared with GLU. There was also a significant difference between time points ($F_{3,31}$=9.728; P<0.05); pairwise comparisons indicated these differences occurred between post-fluid ingestion at half-time and at all time points except following the ingestion of fluid before the start of exercise.

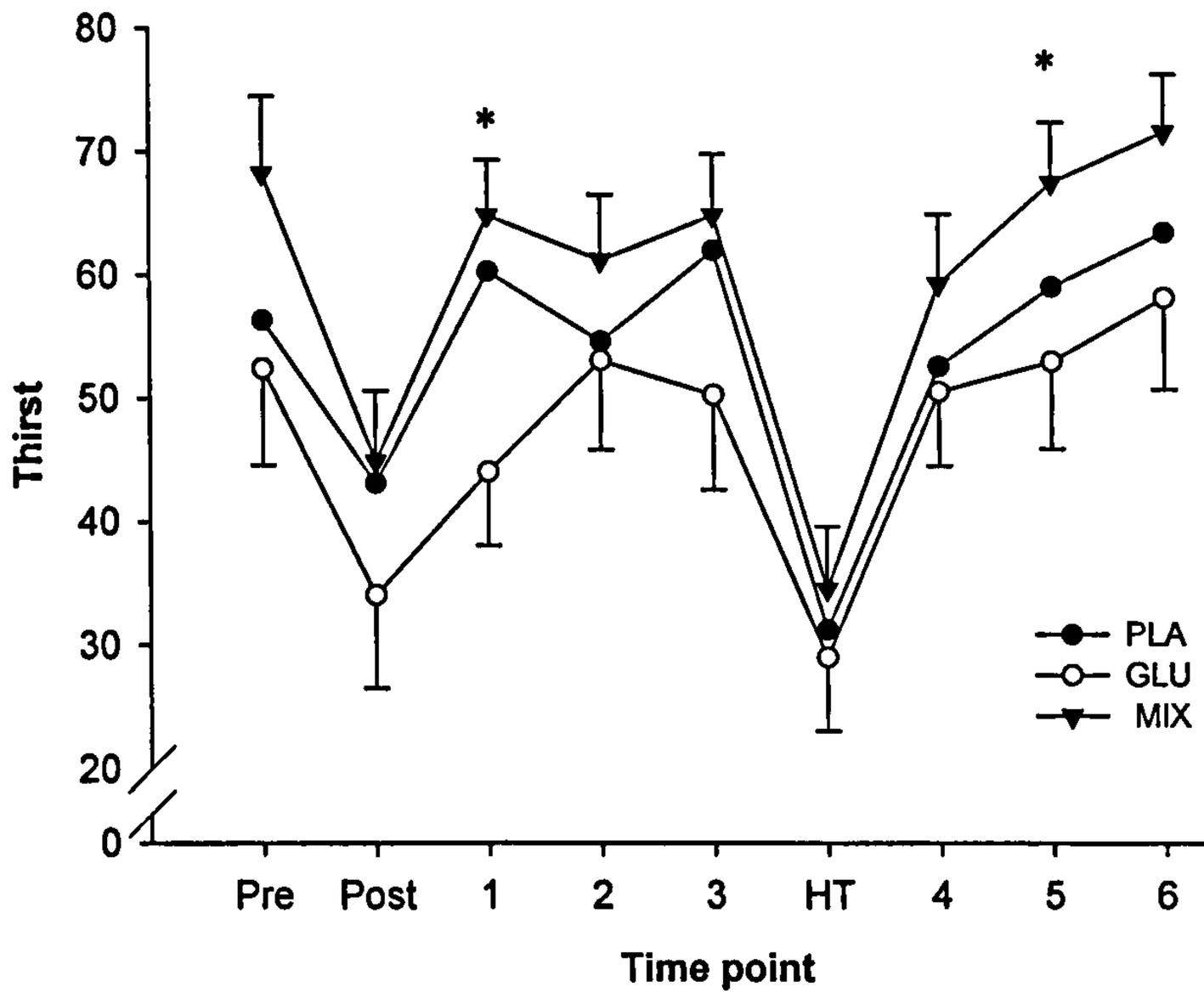

Figure 6.16: Subjective sensation of thirst during the soccer-specific protocol. Pre – pre-fluid, Post – post-fluid, HT – half-time, 1-6 indicates 15-min blocks of the soccer-specific protocol. * MIX significantly higher than GLU.

6.3.8. Core Temperature

There was no significant trial effect on core temperature ($F_{2,17}$=0.654; P>0.05; Figure 6.17). A significant effect of time was observed ($F_{2,24}$=89.672; P<0.05), pairwise comparisons revealed these differences occurred between the first block and the following five blocks. Core temperature increased significantly (P<0.05) during each half of the soccer-specific protocol, but there was no significant difference (P>0.05) between

mean temperature at the end of the first half and the start of the second, i.e. the half-time fall in core temperature did not reach statistical significance.

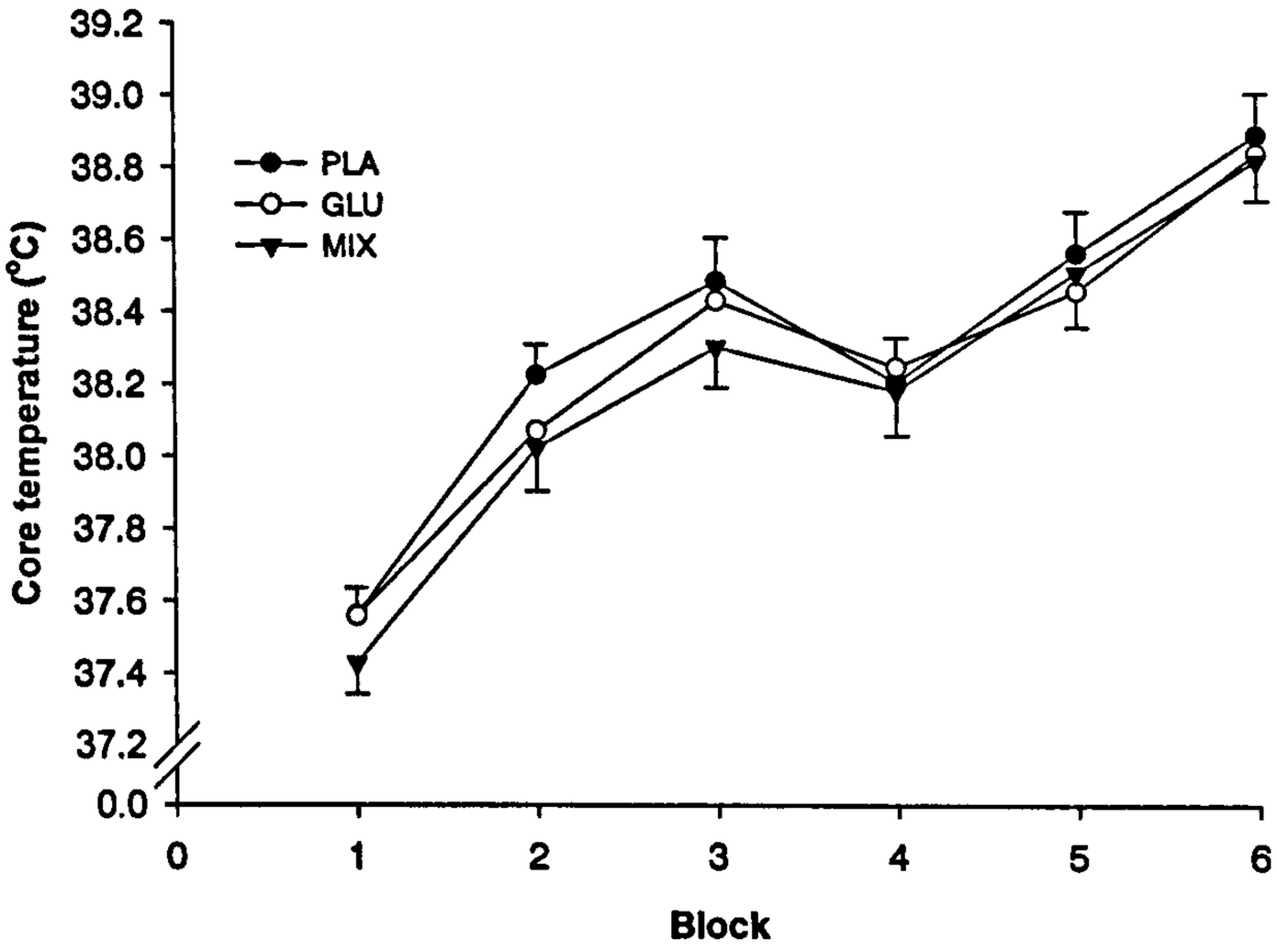

Figure 6.17: Core temperature during soccer-specific protocol.

6.3.9. Thermal sensation

Thermal sensation (Table 6.2) followed the same pattern as core temperature. There was no significant trial effect ($F_{2,17}$=0.083; P>0.05). There was a significant effect of time ($F_{2,22}$=33.860; P<0.05), thermal sensation increasing significantly (P<0.05) during each half of the soccer-specific protocol. Thermal sensation was also significantly lower at half-time compared with blocks 3 and 4.

Table 6.2: Thermal sensation during soccer-specific protocol.

	Time point						
	1	2	3	HT	4	5	6
PLA	5.00±0.1	5.55±0.2	5.95±0.2	4.09±0.2	5.73±0.2	6.27±0.3	6.68±0.3
GLU	5.18±0.2	5.73±0.2	5.91±0.2	4.36±0.1	5.59±0.3	6.05±0.3	6.36±0.3
MIX	5.27±0.2	5.82±0.3	5.95±0.3	4.45±0.1	5.64±0.2	6.02±0.2	6.36±0.3

Note: 1-6 – Blocks; 1-6, HT – Half-time.

6.3.10. Heart rate and RPE

There was no significant trial effect on heart rate ($F_{2,18}$=2.218; P>0.05, Figure 6.18). Heart rate increased significantly ($F_{2,15}$=61.218; P<0.05) throughout each half of the soccer-specific protocol.

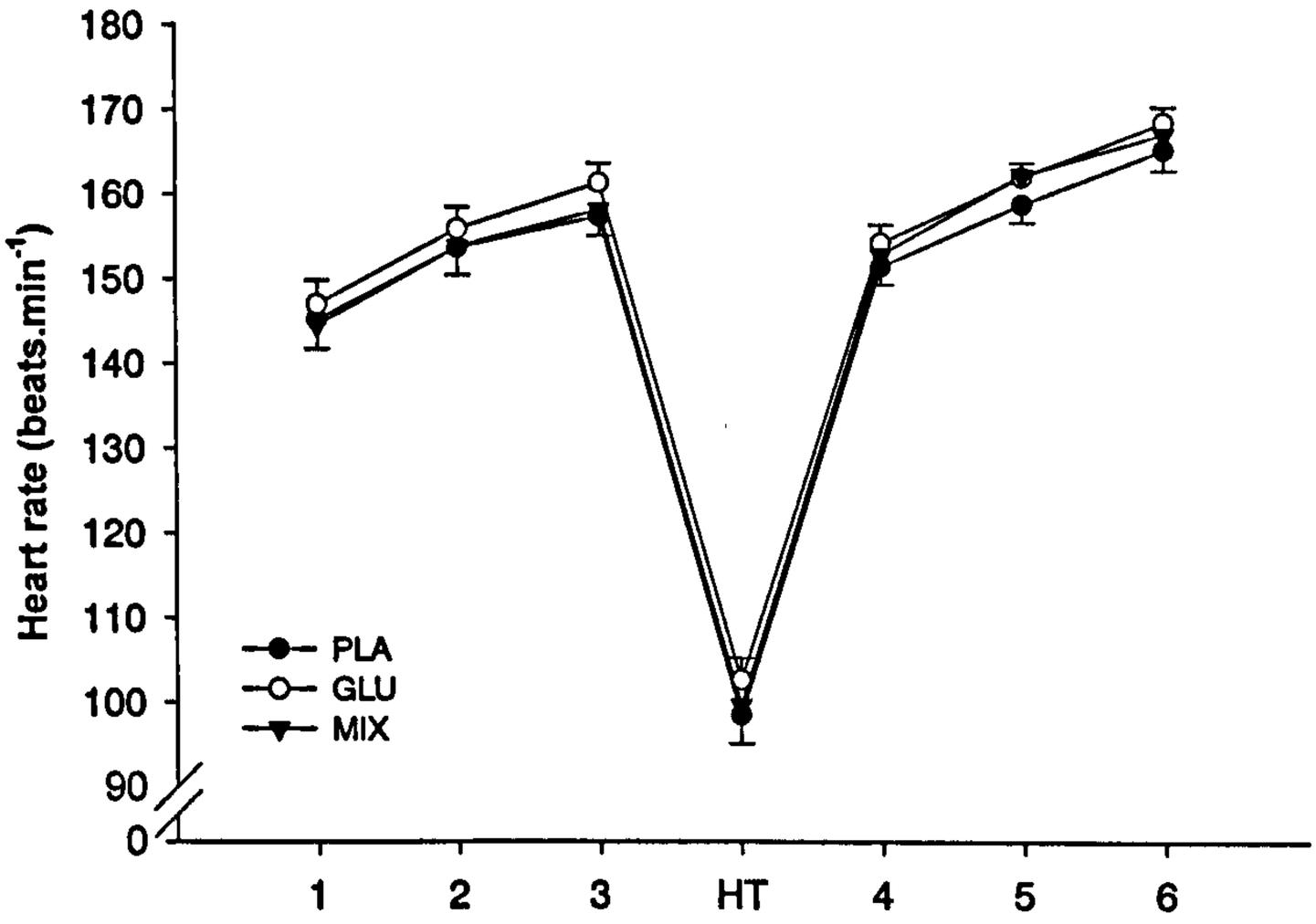

Figure 6.18: Heart rate during soccer-specific protocol.

There was no significant ($F_{2,19}$=1.126; P>0.05) difference in RPE between trials (Figure 6.19). A significant effect of time was detected ($F_{2,23}$=54.101; P<0.05); RPE increased significantly throughout each half of the soccer-specific protocol, following the same pattern as heart rate.

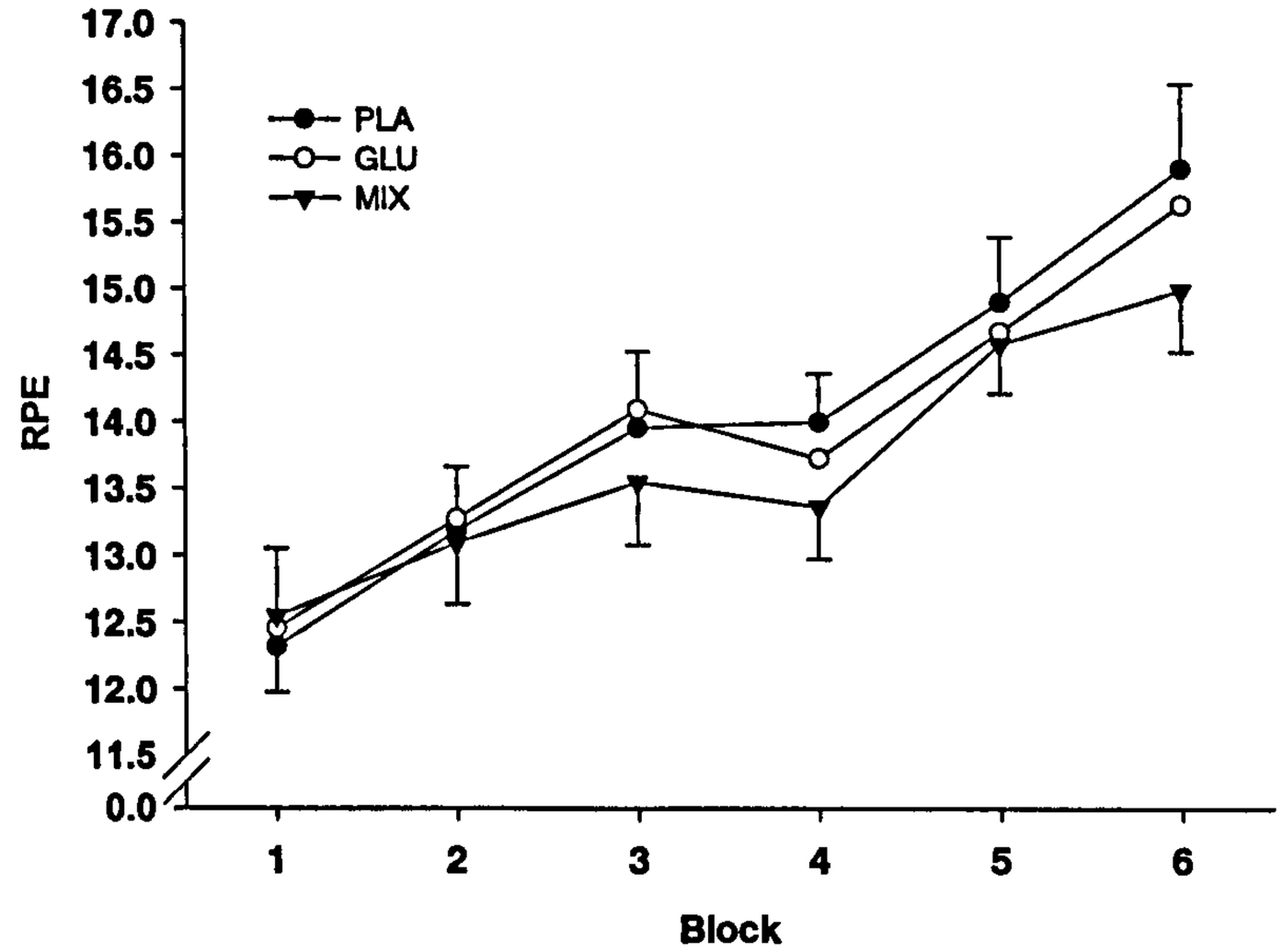

Figure 6.19: RPE during soccer-specific protocol.

6.3.11. Cunningham and Faulkner test

There was no significant trial effect ($F_{2,16}$=1.982; P<0.05) on exercise capacity (Figure 6.20) PLA: 73.62±8.61 s; GLU: 77.11±7.17 s; MIX: 83.04±9.65 s.

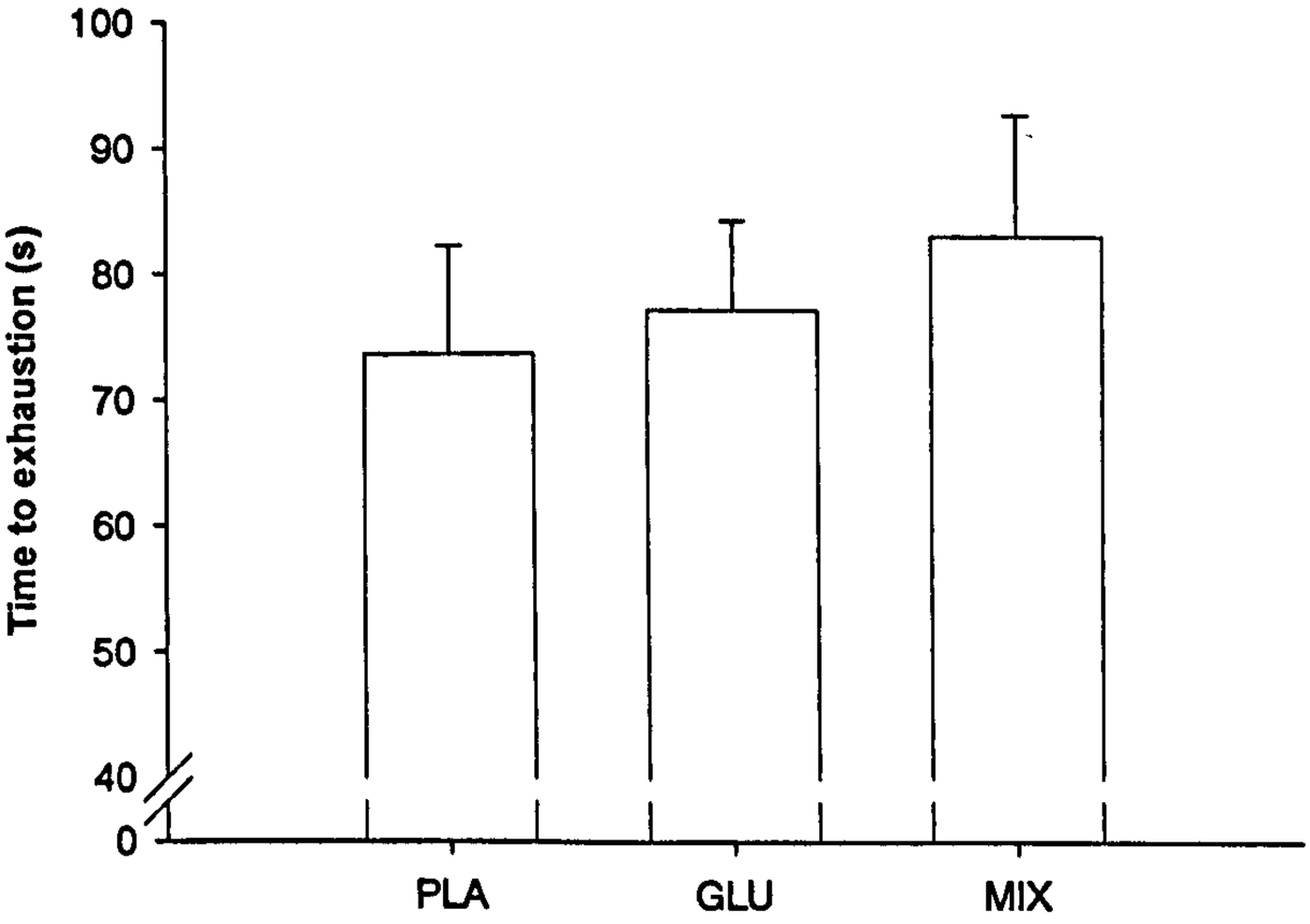

Figure 6.20: Time to exhaustion during the Cunningham and Faulkner test.

6.3.12. Sweat loss

There was no significant difference ($F_{2,17}$=0.019; P>0.05) in sweat loss between the three trials. The mean losses were: PLA (2.08±0.06 kg), GLU (2.11±0.16 kg) and MIX (2.10±0.11 kg). The absolute weight loss (uncorrected for fluid ingestion) was not significantly different between trials ($F_{2,19}$=0.689; P>0.05); PLA: 1.03±0.1 kg, GLU: 1.07±0.2 kg and MIX: 1.02±0.1 kg.

6.1. Discussion

The major finding of this study in relation to exercise capacity was that the ingestion of a solution containing glucose and fructose did not significantly impact on the rate of total carbohydrate oxidation or exercise capacity during a high-intensity exercise test compared with the ingestion of a solution containing only glucose.

Jentjens *et al.* (2006) recently demonstrated that in non-acclimated athletes exercising in the heat the ingestion of a solution containing glucose and fructose resulted in approximately 36% greater exogenous carbohydrate oxidation rates compared with the ingestion of a solution containing only glucose. However, Wallis *et al.* (2005) reported that whilst the ingestion of carbohydrate significantly suppressed endogenous carbohydrate oxidation, there was no significant difference in the sparing of endogenous carbohydrate between a maltodextrin drink and an isoenergetic maltodextrin plus fructose drink. This observation may explain the lack of difference in exercise capacity between GLU and MIX in the present study, as muscle glycogen content, which has been shown to limit exercise capacity, was similar at the end of exercise for both of these treatments and consistent with values observed post competitive match-play (Rico-Sanz *et al.*, 1999). In addition, the pre-exercise concentration of muscle glycogen was similar to previous studies (Graham *et al.*, 1993; Rico-Sanz *et al.*, 1999; Haff *et al.*, 2000)

During exercise in the heat, the onset of fatigue is possibly a consequence of hyperthermia (Nielsen *et al.*, 1993; Gonzalez-Alonso *et al.*, 1999c). It has been suggested that carbohydrate availability is not a limiting factor for exercise in the heat when the heat stress is uncompensable (Febbraio, 2001). This hypothesis is based on the observation that muscle glycogen concentrations are relatively high at the point of fatigue when exercising in the heat (Nielsen *et al.*, 1990; Febbraio *et al.*, 1994b). It also appears that the addition of carbohydrate to a solution has no impact on key thermoregulatory variables such as core temperature and heart rate (Morris *et al.*, 2003).

Nielsen *et al.* (1993) suggested that when fatigue occurs in a hot environment it is ultimately due to an intolerably high core temperature. This observation may explain the lack of difference in exercise capacity between treatments, because despite higher blood glucose during GLU and MIX than in the placebo trial, core temperature and thermal sensation were similar during all conditions. In addition, Nielsen *et al.* (2001) found that alterations in front cortical brain activity correlated with increases in core temperature. Therefore, the motivation to continue to exercise may be reduced with increases in core temperature beyond a critical point. At no point during any of trials did core temperature stabilize, it increased significantly between each time point during the second half of the protocol, suggesting a large thermal strain. However, the final value at the completion of the 90 minutes was lower than previously reported values during similar exercise protocols (Morris *et al.*, 1998; Morris *et al.*, 2003). This observation may be a consequence of different sites being used to assess core temperature, rectal as opposed to intestinal used in the present study.

During prolonged exercise in the heat, it has been suggested that fluid containing more than 2.5% carbohydrate inhibits fluid delivery (Costill and Saltin, 1974). However, Hawley *et al.* (1991) demonstrated that fluid containing as much as 15% carbohydrate was as effective as water in supporting thermoregulation and performance during exercise in the heat. Jentjens *et al.* (2002) reported that the rate of exogenous glucose oxidation was reduced by 10% in the heat compared with a cool environment. They also identified that despite a slower rate of exogenous glucose oxidation, total carbohydrate oxidation was higher in the heat as a result of increased muscle glycogenolysis. As a consequence muscle glycogen utilization was 25% higher during exercise in the heat compared with the cooler condition. Previous studies have demonstrated that the rate of glycogen oxidation was not significantly different between carbohydrate and water ingestion (Angus *et al.*, 2001; Jentjens *et al.*, 2006). Some of the factors that contribute to the reduction in exogenous glucose oxidation in the heat include the reduced carbohydrate absorptive capacity of the intestine as a result of decreased intestinal blood flow (Jentjens *et al.*, 2002), reduced uptake and release of ingested glucose by the liver, decreased glucose transport to the muscle due to reduced muscle blood flow as a consequence of

impaired hydration status (Gonzalez-Alonso *et al.*, 1999a). The rate of gastric emptying is also reduced during exercise in the heat. Studies have shown that hyperthermia and dehydration can impair gastric emptying of carbohydrate solutions or water during treadmill exercise (Owen *et al.*, 1986; Neufer *et al.*, 1989; Rehrer *et al.*, 1990). In addition a negative correlation between final core temperature and the volume emptied from the stomach was observed (Neufer *et al.*, 1989).

The ingestion of carbohydrate during exercise in moderate temperature conditions has been shown to increase blood glucose (Nicholas *et al.*, 1995) and spare muscle glycogen (Yasplekis *et al.*, 1993a), enhancing endurance capacity (Nicholas *et al.*, 1995). In the present study plasma glucose was significantly higher during GLU compared with PLA. This observation was consistent with that of Jentjens *et al.* (2006) and is possibly a consequence of a greater amount of glucose being present in the solution ingested during GLU compared with MIX. The lack of improvement in exercise capacity despite elevated blood glucose concentrations supports the findings of Morris *et al.* (2003) and suggests that carbohydrate availability was unlikely to be the main factor limiting exercise capacity in the present study, especially as there was no significant difference in the muscle glycogen concentration after the soccer-specific protocol.

Recent studies have demonstrated the ingestion of mixed carbohydrate drinks compared with a drink containing an isoenergetic amount of glucose can increase exogenous carbohydrate oxidation in a thermo-neutral environment (Jentjens *et al.*, 2004a; Jentjens *et al.*, 2004b) and in the heat (Jentjens *et al.*, 2006), although total carbohydrate oxidation is unaffected. The present study demonstrated that carbohydrate ingestion significantly increased total carbohydrate oxidation, although there was no difference between GLU and MIX. This observation is consistent with previous studies (Wallis *et al.*, 2005; Jentjens *et al.*, 2006). However, carbohydrate was ingested at higher concentrations (1.8–2.4 $g \cdot min^{-1}$) (Jentjens *et al.*, 2004a; Jentjens *et al.*, 2004b) than in the present study. Therefore the higher carbohydrate oxidation rates observed when the multi-carbohydrate solution was ingested was probably due to the SGLT1 being saturated at these concentrations of glucose, limiting the rate of exogenous carbohydrate oxidation. The

increase in carbohydrate oxidation observed with carbohydrate ingestion can be attributed to the increase in plasma glucose oxidation (Jeukendrup *et al.*, 1999). In contrast, fat oxidation was suppressed during the soccer-specific protocol with carbohydrate ingestion, although there was no significant difference between GLU and MIX. Insulin has been shown to be a powerful inhibitor of lipolysis and the appearance of NEFA in the blood (Horowitz *et al.*, 1997). The increased insulin concentration after carbohydrate ingestion may have reduced whole-body lipolysis, as indicated by the lower fat oxidation and levels of NEFA and glycerol observed during the GLU and MIX trials. The elevated insulin concentration observed during GLU and MIX compared with PLA may also explain the marginally higher (although not significant: P=0.440) adrenaline level shown during PLA. Previous studies (Felig *et al.*, 1982; Fritzsche *et al.*, 2000) indicated that the adrenaline response was blunted when carbohydrate was ingested.

The concentration of serum IL-6 was significantly higher during PLA compared with GLU and MIX. Steensberg *et al.* (2001) demonstrated that IL-6 is released from skeletal muscle in response to low glycogen levels and may be linked to the regulation of glucose homeostasis may function as a sensor of carbohydrate availability (Pedersen *et al.*, 2004). The ingestion of carbohydrate has previously been shown to suppress the release of IL-6 (Nieman *et al.*, 2003; Nieman *et al.*, 2005). Febbraio *et al.* (2003) demonstrated that carbohydrate ingestion had no impact on the increase in muscle IL-6 mRNA when performing semi-recumbent cycling for 2 h at approximately 62% $\dot{V}O_{2max}$. Nieman *et al.* (2005) speculated that the process of IL-6 release but not gene expression for muscle IL-6 is regulated by substrate availability and/ or flux across the contracting muscle. Furthermore, adrenaline has been shown to enhance the release of IL-6 in rats (Yu *et al.*, 2001). These observations would suggest that IL-6 release is dependent on both carbohydrate availability and the corresponding adrenaline response.

Previous studies (Davis *et al.*, 1990; Jentjens *et al.*, 2006) have indicated that fluid availability during exercise in the heat is lower with a glucose drink compared with a combined glucose and fructose drink or water. Shi *et al.* (1995) also demonstrated that the ingestion of a glucose and fructose solution resulted in greater water absorption than a

glucose solution. In addition, Jentjens *et al.* (2006) reported greater (although not statistically significant) changes in plasma volume with glucose compared with a multi-carbohydrate drink and concluded that a glucose drink may be less effective for fluid replacement during exercise in the heat. This conclusion is not supported by the results of the present study, where plasma osmolality and sweat loss were not significantly different between treatments.

Jentjens *et al.* (2006) suggested that gastric emptying and intestinal absorption were lower when a glucose solution was ingested compared with a glucose and fructose solution. Whilst neither were measured in the present study, the subjective sensation of gut fullness was not significantly different between conditions, suggesting the rate of gastric emptying was similar. However, this conclusion is in contrast with the findings of Jentjens *et al.* (2004b) and Wallis *et al.* (2005) who reported an increase in the number of cases of stomach bloating after ingesting a glucose compared with a multi-carbohydrate drink. The ingestion of MIX resulted in a significantly higher rating of thirst compared GLU. One possible explanation for this occurrence is an increase in plasma angiotensin II which has been shown to be elevated following a high fructose diet in rats (Kobayashi *et al.*, 1993) and it has been suggested that angiotensin II is a dipsogenic hormone (McKinley *et al.*, 2004).

There were no significant differences in blood lactate concentration, core temperature, sweat loss, heart rate or RPE between trials during the soccer-specific protocol, indicating a similar level of physiological stress during each trial. These findings are consistent with the results of Davis *et al.* (1988) and Owen *et al.* (1986) who reported no differences in markers of cardiovascular and thermoregulatory stress with and without carbohydrate ingestion at concentrations up to 10% glucose. The results of the present study suggest that any differences in the rate of gastric emptying or intestinal absorption are not sufficient to alter physiological homeostasis during exercise of this nature when performed in the heat.

