The influence of carbohydrate and fluid ingestion on thermoregulation and performance during prolonged, intermittent, high-intensity exercise in hot environmental conditions

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THE INFLUENCE OF CARBOHYDRATE AND FLUID INGESTION ON THERMOREGULATION AND PERFORMANCE DURING PROLONGED, INTERMITTENT, HIGH-INTENSITY EXERCISE IN HOT ENVIRONMENTAL CONDITIONS.

by

Nicholas Gant

A Doctoral Thesis

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

September 2005

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ABSTRACT

When performing team sports such as soccer, rugby and hockey in hot climates, the ingestion of water is often favoured over sports drinks containing carbohydrate. This is because the onset of fatigue under these environmental conditions is typically the result of hyperthermia and dehydration rather than carbohydrate depletion. However, during other modes of exercise conducted in hot environmental temperatures drinking a dilute carbohydrate-electrolyte solution (CES) has been shown to increase exercise performance when compared with plain water. There is currently a dearth of knowledge regarding the benefits that may be derived from consuming these solutions during prolonged, intermittent high-intensity exercise in the heat. One reason for this is the impracticalities associated with measuring core temperature during unconstrained exercise. Therefore, the aim of this thesis was to determine a reliable means of continuously measuring core temperature wirelessly during unconstrained exercise. Thereafter this methodology was used to investigate the influence of ingesting water and a 6% CES during a protocol that closely simulates the demands of intermittent field sports in 30°C.

The first three experimental chapters investigated the suitability of using ingestible temperature sensors as a method of measuring core temperature (Tcore) during unconstrained exercise. It was concluded that intestinal temperature (Tint) is a valid and reliable means of determining Tcore during prolonged, high-intensity intermittent exercise.

The fourth study investigated the effects of ingesting a 6% CES and a flavoured water (FW) during 60 min of intermittent high-intensity exercise in 30°C. The results showed that sprinting performance was improved when the CES was compared with FW, which was accompanied by a higher Tcore.

In study 5 a similar exercise protocol was used to assess the availability of fluid from the two solutions. The rate of gastric emptying was determined using a gastric sampling technique and was found to be similar for both solutions. An increase in performance and elevated Tcore occurred again when the CES was ingested.
The aims of the sixth investigation were to quantify energy expenditure and exercise tolerance in order to determine whether exercise capacity would be improved by either a 6% CES or FW. There were no differences in the time taken to reach the point of fatigue, which occurred at a similar $T_{\text{core}}$ in both trials. Analysis of expired air revealed that an overall increase in energy expenditure and a higher carbohydrate oxidation rate were present during the CES trial. The increased energy expenditure is likely to be due to a higher intensity of sprinting when the CES was ingested.

The final experimental chapter investigated the thermogenesis associated with ingesting a 6% CES and the localised effect of this on $T_{\text{int}}$. The thermic effect of feeding manifested as a small increase in energy expenditure, but no changes were detected in $T_{\text{int}}$ which responded in a similar manner to $T_{\text{rec}}$ during this protocol.

When prolonged high-intensity intermittent running is performed in 30°C, ingesting an appropriate amount of a dilute CES appears to be more advantageous than drinking water alone. The availability of fluid from both beverages is similar, however, the performance of supramaximal exercise is enhanced when an exogenous supply of carbohydrate is available. The rapid onset of exhaustion under these environmental conditions appears to be caused by an intolerable rate of heat storage, to which the ingestion of a CES has no benefit over plain water.

**Keywords:** carbohydrate, fatigue, fluid, hyperthermia, intestinal temperature, intermittent exercise, sports drinks.
CONERENCE PROCEEDINGS


ACKNOWLEDGEMENTS

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**LIST OF ABBREVIATIONS**

List of the abbreviations and acronyms contained in this thesis. Abbreviations are defined in the text in the first instance.

<table>
<thead>
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<th>Description</th>
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<tbody>
<tr>
<td>5-HT</td>
<td>5-hydroxytryptamine</td>
</tr>
<tr>
<td>ANOVA</td>
<td>analysis of variance</td>
</tr>
<tr>
<td>a-v</td>
<td>arterio-venous</td>
</tr>
<tr>
<td>BCAA</td>
<td>branched-chain amino acids</td>
</tr>
<tr>
<td>BM</td>
<td>body mass</td>
</tr>
<tr>
<td>BMI</td>
<td>body mass index</td>
</tr>
<tr>
<td>CES</td>
<td>carbohydrate electrolyte solution</td>
</tr>
<tr>
<td>CHO</td>
<td>carbohydrate</td>
</tr>
<tr>
<td>CI</td>
<td>confidence interval</td>
</tr>
<tr>
<td>CNS</td>
<td>central nervous system</td>
</tr>
<tr>
<td>CV</td>
<td>coefficient of variation</td>
</tr>
<tr>
<td>DM</td>
<td>dry matter (muscle)</td>
</tr>
<tr>
<td>EEG</td>
<td>electroencephalograph</td>
</tr>
<tr>
<td>EMG</td>
<td>electromyograph</td>
</tr>
<tr>
<td>EDTA</td>
<td>potassium ethylenediaminetetraacetic acid</td>
</tr>
<tr>
<td>fMRI</td>
<td>functional magnetic resonance imaging</td>
</tr>
<tr>
<td>FFA</td>
<td>free fatty acids / non-esterified fatty acids</td>
</tr>
<tr>
<td>FW</td>
<td>flavoured water</td>
</tr>
<tr>
<td>G</td>
<td>gravity</td>
</tr>
<tr>
<td>g</td>
<td>gram</td>
</tr>
<tr>
<td>GE</td>
<td>gastric emptying</td>
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<tr>
<td>GI</td>
<td>gastro-intestinal</td>
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<td>h</td>
<td>hour</td>
</tr>
<tr>
<td>HR</td>
<td>heart rate</td>
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<tr>
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<td>maximum heart rate</td>
</tr>
<tr>
<td>ICC</td>
<td>intraclass correlation coefficient</td>
</tr>
<tr>
<td>IMP</td>
<td>inosine 5'-monophosphate</td>
</tr>
<tr>
<td>IMTG</td>
<td>intra-myocellular triacylglycerol</td>
</tr>
<tr>
<td>kJ</td>
<td>kilojoule</td>
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<tr>
<td>kg</td>
<td>kilogram</td>
</tr>
<tr>
<td>l</td>
<td>litre</td>
</tr>
<tr>
<td>LIST</td>
<td>Loughborough intermittent shuttle test</td>
</tr>
<tr>
<td>LOA</td>
<td>limit of agreement</td>
</tr>
<tr>
<td>mg</td>
<td>milligram</td>
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</tr>
<tr>
<td>mOsmol</td>
<td>milliosmole</td>
</tr>
<tr>
<td>MRS</td>
<td>magnetic resonance spectroscopy</td>
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</table>
MSerror  mean square error term
MSFT  multi stage fitness test
MVC  maximal voluntary contraction
n  number of observations
nCI  normalised confidence interval
Nm  newton-meter
nm  nanometre
pmol  picomole
PR  phenol red dye (C₁₉H₁₃O₅SNa)
PV  plasma volume
Q  cardiac output
RER  respiratory exchange ratio
RH  relative humidity
RLOA  ratio limit of agreement
RPE  subjective rating of perceived exertion
s  second
SEM  standard error of measurement
SD  standard deviation
STPD  standard temperature and pressure dry
T_{core}  core temperature
T_{int}  intestinal temperature
T_{mus}  intramuscular temperature
T_{oes}  oesophageal temperature
T_{rec}  rectal temperature
T_{sk}  skin temperature
\bar{T}_{sk}  weighted mean skin temperature
\bar{V}_{O₂ max}  maximum oxygen uptake
\dot{V}_E  expired gas volume
W  watt
\bar{x}  mean
\bar{x}  median
°C  degrees celsius
μl  microlitre

Test statistics

d  Cohen's d calculation
D  Kolmogorov-Smirnov statistic
ε  sphericity epsilon
F  general linear model (ANOVA) statistic
P  critical alpha level
r  Pearson's correlation coefficient
t  Student's t-test statistic
w  Shapiro-Wilks' statistic
z  Wilcoxon's matched pairs statistic
CHAPTER 1

INTRODUCTION

1.1 Introduction
The worldwide popularity of field sports such as soccer and rugby results in the requirement for professional players to regularly take part in matches and compete in tournaments that are held in different climates to their own. Unlike many other athletes preparing to compete in an event overseas, the schedule of domestic leagues often results in teams only having a short period of time to acclimatise to their new surroundings. Therefore interventions that enable individuals to cope with the physiological demands of competing under these circumstances and compensate for the lack of acclimation are of much interest to players and the scientific community alike.

Although there is a wealth of information addressing the demands of prolonged continuous exercise in the heat, the impracticalities of replicating match-play during intermittent sports in controlled environmental conditions has resulted in a lack of published research in this area. Standardising the environmental conditions in laboratories large enough to perform unconstrained exercise is problematic and instrumenting subjects with invasive means of monitoring core temperature ($T_{\text{core}}$) is difficult during free-running. In an attempt to overcome some of these problems a number of treadmill protocols have been developed that replicate the mean exercise intensities observed during prolonged, intermittent high-intensity field sports (Bangsbo et al., 1992; Drust et al., 2000; Nassis et al., 1998; Quanz, 1999; Walton and Rhodes, 1997), but the lack of rapid changes in pace and direction limit the ecological validity of these models. The Loughborough Intermittent Shuttle Running Test (the LIST) is an example of a protocol that overcomes these limitations and accurately elicits physiological demands similar to those encountered in sports such as soccer, rugby and field hockey (Nicholas et al., 2000). This protocol has been successfully used to examine the effects of exogenous carbohydrate ingestion on physical performance and metabolism (Ali et al., 2002; Foskett et al., 2004; 2003b; Nicholas et

Fluid replacement is central in overcoming the additional thermoregulatory demands imposed on the body during intermittent exercise in hot environmental temperatures. The limited opportunity to drink during match-play requires that the composition of an oral rehydration solution is aimed at maximising the volume of fluid ingested and incorporated into the blood and tissues. Currently the two most popular solutions ingested during professional soccer in the UK are flavoured water (usually formulated from a fruit concentrate) and commercially available carbohydrate-electrolyte solutions. Many coaches and players favour the use of water during warm conditions, as they are aware of need to replace fluid losses and the trade-off that exists between the fluid and carbohydrate content of sports drinks. This appears to be a well-informed choice as the energy density of the gastric contents is known to slow the rate of fluid delivery to the duodenum and the fatigue process in the heat is often the consequence of an intolerable rate of heat storage, rather than a lack of available substrate. Nevertheless, a number of studies utilising treadmill running (Millard-Stafford et al., 1997; 1992) and cycling (Below et al., 1995; Carter et al., 2003; Davis et al., 1988b; Fritzsche et al., 2000; Galloway and Maughan, 2000; Murray et al., 1987) protocols have shown that under uncompensable heat stresses exercise performance and capacity can be augmented by ingesting a well formulated carbohydrate-electrolyte solution (CES). On the other hand, a number of investigations that have included periods of maximal sprinting and are therefore more reflective of soccer, rugby and hockey, report an elevation in $T_{\text{core}}$ when a 6% CES is ingested compared with plain water (Fritzsche et al., 2000; Morris et al., 2003). This suggests that players experience a greater thermal strain when ingesting a CES compared with an isovolumetric amount of water. This response is postulated to be the consequence of decreased gastric emptying, fluid absorption, postprandial thermogenesis or simply the result of an increased energy expenditure when ingesting a CES. To date these questions remain to be addressed during an experimental protocol that reflects the demands of prolonged, high-intensity intermittent exercise.

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1 Typically a solution containing a maltodextrin and glucose syrup mixture with a concentration of ~6% and a Na⁺ content of 20 - 30 mmol·l⁻¹. E.g. Lucozade Sport® (GSK), Gatorade® (Quaker Oats Co.), Powerade® (Coca-Cola), Isostar® (Wander).
The aim of this thesis was first to establish a valid and reliable means of monitoring $T_{core}$ during prolonged intermittent high-intensity running. This methodology was then used to investigate performance and the causes of fatigue during the LIST protocol in 30°C, ~30% RH when ingesting either a 6% CES or a flavoured water (FW).

1.2 Organisation of thesis

The experimental work contained within this thesis is presented in 7 chapters, the first three chapters address the measurement of $T_{core}$ during the LIST protocol and the remaining chapters investigate the influence of drink composition when this form of exercise is conducted in 30°C.

A general methods chapter (Chapter 3) outlines the equipment and methodological procedures that are generic to all experimental tests.

The purpose of Chapter 4 was to investigate the validity of measuring $T_{core}$ via the use of ingestible temperature sensor capsules during intermittent field sports. The study compares intestinal temperature ($T_{int}$) alongside rectal temperature during the LIST protocol.

The aim of Chapter 5 was to address the reliability of the $T_{int}$ measurement technique by measuring this variable during the LIST protocol on separate occasions. The study was designed to examine the influence of factors that occur between experimental trials and potentially influence the motility and location of the temperature sensor during exercise.

The intention of Chapter 6 was to determine whether considerable inter-individual variation exists in intestinal temperature between games players. Matched subjects completed the LIST protocol in order to establish if reliable comparisons in $T_{int}$ can be made across a large population.

Chapter 7 examined thermoregulation, metabolism and performance during 60 min of the LIST in 30°C, in order to determine the influence of ingesting a 6% CES and FW over a time period subjects are unlikely to reach the point of exhaustion.
The purpose of Chapter 8 was to measure the rate of gastric emptying during a similar protocol to Chapter 7, in order to quantify the availability of fluid when consuming a 6% CES or FW.

The aim of Chapter 9 was to examine exercise tolerance and extend the findings of the previous studies to the point of exhaustion. Additionally energy expenditure was measured in order to further understand the metabolic mechanisms of fatigue during the LIST protocol in 30°C.

Chapter 10 examined the thermogenic effect of ingesting a 6% CES compared with a FW at rest and the determined the suitability of interpreting T_{int} (compared with T_{rec}) during the postprandial period.

The final chapter (Chapter 11) summarises the results of the experimental chapters and discusses the potential mechanisms behind these findings.
CHAPTER 2

REVIEW OF LITERATURE

The aim of this chapter is to outline and critically examine the pertinent literature that has examined prolonged, intermittent high-intensity exercise in hot environmental conditions. This review will firstly address the physiological demands of such activities and the aetiology of fatigue when they are performed in the heat. The provision of fluid and fuel during exercise in the form of drinks will be discussed, along with the factors affecting their delivery and outcome on the ability to perform exercise. The final section of the chapter provides an analysis of the various methods of measuring deep body temperature during unconstrained exercise and their suitability for use during intermittent high-intensity field sports.

2.1 Intermittent high-intensity exercise in a hot environment

The term intermittent high-intensity exercise is often used to describe the activity patterns observed in sports that involve brief periods of high-intensity activity interspersed with lower intensity exercise or rest. This encompasses field sports such as soccer, rugby and field hockey which are also often referred to as multiple-sprint or stop-go sports. The ability to use a match environment for research purposes is limited and investigators have designed a number of treadmill based laboratory protocols in order to examine these sports in a controlled and repeatable manner (Bangsbo et al., 1992; Drust et al., 2000; Nassis et al., 1998; Quanz, 1999; Walton and Rhodes, 1997). These variable intensity protocols replicate the workloads involved, but are limited by the constrained nature of treadmill exercise that lacks free movement or changes in direction. As an alternative to static exercise shuttle running protocols have been developed that closely simulate the activity patterns and energy demands of match-play and incorporate turning and rapid changes in pace and direction. The Loughborough Intermittent Shuttle-running Test (LIST) is an example of such a model that has been successfully used over the last 10 years to examine performance and fatigue during team sports (see Nicholas et al., 2000 for a comprehensive description of the methodology).
2.1.1 Physiological demands

2.1.1.1 Energy cost

The physiological demands of prolonged high-intensity intermittent field sports will vary considerably depending on the role of each individual within the field of play, the team tactics and the environmental conditions. The majority of understanding of the energy demands of intermittent field sports are based either on estimations made from simple physiological measures recorded during match-play or direct measurements made during practice games or experimental simulations (for a review see Stolen et al., 2005). Miyagi and Ohashi (2003) directly measured oxygen uptake during periods of an elite youth match by collecting expired air using modified Douglas bags. This study reports mean oxygen uptake values of \( \sim 70\% \text{VO}_2\text{max} \) and an average heart rate of 176 beats·min\(^{-1}\) during match-play. Rodregez and Iglesias (1998) estimated the average energy expenditure during a match to be \( \sim 50 \text{kJ·min}^{-1} \) in professional soccer players and around 47 kJ·min\(^{-1}\) in amateurs. Little is known about the degree to which energy expenditure is altered during match-play under heat stress, but the changes in cardiovascular function, blood and muscle metabolites observed in laboratory simulations would suggest the physiological demands are altered significantly.

2.1.1.2 Metabolic demands

High-intensity intermittent exercise places considerable demands on the body's endogenous carbohydrate (CHO) stores. Within field sports this demand is increased with the intensity of play and duration of the game (Coggan and Coyle, 1991) and these stores may play a pivotal role during periods of extra time (Foskett et al., 2003b). At a professional level, multiple-sprint sports are typically played at an intensity of 70 – 80\% \text{VO}_2\text{max}. Proportionally this equates to around 60\% of energy being derived from CHO (glycogen and glucose) and the remaining 40\% from fats (Bangsbo, 1994a). In addition, performing exercise under environmental heat stress has been shown to induce a hyperglycaemic response (Febbraio et al., 1996b; 1994a; 1994b; Fink et al., 1975; Hargreaves et al., 1996a; Jentjens et al., 2002; Yaspelkis et al., 1993) further depleting the reserves of these substrates available when multi-sprint activity is performed in the heat. The additional systemic glucose that appears during heat stress has been shown to originate from the liver. In the absence of heat stress the
liver regulates blood glucose by balancing CHO release from the gut with hepatic glycogenesis or glycogenolysis. However, this homeostatic mechanism has been shown to dysfunction somewhat during hyperthermia, possibly due reduced hepatic blood flow, brought about by high levels of circulating catecholamines (Hargreaves et al., 1996a; Rowell et al., 1968). Elevations in hepatic glucose production have been observed using isotopic tracers (Hargreaves et al., 1996a; Jentjens et al., 2002), dye infusion techniques (Rowell et al., 1968), and a-v difference (González-Alonso et al., 1999a). The complex neural and hormonal regulation of hepatic glucose production during exercise has been shown to be overridden to some degree by the effect of heat stress (Angus et al., 2001). In the feeding study of Angus and co-workers, despite the appearance of glucose in the blood derived from ingested and infused CHO, the high rate of hepatic glucose production remained elevated. This seems to occur because of a reduction in splanchnic blood flow (Rowell, 1974) or an increase in hepatic temperature and highlights the powerful, independent effect of thermal stress on energy metabolism (Febbraio, 2001).

The rate of muscle glycogen utilisation during soccer has been examined during match-play by several authors. Leat and Jacobs (1989) calculated a mean utilisation rate of ~2 mmol glucosyl units·kg DM\(^{-1}\)·min\(^{-1}\) during a game. Similarly Krustrup and co-workers (2003) observed utilisation rates in the order of 2.2 mmol glucosyl units·kg DM\(^{-1}\)·min\(^{-1}\) during a 90 min match. Similar rates of utilisation have been elicited during a simulated experimental model (LIST). A mean rate of 2.1 mmol glucosyl units·kg DM\(^{-1}\)·min\(^{-1}\) has been estimated during 90 min of exercise (Nicholas et al., 1999) and more recently a utilisation rate of 2.5 mmol glucosyl units·kg DM\(^{-1}\)·min\(^{-1}\) was calculated over the same time period (Foskett et al., 2004). During the same experimental protocol at varying ambient temperatures rates of muscle glycogen utilisation have been shown to differ. A rate of 1.4 mmol glucosyl units·kg DM\(^{-1}\)·min\(^{-1}\) was observed in an environmental temperature of 17°C compared with 1.9 mmol glucosyl units·kg DM\(^{-1}\)·min\(^{-1}\) in 33°C (Morris et al., 2005). It is worth noting that in this study the final biopsies were taken at the point of exhaustion in the heat and not after 90 min of exercise, as in the other studies described in this section. Apart from the findings of Morris and colleagues there is a lack of knowledge regarding glycogen utilisation within multiple sprint sports in the heat. However, the influence of heat stress on the rate of muscle energy metabolism has been examined using a number of
alternative experimental models. There is some debate within these studies as to whether climatic heat stress increases glycogen utilisation (Drust et al., 2005; Febbraio et al., 1994a; 1994b; Fink et al., 1975; Jentjens et al., 2002; Morris et al., 2005; Parkin et al., 1999) or has no effect (Maxwell et al., 1999; Nielsen et al., 1990; Yaspelkis et al., 1993; Young et al., 1985). Examination of the experimental designs involved in these studies provides some possible explanations for the conflicting findings. The studies of Maxwell and colleagues (1999) and Yaspelkis et al. (1993) use a difference between environmental temperatures for the hot and moderate conditions that is relatively small (−12°C and −10°C respectively) compared with other studies. This lack of heat stress is reflected in the relatively low core temperatures observed at the end of exercise. The degree of heat stress induced by Yaspelkis and co-workers may have been further reduced by acclimation of the subjects. The negative findings of Nielsen et al. (1990) and Young et al. (1985) are not easily interpreted as differences in the concentration of muscle glycogen existed at the start of exercise in these protocols. Three of the above studies employed protocols involving prolonged intermittent supramaximal exercise (Drust et al., 2005; Maxwell et al., 1996; Morris et al., 2005). Maxwell and co-workers observed no differences in muscle glycogen utilisation and suggest the intensity of their uphill sprint protocol required a maximal rate of energy turnover, to which additional heat stress would have no further influence. The high rate of muscle lactate accumulation recorded supports this conclusion. However, glycogen utilisation has been shown to be greater in a heated leg during intense cycling exercise at 115% ŔO₂ max (Febbraio et al., 1996a; Morris et al., 2005) and therefore it seems more likely that the level of heat stress imposed was too mild to elicit notable changes. The exercise protocol and heat exposure time was also shorter in the study of Maxwell and colleagues compared with other studies. A study examining muscle glycogen metabolism and enzyme activities during the early stages of exercise in the heat (Saunders et al., 2001), concluded that differences in the concentrations of regulatory enzymes caused by thermal stress do not occur during the early stages of exercise as previously suggested (Howlett et al., 1998), but accumulate with exercise duration. This may explain why differences are not observed during relatively short exercise protocols. The available evidence relevant to high-intensity intermittent exercise suggests that the rate of glycogenolysis may be augmented when the exercise duration is prolonged, the environmental
conditions impose sufficient heat stress (conditions equivalent to >30°C and >20% RH) and are accompanied by substantial increase in T_{core}.

The shift toward increased muscle glycogen utilisation in the heat has been attributed to a number of mechanisms. It has been suggested that a possible decreased skeletal blood flow in favour of peripheral blood flow may reduce the availability of O_2 and other substrates hence altering cellular respiration (Febbraio, 2001). However, O_2 availability is not the main mediatory factor increasing glycolysis and lactate accumulation during moderately intense exercise. Additionally, the suggestion of a reduction in O_2 delivery to the active muscle during moderately intense exercise in the heat is questionable (see Section 2.1.1.3). González-Alonso and colleagues (1999a) showed that when muscle blood flow was compromised considerably with dehydration, glucose delivery and uptake into the muscle was unaffected. Subsequently Jentjens et al. (2002) reported a 25% increase in muscle glycogen utilisation in the heat, which was accompanied by a reduction in the rate of exogenous glucose oxidation in the order of 10%. This reduction in exogenous glucose uptake is unlikely to be caused by changes in blood flow to the musculature and therefore the increased glycogenolysis may be due to changes within the muscle. Jentjens and colleagues suggest that this decreased glucose uptake is likely to be due to an elevation in intracellular glucose-6-phosphate caused by an increased rate of glycogenolysis. This could potentially reduce the phosphorylation of glucose, alter the gradient for glucose diffusion and hence inhibit glucose transport. Another possible cause may be increases in intramuscular temperature (T_{mus}), this rises proportionally with exercise workload (Saltin and Hermansen, 1966) and is increased further, independently of exercise intensity under thermal stress (Febbraio et al., 1994a; Febbraio et al., 1994b; González-Alonso et al., 1999a; Hargreaves et al., 1996b; Parkin et al., 1999). It has been suggested that an increase in temperature may up-regulate key enzymes that control and/or alter muscle metabolism (Kozlowski et al., 1985). Febbraio and colleagues (1996a) reported an increased rate of glycolysis and lactate accumulation during high intensity exercise when T_{mus} was altered independently of T_{core} and circulating catecholamines. Similar responses have also been observed during moderate intensity exercise (Starkie et al., 1999). Apart from the studies above, it is difficult to interpret the findings of most investigations that have measured changes in T_{mus} during exercise, as these local temperature changes are
typically accompanied by an elevation in core temperature, whole-body hyperthermia
will typically alter the concentration of plasma catecholamines (Febbraio et al., 1996a;
1994a; González-Alonso et al., 1999a; Hargreaves et al., 1996a). The increase in
adrenaline secretion observed during exercise in the heat has been implicated in
increasing the rate of glycogenolysis (Febbraio et al., 1994a; 1996c; González-Alonso
et al., 1997; Hargreaves et al., 1996b; Morris et al., 2005). During high-intensity
intermittent shuttle running in the heat Morris et al. (2005) report elevated plasma
catecholamines accompanied by an increased rate of glycogen utilisation. Increases in
glycogen utilisation similar in magnitude to those seen by Morris et al. (2005) have
been recorded by infusing comparable doses of adrenalin during cycling (Febbraio et
al., 1998). The intensity and duration of the intermittent exercise model utilised by
Morris and colleagues (LIST) appears to induce changes in metabolites and hormones
that are sufficient to significantly alter carbohydrate metabolism in the heat.