In conclusion the ingestion of a solution containing glucose and fructose compared with an isoenergetic glucose solution did not significantly influence muscle glycogen utilization, the metabolic responses to soccer-specific exercise performed in the heat or exercise capacity measured post-exercise. In addition, carbohydrate ingestion did not significantly improve exercise capacity as reflected in the anaerobic test. These results suggest that core temperature and not carbohydrate availability may be the major fatiguing factor during soccer-specific exercise in the heat.

Chapter 7
Study 4

The previous study demonstrated that fatigue during soccer-specific exercise in the heat may be as a result of elevated core temperature and not substrate availability. Therefore, a reduction in core temperature is a possible means of offsetting fatigue in hot conditions. The combined effect of reducing core temperature and ingesting carbohydrate during soccer-specific exercise and the subsequent impact of exercise capacity was the subject of investigation of the study presented in this chapter.

7.1. Introduction

Dehydration of approximately 2-3% body mass regularly occurs during intermittent high-intensity exercise (activities such as soccer), especially when the ambient temperature is high (Stolen *et al.*, 2005). Studies of the effects of dehydration on exercise performance have reported an increase in core temperature, cardiovascular strain, but a decrease in blood volume, skin blood flow and sweat rate (Sawka *et al.*, 1985; Buono and Wall, 2000; Gonzalez-Alonso *et al.*, 2000). It has been suggested that these factors, either individually or synergistically, contribute to a decreased capacity to perform submaximal exercise in the heat (Kay and Marino, 2000). The ingestion of fluid during exercise in a warm environment has been shown to attenuate hyperthermia (Wimmer *et al.*, 1997) and improve performance (Below *et al.*, 1995). The benefits of fluid ingestion for performance include attenuating the rise in core temperature (Wimmer *et al.*, 1997) and reducing the physiological stress on the cardiovascular, central nervous and muscular systems (Coyle, 2004).

Ingesting carbohydrate in moderate environmental conditions enhances exercise performance and endurance capacity (Coyle *et al.*, 1986; Ball *et al.*, 1995; Nicholas *et al.*, 1995; Vergauwen *et al.*, 1997). The ingestion of glucose has also been shown to enhance cognitive performance (Scholey *et al.*, 2001). However, the ingestion of carbohydrate solutions during exercise in the heat has produced equivocal results. Some authors (Davis *et al.*, 1988; Millard-Stafford *et al.*, 1992) have reported performance benefits, whilst others (Febbraio *et al.*, 1996; Morris *et al.*, 2003) have suggested that carbohydrate availability is not a limiting factor when exercise is performed in the heat, as observed in

the previous study. The effect of ingesting fluid containing carbohydrate during soccer-specific exercise performed in the heat has not been extensively investigated (Morris *et al.*, 2003).

Another fatiguing factor during exercise is when core temperature reaches a critical value (Nielsen *et al.*, 1993), and a number of strategies have been implemented to reduce thermoregulatory strain (Kay and Marino, 2000). The principle of pre-cooling is the reduction of core body temperature prior to performing exercise, thereby increasing the margin for metabolic heat production and the time before reaching a critical limiting temperature when a given exercise intensity can no longer be maintained. Previous research has indicated that pre-cooling can improve endurance exercise (Lee and Haymes, 1995; Booth *et al.*, 1997; Cotter *et al.*, 2001; White *et al.*, 2003; Arngrimsson *et al.*, 2004), although others have demonstrated a negative effect (Kruk *et al.*, 1990; Kay *et al.*, 1999). However, limited research has been conducted using high-intensity exercise (Marsh and Sleivert, 1999; Cotter *et al.*, 2001; Sleivert *et al.*, 2001). Pre-cooling has also been used as a technique prior to performing soccer-specific exercise (Drust *et al.*, 2000a). However, pre-cooling was only performed under "normal" laboratory conditions (20.5°C, 68.3% relative humidity). Therefore, pre-cooling may be of benefit during 90 min of soccer-specific exercise in the heat when core temperature would increase at a faster rate and reach a higher value.

Dehydration causes a decrease in the performance of various cognitive abilities such as decisional or perceptual tasks (Sharma *et al.*, 1986; Gopinathan *et al.*, 1988; Cian *et al.*, 2000; Cian *et al.*, 2001). This reduction in cognitive performance has been shown to occur irrespective of the dehydration mode (exercise or heat stress) (Cian *et al.*, 2000). Furthermore, the reduction is proportionate to the degree of dehydration and becomes significant with a loss of 2% body mass (Gopinathan *et al.*, 1988). Cain *et al.* (2001) reported significant improvements in cognitive performance (long-term memory) when euhydration was restored following dehydration. Ingesting a carbohydrate-electrolyte solution during exercise has also been shown to minimise the negative effect of central fatigue on cognitive performance induced by prolonged exercise at intensities greater

than 60% $\dot{V}O_{2max}$ (Reilly and Lewis, 1985; Collardeau *et al.*, 2001) and intermittent exercise (Winnick *et al.*, 2005). Therefore, reducing the impact of dehydration and maintaining blood glucose may aid cognitive performance during a soccer match and would be beneficial in terms tactical thought and decision making.

At present, the metabolic responses to exercise following pre-cooling have not been widely investigated and have not examined in conjunction with carbohydrate ingestion during exercise. Therefore, the aim of this investigation was to examine the combined effect of carbohydrate supplementation and pre-cooling on the metabolic responses to soccer-specific exercise and a combination of performance measures.

7.2. Methods

7.2.1. Subjects

Twelve male university soccer players (mean age: 25±0.68 years; weight: 73.75±2.55 kg; height: 1.80±0.02 m; $\dot{V}O_{2max}$: 61.3±1.4 $ml \cdot kg^{-1} \cdot min^{-1}$) participated in this study. All subjects provided written informed consent to participate, in accordance with Liverpool John Moores University's ethical procedures. The experiment was approved by the University's Human Ethics Committee.

7.2.2. Experimental Protocol

The subjects undertook two familiarisation sessions, consisting of two blocks of the soccer-specific protocol (i.e. 30 minutes) outlined in section 3.1.4. Subjects completed the full soccer-specific protocol on a motorised treadmill on four occasions in the environmental chamber (30.5±0.1°C and 42.2±0.2% relative humidity). During two sessions a carbohydrate electrolyte solution (Lucozade Sport, GlaxoSmithKline, Gloucestershire, UK) was consumed at 0, 15, 30, 45, 60, 75 min of exercise (CHO). During the other sessions a placebo (a similarly coloured, flavoured and textured electrolyte solution) (GlaxoSmithKline, Gloucestershire, UK) was consumed (PLA).

Table 7.1: Volumes of fluid ingestion.

Trial	Drink volume (ml)	Total volume (ml)
PLA	223±7	1339±41
PLAc	224±7	1344±41
GLU	223±7	1337±41
GLUc	222±7	1333±41

On one occasion for each drink the subject underwent pre-cooling (CHOc and PLAc). The volumes of fluid ingested during each trial are presented in Table 7.1. The pre-cooling strategy involved the subject wearing a cooling vest (Cool Vest, Jackson

Technical Solutions Ltd, Kent, UK) for 60 min prior to exercise (Figure 7.1) and again at half-time. All of the trials were performed in a counter-balanced fashion and where possible, double-blind.

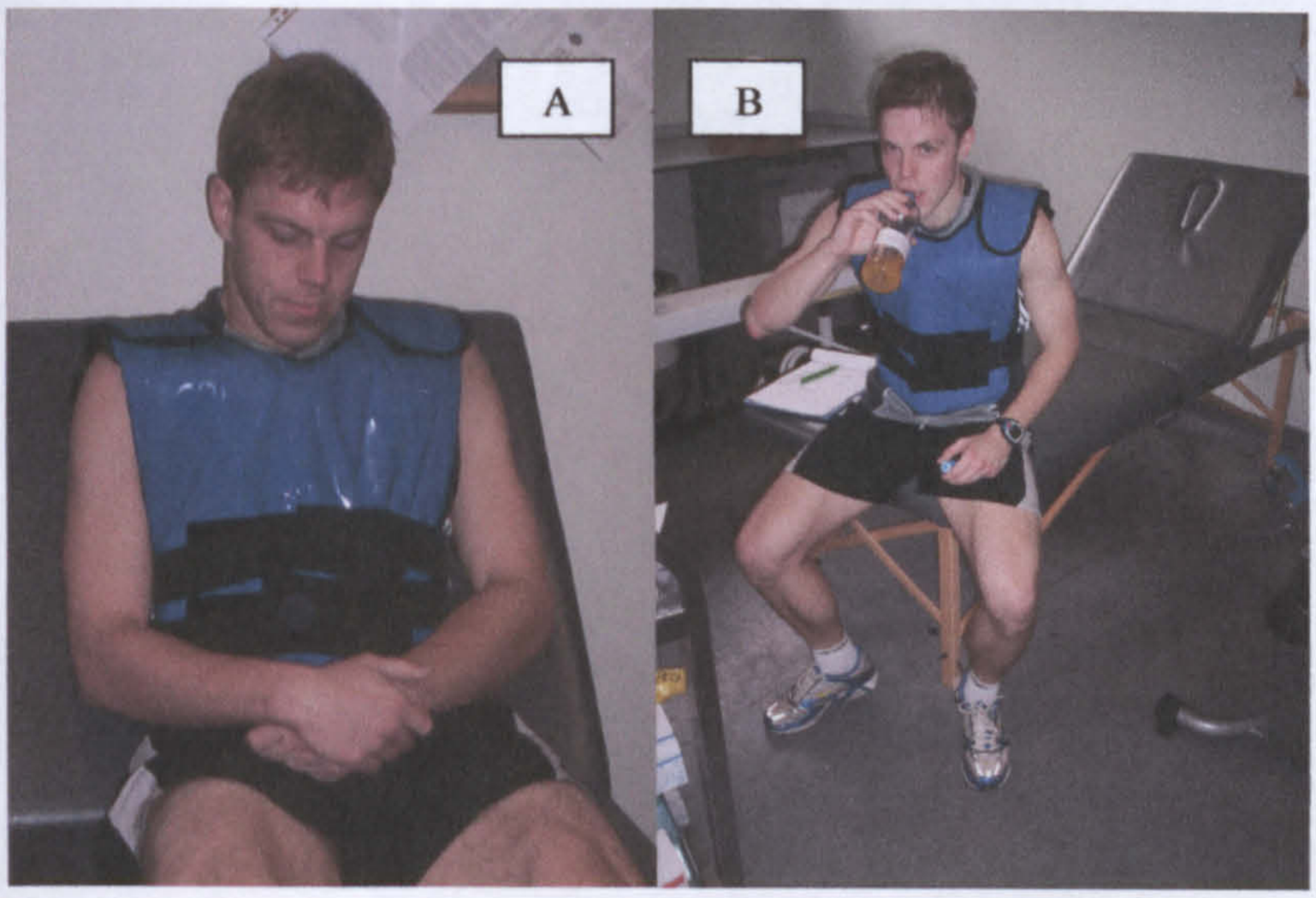

Figure 7.1: Subject undergoing pre-cooling before (A) and at half-time (B).

7.2.3. Mental concentration test

This test was a measure of how well the subject could concentrate on making simple decisions (Hardy and Fazey, 1990) and was performed after 4 min of every 15 min block of the soccer-specific protocol. The test grid (Figure 7.2) was projected onto the wall in front of the treadmill and the task was to read from left to right across the lines of figures and identify each pair of figures that added up to ten. No figure could be used twice, for example, 373 could be used as 37 or 73 but not 373. The subject had 90 seconds to scan all 16 lines and the task was to complete as many lines as possible whilst performing the soccer-specific protocol. When the time was completed the number of "tens" correctly identified was recorded as a percentage of those attempted.

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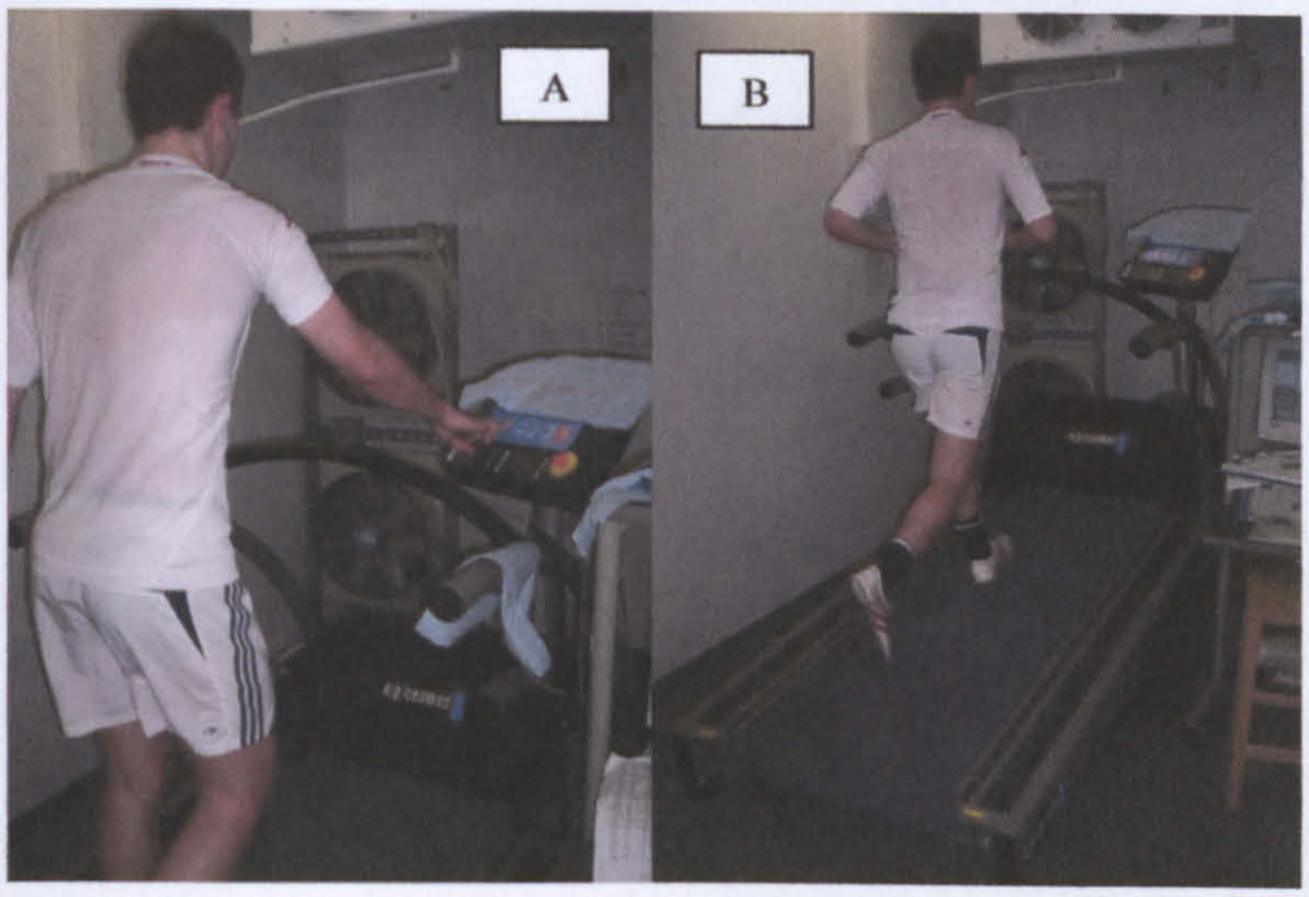

Figure 7.3: Performing the self-chosen work-rate test (A) and Cunningham and Faulkner test (B).

7.2.9. Blood sampling and analysis

Four venous blood samples (16 ml; total 64 ml) were taken from an antecubital vein in the forearm. A blood sample was taken immediately before and after the donning of the cooling vest, at half-time and at the completion of the 90 minutes. The blood samples were later analysed for glucose, lactate, NEFA, glycerol, catecholamines, insulin, cortisol, IL-6 and prolactin (described in section 3.7). Muscle temperature was determined by means of a needle thermistor (Model MKA-A, Ellab, Denmark) inserted into the quadriceps muscle to a depth of 30 mm (Figure 7.4) and was measured at the same time points as the blood samples.

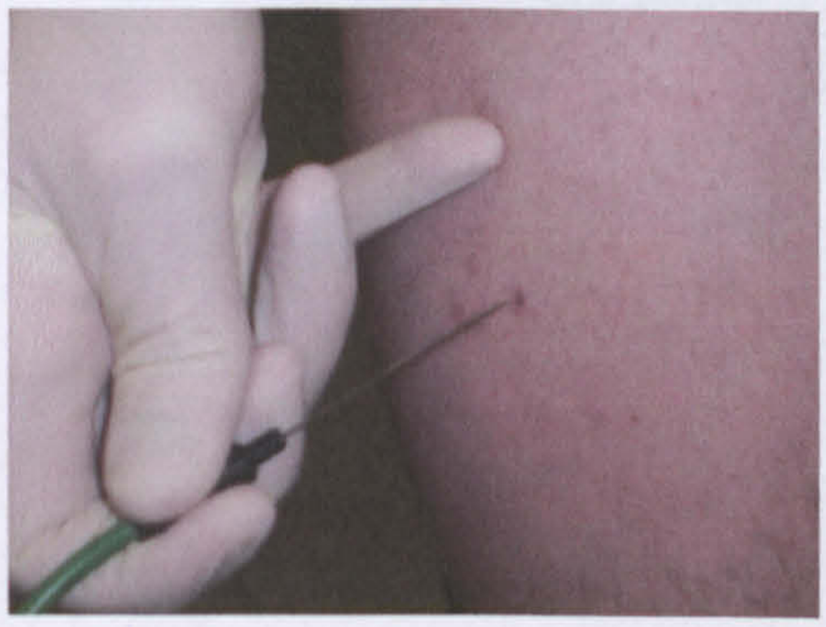

Figure 7.4: Muscle temperature being measured.

7.2.10. Reliability

The subjects, all of whom were familiar with the test, performed the Cunningham and Faulkner test on six occasions 1 week apart on a motorized treadmill (H/P/Cosmos Pulsar 4.0, H/P/Cosmos Sports and Medical GmbH, Nussdorf-Traunstein, Germany). There was no significant difference ($P>0.05$) in time to exhaustion between trials. The coefficient of variation for time to exhaustion was 7.3%. Hence, the Cunningham and Faulkner test was deemed to provide a reliable measure of resistance to high-intensity exercise.

After familiarization the subjects performed the psychophysical test on six occasions 1 week apart on a motorized treadmill (H/P/Cosmos Pulsar 4.0, H/P/Cosmos Sports and Medical GmbH, Germany). There was no significant difference ($P>0.05$) in selected running speed between trials. The CV for running speed was 4.7%. Self-selected running speed was deemed to provide a reliable measure of psychophysical performance.

Following familiarisation the 12 subjects performed the mental concentration test twice whilst performing 30 minutes of the soccer-specific protocol on a motorized treadmill (H/P/Cosmos Pulsar 4.0, H/P/Cosmos Sports and Medical GmbH, Nussdorf-Traunstein, Germany) on six occasions 1 week apart. The two-way ANOVA with repeated measures revealed no significant differences ($P>0.05$) in percentage of correct responses within or between trials. The CV for the percentage of correct responses was 4.8% and the test was deemed to provide a reliable measure of mental concentration.

7.2.11. Statistical analysis

All variables were analysed using except for sweat loss, the performance variables and the reliability of the Cunningham and Faulkner and psychophysical tests, which were analysed using a one-way ANOVA with repeated measures. The Pearson coefficient correlation was used to asses the correlation between the self-chosen work-rate test and the Cunningham and Faulkner test. All results are reported as the mean ± the standard error of the mean (SEM) and a level of $P<0.05$ was considered statistically significant.

7.3. Results

7.3.1. Pre-trial hydration status

Pre-trial hydration status was similar for all of the experimental conditions. Urine colour PLA (2.34±0.22), PLAc (2.42±0.41), GLU (2.37±0.18) and GLUc (2.29±0.23) ($F_{2,24}$=0.468; P>0.05) and osmolality PLA (525.5±109.7 mOsm·kg^{-1}), PLAc (517.2±115.7 mOsm·kg^{-1}), GLU (527.2±101.6 mOsm·kg^{-1}) and GLUc (567.4±106.2 mOsm·kg^{-1}) ($F_{2,22}$=0.569; P>0.05) did not differ significantly between trials.

7.3.2. Core and muscle temperature

There was a significant trial effect on core temperature ($F_{2,20}$=13.482; P<0.05, Figure 7.5). Pre-cooling significantly reduced core temperature; PLAc: 37.2±0.1°C to 36.6±0.1°C; GLUc: 37.3±0.1°C to 36.6±0.1°C before and after pre-cooling, respectively. As a consequence, core temperature during GLUc and PLAc was significantly lower prior to the start of exercise compared with PLA and GLU, and this trend remained throughout blocks 1 and 2. During block 3 core temperature was significantly lower in GLUc and PLAc compared with GLU. During block 4 GLU was significantly higher than GLUc. During blocks 5 and 6 core temperature was significantly higher during PLA, PLAc and GLU compared with GLUc. A significant effect of time was observed ($F_{2,22}$=186.640; P<0.05), with pairwise comparisons showing that core temperature increased significantly (P<0.05) with each block of the soccer-specific protocol and decreased significantly during half-time.

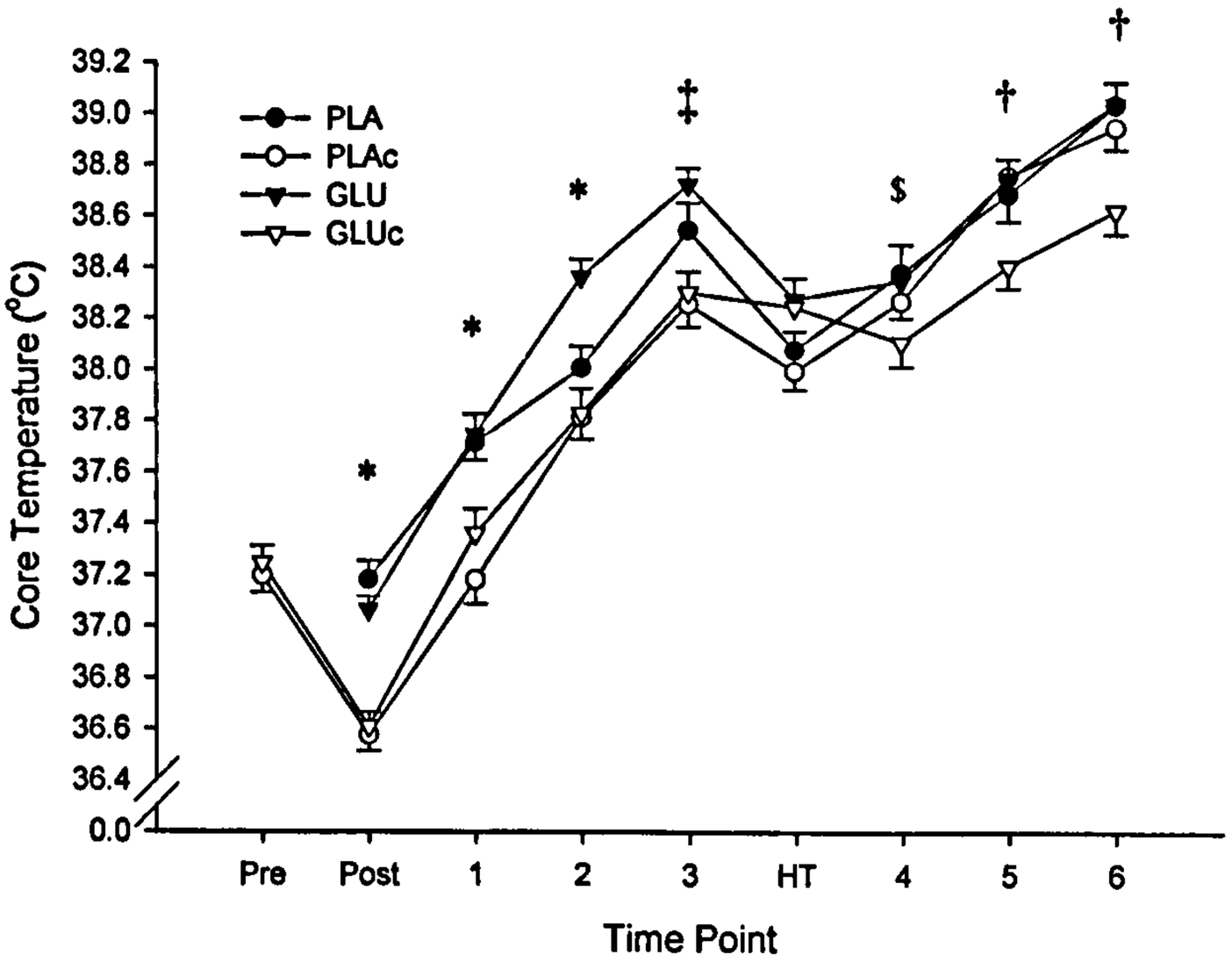

Figure 7.5: Core temperature during soccer-specific protocol.

Pre – before pre-cooling; Post – after pre-cooling; 1-6 – Blocks; 1-6, HT – Half-time.

* PLA and GLU significantly greater than PLAc and GLUc. † PLA, PLAc and GLU significantly greater than GLUc. ‡ GLU significantly greater than PLAc and GLUc. $ GLU significantly greater than GLUc.

Muscle temperature (Figure 7.6) was significantly affected by trial ($F_{2,12}$=3.749; P<0.05). Pre-cooling significantly reduced muscle temperature; PLAc: 36.1±0.2°C to 35.5±0.2°C; GLUc: 36.2±0.1°C to 35.5±0.2°C before and after pre-cooling, respectively. Therefore, muscle temperature during GLUc and PLAc was significantly lower prior to the start of exercise compared PLA and GLU. At half-time and the end of exercise there was no difference between trials. There was a significant effect of time ($F_{1,15}$=199.331; P<0.05), muscle temperature increasing significantly between each time point during exercise.

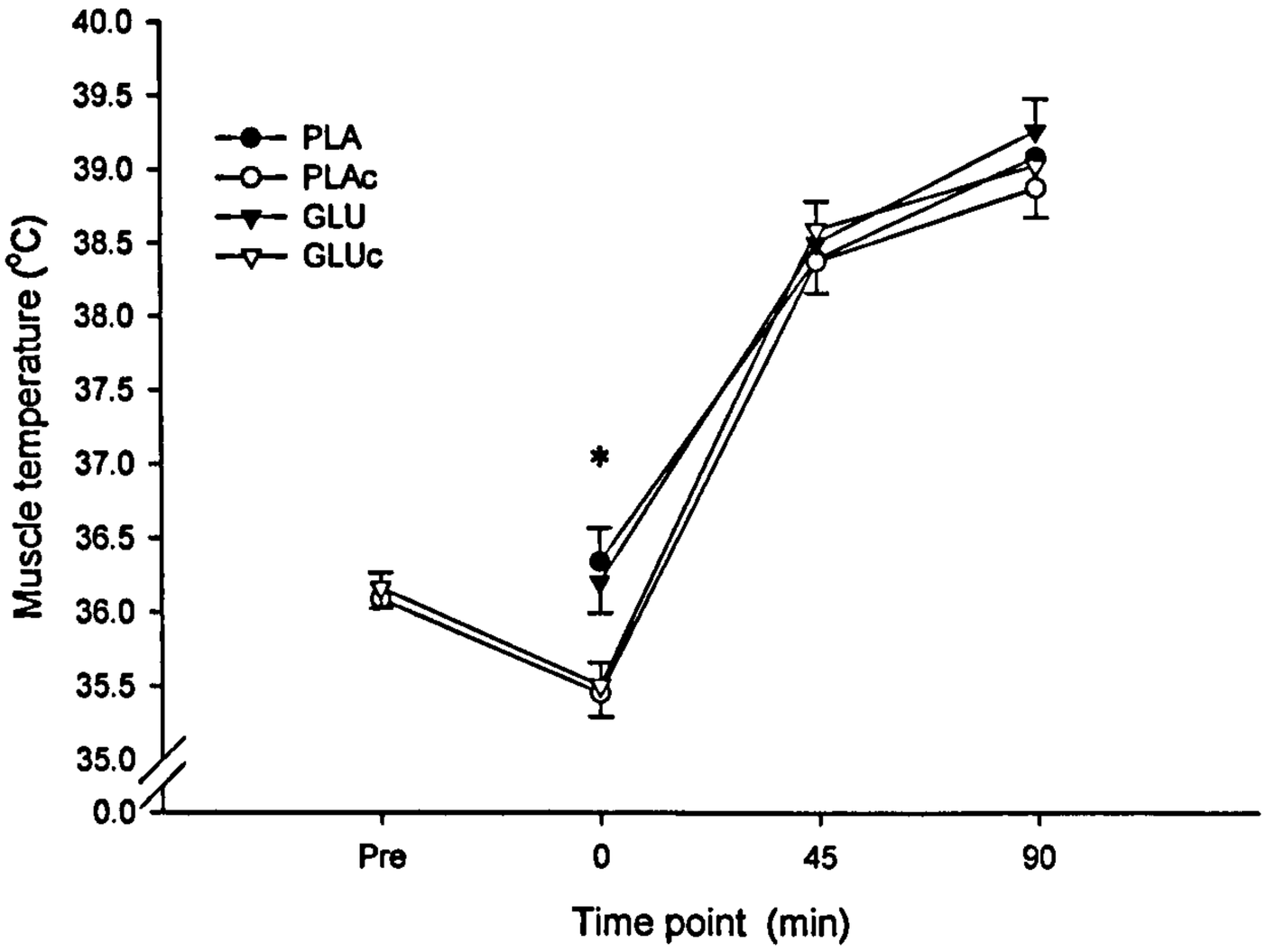

Figure 7.6: Muscle temperature during soccer-specific protocol.

* PLA and GLU significantly greater than PLAc and GLUc.

7.3.3. Thermal sensation

Pre-cooling significantly reduced thermal sensation prior to performing the soccer-specific protocol (Table 7.2) ($F_{1,1}$=45.047; P<0.05). Thermal sensation was significantly lower during GLUc and PLAc at half-time compared with GLU and PLA ($F_{3,4}$=0.721; P<0.05), although during the exercise there was not a significant treatment effect. There was a significant effect of time ($F_{3,31}$=61.053; P<0.05), thermal sensation increasing significantly (P<0.05) during each half of the soccer-specific protocol. It was also significantly lower at half-time compared with blocks 3 and 4.

Table 7.2: Thermal sensation during the soccer-specific protocol.

	Time point								
	Pre-	Post-	1	2	3	HT	4	5	6
PLA		5.1±0.1	6.5±0.2	7.1±0.2	7.3±0.2	5.9±0.2	7.0±0.1	7.3±0.1	7.9±0.2
PLAc	5.0±0.1	3.0±0.2*	6.3±0.1	7.0±0.1	7.3±0.1	5.4±0.1*	7.0±0.2	7.1±0.2	7.8±0.2
GLU		5.1±0.1	6.6±0.2	7.1±0.2	7.2±0.2	5.8±0.1	7.3±0.2	7.4±0.2	7.8±0.2
GLUc	5.2±0.1	3.3±0.1*	6.3±0.1	7.0±0.1	7.2±0.1	5.3±0.1*	7.1±0.2	7.3±0.1	7.8±0.2

Note: Pre – before pre-cooling; Post – after pre-cooling; 1-6 – Blocks; 1-6, HT – Half-time.

* Significantly lower than PLA and GLU

7.3.4. Plasma metabolites

Pre-exercise plasma glucose concentration was similar for all three trials. There was a significant trial effect on the concentration of plasma glucose ($F_{2,17}$=13.255; P<0.05; Figure 7.7). The plasma glucose concentration was significantly higher at half-time and the end of the soccer-specific protocol during GLU and GLUc compared with PLA and PLAc. There was no significant effect of pre-cooling of plasma glucose concentration. There was also a significant effect of time ($F_{2,18}$=16.653; P<0.05). During trials GLU and GLUc, plasma glucose concentrations were elevated significantly (P<0.05) above resting levels at half-time and on completion of the soccer-specific protocol. The repeated measures ANOVA identified a significant time and trial interaction ($F_{3,30}$=5.673; P<0.05); plasma glucose increased during the first half of all trials, markedly so during GLU and GLUc. During the second half, plasma glucose decreased during all of the trials, although no subjects were found to be hypoglycaemic.