The mobilisation of free fatty acids (FFA) has been observed towards the latter stages
of soccer match-play, this is probably due to the compromised availability of CHO
(Bangsbo, 1993). Fat metabolism makes a significant contribution toward energy
metabolism during prolonged exercise and utilisation of this substrate appears to be
unaffected by heat stress during exercise (Fink et al., 1975; Nielsen et al., 1990;
Yaspelkis et al., 1993). However, it is worth noting that concentrations of plasma FFA
only reflect the balance between lipolysis and FFA uptake. It has been shown during
exercise in the heat that FFA concentration may be similar when intra-myocellular
triacylglycerol (IMTG) utilisation is reduced (Fink et al., 1975). There is some
evidence to suggest that during prolonged moderate intensity exercise IMTG may
provide a significant contribution to energy metabolism (for a review see Watt et al.
2000). However, the lack of a clear consensus in the quantification of these changes
appears to be due to discrepancies between findings derived from MRS, biopsy and
isotopic-tracer techniques. There are at present no published data regarding the
involvement of IMTG during high-intensity multiple-sprint exercise.

The relative contribution of protein to metabolism during intermittent exercise is
unclear, but protein has been shown to contribute to less than 10% of the total energy
expenditure during continuous exercise of similar duration and intensity to field sports
(Wagenmakers et al., 1991). Gibala (2001) suggests that the relative contribution may
decline to less than 6% during exercise in the heat. On the other hand, it has been proposed that protein metabolism may be increased when exercising in the heat (Febbraio, 2001). This suggestion has arisen from reports of elevated intramuscular (Febbraio et al., 1994b) and plasma (Marino et al., 2001) ammonia when exercising under heat stress. Ammonia production has been linked with the oxidation of BCAA during exercise (Greenhaff et al., 1991). Aside from the metabolic effects of protein metabolism it is possible that the utilisation of BCAA may have capacity limiting consequences during prolonged exercise in the heat, as BCAA concentration may influence serotonergic activity by altering the free-tryptophan:BCAA ratio.

High-intensity multiple-sprint sports inherently involve a large contribution from anaerobic energy metabolism. Several studies examining blood lactate concentrations in soccer report relatively low concentrations in the range of 4-6 mmol·l⁻¹ during and immediately after a match, suggestive of a rate of energy production that is primarily derived from aerobic sources (Gerisch et al., 1988; Miyagi and Ohashi, 2003; Rohde and Espersen, 1988). In contrast, reports by Bangsbo et al. (1991) and Ekblom (1986) have shown high peak blood lactate concentrations during competitive soccer (10 mmol·l⁻¹ and 12 mmol·l⁻¹ respectively) showing that certain elements of the game rely heavily on energy production via non-oxidative (anaerobic) pathways. When interpreting lactate concentrations during intermittent activity it is worth noting that lactate is a gluconeogenic precursor which is metabolised during low intensity activity (Bangsbo, 1993), therefore even substantial increases may not cause long-term acidosis during prolonged exercise. It appears that the sampling point at which blood lactate concentration is determined during intermittent exercise has a significant influence on the interpretation of this variable. During submaximal steady-state exercise in the heat, blood lactate concentration has been shown to be both elevated (Febbraio et al., 1994a; 1994b; Kozlowski et al., 1985; Savard et al., 1988; Young et al., 1985) and unchanged (Nielsen et al., 1990). Studies that have utilised protocols replicating multi-sprint sports at various ambient temperatures have typically detected no differences in blood lactate concentration between environmental temperatures (Drust et al., 2005; Maxwell et al., 1996; Morris et al., 2005; Sunderland and Nevill, 2005), suggesting that either the oxygen-independent metabolic responses are alike under heat stress when completing repeated bouts of high intensity exercise, or this measure is not sensitive enough to detect any changes. Higher concentrations of blood
lactate during prolonged exercise are usually indicative of an elevated rate of glycolysis. Authors that have published these observations suggest that an increased catecholamine concentration (Marino et al., 2001; Nielsen et al., 1993) or possible reductions in blood flow to the active muscle (Young et al., 1985) may be responsible. But the evidence supporting reduced muscle blood flow and blood-borne substrate delivery observed in the study of Young and associates is equivocal (see Section 2.1.2.2).

Steady state studies conducted in the heat that have detected higher concentrations of lactate in the blood (Yaspelkis et al., 1993; Young et al., 1985) and muscle (Young et al., 1985) report the lack of an accompanying increase in glycogen utilisation. As the appearance of lactate in the blood during prolonged exercise reflects a balance between lactate entry and clearance this could possibly indicate a reduced clearance by organs such as the liver or release of lactate from inactive tissue with increased sympathetic stimulation (Yaspelkis et al., 1993).

The weight of the available evidence shows that prolonged high-intensity exercise in a hot environment results in an increased reliance upon CHO oxidation, which accelerates muscle glycogen utilisation. This has the potential to result in a greater accumulation of intramuscular lactate. Changes in muscle temperature and its influence on key enzymes and/or elevated plasma catecholamines may be responsible for these metabolic alterations.

2.1.1.3 Thermoregulatory demands
Competitive team sports are performed at intensities that result in considerable metabolic heat production and therefore players require efficient routes of heat dissipation to the environment in order to prevent a progressive storage of heat. Even in temperate conditions $T_{core}$ may rise by $\sim 2^\circ C$ during 90 min of match-play (Reilly, 1997) and when field sports are played in hot conditions circulatory and thermoregulatory function will be further challenged. Soccer is the most widely studied of the popular team sports and Table 2.1 summarises a number of typical post-match $T_{core}$ values recorded following competitive games. It is clear from these values that over a range of environmental temperatures commonly encountered in this sport a considerable degree of hyperthermia is evident in players after 90 min. It is common
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Table 2.1 Thermoregulation and fluid balance during competitive soccer matches. Studies are ranked according to ambient conditions and all values reported are for 90 min competitive soccer matches. The sweat loss shown is actual reduction in body weight after correction for fluid intake, in absolute terms (litres) and relative to body mass (%). Updated from Maughan and Leiper (1994). * denotes T<sub>int</sub> was used as the measure of T<sub>core</sub>. 
for $T_{\text{core}}$ values in excess of $40^\circ\text{C}$ to be recorded following a soccer match (Ekblom, 1986; Smolilaka, 1978) and these are similar to those seen after prolonged endurance events such as marathon running (Maughan, 1984; Maughan et al., 1985).

When the metabolic and thermoregulatory demands are both high, a finite blood volume must be distributed to both the active muscle tissue and the skin. Under these circumstances central blood volume and cardiac filling pressure will be significantly challenged. In order to preserve cardiac output ($Q$) a reduction in splanchnic and renal blood flow (Rowell, 1974; Rowell et al., 1968) and an increase in heart rate will occur. During severe thermoregulatory challenges caused by heat storage, the maintenance of $Q$ has been observed up until the point when maximum heart rate ($HR_{\text{max}}$) is achieved (González-Alonso and Calbet, 2003). In a number of studies employing moderately intense continuous exercise, successful maintenance of $Q$ has been observed up to an average level of hyperthermia equivalent to a $T_{\text{core}}$ of $\sim39.3^\circ\text{C}$ (González-Alonso et al., 1995, 1997; Nadel et al., 1979; Nielsen et al., 1990). These investigations demonstrate that during continuous exercise the cardiovascular system of well trained individuals responds adequately to heat stress at intensities below $HR_{\text{max}}$. However, the cardiovascular and thermoregulatory responses to intermittent high-intensity exercise are not as well understood. It has been shown that intermittent exercise imposes greater energy and thermoregulatory demands compared with continuous exercise of a similar intensity (Drust et al., 2000; Lind, 1963; Nevill et al., 1995). In a series of experiments using a free-running laboratory model (LIST), Morris and co-workers (Morris et al., 2005; 1998a; 2000) showed that well trained games players exhibit an increase in cardiac frequency of approximately 5 beats·min$^{-1}$ in the heat compared with the same exercise in an environment of moderate temperature. During these studies hydration status remained essentially constant between trials and there were no differences in performance, suggesting that the demands of this form of exercise in air temperatures of $\sim30^\circ\text{C}$ are within the circulatory capacity of games players.

Endurance athletes appear to be able to maintain cardiovascular stability when hyperthermic or mildly dehydrated during exercise at mean exercise intensities similar to those encountered during intermittent team sports. However, in a well controlled
series of studies González-Alonso and colleagues illustrated that the synergistic
effects of hyperthermia and dehydration have profound consequences for
cardiovascular function. González-Alonso et al. (1997) showed that a set level of
hyperthermia or dehydration independently decreased stroke volume by 7-8 % and
increased heart rate by 5% with no overall change in $\dot{Q}$. But when these two were
combined, a 20% reduction in stroke volume was reported alongside a 9% elevation
in heart rate, leading to overall deficit in $\dot{Q}$ of 13%. Dehydration appears to have a
powerful compromising influence on thermoregulatory function and during cycling
and running exercise in hot environments a level of dehydration equivalent to 3-4.1 %
BM results in reduction in $\dot{Q}$ of 7-11 % (Montain and Coyle, 1992a; Montain and
Coyle, 1992b; Sawka et al., 1979). Within a range of BM losses of 1.1 – 4.2% it has
been shown that $\dot{Q}$ is directly related to the magnitude of dehydration (Montain and
Coyle, 1992b). This compromising of circulatory function generally manifests as an
increase in heart rate of 5-8 beats·min$^{-1}$ and is accompanied by an increase in $T_{core}$ of
0.2-0.3°C for every 1 % of BM lost (Coyle, 2004).

The first compensatory outcome of overreaching the capacity of the circulatory
system appears to be an increase in systemic vascular resistance. González-Alonso et
al. (1995) showed that for a 4.9% decrease in BM with an 18% reduction in $\dot{Q}$, there
was an increase in systemic vascular resistance of 17%. It seems that when
dehydrated to levels commonly encountered during field sports such as soccer (Table
2.1) the preservation of arterial pressure in order to maintain $O_2$ and substrate delivery
to the muscle occurs at the expense of blood flow to the skin. Whether or not over
time the reduced skin blood flow is accompanied by a significant restriction of blood
supply to the active musculature, which would alter force generation, is uncertain.

A number of studies utilising walking and cycling protocols have reported no
differences in blood flow to the legs and muscle when a significant degree of
hyperthermia and/or hypohydration is imposed upon subjects (Kirwan et al., 1987;
Nielsen et al., 1993; 1990; 1997; Savard et al., 1988). However conversely more
recent work by González-Alonso and colleagues (2003; 1998; 1999a), has shown that
muscle blood flow is reduced under conditions of thermal stress and dehydration. The
group that conducted the recent work argue that the previous studies failed to stress
the cardiovascular system to an adequate degree for this to occur. This may be the case as in earlier studies venous pressure appears unaltered, which is reflected in the maintenance or increase in \( \dot{Q} \) during these protocols. In the findings of González-Alonso and co-workers despite a decline in muscle blood flow with progressive dehydration and hyperthermia there were no reductions in \( O_2 \) delivery or leg \( \dot{VO}_2 \) (González-Alonso et al., 1998). It appears that reductions in blood flow during exercise do not occur to an extent that compromises muscle function. The authors did not find any alteration in vascular conductance during these studies, which suggests that reduced perfusion pressure and systemic blood availability are responsible for blood flow changes, rather than neural vasoconstriction. This implies that the body attempts to preserve the function of the active muscle tissue despite significant reductions in central blood pressure. Therefore it may be the case that the skin and visceral organs are the primary target of the baroreflex regulation of blood volume during exercise under conditions of hypohydration and hyperthermia (González-Alonso et al., 1998). Similarly no changes were found in the delivery of glucose and FFA or the removal of lactate during this exercise model (González-Alonso et al., 1999a). These results suggest that hormonal influences rather than \( O_2 \) availability may be responsible for the changes in muscle metabolism discussed earlier. Whether or not muscle blood flow is reduced during high-intensity intermittent exercise is uncertain, it seems likely considering similar levels of dehydration (-3.9 % BM) and hyperthermia (39.7 °C) to those imposed in the studies above have been observed during soccer matches in warm environments (Table 2.1). The greater mass of metabolically active muscle used during free-running may also present additional demands to the available blood flow compared with the cycle ergometry protocols discussed above.

2.1.2 Performance and fatigue

In hot environmental conditions exercise performance and the capacity to perform prolonged, intermittent high-intensity running is significantly reduced in comparison with temperate conditions (Morris et al., 1998a; 2000; Sunderland and Nevill, 2005). During prolonged high-intensity intermittent exercise in cool environmental conditions, the point of fatigue has been shown to coincide with a number of peripheral factors, the most common being the depletion of glycogen. However, in hot
environments fatigue is often a direct consequence of a rapid rate of heat storage. International competitions are frequently held in hot and/or humid environments, therefore it seems pertinent to examine the causes of fatigue when fields sports such as soccer, rugby and hockey are played in a hot environment.

2.1.2.1 Circulatory limitations

During competitive team sports in hot environmental conditions well trained players should, on the whole, be able maintain central blood pressure and $\dot{Q}$ at a level that is not detrimental to performance (discussed in Section 2.1.1.3). However, when total blood volume is further compromised by fluid losses the cardiovascular system may be rapidly pushed to it's absolute regulatory limit, where $\dot{Q}$ and $O_2$ transport to the active muscle tissue can no longer be maintained (González-Alonso and Calbet, 2003). This phenomenon was observed by Adams and colleagues (1975), who suggested that fatigue observed during running in a hot environment was caused by 'thermoregulatory failure' due to the demands of cutaneous circulation exacerbated by fluid loss. This was subsequently examined in a more controlled manner by Sawka et al. (1992). Their study involved treadmill walking to exhaustion in the heat. When hypohydrated subjects reached the point of exhaustion more rapidly, despite having a considerably lower $T_{core}$ at the point of fatigue compared with euhydrated subjects (38.7 vs. 39.1°C). Similar core temperatures to this (~38.6°C) are reported at the point of fatigue by Adams et al. (1975) and Suzuki (1980). These studies demonstrate that when dehydrated, 'circulatory failure', in other words fatigue that is ischemic in nature, may occur at core temperatures that would not be considered critical in euhydrated subjects.

The affect of dehydration on exercise performance has been investigated extensively during prolonged continuous exercise. Fluid deficits equivalent to as little as 1% of body mass have been shown to decrease performance (Ekblom, 1986) and due to the increased circulatory demands, lesser fluid losses are required to detrimentally effect performance in the heat. The results of the treadmill walking protocol used by Sawka et al. (1992) showed that a 3% loss in body mass was required to alter performance in a temperate environment, however, only a 2% loss in body mass reduced performance in hot environmental conditions. Both Walsh et al. (1994) and Below et al. (1995)
observed decrements in exercise performance over 60 min in the heat with similar levels of dehydration (1.8% BM and 2% BM respectively). During supramaximal intermittent running exercise, Maxwell and colleagues (1996) found that only marginal sweat-induced decreases in body mass (1-2%) were required to cause considerable decrements in performance.

Reports from moderate intensity, ergometer exercise could underestimate the body mass deficits required to curtail performance during high-intensity intermittent running. This is because potentially in comparison with steady-state exercise, a larger mass of muscle tissue is active and for brief periods workloads are disproportionately high. Table 2.1 includes a number of mean relative fluid losses recorded during competitive soccer matches in the heat. These levels of dehydration appear sufficient to significantly impair cardiovascular and thermoregulatory function. In addition to the values reported in the table, Mustafa and Mahmoud (1979) observed losses in players of up to 4 l during a competitive soccer game. Similarly Bangsbo (1994b) reports losses in some individuals of up to 3.5 l. Levels of fluid deficit equivalent to these (~5% BM) are likely to seriously compromise the ability of players to perform successfully during a match and have been shown to decrease exercise capacity by around 30% during other forms of exercise (Saltin and Costill, 1988).

Team sports not only require a capacity to perform at high exercise intensities but also include complex motor skills. Dehydration will therefore have a greater impact on the outcome of team sports than other forms of continuous exercise as optimal cognitive function is required throughout matchplay in addition to the maintenance locomotor function (Burke, 1997). Gopinathan et al (1988) noted that performance in a variety of cognitive tasks was adversely affected when the level of dehydration during exercise in the heat reached 2% of initial bodyweight. During an intermittent exercise protocol (LIST), McGregor et al. (1999) also showed that soccer specific skill deteriorated by 5% without the provision of fluid, but was maintained when fluid was ingested.

2.1.2.2 Substrate availability
In cool and moderate ambient conditions (≤20°C) the point of exhaustion during prolonged exercise has been shown to coincide with low concentrations of muscle glycogen. This has been reported during cycling exercise (Bergstrom et al., 1967;
Hermansen et al., 1967), treadmill running (Tsintzas et al., 1996) and recently in the
capacity to perform prolonged high-intensity intermittent exercise (Foskett et al.,
2004; Nicholas et al., 1999). These laboratory findings are in agreement with the
earlier field observations of Saltin (1973) who noted that total distance covered during
a soccer match was strongly dependent on the concentration of glycogen within \textit{m. vastus lateralis}. A recent report illustrated that similar depletion patterns to those seen
in the laboratory studies above were apparent during a soccer match (Krustrup et al.,
2003). The results show that during 90 min of match-play 35% of type 2 fibres are
almost depleted of glycogen and 12% are completed depleted.

During exercise in a hot environment the involvement of muscle glycogen in the
fatigue process is less well understood. A number of studies have detected elevated
glycogen utilisation rates in the heat compared with cooler environmental
temperatures, however, because of the rapid onset of hyperthermia during exercise in
hot environments the point of fatigue does not routinely coincide with the depletion of
skeletal muscle glycogen. Morris and colleagues (2005), report significantly higher
glycogen concentrations in \textit{m. vastus lateralis} at the point of exhaustion during
exercise in a environmental temperature of 33°C compared with 20°C (200 mmol
glycosyl units·kg DM\(^{-1}\) vs. 145 mmol glucosyl units·kg DM\(^{-1}\)). This has also been
observed during a cycling protocol conducted by Parkin and co-workers (1999). They
report that the muscle glycogen concentration at the point of fatigue in 40 °C was
twice that recorded during exercise in 20°C. A similar trend was revealed by Nielsen
and associates (1990), but in this investigation a number of the subjects did not reach
the point of fatigue before the end of the exercise protocol. In the studies discussed
above fatigue occurs considerably earlier in the heat and it appears that under these
circumstance the early onset of fatigue may not be a metabolically driven
phenomenon.

\subsection{2.1.2.3 High temperature \textit{per se}}
The rate and magnitude of heat production and storage during exercise is likely to
differ considerably between individuals (van Marken Lichtenbelt et al., 2001).
However, it has been demonstrated that under uncompensable heat loads exhaustion
occurs at a high T\text{core} (González-Alonso et al., 1999b; Nielsen et al., 1993; Walters et
al., 2000) independently of heat storage, acclimation, initial T\text{core} or skin temperature.
(T_{sk}) \ (Bruck \ and \ Olschewski, \ 1987; \ González-Alonso\ et\ al.,\ 1999b). Sawka \ and 
colleagues (1992) reported that 75\% of subjects taking part in intermittent walking 
exercise in the heat reached exhaustion at a T_{core} around 39.1°C. In several studies 
using a simulation In several studies using a simulation model of intermittent field 
sports (LIST), fatigue in hot environmental conditions (30°C) was shown to occur at 
an average of 39.5°C and 39.6°C in well trained males games players (Morris et al., 1998b; 2003 respectively) and similar temperatures of 39.4°C, 39.5°C and 39.6 °C in 
female games players (Morris et al., 2000; Sunderland, 2001; Sunderland and Nevill, 2005 respectively) regardless of fluid ingestion or acclimation. A recent analysis of 
131 field trials indicates that 50\% of individuals will reach exhaustion at a T_{core} of no 
more than 39.5°C (Sawka et al., 2001). When these observations are compared with 
the T_{rec} following competitive soccer matches (Table 2.1) it is clear that core 
temperatures regularly reach levels that would potentially limit capacity and 
performance in the majority of individuals. Endurance trained athletes may be able to 
tolerate higher core temperatures before volitional exhaustion and will fatigue at 
temperatures close to 40°C with little inter-individual variation (González-Alonso et 
al., 1999b; Nielsen et al., 1993; 1997). Additionally none of the endurance athletes in 
the studies above reached the point of exhaustion with T_{core} values of less than 
39.5°C. The ability to achieve these relatively high temperatures may be due to the 
very high aerobic capacity and other training adaptations of this elite cohort. 
However, the attainment of these higher core temperatures could be attributed to the 
exercise mode used, for example cycle ergometry facilitates venous return due to the 
seated posture and leg cranking (Sawka et al., 2001), this possibly allows the 
maintenance of Q for a relatively longer period compared with other modes of 
exercise.

An alternate cause of fatigue during hyperthermia is a high temperature within the 
active muscle tissue. It has been suggested that high temperatures within the active 
musculature may contribute to fatigue by altering motor function directly and 
independently of the effects on substrate utilisation. If this was to occur then the 
functional capacity of the motor unit would be reduced due structural alterations in a 
number of proteins (Nielsen et al., 1990). The proteins that are potentially effected play key roles in the distribution of electrolytes across cell membrane, the release and
uptake of Ca$^{2+}$ by the sarcoplasmic reticulum, and are pivotal in the interaction between actin and myosin and the mitochondrial respiratory chain (Hargreaves and Febbraio, 1998).

Some controversy exists as to whether the force generating capacity of muscle tissue is decreased in the range of temperatures seen during exercise. One of the first studies to highlight a potential decrement in isometric force production was conducted by Edwards and colleagues (1972). They reported decrements in force production at $T_{\text{mus}}$ values as low as 38.6°C and at higher temperatures (>40°C) it has been shown that mitochondrial function can be determinately affected (Brooks et al., 1971). During prolonged cycle ergometry Parkin et al. (1999) noted higher muscle temperatures at the point of exhaustion in the heat compared with exercise in cooler air temperatures. Morris and co-workers (2005) reported similar findings during prolonged intermittent exercise. González-Alonso et al. (1999b) noted that fatigue coincided with a temperature in m. vastus lateralis of 40.7 - 40.9 °C. In the study of Morris et al. (2005), muscle temperature was 40.2 °C at the point of fatigue. The interpretation of $T_{\text{mus}}$ recorded during prolonged exercise tests is difficult, as under these circumstances alterations in $T_{\text{mus}}$ are accompanied by cardiovascular strain and increases in $T_{\text{core}}$. In addition to changes in temperature Parkin and colleagues (1999) discovered significant elevations in IMP concentration at the point of fatigue when glycogen concentrations were adequate. As this would only usually be apparent in muscle with depleted glycogen, the authors suggest this could provide evidence of a temperature induced metabolic perturbation. More recent studies have demonstrated that no change in isometric (Thornley et al., 2003) or isokinetic (Cheung and Sleivert, 2004) maximum voluntary contraction (MVC) of the knee extensors is evident following hyperthermia. However, the inherent limitation in measuring MVC in this manner is that central factors are involved in the generation of force. This was highlighted in two recent studies that either stimulated the muscles electrically (Nybo and Nielsen, 2001b) or employed motor cortex stimulation (Todd et al., 2004) during hyperthermia. In both of these studies a decline in MVC following hyperthermia was detected, but this deficit in force generation was eliminated to a large degree when the direct stimuli were imposed. Todd and colleagues (2004) suggest that although the majority of the decrement in voluntary drive to produce muscular force occurs at or
above the level of the motor cortex, some of reduction can possibly be accounted for by temperature-related changes in the contractile properties of muscle.