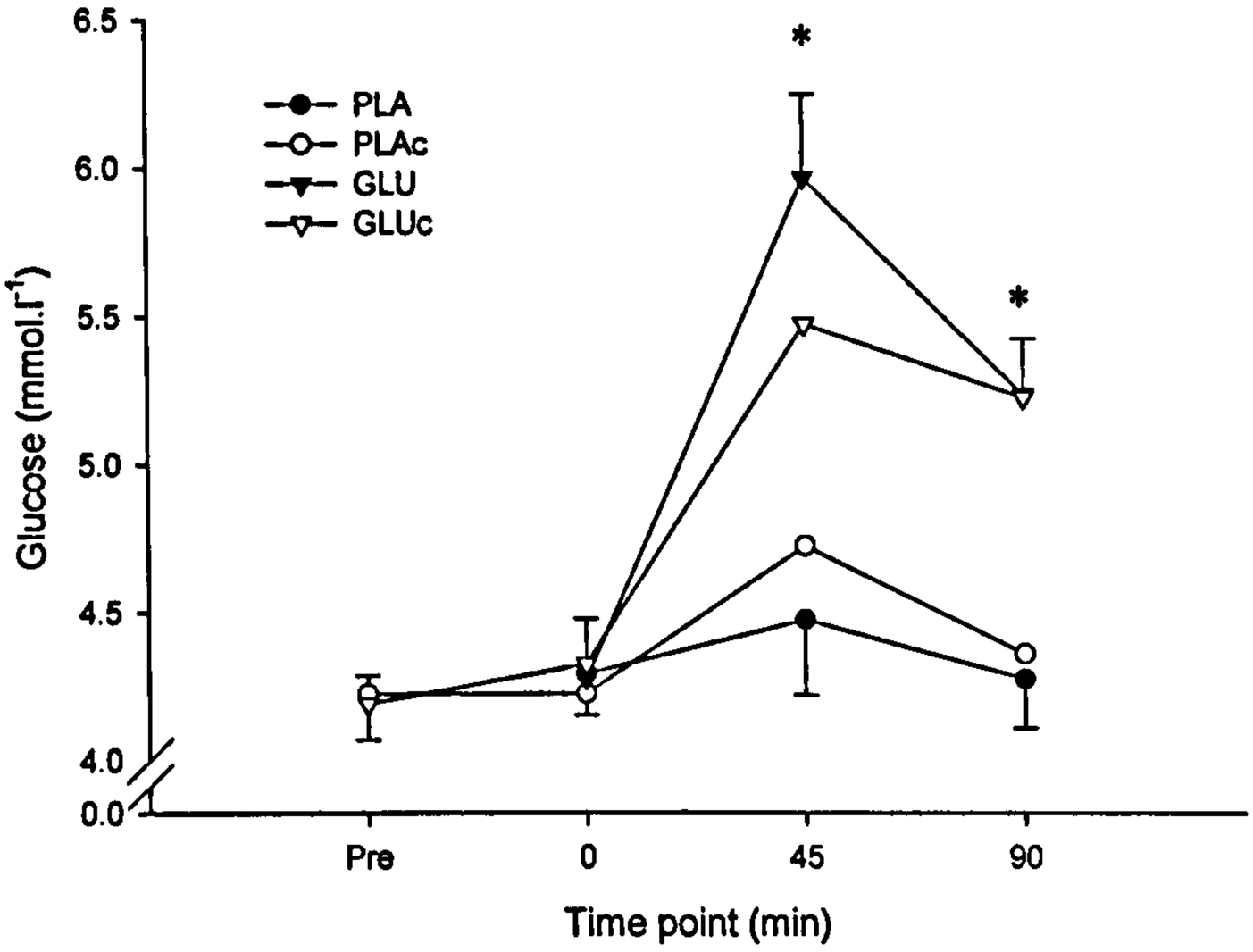

Figure 7.7: Plasma glucose concentration during the soccer-specific protocol.
* GLU and GLUc significantly greater than PLA and PLAc.

The repeated measures ANOVA revealed that there was a significant trial effect on the plasma concentration of NEFA ($F_{2,26}$=4.514; P<0.05), although there was no significant effect of pre-cooling. The concentration of NEFA was significantly (P<0.05) higher during PLA and PLAc compared with GLU (Figure 7.8). There was a significant effect of time on the concentration of plasma NEFA ($F_{3,33}$=166.683; P<0.05), which increased significantly between each time point as exercise progressed. There was also a significant ($F_{2,25}$=4.432; P<0.05) trial by time interaction; after half-time NEFA concentration increased markedly more during PLA and PLA compared with GLU and GLUc, which increased at a steady rate.

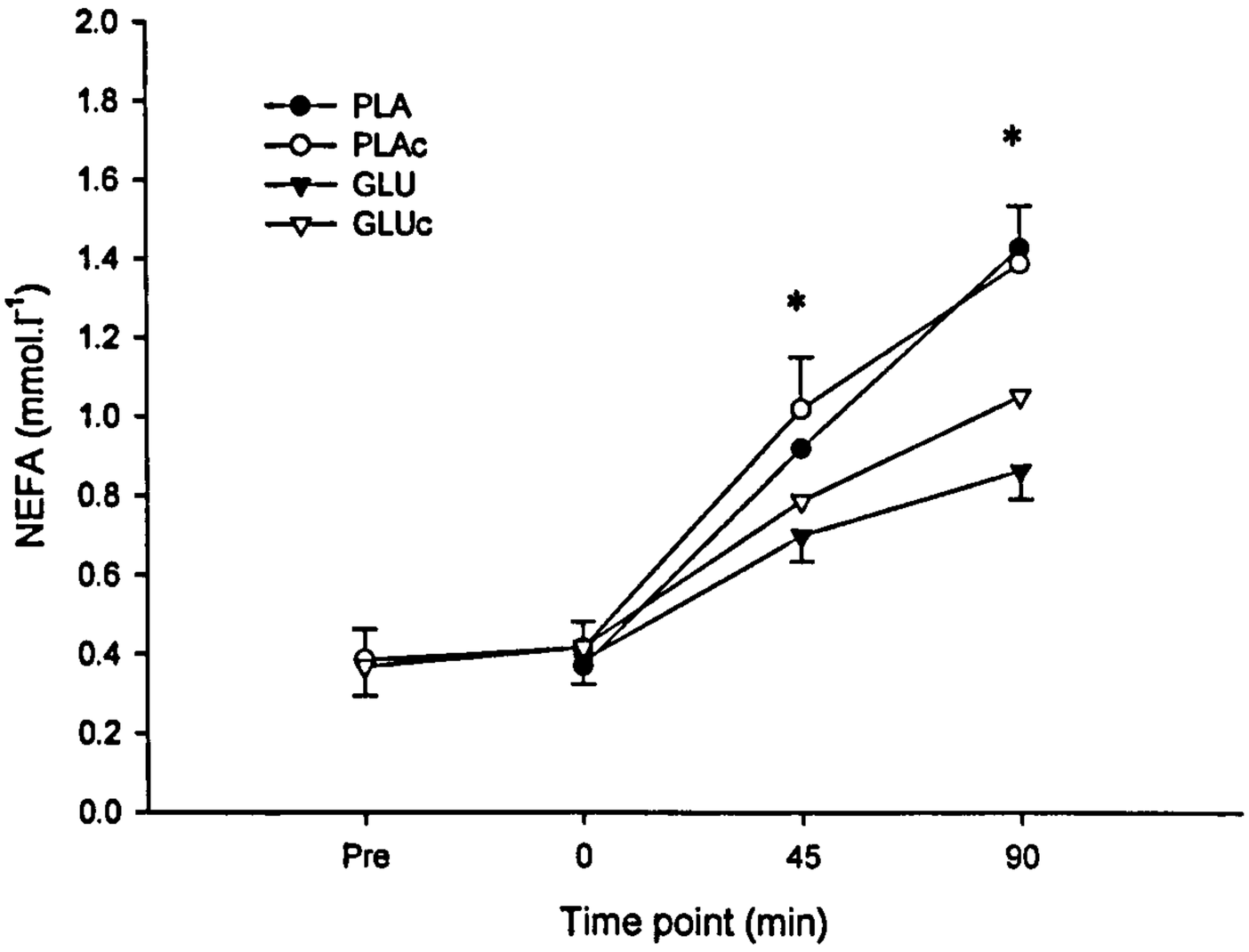

Figure 7.8: Plasma NEFA concentration during the soccer-specific protocol.

* PLA and PLAc significantly greater than GLU.

The plasma concentration of glycerol was significantly affected by the trial ($F_{1,15}$=10.787; P<0.05), with the concentration significantly (P<0.05) higher during the PLA trial compared with GLU and GLUc throughout the protocol. There was no significant effect of pre-cooling. Glycerol concentration was also significantly higher in PLAc than GLU and GLUc at 90 min (Figure 7.9). Plasma glycerol concentration increased significantly between each time point ($F_{1,14}$=123.866; P<0.05). There was also a significant trial and

time interaction ($F_{2,18}$=15.066; P<0.05); glycerol concentration increased markedly more during PLA and PLAc compared with GLU and GLUc.

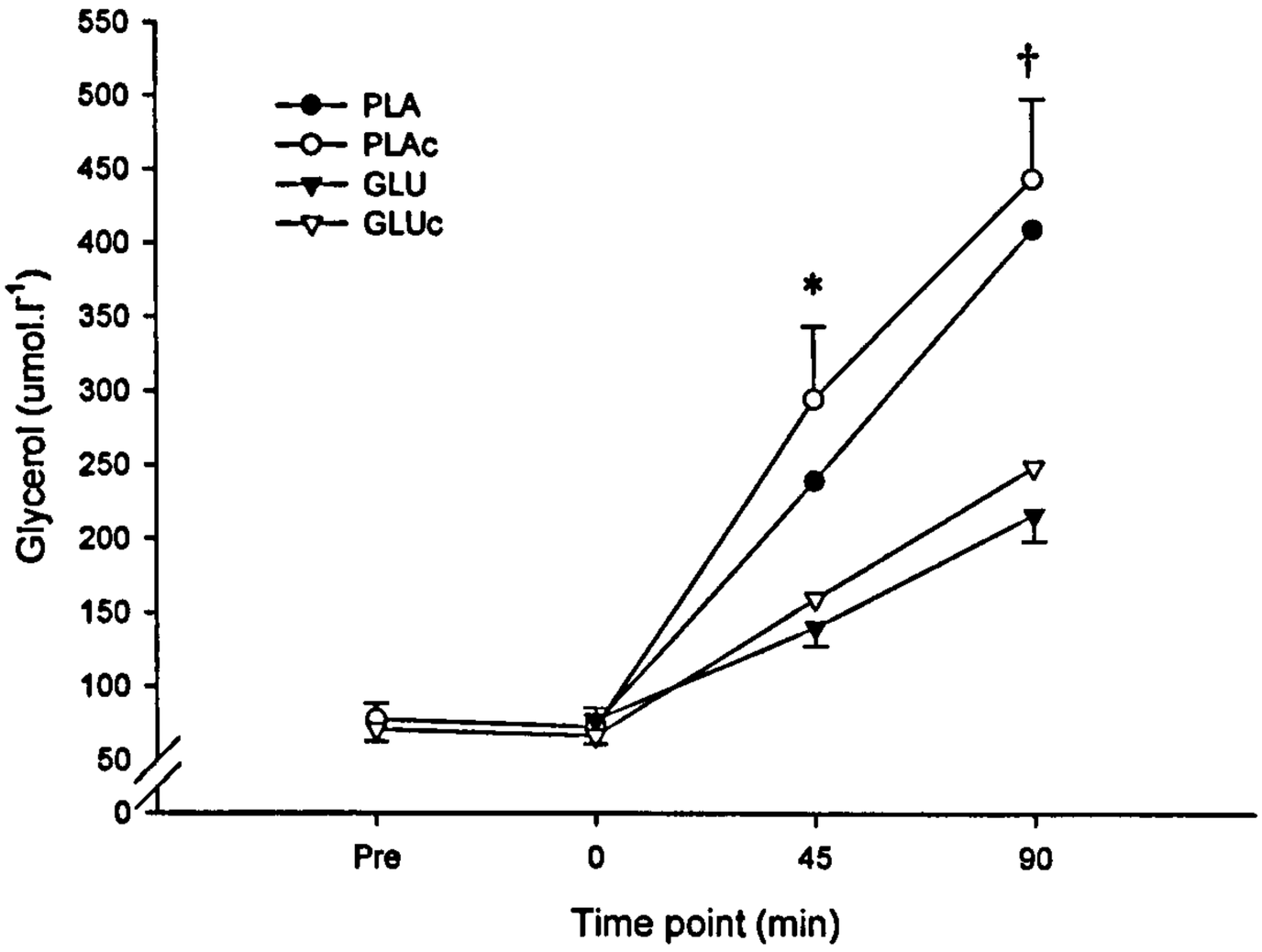

Figure 7.9: Plasma glycerol concentration during the soccer-specific protocol.

* PLA significantly greater than GLU and GLUc.

† PLA and PLAc significantly greater than GLU and GLUc.

The repeated measures ANOVA revealed that there was no significant trial effect on the plasma concentration of lactate ($F_{2,24}$=0.981; P>0.05; Figure 7.10). There was a significant effect of time on plasma lactate during the soccer-specific protocol ($F_{1,13}$=48.308; P<0.05), lactate concentration increasing significantly between rest and half-time.

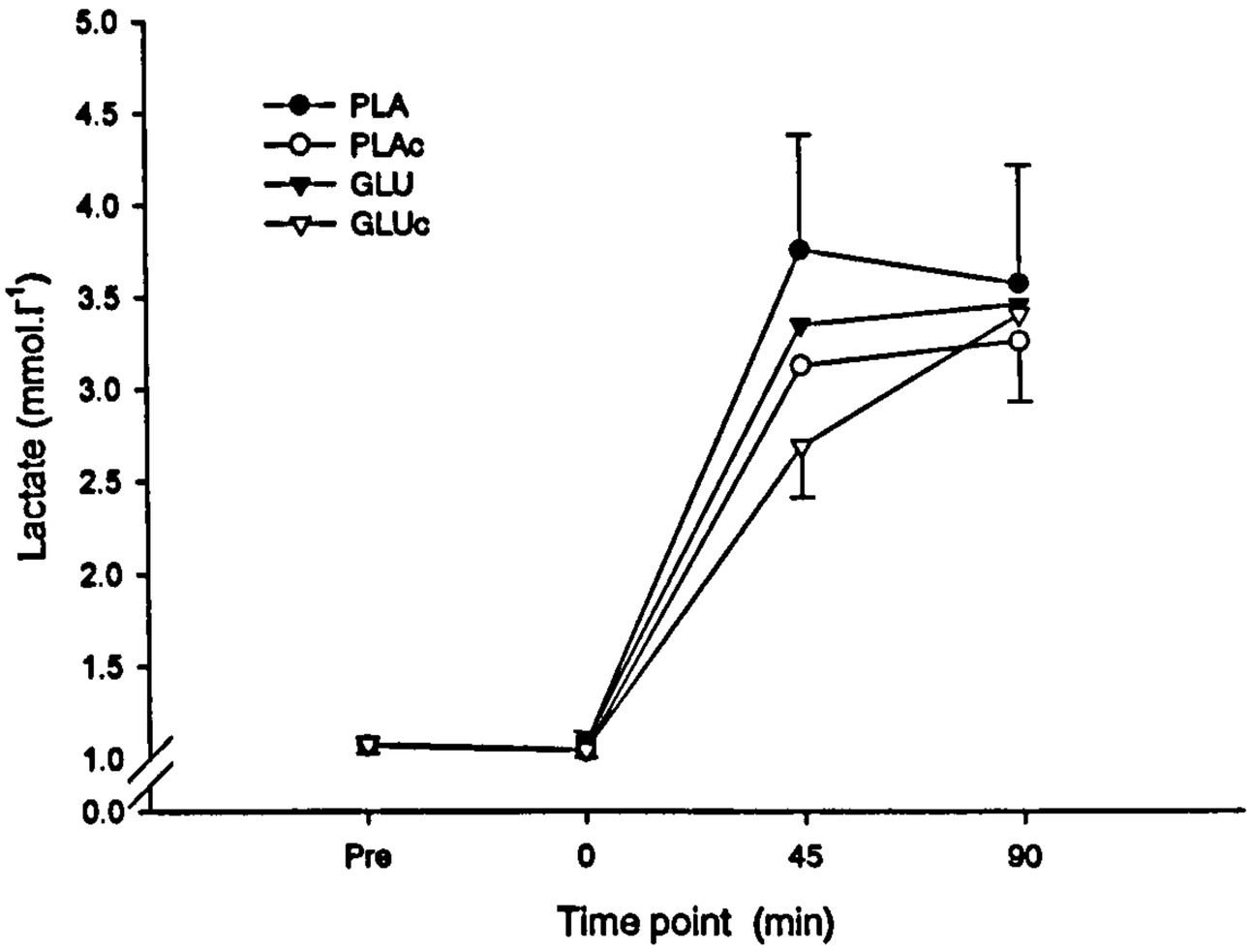

Figure 7.10: Plasma lactate concentration during the soccer-specific protocol.

7.3.4. Hormones

The concentration of adrenaline was found to be similar during all trials ($F_{2,21}$=2.809; P>0.05, Figure 7.11), and increased significantly ($F_{2,19}$=18.764; P<0.05) between each time point during the exercise period. There was also a significant interaction ($F_{3,30}$=3.065; P<0.05); the adrenaline concentration increased markedly during the second half of PLA and PLAc, in contrast adrenaline increased during GLU and GLUc at a constant rate throughout the protocol. There was a trend for adrenaline to be lower during PLAc compared with PLA, although this change was not significant (P=0.086).

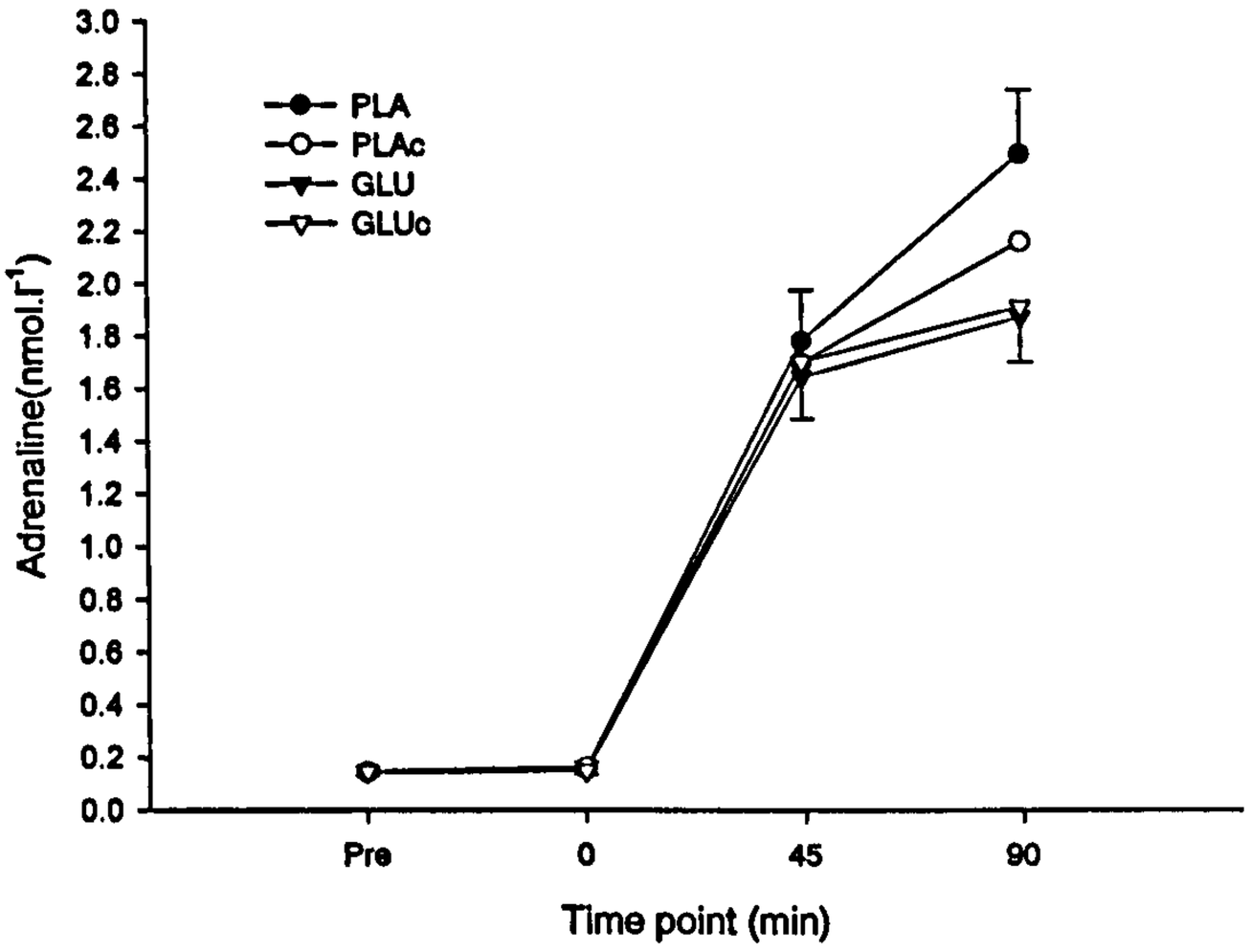

Figure 7.11: Plasma adrenaline concentration during the soccer-specific protocol.

The concentration of noradrenaline was found to be similar during all trials ($F_{2,21}$=2.809; P>0.05, Figure 7.12), and increased significantly ($F_{1,15}$=26.172; P<0.05) between each time point during the exercise period. There was a trend for noradrenaline concentration to be lower during the pre-cooling trials, although this effect was not significant (P=0.252).

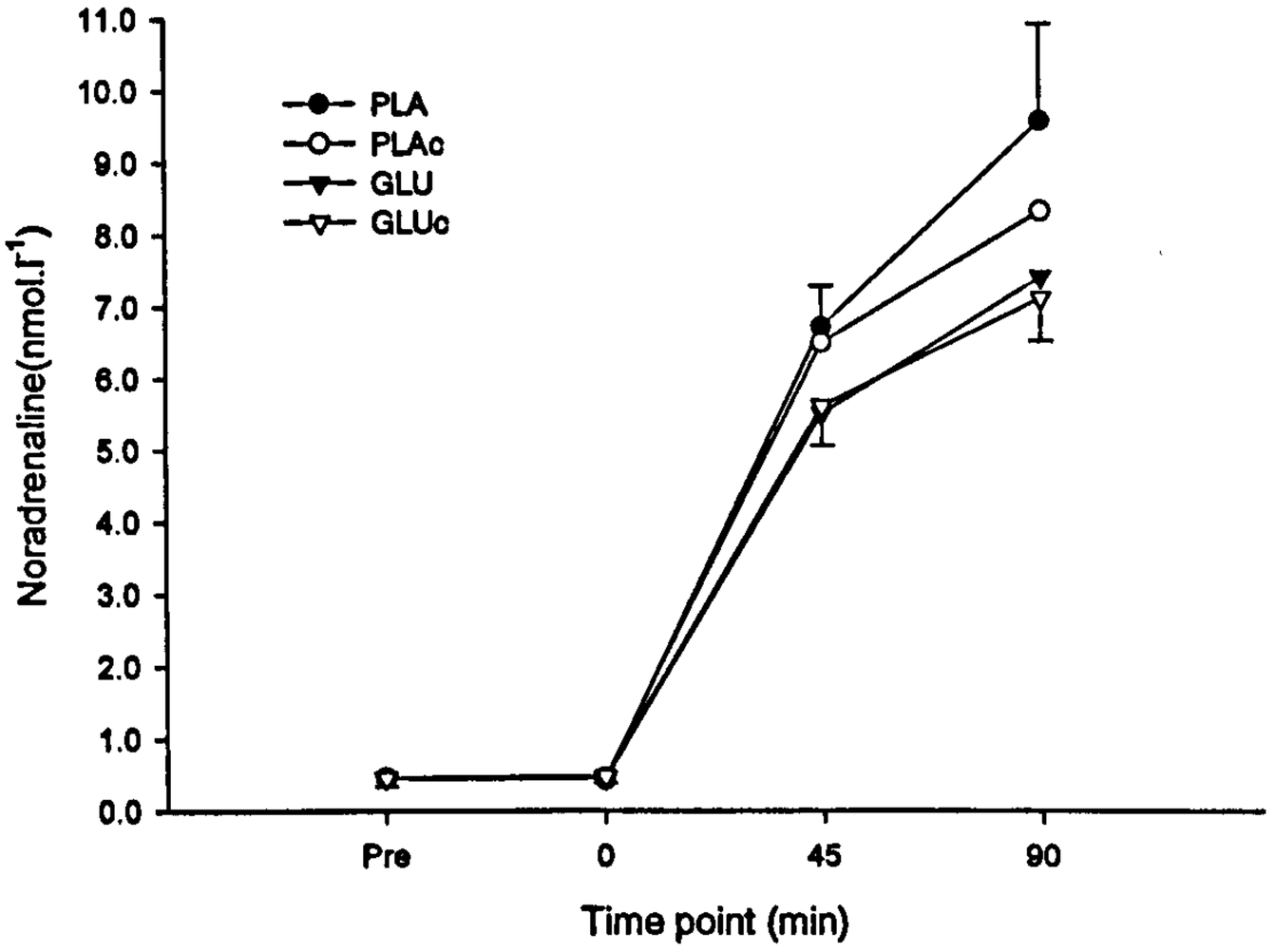

Figure 7.12: Plasma noradrenaline concentration during the soccer-specific protocol.

There was not a significant trial effect of the concentration of cortisol ($F_{2,26}$=0.259; P>0.05; Figure 7.13). The repeated measures ANOVA revealed that there was not a significant effect of time ($F_{1,16}$=2.034; P>0.05). Pre-cooling resulted in an increase in cortisol concentration, although this effect did not reach statistical significance (P=0.073).

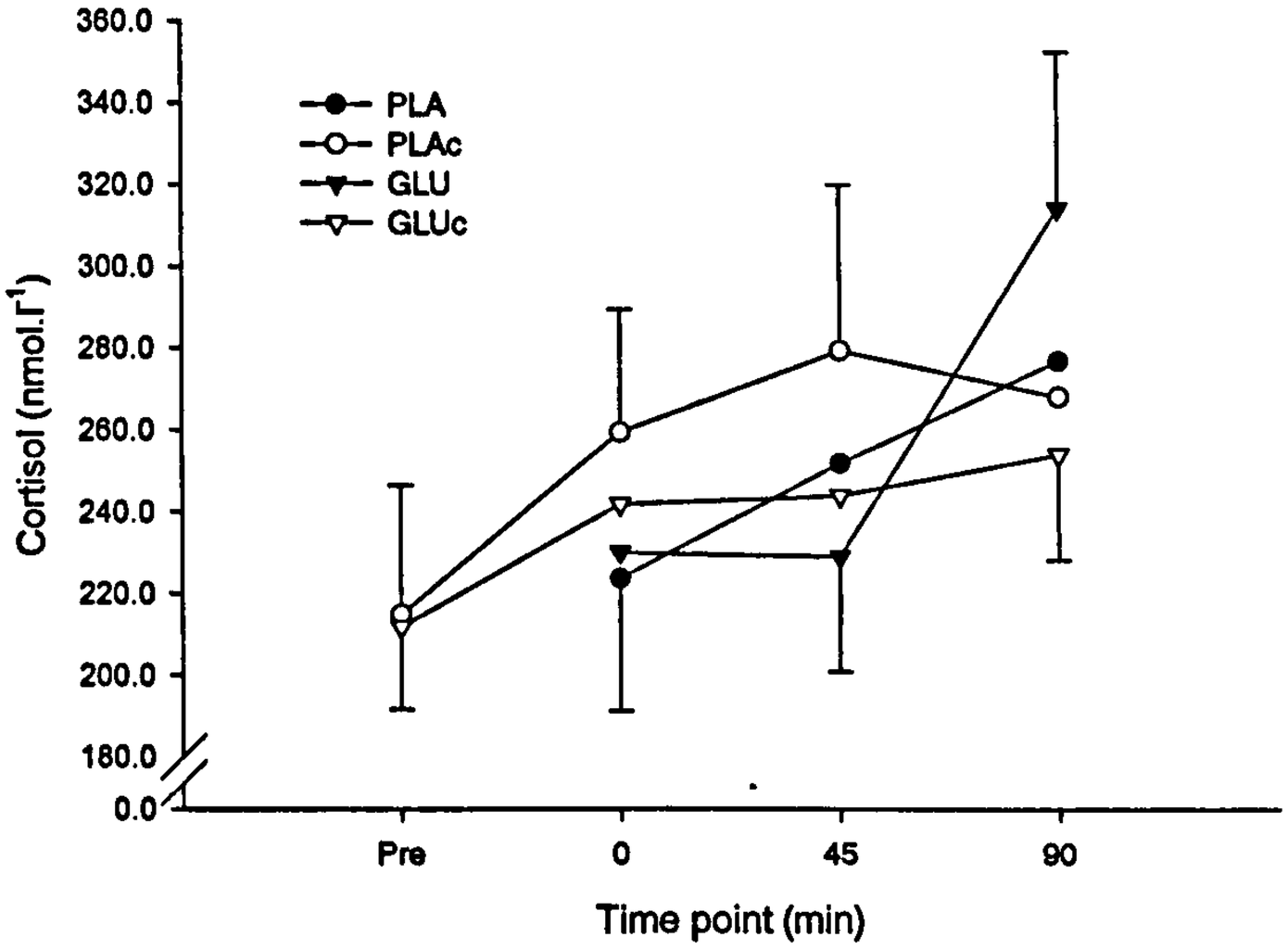

Figure 7.13: Serum cortisol concentration during the soccer-specific protocol.

There was a significant trial effect on the concentration of serum insulin ($F_{2,22}$=21.830; P<0.05; Figure 7.14). The serum insulin concentration was significantly higher during GLU and GLUc than during the PLA and PLAc (P<0.05). The repeated measures ANOVA revealed that there was not a significant effect of time ($F_{1,13}$=2.723; P>0.05). The repeated measures ANOVA identified a significant time and trial interaction ($F_{2,25}$=3.571; P<0.05); serum insulin concentration increased during the first half of GLU and GLUc, in contrast it decreased during PLA and PLAc. All trials demonstrated decreased insulin response during the second half, markedly so during GLU.

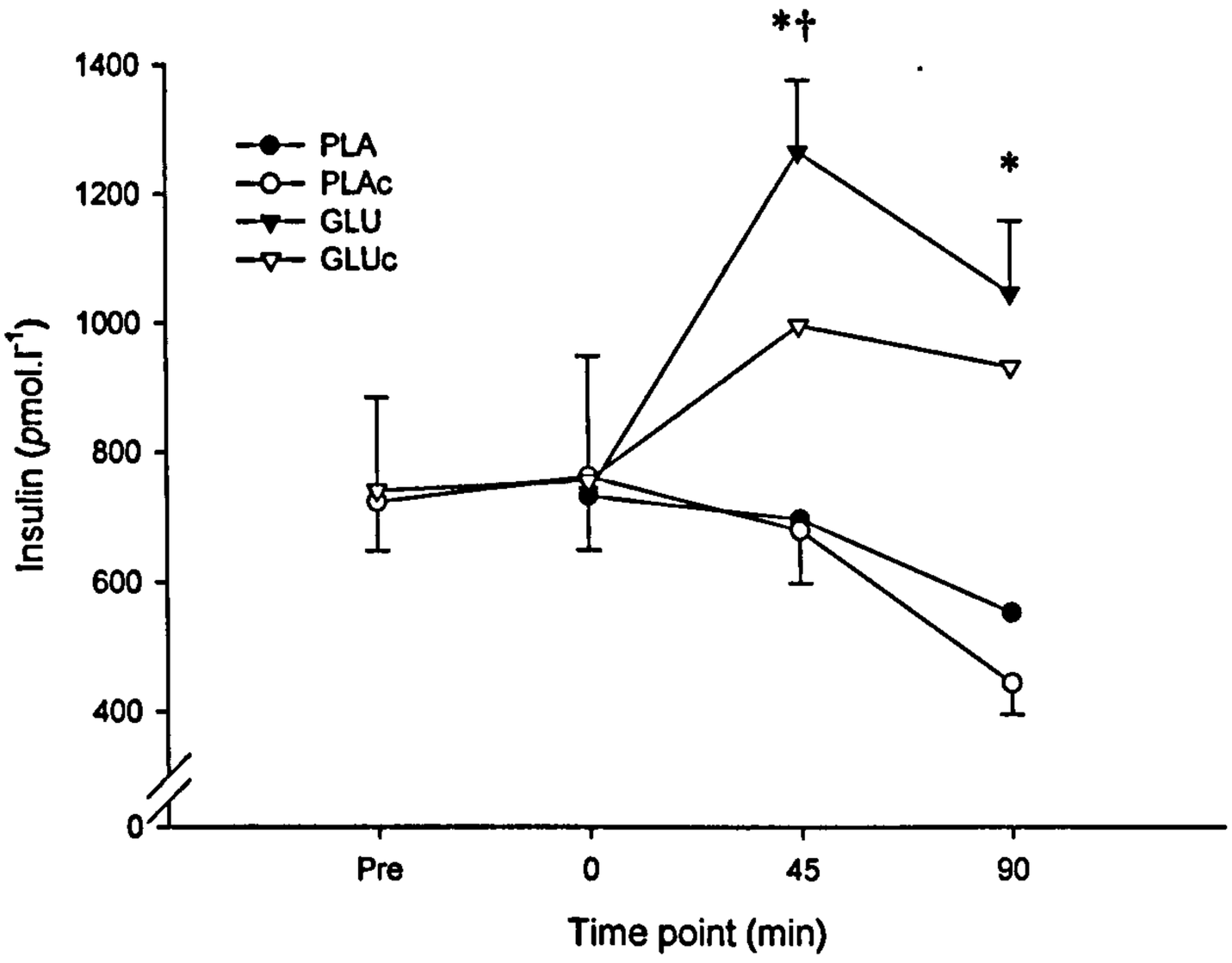

Figure 7.14: Serum insulin concentration during the soccer-specific protocol.

* GLU and GLUc significantly higher than PLAc.

† GLU significantly higher than PLA.

There was a significant trial effect on the concentration of IL-6 ($F_{2,24}$=3.731; P<0.05; Figure 7.15). At half-time, the IL-6 concentration was significantly higher in PLA and PLAc compared with GLU and GLUc (P<0.05). The repeated measures ANOVA revealed that there was a significant effect of time ($F_{2,19}$=233.889; P<0.05), with IL-6 concentration increasing significantly during both halves of the protocol, although the period of pre-cooling had no significant effect (P>0.05).

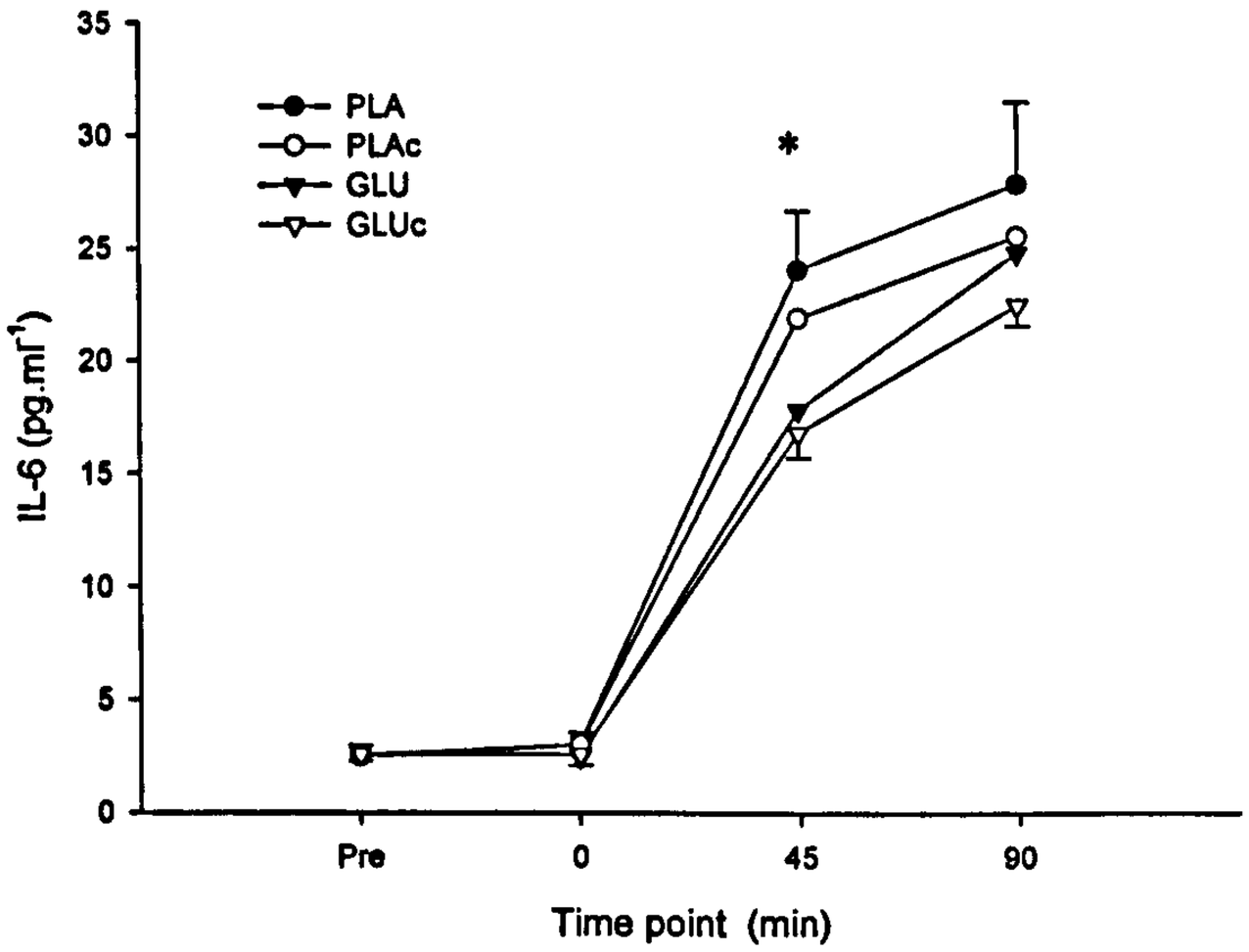

Figure 7.15: Interleukin-6 concentration during the soccer-specific protocol.

* PLA and PLAc significantly higher than GLU and GLUc,

There was not a significant trial effect on the concentration of prolactin ($F_{2,27}$=0.402; P>0.05; Figure 7.16). The repeated measures ANOVA revealed that there was a significant effect of time ($F_{2,21}$=42.937; P<0.05); prolactin values increased significantly during the first half of the protocol. Pre-cooling resulted in a significant decrease in prolactin concentration ($F_{1,11}$=7.397; P<0.05).

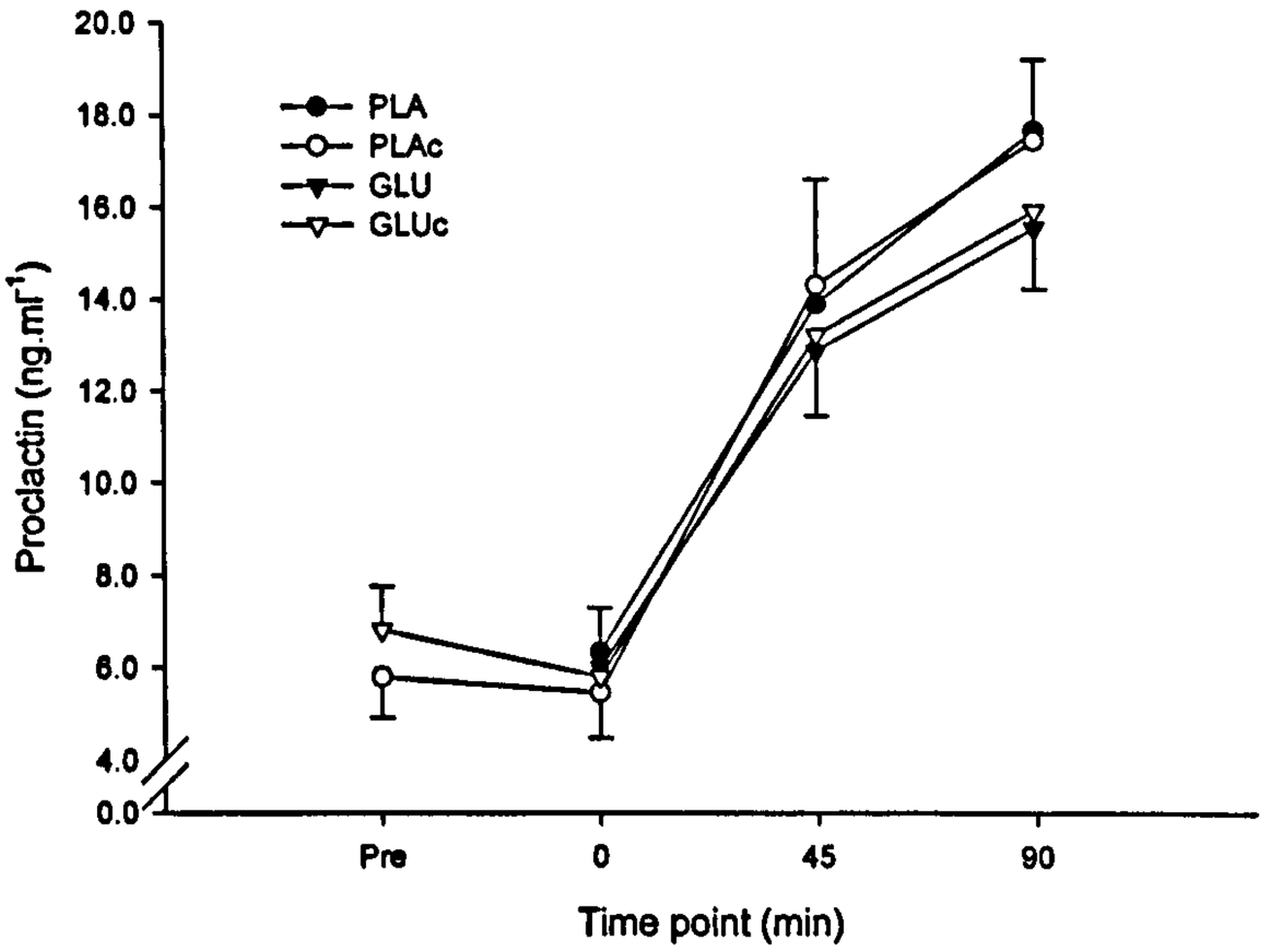

Figure 7.16: Serum prolactin concentration during the soccer-specific protocol.

7.3.5. Plasma osmolality

There was no significant difference in plasma osmolality (Figure 7.17) between the three trials ($F_{2,20}$=0.106; P>0.05). Plasma osmolality was significantly higher ($F_{2,22}$=25.293; P<0.05) at half-time and at the completion of the soccer-specific protocol, compared with pre-exercise values. There was no significant effect of pre-cooling ($F_{1,11}$=0.040; P>0.05).

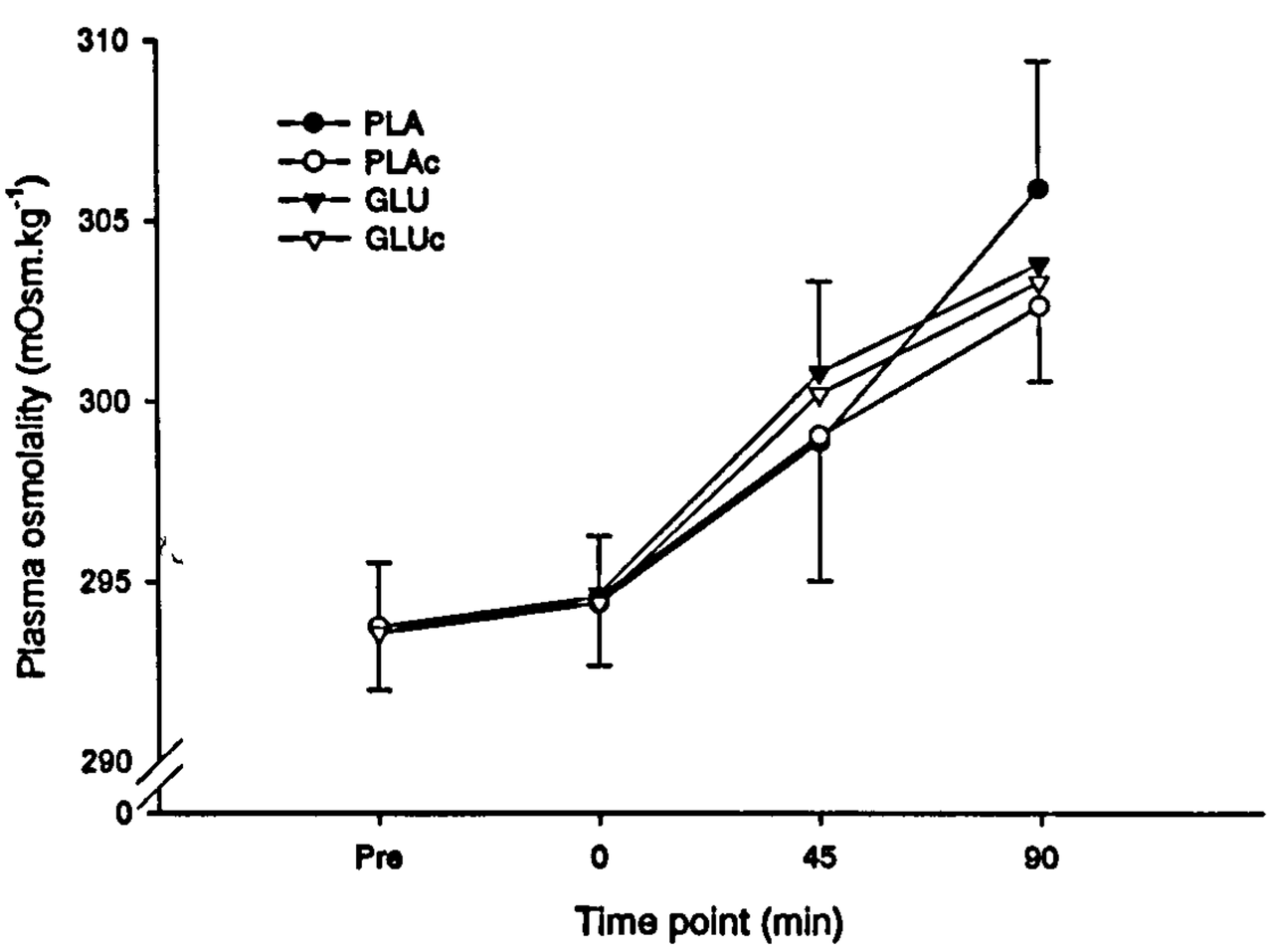

Figure 7.17: Changes in plasma osmolality during the soccer-specific protocol.

7.3.6. Indirect calorimetry

There was a significant difference in RER between the trials ($F_{2,26}$=2.254; P<0.05, Figure 7.18). During GLUc, RER was higher than during PLA or PLAc at block 5, while at block 6 both GLU and GLUc were significantly higher than PLA and PLAc (P<0.05). There was a significant effect of time on RER ($F_{3,28}$=23.147; P<0.05); blocks 1 and 2 showed significantly (P<0.05) higher values than the subsequent 60 min, indicating that a greater proportion of carbohydrate was oxidised during this time. There was a significant interaction ($F_{5,59}$=4.174; P<0.05); RER during GLU and GLUc remained relatively constant throughout the soccer-specific protocol, whereas during PLA it decreased steadily throughout. In contrast during PLAc, RER was consistent for the first 30 min (blocks 1 and 2), before decreasing throughout the remainder of the protocol.

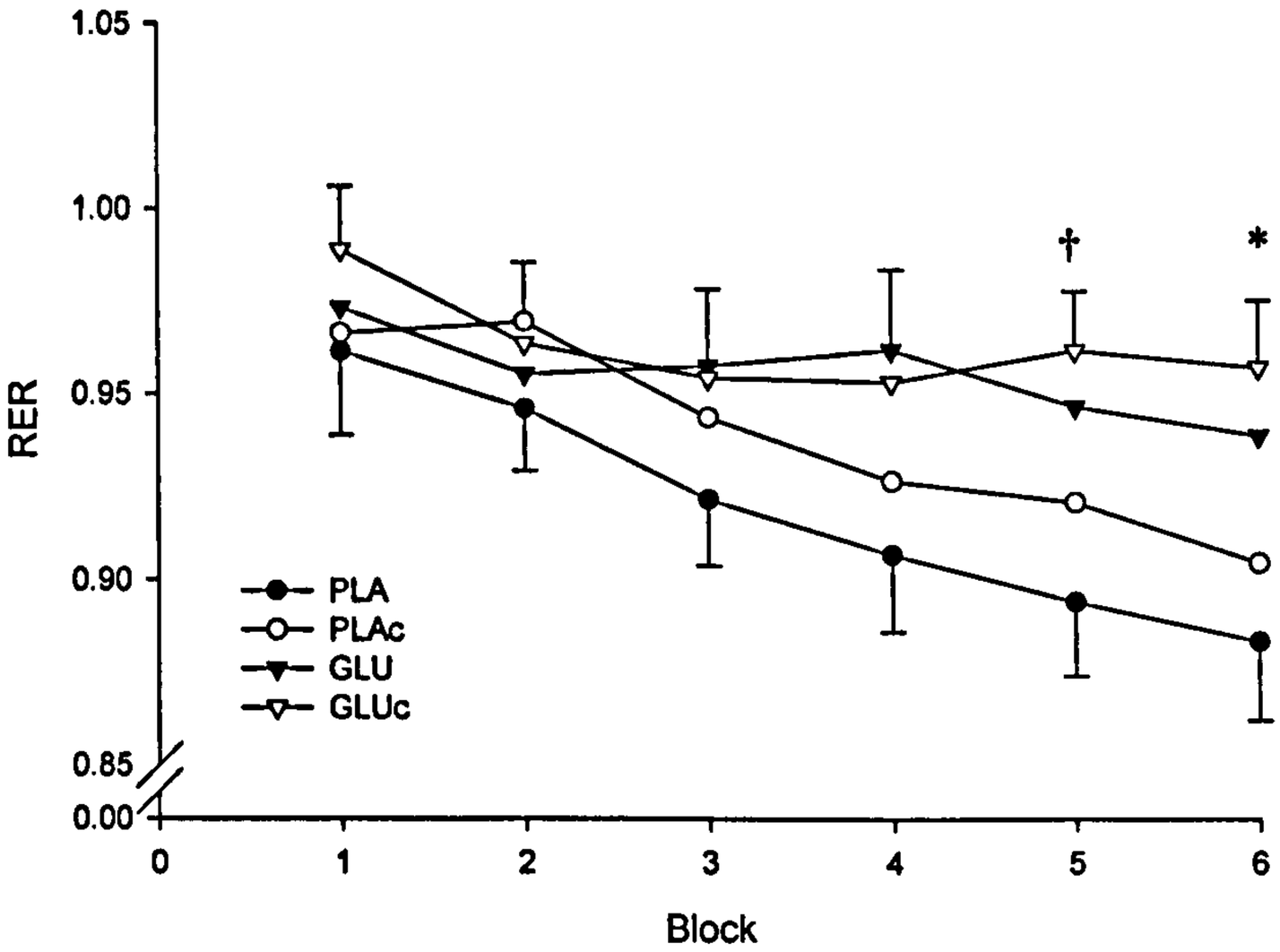

Figure 7.18: Respiratory exchange ratio during the soccer-specific protocol. † GLUc significantly higher than PLA and PLAc. * GLU and GLUc significantly higher than PLA.

Total carbohydrate oxidation (Figure 7.19) was significantly ($F_{2,23}$=1.588; P<0.05) affected by the trials. Carbohydrate oxidation was greater during GLU and GLUc during block 6 compared to PLA (P<0.05). There were no significant differences between GLUc and GLU (P>0.05), or PLAc and PLA (P>0.05). Carbohydrate oxidation was significantly ($F_{2,26}$=26.410; P<0.05) higher during block 1 (1 – 15 min) compared with the rest of the protocol (16 – 90 min). There was a significant interaction between trial and time ($F_{5,53}$=5.900; P<0.05); carbohydrate oxidation was stable during GLU and GLUc, whereas in contrast it declined steadily during PLA and PLAc.

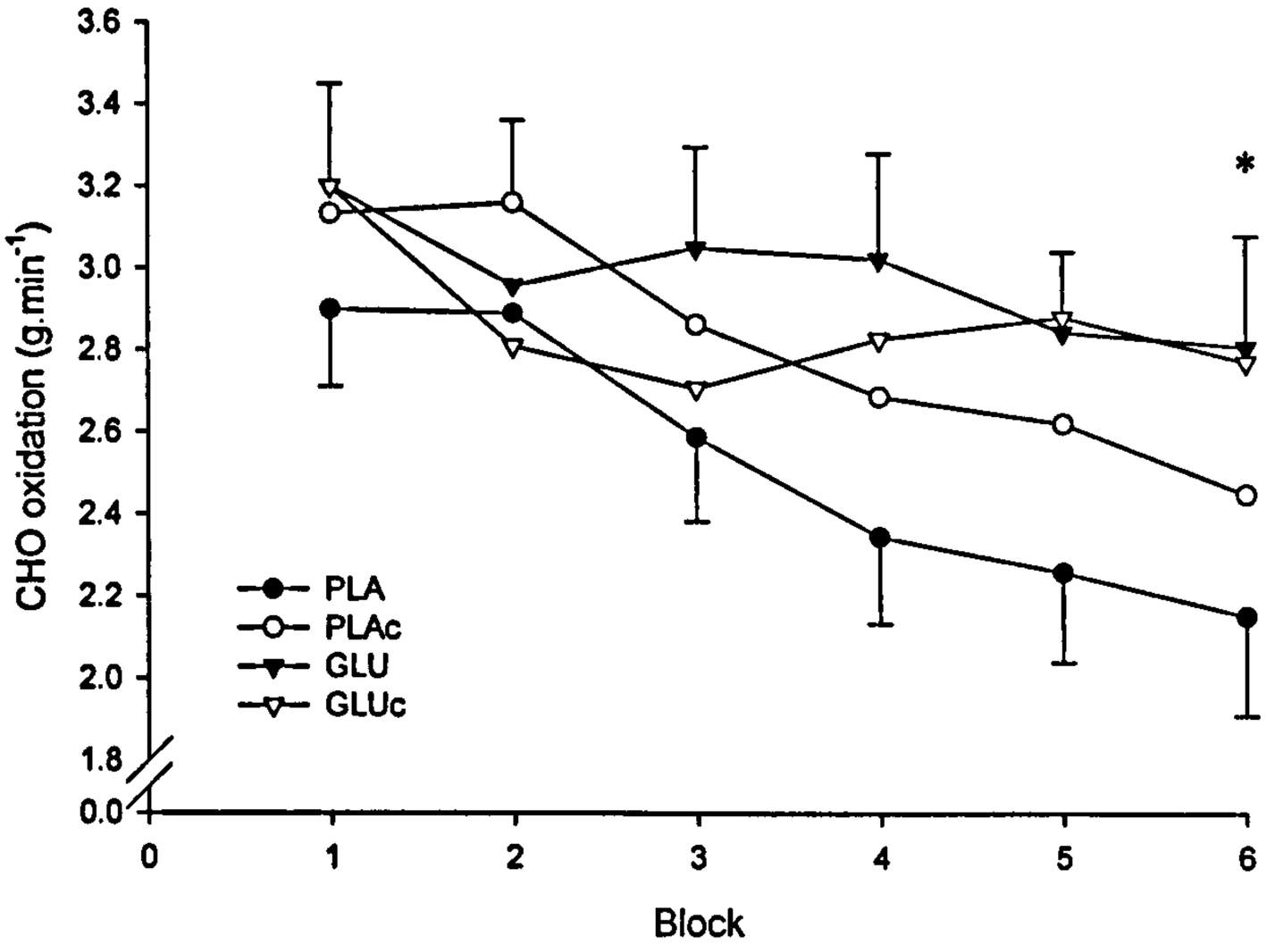

Figure 7.19: Carbohydrate oxidation during the soccer-specific protocol.

* GLU and GLUc significantly higher than PLA.

Repeated measures ANOVA showed that the rate of fat oxidation during PLA was significantly higher ($F_{2,25}$=3.380; P<0.05, Figure 7.20) compared with GLUc during blocks 4 to 6 (45 to 90 min). There was no significant difference between the other trials (P>0.05) The repeated measures ANOVA identified a significant effect of time ($F_{2,27}$=36.924; P<0.05). The Bonferroni corrected pairwise comparisons showed that fat oxidation was significantly lower (P<0.05) during block 1 (1 – 15 min) compared with the remainder of the protocol (16 – 90 min). There was a significant interaction effect ($F_{15,165}$=9.691; P<0.05); fat oxidation increased steadily during PLA, whereas during the carbohydrate trials, in particular GLUc, fat oxidation was relatively constant.

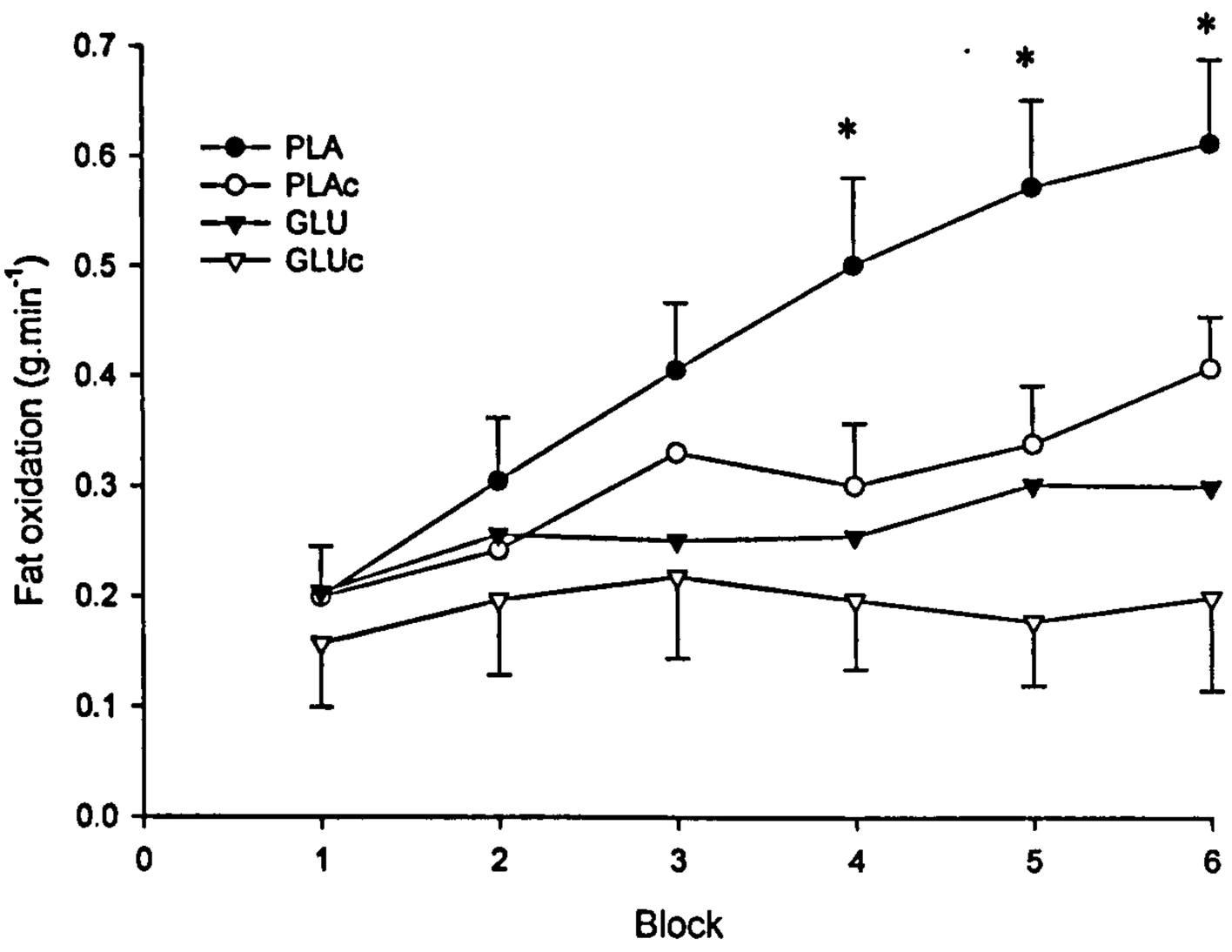

Figure 7.20: Fat oxidation during the soccer-specific protocol.

* PLA significantly higher than GLUc.

7.3.7. Gut Fullness and thirst

Gut fullness was not significantly ($F_{2,20}$=0.896; P>0.05) affected by the trials (Table 7.3). There was a significant effect of time ($F_{2,27}$=6.694; P<0.05); pairwise comparisons disclosed that gut fullness was significantly greater post-fluid ingestion at half-time compared to pre-fluid ingestion at the start of the protocol.

Table 7.3: Subjective sensation of gut fullness during the soccer-specific protocol.

	Time point								
	Pre-	Post-	1	2	3	HT	4	5	6
PLA	25.6±4.6	34.9±5.4	29.0±5.3	38.9±6.5	36.5±5.1	44.3±6.7	38.6±5.0	39.9±5.3	43.5±5.6
PLAc	20.0±4.3	29.9±4.3	26.9±2.6	27.2±4.2	31.0±4.7	37.1±5.5	36.3±5.0	36.5±4.7	35.9±6.0
GLU	21.8±4.7	31.8±4.7	26.5±4.1	33.5±4.2	35.3±4.5	45.1±4.6	32.8±4.4	33.7±4.3	34.7±5.3
GLUc	21.8±4.7	31.8±4.7	26.5±4.1	33.5±4.2	35.3±4.5	45.1±4.6	32.8±4.4	33.7±4.7	28.3±3.2

Note: Pre – Before fluid ingestion; Post – After fluid ingestion; 1-6 – Blocks; 1-6, HT – Half-time.

There was not a significant difference in thirst between the trials ($F_{3,32}$=0.266; P>0.05, Table 7.4). There was also a significant difference between time points, ($F_{2,25}$=12.323;

$P<0.05$); pairwise comparisons revealed these differences occurred between pre- and post-fluid ingestion at the start of the protocol. Thirst was also significantly lower at half-time compared with pre-fluid ingestion at the start of the protocol. The half-time value was also significantly lower than all time points except immediately following fluid ingestion before the start of exercise.

Table 7.4: Subjective sensation of thirst during the soccer-specific protocol.

	Time point								
	Pre-	Post-	1	2	3	HT	4	5	6
PLA	54.5±6.4	41.5±5.7	62.1±7.2	58.8±7.6	61.1±7.1	35.9±5.5	51.0±6.2	52.9±6.8	57.2±6.6
PLAc	55.4±5.2	43.7±5.5	55.1±5.7	52.0±6.2	54.9±7.4	31.0±4.5	50.0±6.3	54.2±6.7	56.7±7.5
GLU	57.1±6.3	48.0±7.0	58.5±6.7	57.7±6.6	53.4±6.9	34.0±6.8	52.7±7.1	50.0±5.9	52.3±6.7
GLUc	52.8±5.9	45.5±5.1	58.8±4.7	60.3±6.5	58.3±6.5	33.3±5.7	53.5±5.7	55.3±6.5	55.2±7.0

Note: Pre – Before fluid ingestion; Post – After fluid ingestion; 1-6 – Blocks; 1-6, HT – Half-time.

7.3.8. Heart rate and RPE

There was no significant trial effect on heart rate ($F_{2,22}=3.842$; $P>0.05$, Figure 7.21). Heart rate increased significantly ($F_{3,28}=656.231$; $P<0.05$) between each block of the soccer-specific protocol and decreased significantly during half-time.