2.1.2.4 Central Factors

The potential peripheral causes of fatigue discussed so far in this chapter do not adequately explain the phenomenon of a critical core temperature (Section 2.1.2.3). The most popular hypothesis is that the exercise-critical $T_{\text{core}}$ may be a CNS mediated protective mechanism that prevents potential damage that may be inflicted by further heat storage. It is thought that this mechanism manifests as a substantial change or dysfunction within the CNS at high temperatures (Nielsen and Nybo, 2003). Nielsen and associates (2001) were the first to use electroencephalograph (EEG) recordings in the examination of brain activity and fatigue during exercise in humans. Their cycling study comparing hot and moderate environmental temperatures reports a progressive reduction in activity from the prefrontal cortex as progressive hyperthermia is induced. An increase in the ratio of $\alpha$ to $\beta$ frequency bands appears to occur in parallel with the rise in oesophageal temperature ($T_{\text{oes}}$). Normally a shift in $\beta$-bands toward $\alpha$-bands is seen in the transition in arousal from alert to sleep or drowsiness. The authors suggest that signals arising from the hypothalamus could be responsible for these changes, but the specific location or basis of the alterations of brain function is not currently understood. It has recently been shown that hyperthermia during exercise is associated with a reduction in cerebral blood flow (Nybo et al., 2002a). The subjects’ cardiac output remained unchanged during this protocol, which implies these alterations may be due to deliberate regional changes in blood flow to certain areas of the brain. Nunneley and colleagues (2002) addressed this possibility using positron emission tomography, they examined regional cerebral metabolism at rest during hyperthermia. Areas of decreased metabolism were apparent in certain parts of the brain and regions of increased metabolic activity were visible in the preoptic area of the anterior hypothalamus, presumably as this controls thermoregulatory processes. These changes that occur in the brain during exercise with hyperthermia appear to result in an impairment of heat removal. A recent study in which thermocouples were placed in the jugular vein and the aorta, reported a reduced rate of heat removal from the brain and hypothesised that this was primarily the result of a 20% decrease in cerebral blood flow (Nybo et al., 2002b).
The volitional cessation of prolonged exercise in the heat often coincides with a high level of perceived exertion. An increase in RPE has been shown to correlate with the frequency index of EEG obtained over the prefrontal cortex (Nybo and Nielsen, 2001b). Subjective ratings of perceived exertion have also been reported to increase in parallel with T_{core} and heart rate (Nielsen et al., 2001; Nybo and Nielsen, 2001a). A number of studies illustrate the trend for RPE to increase in line with T_{core} during both static ergometry and intermittent free-running exercise, with hyperthermia and the combination of hyperthermia and dehydration (González-Alonso et al., 1998; 1999a; 2000; 1999b; Morris et al., 1998a; 1998b; 2003; Nielsen et al., 1993; 1997). These findings suggest that there is a greater reduction in the motivational drive to continue exercise under thermoregulatory stress regardless of the exercise intensity. The changes of mind state in the heat are likely to be due to biochemical alterations occurring at both neuronal and regional brain level (Nybo and Secher, 2004; Racagni and Brunello, 1999), however these neurochemical adjustments are beyond the scope of this discussion.

Setting aside the volitional causes of fatigue, the consequence of attaining a critical brain temperature may be an inability to voluntarily recruit a sufficient number of motor units. The majority of studies published recently in this area suggest that neuromuscular function is markedly impaired during hyperthermia. Nybo and Nielsen (2001b) reported reduced MVC of the knee extensors following cycle exercise in the heat. But when the muscle was electrically stimulated there was no change between thermal conditions. Decreased central activation was suggested as the cause of the loss in voluntary activation and in this study it appears to account for all of the loss in force development. More recently Morrison et al. (2004) reported similar findings when examining MVC in the same musculature after a passive heating protocol. The lower level of voluntary activation was fully restored when T_{core} recovered to basal levels. Because of the lack of any significant exercise induced cardiovascular strain in their protocol the authors suggest that T_{core} alone is the primary thermal input responsible for these alterations in motor recruitment. However, unlike the previous study no involuntary contraction was imposed, so the loss of any function due to direct alterations of motor function cannot be determined. Todd et al. (2004) observed a decline in brief and sustained MVC performed when T_{core} was elevated to ~38.5°C. The decline was significantly less when the motor cortex of the brain was stimulated,
suggesting that the effects of hyperthermia act on or above the level of the motor cortical output. The authors of this study propose that the motor pathways involved may be altered by output from the thermoregulatory centres of the hypothalamus acting upon other structures including the cerebral cortex. Contrary to the findings above Cheung et al. (2004) observed no decrement in isokinetic MVC following passive heating. This experimental protocol is subject to the same methodological flaws as Morrison and associates’ study, as an involuntary stimulus was not imposed. Studies that have examined electromyographic (EMG) activity during exercise in the heat also report inconsistent findings. Faiti and co-workers (2001) observed no changes in EMG activity of the knee extensors or flexors whilst treadmill running under thermal stress. On the other hand Tucker and associates (2004) recorded a reduction in EMG activity of the quadriceps during exercise in the heat, compared with a temperate trial. This down-regulation occurred relatively early in the hot environment, at a time when $T_{\text{core}}$ was not significantly elevated. The authors suggest that this is an anticipatory response, which reduces muscle recruitment in order to curtail heat production. This idea may support recent suggestions that a central controller regulates the number of motor units that are recruited during exercise in the heat, in order to prevent excessive heat storage (Marino, 2004). This is a hypothetical mechanism whereby a continual adjustment of muscle recruitment is regulated by the CNS in order to avoid the catastrophic outcome of a high $T_{\text{core}}$. The model is conceptually similar to other models describing the central neural regulation of effort recently proposed by the same research group (Lambert et al., 2005; Noakes et al., 2005b; St Clair Gibson et al., 2005).

The anticipatory down regulation of skeletal muscle during self-paced exercise is suggested by these authors to occur during the early stages of exercise and respond relative to the rate of rise of $T_{\text{core}}$, rather than be initiated at a critical temperature. A number of studies using self-paced running and cycling provide evidence to support this concept. These studies all report that regardless of $T_{\text{core}}$ prior to exercise (Arngrimsson et al., 2004) or rates of heat storage (Marino et al., 2004; Tatterson et al., 2000) an exercise intensity is chosen that results in a similar rate of rise in $T_{\text{core}}$ between conditions/groups. These findings are in opposition to the critical limiting temperature hypothesis as regulatory responses in the continual adjustment model would occur when $T_{\text{core}}$ is relatively low.
A potential factor underpinning the development of central fatigue during prolonged exercise in the heat is altered serotonergic and catecholaminergic neurotransmission. Increases in the neurotransmitter serotonin (5-hydroxytryptamine; 5-HT) have been implicated for some time as a potential factor in the development of fatigue during prolonged exercise (Newsholme et al., 1987). This proposed mechanism appears applicable to the cessation of prolonged exercise as at rest an elevation in 5-HT is associated with perceived pain, fatigue and sleep (Dunn and Dishman, 1991; Young, 1986). There is currently some indirect evidence in humans suggesting that exercise in hyperthermic circumstances results in increased serotonergic neurotransmission (Bridge et al., 1999; Pitsiladis et al., 2002). It has been shown in rats that a pharmacologically induced increase in serotonergic activity will transiently increase Tcore (Lin et al., 1998). Also supporting the involvement of 5-HT in fatigue are a number of studies that have administered pharmaceuticals with 5-HT agonistic properties (Davis et al., 1993; Marvin et al., 1997; Wilson and Maughan, 1992). These studies generally report a reduction in exercise capacity under the influence of number of different 5-HT agonists. Limited information is available regarding the effects of these pharmaceuticals in the heat, one study has shown that during exercise and hyperthermia paroxetine (a 5-HT re-uptake inhibitor) had no influence on exercise capacity or markers of 5-HT activity (Strachan et al., 2004).

During cycling exercise in the heat the activity of the neurotransmitter dopamine also appears to be a reliable predictor of exercise tolerance (Bridge et al., 2003) and has been shown to correlate with the increase in perceived effort (Pitsiladis et al., 2002). In animals a clear relationship has been demonstrated between dopaminergic activity in the preoptic hypothalamus and changes in core temperature (Hasegawa et al., 2000).

An easily obtainable marker of serotonergic activity that is frequently measured in exercise physiology is the blood-borne neuroendocrine prolactin. Serotonergic neurones project to hypothalamic sites and stimulate prolactin secretion through the activation of 5-HT receptors (van de Kar et al., 1996), hence circulating concentrations of this hormone reflect changes in central 5-HT activity. Elevations in prolactin concentrations have been found at the point of fatigue during treadmill
running exercise (Daly et al., 2005) and after both 90 min (Ali, 2002) and exhaustive (Foskett, 2003) intermittent exercise (LIST). In both running and cycling investigations conducted in hot environmental conditions increased serum prolactin concentration have been shown to coincide with increased $T_{\text{core}}$ and $T_{\text{sk}}$ (Bridge et al., 1999; Brisson et al., 1986; Melin et al., 1988). It is worth noting that comparisons of prolactin concentrations between thermoneutral and hot environments may be confounded by decreased prolactin clearance from circulation in hyperthermia, due to decreased renal blood flow (Rowell et al., 1968).

Current understanding of how projections of these neurotransmitters alter the hypothalamus is limited and considering the complexity of CNS as a central governor implicating a single neurotransmitter may be an overly reductionist approach. However, it does appear that the ratio of 5-HT to dopamine may be pivotal in the tracking the development of fatigue during prolonged exercise in the heat.

During prolonged high-intensity intermittent exercise in the heat the development of fatigue and subsequent exhaustion may be due to a number of mechanisms or the synergistic effect of several. The potential interaction of these mechanisms is represented schematically in Figure 2.1. Stroke volume is likely to be reduced in the heat and if hyperthermia is severe or dehydration is imposed, cardiac output may be compromised. Under these circumstances systemic vascular resistance may be elevated, constraining the loss of heat from the periphery and further exacerbating hyperthermia. It is also possible that blood flow and hence $O_2$ and substrate delivery to active muscle may be decreased, this or the effects of an elevated muscle temperature could accelerate the utilisation of glycogen and lactate production in the active musculature. However, results from a number studies examining fatigue in the heat show that muscle contains a sufficient concentration of glycogen and that lactate resides in concentrations that would not limit locomotion. Furthermore, skeletal muscle has been shown to be capable of generating sufficient force when externally stimulated. These findings suggest that the cause of fatigue may lie "up-stream" of muscle metabolism and is possibly related to neurobiological changes within the CNS that are associated with the attainment of a critical core temperature.
Figure 2.1 The potential interaction of physiological mechanisms that lead to fatigue during exercise in the heat. Adapted from Cheuvront et al. (2003) and Hargreaves & Febbraio (1998).

2.1.3 Fluid intake

The magnitude of fluid lost as sweat during sports similar to soccer in hot environments is substantial during both competitive match-play (Table 2.1) and training (Maughan et al., 2004; Shirreffs et al., 2003). These studies report wide individual variability in the relative amount of BM lost, but all note a tendency for players to voluntarily dehydrate when fluid is provided ad libitum (within the constraints of the game). In a well controlled experimental exercise model (LIST) conducted in 30°C, Morris et al. (1998b) showed that when subjects were provided with either water ad libitum and no water they exhibited similar sweat rates (1.7 l·h⁻¹) but lost approximately 1% or 2% of pre-exercise body weight under the two conditions respectively. In this study participants were provided with considerably more opportunities to drink than is likely during actual match-play. The fluid deficit seen during this study suggests that self-selected fluid intake fails to adequately replace fluid losses, which are likely to be more substantial during a competitive game. The volume of fluid required to fully replace water loss through the sweat and urine under these conditions is often more than can be tolerated and if prescribed
would potentially cause gastro-intestinal (GI) distress during exercise. Using prolonged moderately intense cycling exercise (60% \( \dot{V}O_2 \text{ max} \)), Galloway and colleagues (2000) determined that the maximum tolerable drinking schedule for CHO based rehydration solutions was a 7.1 ml·kg BM\(^{-1}\) as a bolus before exercise followed by serial feedings of 3.6 ml·kg BM \(^{-1}\)·10min\(^{-1}\). During intermittent high-intensity exercise more fluid is likely to be retained in the stomach (for discussion see Section 2.1.6.1). Despite this, Morris and colleagues (2003) successfully administered a 6.5 ml·kg BM\(^{-1}\) bolus of a 6.5% CES followed by 4.5 ml·kg BM \(^{-1}\)·15 min\(^{-1}\) during the LIST protocol in an environmental temperature of 30°C. In their study the degree of hypohydration following exercise was equivalent to between 0.7 – 1.3% of pre-exercise body mass. This appears to be a suitable drinking regime during this mode of exercise and the magnitude of fluid lost is unlikely to have a critical impact on exercise capacity and performance. During prolonged high-intensity exercise in the heat the limiting factors determining fluid replacement appear to be the gastric volume that can be tolerated and the availability of drinks during match-play. It has been shown that in order to replace all fluid lost during exercise, around 150% of the total fluid deficit must be replaced (when ingesting a well formulated sports drink). This has been shown to return subjects to a euhydrated state and to compensate for ongoing fluid losses in urine that occur after exercise (Maughan and Leiper, 1995; Shirreffs and Maughan, 1998a; Shirreffs et al., 1996). During a professional football match in a hot environment it is unlikely that players would have the opportunity or the capacity to ingest enough fluid to prevent the development of dehydration.

2.1.4 Electrolyte replacement

Electrolytes are considered an important addition to sports drinks and an essential component of oral rehydration solutions (American Physiological Society, 2003). During exercise, salts (mainly Na\(^+\)) are lost along with water via sweating and hence replacing both water and electrolytes during and after exercise will help maintain the osmolality of the extracellular fluid. Sodium is the major ion in plasma and a considerable amount may be lost when sweat rates are high and prolonged. During a 90 min soccer training session at 32°C Shirreffs and colleagues (2003) recorded net Na\(^+\) losses in players from a professional Spanish team of 1.5 g (0.6 – 3.0). Similarly, the same research group (Maughan et al., 2004) noted Na\(^+\) losses of 2.3 g (1.2 – 3.1) during a training session (27°C, 55 %RH) of similar duration in professional English
players. Replacing Na\(^+\) losses during and after competition will aid players to return to and maintain a euhydrated state. A number of well controlled studies have demonstrated that when drinks containing Na\(^+\) are compared with water alone, plasma volume is better maintained (Maughan and Leiper, 1995; Nielsen et al., 1986), the decline is plasma osmolality is less (Nose et al., 1988b, 1988a) and smaller volumes of urine are excreted (Maughan et al., 1994; Shirreffs and Maughan, 1998a; Shirreffs et al., 1996). A decrease in plasma osmolality caused by haemodilution has been associated with lower concentrations of circulating antidiuretic hormone and elevations in aldosterone which result in diuresis (Nose et al., 1988b). It is also thought that low plasma osmolality results in a reduction in the drive to drink (Maughan and Leiper, 1995; Nose et al., 1988a). When large volumes of fluid are ingested the addition of Na\(^+\) to the drink will prevent haemodilution and provide better maintenance of plasma osmolality. By utilising solutions with a range of Na\(^+\) concentrations, Maughan and Leiper (1995) showed that individuals who were hypohydrated to 2% of BM and subsequently ingested fluid volumes equivalent to 150% of losses, required a rehydration solution containing 50 mmol·l\(^{-1}\) of Na\(^+\) in order to achieve and maintain fluid balance. Similar findings were published subsequently by Shirreffs et al. (1996), reporting that a 61 mmol·l\(^{-1}\) solution was required to completely replace fluid losses under the same conditions. This investigation also demonstrated that when a solution containing 23 mmol·l\(^{-1}\) of Na\(^+\) was ingested in a volume equivalent to twice the fluid losses, subjects did not remain in positive fluid balance. Therefore it appears that the optimal Na\(^+\) concentration for rehydration solutions is higher than that found in the majority of sports drinks. Ideally this should be similar to that of the individual’s sweat (Shirreffs and Maughan, 1998a), however, solutions with Na\(^+\) concentrations similar to sweat have been shown to be unpalatable (Maughan and Leiper, 1993).

Other electrolytes such as K\(^+\) and Mg are often added to rehydration solutions. Under certain circumstances KCl may be equally as effective as NaCl in retaining water (Maughan et al., 1994). There is also anecdotal evidence that suggests replacing Mg losses can reduce the incidence of muscle cramping (Roffé et al., 2002; Schindler et al., 1998). However, the addition of these electrolytes to sports drinks is thought to be of lesser importance than Na\(^+\) (Coyle, 2004; Shirreffs et al., 2004).
The addition of electrolytes to sports drinks and oral rehydration solutions is also important in the prevention of dilutional hyponatraemia. This condition develops when large volumes of hypotonic solutions are ingested over prolonged periods of time and is accelerated by the loss of Na⁺ through sweat. (for a position statement see Hew-Butler et al., 2005) The clinical consequences of hyponatraemia (and ultimately the development of hyponatraemic encephalopathy) are typically more severe than the effects of exercise induced dehydration, which in some respects acts to prevent the onset of this condition (Noakes et al., 2005a).

2.1.5 Carbohydrate Ingestion

It is well established that the point of fatigue during prolonged exercise in cool or temperate environmental conditions coincides with the depletion of CHO in the working muscles and liver. In these situations providing an exogenous supply of carbohydrate may delay the onset of fatigue and increase exercise performance by maintaining a high rate of CHO oxidation. During prolonged high-intensity intermittent running in cool environmental conditions (<25°C), twenty studies have reported augmentations in capacity or performance during either actual match-play² or laboratory simulations³ when CHO solutions were ingested. The feeding strategies adopted in these studies primarily concentrate on substrate provision rather than fluid replacement, as the thermoregulatory demands of exercise under these conditions are not substantial. The evidence suggesting that CHO ingestion improves exercise performance and capacity in the heat is less convincing. Table 2.2 summarises the 10 published studies that have found improvements in exercise performance and capacity in stressfully hot environmental conditions (≥25°C or equivalent to a wet bulb globe temperature ≥23°C) when ingesting CHO. Similarly Table 2.3 outlines the 4 studies that have reported no improvement in capacity or performance when ingesting CHO solutions during exercise in the heat.

² Foster et al. (1986); Kirkendall et al. (1988); Reilly and Keane (2002); Simard et al. (1988).
³ Ali et al. (2002); Davis et al. (2000); Davis et al. (1999); Foskett et al. (2004), (2003); Ingle et al. (2000); Leatt and Jacobs (1989); MaClaren and Close (2000); Nicholas et al. (1999), (1995); Northcott et al. (1999); Quanz (1999); Shirreffs and Merson (2003); Walton and Rhodes (1997); Welsh et al. (2002); Winnick et al. (2005).
2.1.5.1 Exercise Capacity

When moderately intense exercise is conducted under compensable heat stresses, glycogen reserves are likely to become depleted before $T_{core}$ approaches 40°C. However, under circumstances where the body is unable to dissipate heat as rapidly as required (such as when considerable fluid losses have occurred), $T_{core}$ may reach a level at which blood flow and $O_2$ delivery is compromised and/or where motor activity is inhibited and central causes of fatigue limit exercise capacity. In addition, heat stress during exercise has been shown to elicit an elevated hepatic glucose production that supersedes the required utilisation rate and hence results in hyperglycaemia (Angus et al., 2001; Febbraio, 2001). Under thermally stressful environmental conditions, exercise is predominantly terminated before any potential benefits of exogenous CHO are evident and therefore in these circumstances it may be more important to aim drinking strategies primarily at fluid replacement rather than energy provision. This suggestion has been explored by only 4 peer reviewed studies, these investigations assessed exercise capacity while subjects ingested CHO solutions or flavoured water. Two of these investigations employing cycling protocols reported improvements in performance when CHO was ingested (Carter et al., 2003; Galloway and Maughan, 2000), on the other hand both a cycling study (Febbraio et al., 1996b) and a LIST protocol (Morris et al., 2003) reported no benefit from ingesting CHO. The different findings of these studies can to some extent be explained by methodological differences in the protocols. In a well designed cycling study, Carter and colleagues (2003) reported improvements in exercise capacity when their subjects ingested CHO at both an exercise intensity that the authors envisaged would be compensable (60% $\dot{VO}_2_{max}$) and an intensity that was uncompensable (70% $\dot{VO}_2_{max}$). The time taken to reach the point of exhaustion under these two conditions would suggest that peripheral causes of fatigue dominate during the lower exercise intensity compared with the higher exercise intensity (~2 h at 60% $\dot{VO}_2_{max}$ vs. ~1 h at 70% $\dot{VO}_2_{max}$). However, at both exercise intensities fatigue occurred at a similar level of hyperthermia, so it appears that although less stressful, the lower workrate was not thermally compensable and therefore a high $T_{core}$ was probably the cause of fatigue at the two intensities. At both intensities a similar improvement in capacity was seen when ingesting a 6.4% CES compared with flavoured water. As substrate availability
<table>
<thead>
<tr>
<th>Study</th>
<th>Ambient Temp &amp; RH</th>
<th>Protocol</th>
<th>Post $T_{core}$</th>
<th>Test drinks</th>
<th>Volume ingested</th>
<th>Effect of CES</th>
</tr>
</thead>
<tbody>
<tr>
<td>Below et al. (1995)</td>
<td>31°C 54%</td>
<td>50 min cycling</td>
<td>38.7°C</td>
<td>6% CES FW</td>
<td>calculated to replace 100% of estimated sweat loss 7% increase in performance during a subsequent 10 min performance test</td>
<td></td>
</tr>
<tr>
<td>Carter et al. (2003)</td>
<td>35°C 30%</td>
<td>cycle to exhaustion</td>
<td>39.1°C</td>
<td>6.4% CES FW</td>
<td>8 ml·kg BM$^{-1}$ (pre) 3 ml·kg BM$^{-1}$.15 min$^{-1}$ 14.5% increase in capacity at 60% $\text{VO}_2$ max (23 min improvement) and 13.5% increase (10 min improvement) at 70%</td>
<td></td>
</tr>
<tr>
<td>Davis et al. (1988b)</td>
<td>27°C 68%</td>
<td>120 min cycling</td>
<td>39°C 39.2°C</td>
<td>2.5% CES FW</td>
<td>275 ml·20 min$^{-1}$ faster performance with 6% CES during a test administered 30 min after the 2h of cycling. No differences with 2.5% CES.</td>
<td></td>
</tr>
<tr>
<td>Fritzsche et al. (2000)</td>
<td>35°C 50%</td>
<td>122 min cycling</td>
<td>38.8°C 38.5°C</td>
<td>6% CES FW</td>
<td>$\sim$1100 ml (pre) $\sim$600 ml·30 min$^{-1}$ Maximal power measured every 30 min CES - 7% decline in maximal power. FW – 10% decline in maximal power.</td>
<td></td>
</tr>
<tr>
<td>Galloway and Maughan (2000)</td>
<td>30°C 71%</td>
<td>cycle to exhaustion</td>
<td>39.5°C 39.5°C</td>
<td>2% CES 15% CES no fluid</td>
<td>7.14 ml·kg BM$^{-1}$ (pre) 3.57 (2%) or 1.79 ml·kg BM$^{-1}$.10 min$^{-1}$ (15%) improved capacity with 2% CES (35 min) and lesser improvement with 15% CES (14 min), compared with no fluid</td>
<td></td>
</tr>
<tr>
<td>Millard-Stafford et al. (1992)</td>
<td>29°C 72%</td>
<td>treadmill race</td>
<td>39°C 39.4°C</td>
<td>7% CES FW</td>
<td>400 ml (pre) $\sim$750 ml·h$^{-1}$ (every 5km) increased performance over last 5km (2.5 min faster)</td>
<td></td>
</tr>
<tr>
<td>Millard-Stafford et al. (1997)</td>
<td>28°C 69%</td>
<td>treadmill race</td>
<td>39°C 39.5°C</td>
<td>6% CES 8% CES ad libitum 10 - 706 rnl drinks, no differences in performance between 6% &amp; 8% CES.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Millard-Stafford et al. (2005)</td>
<td>WBGT 26°C</td>
<td>outdoor running</td>
<td>39.8°C 40.1°C</td>
<td>6% CES 8% CES FW</td>
<td>400 ml (pre) 250 ml·5 km$^{-1}$ Over 5 km self-paced phase performance increased by 8% with 8% CES and 7% with 6% CES compared with FW.</td>
<td></td>
</tr>
<tr>
<td>Murray et al. (1987)</td>
<td>33°C 44%</td>
<td>intermittent cycling</td>
<td>$\sim$38.6°C</td>
<td>5% CES 2 ml·kg BM$^{-1}$.15 min$^{-1}$ Increased performance with 6% and 7% CES during the self-paced phases of the test.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ostojic and Mazic (2002)</td>
<td>25°C 57%</td>
<td>soccer match-play</td>
<td>$\sim$38.8°C</td>
<td>7% CES FW</td>
<td>5 ml·kg BM$^{-1}$ (pre) 2 ml·kg BM$^{-1}$.15 min$^{-1}$ improved performance in a number of post-match soccer skill and power tests</td>
<td></td>
</tr>
</tbody>
</table>

Table 2.2 Summary of studies that have reported improvements in exercise capacity or enhanced exercise performance when ingesting CHO solutions in hot environmental conditions. Post-exercise $T_{core}$ values relate to the respective test drink in the adjacent column.
<table>
<thead>
<tr>
<th>Study</th>
<th>Ambient Temp &amp; RH</th>
<th>Protocol</th>
<th>Post T&lt;sub&gt;core&lt;/sub&gt;</th>
<th>Test drinks</th>
<th>Volume ingested</th>
<th>Effect of CES</th>
</tr>
</thead>
<tbody>
<tr>
<td>Davis et al. (1988a)</td>
<td>27°C 68%</td>
<td>120 min cycling</td>
<td>38.8 °C</td>
<td>6% CES</td>
<td>275 ml·20 min&lt;sup&gt;-1&lt;/sup&gt;</td>
<td>no differences between drinks in performance tests administered during and after the protocol.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>65% VO&lt;sub&gt;2&lt;/sub&gt;&lt;sub&gt;max&lt;/sub&gt;</td>
<td>39 °C</td>
<td>12% CES</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>38.8 °C</td>
<td>FW</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Febbraio et al. (1996b)</td>
<td>33°C 20-30%</td>
<td>cycle to exhaustion</td>
<td>39.3 °C</td>
<td>4.2% CES</td>
<td>250 ml·15 min&lt;sup&gt;-1&lt;/sup&gt;</td>
<td>no differences in endurance capacity between fluid conditions.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>70% VO&lt;sub&gt;2&lt;/sub&gt;&lt;sub&gt;max&lt;/sub&gt;</td>
<td>39.2 °C</td>
<td>7% CES</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>39.2 °C</td>
<td>14% CES</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>39.2 °C</td>
<td>Placebo</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Millard-Stafford et al. (1990)</td>
<td>30°C 68%</td>
<td>simulated triathlon</td>
<td>39.8 °C</td>
<td>7% CES</td>
<td>2 ml·kg BM&lt;sup&gt;-1&lt;/sup&gt; post swim</td>
<td>no differences in performance during the three exercise modes.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(swim 5 km, cycle 40 km, run 10 km)</td>
<td>39.8 °C</td>
<td>FW</td>
<td>2 ml·kg BM&lt;sup&gt;-1&lt;/sup&gt;-8 km&lt;sup&gt;-1&lt;/sup&gt; cycle</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2 ml·kg BM&lt;sup&gt;-1&lt;/sup&gt;-3.2 km&lt;sup&gt;-1&lt;/sup&gt; run</td>
<td></td>
</tr>
<tr>
<td>Morris et al. (2003)</td>
<td>30°C 42%</td>
<td>shuttle running to exhaustion (LIST)</td>
<td>39.6 °C</td>
<td>6.5 % CES</td>
<td>6.5 ml·kg BM&lt;sup&gt;-1&lt;/sup&gt; (pre)</td>
<td>No differences in exercise capacity or sprint performance between test drinks.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>39.4 °C</td>
<td>FW</td>
<td>4.5 ml·kg BM&lt;sup&gt;-1&lt;/sup&gt;-19 min&lt;sup&gt;-1&lt;/sup&gt;</td>
<td></td>
</tr>
</tbody>
</table>

Table 2.3  Summary of studies that have reported no exercise capacity or performance benefits of ingesting CHO solutions in hot environmental conditions. Post-exercise T<sub>core</sub> values relate to the respective test drink in the adjacent column.
is unlikely to be the cause of fatigue during these trials, the authors speculate that the CNS may exhibit an improved tolerance to a raised T<sub>core</sub> when CHO is consumed. Galloway and Maughan (2000) report comparable exercise capacities when ingesting a CES using a similar experimental protocol to Carter and colleagues. In this study subjects were able to exercise for longer when ingesting a 2% CES compared with a 15% CES. However, these drinks were not isovolumetric and were aimed at replacing 150% of BM loss and 100% of BM lost in the 2% and 15% trials respectively. Therefore the benefits of CHO provision cannot be determined independently of fluid provision. Their findings show that larger volumes of dilute CHO solutions may be more beneficial in the heat compared with smaller volumes with higher carbohydrate concentrations, perhaps due to fluid provision or the gastric emptying characteristic of the two drinks. This highlights the primary importance of fluid replacement rather than substrate provision when the thermoregulatory demands of exercise are great.

Opposing the conclusions of the studies discussed above is the work of Febbraio and colleagues (1996b). Their study showed that four different CHO solutions with concentrations over the range encountered in commercially available sports drinks had no effect on cycling capacity at 70% V<sub>O</sub><sub>2</sub><sub>max</sub>. The range of concentrations examined, the volume ingested and the level of hyperthermia achieved were comparable with the previous studies discussed above. However the same cohort of subjects was not used for all trials, subjects only completed the test under 2 of the 4 fluid conditions so the possibility of inter-individual variation must be taken into consideration when interpreting these results. Interestingly the authors also repeated all trials at 5°C and found that the CHO solutions did increase time to exhaustion when compared with the FW under these colder conditions. A recently published study that is more applicable to field sports (Morris et al., 2003) examined the effects of drinking a 6.5% CES and a FW during a match-play simulation model (LIST) performed until exhaustion in 30°C. This study also found that the CES failed to extend the time taken to fatigue when players reach limiting levels of hyperthermia rapidly. However, the case made by these authors is weakened somewhat by a trial order effect that manifests as a lower HR, a trend toward a lesser ΔT<sub>rec</sub> (P=0.07) and an increased exercise capacity. The changes seen in these variables are indicative of heat acclimation, which could have potentially been caused by the earlier exposure to the protocol or activities performed between trials. A further methodological flaw in
the study was the decision to terminate the exercise test at a $T_{rec}$ of 39.5°C. This safety measure resulted in 9 subjects being removed from the test; presumably these individuals would have been capable of continuing exercise and hence altered the outcome of the study.

Throughout the trials conducted during all four of the studies described in this section mean plasma glucose concentration remained above 4.3 mmol.l$^{-1}$. The investigations that made comparisons of CHO oxidation rates (Carter et al., 2003; Febbraio et al., 1996b; Galloway and Maughan, 2000), also report no differences between CHO solutions and FW. These data rule out the possibility that fatigue may be due to hypoglycaemia or that endogenous substrate availability was a limiting factor in exercise lasting <2.5 h in the heat. No differences were found in plasma volume in the studies that compared this variable between a CES and FW solution. Similarly other markers of hydration status were unchanged between solutions, suggesting that the rate of fluid delivery was not altered by the energy content or osmolality of drinks.

The study of Galloway and Maughan (1998) shows that intravascular volume is maintained better with a 2% CES compared with the 15% solution, however, considerably differing fluid volumes were ingested (3.1 l vs. 1.5 l) so the effects of the CHO on gastric emptying and fluid absorption cannot be determined.

The core temperatures observed at the termination of exercise in the above studies are within the range which is considered critical to the onset volitional fatigue and reduced neuromuscular function (see Section 2.2.3 for discussion). The lack of any apparent peripheral causes of fatigue in these investigations suggests that a central mechanism may be responsible for the cessation of exercise in these environmental temperatures, and therefore when ingested CHO increases capacity it may do so by eliciting an ergogenic effect upon the brain. There is some emerging evidence that supports this suggestion and proposes that brain metabolism may potentially be enhanced by the ingestion of CHO (Nybo, 2003; Nybo et al., 2003). In the first of these studies prolonged cycling exercise was performed with or without the ingestion of a 6% CES. The outcome without the ingestion of CHO was an impaired performance during sustained MVC of the knee extensors after exercise, compared with force evoked by electrical stimulation. In the second investigation Nybo and colleagues used a similar exercise protocol to calculate the a-v difference of glucose
and other metabolites across the brain. During the trial without CHO supplementation a reduced glucose uptake was detected, which was accompanied by a decreased cerebral uptake of O2 that was not adequately compensated for by ketone bodies or other substrates. A continuous systemic glucose supply to the CNS is essential during exercise as glucose storage in neural tissue is minimal, hence hypoglycaemia may limit cerebral energy turnover. The authors suggest that ingested CES stabilises cerebral glucose uptake during hypoglycaemia and so prevents/reverses central fatigue (Nybo and Secher, 2004). There is existing evidence to support this suggestion from a canine model (Kozlowski et al., 1981); direct infusion of glucose through the carotid arteries in exercising dogs has been shown to delay fatigue. In the studies of Nybo and colleagues discussed above, the effects of inhibited neuromuscular function and decreased cerebral energy turnover became evident at plasma glucose concentrations around 3 mmol·l⁻¹. This is a considerably more severe degree of hypoglycaemia compared with that evident in any of the feeding studies in the heat discussed earlier. The investigations summarised in Tables 2.2 and 2.3 found no severe fall in plasma glucose concentration and indeed some have noted a degree of elevation due to the hyperglycaemic effect of hyperthermia. In the studies of Nybo et al., the reductions in neuromuscular performance observed when subjects were hypoglycaemic are similar to those seen by the same authors in a study that involved hyperthermic subjects (Nybo and Nielsen, 2001b). One might speculate that if the influence on the CNS of hypoglycaemia and hyperthermia are somehow synergistic then decrements in exercise capacity may become apparent at lesser magnitudes of hypoglycaemia and hyperthermia. Within the studies discussed earlier that report longer exercise durations with CHO ingestion, there is a trend toward a higher (~0.2°C overall) Tcore at the point of fatigue in the CES trials. As speculated recently by Carter and colleagues (2003), this may indicate that the subjects’ heat tolerance is altered by the ingestion on CHO.

Without evidence of hypoglycaemia the ergogenic effect of CHO on the CNS may be related to alterations in ammonia concentration or central neurotransmitter activity. During prolonged exercise the elevation of systemic glucose often blunts the rise in plasma FFA. As the concentration of nonesterified fatty acids can determine the concentration of free tryptophan this may attenuate the increase in 5-HT and hence serotonin mediated fatigue. In support of this proposition, recent work has shown that
glucose infusion blunts the rise in extracellular brain serotonin in exercising rats (Béquet et al., 2001, 2002).

The weight of available evidence supports the concept of a capacity-critical core temperature (discussed in Section 2.1.2.3) and demonstrates that in the heat (~30°C) prolonged high-intensity exercise can only be sustained for around 2 h. During this relatively short duration exercise capacity is not limited by substrate availability and the potential benefits afforded by CHO ingestion may be mediated through a central rather than peripheral mechanism. This may be altered by fuel delivery to the brain or 5-HT, ammonia and other homeostatic changes which remain speculative at this time (Nybo and Secher, 2004). Due to a limited amount of published work and the flaws in the study of Morris et al (2003), the knowledge within this area is limited to cycling exercise and therefore cannot be confidently used to explain fatigue during prolonged, intermittent high-intensity exercise in the heat.

2.1.5.2 Exercise Performance

Under uncompensable heat loads whether or not the addition of CHO to rehydration solutions increases the time taken to fatigue is debatable. However, the available evidence demonstrating that performance may be increased before the point of fatigue is reached is more abundant. By and large these studies (summarised in Table 2.2) have employed both fixed distance, self-paced treadmill races (Millard-Stafford et al., 1997; 1992) and cycle ergometer time trials (Below et al., 1995; Davis et al., 1988b). To the author’s knowledge all published research that has used fixed distance treadmill or cycling time trials in the heat have reported an improvement in performance, when a well formulated CES was compared with water alone (when volumes of CES are ingested that adequately replace fluid losses and that contain CHO in concentrations up to 8% by volume). An alternative method used to assess performance during fixed workload cycling exercise is to routinely include self-paced performance tests at set intervals during the protocol. In a study designed to replicate the mean exercise intensity of high-intensity intermittent field sports and reproduce the number of sprints attempted in a match, Fritzche and colleagues (2000) incorporated maximal power tests into 110 min of cycling exercise. The authors found that ingesting a 6% CES improved maximal neuromuscular power compared with a similar volume of FW. In addition to hyperglycaemia, hyperinsulinemia, an increased
rate of CHO oxidation and an elevated $T_{core}$ were observed when the CES was compared with a FW. The investigators provide no explanation for the increase in $T_{core}$, but it is possible that the additional work performed during sprinting may have increased heat storage. The results of this study should be interpreted with some caution as the volumes of fluid ingested, although similar, were not isovolumetric (CES $3.39 \pm 0.23$ l vs. FW $3.28 \pm 0.21$ l). This is because these volumes were determined by the maximum volume of each solution that each subject could tolerate during preliminary tests. A similar method of incrementally assessing performance was used in the recent study conducted by Millard-Stafford et al. (2005). During an outdoor running protocol subjects completed 32 km with the inclusion of 2 maximal efforts, one at the midpoint and one in the final 5 km of the total distance. These authors report an 8.5% and 7% improvement in performance with an 8% and 6% CES respectively when compared with flavoured water. Interestingly an increase in $T_{core}$ was detected when the CHO solutions were compared with water. As with the previous study it seems likely the additional work performed during the performance tests may be accountable for the higher core temperatures.

There are several published studies that have failed to find any performance differences between CHO solutions and FW in the heat (Davis et al., 1988a; Millard-Stafford et al., 1990) (Table 2.3). In the study of Davis and colleagues subjects cycled for 1 hour at 65% $VO_2_{max}$ and the protocol incorporated 3 brief performance tests, one of which was administered 30 min after exercise. Before the final performance test had commenced many of the physiological variables measured had recovered considerably in comparison with basal levels. This perhaps provides some explanation as to why no overall differences were found in performance. The study of Millard-Stafford and associates was a rather complex multi-model triathlon simulation. During this protocol subjects completed a swim (1.5 km), a cycle (40 km) and a treadmill running phase (10 km). Test solutions were not ingested until after the swimming phase and the overall heat stress imposed by the protocol may have been decreased considerably by the pool swim that preceded the static modes of exercise. These methodological factors may go some way to explaining why no enhancement in performance was observed in these two investigations in contrast to the studies summarised in Table 2.3. Similarly, Morris and colleagues (2003) observed no changes in sprint performance over the 15 m sprint phases of their field sport specific
exercise capacity test (LIST). However the interpretation of these findings may be limited by fundamental flaws in this study that have been outlined previously.

When CHO drinks are ingested during exercise in the heat, reports of increased exercise performance due to high voluntary workrate appear to occur over a timescale when metabolic evidence would suggest that muscle glycogen concentration is not limited. Several authors that have reported enhanced performance in this way have postulated that a higher blood glucose concentration may lead to an increased CHO oxidation rate within the active musculature. But an increase in the oxidation rate of CHO is not a frequently reported occurrence, in fact only two studies (Davis et al., 1988b; Fritzsche et al., 2000) have detected any change in CHO metabolism between CHO and FW solutions via expired air analysis. Using a similar protocol to the two studies mentioned above, Jentjens et al. (2002) recently quantified glucose oxidation using isotopic tracer techniques. Their investigation found that glucose oxidation was reduced by ~10% in the heat regardless of whether a CHO or placebo solution was ingested. This suggests that the increases in human performance that occur in the heat over durations of less than 2 h, potentially happen independently of any additional glucose supplied from an exogenous source.

The mechanism by which CHO enhances voluntary workrate in the heat may involve factors other than substrate delivery to the muscle. Carter and colleagues have recently proposed a CNS-mediated ergogenic effect of CHO in the heat that occurs independently of glucose appearance in the blood (Carter et al., 2004a; 2004b). In an initial study (Carter et al., 2004b) it was noted that performance during a 1 h time trial in the heat was unaffected by the infusion of glucose at a rate of 1 g·min⁻¹. This was followed by a study involving a similar exercise test during which subjects rinsed their mouth with a 6.4% CES or a FW without ingesting either solution (Carter et al., 2004a). Surprisingly performance was enhanced by 2.9% when the CES solution was swilled in the mouth, suggesting that putative CHO receptors in the oral cavity may modulate central pathways associated with motivation and reward. A feeding study in humans examining temporal responses to glucose intake provides some potential support to the theory of Carter and colleagues (Liu et al., 2000). This investigation used functional magnetic resonance imaging (fMRI) techniques to map dynamic brain activity following glucose ingestion. An immediate increase in hypothalamic activity
was observed in parallel with CHO ingestion, this occurred independently of the
effects of swallowing and other artefacts. This was followed (~10 min later) by
increased cerebral activation that coincided with measured changes in plasma glucose
concentration. These findings suggest that early biochemical changes in the brain occur
rapidly and independently of the hormonal changes associated with blood-borne
energy substrates.

In addition to gross motor performance, sports involving intermittent exercise
typically encompass a large number of movements that require fine motor control.
Skill and technique have been shown to deteriorate during the course of exercise and
match-play. The effect on skill of ingesting CHO during exercise is not adequately
understood at present. A number of investigations have addressed this issue by using
various skill and technique tests (Ali et al., 2002; McGregor, 1999; Northcott et al.,
1999; Ostojic and Mazic, 2002; Zeederberg et al., 1996). Using soccer skill tests
following match-play Zeederberg and colleagues (1996) report no improvement in
motor skill proficiency of soccer players when ingesting 5 ml·kg BM⁻¹ of a 6.9 % CES
every 15 minutes during the match. Using a simulation protocol (LIST) McGregor et
al. (1999), and Ali et al. (2002) found no changes in soccer specific skill performance
after exercise when players ingested a 6.4% CES vs. a placebo (5 ml·kg BM⁻¹ before
exercise and 2 ml·kg BM⁻¹·15 min⁻¹) during the protocol. In a different type of
laboratory simulation Northcott and associates (1999) report enhanced skill
performances when an 8% CHO solution (5 ml·kg BM⁻¹) was ingested before and at
half-time and after the simulated 90 min match. The research studies discussed above
were conducted in low ambient temperatures, only one study has examined games
related skill performance in the heat. This was achieved by measuring skill
performance following an exhibition soccer match played in high environmental
temperatures (Ostojic and Mazic, 2002) (Table 2.2). Players ingested either a 7% CES
or water alone, both before (5 ml·kg BM⁻¹) and during (2 ml·kg BM⁻¹·15 min⁻¹) the
match. The outcome was a 5% improvement in dribbling skill when ingesting the
CES. However, the use of separate groups in this study design does not allow changes
within subjects to be compared against a baseline value. The aetiology of the fatigue
of specific skills is likely to be complex compared with gross motor performance, but
it seems plausible that the metabolic and central changes discussed earlier in this
section will play a significant role.
2.1.6 Gastrointestinal function

In order for ingested fluids and nutrients to be incorporated into the appropriate body pools, they must first pass from the stomach into the small intestine where the process of absorption occurs. The rate at which the stomach contents empties into the duodenum and the speed at which the contents of the intestine are absorbed are therefore critical for rapid replacement of fluid and substrate during intense exercise, especially in the heat.

2.1.6.1 Gastric emptying

A strong determinate of the rate of gastric emptying is the volume of fluid within the stomach. Rapid emptying rates occur at larger gastric volumes and this relationship typically follows an exponential pattern (Simpson, 2003) (see Figure 2.2). Elevated intragastric pressure leads to increased motility until a threshold is reached at which excessive distension of the stomach restricts emptying. Enhanced emptying rates have been observed up to volumes of ~600 ml (Costill and Saltin, 1974), ~750 ml (Hunt and Spurrell, 1951) and ~1600 ml (Mitchell and Voss, 1990), after which the emptying rate plateaus or is impaired. Inter-individual anatomical differences will determine the upper limit of emptying and tolerance volume. It is therefore advantageous to maintain a high gastric volume throughout exercise in the heat, in order to ensure high rates gastric emptying and fluid delivery. It has been demonstrated that by repeatedly ingesting fluid, emptying can be maintained at rates observed during the initial fast phase, when the volume of fluid within the stomach is high (Mitchell and Voss, 1991; Noakes et al., 1991; Rehrer et al., 1990b). Typically during fields sport and in the laboratory protocols that simulate these activity patterns, an initial bolus will be ingested prior to exercise (~500-600 ml) followed by serial feedings (100-200 ml) at regular intervals dictated by breaks in play (~15-20 min). A typical emptying pattern observed in response to repeated feeding is shown in Figure 2.2.

The rate of gastric emptying has been shown to be closely regulated by the energy content of CHO solutions (Brener et al., 1983; Costill and Saltin, 1974; Mitchell et al., 1989; Rehrer et al., 1989). The findings of Vist and Maughan (1994) illustrate this principle by using a double sampling technique to examine the emptying characteristics of 2%, 4% and 6% CHO solutions and water. The study found that the
6% and 4% solutions emptied considerably slower than the 2% solution and water, with the 6% solution delaying emptying the longest. There were no detectable differences between the 2% solution and water. This observation supports the general consensus that gastric emptying is only significantly delayed when CHO solutions have an energy content in excess of 2.5% (Costill and Saltin, 1974; Houmard et al., 1991; Neufer et al., 1986; Sole and Noakes, 1989). At relatively low energy densities it may be the case that a restriction in emptying is absent or that the traditional gastric sampling techniques employed are not precise enough to detect any change. Nevertheless, it is well established that the concentrations of CHO encountered within popular sports drinks (~6%) notably reduce the rate of emptying (Maughan, 1997).

When ingested at similar energy densities to those encountered in commercially available sports drinks there is some evidence suggesting that polymerised solutions may enhance the rate of emptying. Foster et al. (1980) noted that a 5% glucose
polymer emptied more rapidly than a 5% glucose solution. However, other reports have failed to confirm these differences (Brouns et al., 1995; Murray et al., 1994).

The osmolality of drinks that are free of energy is a major contributing factor to their gastric emptying characteristics (Leiper, 2001). But energy density is thought to be a more powerful overriding regulator of emptying across the range of osmolalities encountered in the majority of sports drinks. Several studies support this suggestion showing that the osmolality is unlikely to have any additional influence on delaying gastric emptying when solutions contain ~6% CHO (Brouns et al., 1995; Shi et al., 2000; Simpson et al., 2002). Brouns and colleagues (1995) examined six isoenergetic solutions (6% CHO) with osmolalities between 245-375 mOsmol·kg⁻¹ and found no differences in the speed of emptying. Similarly, Shi et al. (2000) report negligible differences in the emptying rate of four 6% solutions varying in osmolality (1-424 mOsmol·kg⁻¹). Recently a number of 6.4% CES with a range of solute concentrations (25-390 mOsmol·kg⁻¹) have also been shown to exhibit similar emptying characteristics (Simpson et al., 2002). There are other variables of ingested solutions that effect gastric emptying, however these often contribute to a lesser extent than those outlined so far in this section. The temperature of beverages has been shown to delay emptying when the thermal gradient between the fluid and the stomach is large (Costill and Saltin, 1974; Sun et al., 1988). But on the other hand drinks served at temperatures cooler than Tcore have been shown to increase palatability and so encourage larger volumes to be ingested (Leiper, 2001). The concentration of certain acids within test solutions have been shown to alter the rate of gastric emptying (Hunt and Knox, 1969), although the organic acids encountered in sports drinks are unlikely to have a significant influence (Leiper, 2001).