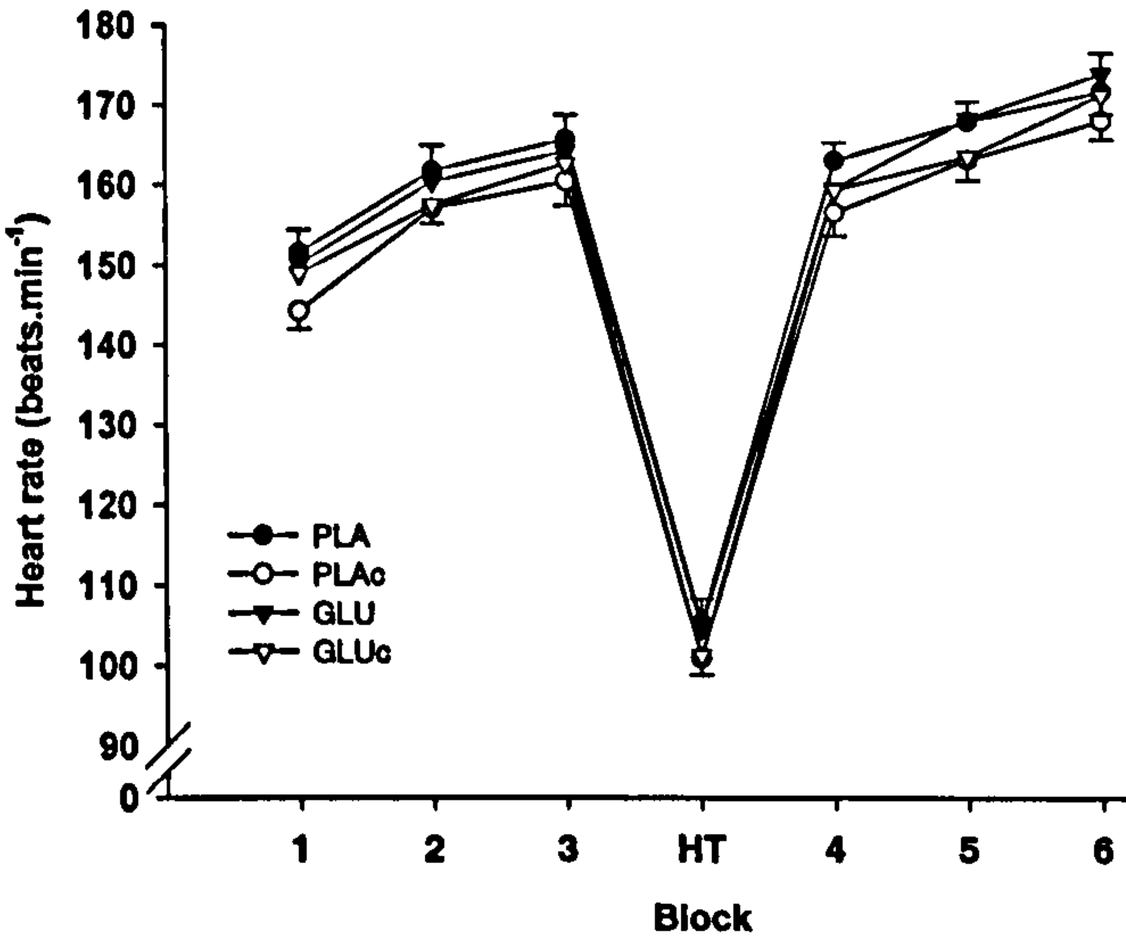

Figure 7.21: Heart rate during the soccer-specific protocol.

There was no significant ($F_{3,33}$=2.392; P>0.05) difference in RPE between trials (Figure 7.22). A significant effect of time was detected ($F_{1,16}$=90.721; P<0.05), RPE increased significantly between each block of the soccer-specific protocol, with the exception of between blocks 3 and 4, before and after half-time.

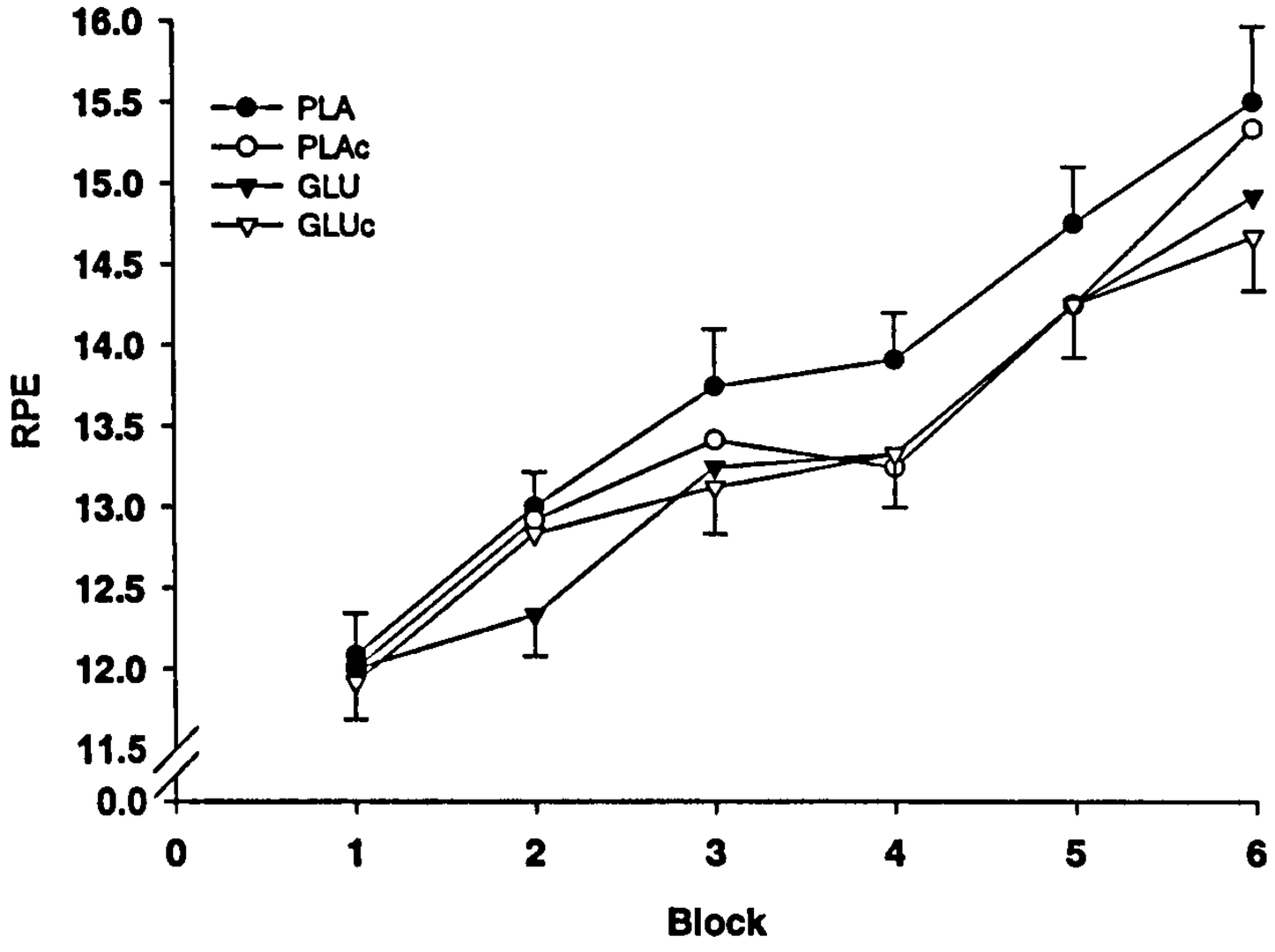

Figure 7.22: RPE during soccer-specific protocol.

7.3.9. Mental concentration

Mental concentration was significantly influenced by trial ($F_{2,26}$=0.740; P>0.05; Figure 7.23); in blocks 1, 3 and 4 the percentage of correct response was significantly higher during GLUc compared with PLA. There was no significant effect of time ($F_{2,20}$=2.180; P>0.05), the percentage of correct answers was relatively constant throughout the protocol.

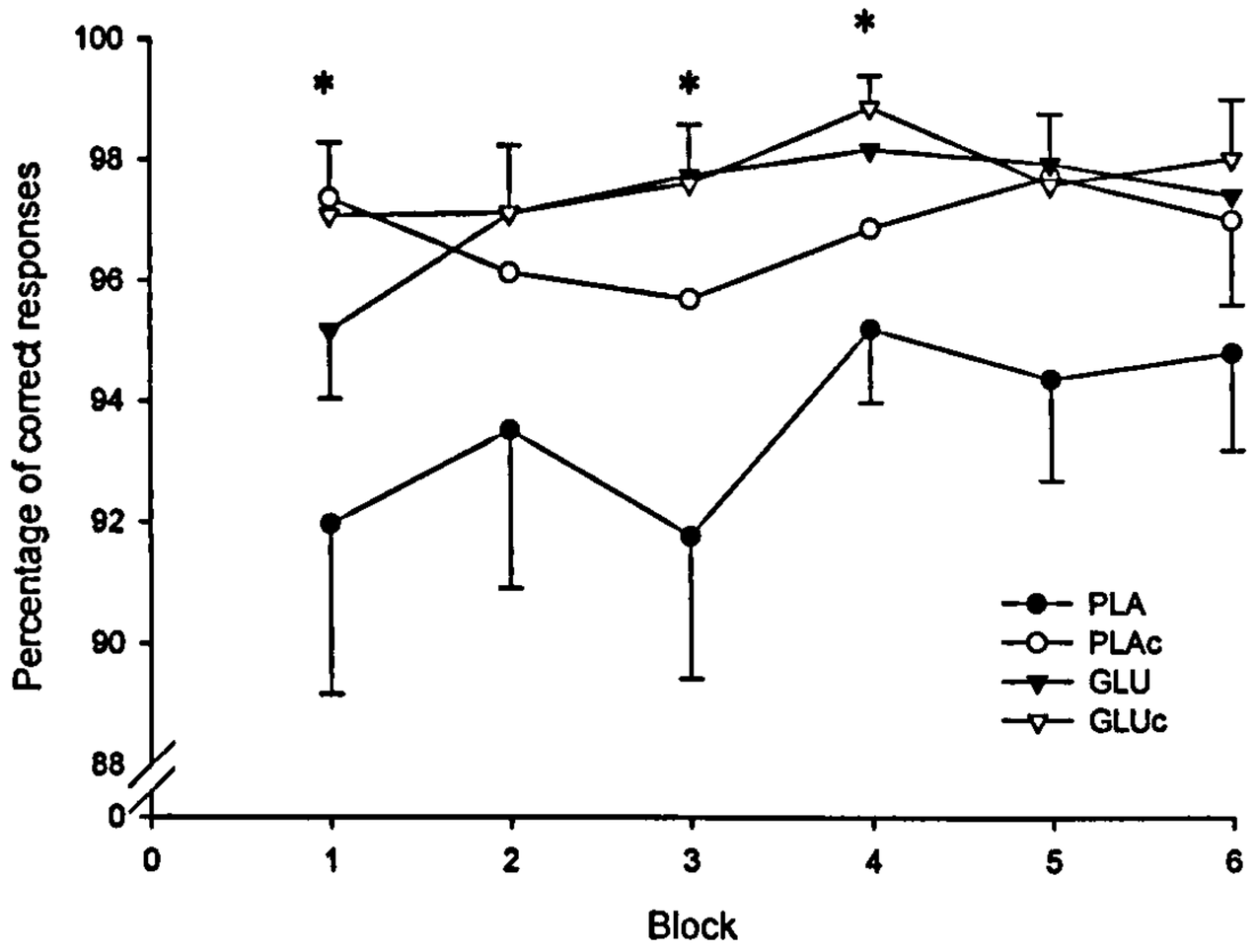

Figure 7.23: Mental concentration during soccer-specific protocol.

* GLUc significantly greater than PLA.

7.3.10. Self-chosen work-rate test

The self-chosen speed ($F_{2,22}$=5.315; P<0.05; Figure 7.24) was significantly higher during GLUc compared with the other trials (PLA: 11.3±0.4 km·h^{-1}; PLAc:11.6±0.5 km·h^{-1}; GLU: 11.3±0.4 km·h^{-1}; GLUc: 12.5±0.5 km·h^{-1}).

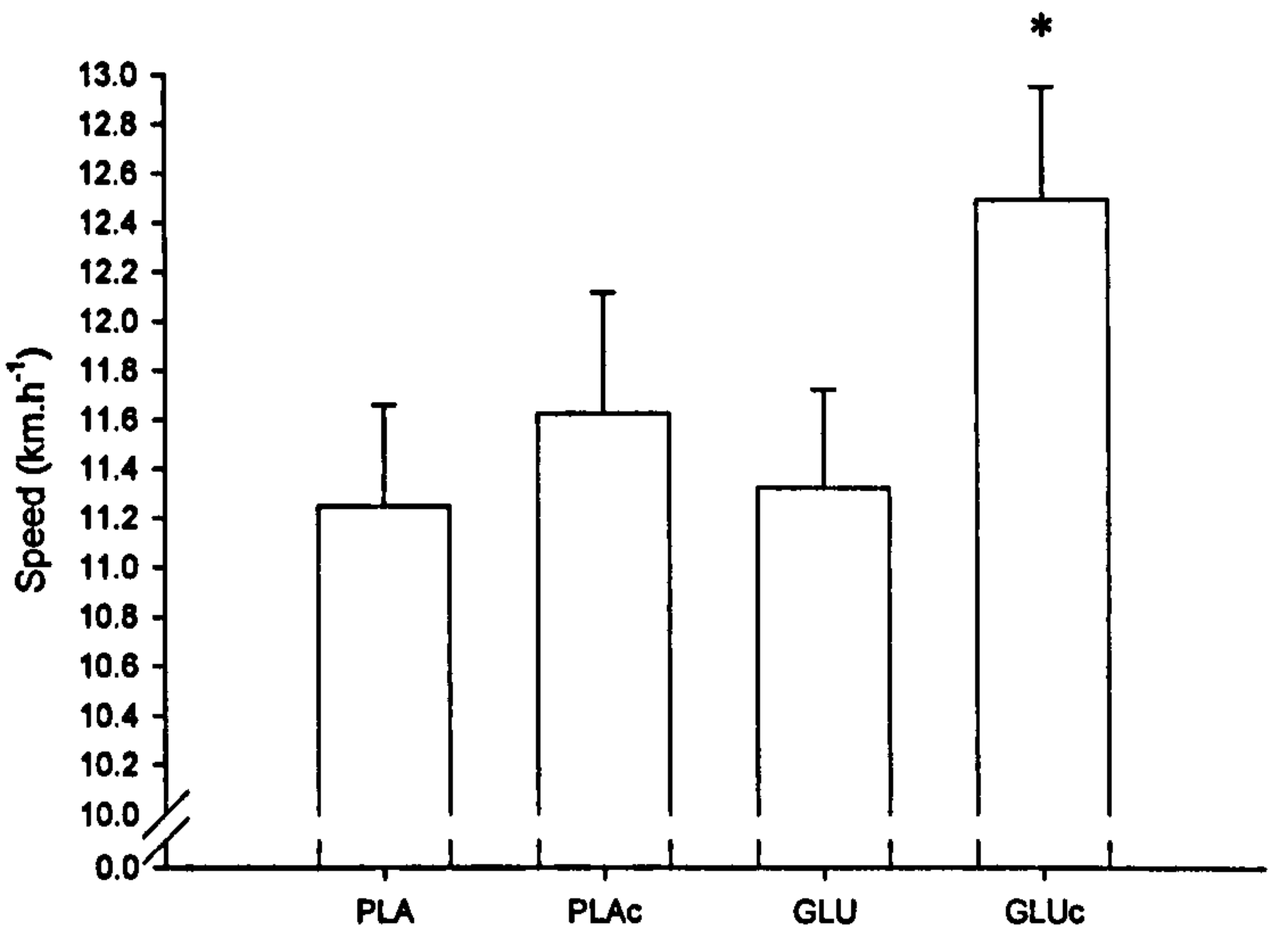

Figure 7.24: Running speed during the self-chosen work-rate test.

* significantly higher than PLA, PLAc and GLU.

7.3.11. Cunningham and Faulkner test

There was a significant treatment effect ($F_{2,24}$=11.171; P<0.05) on exercise capacity during the Cunningham and Faulkner test (Figure 7.25); GLU and GLUc were significantly higher compared to PLA; PLA: 57.09±5.25 s; PLAc: 70.05±7.74 s; GLU: 72.06±5.16 s; GLUc: 79.82±6.93 s. There was a trend for performance to be improved with pre-cooling during the placebo trials, but this effect was not significant (P=0.072). In addition, the Person coefficient revealed a significant correlation between the self-chosen work-rate test and the Cunningham and Faulkner test (Speed: r=0.48; P<0.05).

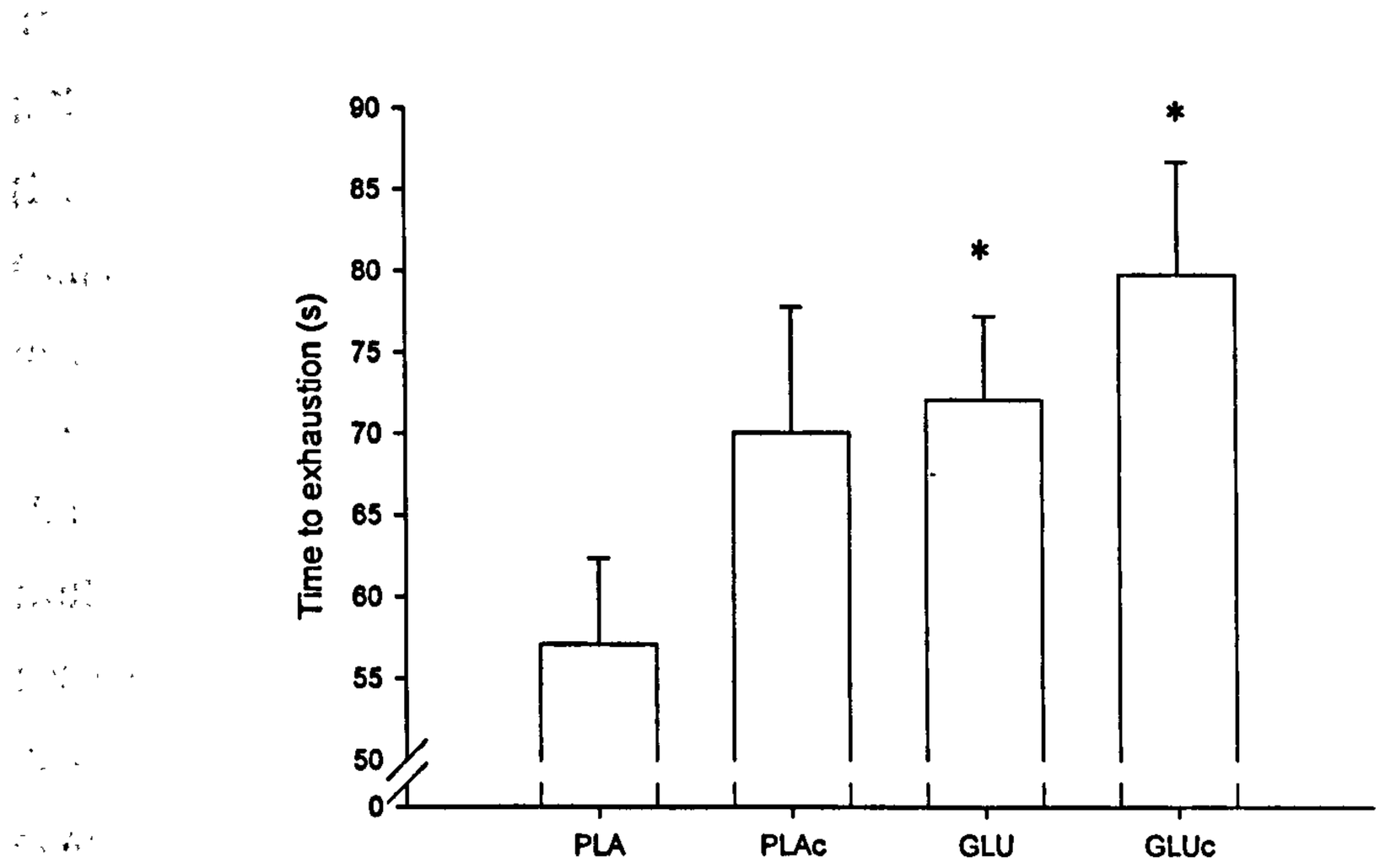

Figure 7.25: Time to exhaustion during the Cunningham and Faulkner test.

* significantly higher than PLA.

7.3.12. Sweat loss

There was no significant difference ($F_{2,26}$=1.654; P>0.05) in sweat loss between the four trials. The mean losses were: PLA (2.09±0.15 kg), PLAc (2.33±0.12 kg), GLU (2.12±0.15 kg) and GLUc (2.23±0.10 kg). The absolute weight loss (uncorrected for fluid ingestion) was not significantly different between trials ($F_{2,27}$=2.177; P>0.05); PLA: 1.02±0.1 kg, PLAc: 1.29±0.1 kg, GLU: 1.06±0.1 kg and GLUc: 1.22±0.1 kg.

7.4. Discussion

The major findings of this study were i) a 1 h period of pre-cooling significantly reduced core temperature, ii) exercise capacity following 90 min of soccer-specific exercise in the heat was significantly improved with the ingestion of carbohydrate, iii) the combined effect of carbohydrate ingestion and pre-cooling significantly enhanced self-chosen running speed and exercise capacity and iv) the combined effect of carbohydrate ingestion and pre-cooling significantly improved mental concentration.

The present study demonstrated that exercise capacity was significantly improved following the ingestion of carbohydrate, supporting the findings of previous studies (Nicholas *et al.*, 1995). The increase in exercise capacity, measured using the Cunningham and Faulkner test was possibly due to the ingestion of carbohydrate increasing blood glucose levels (Figure 7.7) enabling a higher rate of carbohydrate oxidation to be maintained (Figure 7.19). Carbohydrate ingestion has been repeatedly shown to improve exercise capacity (Coyle *et al.*, 1986; Coggan and Coyle, 1987; Millard-Stafford *et al.*, 1992; Yasplekis *et al.*, 1993a), possibly due to increased availability of blood glucose to replace that utilized by the muscle during exercise (Coyle *et al.*, 1983). Other potential mechanisms include, sparing of muscle glycogen (Yasplekis *et al.*, 1993a), maintaining a higher rate of carbohydrate oxidation at a time when muscle glycogen levels are low (Coggan and Coyle, 1987) and reduced central fatigue (Davis *et al.*, 1992). Nicholas *et al.* (1995) concluded that the most probable cause of increased exercise capacity was reduced muscle glycogen utilization during exercise when consuming carbohydrate. However, in a previous study, Morris *et al.* (2003) examined the effect of carbohydrate ingestion on performance of intermittent exercise in the heat and failed to show any benefit of carbohydrate ingestion. The authors concluded that hyperthermia may have been the main fatiguing factor and not dehydration or energy depletion. In this study, (Morris *et al.*, 2003) all but one subject failed to complete the first part of the protocol, so exercise capacity was not assessed.

It is worth noting that in the present study the ingestion of carbohydrate significantly improved time to exhaustion during the Cunningham and Faulkner test. This observation is in contrast to the findings of the previous study. One possible explanation for this occurrence is that in study 3 muscle biopsies were performed prior to performing the Cunningham and Faulkner test which may have caused muscle soreness and pain which then influenced time to exhaustion. However the exact mechanism remains to be identified.

Pre-cooling has previously been reported to improve the performance of endurance exercise (Lee and Haymes, 1995; Booth *et al.*, 1997; Cotter *et al.*, 2001; White *et al.*, 2003; Arngrimsson *et al.*, 2004) and high-intensity exercise (Marsh and Sleivert, 1999; Sleivert *et al.*, 2001). In the present study the combined effect of pre-cooling and carbohydrate significantly improved self-chosen pace and exercise capacity when compared with placebo, placebo with pre-cooling and carbohydrate ingestion. There was a trend for self-chosen running speed and high-intensity exercise capacity to be improved with placebo and pre-cooling compared with placebo. Pre-cooling has previously been shown not to impact on muscle metabolism (Kay *et al.*, 1999; Booth *et al.*, 2001) Therefore it is unlikely that the increased exercise capacity and self-chosen running speed observed in the present study and exercise performance in previous studies (Booth *et al.*, 1997) can be explained on the basis of altered muscle metabolism. One possible explanation for the faster self-chosen pace during GLUc is the lower core temperature at the completion of the soccer-specific protocol as hyperthermia may reduce the central drive for exercise by influencing the motor control centre in the brain (Nielsen *et al.*, 1993). It has also been shown that during hyperthermic exercise cerebral temperature increases in parallel with core temperature (Nybo *et al.*, 2002b) and during treadmill exercise goats reduce their speed, or refuse to move, when the brain temperature exceeds 42°C (Caputa *et al.*, 1986). In addition, exercise-induced hyperthermia reduces voluntary activation of motorneurons during a sustained maximal muscle contraction in humans (Nybo and Nielsen, 2001).

Thus far the metabolic responses to exercise after pre-cooling have not been extensively investigated. It has been proposed that pre-cooling may enhance exercise performance by reducing the metabolic perturbation often observed with increased core and muscle temperatures (Marino, 2002). Pre-cooling did not have a significant effect on metabolism. One potential explanation for the lack of difference in metabolism in the present study is the failure of pre-cooling to affect muscle temperature during exercise. Pre-cooling significantly reduced muscle temperature prior to exercise but there was not a significant difference at subsequent time points during exercise. Recently, Booth *et al.* (2001) demonstrated that pre-cooling only had a negligible effect on substrate utilization during exercise in the heat. Despite lower muscle and core temperatures with pre-cooling, muscle glycogen utilization was not significantly different from the control trial. The authors suggested that muscle temperature needed to reach a critical value before muscle energy metabolism is sufficiently affected to influence exercise performance. The present study demonstrated that pre-cooling significantly reduced core temperature but not muscle temperature during exercise, suggesting that muscle temperature is an important factor in regulating muscle metabolism.

The lack of a difference in metabolism between trials may have been due to there being no difference in muscle temperature, once exercise had began. Starkie *et al.* (1999) suggested that muscle temperature was involved in the regulation of intramuscular carbohydrate utilization and was responsible, in part, for the increase in muscle glycogen utilization frequently observed during exercise in the heat. Oxygen uptake was unaffected by pre-cooling, as demonstrated in previous studies. Most studies (Lee and Haymes, 1995; Booth *et al.*, 1997; Kay *et al.*, 1999; Drust *et al.*, 2000a) have shown that oxygen consumption during exercise remains unchanged with pre-cooling.

The period of pre-cooling significantly reduced core and muscle temperatures and consequently thermal sensation. Core temperature remained significantly lower during PLAc and GLUc throughout the first 45 min of exercise. This observation is in contrast to a number of previous studies (Kruk *et al.*, 1991; Lee and Haymes, 1995; Bolster *et al.*, 1999), which demonstrated that core temperature was significantly lower after pre-

cooling for between 10 and 26 min. However, Drust *et al.* (2000a) reported that pre-cooling significantly reduced rectal temperature during the first half of soccer-specific exercise. One possible explanation for these discrepancies is that a wide range of cooling techniques and exercise intensities and durations have been used to assess the impact of pre-cooling (Lee and Haymes, 1995). The reduced core temperature associated with pre-cooling allows for a greater heat storage before core temperature reaches a level high enough to stimulate heat dissipation (Drust *et al.*, 2000a), reducing the physiological strain. However, in the present study there was not a significant reduction in the heart rate and RPE during the pre-cooling trials. Thermal sensation differed between trials only when the cooling vest was being worn, similar to the findings of Bolster *et al* (1999). Pre-cooling did not significantly affect total sweat loss during the soccer-specific protocol. This observation may be as a consequence of the amount of heat lost through the evaporation of sweat is largely determined by the metabolic heat production ($\dot{V}O_2$) and the environmental heat load (Nielsen, 1996), which was the same for all trials. This observation was consistent with the findings of Booth *et al.* (1997) and Drust *et al.* (2000a).

Circulating levels of IL-6 were significantly elevated during the placebo trials at half-time. Endurance exercise (Nieman *et al.*, 1998; Suzuki *et al.*, 2003) and repeated bouts of high-intensity cycling (Meyer *et al.*, 2001a) have been shown to increase IL-6 levels in response to skeletal muscle inflammation. In addition, adrenaline has been shown to increase the appearance of IL-6 in rats (Yu *et al.*, 2001), enhancing the stimulation of lipolysis. Stouthard *et al.* (1996) found an increase in circulating NEFA with IL-6 infusion although it was not possible to establish weather IL-6 acted directly on the adipocytes due to the elevated adrenaline levels. The release of IL-6 has been shown to be attenuated by the ingestion of carbohydrate during prolonged cycling at 70-75% $\dot{V}O_{2max}$ (Nieman *et al.*, 2003; Nieman *et al.*, 2005). These findings suggest that the release of IL-6 is dependent on carbohydrate availability and the associated diminished adrenaline response and may have contributed to the elevated fat oxidation rates observed during the placebo trials.

The prolactin values at the completion of the soccer-specific protocol observed in the present study are similar to those reported by Purvis *et al.* (2001) following soccer-specific exercise in the heat. Despite the absence of a significant difference between conditions there was a trend for prolactin levels to be lower during the carbohydrate trials. This may have been as a consequence of the increased availability of carbohydrate (Chan *et al.*, 2004), or the elevated NEFA concentration observed during the placebo conditions. High NEFA concentrations during exercise are associated with increased cortisol, serotonin (5-HT) and adrenaline levels, which have been shown to increase the synthesis and release of prolactin (Struder and Weicker, 2001b). Prolactin production is mediated by the adrenal stimulation of f-TRP, which is released from albumin (Struder and Weicker, 2001a). Trytophan further stimulates 5-HT production (Chaouloff, 1997) which stimulates the lactotrophic cells within the anterior pituitary (Struder and Weicker, 2001a). An elevated core temperature (Brisson *et al.*, 1991) and high-intensity exercise (Brisson *et al.*, 1981) have also been shown to increase prolactin levels. However, it has also been suggested that core temperature regulates in the release of prolactin. Low *et al.* (2005b) reported that core temperature, and not cardiovascular afferents was the key stimulus for prolactin and may be a marker of central serotonergic and dopaminergic activity relating to central fatigue during exercise in hot environments. Previous studies (Brisson *et al.*, 1991; Low *et al.*, 2005a) have indicated that there is a threshold level in core temperature above which the release of prolactin increases and this temperature is 38°C. This threshold for prolactin release would explain the high levels of prolactin observed in the present study, as core temperature at the completion of the soccer-specific protocol exceeded 38.5°C in all trials, despite differences in carbohydrate availiabilty.

The present study demonstrated that during the soccer-specific protocol, mental concentration was significantly improved following carbohydrate ingestion and pre-cooling compared with placebo without pre-cooling. This observation may have been a consequence of increased blood glucose availability. Glucose is the predominant energy source of the central nervous system (Welsh *et al.*, 2002) and therefore, maintaining blood glucose could have beneficial effects in sports such as soccer, which require tactical decision-making. Carbohydrate supplementation can maintain blood glucose

levels and potentially prevent a decrease in cognitive functioning. A number of authors have demonstrated that glucose administration is capable of enhancing cognitive performance in healthy young adults at rest (Benton *et al.*, 1994; Kennedy and Scholey, 2000; Scholey *et al.*, 2001) and after exercise (Reilly and Lewis, 1985; Collardeau *et al.*, 2001). However, the mechanisms underlying the cognition-enhancing effects of glucose are unknown (Scholey *et al.*, 2001).

The present study also reaffirms previous reports (Coyle *et al.*, 1986; Coggan and Coyle, 1987; Wright *et al.*, 1991; Millard-Stafford *et al.*, 1992; Yasplekis *et al.*, 1993a) that the ingestion of carbohydrate during exercise significantly increases carbohydrate oxidation, and glucose and insulin concentrations. Carbohydrate ingestion also suppresses fat oxidation, and NEFA and glycerol concentrations during exercise.

In conclusion, these results suggest that carbohydrate ingestion can improve exercise capacity following soccer-specific exercise performed in the heat. In addition, pre-cooling in conjunction with the ingestion of carbohydrate during exercise increased mental concentration and exercise capacity. Whilst this combination is a suitable strategy for soccer players when performing in the heat, the mechanisms for this improvement remain to be identified.