There are several mechanisms by which running exercise may alter the rate of gastric emptying. These are: changes in gastric motility, visceral blood flow, elevated catecholamines and other hormones that may compromise emptying (Murray, 1987; Neufer et al., 1989b), but as yet it is not known which of these plays the pivotal role during exercise. When the exercise intensity during running is less than 70% VO₂ max it appears unlikely that the rate of gastric emptying is markedly reduced. Indeed it has been reported that emptying is increased during walking and running at intensities
between 28% - 70% $\dot{V}O_2$\textsubscript{max} (Neufer et al., 1989b). This increase in the rate of emptying may be due to the biomechanical vibration of the abdominal region that occurs during running (Rehrer and Meijer, 1991) and a shift of the gastric contents toward the antrum (Costill, 1990). An early study carried out by Fortran and Saltin (1967) observed no effect on gastric emptying of running exercise at 70% $\dot{V}O_2$\textsubscript{max}, the general consensus since then appears to be that most forms of steady state exercise will have little effect of gastric emptying until the exercise intensity exceeds 70-80% (Costill and Saltin, 1974; Gisolfi, 2000; Leiper, 2001). During steady-state exercise at very high intensities, the slowing of emptying may be of little consequence as the exercise duration is likely to be too short for any benefit to be derived from an ingested sports drink (Maughan, 1991). However, team sports that involve supramaximal exercise interspersed with periods of low intensity exercise last considerably longer and are likely to be affected by the consequences of delayed emptying.

The average intensity at which soccer matches are played is around 70% $\dot{V}O_2$\textsubscript{max} (Bangsbo, 1994a), the findings from constant workrate studies suggest that running at this intensity would have little effect on gastric emptying. However, for short periods of time players run at maximal intensities. It has previously been proposed that the periods of play spent at low workrates would afford sufficient time for drinks to empty from the stomach (Brouns et al., 1993). Recently this theory was examined by Leiper and colleagues (2001a; 2005; 2001b) using a series of investigations involving high-intensity intermittent forms of exercise. The first of these studies employed a variable intensity intermittent cycling protocol and showed that emptying of a 6% CES was slowed when compared with steady state exercise (66% $\dot{V}O_2$\textsubscript{max}) (Leiper et al., 2001a). These findings support similar observations reported during fixed workload interval cycling (Mitchell et al., 1989). In a subsequent soccer specific investigation (Leiper et al., 2001b) the emptying characteristics of a 6% CES were investigated during a thirty minute 5-a-side soccer game and a separate walking exercise trial. The soccer match-play significantly delayed emptying of the CES when compared with walking only. The estimated mean exercise intensity during the two periods of the match were 54% $\dot{V}O_2$\textsubscript{max} and 63% $\dot{V}O_2$\textsubscript{max}, considerably lower than the mean exercise intensity required to restrict emptying during steady-state exercise.
This finding was readdressed in a more controlled manner using a standardised shuttle running test (LIST) (Leiper et al., 2005). The emptying rate of a 6% CES and a FW was found to be slower during 30 min of the LIST compared with walking exercise. Collectively these studies suggest that the sprinting or the supramaximal component of these activities may be the main factor constraining gastric emptying. Although the exact amount of high-intensity exercise required to inhibit gastric emptying is unknown, the sprinting performed during high-intensity, intermittent field sports seems to be sufficient to notably restrict delivery of fluids, even when the mean intensity of the exercise is relatively low. It is worth noting that in the study above Leiper and co-workers detected no differences in the emptying rates of the different test solutions during the LIST protocol (CES vs. FW), but found that the 6% CES solution emptied less readily than FW during the walking exercise. This demonstrates that the powerful inhibitory effect of exercise on emptying is considerably more influential than the energy content or osmolality of the stomach contents.

Moderate increases in $T_{\text{core}}$ have been shown to have no effect on the rate of gastric emptying at rest (Bowen and Shirreffs, 2001), but when combined with strenuous exercise it appears the rate of emptying may be suppressed. This was initially demonstrated by Owen and associates (1986), they noted a slower rate of gastric emptying when subjects completed 2 h of treadmill running at 65% $\dot{V}O_2\text{max}$ in 35°C compared with 25°C. This change occurred independently of alterations in hydration status and appeared to reflect the increase in $T_{\text{core}}$. Similarly Neufer and co-workers (1989a) reported slower rates of gastric emptying when ingesting water during exercise (50% $\dot{V}O_2\text{max}$) in ambient temperatures of 18°C and 49°C, but found no differences between 18°C and 35°C. The reasons for the lack of any differences at 35°C in this study may be explained by the lower energy expenditure and relative humidity than that of Owen and co-worker’s study (1986). It seems unlikely that this inhibition of gastric emptying is the consequence of changes in $T_{\text{core}}$ per se, but rather a decline in blood volume perfusing the visceral organs and/or changes in the concentration of circulating catecholamines. Potentially through similar mechanisms dehydration in combination with exercise is thought to contribute to a reduction in the rate of gastric emptying. Neufer et al. (1989a) and van Nieuwenhoven et al. (2000) have both reported reductions in the rate of emptying during moderately intense
treadmill running (50% \( \dot{V}O_2 \text{max} \)) and cycling (70% \( \dot{V}O_2 \text{max} \)) when individuals were dehydrated by 5% and 3% of bodyweight respectively. At lower exercise intensities dehydration may have less of an impact on gastric function. It has been demonstrated than when dehydrated to a level equivalent to \(-3\% \) BM, exercise at 65% \( \dot{V}O_2 \text{max} \) has no detectable effect on gastric emptying (Ryan et al., 1998). However Rehrer et al. (1990a) showed that when dehydration (3.7% BM) was combined with the effects of heat stress (30°C), the outcome was a restriction in emptying even at relatively low exercise intensities (60% of maximum running velocity). It appears that dehydration or moderate hyperthermia are unlikely to effect the rate of gastric emptying independently, but when these two conditions are combined during exercise, gastric function may be compromised significantly.

The redistribution of blood flow away from visceral organs during exercise is likely to occur proportionally according to the synergistic or separate affects of exercise, hyperthermia and hypohydration (for discussion see Section 2.1.1.3). These changes alone may be responsible for alterations in gastric function under the physiological stresses discussed earlier. It is well established that visceral circulation is substantially reduced during exercise. Rowell et al. (1964) noted a 60-70% reduction in splanchnic blood flow during similar exercise intensities to those encountered in sports such as soccer, rugby and hockey (70% \( \dot{V}O_2 \text{max} \)). Extending the scope of these findings to higher workloads Clausen (1977) calculated that there was up to an 80% reduction in splanchnic blood flow during maximal exercise. The ischemic consequences of these considerable cardiovascular alterations may have profound effects on gastric emptying.

In addition to the volume of blood available it has been suggested that exercise coupled with heat stress may slow the contractile activity of the stomach (Neufer et al., 1989a). Elevations in plasma endorphins (Konturek, 1980) and other GI hormones (O'Connor et al., 1995) have been shown to decrease the contraction rate of the proximal region of the stomach. This would also have a profound effect on emptying as the increase in intragastric pressure caused by this contractile activity is considered to be the primary mechanism for emptying liquids from the stomach (Minami and McCallum, 1984).
2.1.6.2 Intestinal absorption

In order to allow adequate replacement of fluid losses and delivery of substrate, the small intestine must be able to absorb fluids and nutrients at a sufficient rate after they have emptied from the stomach. Intestinal absorption of water across the epithelial membrane is a passive process that is primarily driven by local osmotic and hydrostatic gradients. It is well known that the osmolality of fluids is key in determining the direction and magnitude of net water flow between the gut and blood plasma (for a review see Leiper, 2001). When fluids that cause the luminal contents to become hypotonic with respect to serum are ingested, water absorption is enhanced. On the other hand ingesting drinks that cause hypertonicity are known to promote water secretion into the small intestine (Sladen, 1972). This is a very simple relationship but its interpretation is confounded somewhat when a number of solutes (such as CHO) are added to sports drinks.

It has been shown that 6% CHO solutions with a range of osmolalities (186 – 403 mOsmol·kg⁻¹) induce similar changes in plasma volume at rest (Shi et al., 1994; Simpson, 2003). Similar rates of water absorption have also been reported between hypotonic, isotonic and hypertonic 6% solutions during cycling (Gisolfi et al., 2001; 1998; Leiper et al., 1994b). However, a number of other studies have reported that water absorption occurs more rapidly from hypotonic than isotonic CHO solutions (Shi et al., 1995; Thillainayagam et al., 1998; Wapnir and Lifshitz, 1985). Although it seems that hypertonic CHO solutions tend to restrict water absorption it is currently unclear whether hypotonic solutions are able to deliver water from the gut more rapidly than isotonic CHO beverages.

The energy containing solutes commonly added to sports drinks are thought to increase intestinal absorption of water compared with energy-free solutions (Schedl et al., 1994). Gisolfi and co-workers (1992) report increased absorption of water after the ingestion of 2, 4 and 6% glucose solutions compared with a isotonic saline drink. Similarly, Rehrer and associates (1992) noted enhanced net water absorption when subjects ingested a 4.5% CHO solution compared with water alone. A number of potential absorptive routes have been implicated in this enhancement of water delivery. Transporter proteins involved in carrying solutes such as glucose are thought to provide low-conductance water channels allowing the passage of solute and water.
against electrical or concentration gradients (Loo et al., 1999). Madara and Pappenheimer (1987) observed in rodents a solvent-drag mechanism by which the Na⁺-coupled active transport of glucose provided an osmotic driving force enabling additional water and solute absorption. The protein implicated in this process is SGLT1 which has been shown to be unsaturated up to luminal glucose concentrations of ~200 mmol·l⁻¹ (Leiper, 2001). The activity of the high capacity transporter protein GLUT2 is thought to respond in a similar way to increases in luminal glucose concentration, however an increased activity of GLUT2 has been noted at concentrations in excess of those at which SGLT1 becomes saturated.

It proposed by some that the rate of appearance of glucose from the intestine is not the rate-limiting step in its disposal from the blood. The maximal rate of glucose appearance in arterial blood from the gut has shown to be ~1.3 g·min⁻¹ (Duchman et al., 1997), which is in excess of maximum oxidation rates measured from glucose derived from CHO ingested during exercise (~1.1 g·min⁻¹) (Jeukendrup and Jentjens, 2000; Moodley et al., 1992; Rehrer et al., 1992). However more recently maximum rates of glucose oxidation of up to 1.75 g·min⁻¹ have been observed when glucose is combined with fructose and sucrose (Jentjens and Jeukendrup, 2005; Jeukendrup and Moseley, 2005; Jentjens et al., 2004a; 2004b; 2004c). These authors, from the same group, propose that because fructose and sucrose can be absorbed by separate facilitative transporters to glucose, the rate of exogenous glucose disposal has the potential to exceed 1.3 g·min⁻¹ without the restrictions imposed by intestinal absorption. Other substrate combinations that do not compete for the same transporters, such as fructose and maltodextrins, have also been combined in CHO solutions. It has recently been shown that this combination will result in a rate of CHO oxidation during exercise that is ~40% higher than when consuming a isoenergetic amount of maltodextrins (Wallis et al., 2005). The findings in this area are equivocal and the substitution of monomers with disaccharides and maltodextrins has been shown to both increase glucose absorption (Jones et al., 1987; Leiper et al., 1996) and have no effect (Shi et al., 1995). It seems the main benefit of using mixtures of maltodextrins in sports drinks is derived from the lower osmolality of these solutions and hence the greater potential for fluid absorption (Leiper et al., 1994a; Merson et al., 2002; Rehrer et al., 1992).
The available evidence suggests that during moderately intense exercise there is little change in intestinal absorption compared with rest. Several studies employing sound methodologies have reported no differences in the rate of intestinal absorption of water and CHO solutions, during both moderately intense treadmill running (Fordtran and Saltin, 1967) and cycling (Gisolfi et al., 1991; Spranger et al., 1989) when compared with rest. Blood flow to the small intestine does not appear to be reduced to a level that restricts the rate of active or passive transport during moderately intense exercise (Fordtran and Saltin, 1967; Moses, 1990). Additionally Perko et al. (1998) have shown that the vasoconstriction that typically occurs during exercise in the superior mesenteric artery is less pronounced when subjects are in a fed state. It has been suggested recently that the major signal in this response is insulin (Grossini et al., 2004). This increase in blood supply may provide an enhancement in water absorption when CHO is ingested that occurs in addition to the solute-driven absorptive routes discussed previously. Findings during exercise that support these potential mechanisms are provided by Spranger and co-workers (1989), who observed a more rapid rate of fluid absorption from a CES than water during cycling exercise. But subsequently Gisolfi and colleagues (1998) found no differences in fluid absorption between water and a range of CHO solutions that differed in osmolality.

Emerging evidence from a rodent model suggests that exercise training may also enhance the function of the intestine (Chies et al., 2004). This adaptation may occur by reducing the catecholamine-induced vasoconstriction of the superior mesenteric artery that occurs during exercise. The in vitro tissue analysis performed in this study shows that the adaptation arises due to alterations in the vasomotor potency of catecholamines on the vascular tissue in this region.

At present it is unknown whether supramaximal exercise alters the rate of intestinal absorption or whether the efficiency of intestinal function is compromised further when exercise is performed under the additional stress of hyperthermia. It is possible that further vasoconstriction-induced reductions in splanchnic blood flow and changes in catecholamine levels associated with hyperthermia may suppress intestinal absorption. It has also been suggested that sprinting may curtail intestinal absorption to a similar degree as it does gastric emptying (Leiper, 2001). Whether or not intestinal function is altered significantly during high-intensity intermittent activity in
the heat is yet to be established, but considering the magnitude to which gastric emptying is restricted during multi-sprint exercise, it seems unlikely that the rate of intestinal absorption will be the rate-limiting factor in the delivery of water and substrate to the body interior from a well formulated sports drinks.

2.2 Measurement of core temperature during unconstrained exercise

The concept of a deep body or 'core' temperature is somewhat ambiguous as it implies a common temperature for all tissues within its definition. In reality the temperature of the internal organs will vary depending on the area of the body being considered (Parsons, 2003). Nielsen and Nielsen (1962) suggested that the term should reflect the temperature of aortic blood leaving the heart to perfuse the body core. This definition appears applicable to the study of thermoregulation as it is a branch of this vessel that perfuses the anterior and preoptic regions of the hypothalamus. Within the field of exercise physiology the use of core temperature is generally extended to encompass the vital organs within the trunk and head. This is a reasonable generalisation as the temperature within the organs of the abdominal cavity and skull are maintained within a few tenths of a degree of the blood inside the major vessels (Gisolfi and Wenger, 1984). The following discussion will address the commonly used invasive sites of $T_{core}$ measurement that can be used practically during prolonged, intermittent free-running exercise.

2.2.1 Oesophageal temperature

The oesophagus is the preferred site of $T_{core}$ measurement by many due to its proximity to the left ventricle and aorta (Cooper and Kenyon, 1957), reflecting the temperature of direct blood flow to the hypothalamus (Gerbrandy et al., 1954). However recently Nybo et al. (2002b) have shown that brain temperature may be at least $0.2^\circ C$ higher than this measure. Oesophageal temperature also responds rapidly to changes in $T_{core}$ and is considered the most suitable site during exercise when rapid detection of changes in heat storage is necessary. The drawback of this technique is that the presence of a $T_{oes}$ thermistor frequently causes discomfort and irritation of the nasal passages and throat, which are often exacerbated during exercise (Moran and Mendal, 2002; Stitt, 1993). The temperature of ingested fluids and solids may also briefly alter temperature measured at this site. Therefore this technique is often
impractical during dynamic exercise, particularly when performance is critical or when feeding occurs.

2.2.2 Rectal Temperature
The rectum is the most commonly used site of $T_{\text{core}}$ measurement during prolonged exercise due to ease of measurement and relative stability. The response time of rectal temperature ($T_{\text{rec}}$) during rapid changes in $T_{\text{core}}$ has been reported to be slower than other techniques (Cooper and Kenyon, 1957; Gerbrandy et al., 1954), this is due to the relatively low level of systemic circulation in the region. This location can also be susceptible to changes between exercise and rest that are an artefact of venous return from the legs via the haemorrhoidal veins (Mead and Bonmarito, 1949). During prolonged free-running, where it is often necessary for a thermistor to reside in the rectum, measures must be taken to ensure the thermistor remains in position. This usually requires the attachment of bulb or bung to the thermistor of sufficient diameter so that the anal sphincter is not breached.

2.2.3 Tympanic and aural temperature
Measuring temperature on the surface of the tympanic membrane and within the aural canal have become popular methods of determining $T_{\text{core}}$ during exercise due to ease of access and correlation with hypothalamic temperature. However, tympanic and aural temperature have been shown to change with local heating (Marcus, 1973; McCaffrey et al., 1975) and cooling (Brengelmann, 1993; Marcus, 1973; McCaffrey et al., 1975; Shiraki et al., 1988; Thomas et al., 1997) of the face and head independently of changes in $T_{\text{core}}$. Measurements using emerging infra-red techniques may also be inaccurate during dynamic exercise (Yeo and Scarbough, 1996). Instrumenting these sites is often problematic (Moran and Mendal, 2002) and measuring tympanic temperature risks membrane perforation (Wallace et al., 1974). Additionally, the presence of aural foreign bodies during free-running and field sports can cause disorientation, nausea and hearing impairment.

2.2.4 Intestinal temperature
The potential use of orally administered temperature sensors was first brought to the attention of the scientific community in 1961 (Fox et al., 1961; Wolff, 1961); subsequently ingestible sensors were used to monitor intestinal temperature ($T_{\text{int}}$) in
astronauts during spaceflight. In recent years the use of disposal versions of these sensors has become popular in research and the professional sporting environment. The major benefit of this technique is the absence of cables and data logging equipment, which are often incompatible with many free-running applications and may be cumbersome in situations where performance is critical. The long-term presence of hard-wired probes may also cause discomfort and present sanitary problems during long periods of data collection. Despite these obvious advantages relatively little is known about the validity and reliability of this measure compared with other techniques, particularly during dynamic upright exercise.

A number of studies have attempted to evaluate the validity of $T_{int}$ during exercise and rest (Table 2.4) using Cor-100 disposable sensors (HQinc, Florida, USA) and an ambulatory data recorder (CorTemp 2000, HQinc, Florida, USA). Several alternative designs of ingestible sensor are available from other manufacturers, however, the equipment mentioned above appears to be the most robust (Stephenson et al., 1992) and is currently the most widely used.

Intestinal temperature has been shown to represent $T_{oes}$ more closely than $T_{rec}$ during interval cycle ergometry (Kolka et al., 1993; Lee et al., 2000; Stephenson et al., 1992). These studies conclude that $T_{int}$ and $T_{oes}$ consistently measure hotter temperatures than at the rectum during seated exercise (an average increase of 0.15 °C across studies). However, during steady-state cycle ergometry O’Brien and colleagues (1998) demonstrated that $T_{int}$ was a closer representation of $T_{rec}$ than $T_{oes}$. The agreement with regard to $T_{oes}$ during upright exercise is less clear, the study of Kolka et al. (1997) noted slightly higher $T_{int}$ values (0.04 °C) than $T_{oes}$ during treadmill exercise. Three of the studies discussed above examined the speed of response or time to a threshold of each site of measurement. The findings were that $T_{int}$ was either intermediate to $T_{oes}$ and $T_{rec}$ or no different to $T_{oes}$. This highlights the degree of thermal inertia within the rectum described previously. It is worth noting that when determining rate of temperature response, the thermal dynamics of the sensor itself may result in slower changes in $T_{int}$. This has been noted during calibration by several authors (Kolka et al., 1993; Lee et al., 2000).
<table>
<thead>
<tr>
<th>Study</th>
<th>Exercise mode</th>
<th>Ingestion interval</th>
<th>Calibration technique</th>
<th>Sites of measurement</th>
<th>Speed of response (ranked)</th>
<th>Absolute differences</th>
</tr>
</thead>
<tbody>
<tr>
<td>Castle et al. 2003</td>
<td>Interval cycling (20°C)</td>
<td>------</td>
<td>------</td>
<td>$T_{rec} \ vs \ T_{int}$</td>
<td>------</td>
<td>$\bar{x} \ T_{int} - T_{rec}$ $= -0.2 \pm 0.3 ^\circ C$</td>
</tr>
<tr>
<td>Edwards et al. 2002</td>
<td>Normal daily activities</td>
<td>$\geq 8h$</td>
<td>None</td>
<td>$T_{rec} \ vs \ T_{int}$</td>
<td>------</td>
<td>$\bar{x} \ T_{int} - T_{rec}$ $= 0.2 \pm 0.4 ^\circ C$</td>
</tr>
<tr>
<td>Kolka et al. 1997</td>
<td>Treadmill walking (30°C) ♀</td>
<td>2h (±0.5)</td>
<td>None (screened)</td>
<td>$T_{oes} \ vs \ T_{int}$</td>
<td>------</td>
<td>$\bar{x} \ T_{int} - T_{oes}$ $= 0.04 ^\circ C$</td>
</tr>
<tr>
<td>Kolka et al. 1993</td>
<td>Interval cycling (30°C)</td>
<td>2h (±0.5)</td>
<td>None (screened)</td>
<td>$T_{rec} \ vs \ T_{oes} \ vs \ T_{int}$</td>
<td>1. $T_{oes}$ 2. $T_{int}$ 3. $T_{rec}$</td>
<td>$T_{int} \ &amp; \ T_{oes}$ higher than $T_{rec}$</td>
</tr>
<tr>
<td>Lee et al. 2000</td>
<td>Interval cycling (room temperature)</td>
<td>6h</td>
<td>Linear regression</td>
<td>$T_{rec} \ vs \ T_{oes} \ vs \ T_{int}$</td>
<td>1. $T_{oes}$ 2. $T_{int}$ 3. $T_{rec}$</td>
<td>Peak $T_{int} \ &amp; \ T_{oes} - T_{rec}$ $= 0.2 ^\circ C$</td>
</tr>
<tr>
<td>O’Brien et al. 1998</td>
<td>Cycling (36°C)</td>
<td>12h</td>
<td>Linear regression</td>
<td>$T_{rec} \ vs \ T_{oes} \ vs \ T_{int}$</td>
<td>------</td>
<td>$T_{int} \ &amp; \ T_{rec}$ lower than $T_{oes}$</td>
</tr>
<tr>
<td>Sparling et al. 1993</td>
<td>Cycling or treadmill running (21°C)</td>
<td>3h – 9h</td>
<td>None</td>
<td>$T_{rec} \ vs \ T_{int}$</td>
<td>------</td>
<td>Peak $T_{int} - T_{rec}$ $= -0.8 \pm 0.5 ^\circ C$</td>
</tr>
<tr>
<td>Stephenson et al. 1992</td>
<td>Interval cycling (29°C)</td>
<td>2h</td>
<td>Linear regression</td>
<td>$T_{rec} \ vs \ T_{oes} \ vs \ T_{int}$</td>
<td>1.$T_{oes} \ &amp; \ T_{int}$ 2.$T_{rec}$</td>
<td>Peak $T_{int} \ &amp; \ T_{oes} - T_{rec}$ $= 0.1 ^\circ C$</td>
</tr>
</tbody>
</table>

Table 2.4 Previous studies examining the validity of $T_{int}$ (Cor-100, HQinc, Florida, USA) compared with other sites in humans. Speed of response for each technique is reported in rank order (most rapid first) and absolute differences in temperature (either overall mean or at peak temperature) are included where available (\(\bar{x} \pm SD\)). The calibration technique ‘screened’ indicates that pill output was confirmed against a single known temperature (no calibration factor applied). ------ indicates no report of this variable.
The literature that has compared solely $T_{rec}$ with intestinal temperature reports mixed findings. Two of these studies, that used cycling as the mode of exercise (Castle et al., 2003; Sparling et al., 1993) report lower intestinal temperatures compared with $T_{rec}$. On the other hand the work of Edwards and co-workers (2002), monitoring the routine daily activities of subjects noted higher intestinal temperatures.

A major drawback inherent in a number of published studies using this technique is the time interval between ingesting the capsule and collecting measurements. If the sensor resides within the stomach or an area of the small intestine in close proximity to the stomach, $T_{int}$ may be influenced by the temperature of ingested material. Erroneous measurements will increase in magnitude and duration when the temperature gradient between the ingested material and the core is large. This phenomenon can be seen in the results of Kolka et al. (1993) and has been evident up to 4 hours after the ingestion of the sensor when fluid (500 ml, 4°C) was consumed in our laboratory (unpublished findings). It seems reasonable to suggest that the rate of gastric emptying and orocecal transit time (mouth - cecum) at rest must be estimated for a cohort of subjects when calculating the time interval between pill ingestion and temperature measurement. Orocecal transit time at rest is controlled by numerous neural and hormonal factors which vary between individuals. A particularly important modifiable factor is daily energy intake. Harris et al. (1991) showed a correlation between orocecal motility at rest and the daily energy intake of trained active men. These data suggest that the findings of a number of the validity studies summarised in Table 2.4 (Kolka et al., 1997; 1993; Sparling et al., 1993; Stephenson et al., 1992) may be inaccurate if the daily energy intakes of subjects was $\leq$10.5 MJ·day$^{-1}$ (the daily energy intake of subjects was not mentioned by these authors). The adequate passage of a sensor through the GI tract before the start of measurement is a major consideration, however, there is no mention of the specific points discussed above in any investigations this author has encountered.

A further limitation of measuring $T_{int}$ is the potential movement of the measurement site during exercise. The sensor will progress within the intestine during a given period of measurement. Portions of the GI tract may vary in temperature slightly due to their proximity to major organs and vessels. If the sensor has not reached the descending or sigmoid colon, the potential distance that is moved during the exercise
protocol may be significant and have a notable effect on temperature measurements recorded.

Although high-intensity exercise modifies a number of factors involved in the control of intestinal motility (e.g. concentration of plasma catecholamines, endorphins and intestinal blood flow) and involves rhythmical mechanical forces, it’s affect on gut motility is still uncertain. Soffer and colleagues (1991) noted influences of interval cycle ergometry (60, 80 and 90% VO$_2$max) on intestinal postprandial motor activity, but no significant effect on orocecal transit. Similarly no influence on motility was reported during interval cycling by Cammack et al. (1982) and Rao et al. (1999). Conversely, Keeling and Martin (1987) found the orocecal transit time was accelerated by 20-25% during treadmill walking exercise, possibly due to the rhythmic vibration of the abdomen that occurs during walking. No studies to date have addressed the repeatability of T$_{int}$ measurements made on separate occasions in either a within-subject or between-subject manner. Depending on the activity and dietary habits of individuals, intestinal motility may be altered between repeated experimental trials, which may lead to observable changes in T$_{int}$ which are independent of the experimental treatment.

It appears that the manufacturer’s calibration of the sensor capsules, which takes place before transit and storage, may not be adequate for precise T$_{core}$ measurement in human subjects (Stephenson et al., 1992). The investigations of Lee and colleagues (2000), O’Brien et al. (1998) and Stephenson et al. (1992) describe precise bench-calibration techniques that were performed before pills were ingested. However, the findings of a number of the validity studies fail to consider this and may be discredited to some degree by the lack of prior screening and calibration (Castle et al., 2003; Edwards et al., 2002; Sparling et al., 1993) or the use of questionable calibration techniques (Kolka et al., 1997; 1993).

If the correct degree of experimental control is implemented, T$_{int}$ may be a good alternative to T$_{oes}$ and superior to T$_{rec}$ during exercise. It appears from analysis of the studies reported in Table 2.4 that trends in bias between methods of measurement may be related to both the position of the body during exercise (seated vs. standing activities) and the mode of the exercise (intermittent vs. continuous). This seems
plausible considering the differing amount of muscle mass involved in each form of exercise (Lewis et al., 1983) and the unique circulatory responses to these different activities. Clearly more investigation is required, particularly with respect to unconstrained running, where dynamic forces occurring through numerous planes of motion are likely to have a more pronounced effect on the position and mobility of the sensor.
CHAPTER 3

GENERAL METHODS

3.1 Introduction
This chapter outlines the methodological procedures that are generic to all studies throughout this thesis. Study-specific protocols and procedures are described in the methods section of the respective experimental chapter.

All generic procedures and experimental studies received prior approval from the Loughborough University Ethical Advisory Committee and were conducted in accordance with the Declaration of Helsinki (2004) along with a local code of practice for workers having contact with body fluids. All recorded personal data were treated in accordance with the Data Protection Act (1998).

3.2 Subjects
The subjects who participated in the studies described in this thesis were all healthy male volunteers, typically recruited from the Loughborough University Students Football Club and local semi-professional soccer teams. All subjects were involved in physical training programmes of which soccer was the central feature and were familiar with strenuous exercise. Potential subjects were screened to ensure they had not visited, trained or competed in countries with climates that differed substantially from the UK within three months of the study. Volunteers were informed of the aims, procedures, demands and any potential risks and discomforts that the study may entail. Written consent was then obtained (Appendix A) and subjects were fully informed of their right to withdraw from the study, without reason, at any stage. Subjects were asked to complete a general health history questionnaire (Appendix B) and a health questionnaire concerned with the use of ingestible temperature sensors (Appendix C). Individuals who reported any current or past medical conditions indicated on these questionnaires were excluded from participation. Additionally, subjects completed a physical activity status questionnaire (Appendix D) and an acute health status questionnaire (Appendix A) on arrival at each visit to the laboratory.
3.2.1 Subject controls

Subjects were required to report for each experimental trial following a 10 h overnight fast. They were asked to drink 0.5 l of water 10 h before arrival at the laboratory and a further 0.5 l, 1.5 h before reporting to the laboratory (in accordance with the guidelines for ingesting the temperature sensor, Section 3.3.6.2 and the recommendations of Shirreffs et al. (2004)). Shortly after rising and before ingesting the 0.5 l of fluid (if possible), subjects collected a 10 ml sample of their first urine of the day. When the osmolality of this sample was \( \leq 900 \) mOsmol·kg\(^{-1}\) subjects were considered euhydrated (Shirreffs and Maughan, 1998b) and were permitted to take part in the investigation. In the 48 hour period before the first experimental trial subjects weighed and recorded their food and drink intake using a food record diary (Appendix F) and precision strain-gauge weight sensing scales (± 1g; Salter, Model 3010, England). This 48 hour diet was then replicated for the following experimental trial and diets were analysed for energy intake and nutritional content using dietetic software (Comp-Eat, version 5; Com-Eat Nutrition Systems, Grantham, UK). Participants were asked to refrain from heavy physical activity in the 48 hours preceding each experimental trial and to record any physical activity peculiar to their normal daily routine (Appendix F). Subjects were required to abstain from alcohol and caffeine in this 48 hour period and pharmacological substances for the duration of their involvement in the study. Experimental trials were separated by 7 days to allow sufficient recovery and in the case of the studies conducted in the heat, to limit the development of heat acclimation (Barnett and Maughan, 1993). Repeated experimental trials were conducted by the same experimenters to minimise the influence of social facilitation and subjects wore the same shorts and footwear on each occasion. All main trials were conducted at 08:00 to minimise the influence of circadian variation (Reilly and Brooks, 1986), on days between Tuesday and Friday to account for varying weekend routines.

3.3 Experimental testing procedures

3.3.1 Testing facility

Experimental trials and preliminary tests were conducted in the School of Sport and Exercise Sciences' Sports Hall at Loughborough University. The hall is a large (~1625 m\(^3\)) unfurnished area, with a flat, non-slip sprung wooden surface. Non-slip tape was used to mark lines representing 0 m, 10 and 20 m for the Multi-Stage Fitness
Test (Ramsbottom et al., 1988) and the Loughborough Intermittent Shuttle Test (LIST) (Nicholas et al., 2000).

3.3.1.1 Raising the environmental temperature

For the studies conducted at an environmental temperature of 30°C (Chapter 7, 8 and 9), the Sports Hall was raised to the appropriate temperature over a 5-8 hour overnight period before testing. This was achieved using three, 13 kW electric fan heaters (DE65 Andrew Sykes Ltd, Nottingham, U.K.), three 3 kW electric fan heaters and an external, 61 kW indirect gas fired heater (IG60 Andrew Sykes Ltd, Nottingham, U.K.). Heaters were distributed throughout the hall and the external ducted heater provided an air exchange rate of 5000 m³·h⁻¹.

3.3.1.2 Monitoring and maintaining the environmental conditions

Four ambient temperature thermistors (VH-G-23, Grant instruments Ltd, Cambridge, UK) were positioned at head-height and distributed at 5 meter intervals adjacent to the running lanes. A further sensor station comprising of black globe radiant temperature (AG, Grant instruments Ltd, Cambridge, UK), relative humidity (RH) and air temperature (RH-G-23, Grant instruments Ltd, Cambridge, UK) sensors was positioned at the same elevation in the centre of the hall (see Figure 3.1 for plan). The seven environmental sensors were continually monitored by computer software (SquirrelView, Grant instruments Ltd, Cambridge, UK) and an audio signal indicated if sensors recorded values outside the desired temperature (± 0.5 °C). When alerted to changes in regional temperature, the respective heaters were adjusted accordingly by an investigator. Relative humidity was not specifically controlled, however, due to the large volume of the hall RH remained essentially the same at any given temperature. For the studies described in Chapters 4, 5 and 6, a sling psychrometer (whirling hygrometer, P2520, Zeal Ltd, London, UK) was used to measure dry and wet bulb temperatures at the same locations at 15 minute intervals. Relative humidity was calculated from these variables using the equation of Murray (1967). Barometric pressure was determined using a wall mounted barometer (BHL-340-X Griffin and George, UK) 15 min before commencement of experimental trials.
Figure 3.1 A plan view diagram of hall in which subjects performed the LIST protocol. Symbols represent: ♨ Heater, ⬜ Arc of heater movement, ⡅ Environmental monitoring point, 🔊 LIST protocol audio speaker, ⚑ Running track area, ⏰ Table, ⭨ Sprint arresting crash mats, ⌛️ Photoelectric timing gate, 🍀 static hot-water radiator.
3.3.2 Anthropometry

Subject height was measured using a fixed stadiometer and recorded to the nearest 0.1 cm (Holtain Ltd, Crymych, UK). The investigator ensured the subjects’ feet were in contact with the heel board and the headboard was lowered until contact was made with the superior point of the head. The head was then maintained in the Frankfort plane whilst gentle traction was then applied to the mastoid process to compensate for any inter-vertebral disc shrinkage. Nude body mass was measured using a beam balance (3306 ABV, Avery Ltd, Birmingham, UK) to the nearest 0.1 kg. Subjects were towel dried before post-exercise body mass calculations; cannulas and other affixed instrumentation were accounted for when determining body mass. All body mass calculations were corrected for fluid intake and urine production. Respiratory water losses and losses due to substrate oxidation were not accounted for.

3.3.3 Estimation of maximal oxygen uptake

Maximal oxygen uptake (\(\dot{V}O_2\)\(_{max}\)) was estimated using The Multistage Fitness Shuttle Run Test (Ramsbottom et al., 1988), based upon the original protocol of Leger and Lambert (1982). The test requires subjects to run back and forth between a 20 m distance marked with tape lines. This continues at progressively increasing speeds, dictated by an audio signal generated via a compact disc (The National Coaching Foundation, Leeds, UK). The test began at a running speed of 2.2 m·s\(^{-1}\) which increased by 0.14 m·s\(^{-1}\) every 60 s as the intervals between signals were progressively shortened. Subjects were required to place one foot on or over the marked line in time with the audio signal. Subjects were encouraged verbally until they reached volitional exhaustion or were unable to maintain the required intensity on three consecutive shuttles. The level and shuttle number attained were recorded and used to predict \(\dot{V}O_2\)\(_{max}\) according to the regression equation of Ramsbottom et al. (1988).

Predicted \(\dot{V}O_2\)\(_{max}\) scores were used to calculate running speeds required to elicit 55% and 95% of \(\dot{V}O_2\)\(_{max}\) for use during the LIST. Subject pairs were matched for \(\dot{V}O_2\)\(_{max}\) values and 15 m sprint performance where possible, completing all experimental trials together in order to increase motivation.
On a separate visit to the laboratory, no less than 1 week before main experimental trials, subjects completed 3 blocks of the LIST protocol. The purpose of this test was to familiarise subjects with the demands and activity patterns of the protocol and to allow them to become accustomed to the sampling procedures and instrumentation used during the main experimental trials. A mean 15 m sprint time was calculated during this test and was subsequently used to ensure subjects maintained the required sprint intensity during main trials.

3.3.4 The Loughborough Intermittent Shuttle Test (LIST)

The LIST is a free-running protocol designed to simulate the activity patterns and energy demands of sports such as soccer, rugby and field hockey (Nicholas et al., 2000). The protocol used throughout this programme of research is based on a modification of the original protocol, in that the order of the Running (95% \( \dot{V}O_2 \max \)) and Jogging (55% \( \dot{V}O_2 \max \)) phases are switched. This modification has been used in a number of recent studies (Ali et al., 2002; Bishop et al., 2002; Foskett et al., 2003a; 2004; 2003b; Leiper et al., 2005; McGregor et al., 1999; Morris et al., 1998a; 1998b; 2003; 2000; Sunderland and Nevill, 2003; 2005) and allows subjects to differentiate between intensities more reliably.

The LIST requires subjects to complete repeated shuttles between two lines marked 20 m apart. In the studies reported within this thesis the intensity of shuttles was dictated by varying audible signals, verbal cues and a moving video image; all generated by computer software (Nicholas Gant, Loughborough University, UK). This is a modification of the standard equipment in which only one audible tone is generated by microcomputer. The audio signals comprised of a long signal indicating contact with the 20 m lines and a short signal indicating the midway point (10m). These signals were produced at increasing pitches for the walk, jog and run phases respectively. In addition the following spoken words and countdowns were generated to indicate the transition between activities: “walk”, “jog”, “run in 3 2 1”, “sprint in 3 2 1”. This audio sequence was transmitted to speakers distributed along the hall (shown in Figure 3.1) using an FM transmitter. Investigators viewed the protocol via a video screen (screenshots shown in Figure 3.2), the moving representation of a
participant indicated the subjects' required position on the running track and displayed their status within the protocol.

The sequence and relative/absolute intensity of shuttles is represented in Table 3.1. Each of these sequences forms a cyclic activity pattern, of which there are 11 per block of exercise. Each block of exercise lasted for approximately 15 min (depending on the VO2max of participants) and blocks were separated by 3 minutes of rest.

![Figure 3.2 Captured frames from the LIST protocol video stream. © N.Gant 2002.](image)

Subjects remained standing during these 3 minute rest periods, during which time solutions were ingested. An exercise protocol consisting of 6 blocks of exercise is represented schematically in Figure 3.3. A number of the physiological, performance and subjective measures sampled at discrete phases within the LIST protocol have been expressed in this thesis relative to total exercise time. However, the duration of each cycle changes according to the VO2max of participants by 2.53 s per 10 ml·kg⁻¹·min⁻¹. The range of subjects' VO2max scores within this thesis vary by a relatively small amount, allowing acceptable accuracy when presenting variables relative to time. Where chronological time is presented relative to LIST-dependent results, the time scale is labelled as an approximate value.

Time taken to complete each 15 m sprint was measured uni-directionally over the first 15 m of the running lanes. This was achieved using infra-red photoelectric cells (RE Components Ltd, Switzerland) interfaced with computer software.
The standard pattern of exercise blocks and rest periods described in this section was used throughout the experimental studies within this thesis. For individual studies the duration of exercise was selected by modifying the number of exercise blocks. Chapters 4, 7 and 8 use 4 blocks equating to approximately 60 min of exercise. The protocol in Chapters 5 and 6 comprises of 6 blocks, an exercise duration of approximately 90 minutes. In the final LIST study (Chapter 9) the standard repetition of blocks of exercise and rest was continued until volitional fatigue or the point at which subjects were withdrawn according to the criteria explained in Chapter 9.2.2. Modifications to the duration of the LIST protocol are illustrated schematically in the methods section of each experimental chapter.

<table>
<thead>
<tr>
<th>Sequence</th>
<th>Activity</th>
<th>Distance (m)</th>
<th>Intensity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Walking</td>
<td>$3 \times 20$</td>
<td>$1.54 \text{ m·s}^{-1}$</td>
</tr>
<tr>
<td>2</td>
<td>Maximal Sprint</td>
<td>$1 \times 15$</td>
<td>Maximum</td>
</tr>
<tr>
<td>3</td>
<td>Recovery</td>
<td>3</td>
<td>$\sim 1 \text{ m·s}^{-1}$</td>
</tr>
<tr>
<td>4</td>
<td>Running</td>
<td>$3 \times 20$</td>
<td>$95% \text{ } \dot{V}O_2\text{max}$</td>
</tr>
<tr>
<td>5</td>
<td>Jogging</td>
<td>$3 \times 20$</td>
<td>$55% \text{ } \dot{V}O_2\text{max}$</td>
</tr>
</tbody>
</table>

Table 3.1 The sequence and intensity of shuttles that form 1 cycle of the LIST.

3.3.5 Heart rate monitoring

Heart rate was measured at 5 s intervals during rest and exercise via short-range telemetry. Coded chest transmitter belts (Polar Electro, Kempele, Finland) were worn along with wristwatch receiver-loggers (Polar Sports Tester, Polar Electro, Kempele, Finland), data was downloaded post-exercise to computer software (Polar HR Analysis software, v5.04, Polar Electro, Kempele, Finland).
Figure 3.3  A schematic representation of the 6-block LIST protocol. The relative time scale shown is equivalent to a $\dot{V}O_2_{\text{max}}$ value of 60 ml·kg$^{-1}$·min$^{-1}$. The six blocks of exercise lasting approximately 15 min comprise of 11 repeated cycles of the activity pattern represented in the diagram. The relative or absolute speeds of each activity are shown in Table 3.1.
3.3.6 Deep body temperature

Deep body temperature was measured via ingestible temperature sensors throughout this thesis. In Chapters 4 and 10 rectal temperature was used as an additional measure to provide a comparison of techniques, the relevant methodology is described in the methods section of Chapter 4.

3.3.6.2 Intestinal temperature

Intestinal temperature was measured using disposable temperature sensor capsules (Cor-100, HQinc, Florida, USA) ingested 8-10 hours before experimental trials. This time period allowed sensors to pass through the stomach and regions of the gastrointestinal tract susceptible to changes in temperature caused by ingested fluids. Intestinal temperature was measured pre, post and during each walking phase of the LIST protocol (~ every 80 s) by positioning the ambulatory data recorder (CorTemp 2000, HQinc, Florida, USA) near to the posterior lumbar region of the subject’s back. The electromagnetic flux generated by the sensors extends to a radial proximity of around 60 cm, allowing measurements to be made whilst subjects walk without interrupting the exercise test.

Subjects were required to ingest the sensor on the evening before experimentation in accordance with the guidelines included in Appendix E. After ingesting the sensor subjects were asked to refrain from defecating until after the experimental trial, to prevent the loss of the sensor with the faeces.

3.3.6.3 Sensor capsule calibration

Ingestible sensors were calibrated less than 1 week before experimental trials in a stirred water bath (Thermed 5001, GFL, Hannover, Germany) over a range of temperatures, using a calibrated mercury thermometer (15 - 45°C, LW Scientific, Georgia, USA). The technique used was similar to that employed by Lee et al. (2000), O’Brian et al. (1998) and Stephenson et al. (1992). Sensors were individually attached to the bulb of the thermometer with a small rubber o-ring and immersed in the water bath. Sensor and thermometer were immersed for 4 minutes before comparisons were made (at the highest temperature in the range it took on average 2.5 min for pills to achieve thermal equilibrium with the bath). The raw sensor output (discarding factory calibration) and thermometer temperature were compared at bath temperatures of 37, 38 and 39°C (Chapters 4, 5 and 6) or 37, 39 and 41°C (Chapters 7, 8 and 9). Sensors
with a Pearson’s correlation coefficient (r) of < 0.999 were not used in experimental trials. A three-point linear regression was than applied and the slope of the line was used to transform raw output from the sensors. Raw sensor readings were transformed by an experimenter shortly after being collected using computer software. This method allowed experimenters to communicate sensor readings without subjects becoming aware of their deep body temperature.

3.3.7 Subjective ratings

Four subjective rating scales (Appendix G) were administered pre, post and during the last walking phase of each exercise block. Subjects indicated a number or descriptor by pointing to the scale without revealing their choice to the other participant; this prevented any bias in reporting. Perceived exertion was assessed during exercise using a 15-point scale (Borg, 1982). A 15-point thermal sensation scale was used to indicate perceived thermal stress. This was a modification of the 7-point scales of Bedford (1936) and ASHRAE (1966), a neutral sensation represents a value of zero. Fifteen-point ratings were also used to indicate perceived gut fullness and thirst.

3.3.8 Test solutions

During each experimental study the same relative volumes of fluid calculated according to body mass (BM) were ingested at similar time intervals. All drinks were served in clear plastic drinking bottles at a temperature of 4°C and were administered in a double-blind fashion, with the treatment order randomised. Solutions were described to subjects as equally beneficial ergogenic aids. A pre-exercise bolus volume equivalent to 6.5 ml·kg BM\(^{-1}\) was rapidly ingested (< 180 s) 3 minutes before the exercise protocol. Further drinks were then given during the rest periods between exercise blocks. Volumes of these drinks were equivalent to 3.5 ml·kg BM\(^{-1}\) and were ingested whilst standing upright within 150 s. In Chapters 4, 5 and 6 the fluid ingested was plain water (Swithland Spring, Leicester, UK). During subsequent studies (Chapters 7, 8, 9 and 10) two test solutions were used, a carbohydrate electrolyte solution (CES) and a taste-matched flavoured water (FW).

3.3.8.1 Composition of test solutions

The CES used in this programme of research was a commercially available, orange flavoured, isotonic sports drink (Lucozade sport, GlaxoSmithKline, Brentford, UK),
containing a maltodextrin and glucose syrup. The CHO content of this drink was approximately 6.4%. To minimise production variation the CES used in each study was taken from a single manufacturing batch. The FW consisted of water (Swithland Spring, Leicester, UK) with an added low-sugar orange flavouring (Robinsons Ltd, Chelmsford, UK) (200 ml·l⁻¹) and an artificial sweetener (Sweetex, Crooks Laboratories, Basingstoke, UK) (27 mg·l⁻¹). The FW was formulated in order to have an identical appearance, taste and mouth-feel to the CES. The flavoured water was formulated in our laboratory as a single batch for each study and stored at 4°C.

Samples of the test solutions were taken from each batch and analysed in our laboratory for pH, osmolality, Na⁺ and K⁺ concentration and by the Leicestershire Public Analyst Laboratory for total CHO and energy content. Table 3.2 reports the results of these analyses along with the typical values provided by the manufacturer of the CES.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Typical CES</th>
<th>Chapter 7 CES</th>
<th>FW</th>
<th>Chapter 8 CES</th>
<th>FW</th>
<th>Chapter 9 CES</th>
<th>FW</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy content (KJ·100 ml⁻¹)</td>
<td>118</td>
<td>128</td>
<td>6</td>
<td>120</td>
<td>4</td>
<td>125</td>
<td>5</td>
</tr>
<tr>
<td>Total CHO (g·100 ml⁻¹)</td>
<td>6.4</td>
<td>6.4</td>
<td>0.1</td>
<td>6.2</td>
<td>0.1</td>
<td>6.3</td>
<td>0.1</td>
</tr>
<tr>
<td>Na⁺ (mmol·l⁻¹)</td>
<td>27</td>
<td>21</td>
<td>6</td>
<td>24</td>
<td>5</td>
<td>22</td>
<td>6</td>
</tr>
<tr>
<td>K⁺ (mmol·l⁻¹)</td>
<td>0.57</td>
<td>0.56</td>
<td>0.07</td>
<td>0.62</td>
<td>0.06</td>
<td>0.60</td>
<td>0.06</td>
</tr>
<tr>
<td>pH</td>
<td>3.45</td>
<td>3.34</td>
<td>3.52</td>
<td>3.88</td>
<td>3.42</td>
<td>3.37</td>
<td>3.35</td>
</tr>
<tr>
<td>Osmolality (mOsmol·kg⁻¹)</td>
<td>286</td>
<td>288</td>
<td>45</td>
<td>292</td>
<td>47</td>
<td>290</td>
<td>44</td>
</tr>
</tbody>
</table>

Table 3.2 Composition of test solutions used during experimental trials. Typical values provided by the manufacturer of the CES are reported alongside analyses of the batches of CES and FW used in each chapter.
3.4 Blood sample collection, treatment, storage and analysis.

3.4.1 Sample Collection
Serial venous blood samples were obtained via an indwelling cannula (Venflon, 18G, Becton-Dickinson, Helsingborg, Sweden), which was kept patent by flushing at every sampling period with 10 ml of sterile saline (Stenpak Ltd, Runcorn, UK). The cannula was inserted into an antecubital forearm vein and connected to a three-way tap (Becton-Dickinson, Helsingborg, Sweden), which was secured to the arm with medical tape (Transpore, 3M, Loughborough, UK). After 15 min of standing an 11ml resting sample was drawn into a syringe (Becton-Dickinson, Helsingborg, Sweden), further 11ml samples were taken during the first minute of rest periods separating LIST blocks.