Chapter 8
Synthesis of findings

8.1. Synthesis of findings

The studies described in the present thesis were designed to investigate the effect of ingesting sports drinks on the physiological, metabolic and hormonal responses to soccer-specific exercise. The exercise protocol was designed to simulate the work-rate in competitive soccer match-play. The first two studies consisted of investigations into the metabolic and performance responses during soccer-specific exercise. In these studies the provision of carbohydrate-electrolyte solutions was manipulated so that their effects could be examined with respect to their influence on the metabolic responses to soccer-specific exercise (motorised treadmill) and on performance during the soccer-specific protocol (reflected in the sprint portion of the simulated work-rate on a non-motorised treadmill). A further study was conducted to investigate the effect of carbohydrate formulation on the metabolic responses and performance of soccer-specific exercise in the heat. The effect of ingesting a multi-carbohydrate solution has previously only been examined during low-intensity exercise. The final study examined the impact of pre-cooling in conjunction with carbohydrate ingestion on the performance of soccer-specific exercise in the heat.

8.1.1. Carbohydrate ingestion and soccer-specific exercise

The performance of soccer-specific exercise is dependent upon a number of factors including substrate availability, hydration status and core temperature. The schematic model in Figure 8.1 illustrates the inter-relationship between fluid replacement, core temperature, metabolism and substrate availability and oxidation and their subsequent impact on the performance of soccer-specific exercise.

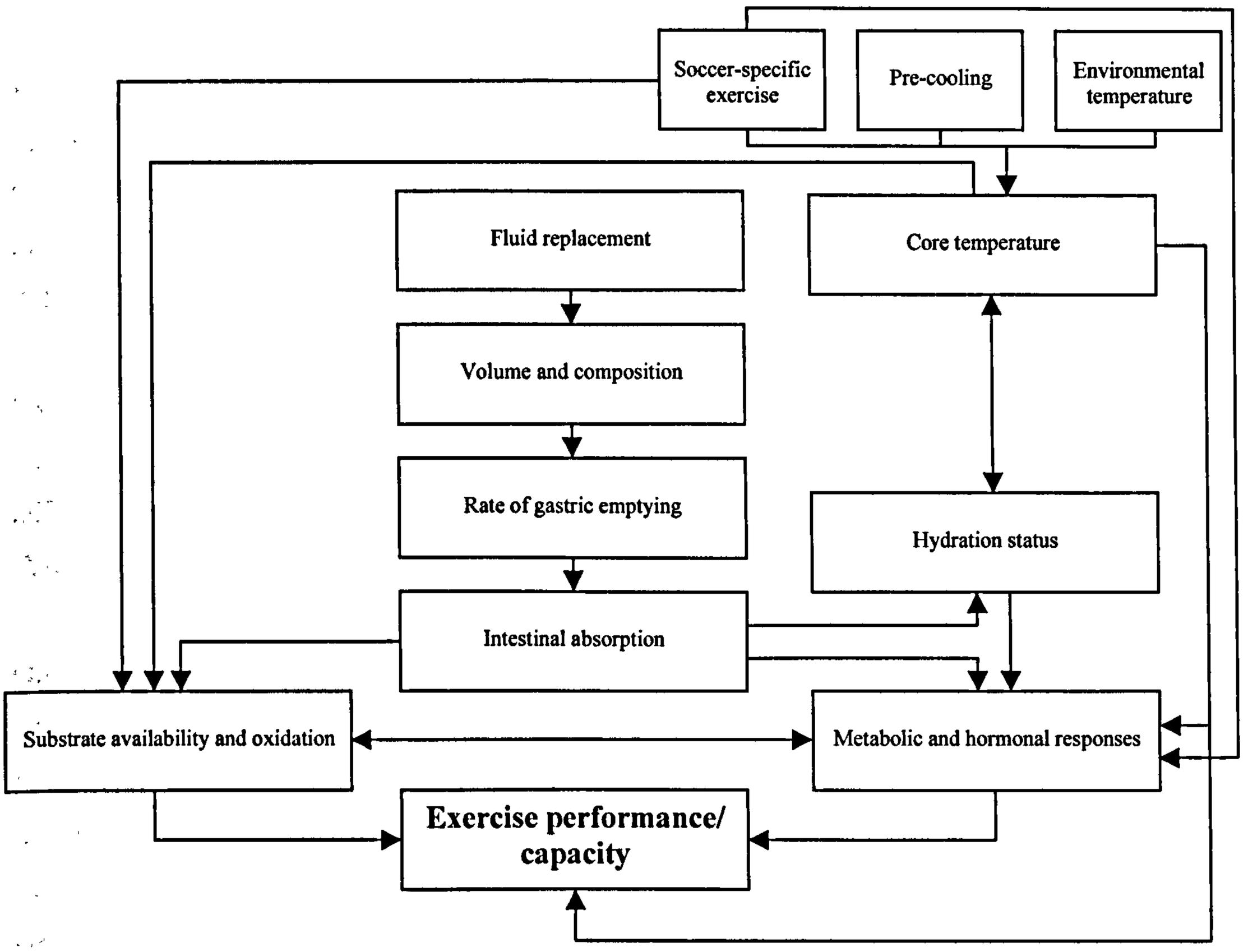

Figure 8.1: Schematic model representing the factors that influence exercise performance and individual capacity during and after soccer-specific exercise.

The ingestion of a carbohydrate-electrolyte solution significantly affected metabolite concentration, whereby plasma glucose was significantly increased and the rise in glycerol and NEFA levels were attenuated, although lactate levels were not different between trials. The elevated glucose levels were associated with significantly increased insulin concentrations whilst there was a tendency for the catecholamine and IL-6 response to be attenuated.

The alterations in substrate availability had a significant effect on substrate oxidation rates. Carbohydrate oxidation was significantly higher when plasma glucose was

elevated. In contrast, fat oxidation was suppressed during the soccer-specific protocol with carbohydrate ingestion. Insulin has been shown to be a powerful inhibitor of lipolysis and the appearance of NEFA (Horowitz *et al.*, 1997). The increased insulin concentration after carbohydrate ingestion reduces whole-body lipolysis, as indicated by the lower fat oxidation and NEFA and glycerol levels observed during the carbohydrate trials. The elevated insulin concentration observed during the carbohydrate trials compared with placebo may also explain the higher, although not significant adrenaline levels shown during the placebo trials. Previous studies reported that when carbohydrate was ingested the adrenaline response was blunted (Felig *et al.*, 1982; Fritzsche *et al.*, 2000). In addition, adrenaline has been shown to increase the appearance of IL-6 in rats (Yu *et al.*, 2001), the consequence of which increase lipolysis.

One of the most important factors regulating fluid replacement is the rate of gastric emptying which is influenced by the volume within the stomach. The larger the volume the faster the rate of gastric emptying and as the volume of fluid within the stomach declines so does the gastric emptying although the ingested were relatively small. Therefore, it would have been interesting to have investigated the impact of consuming larger volumes, which may be necessary in order to prevent dehydration. Furthermore, a limitation of the presented studies is that a placebo was not ingested at frequent intervals. However, altering the timing and volume of carbohydrate ingestion or the carbohydrate formulation did not have a significant impact on hydration status, substrate availability and oxidation or hormonal and metabolic responses. This result was presumably a consequence of the same total amount of carbohydrate and fluid being ingested after 30 min of each half of the soccer-specific protocol. In addition, it may have been that the exercise intensity was the overriding factor controlling the rate of gastric emptying and intestinal absorption and not the volume or composition of the ingested fluid. The exercise intensity associated with soccer has been shown to be sufficient to slow gastric emptying (Leiper *et al.*, 2001; Leiper *et al.*, 2005) and consequently the intestinal absorption of carbohydrate and fluid, which in turn influences the appearance of substrates in the blood and oxidation rates. The reduced rate of gastric emptying during soccer (exercise) is possibly due to vasoconstriction of the gastrointestinal tract,

diminishing gastric blood flow. However, ingesting a small volume frequently does reduce the discomfort associated with gastric fullness, although this may not be a practical strategy as there are not any breaks scheduled into soccer matches where fluid could be ingested.

A possible explanation for the failure of carbohydrate ingestion to influence peak power output during sprinting is that during short duration high-intensity exercise, PCr is the major energy source. It is the availability of PCr, and its rates of resynthesis, which determines maximal sprinting performance (Greenhaff *et al.*, 1994). Therefore reduction in muscle glycogen or blood glucose may not influence PCr degradation during exercise. Balsom *et al.* (1992) demonstrated that forty 15-m sprints could be performed at 30-s intervals unimpaired without carbohydrate supplementation. In addition Nevill *et al.* (1993) reported that increasing the carbohydrate content of the normal diet did not improve sprint performance during 1 h of maximal, intermittent exercise. In study 2 a total of 18 sprints were performed separated by approximately 200 s of lower-intensity exercise, suggesting there was sufficient time for PCr resynthesis between sprints and that the PCr-phosphokinase system was able to buffer the energy demands during the sprints.

8.1.2. Carbohydrate ingestion and soccer-specific exercise in the heat

Jentjens *et al.* (2002) reported that the rate of exogenous glucose oxidation was reduced by 10% in the heat compared with a cool environment. Some of the factors that contribute to the reduction in exogenous glucose oxidation in the heat include the reduced carbohydrate absorptive capacity of the intestine as a result of decreased intestinal blood flow (Jentjens *et al.*, 2002), reduced uptake and release of ingested glucose by the liver, decreased glucose transport to the muscle due to reduced muscle blood flow as a consequence of impaired hydration status (Gonzalez-Alonso *et al.*, 1999a). Studies have also shown that hyperthermia and dehydration can impair gastric emptying of carbohydrate solutions or water during treadmill exercise (Owen *et al.*, 1986; Neufer *et al.*, 1989; Rehrer *et al.*, 1990). Another potential limiting factor for the oxidation of

exogenous carbohydrate and subsequent exercise performance is the rate of intestinal carbohydrate absorption. The time course for the appearance of sugars in the blood and subsequent oxidation is dependent on the rate at which they leave the stomach and are absorbed from the intestinal region. Furthermore, increased intestinal solute (i.e. carbohydrate) transport has been shown to promote greater fluid absorption (Shi *et al.*, 1995) which may reduce the impact of dehydration associated with exercising in the heat. However, the intestinal absorption of a 6.6% carbohydrate solution does not appear to be a limiting factor in the oxidation of exogenous carbohydrate during soccer-specific exercise in the heat as ingesting a single- or multi-carbohydrate did not significantly influence muscle glycogen utilization, carbohydrate oxidation and exercise capacity. muscles (McCully *et al.*, 2002).

Three of the most regularly cited physiological causes of fatigue are substrate depletion, dehydration and hyperthermia. It is unlikely that dehydration was the key factor in the development of fatigue as sweat loss was similar for each trial. It is also improbable that carbohydrate depletion was the cause as muscle glycogen content was similar for all trials after completing the soccer-specific protocol, plasma glucose concentration was above 4 $mmol.l^{-1}$ in all conditions, and during the carbohydrate trials, carbohydrate oxidation had been maintained throughout the soccer-specific protocol. Therefore, a possible cause of fatigue during the Cunningham and Faulkner test was hyperthermia. Nielsen *et al.* (1993) suggested that when fatigue occurs in a hot environment it is ultimately due to an intolerably high core temperature being reached based on the observation that muscle glycogen concentrations are relatively high at the point of fatigue when exercising in the heat (Nielsen *et al.*, 1990). Nielsen *et al.* (2001) found that alterations in front cortical brain activity correlated with increases in core temperature. Therefore, the motivation to continue to exercise may be reduced when core temperature increases beyond a critical point. As with all tests of exercise capacity, motivation plays an important role when performing the test of Cunningham and Faulkner, and if the motivation to continue to exercise is reduced so will time to exhaustion. This may have been the case in the present study as core temperature and thermal sensation were similar during each condition. However, the final core temperature values at the completion of the 90 minutes observed

in studies 3 and 4 were lower (38.6°C – 39.1°C) than previously reported values during similar exercise protocols in the heat (38.9°C – 39.6°C) (Morris *et al.*, 1998; Morris *et al.*, 2003). This observation may be a consequence of different sites being used to assess core temperature, rectal as opposed to intestinal used in the present study or the fitness of the subjects, as a higher level of aerobic fitness is associated with an increased tolerance to a high core temperature (Selkirk and McLellan, 2001).

In study 4 the ingestion of carbohydrate with and without pre-cooling significantly improved exercise capacity, possibly due to the higher blood glucose availability. This was an unexpected result as exercise capacity was unaffected by carbohydrate ingestion in study 3. A possible explanation for this occurrence is that the muscle biopsies performed in study 3 influenced the ability to perform high-intensity exercise. There are limited data suggesting that the concentrations of stress hormones such as cortisol and noradrenaline may remain elevated after a biopsy (Holck *et al.*, 1994; Helge *et al.*, 1999), which may hasten fatigue (Davis and Brown, 2001). In addition, a percutaneous needle biopsy of vastus lateralis muscle has been shown to significantly reduce insulin-stimulated glucose uptake (Holck *et al.*, 1994). Although the mechanism of this phenomenon is not known, the biopsy may have impaired the uptake and subsequent oxidation of blood glucose.

Figure 8.2 the highlights the potential relationships between pre-cooling, carbohydrate ingestion, central fatigue and exercise capacity.

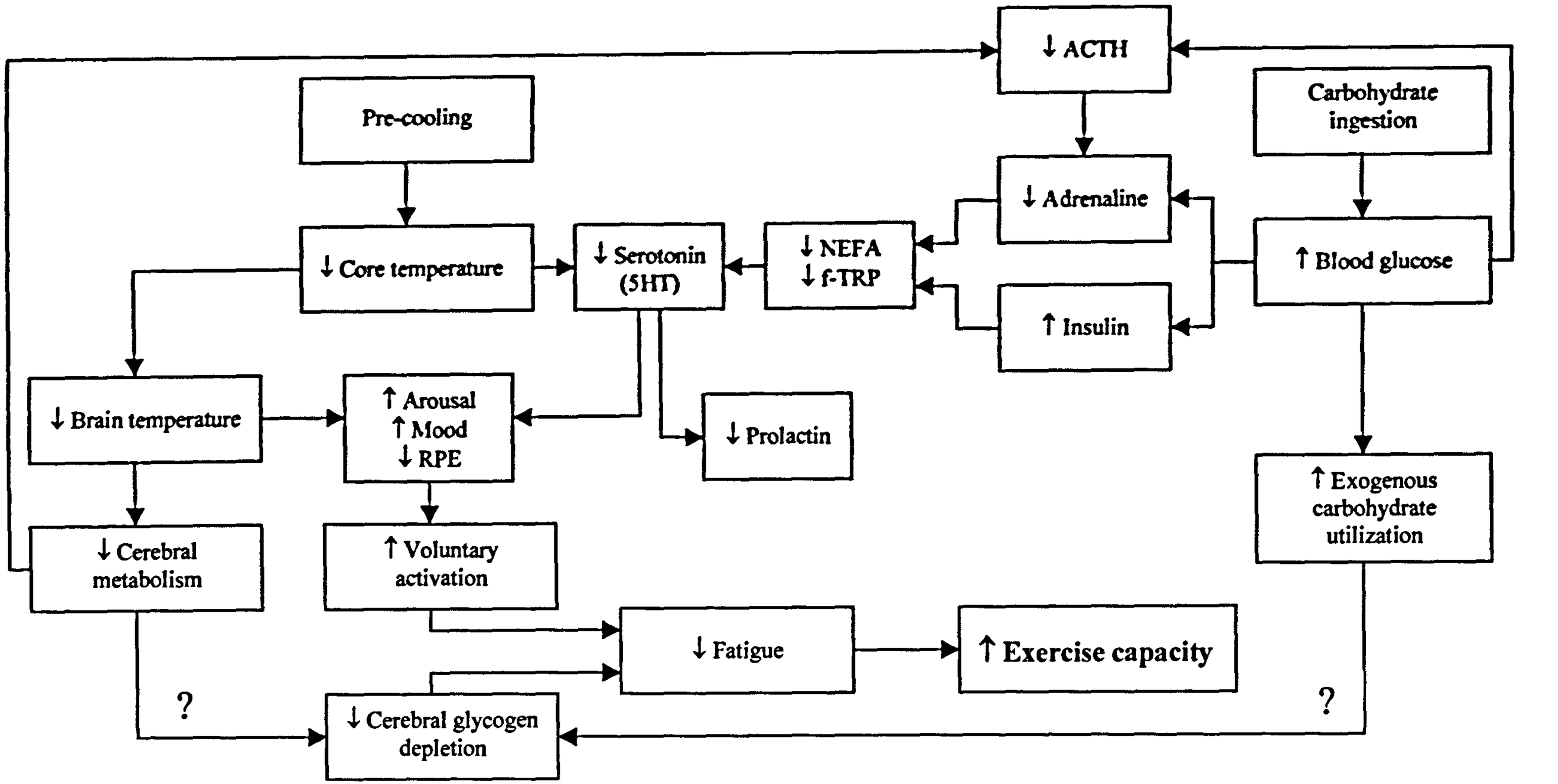

Figure 8.2: Proposed interaction between carbohydrate ingestion, pre-cooling and central fatigue.

The ingestion of carbohydrate has been shown to increase blood glucose and exogenous carbohydrate oxidation and spare the body's glycogen stores. Furthermore, the ingestion of carbohydrate attenuated the exercise induced rise in plasma NEFA concentration, preventing a rise in f-TRP. When carbohydrate was ingested without pre-cooling, a lower plasma concentration of NEFA was observed, resulting from decreased adrenaline (Fritzsche *et al.*, 2000) and increased insulin (Nieman *et al.*, 1998). When the amount of NEFA in the blood is reduced the concentration of f-TRP also declines. As a consequence there is less TRP available to be converted into serotonin, which has been associated with central fatigue (Davis *et al.*, 1992). Therefore fatigue may be delayed by improved mood and motivation, reduced sensation of effort. Carbohydrate ingestion has been shown to decrease RPE during exercise (Utter *et al.*, 1999) and increase voluntary activation (Nybo, 2003).

Performing soccer-specific exercise in a hot environment significantly increased core temperature. Low *et al.* (2005b) reported that an elevated core temperature was the key stimulus for prolactin, which may be a marker of central serotonergic activity. An increase in serotonin (5-HT) has been suggested to decrease arousal, mood and motivation and increase RPE and in turn reduce voluntary activation causing fatigue and impaired exercise capacity (Cheung and Sleivert, 2004). Watson *et al.* (2005) suggested that blood-brain barrier permeability may be altered during prolonged exercise in a warm environment. Therefore, the combined effect of carbohydrate ingestion and pre-cooling which significantly reduced core temperature potentially reduced serotonergic activity due to decreased serotonin formation. Consequently there may be an increase in arousal and a reduction in RPE, resulting in an increase in voluntary activation. These factors may delay the onset of fatigue, increasing exercise capacity. In study 3 it was suggested that an elevated core temperature may have contributed to fatigue following the performance of soccer-specific exercise in the heat. In study 4 the effect of lowering core temperature prior to soccer-specific exercise was examined. The results show pre-cooling allows for a greater degree of heat storage, with the effect of attenuating the rate of the rise in core temperature. Although the mechanisms for the improved exercise

capacity associated with pre-cooling are not yet known, it is possible that the lower core temperature reduces the effect of central and hyperthermic fatigue.

8.1.3. Recommendations

Based on the results of the studies contained within this thesis, some practical recommendations for soccer players can be made. The results suggest that carbohydrate ingestion may benefit the performance high-intensity exercise, but not short duration sprints. The hydration strategy would not appear to influence performance providing that sufficient fluid is ingested, although an important factor to consider would be what volume and carbohydrate formulation the player finds most comfortable to ingest. However, the volume would need to be changed to suit the environmental conditions. If too large a volume is ingested the player may suffer discomfort which could adversely influence performance. Additionally, the findings from study 4 suggest that when matches are played in a hot environment it would be beneficial if core temperature could be reduced prior to kick off.

Whilst the combination of pre-cooling and carbohydrate ingestion appears to be a strategy that could be used by soccer players to improve performance when playing in the heat, it seems unlikely that the particular pre-cooling manoeuvre will be employed prior to competitive matches. The pre-cooling regime in this study involved the subject wearing the cooling vest for 60 min whilst remaining seated. This would not be a practical strategy for professional players as they would require a warm up. A compromise would be to wear the cooling vest during the warm up. This would have the added advantage that muscle temperature would not decrease to the level observed in this study. However, this strategy would require further investigation to establish whether it is possible to achieve sufficient cooling to produce an advantage.

8.1.4. Limitations

A limitation of the studies presented in the thesis is that the findings may not transfer directly to professional soccer where the players operate at a higher tempo than is possible in laboratory conditions. Furthermore the pattern of activity in a match is dictated by the progress of the contest. In addition it is likely that professional players will have higher aerobic and anaerobic power capacities than the current subjects. However, the $\dot{V}O_{2max}$ of the subjects was close to that displayed among professional players and the intermittent exercise protocol simulates the pattern of activity in matches. Whilst the soccer-specific protocols employed in the four studies in this thesis were designed to simulate the work-rate of competitive soccer, it is not possible to make a complete representation. Activities such as tackling, jumping and shooting require additional energy to perform, and these activities were not included in the energy demands of the protocol. Additional factors that influence energy expenditure during a match include the condition of the pitch i.e. waterlogged and environmental factors such as wind and rain. Therefore, it is not possible to simulate completely the demands of a soccer match on a running treadmill. There is a large amount of psychological factors associated with a competitive soccer match. It has been shown that matches increase psychological stress as indicated by cortisol and catecholamines (Carli *et al.*, 1986), and these increases may depend on the importance of the match. However, it is not possible to recreate this level of stress in a laboratory, although the catecholamine responses were similar to those observed by Bangsbo (1994b) during competitive match-play.

One of the advantages of simulating the work-rate that represents that of a competitive soccer match on a treadmill in a laboratory is that the external factors such as temperature can be set, thereby allowing for controlled experimentation. In addition, if the study was field-based there would be an added factor of the opposition, which would not be consistent for each trial. Possibly the biggest advantage of using a soccer-specific protocol in a laboratory is the fact that more variables can be measured than would be possible on a soccer pitch. When the effect of interventions are investigated in terms of match-play only a few variables are usually measured i.e. heart rate, total distance

covered, distance and number of sprints. Recently core temperature has been measured during an amateur match using the temperature pill (Edwards and Clark, 2006). However, in the laboratory it was possible to measure these variables and others such as substrate oxidation rates, mental concentration and thirst.

8.2. Conclusions

The aims of this thesis have been fully realised and the overall conclusions are that:

- Altering the timing and volume of carbohydrate ingestion does not:
  - Significantly affect metabolism during soccer-specific exercise
  - Significantly influence peak sprint power output during soccer-specific exercise

- Compared with placebo, ingesting carbohydrate significantly affects plasma metabolites and increases carbohydrate oxidation but fails to impact on performance of short sprints during soccer-specific exercise.

- When performing soccer-specific exercise in the heat ingesting a multi-carbohydrate solution compared with a single-carbohydrate solution does not significantly influence metabolism or exercise capacity

- Ingesting carbohydrate compared with a placebo during soccer-specific exercise performed in the heat significantly alters metabolism and enhances exercise capacity

- Pre-cooling in conjunction with carbohydrate ingestion during soccer-specific exercise significantly increases exercise capacity.

8.3. Recommendations for future research

After conducting the research presented in this thesis, and from the comprehensive review of the literature, the following recommendations for future research can be made:

- All of the studies presented in this thesis were conducted in the controlled environment of a laboratory. Therefore, a future development would be to investigate the effects of manipulating the provision of drinks on actual match-play performance. Similarly it would be possible to examine the influence of pre-cooling on performance during competitive match-play.

- One of the factors that limit the effectiveness of hydration strategies is the rate of gastric emptying and intestinal absorption. However, this was not measured in any of the studies conducted. Therefore, measuring the rate of gastric emptying and intestinal absorption would provide a more detailed assessment of the mechanisms involved.

- Study 3 demonstrated that the ingestion of multi-carbohydrate solutions has no effect on soccer-specific exercise performed in the heat and it was concluded that a high core temperature was a limiting factor for exercise capacity. Therefore, investigating the impact of ingesting multi-carbohydrate solutions in temperate conditions, where core temperature is less likely to be the limiting factor, would establish whether the intestinal absorption of the type of carbohydrate solution limits exogenous carbohydrate oxidation during soccer-specific exercise, as has been reported during continuous exercise.

- The combination of pre-cooling and carbohydrate ingestion in study 4 was a novel investigation. Therefore, before the strategy could be recommend as suitable regime for soccer players the study would need to be repeated to reaffirm these results. In addition, study 4 could be repeated using a non-motorised treadmill so

that the effect of reducing muscle temperature as a consequence of pre-cooling could be assessed in terms of sprint performance during soccer-specific exercise.

- Study 4 demonstrated that the combined effect of pre-cooling and carbohydrate ingestion significantly improved exercise capacity. However, it was not possible to establish the mechanisms. Future research could examine the effect of pre-cooling and carbohydrate ingestion on muscle glycogen utilization by means of using stable isotopes and measuring glycogen content to identify mechanisms for the improved exercise capacity.

- The pre-cooling regime in study 4 involved the subject wearing the cooling vest whilst resting. Wearing the cooling vest prior to and during the warm up before a match would be more representative of usual practices and may be a more suitable strategy in competitive situations. In addition, future research could also focus on alternatives to the cooling vest. External pre-cooling of the head, neck or hands and internal pre-cooling via ingestion of crushed ice or intravenous administration of cool fluids are possibilities.

- The participants in the studies presented in this thesis were unacclimatized, which may have contributed to hyperthermia limiting exercise capacity. Therefore, a future investigation could examine the impact of heat acclimatization on the performance of soccer-specific exercise and heat shock protein gene expression such as HSP70 and HSP72. Such observations would provide information about the physiological stress responses to such activities.

- The effect of performing soccer could be assessed in terms of its impact on mental performance and consequently tactical thought could be investigated. Prior to, and after the participants complete 90-min of soccer-specific exercise neuro-imaging by means of Functional Magnetic Resonance Imaging (fMRI) could be used to assess any changes in cerebral activity.

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Appendices

Appendix A

Publications

International Journal of Sport Nutrition and Exercise Metabolism, 2005, 15, 625-640

Strategies for Hydration and Energy Provision During Soccer-Specific Exercise

N.D. Clarke, B. Drust, D.P.M. MacLaren, and T. Reilly

The aim of the present study was to investigate the effect of manipulating the provision of sports drink during soccer-specific exercise on metabolism and performance. Soccer players ($N = 12$) performed a soccer-specific protocol on three occasions. On two, 7 mL/kg carbohydrate-electrolyte (CHOv) or placebo (PLA) solutions were ingested at 0 and 45 min. On a third, the same total volume of carbohydrate-electrolyte was consumed (CHOf) in smaller volumes at 0, 15, 30, 45, 60, and 75 min. Plasma glucose, glycerol, non-esterified free fatty acids (NEFA), cortisol, and CHO oxidation were not significantly different between CHOv and CHOf ($P > 0.05$). Sprint power was not significantly affected ($P > 0.05$) by the experimental trials. This study demonstrates when the total volume of carbohydrate consumed is equal, manipulating the timing and volume of ingestion elicits similar metabolic responses without affecting exercise performance.

Key Words: fluid, carbohydrate, performance, metabolism

During a soccer match, players perform a wide range of different exercises ranging from standing still to sprinting, and so the intensity of effort alters frequently. The energy cost of a competitive soccer match has been estimated to be approximately 6700 kJ (3). During a match, energy is provided predominantly by aerobic metabolism (35), with a rise in circulating free fatty acids as the match progresses (3). Crucial components of activity, however, e.g., tackling, jumping, and sprinting, rely on anaerobic energy production of which carbohydrate is an important fuel.

Apart from energy expenditure, the intensity of exercise associated with a competitive match is high enough to induce appreciable heat load, causing players to lose up to 3 L of sweat in a game (16). The elevation in core temperature is greater during intermittent exercise compared with continuous exercise at the same average intensity (17). A mild level of dehydration (40) and an elevated core temperature (19, 32) can limit exercise performance. It is, therefore, important that athletes consume fluid during prolonged exercise. The addition of carbohydrate to this fluid can further improve exercise capacity (29), possibly due to the sparing of muscle glycogen and delaying the onset of fatigue (23). Therefore, an optimal

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refueling and rehydration regime might be key in enhancing performance during a game.

Some authors have investigated the impact of carbohydrate ingestion on exercise capacity during simulated soccer (27, 29, 41) and actual match-play (21, 23, 44). The drinking strategy employed in these studies has been to ingest a large volume before the activity and again at half-time. No previous study has focused on the effect of consuming small repetitive doses on performance of soccer-specific exercise.

Gastric emptying is deemed to be a limiting factor in fluid replacement (37) and is an important aspect in determining the rate at which nutrients enter the duodenum where glucose and water can be absorbed into the bloodstream (8). Studies using a single large ingestion (10) or repetitive smaller ingestions (15) demonstrate that the maximum rate at which water and carbohydrate can be delivered from an ingested solution is influenced by the average volume of fluid in the stomach, which in turn is determined by the volume ingested and the drinking pattern. Gastric emptying is also influenced by exercise intensity. Leiper et al. (24) demonstrated that the intensity associated with a soccer match is sufficient to slow gastric emptying.

Given the acyclic nature of activity in soccer, there are no scheduled breaks where fluid can be consumed; besides, gastric tolerance and the perception of gut fullness do not allow for suitable rehydration for soccer players. Due to the continuous nature of play, with infrequent, unscheduled stoppages, the only two occasions that a player is guaranteed to be able to consume fluid are before the game and at half-time. The American College of Sports Medicine position stand on exercise and fluid replacement (9) has stated, however, that during exercise, athletes should start drinking early and at regular intervals in an attempt to consume fluids at a rate sufficient to replace the water lost through sweating, or consume the maximal amount that can be tolerated. Most advice regarding rehydration during exercise has been based on continuous exercise, e.g., cycling and road-running or sports where there are opportunities for breaks when fluid can be consumed, e.g., American football and basketball.

The aim of the present study was to investigate the effect of manipulating the provision of sports drink during soccer-specific exercise on the metabolic responses and exercise performance.

Methodology

Subjects

Twelve male university soccer players participated in this study. Mean (± standard error) age: 25 ± 3 y; height: 1.77 ± 0.1 m; body mass: 74.5 ± 6 kg; maximal oxygen uptake (VO_{2max}): 59.37 ± 6 mL · kg^{-1} · min^{-1}. Before the subjects performed any exercise they were screened for contra-indicators to participate, using the Physical Activity Readiness Questionnaire (PAR-Q) (38). Subjects were tested in a post-absorptive state, having performed no vigorous exercise, i.e., competitive match or intense training for 48 h prior to testing. Each subject performed all of the exercise sessions at the same time of day (14:00 to 18:00 h) to minimize the circadian variation of the measured variables (34). All subjects provided written informed consent to participate, in accordance with Liverpool John Moores

University's ethical procedures. The test procedures were approved by the Human Ethics Committee of Liverpool John Moores University.

Experimental Protocol

Each subject attended the laboratory on six separate occasions. During the first visit, the subject's VO_{2max} was assessed while exercising on a motorized treadmill (Woodway, Auf Schrauben, Germany) using a graded exercise test to volitional exhaustion. During this session height and body mass were recorded using standard laboratory measurement techniques.

The subjects also undertook two familiarization sessions, consisting of two blocks of the soccer-specific protocol (i.e., 30 min). The soccer-specific protocol, arranged around a 15-min activity block, incorporated 9 static periods (15.3 s), 9 walks (46.8 s), 9 jogs (33.0 s), 3 cruises (11.4 s), and 3 sprints (3.3 s) (Figure 1) (13), performed on a modified non-motorized treadmill (Woodway, Auf Schrauben, Germany). Before the first familiarization session, 500 mL of carbohydrate electrolyte solution [Still Lucozade Sport (6.35 ± 0.05 g/100 mL CHO, 48 ± 1 mg/100 mL Na) GlaxoSmithKline, Gloucestershire, UK] was consumed whereas 500 mL of a similarly colored, flavored, and textured placebo (GlaxoSmithKline, Gloucestershire, UK) was consumed before the start of the second familiarization trial. These procedures ensured that there were no adverse gut reactions to the volume and composition of the fluid consumed and reliable sprint power outputs were obtained (CV = 6.9%) within this 30-min period.