3.4.2 Treatment, Storage and Analysis of venous blood samples
Samples were stored and analysed in a biochemistry laboratory within the School of Sport and Exercise Sciences, Loughborough University. Radio-immunoassays were performed in a radiochemistry laboratory within the Department of Chemistry, Loughborough University. All biochemical analyses described in this thesis were performed in duplicate, unless otherwise stated.

Five ml of venous whole blood was dispensed into tubes containing potassium ethylenediaminetetraacetic acid (EDTA) (49.355.001; Sarstedt, Nümbrecht, Germany). Duplicate 20μl aliquots were aspirated from the sample and rapidly deproteinised in 200μl of 2.5% perchloric acid at 4°C, centrifuged for 3 min at 7000 ×G (Model 5414C, Eppendorf, Hamburg, Germany) and stored at -80°C. The supernatant was used to fluorimetrically determine blood lactate concentration (Locarte, Model 8-9, London, UK) using the enzymatic method outlined by Maughan (1982).

Triplicate 50 μl samples of whole blood were collected using heparinised pipettes (Brand GMBH, Wertheim, Germany) and micro-centrifuged (Gelman Hawksley Ltd, Sussex, UK) for 15 min at 6700 ×G. Packed cell volume (PCV) was determined using a sliding micro-haematocrit reader (Gelman Hawksley Ltd, Sussex, UK). Duplicate 20 μl aliquots (Brand GMBH, Wertheim, Germany) of whole blood were collected and used for the determination of haemoglobin concentration by the cyanmethaemoglobin
method. Changes in plasma volume (Δ%) were estimated using the method of Dill and Costill (1974).

The remaining EDTA-treated whole blood was centrifuged for 10 min at 1700 ×G at a temperature of 4°C (Allegra X-22R, Beckman Coulter, California, USA). The resulting supernatant was stored in untreated Eppendorf tubes at -80°C and subsequently used to determine concentrations of plasma glucose, free fatty acids (FFA) and glycerol on an automated system (COBAS Mira Plus, Roche Diagnostics Systems, Switzerland) using the respective commercially available kits (GOD-PAP method, Randox Ltd, Co Antrim, UK; NEFA, Wako Chemicals Inc, Virginia, USA; GPO-PAP, Randox Ltd Co Antrim, UK).

Five ml of whole blood was dispensed into tubes containing a clotting activator (49.351.001 Sarstedt, Nümbrecht, Germany) and allowed to clot for 1 hour at room temperature to obtain serum. The coagulated sample was then centrifuged for 10 min at 1700 ×G in a temperature of 4°C (Allegra X-22R, Beckman Coulter, California, USA). The visible plasma was transferred into untreated Eppendorf tubes which were stored at 4°C and -80°C for later analysis. Frozen samples were assayed to determine serum insulin concentration using a solid-phase 125Iodine radio-immunoassay (Coat-Count, Diagnostica Products Corporation, Caernarfon, UK) and counted using an automated gamma counter (Cobra 5000, Packard, Pangbourne, UK). Serum, test drink and urine osmolality were determined for samples stored at 4°C using freezing point depression (Osmomat 030, Gonotec, Berlin, Germany). Serum and test drink sodium and potassium concentrations were established by flame photometry (480 Flame Photometer, Corning, Halstead, UK) and chloride concentration was determined via coulometric titration (Jenway PCLM 3, Dunmow, UK).

An intra-assay coefficient of variation (CV) was calculated for the biochemical assays used throughout this thesis. Table 3.3 reports the within-batch CV \( \left( \frac{SD}{\bar{x}} \times 100 \right) \) for repeated measurements (n=20) within the physiological range.
<table>
<thead>
<tr>
<th>Assay</th>
<th>Method</th>
<th>Mean</th>
<th>CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haemoglobin</td>
<td>Cyanmethaemoglobin</td>
<td>14.4 g·100 ml⁻¹</td>
<td>0.9</td>
</tr>
<tr>
<td>Packed Cell Volume</td>
<td>Microcentrifugation</td>
<td>44 %</td>
<td>1.1</td>
</tr>
<tr>
<td>Blood Lactate</td>
<td>Maughan (1982)</td>
<td>3.4 mmol·l⁻¹</td>
<td>1.7</td>
</tr>
<tr>
<td>Plasma Glucose</td>
<td>GOD-PAP (Randox)</td>
<td>4.8 mmol·l⁻¹</td>
<td>1.2</td>
</tr>
<tr>
<td>Plasma FFA</td>
<td>NEFA (Wako Chemicals)</td>
<td>0.3 mmol·l⁻¹</td>
<td>2.0</td>
</tr>
<tr>
<td>Plasma Glycerol</td>
<td>GPO-PAP (Randox)</td>
<td>0.1 mmol·l⁻¹</td>
<td>5.3</td>
</tr>
<tr>
<td>Serum Insulin</td>
<td>Radio-immunoassay</td>
<td>143 pmol·l⁻¹</td>
<td>6.6</td>
</tr>
<tr>
<td>Serum Sodium</td>
<td>Flame Photometry</td>
<td>140 mmol·l⁻¹</td>
<td>0.7</td>
</tr>
<tr>
<td>Serum Potassium</td>
<td>Flame Photometry</td>
<td>4.7 mmol·l⁻¹</td>
<td>1.5</td>
</tr>
<tr>
<td>Serum Chloride</td>
<td>Coulometric Titration</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Serum Osmolality</td>
<td>Freezing point depression</td>
<td>295 mOsmol·kg⁻¹</td>
<td>0.9</td>
</tr>
</tbody>
</table>

Table 3.3 Within-batch coefficients of variation for biochemical assays.

3.5 Statistical analyses

Statistical analyses of the physiological and subjective data within this thesis were performed using the Statistical Package for the Social Sciences software (SPSS Inc, Illinois, USA). All data were tested for normality of distribution either using the Shapiro-Wilk test (w) for sample sizes <50 or the Kolmogorov-Smirnov (d) test for larger sample sizes. Data differing substantially from Gaussian distribution were analysed using non-parametric analyses or logarithmically transformed (Altman, 1991) and re-analysed.

To detect systematic bias in comparisons over time, two-factor (trial × time) general linear models (ANOVA) (F) with repeated measures on both factors (Atkinson, 2002) were used to analyse main effects and the interaction of these two factors. Analyses were controlled for violation of the sphericity assumption using Mauchly’s test of sphericity according to Atkinson (2001). Where asphericity was detected the Greenhouse-Geisser correction was applied for epsilon (ε) <0.75 and for higher ε values the Huyn-Feldt correction was used (as suggested by Girden, 1992 and Atkinson, 2001). The presence of a significant interaction between the trial and time
factors in the ANOVA was explored using the Holm-Bonferroni step-wise method as outlined by Atkinson (2002) and (Ludbrook, 1998). For variables comprising of a large numbers of multiple comparisons this method may raise the significance threshold to prohibitive levels resulting in a type 2 error (Perneger, 1998). In these instances the incremental area under curve as outlined by Wolever (2004), was also used to explore the cumulative magnitude of differences detected in serial measurements.

For single comparisons between normally distributed data, paired Student’s t-tests (two-tailed) (t) and Pearson’s correlation coefficient (r) were used to examine bias between trials or treatments. Non-normal data were analysed using the Wilcoxon matched-pairs test (z).

For experimental investigations involving manipulation of an independent variable, a priori power analyses were undertaken in order to predict required sample and effect sizes. These tests were based on similar published findings using a modified form of Cohen’s (d) test (Cohen, 1988).

Null hypotheses were rejected at the alpha level of $P<0.05$, except for comparisons where the appropriate Holm-Bonferroni adjustment was applied. Central tendency is reported as mean ($\overline{x}$) ± standard deviation (SD) for normally distributed data and median ($\overline{x}$) ± range when non-Gaussian distribution is present.

### 3.6 Graphical representation of error
The Normalised 95% Confidence Interval (nCI) was used to represent error graphically for the studies in which treatments were administered in a within-subjects design (Chapters 7, 8 & 9). This was achieved using the method described by Masson and Loftus (2003) and Loftus and Masson (1994), which are extensions of the suggestions of Anderson and McLean (1974). Plotting means along with their nCI provides an initial, intuitive assessment of the underlying population mean and the degree to which the pattern of sample means reflects the underlying pattern of the population mean, i.e. the degree of statistical power. In other words the nCI provides information about the amount of statistical noise that obscures the conclusions that one can draw about a pattern of means. Because the nCI is based on the mean square
error term (MSerror) of the general linear model it leads to comparable conclusions, this allows the nCI to be used as an alternative to null hypothesis significance testing. Within this thesis the graphical representation of the nCI will be used to supplement hypothesis-testing procedures.

When interpreting within-subject designs the between-subject variability is not relevant to the evaluation of the pattern of means, therefore the data can be normalised to omit this. Individual scores were normalised using the method described by Masson and Loftus (2003), this method leaves the pattern of scores unchanged and between-subject variability eliminated whilst between-condition interaction and variability remain unchanged. The nCI computed for each contrast was calculated according to the following equation:

\[ \text{nCI} = \bar{x} \pm \sqrt{\frac{\text{MSerror}}{n}} \cdot (t - \text{distribution}) \]

Where \( \bar{x} \) is the mean of the group, \( n \) is the number of subjects associated with each mean, \( \text{MSerror} \) is the GLM error term for the contrast-specific F-ratio and \( t\text{-distribution} \) is the students t-distribution based on the degrees of freedom from the effect (at the 0.05 critical value).

If the nCI for the difference between subjects is larger than the effect of the treatments this indicates that there is not adequate power to detect a difference in means. In other words where error bars representing the nCI do not overlap we can be confident that the sample means are significantly different. If bars overlap by less than half of their length there is a strong possibility that the means are significantly different.

For readers more au fait with the use of SD as a graphical representation of error, these data are reported in Appendix H for all figures presented within this thesis.
CHAPTER 4

A COMPARISON OF INTESTINAL AND RECTAL TEMPERATURE DURING HIGH-INTENSITY INTERMITTENT EXERCISE.

4.1 Introduction

During prolonged, high-intensity shuttle running involving feeding, the most reliable predictor of $T_{core}$ is likely to be rectal temperature (for discussion see Chapter 2.2.2). However, there are a number of impracticalities associated with using this measurement technique during the LIST. The presence of a rectal thermistor often results in some discomfort that has the potential to negatively influence self-selected sprinting performance and exercise capacity. In certain individuals a flexible rectal thermistor moves or is expelled from the rectum during sprinting or the deceleration from this phase of the LIST protocol. In addition to these practical issues, a large number of games players are not willing to undergo an instrumentation procedure that is invasive in this manner. Therefore the aim of this investigation was to compare $T_{rec}$ with ingestible temperature sensors, an alternative method of $T_{core}$ estimation during the LIST that overcomes these issues.

The validity of measuring deep body temperature using ingestible sensors (Cor-100, HQinc, Florida, USA) has been previously examined during interval cycling (Castle et al., 2003; Kolka et al., 1993; Stephenson et al., 1992), treadmill walking (Kolka et al., 1997) and whilst performing normal daily activities (Edwards et al., 2002). However, to date no study has compared this technique with others during forms of upright unconstrained exercise. During running exercise the distribution of blood flow and the amount of metabolically active tissue is somewhat different to seated exercise. Despite the lack of applicable research ingestible temperature sensors now see widespread use in sporting (Clark et al., 2004; Fowkes Godek et al., 2004; Guerra et al., 2004; Leclerc et al., 2000; Schnirring, 2004), military (Bryne et al., 2004; Cotter et al., 2000; Fortney et al., 1998), occupational (Kolka et al., 1997; OPL, 2004; White et al., 1998) and medical settings (Freedman, 2001; Hamilos et al., 1998). Considering that gut motility may also be increased by both upright weight-bearing exercise (Keeling and Martin, 1987) and high intensity interval exercise (Soffer et al., 1991), it
seems pertinent to investigate whether $T_{int}$ measurement is valid during this form of exercise.

An important consideration that is often overlooked is the time interval between sensor ingestion and the commencement of temperature measurement. Several previous validity studies have used intervals between sensor ingestion and exercise that may be insufficient for sensors to progress to areas of the large intestine that are not sensitive to changes caused by ingested material (Kolka et al., 1997; Kolka et al., 1993; Sparling et al., 1993; Stephenson et al., 1992). In the present study sensors were ingested 10 h before exercise and the daily energy intake of subjects was monitored over a 48 h period before exercise to ensure daily energy intake was sufficient to represent the hyperphagic eating habits of athletes.

The major conceptual flaw in this measurement technique is the lack of standardisation of the measurement site; this changes as the sensor progresses along the GI tract during exercise. The protocol used in the present study is dynamic in nature with regular changes in motion that occur across several planes. The mechanical vibration if the abdomen associated with this activity pattern is likely to elicit movement of temperature sensors if insufficient progression through the GI tract has not been made in the 10h prior to the test.

Rectal temperature is the only reliable measurement of $T_{core}$ that has been successfully administered during the LIST protocol (Morris et al., 1998a; 1998b; 2003; 2000; Sunderland and Nevill, 2003; 2005). Therefore, solely rectal temperature was compared with intestinal temperature in a population of subjects with a range of $\dot{V}O_2\text{max}$ values seen in trained sportsmen, whilst completing 4 blocks of the LIST protocol.

4.2 Methods
4.2.1 Subjects
Ten male games players aged 24 (21 - 26) years with a body mass of 76 ± 12 kg and height of 1.8 ± 0.1 m took part in this study. The mean predicted $\dot{V}O_2\text{max}$ of the group was $57 \pm 4 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ with a range of 50 - 63 ml·kg⁻¹·min⁻¹. Subjects habitually
spent 280 min·week\(^{-1}\) (± 111) engaged in training activities applicable to the exercise protocol and were familiar with the technique for measuring \(T_{rec}\) during exercise.

4.2.2 Experimental design

Prior to the main trial subjects completed The Multistage Fitness Test (Ramsbottom et al., 1988) as previously described in Chapter 3.3.3 in order to estimate \(\dot{V}O_2\max\). The resultant predicted \(\dot{V}O_2\max\) scores were used to calculate shuttle speeds corresponding to 55% and 95% of \(\dot{V}O_2\max\) for appropriate phases of the LIST protocol. The mean duration of each LIST activity cycle for the population of subjects was 80.8 ± 1.2 s (range (79 - 83 s). On a second visit to the laboratory a familiarisation session consisting of 3 blocks of the LIST protocol was completed under experimental conditions at an ambient temperature of –15°C. This session was conducted in order to accustom subjects to the demands and activity patterns of the protocol. The instrumentation used in the main trial was used during this familiarisation session. The above preliminary tests were performed 1 – 2 weeks before main experimental trials.

Main experimental trials were conducted at 08:00 between Tuesday and Friday. Subjects arrived at the laboratory following an overnight fast (10 h) bringing with them a sample of their first morning urine. On arrival serial \(T_{int}\) measurements were taken to establish that the temperature sensor was working correctly and to confirm that \(T_{core}\) was in a steady state. If necessary the subject sat quietly for 10 min in an environmental temperature of 25°C in order for this to be achieved. In a private area the subject then inserted a flexible rectal thermistor (401, YSI, Ohio, USA) to a depth of 10cm beyond the anal sphincter, indicated by a bulb attached to the cable. A smooth plastic marble (15 mm diameter) was used in order to retain the probe during exercise and as an indicator of the correct insertion depth. Rectal thermistors were calibrated against using a mercury thermometer (15 - 45°C, LW Scientific, Georgia, USA) in a stirred water bath (Thermed 5001, GFL, Hannover, Germany). Thermistor and thermometer temperature were compared at bath temperatures of 37, 38 and 39°C. Thermistors with a Pearson’s correlation coefficient (r) of < 0.999 were not used in experimental trials. If comparisons were linear in nature a three-point regression was applied and the slope of the line was used to transform \(T_{rec}\)
measurements. Intestinal temperature sensors were calibrated according to the method described in Chapter 3.3.6.3.

Rectal temperature was measured by connecting the rectal thermistor to a light-weight (25g), portable data logger (ML2002, Mini-Mitter Inc, Oregon, USA). The temperature of the intestinal sensor was measured using a portable ambulatory data recorder (CorTemp 2000, HQinc, Florida, USA) (218g). Both data loggers were packed into a low-profile neoprene waist pouch which was tightly secured to the posterior lumbar region of the subject's torso. The rectal thermistor cable was secured to the skin superior to the natal cleft with medical tape (Transpore, 3M, Loughborough, UK) to prevent any movement.

The subject then entered the sports hall adjacent to the laboratory (~ 15°C, ~60% RH) and was given a volume of water equivalent to 6.5 ml·kgBM⁻¹, which was consumed within 180 s. During this period, light, static stretching of the lower limbs was permitted. Following the consumption of the water the exercise protocol consisting of 4 blocks of LIST activity (outlined in Chapter 3.3.4) commenced. The Tint and Tree data loggers were activated simultaneously at the start of the exercise, and data collected with a sampling interval of 5 s and resolutions of 0.01°C and 0.05°C respectively. During each rest period between LIST blocks a volume of water equivalent to 3.5 ml·kgBM⁻¹ was ingested within 150s and the data loggers were examined to check that they were working correctly. Throughout the protocol environmental variables were monitored and adjusted accordingly (as described in Chapter 3.3.1.2). At the end of the exercise test the data loggers were stopped and subjects removed the rectal thermistor in a private area.

4.2.3 Statistical analyses
All data were tested for normality of distribution and for homogeneity of variance as described in Chapter 3.5. Presence of heteroscedastic error was investigated using correlated variables as outlined by Nevill and Atkinson (1997). Paired Student's t-tests (two-tailed) (t) and Pearson's correlation coefficients (r) were used to determine significant bias between methods of measurement. Intraclass correlation coefficients (ICC) were calculated using the method suggested by Atkinson & Nevill (1998), as an indicator of bias and random variation between data sets. Standard error of
measurement (SEM) was calculated to indicate absolute reliability (68% of population) using the following equation: $SEM = SD \sqrt{1 - ICC}$ (Atkinson and Nevill, 1998). The coefficient of variation was used to assess absolute reliability and was calculated using the method suggested by Atkinson and Nevill (1998):

$$\frac{SD \ (of \ difference \ between \ data \ sets)}{x \ (of \ both \ data \ sets)} \times 100$$

Bland and Altman's method of calculating 95% levels of agreement was used to indicate absolute reliability (Bland and Altman, 1986). Absolute bias and limits of agreement (LOA) were calculated along with the dimensionless ratio limits of agreement (RLOA). Ratio limits of agreement were calculated from log transformed data in order to account for heteroscedastic errors. The Bland-Altman comparisons representing absolute (untransformed) differences are included in Appendix H. Inferential statistics are based on a population of 10.

The CorTemp 2000 ambulatory data recorder (HQinc, Florida, USA) is prone to recording occasional, short-term erroneous temperatures. This occurs due to malalignment of telemetry and external electromagnetic interference. In the present investigation and Chapter 10, automatically logged data that contains a maximum of three consecutive erroneous data points (<20 s), which are outside of the expected physiological range, were corrected using an interpolation analysis as recommended by Streiner (2002). Incorrect data collected for more prolonged periods were removed using listwise deletion.

### 4.3 Results

Table 4.1 reports the results of a number of statistical tests applicable to the examination of physiological method comparison data. There are a number of potential flaws associated with employing each technique in this particular study design and currently no agreement to which is the most suitable test (for a discussion see Atkinson & Nevill 1998). Therefore these variables are all presented in order that the reader interprets whichever he/she in most familiar with. For the comparison of $T_{rec}$ and $T_{int}$ the use of the coefficient of variation technique supplemented with the Bland-Altman's limits of agreement may provide the best absolute indicator of reliability.
4.3.1 Systematic bias
A significant bias between methods of measurement (\(\bar{x} - 0.24^\circ C\)) was found throughout the protocol \((P<0.0001; \text{Table 4.1})\) and these absolute differences are explored graphically using a Bland-Altman plot (Figure 4.2). The shift in central tendency between methods of measurement is also apparent in the means plot of temperature vs. time (Figure 4.1). Comparisons between methods of measurement are represented in Figure 4.3 as separate Bland-Altman plots for each block of exercise. The systematic bias between methods of measurement appears to decrease slightly in magnitude with the duration of the exercise protocol (-0.17 \(^\circ\)C Block 1 vs. -0.12 \(^\circ\)C Block 4; Table 4.1).

4.3.2 Relative reliability
A mean intraclass correlation coefficient of 0.99 suggests excellent consistency of comparison between methods (Vincent, 1994). This is supported by a strong correlation between methods \(r = 0.98 (P<0.0001)\), calculated from log transformed measurements. There are no statistical trends in these variables in relation to exercise time. It should be noted that these comparisons were not performed on independent observations, a fundamental assumption in the computation of this variable. These coefficients are also capable showing strong correlations when significant bias exists between methods, and hence should only be interpreted alongside the systematic bias.

4.3.3 Absolute reliability
The mean standard error of measurement between methods was ± 0.06 \(^\circ\)C. This is an acceptable amount of error between methods as meaningful differences when comparing \(T_{\text{core}}\) during separate exercise trials are likely to be considerably greater than this (differences detected between trials during the pilot investigation were 4 times larger than this). However, the presence of heteroscedastic error may render this test less useful than a ratio static (such as CV). The mean coefficient of variation between methods of measurement for all measurements combined is 0.29\% (Table 4.1). This statistic is perhaps the most suitable measure of absolute reliability for this data set and shows that an excellent degree of agreement exists between the techniques. Limits of agreement (± 2 SD) are shown graphically for all measurements in Figure 4.2 and for each block of exercise in Figure 4.3. This sample of temperature measurements is not normally distributed \((w = 0.081, P<0.0001)\), therefore it is more
appropriate (although perhaps not graphically) to examine the variables computed from logarithmically transformed measurements. Antilogs taken from the differences between LOA show that in 95% of cases, intestinal temperature differs by 1% above and 0.2% below rectal temperature.

The amount of random error may be magnified in the present study as temperature measurements were automatically logged. Automatic logging leads to infrequent erroneous data points caused by antenna alignment difficulties, this irregularity has been previously observed by Kolka et al. (1997). In subsequent studies manual measurements that lie outside these limits will generally be identifiable as erroneous by an investigator with knowledge of the expected range and would be repeated at various antenna alignments until an acceptable value was obtained.

4.3.4 Environmental conditions
Ambient temperature (15.3 ± 0.9 °C) and relative humidity (59.9 ± 13.7 %) remained constant and no differences were detected between trials or over time in these environmental parameters.

4.3.5 Dietary energy intake
Subjects were asked to eat the diet that they would normally consume 48 h before taking part in strenuous activity/match-play. The mean consumption of CHO, fat and protein over this period was 7.4 ± 2 g·kg BM⁻¹·day⁻¹, 1.1 ± 0.6 g·kg BM⁻¹·day⁻¹ and 1.8 ± 1 g·kg BM⁻¹·day⁻¹ for each macronutrient respectively. No caffeine was detected in the dietary intake of the subjects and the osmolality of the first morning urine was <900 mOsmol·kg⁻¹. Mean energy intake over the 48 h period prior to exercise was 13.6 ± 2.9 MJ·day⁻¹. By applying linear regression analysis to the findings of Harris et al. (1991), the orocecal transit time of the temperature sensor can be approximated to 83 ± 20 min at the energy intake recorded by this cohort of subjects.
Figure 4.1  Mean rectal and intestinal temperature (\( \bar{x} \pm SD; ^\circ C \)) during 4 blocks of the LIST protocol.