The full soccer-specific protocol was performed on three occasions, and consisted of 90 min of activity. The 90-min period was divided into two identical 45-min blocks, separated by a 15-min half-time. On two occasions either 7 mL/kg BM of carbohydrate-electrolyte (CHOv) or placebo (PLA) solution was ingested before and at half-time (mean 533 ± 11 mL; i.e., mean total 1065 ± 22 mL). On

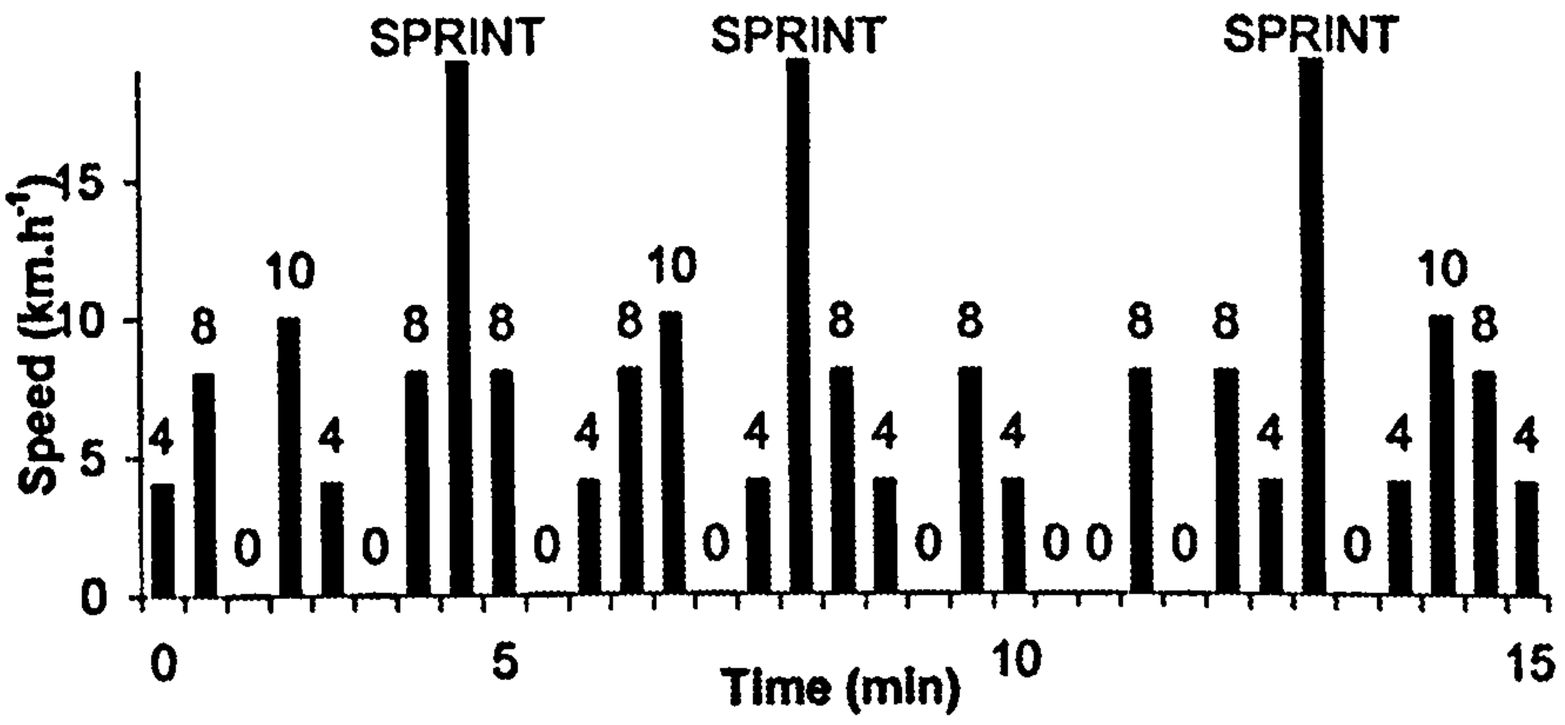

Figure 1 — Activity profile of experimental protocol; sprint = maximal effort.

a third occasion the same volume of carbohydrate-electrolyte solution was consumed (CHOf) but in smaller volumes at 0, 15, 30, 45, 60, and 75 min (mean 177 ± 4 mL) during the final walking phase of each block. During the carbohydrate trials the total amount of carbohydrate ingested was 67.71 ± 1.40 g CHO. Subjects acted as their own controls in a double-blind repeated-measures crossover design with the order randomly assigned. The soccer-specific protocol was performed in "normal" laboratory conditions (mean temperature: 18.9 ± 0.4 °C; relative humidity: 59.3 ± 4%).

For the 3 d prior to the first test session, subjects completed a diet diary, which provided a dietary template prior to subsequent trials. Subjects arrived at the laboratory approximately 3 to 4 h before they were due to commence testing, where they consumed a standard snack (65% CHO; 20% fat; 15% protein; 2500 to 3000 kJ). At this time a urine sample was collected, and color (1) and osmolality (Advanced Micro-osmometer model 3300, Advanced Instruments, Inc., Norwood, MA) were measured to ensure constant hydration status for each trial. Thirty minutes before the subject was due to commence exercising, a venous blood sample was drawn from an antecubital vein in the forearm using the Vacutainer collection system (Becton Dickinson Vacutainer Systems Europe, Meylan, France). A standard 15-min warm-up was performed, consisting of jogging, sprinting, and stretching before the subject began the 90 min of exercise.

Physiological Measurements

During the soccer-specific intermittent protocol, heart rate was measured continuously using short-range radio telemetry (Polar Coach, Polar Electro, Kempele, Finland). Between 11 to 13 min, 26 to 28 min, 41 to 43 min, 56 to 58 min, 71 to 73 min, and 86 to 88 min respiratory gases (VO_2 and VCO_2) were monitored using an on-line automated gas analyzer (Metalyzer3B, Cortex Biophysic GmbH, Leipzig, Germany). These data were used to calculate substrate oxidation rates (18).

Rating of perceived exertion (RPE) (7), gut fullness, and thirst (43) were recorded during the double static period of each 15 min block. Gut fullness and thirst were also recorded immediately before and after fluid ingestion prior to commencing exercise. During each sprint phase, the peak power output was recorded to monitor performance and indicate the occurrence of fatigue. Peak power output was defined as the maximum value obtained during each sprint and was calculated using the horizontal component of applied force and the treadmill belt speed (22).

Blood Sampling and Analysis

Venous blood samples (14 mL) were drawn prior to exercise, at half-time (immediately after the completion of the 45 min), and at completion of each trial. Samples were collected in plastic tubes containing EDTA [for analysis of non-esterified free fatty acids (NEFA), glycerol, catecholamines, and cortisol] and lithium heparin (for analysis of glucose, lactate, and plasma osmolality), centrifuged and frozen at –80 °C for later analysis. Plasma samples were analyzed for glucose, lactate (GEM Premier 3000, Instrumentation Laboratory Co., Warrington, UK), NEFA (NEFA-C, Wacko Chemicals GmbH, Neuss, Germany), glycerol (Randox Laboratories Ltd., Co. Antrim, UK), catecholamines (Catcombi ELISA, IBL GmbH, Hamburg, Germany) and cortisol (Cortisol ELISA, IBL GmbH, Hamburg, Germany). The

change in body mass was calculated from the difference in nude body mass between pre- and post-exercise and values were corrected for the volume of fluid ingested and urine loss to calculate sweat loss.

Statistical Analysis

All variables were analyzed using two-way ANOVAs with repeated measures except for body mass loss, which was analyzed using a one-way ANOVA with repeated measures. Where sphericity was found to be violated the Greenhouse-Geisser adjustment was used to determine statistical significance. Where differences were noted, pairwise comparisons (Bonferroni adjusted) were used to identify exactly where they lay. All statistical analyses were performed using SPSS for Windows version 11 (SPSS, Inc., Chicago, IL) and a level of $P < 0.05$ was considered statistically significant. All results are reported as the mean ± the standard error of the mean.

Results

Pre-Trial Conditions

The pre-trial conditions were similar for all trials (Table 1). There were no significant differences in the carbohydrate [$F(1.036, 11.400) = 1.920$; $P > 0.05$] or energy [$F(1.108, 12.191) = 0.122$; $P > 0.05$] content of the participant's pre-trial diet. Pre-trial hydration status was similar for all conditions, urine color [$F(2, 22) = 1.055$; $P > 0.05$] and osmolality [$F(2, 22) = 1.311$; $P > 0.05$] were not significantly different.

Plasma Metabolites

Plasma glucose concentration (Figure 2) was not significantly different between CHOv and CHOf pre-exercise, at half-time, or post-exercise. Plasma glucose, however, was significantly higher during CHOf compared with PLA [$F(2, 22) = 4.909$; $P < 0.05$) at half-time and post-exercise. For all trials plasma glucose was significantly lower post-exercise than at half-time [$F(2, 22) = 21.197$; $P < 0.05$). During all of the trials no subjects were found to be hypoglycemic.

Table 1 Pre-Trial Dietary and Hydration Status

	Diet		Urine	
Trial	CHO (%)	Energy (MJ/d)	Color	Osmolality (mOsm/kg)
PLA	54.58 ± 1.62	7.09 ± 0.39	2.42 ± 0.26	354.75 ± 53.42
CHOv	53.49 ± 1.67	7.08 ± 0.46	2.08 ± 0.26	299.58 ± 47.19
CHOf	53.71 ± 1.63	7.14 ± 0.45	2.00 ± 0.33	293.42 ± 58.83

The concentration of NEFA was not significantly different between CHOv and CHOf (Figure 3), although it was significantly higher during PLA at half-time and post-exercise [$F(2, 22) = 22.802$; $P > 0.05$]. Plasma NEFA concentration increased significantly between each time point [$F(1.19, 13.04) = 35.809$; $P < 0.05$]. Glycerol concentration was significantly higher post-exercise following PLA compared to CHOv and CHOf (Figure 4), and increased significantly between each time point [$F(1.14,12.50) = 61.592$; $P < 0.05$].

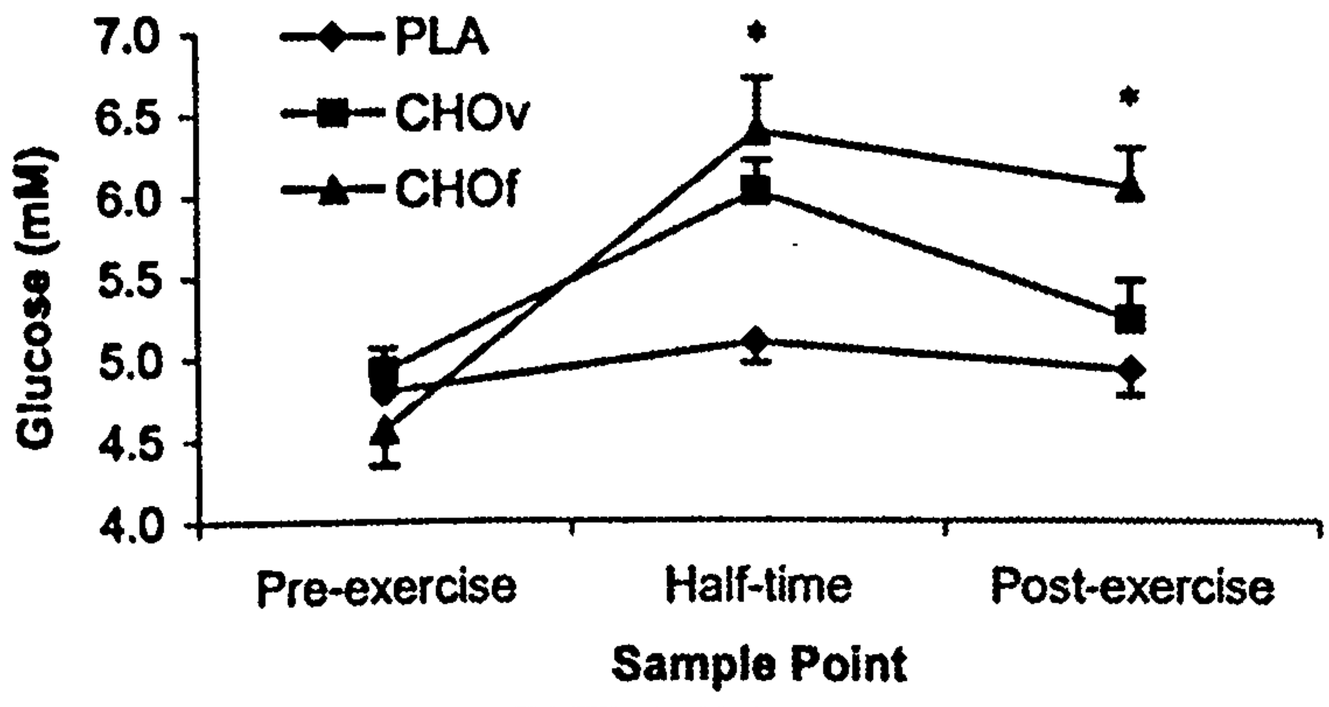

Figure 2 — Plasma glucose concentration during the soccer-specific protocol; *CHOf significantly greater than PLA.

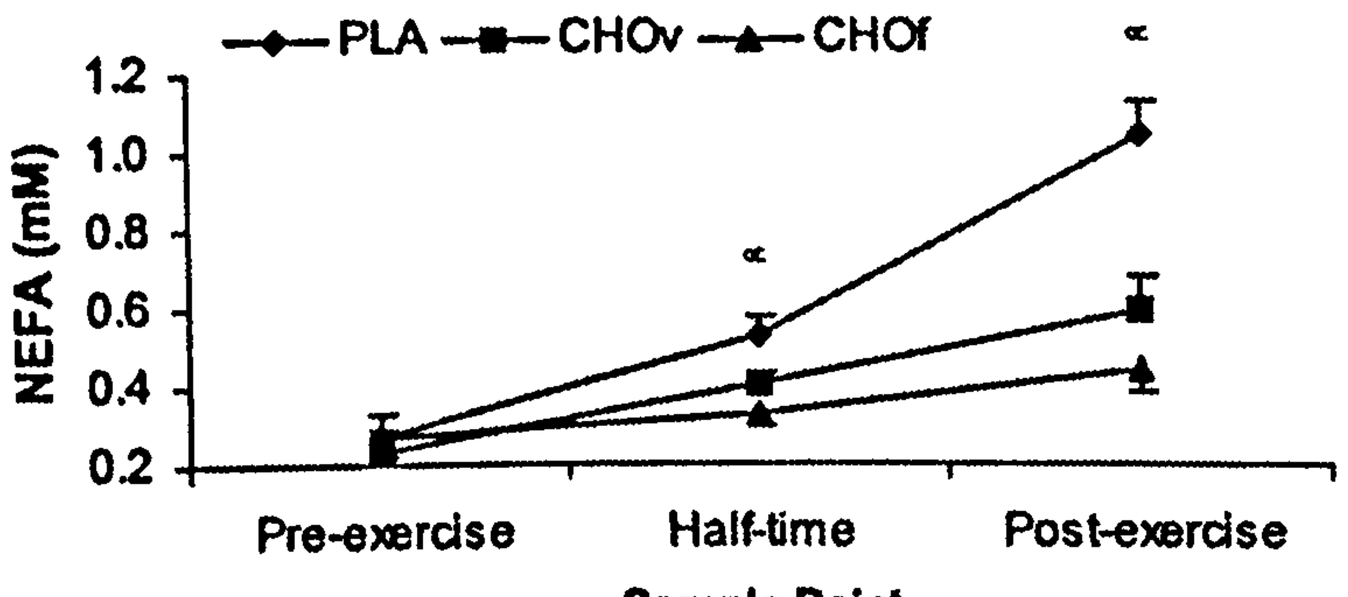

Figure 3 — Plasma NEFA concentration during the soccer-specific protocol; ∝ PLA significantly greater than CHOf and CHOv.

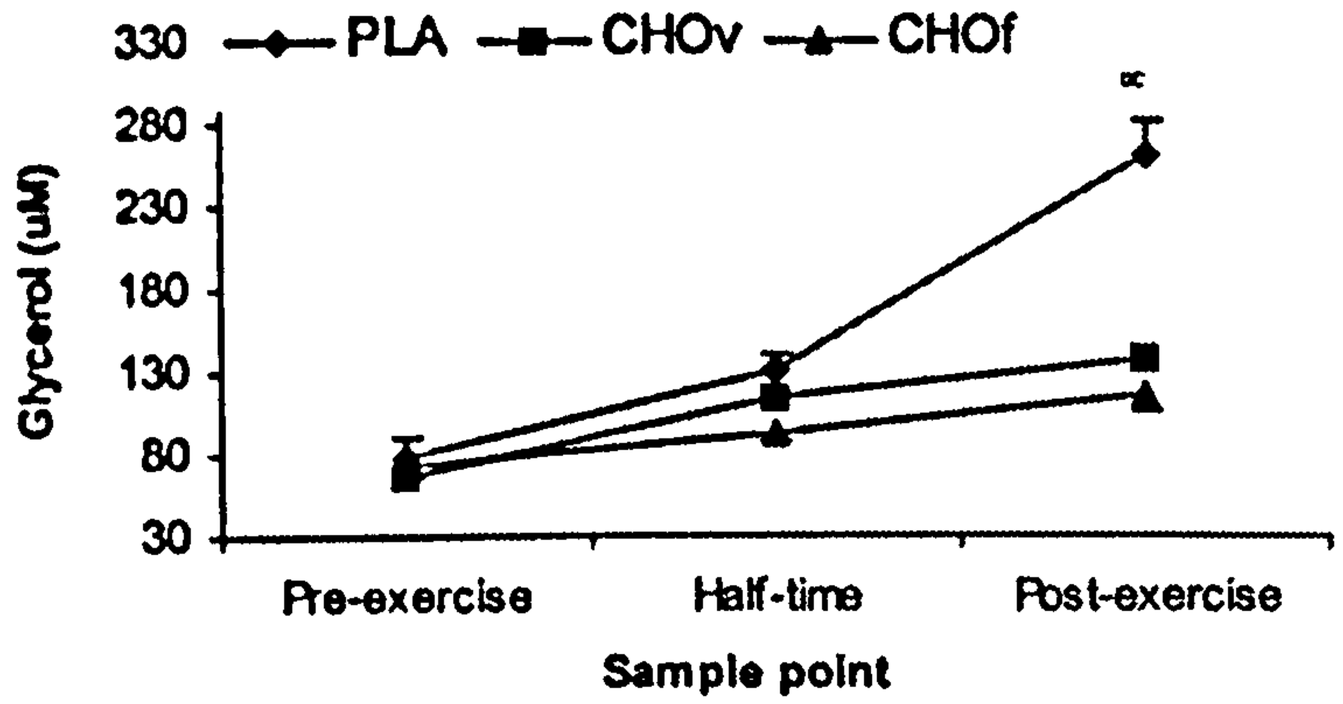

Figure 4 — Plasma glycerol concentration during soccer-specific protocol; ∝ PLA significantly greater than CHOf and CHOv.

Lactate concentration was not significantly different between any of the trials [$F(2, 22) = 0.583$; $P > 0.05$], although it increased significantly above resting levels after the onset of exercise, with peak values at half-time (PLA: 2.83 ± 0.6 mM; CHOv: 3.30 ± 0.4 mM; CHOf: 2.95 ± 0.6 mM) [$F(1.10,12.14) = 10.592$; $P < 0.05$]. Mean plasma osmolality during the soccer-specific protocol was not significantly affected by the trials (PLA: 279.67 ± 3 mOsm/kg; CHOv: 278.31 ± 3 mOsm/kg; CHOf: 279.58 ± 3 mOsm/kg) [$F(2, 22) = 0.071$; $P > 0.05$]. There were no significant differences between trials in plasma volume changes (PLA: −1.32 ± 0.3%; CHOv: −1.71 ± 0.2%; CHOf: −1.24 ± 0.3%) [$F(2, 22) = 1.447$; $P > 0.05$] or change in body mass (PLA: 1.80 ± 0.2 kg; CHOv: 1.62 ± 0.1 kg; CHOf: 1.58 ± 0.1 kg) [$F(1,11) = 0.605$; $P > 0.05$].

Hormones

Adrenaline levels were found to be similar during all trials [$F(2, 22) = 0.609$; $P > 0.05$] with significant increases between each time point (half-time: PLA: 1.47 ± 0.1 nM; CHOv: 1.48 ± 0.1 nM; CHOf: 1.42 ± 0.1 nm; post-exercise: PLA: 1.85 ± 0.1 nM; CHOv: 1.92 ± 0.2 nM; CHOf: 1.85 ± 0.2 nm) [$F(1.14, 12.55) = 127.216$; $P < 0.05$]. A similar pattern was observed for noradrenaline levels (half-time: PLA: 12.41 ± 0.9 nM; CHOv: 11.61 ± 1.0 nM; CHOf: 12.03 ± 0.8 nM; post-exercise: PLA: 15.98 ± 1.1 nM; CHOv: 16.12 ± 1.1 nM; CHOf: 16.22 ± 0.9 nM) [$F(2, 22) = 185.51$; $P < 0.05$], with no significant effect of the treatments [$F(2, 22) = 0.091$; $P > 0.05$]. Cortisol was significantly higher post-exercise for CHOf (257.9 ± 27.4 nM) compared with PLA (186.4 ± 24.4 nM) [$F(2, 22) = 4.053$; $P < 0.05$]; Figure 5), although no significant difference was identified between CHOv and CHOf. A significant time effect [$F(2, 22) = 4.937$; $P < 0.05$] was observed, values were significantly lower at full time, compared with pre-exercise and half-time.

Substrate Oxidation Rates

Carbohydrate oxidation (Figure 6) was not significantly different between CHOf and CHOv ($P > 0.05$), although it was significantly [$F(2, 22) = 3.759$; $P < 0.05$] greater during CHOf and CHOv compared to PLA. In contrast, fat oxidation was not significantly [$F(2, 22) = 2.428$; $P > 0.05$] different between trials (PLA: 0.51 ± 0.03 g/min; CHOv: 0.50 ± 0.04 g/min; CHOf: 0.42 ± 0.04 g/min).

Perceived Thirst and Gut Fullness

There were no significant differences [$F(2, 22) = 0.573$; $P > 0.05$] in rating of thirst between the three trials (Table 2). There was a significant [$F(2.92, 32.14) = 25.425$; $P < 0.05$] effect of time on rating of thirst. Thirst decreased significantly

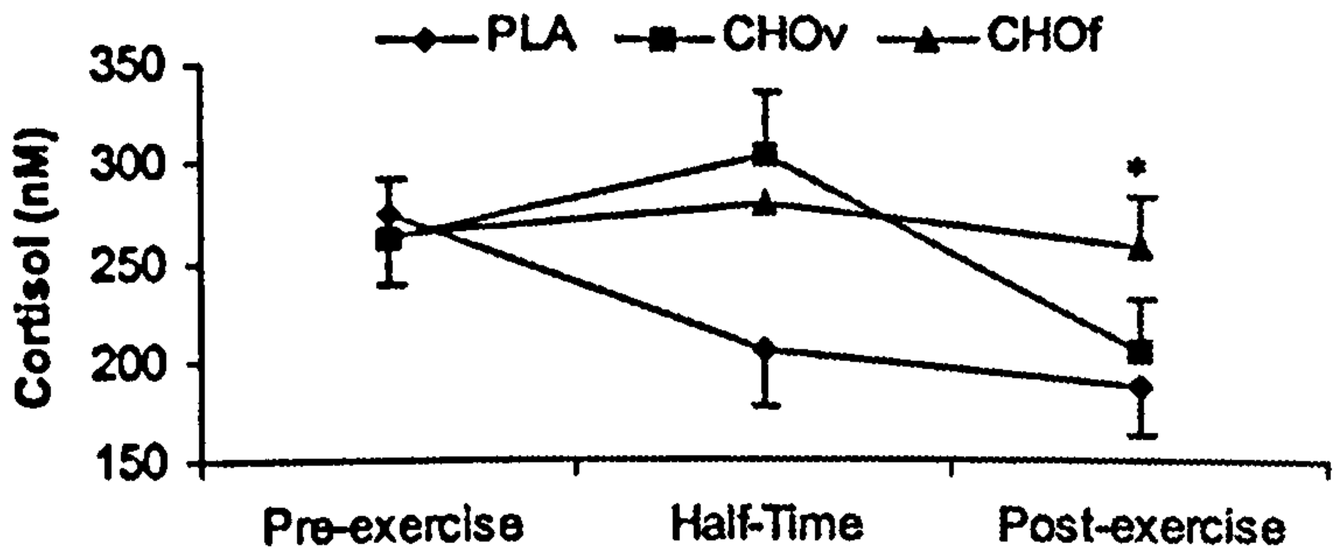

Figure 5 — Plasma cortisol concentration during soccer-specific protocol; * CHOf significantly greater than PLA.

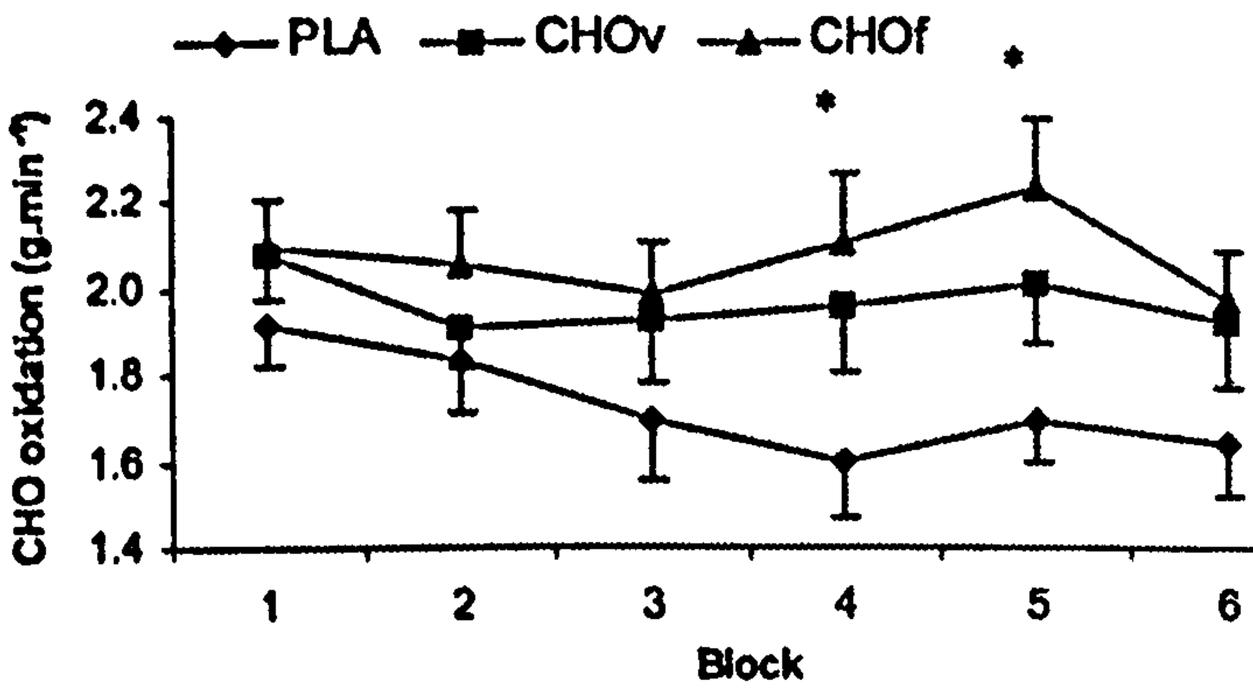

Figure 6 — Carbohydrate oxidation during the soccer-specific protocol; * CHOf significantly greater than PLA.

Table 2 Perceived Thirst Throughout the Soccer-Specific Protocol

Trial	Pre-fluid	Post-fluid	Block 1	Block 2	Block 3	Half-time	Block 4	Block 5	Block 6
PLA	12.67 ± 0.4‡†	8.00 ± 0.3	10.33 ± 0.3‡†	11.00 ± 0.4‡†	11.58 ± 0.5‡†	8.25 ± 0.6	9.25 ± 0.5	9.67 ± 0.3‡†	11.75 ± 0.6‡†
CHOv	11.42 ± 0.6‡†	7.83 ± 0.2	9.58 ± 0.5‡†	10.83 ± 0.4‡†	11.08 ± 0.6‡†	8.00 ± 0.4	9.42 ± 0.4	10.50 ± 0.4‡†	11.25 ± 0.5‡†
CHOf	12.17 ± 0.9‡†	9.42 ± 0.8	11.08 ± 0.5‡†	10.50 ± 0.6‡†	10.75 ± 0.5‡†	8.92 ± 0.4	10.00 ± 0.4	10.33 ± 0.5‡†	10.25 ± 0.5‡†

Note. ‡ significantly higher than post-fluid; † significantly higher than half-time.

($P < 0.05$) following the consumption of fluid pre-exercise but was significantly ($P < 0.05$) higher during the first half of the soccer-specific protocol compared with immediately post-fluid ingestion. Subjective rating of thirst also increased steadily throughout the second half of the soccer-specific protocol and during block 6 was significantly ($P < 0.05$) higher than at half-time.

There was a significant [$F(2.16, 28.79) = 16.445$; $P < 0.05$] effect of time on rating of gut fullness (Table 3), which was significantly ($P < 0.05$) lower immediately prior to fluid consumption pre-exercise compared with any stage during the first half or at half-time. Gut fullness increased significantly ($P < 0.05$) following the consumption of fluid at the start of the first half and was significantly ($P < 0.05$) higher at half-time following fluid ingestion, compared with any stage of the second half. Subjective rating of gut fullness decreased steadily throughout each half of the soccer-specific protocol. There were no significant differences [$F(2, 22) = 1.061$; $P > 0.05$] in rating of gut fullness between the three trials.

Power Output

There was no significant effect of the treatments on peak power output during sprinting [$F(2, 22) = 0.133$; $P > 0.05$] (PLA: 1080 ± 70 W; CHOv: 1104 ± 66 W; CHOf: 1091 ± 39 W) (Figure 7). Peak power output remained constant throughout each trial [$F(5, 55) = 1.379$; $P > 0.05$].

Discussion

The main findings of the present study were 1) the timing and volume of carbohydrate ingestion did not significantly affect metabolism or sprint power output during CHOv or CHOf, and 2) although consuming a carbohydrate-electrolyte solution when compared to placebo significantly increased plasma glucose concentration and carbohydrate oxidation, and suppressed NEFA and glycerol, there was no impact on sprint power output during sprinting.

One of the key factors in sustaining prolonged exercise at intensities ranging from 65 to 85% VO_{2max} is the concentration of muscle glycogen (4). Fatigue during exercise of this nature is associated with the depletion of the muscle's limited glycogen stores (27) and a reduction in plasma glucose (10). In the present study, plasma glucose concentration during CHOv and CHOf was not significantly affected by manipulating the timing and volume of ingesting a carbohydrate-electrolyte solution. This might have been as a consequence of the same total volume being ingested, and the same amount of glucose being made available. This study also reaffirmed the findings that consuming carbohydrate during exercise increases plasma glucose concentration (12, 29).

Plasma NEFA and glycerol concentration increased during the soccer-specific protocol, with a greater increase occurring during the second half, supporting the findings of Bangsbo (3). The largest increase occurred during PLA, suggesting that consuming a carbohydrate solution during exercise suppresses the release of NEFA and glycerol during CHOv and CHOf, possibly as an effect of an elevated insulin concentration, which has been shown to occur following carbohydrate ingestion during a simulation of the exercise intensity of soccer (29).

Table 3 Perceived Gut Fullness Throughout the Soccer-Specific Protocol

Trial	Pre-fluid	Post-fluid	Block 1	Block 2	Block 3	Half-time	Block 4	Block 5	Block 6
PLA	7.83 ± 0.2	11.58 ± 0.7‡	9.92 ± 0.5‡†	9.75 ± 0.4‡†	9.50 ± 0.6‡†	11.67 ± 0.8‡	10.25 ± 0.8†	9.67 ± 0.5†	8.92 ± 0.5†
CHOv	8.00 ± 0.3	12.33 ± 1.0‡	10.58 ± 0.7‡†	10.25 ± 0.5‡†	9.83 ± 0.5‡†	12.92 ± 0.9‡	10.67 ± 0.7†	10.67 ± 0.7†	10.08 ± 0.8†
CHOf	8.08 ± 0.4	10.42 ± 1.0‡	9.50 ± 0.8‡†	9.50 ± 0.5‡†	9.25 ± 0.4‡†	11.00 ± 1.0‡	10.25 ± 0.9†	9.67 ± 0.7†	9.75 ± 0.7†

Note. ‡ significantly higher than pre-fluid, † significantly lower than half-time.

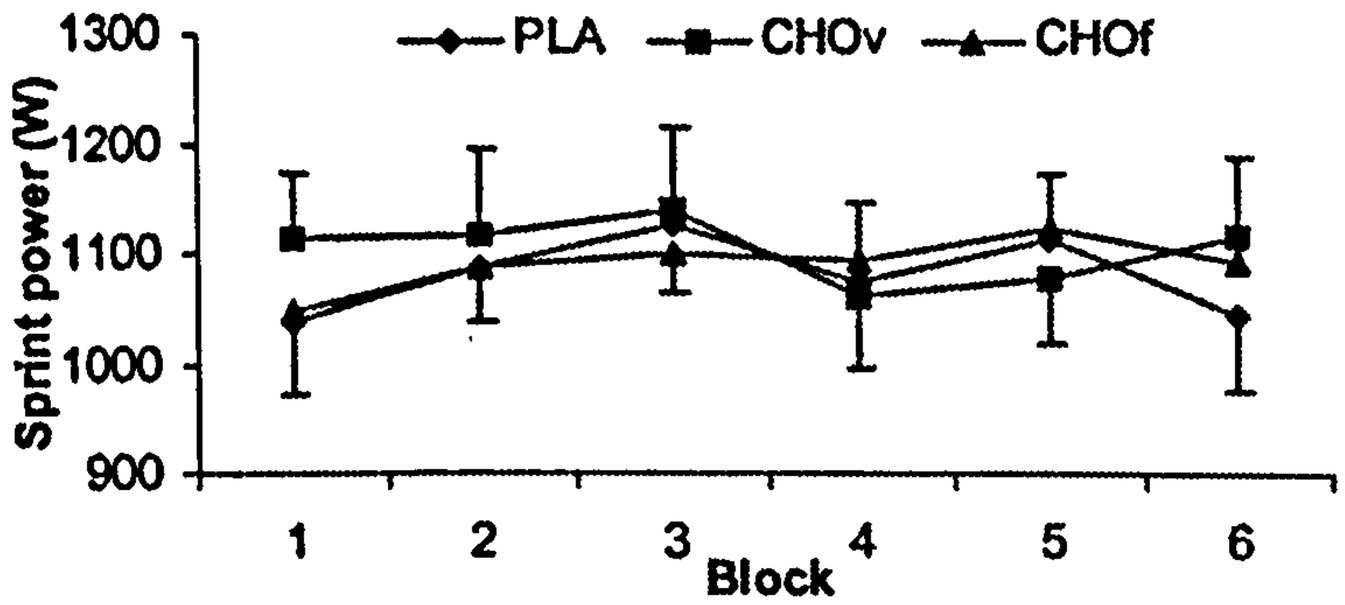

Figure 7 — Peak sprint power output during the soccer-specific protocol.

A similar pattern of carbohydrate oxidation was observed when carbohydrate was consumed during exercise, irrespective of timing and volume, possibly due to the total amount of carbohydrate ingested being the same during both trials. Carbohydrate oxidation was significantly higher, however, during CHOf and CHOv compared with PLA. Previous studies have demonstrated that consuming carbohydrate during exercise maintains high rates of plasma glucose oxidation late in exercise (11). The higher carbohydrate oxidation after ingesting carbohydrate has been attributed to either increased muscle glycogenolysis or elevated glucose uptake and oxidation (42).

High-intensity exercise has been associated with elevated plasma cortisol concentration to maintain plasma glucose concentration, and this rise can be attenuated by the consumption of carbohydrate drinks (20). In the present study, plasma glucose concentration did not fall significantly below resting levels, which could explain why plasma cortisol concentration was maintained during the carbohydrate trials. The failure of cortisol to increase during PLA might have been due to the overall exercise intensity not being high enough (67 ± 1% VO_{2max}) to elicit a cortisol response, although this intensity is close to values observed during a match (70% VO_{2max}) (38). Nieman et al. (30) found that high-intensity exercise (80% VO_{2max}) produced a significantly greater cortisol response compared to moderate-intensity exercise (50% VO_{2max}). It has been demonstrated (5, 26) that when carbohydrate ingested during exercise fails to affect insulin concentration, the adrenaline response does not seem to be affected, and so might explain the lack of a difference between the carbohydrate trials and PLA in the present study. Bishop et al. (6) concluded that during soccer-specific exercise the change in stress hormones was minimal and carbohydrate supplementation had a negligible effect.

Plasma osmolality increased during all trials of the soccer-specific protocol, but remained within normal values, indicating that the subjects did not suffer from severe dehydration. There were no significant differences between the trials, suggesting that the overall rate of gastric emptying was the same and it is the total volume of fluid ingested, and not the timing of ingestion that is important in preventing dehydration during soccer-specific exercise. A possible explanation

for this occurrence is that the time-course for the volume of ingested fluid to be distributed throughout the body after gastric emptying, intestinal absorption, and osmotic flow is 40 to 60 min (31, 36) and within this time scale a similar volume of fluid would have emptied from the stomach. Total sweat loss was comparable with previous studies (6, 28, 29), which have simulated the exercise intensity of soccer. The value was relatively low, indicating that the volume of fluid intake was appropriate for the environmental conditions and fitness of the subjects. The volume consumed, however, might need to be adjusted to match environmental conditions, i.e., a larger volume in warmer environments.

Despite the different timings and volume of fluid consumed, there were no significant differences in either thirst or gut fullness between trials. Irrespective of how the total volume was consumed, thirst decreased significantly following the consumption of fluid at rest, prior to the soccer-specific intermittent protocol, and at half-time. In contrast, gut fullness increased significantly at these time points. Gastric emptying is affected by the volume of fluid ingested, the larger the volume the faster the rate, i.e., drinking a large volume prior to a match (CHOv). As the volume in the stomach declines the rate of gastric emptying decreases proportionally. If, however, the volume is maintained by repeated ingestion of smaller volumes of fluid, i.e., every 15 min (CHOf), the rate of gastric emptying remains relatively constant (33). This could explain the small differences, although not significant, in plasma metabolites and carbohydrate oxidation between CHOf and CHOv. During the CHOf trial carbohydrate might have passed from the stomach into the small intestine at a constant rate, enabling glucose absorption to occur at a constant rate and plasma glucose and carbohydrate oxidation to be maintained at higher levels. When fluid was consumed the subjective rating of gut fullness increased significantly, especially during PLA and CHOv. This finding agrees with Mitchell and Voss (25), who demonstrated that ingesting large volumes caused an increased frequency of complaints of gastric fullness.

The increases in plasma glucose and carbohydrate oxidation following carbohydrate ingestion were not reflected in performance as peak sprint power output was relatively constant throughout all of the trials and not significantly different between trials. This finding is similar to that of Nicholas et al. (29), who demonstrated that the ingestion of a carbohydrate-electrolyte solution significantly improved a run to exhaustion following a period of intermittent exercise, but had no impact on the performance of high-intensity exercise, 15-m sprints. It has also been demonstrated that ingesting carbohydrate during a soccer match has no impact on the ability to perform high-intensity skills such as tackling and heading (44).

The mean sprint duration in a competitive match is 3.7 s (14). In the present study the duration of each sprint was 3.3 s. At high-intensity exercise of these durations, phosphocreatine (PCr) is the major energy source, and a reduction in carbohydrate availability might increase PCr degradation during exercise (39). Balsom et al. (2) demonstrated, however, that 15-m sprints could be performed at 30-s intervals without impaired performance without carbohydrate supplementation. In the present study, 18 sprints were performed separated by approximately 200 s of lower-intensity exercise, suggesting there was sufficient time for PCr resynthesis between sprints and that the PCr system was able to meet the energy demands during the sprints. Also during PLA no signs of hypoglycemia were displayed, which is associated with fatigue and reduced performance (11), suggesting

carbohydrate availability was not a factor limiting peak sprint power output in the present study.

The general recommendation for fluid ingestion during exercise is that fluid should be consumed early, and at regular intervals in an attempt to replace the water lost through sweating, or to consume the maximal amount that can be tolerated (9). This study indicates that if sufficient carbohydrate-electrolyte solution is ingested before and at half-time, sprint performance and metabolism are not significantly affected when compared to consuming the same total volume ingested at the recommended 15-min intervals. The absence of scheduled breaks in soccer prevents players taking regular feedings of carbohydrate other than at half-time. These findings indicate that consuming a carbohydrate-electrolyte solution before a match and at half-time is a practical strategy for fluid provision during soccer at normal, ambient temperatures. When employing this strategy, extra fluid could be consumed during a match when the opportunities arise as a consequence of the natural breaks that typically occur, e.g., injuries, as the rules require that players must go to the perimeter lines to avail themselves of any drinks provided by support staff during the game.

A limitation of the current study is that the findings might not transfer directly to professional soccer where the players operate at a higher tempo than is possible in laboratory conditions and are likely to have higher aerobic and anaerobic power capacities than the current subjects. The VO_{2max} of the subjects, however, was close to that displayed among professional players and the intermittent exercise protocol simulates the pattern of activity in matches.

In conclusion, providing that the total volume of fluid consumed is equal, manipulating the timing and volume of carbohydrate ingestion does not influence exercise performance and elicits the same metabolic responses. Furthermore, ingesting a carbohydrate-electrolyte solution compared with a flavored placebo during a soccer-specific protocol significantly alters metabolism, although it has no impact on peak sprint power output.

Acknowledgments

This study was supported by GlaxoSmithKline.

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Isaac Sorinola is sponsored by the Association of Commonwealth Universities PhD studentship

All procedures accord with current local guidelines and the Declaration of Helsinki

PC98

Hydration and energy provision during soccer-specific exercise

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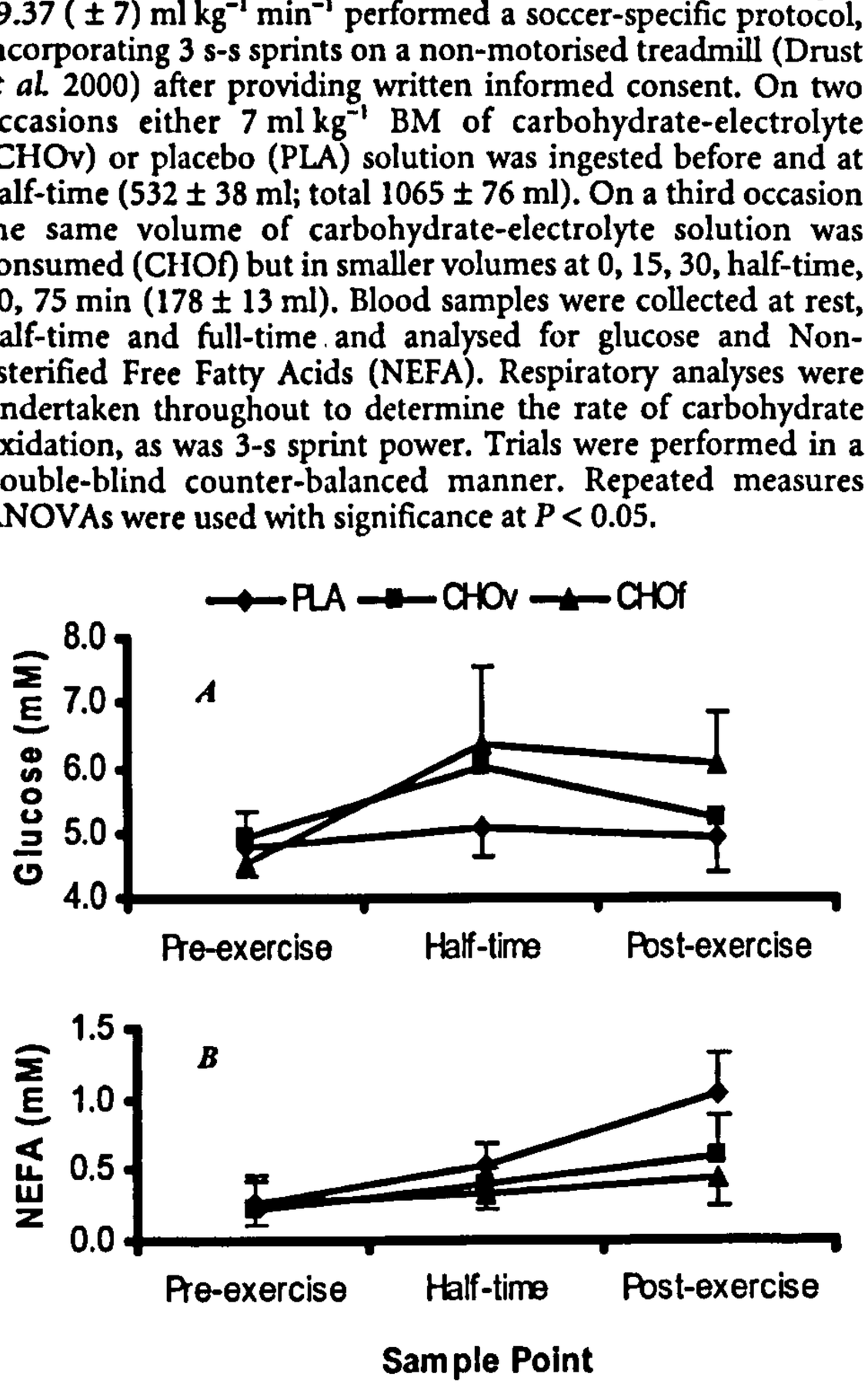
During soccer play there is a net depletion of muscle glycogen and players may lose 2–3 L of sweat. Therefore there are opportunities for enhancing performance during a game by adopting refuelling and rehydration regimes. The present aim was to manipulate the provision of sports drinks during soccer-specific exercise and to investigate the effect on metabolic responses and on components of performance.

Twelve male soccer players of mean (± S.D.) age 24.5 (± 3) y; height 1.77 (± 0.1) m; body mass 74.5 (± 7) kg; $\dot{V}_{O_2max}$ 59.37 (± 7) ml kg^{-1} min^{-1} performed a soccer-specific protocol, incorporating 3 s-s sprints on a non-motorised treadmill (Drust *et al.* 2000) after providing written informed consent. On two occasions either 7 ml kg^{-1} BM of carbohydrate-electrolyte (CHOv) or placebo (PLA) solution was ingested before and at half-time (532 ± 38 ml; total 1065 ± 76 ml). On a third occasion the same volume of carbohydrate-electrolyte solution was consumed (CHOf) but in smaller volumes at 0, 15, 30, half-time, 60, 75 min (178 ± 13 ml). Blood samples were collected at rest, half-time and full-time and analysed for glucose and Non-esterified Free Fatty Acids (NEFA). Respiratory analyses were undertaken throughout to determine the rate of carbohydrate oxidation, as was 3-s sprint power. Trials were performed in a double-blind counter-balanced manner. Repeated measures ANOVAs were used with significance at $P < 0.05$.



Plasma glucose (Fig. 1A) and carbohydrate oxidation (Table 1) were higher ($P < 0.05$) during CHOf compared with PLA. The concentration of NEFA (Fig. 1B) was reduced ($P < 0.05$) with CHOv and CHOf compared with PLA.

Table 1: Carbohydrate oxidation (g·min^{-1}).

	15 min	30 min	45 min	60 min	75 min	90 min
PLA	1.91±0.3	1.82±0.4	1.69±0.5	1.59±0.4	1.69±0.3	1.64±0.4
CHOv	2.08±0.4	1.91±0.3	1.92±0.5	1.96±0.5	2.01±0.5	1.92±0.6
CHOf	2.09±0.4	2.05±0.4	1.98±0.4	2.10±0.6	2.23±0.6	1.97±0.4

Mean sprint power was not affected ($P > 0.05$) by the experimental treatments (PLA: 1080.42 ± 241 W; CHOv: 1103.67 ± 228 W; CHOf: 1090.59 ± 136 W).Ingesting carbohydrate-electrolyte solution significantly affected plasma metabolites and increased carbohydrate oxidation but failed to impact on performance of short sprints during soccer-specific exercise. Furthermore, the timing and volume of ingestion did not significantly affect metabolism or sprint power.

Drust B *et al.* (2000). *Eur J Appl Physiol* 81, 11–17.

This study was sponsored by GSK.

All procedures accord with current local guidelines and the Declaration of Helsinki

PC99

Effect of wearing protective clothing and self contained breathing apparatus on heart rate, temperature and oxygen consumption.

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Fire fighters must possess the ability to respond to both extrinsic stress and stress from wearing protective clothing (PC) and self-contained breathing apparatus (SCBA) (White *et al*, 1991, Richardson & Capra, 2001). The effects of wearing PC+SCBA (20.42 ± 1.5 kg mean ± S.D.) on heart rate (HR), temperature responses and oxygen cost in six subjects (age 20.3 ± 0.8 years, weight 77.7 ± 7.0 kg and height 180.3 ± 4.3 cm) were observed. Ethical approval was obtained from the University College Chester Ethics Committee and the Health and Safety Officer from Greater Manchester Fire Service.

Table 1 –Mean HR (bpm) responses to the CST whilst dressed in GK, WGK and PC+SCBA

CST Level	Gym Kit	Gym Kit and Weighted Rucksack	PC+SCBA
	HR (bpm)	HR (bpm)	HR (bpm)
1 (Low)	106 (±4 1)	123 (±7 6)	126 (±7 8)
5 (High)	163 (±3 7)	185 (±6 6)	188 (±5 6)

There were significant increases in HR when carrying out the Chester Step Test (CST) (Sykes, 1995) wearing gym kit (GK), gym kit and weighted rucksack (WGK) (weighted to PC+SCBA equivalent) and PC+SCBA (thermoneutral conditions) (Table 1). Data was analysed using a one way ANOVA with post hoc Tukey analysis. Significant increases ($P < 0.05$) at CST level 5 were observed between GK and WGK for HR (Δ 23.3 ± 5.8bpm) and GK and SCBA+PC for HR (Δ 25.2 ± 5.2bpm) and for O_2 cost (Δ 6.1 ± 3.8 ml O_2 kg^{-1} min^{-1}). Thus, cardiovascular responses are elicited both from the workload and weight of the PC+SCBA (Table 1). Skin temperature significantly increased ($P < 0.05$) between GK and PC+SCBA (Δ 3.1 ± 1.3°C) and also WGK and PC+SCBA (Δ 3.5 ± 1.7°C). This may suggest therefore that the

ficant difference in gross efficiency (P<0.01) between SFs of 24 and 32 (P<0.01), and SFs of 28 and 32 (P<0.05) were identified. Significant differences in work efficiency between SFs of 24 and 32 (P<0.01), and between 24 and 28 (P<0.001) were also identified (Table 1). Significant differences (P<0.05) in heart rate between SFs of 24 and 32 were observed. There were no significant differences (P>0.05) in plasma lactate levels for power output or SF. Oxygen consumption, gross and work efficiency are affected by SF. To ensure that rowing ergometer tests are reliable, SF must remain consistent for all physiological experiments.

Table 1. Mean (±SEM) VO_2, gross and mechanical efficiency during 10 min simulated rowing at SFs of 24, 28 and 32, with and without resistance (*significant difference between 24spm and 32spm, ** 28spm and 32spm, <#\160>24spm and 28spm)

	Power	24spm	28spm	32spm
VO_2 (l min^{-1})	NR	1.37* (0.06)	1.60** (0.05)	1.80 (0.05)
	Resistance	4.13* (0.06)	4.16** (0.06)	4.29 (0.07)
Gross Efficiency (%)	Resistance	15.8* (0.26)	15.7** (0.21)	15.2 (0.20)
Work Efficiency (%)	Resistance	22.1* (0.45)	25.1 † (0.48)	24.3 (0.71)

Gaesser, G and Brooks, G. (1975). J Appl Physiol, 38:1132-1139.

Where applicable, the experiments described here conform with Physiological Society ethical requirements.

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Fluid Provision and Metabolic Responses to Soccer-Specific Exercise

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During a competitive soccer match there is a net reduction in muscle glycogen, and the exercise intensity is high enough to induce appreciable heat load, causing players to lose up to 3 litres of sweat. The aim of this study was to manipulate the administration of sports drinks during soccer-specific exercise and to investigate the effect on metabolic responses.
After providing written informed consent and undergoing familiarisation, 12 male soccer players of mean (±S.D.) age: 24 ±4 years; height: 1.80 ±0.1 m; body mass: 76.5 ± 9) kg; VO_{2max}: 61.08 ± 4 $ml.kg^{-1}.min^{-1}$ performed a soccer-specific protocol, incorporating static periods, walking, jogging, cruising and sprinting on a motorised treadmill on three occasions. On two occasions either 7 $ml.kg^{-1}$ BM of carbohydrate-electrolyte (CHOv) or placebo (PLA) solution was ingested 0 and 45 min (538 ± 66 ml; total 1075 ± 132 ml). On a third occasion the same volume of carbohydrate-electrolyte solution was consumed (CHOf) but in smaller volumes at 0, 15, 30, 45, 60, 75 min (179 ± 22 ml). Blood samples were collected at 0, 45 and 90 min and analysed for glucose, glycerol and insulin. Respiratory analyses were undertaken throughout to determine the rate of carbohydrate oxidation (Frayn, 1983). Trials were performed in a double-blind counter-balanced manner. Repeated measures ANOVAs were used to identify differences and significance was accepted at P<0.05. Glucose and insulin concentration (Table 1), and carbohydrate oxidation (PLA: 3.02 ±0.7 $g.min^{-1}$; CHOv: 3.60 ± 0.8 $g.min^{-1}$; CHOf: 3.50 ± 0.6 $g.min^{-1}$) were higher (P<0.05) during CHOv and CHOf compared with PLA and there were no differences between CHOv and CHOf. Glycerol (Table 1) was higher (P<0.05) during PLA compared with CHOv and CHOf.
Ingesting carbohydrate-electrolyte solution significantly affected plasma metabolites and increased carbohydrate oxidation. The timing and volume of ingestion did not significantly affect metabolism.

Table 1: Glucose, glycerol and insulin concentration (means ± S.D.)

	0 min	45 min	90 min
Plasma glucose (mM)			
PLA	5.08 ± 1	5.23 ± 1	4 94 ± 1
CHOv	5.14 ± 1	5.76 ± 1	5 64 ± 1
CHOf	5.16 ± 1	6.52 ± 1	6 16 ± 1
Plasma glycerol (uM)			
PLA	67.5 ± 27	112.4 ± 44	181.4 ± 75
CHOv	63.7 ± 32	82.1 ± 30	114.3 ± 32
CHOf	65.2 ± 30	83.8 ± 32	131.5 ± 50
Serum insulin (mIU/l)			
PLA	32.1 ± 19	25.7 ± 15	21.4 ± 18
CHOv	33.4 ± 19	31.7 ± 21	28 6 ± 33
CHOf	32.0 ± 19	37.3 ± 18	31.9 ± 19

Frayn, K.N. (1983). J. Appl. Physiol. 55, 628-634.

This study was supported by GlaxoSmithKline

Where applicable, the experiments described here conform with Physiological Society ethical requirements.

PC27

Human knee-extensors architecture: diurnal rhythmicity and torque characteristics

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Time of day increments in strength have previously been reported (Deschenes et al. 1998; Martin et al. 1999), but no attempt to study this effect in conjunction with internal muscle structure has been made, even though in vivo, muscle architecture (fibre pennation angle (θ) and muscle size), are determinants of the capacity of a muscle to generate torque (Maganaris et al. 2001). The aim of the current study was therefore to examine whether muscle architecture can account for a) force changes or b) any changes in the torque-angle relationship with time of day. Sixteen healthy young men (aged 23 ± 5.7 years) were tested at 7h45am and 5h45pm. To prevent order effects, seven subjects were tested in the order am to pm, and the rest in the order pm to am. Knee extensors test angle was randomised and the best of 3 contractions (peak isometric extension torque (PIET)) at each angle was used for analysis. The investigation was approved by the Manchester Metropolitan University Institutional Ethics Committee and all subjects gave their written informed consent to participate in the study.
PIET showed an average 7.0 ± 1.8% (p=0.023) upward shift throughout the range (30-90 deg) in the evening compared to the morning. The polynomial regressions fitted through the torque/angle relationship data showed a 10 deg shift of the angle at which PIET occurs (from ~80 deg at am to ~70 deg at pm) so

Appendix B

Example of informed consent form and additional information sheet

Participant Information Sheet

Project title: The effect of pre-cooling and the ingestion of sports drinks on the performance of soccer specific exercise in the heat.

Researchers: Neil Clarke, Profs. T. Reilly and D. MacLaren and Dr B. Drust.

The purpose of this study is to investigate the effect of pre-cooling and consuming sports drinks on performance on a treadmill during a simulation of the exercise intensity equal to a soccer match in the heat (30°C).

After completing a standard questionnaire about your physical activity you will be required to attend the laboratories at the Research Institute for Sport and Exercise Sciences, Liverpool John Moores University on 8 occasions.

First session: You will run on a treadmill at increasing intensities until you cannot continue; this will last about 15 minutes and will be no harder than a normal training session. During this session your height and weight will also be measured.

Next session: You will be required to exercise on the treadmill for 30 minutes at intensities ranging from standing still to sprinting. This is to ensure that you are familiar with running on the treadmill.

Next 6 sessions: For 3 days before each session you will keep a diary of what you have eaten and drunk and it will be necessary that you do not consume caffeine from 18:00 onwards the day prior to each trial. The test sessions will take place between 14:00 and 18:00, at your convenience and at least 48 hours after a competitive match or hard training. Before testing, it will also be necessary to swallow a pill, which is the size of a "cod-liver oil" capsule and will cause no discomfort and is used to measure body temperature. Before you start to exercise your body weight will be recorded. When you have completed the exercise your body weight will also be recorded. The test sessions will involve exercising in repeated 15-minute cycles of activity on a motorised treadmill for 90 minutes at intensities ranging from walking through to sprinting in the environmental chamber set at 30°C. This entire session is separated into two 45-minute halves, with a 15-minute break to represent half-time. During each session you will drink either flavoured water or two types of sports drink. On 3 occasions, before you perform the soccer-specific protocol you will be required to wear a cooling vest, that has pockets for ice packs, until your body temperature decreases by 0.6°C, this procedure is not unduly uncomfortable. The fluid consumed contains a source of phenylalanine and therefore you will not be able to participate if you suffer from phenylketonuria.

Before you start to exercise it will be necessary to collect a urine sample to ensure that you are adequately hydrated. It will also be necessary to take four 16 ml venous blood samples from an arm vein and measure muscle temperature; these procedures will feel like pinprick and will take place at the following times:

- Before you wear *the cooling* jacket
- Before you start to exercise
- At half-time
- When you have finished the 90 minutes of exercise

Also you will be required to wear a heart rate monitor during all exercise sessions. During all of the sessions you will need to wear suitable clothes e.g. shorts, T-shirt and trainers.

You will have the discretion to terminate the exercise test at any point should you feel the need.

Thank you very much for your time and assistance

LIVERPOOL JOHN MOORES UNIVERSITY
FORM OF CONSENT

The effect of pre-cooling and the ingestion of sports drinks on the performance of soccer specific exercise in the heat.

I.....**(Subject's full name*)** agree to take part in the above named project/procedure, the details of which have been fully explained to me and described in writing and I am free to withdraw at any time without giving a reason and without loss of benefits to which you are otherwise entitled

Signed ..... Date.....
(Subject)

I...NEIL CLARKE... (Investigator's full name*) certify that the details of this project/procedure have been fully explained and described in writing to the subject named above and have been understood by him/her.

Signed ..... Date .....
(Investigator)

I.....**(Witness' full name*)** certify that the details of this project/procedure have been fully explained and described in writing to the subject named above and have been understood by him/her.

Signed ..... Date .....
(Witness)

NB The witness must be an independent third party.

* Please print in block capitals

Appendix C

Phlebotomy certificate

Royal Liverpool Children's
NHS Trust

NHS

ETL
Excellence through Learning

Certificate of Attendance

This is to certify that

....NEIL CLARKE.....

has attended the
Venepuncture and Cannulation workshop

Signed by: *Pauline Brown*

Date: 06/05/03

Appendix D

Method for determining muscle glycogen content

Muscle glycogen analysis

Glycogen is the storage form of carbohydrate in the body and is a macromolecule of glucose residues linked together by alpha 1-4 and 1-6 carbons. The glycogen is hydrolyzed in HCl into it constituent glucose residues and measured enzymatically. The method is the hexokinase/ glucose-6-phosphate dehydrogenase reaction for measuring glucose based on the method developed by Lowry and Passonneau (1972).

$$\text{Glucose} + \text{ATP} \xrightarrow{\text{Hexokinase}} \text{Glucose-6-phosphate} + \text{ADP}$$

$$\text{G-6-P} + \text{NADP} \xrightarrow{\text{G-6-P dehydrogenase}} \text{6-phosphogluconic acid} + \text{NADPH}$$

The NAPDH produced can be read on a spectrophotometer at 340 nm if the concentration of glucose is high enough, or on a fluorometer at low concentrations.

The concentrations and volumes of the solutions used in this assay were:

Tris buffer	0.05 g $MgCl_2$ + 1.51 g Trizma in 500 ml H_2O (pH 8.1)
Glucose reagent	7 mg ATP + 10 mg NADP + 5 µl G-6-PDH in 25 ml Tris buffer
1 M NaOH	4 g NaOH in 100 ml H_2O
1.5 M acetic acid	1.17 ml H_2O + 100 µl concentrated stock acetic acid
0.1 M acetate buffer	2.86 ml stock acetic acid + 6.8 g sodium acetate in 1000 ml H_2O (pH 4.5)
0.02 M HCl	20 µl stock HCl + 11.5 ml H_2O
Amyloglucosidase solution	2 ml acetate buffer + 100 µl stock amyloglucosidase (10 µg/ml)
Hexokinase (0.1 dilution)	2 µl hexokinase + 18 µl Tris buffer
Glucose standard	0.5 mM glucose solution

All reagents were obtained from Sigma or Fluka (Sigma-Aldrich Company Ltd, UK).

Once the muscle was obtained it was immediately frozen in liquid nitrogen and stored in an Eppendorf container at -80°C. The frozen samples were ground under liquid nitrogen and placed into polypropylene tube. Each tube was weighed before and after the muscle was added using an analytical fine balance (Mettler AE 200) and recorded to the nearest milligram. The following procedure was followed for measuring muscle glycogen.

1. The muscle was homogenized on ice in 1 ml of 0.02 M HCl.
2. The tubes were capped and placed in boiling water for 10 minutes.
3. Following the addition of 100 µl of 1 M NaOH the samples were immersed in boiling water for a further 10 minutes.
4. 100 µl of 1.5 M acetic acid and 200 µl of 0.1 M acetate buffer were added to the samples and mixed.
5. 500 µl of the muscle extract was transferred to an Eppendorf tube.
6. 500 µl of acetate buffer as added to two Eppendorf tubes (blanks) and 500 µl of the 0.5 mM glucose solution was added to another two tubes (standards).
7. 100 µl of the amyloglucosidase solution was added to all blanks, standards and samples and left to incubate at room temperature for 2 hours.
8. The tubes were centrifuged at 4°C for 5 minutes at 14,000 $rev \cdot min^{-1}$.
9. 200 µl of the supernatant was transferred to another Eppendorf tube. Then 15 µl of the 1 M NaOH and 400 µl of the glucose reagent were added, mixed and left at room temperature for 5 minutes.
10. 200 µl was into the wells of a microplate and absorbance was measured at 340 nm (Powerwave X340, BioTek Instruments Inc, USA).
11. 2µl of the hexokinase solution was then added, mixed and the reaction proceeded to its endpoint (20 minutes) at room temperature.
12. After the hexokinase reaction was complete absorbance was again measured and the initial value of blank was subtracted from the final value of the samples and standards. The following equation was used to calculate the values.

$$\left(\frac{(\Delta Abs_{sample} / \Delta Abs_{std}) \times (mM\ Conc_{std} \times ml\ Vol_{std}) \times (dilution_{muslce})}{mg\ weight_{muscle}} \right) X\ 1000$$

= µmoles glucosyl units·gram wet $weight^{-1}$ or mmol·kg wet $weight^{-1}$