Figure 4.2  A Bland-Altman plot representing comparisons between rectal and intestinal temperature (°C), during 4 blocks of the LIST protocol. Bias and random error lines (95% limits of agreement) are included.
Figure 4.3 A Bland-Altman plot representing comparisons between rectal and intestinal temperature (°C), during 4 blocks of the LIST protocol. Bias and random error lines (95% limits of agreement) are included. Each block is presented on a separate ordinate.
Table 4.1 Results of statistical comparisons between rectal and intestinal temperature (°C), during each approximately 15 min block of the LIST protocol. Table reports two-way paired t-test statistic (t), Pearson’s product-moment correlation (r), intraclass correlation coefficient (ICC), standard error of measurement (SEM), coefficient of variation (CV), Bland-Altman’s absolute limits of agreement (LOA) and ratio limits of agreement (RLOA). (ln) denotes tests have been performed on logarithmically transformed data. Σ row indicates data from the entire protocol.* denotes $P<0.01$; ** denotes $P<0.0001$.
4.4 Discussion

A systematic bias between the two techniques is evident throughout the protocol, this difference is similar in direction and magnitude to that reported by Stephenson and co-workers (1992). The consistently higher intestinal temperature observed in the present study is in agreement with other studies that report higher temperatures in $T_{\text{int}}$ vs. $T_{\text{rec}}$ at a given time (Edwards et al., 2002; Kolka et al., 1993; Lee et al., 2000; Stephenson et al., 1992). No significant differences were detected in the relative response time of the two measures and the decline in temperature following the rest periods occurred at essentially the same time. This is in agreement with the findings of Castle et al. (2003), and suggests that the bias during the LIST protocol is possibly not attributable to a greater thermal inertia at the rectum, but rather an absolute difference of 0.14 °C between the colon and the rectum. However, as emphasised by the results of Lee and colleagues (2000), absolute differences should be interpreted with caution if the exercise intensity is not constant, as the thermal inertia of the instruments themselves differs. A posture-related distribution in blood flow to metabolically active tissue may explain why response time was different during this upright form of weight-bearing exercise compared with the majority of previous research, which has used seated or supine cycling.

The bias between methods of measurement declines slightly over time, a total of 0.05 °C during the protocol. This reduction in bias is mirrored in an increase in the absolute reliability of comparison. This may suggest that any progression of the sensor along the GI tract occurs early on in the exercise protocol, the position of the sensor may become normalised between subjects as the duration of exercise increases. This potentially supports the anecdotal observations that sensors are passed at a similar time shortly after exercise. Alternatively this shift in bias could be representative of a change in blood flow at these sites with exercise duration. It has been shown that as central venous pressure drops during prolonged exercise a greater fraction of cardiac output is diverted away from the hepatic-splanchnic organs and the kidneys (Rowell et al., 1968). This mesenteric ischemia may lead to $T_{\text{int}}$ becoming less sensitive to small changes in blood temperature and hence more reflective of $T_{\text{rec}}$. Another hypothesis might be that changes in the amount of metabolically active tissue in leg blood flow could lead to increased venous return via the haemorrhoidal veins increasing the sensitivity of $T_{\text{rec}}$. 

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The ingestion period of 10h appears to have enabled sufficient progress of the sensor through the GI tract, as no differences between $T_{\text{int}}$ and $T_{\text{rec}}$ are apparent after the repeated ingestion of 4°C water. This is in agreement with the studies in which the ingestion time has ranged from 6 – 12 h (Edwards et al., 2002; Lee et al., 2000; O'Brien et al., 1998).

The literature that has previously examined the validity of $T_{\text{int}}$ measurement routinely reports only absolute differences in techniques and response times. There is generally a limited use of inferential statistics or statistical methods for assessing reliability. Castle and co-workers (2003) did include a limited analysis of error and their findings suggest that the range of temperatures detected in $T_{\text{int}}$ is not clinically acceptable. However, the bias and LOA detected in their study ($0.2 \pm 0.3 \, ^\circ\text{C}$) were calculated from heteroscedastic data. Additionally, the overall correlation of comparison recorded ($r=0.82$) was somewhat less than in the present study ($r=0.98$).

Considering that the temperature throughout the body core is generally not uniform, the differences observed in the present study suggest that $T_{\text{int}}$ provides an acceptable level of agreement with $T_{\text{rec}}$ and consistency during serial measurements. This site of measurement appears to provide a reliable and accurate index of core temperature for use in exercise physiology where measurement at other sites is not feasible.

The close agreement between $T_{\text{int}}$ and $T_{\text{rec}}$ may also have implications in sporting and occupational settings, where long-term measurement is often problematic as sensors are not retained for a sufficient period. In this situation comparable results could potentially be obtained by administering additional sensors rectally.

This study additionally highlights the issue of rectal probes being expelled during shuttle-running. This occurred predominately during the sprinting phases of the LIST protocol and three of the subjects originally recruited were withdrawn from the study due to reoccurrences of this throughout the protocol.
CHAPTER 5

THE RELIABILITY OF INTESTINAL TEMPERATURE DURING HIGH-INTENSITY INTERMITTENT EXERCISE.

5.1 Introduction
The validity of $T_{int}$ measurements has been examined in previous research and in Chapter 4 by comparing the technique with various other sites of measurement. However, the reproducibility of this technique in the same individuals on separate occasions remains to be addressed. Repeated measures study designs are popular within the field of exercise physiology and although a suitable level of control is usually imposed before separate trials, there are many factors that can alter orocecal transit time (and hence the location of temperature sensors) independently of physical activity and food intake.

Temperature throughout the length of the GI tract is not uniform and varies relative to the proximity of major vessels and organs. If temperature sensors are not in a similar location at the start of an exercise protocol, or progress along the GI tract at a varying speed in separate trials, differences in $T_{int}$ may be detected that are independent of the experimental treatment.

In the present study the reliability of $T_{int}$ during an exercise test was addressed by controlling the level of external influences between trials to the extent one would expect in a robust study design. This comprised of a weighed and then repeated 48 h diet and a replication of physical activity not related to normal daily living. The number of hours awake and time of rising and retiring was also broadly controlled during this 48 h period.
5.2 Methods

5.2.1 Subjects

Nine competitive male games players aged 21 (19 - 23) years with a mean body mass of 74 ± 4 kg and average height of 1.8 ± 0.1 m took part in this study. The mean predicted $\dot{V}O_2_{\text{max}}$ of the cohort was 58 ± 4 ml·kg$^{-1}$·min$^{-1}$ Subjects were of a semi-professional, ex-professional or of university 1st team soccer standard and were selected from a range of outfield playing positions. Subjects habitually spent 211 min·week$^{-1}$ (± 96) engaged in training activities applicable to the exercise protocol.

5.2.2 Experimental Design

Subjects completed The Multistage Fitness Test (Ramsbottom et al., 1988) as previously described in Chapter 3.3.3 in order to estimate $\dot{V}O_2_{\text{max}}$. This value was used to calculate shuttle speeds corresponding to 55% and 95% of $\dot{V}O_2_{\text{max}}$ for appropriate phases of the LIST protocol and to group experimental subject pairs. The duration of the LIST activity cycle for the cohort of subjects was 80.4 ± 1.2 s (range 78 – 82 s). On a separate visit to the laboratory 3 blocks of the LIST protocol were completed under experimental conditions. This familiarisation session was conducted in order to accustom subjects to the demands and activity patterns of the protocol, sampling procedures and instrumentation. The above preliminary tests were performed 1 – 2 weeks before main experimental trials.

Main experimental trials were conducted at 08:00 between Tuesday and Friday, repeated trials were conducted on the same weekday 7 days later. Subjects arrived at the laboratory following an overnight fast (10 h) bringing with them a sample of their first morning urine. On arrival serial $T_{\text{int}}$ measurements were made to establish that temperature sensors were working correctly and to confirm that $T_{\text{core}}$ was in a steady state and similar before both trials. If necessary subjects sat quietly for 10 minutes in an environmental temperature of 25°C in order for this to be achieved. Subjects then voided and nude body mass was measured before heart rate monitoring equipment was attached.

Subjects then entered the sports hall adjacent to the laboratory and were given a volume of water equivalent to 6.5 ml·kg BM$^{-1}$, which was consumed within 180 s.
During this period, light, static stretches of the lower limbs were permitted. Subjects then began to exercise, the protocol used consisted of 6 blocks of LIST activity (outlined in Chapter 3.3.4) Intestinal temperature was measured pre-exercise, immediately post-exercise and during the walking phase of each cycle. After each exercise block a mean heart rate was obtained and volumes of water equivalent to 3.5 ml·kg BM⁻¹ were ingested within 150s. Throughout the exercise protocol environmental temperature and RH were monitored and adjusted accordingly as described in Chapter 3.3.1.2.

5.2.3 Statistical Analyses

The statistical analyses performed in Chapter 4.2.3 were repeated for this study and the factors that should be considered when interpreting these variables (discussed in Chapter 4.3) apply to the present chapter. In addition an independent two-way analysis of variance (ANOVA) with repeated measures and a correction for sphericity was used to determine if any systematic bias existed between repeated trials over time. Differences were explored using the Holm-Bonferroni step-wise method. Inferential statistics are based on a population of 9.
5.3 Results

5.3.1 Exercise intensity

Mean heart rate was essentially the same between repeated trials (Figure 5.1). No statistical differences were detected between trials in mean heart rate before exercise, or during each exercise block ($F_{1,8} = 0.068; P=0.802$). Sprint performance was similar between repeated tests, no differences were found in mean 15 s sprint times between trials ($F_{1,8} = 0.555; P=0.837$; Figure 5.2).

5.3.2 Systematic bias

An analysis of variance with repeated measures did not detect any systematic bias between repeated trials ($F_{1,8} = 0.766; P=0.407$). A paired students t-test detected significant differences between repeated trials in 3 of the 6 blocks of exercise (Table 5.1). An overall comparison of means reported a significant bias between tests ($t_{(602)} = -2.98, P<0.01$). However, the above tests are not sensitive to the presence of random error. A small shift in bias is evident between trials (-0.01 °C; Figure 5.3), this remains similar in direction and comparable in magnitude during each exercise block (Figure 5.5) Basal intestinal temperature was 37.18 ± 0.26 °C and 37.15 ± 0.28 °C in trials 1 and 2 respectively, at the end of exercise these were 38.43 °C ± 0.38 and 38.46 ± 0.37 °C.

5.3.3 Relative reliability

Pearson's product-moment correlation co-efficients calculated from logarithmically transformed data show a high correlation between repeated trials in each approximately 15 min period of exercise (Table 5.1). The agreement between all measurements combined is also strong ($r = 0.971, P<0.01$). Intraclass correlation co-efficients indicate excellent reliability, the ICC for all comparisons combined was 0.99.

5.3.4 Absolute reliability

The overall standard error of measurement was ± 0.06 °C. However, heteroscedastic error was detected which may confound the interpretation of this variable. The coefficient of variation for each approximately 15 min block of exercise is shown in Table 5.1, the overall CV for all measurements was 0.17%. Limits of agreement for repeated trials are represented graphically in Figure 5.4, antilogs taken from the
differences between LOA show that in 95% of cases intestinal temperature differs between repeated trials by ± 0.6%. All measures of absolute reliability reported in Table 5.1 exhibit a trend toward better reliability with exercise duration. This trend can be seen graphically as a contraction of the LOA in the multiple Bland-Altman plots (Figure 5.5).

5.3.5 Environmental conditions and trial order effects
Environmental temperature (14.9 ± 0.7 °C) and relative humidity (62.4 ± 7.9 %) remained constant and no differences were detected between trials or over time in these parameters. No trial order effects were detected in any of the reported measures.

5.3.6 Habitual dietary food intake
Mean energy intake over the 48 h period prior to the first exercise trial was 13.5 ± 3.7 MJ·day⁻¹. The mean mass of CHO, Fat and protein consumed during the recording period was 7.2 ± 2.6 g·kg BM⁻¹·day⁻¹, 1.0 ± 0.7 g·kg BM⁻¹·day⁻¹ and 1.7 ± 0.4 g·kg BM⁻¹·day⁻¹ respectively. By applying linear regression analysis to the findings of Harris et al. (1991), the orocecal transit time of the temperature sensor can be approximated to 84 ± 25 min at the energy intake recorded by this cohort of subjects. The average amount of caffeine detected in subjects diets was 3.4 ± 8.3 mg·day⁻¹.
Figure 5.1 Mean heart rate ($\bar{x} \pm nCI$; beats·min$^{-1}$) for repeated trials of the 6 block LIST protocol.

Figure 5.2 Mean 15 m sprint times ($\bar{x} \pm nCI$; s) for repeated trials of the 6 block LIST protocol.
Figure 5.3  Mean intestinal temperature ($\bar{x} \pm SD; ^\circ C$) during repeated trials of the LIST protocol.

Figure 5.4  A Bland-Altman plot representing comparisons of intestinal temperature ($^\circ C$), between repeated LIST trials. Bias and random error lines (95% limits of agreement) are included.
Figure 5.5  Bland-Altman plots representing comparisons of intestinal temperature (°C), between repeated LIST trials. The 6 blocks of exercise are presented on separate ordinates to the same scale. Bias and random error lines (95% limits of agreement) are included.
Table 5.1 Results of statistical comparisons of intestinal temperature (°C) between repeated trials, during each approximately 15 min block of the LIST. Table reports two-way paired t-test statistic (t), Pearson’s product-moment correlation (r), intraclass correlation coefficient (ICC), standard error of measurement (SEM), coefficient of variation (CV), Bland-Altman’s absolute limits of agreement (LOA) and ratio limits of agreement (RLOA). (In) denotes tests have been performed on logarithmically transformed data. \( \Sigma \) row indicates data from the entire test.* denotes \( P<0.05; \) ** denotes \( P<0.01. \)

<table>
<thead>
<tr>
<th>Block</th>
<th>( t (n) )</th>
<th>( r (\text{ln}) )</th>
<th>ICC</th>
<th>SEM (°C)</th>
<th>CV (%)</th>
<th>LOA</th>
<th>RLOA (ln)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Block 1</td>
<td>( t (98) = -1.14 )</td>
<td>( r = 0.914^{**} )</td>
<td>0.95</td>
<td>±0.08</td>
<td>0.23</td>
<td>0.02 ± 0.30</td>
<td>1.000×/÷ 1.008</td>
</tr>
<tr>
<td>Block 2</td>
<td>( t (98) = 0.86 )</td>
<td>( r = 0.949^{**} )</td>
<td>0.93</td>
<td>±0.07</td>
<td>0.19</td>
<td>-0.01 ± 0.26</td>
<td>1.000×/÷ 1.007</td>
</tr>
<tr>
<td>Block 3</td>
<td>( t (98) = -0.34^{**} )</td>
<td>( r = 0.950^{**} )</td>
<td>0.97</td>
<td>±0.05</td>
<td>0.15</td>
<td>0.03 ± 0.18</td>
<td>1.000×/÷ 1.005</td>
</tr>
<tr>
<td>Block 4</td>
<td>( t (98) = -3.46^{**} )</td>
<td>( r = 0.877^{**} )</td>
<td>0.97</td>
<td>±0.05</td>
<td>0.14</td>
<td>0.03 ± 0.19</td>
<td>1.000×/÷ 1.005</td>
</tr>
<tr>
<td>Block 5</td>
<td>( t (98) = -2.10^{*} )</td>
<td>( r = 0.975^{**} )</td>
<td>0.97</td>
<td>±0.06</td>
<td>0.17</td>
<td>0.02 ± 0.22</td>
<td>1.000×/÷ 1.006</td>
</tr>
<tr>
<td>Block 6</td>
<td>( t (107) = 0.77 )</td>
<td>( r = 0.963^{**} )</td>
<td>0.98</td>
<td>±0.05</td>
<td>0.14</td>
<td>-0.01 ± 0.20</td>
<td>1.000×/÷ 1.005</td>
</tr>
<tr>
<td>( \Sigma )</td>
<td>( t (602) = -2.98^{**} )</td>
<td>( r = 0.971^{**} )</td>
<td>0.99</td>
<td>±0.06</td>
<td>0.17</td>
<td>-0.01 ± 0.23</td>
<td>1.000×/÷ 1.006</td>
</tr>
</tbody>
</table>
5.4 Discussion

The exercise intensity between repeated trials appears to have been standardised to an acceptable degree, the heart rate and sprint performance indicate that during the sprinting phases of the protocol, self-selected exercise intensity was repeated adequately. There was no significant bias detected in $T_{int}$ or sprint performance between repeated trials, this supports the evidence showing that there is no notable learning or training effect during this protocol when trials are separated by 7 days (Nicholas et al., 2000). In addition, the similar heart rate and core temperature responses suggest that there is no acclimation effect when repeating the LIST protocol in 15°C.

The magnitude of random error between the trials decreases notably during the first three blocks of exercise. To some degree this reduction in error may be due to the relatively large amount of heat stored during exercise, which could possibly normalise any small differences that were present in $T_{core}$ the start of the protocol (Gant et al., 2004). However, considering that basal temperature was similar at the start of the protocol, the majority of the error is likely to be due to movement of the sensor. One suggestion may be that the sensor typically resides within an upper portion on the large intestine before exercise. The increased motility during the early stage of the protocol possibly moves the sensor until it reaches an area of the descending or sigmoid colon in which more compacted faecal material restricts movement. This suggestion is supported by the x-ray films published by Wang et al. (2005), that show endoscopy sensors (of similar dimensions to the $T_{int}$ sensor) residing in the transverse and descending regions of the colon 10 h after ingestion at rest. Furthermore this suggestion appears to agree with the reduction in systematic bias over time seen between $T_{int}$ and $T_{rec}$ in Chapter 4 and may be a more valid conclusion than those associated with circulatory changes.

Elements of the LIST protocol and the environmental conditions are not as controlled as would be achieved during static exercise in an environmental chamber. The proportion of random error attributable to changes in temperature that are independent of exercise intensity or environmental conditions cannot be determined in the present study. The inclusion of $T_{rec}$ measurement during this study or future work would
enable the degree of biological variability and systematic error to be isolated from the accuracy of the sensor.

It is also likely that a proportion of the biological variability between trials occurs as a result of differing within-subject temperature changes. The T\text{core} response when repeating a fixed intensity exercise test may vary notably on separate occasions (Livingstone et al., 1992), regardless of standardising basal T\text{core} before exercise (Jette et al., 1995). Jette and colleagues (1995) examined the reproducibility of moderate increases in T\text{core} during treadmill exercise in varying environmental temperatures. They report that whilst the change in T\text{rec} was reproducible when exercising in athletic clothing at 20°C (increase in T\text{rec} of \textasciitilde 0.3°C), it was dissimilar when comparing trails at 40°C (increase in T\text{rec} of \textasciitilde0.5°C, significant difference of 0.04°C between trails). It seems that regardless of the reliability of the T\text{int} technique, there will be a certain degree of the underlying systemic bias between trails that cannot be accounted for when comparing T\text{core} responses. However, this discrepancy between trials is likely to be small in quantifying with the magnitude of differences that would be deemed meaningful as an intervention effect.

It is worth considering that the LOA and dispersion of results reported here are for a population of subjects, typically individual variation is of considerably lesser magnitude and often unidirectional (within-subject comparisons are presented in Appendix H). Although inferential statistics give an idea of the distribution of measurements over a population of subjects the reliability within individual subjects is somewhat greater.

In summary it appears that ingestible temperature sensors reproduce individual T\text{core} measurements an acceptable accuracy to examine internal heat storage during repeated trials. The precision of this site of measurement appears to increase once a steady-state exercise core temperature is achieved.
CHAPTER 6

THE REPEATABILITY OF INTESTINAL TEMPERATURE DURING HIGH-INTENSITY INTERMITTENT EXERCISE

6.1 Introduction

The previous two chapters have addressed the validity of $T_{\text{int}}$ and the repeatability of this technique in a within-subjects design. What remained to be addressed was whether considerable variation exists in this measure between individuals. This is particularly relevant to sporting applications where $T_{\text{int}}$ is routinely used to compare absolute temperatures between athletes and within team squads. Intestinal temperature and transit time may not necessarily be the same in subjects that are matched for other physiological characteristics, this may vary depending on the physical characteristics and central control of the organs within the abdominal cavity. One published report includes data from individual subjects during exercise (Stephenson et al., 1992). These data for 8 subjects exercising at 40% peak $\dot{V}O_2$ report steady-state intestinal temperature differing over a range of $0.84 \, ^\circ C$. However, this group was not homogeneous with respect to peak $\dot{V}O_2$ (which varied by $\pm 5 \, \text{ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) or other physiological characteristics that may determine the rate of heat storage.

It has been shown that the major determinate of $T_{\text{core}}$ at relative exercise intensities is cardiovascular fitness (Gant et al., 2004; Saltin and Hermansen, 1966). Another major influence is the recent training history of individuals, this will effect $T_{\text{core}}$ independently of cardiovascular fitness. Trained individuals have been shown to establish thermal equilibriums more rapidly (Henane et al., 1977) and have a higher sensitivity to sweating at relative workloads than untrained subjects (Nadel et al., 1974). Additionally anthropometric properties including body mass, surface and body composition will determine the extent and rate of heat storage. Therefore, in the present study a large number of subjects were pair matched considering the above variables and completed an exercise test at the same relative intensity.

Primarily pairs were matched according to the physiological characteristics that determine the energy expenditure during the exercise protocol. Predicted $\dot{V}O_2_{\text{max}}$ and
mean sprint performance (as this will affect the amount of energy expended during the sprinting phases) were matched along with additional measures of leg extension and flexion function, as this has been shown correlate with sprint performance in soccer players (Cometti et al., 2001). Variables that determine heat storage - training status, individual's body mass index and skinfold measures were also controlled.

6.2 Methods
The experimental design and methodological procedures involved in this study were essentially the same as those used in Chapter 5. The experimental design differed in that subjects were recruited to form experimental matched pairs which were assigned to two separate matched groups. Subjects completed only one main LIST trial, running with their appropriate partner.

6.2.1 Subjects
Thirty competitive male games players aged 21 (18 - 32) years with a mean body mass of 79.2 ± 11.4 kg and average height of 1.8 ± 0.1 m took part in this study. Potential subjects were selected according to their suitability to fit the matched pairs study design. The mean predicted \( \dot{V}O_2 \) max of the cohort was 55.8 ± 3.6 ml·kg\(^{-1}\)·min\(^{-1}\). Subjects habitually took part in 5 (1 – 8) training sessions per week that involved activities applicable to the exercise protocol. The duration of the LIST activity cycle for the population of subjects was 81.1 ± 1.1 s (range (78 – 85 s).

Skinfold thickness was measured according to ACSM guidelines (ACSM, 2000) on the right-hand side of the body using calibrated calipers (Holtain Ltd, Crymych, UK). Measurements were taken at 4 sites: biceps, triceps, subscapular and suprailiac. Skinfold thickness is reported as the sum of these four sites, the mean 4 site skinfold thickness was 31 mm (± 9).

Function of the leg extensor and flexor muscles was accessed using an isokinetic dynamometer (770, Cybex Ltd, Northampton, UK). Isometric Maximal Voluntary Contraction (MVC) of leg extension and flexion was assessed through the sagittal plane with an axis of rotation through the femoral condyles. After a familiarisation session (≤ 4 days prior), two isometric repetitions of extension were performed with the left leg at 60° for 5 s. This was followed by two repetitions of isometric flexion for
5 s at 35°. The highest torque achieved in both exercises was recorded as the peak isometric MCV at each respective angle (Nm). The mean force of MVC produced by the cohort for leg extension was 348 ± 7 Nm and 204 ± 45 Nm for flexion.

Following preliminary testing subjects were assigned to matched pairs, grouped according to predicted LIST energy expenditure (using \( \text{VO}_2 \text{max} \) and sprint performance as a surrogate measure), training status and physical dimensions. The characteristics of the two matched-pairs groups are shown in ranked order within table 6.1.

### 6.2.2 Statistical Analyses

The statistical analyses performed in this chapter are described in Chapter 5.2.3. Inferential statistics are based on a population of 30. Error is represented graphically as SD.

<table>
<thead>
<tr>
<th>Grouping characteristics</th>
<th>Matched groups</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>1 Estimated ( \text{VO}_2 \text{max} ) (ml·kg(^{-1} )·min(^{-1} ))</td>
<td>55.8 ± 3.3</td>
<td>55.8 ± 3.9</td>
</tr>
<tr>
<td>2 Mean 15 m sprint time</td>
<td>2.69 ± 0.08</td>
<td>2.72 ± 0.13</td>
</tr>
<tr>
<td>3 Training status (sessions·week(^{-1} ))</td>
<td>5 (1 - 7)</td>
<td>4 (1 - 8)</td>
</tr>
<tr>
<td>4 Body Mass (kg)</td>
<td>77.9 ± 10.6</td>
<td>80.5 ± 11.6</td>
</tr>
<tr>
<td>5 Surface Area (m(^2 ))</td>
<td>2.0 ± 0.15</td>
<td>2.0 ± 0.20</td>
</tr>
<tr>
<td>6 Sum of skinfolds (mm)</td>
<td>29.4 ± 5.9</td>
<td>32.3 ± 10.7</td>
</tr>
<tr>
<td>7 MVC extension (Nm)</td>
<td>362 ± 80</td>
<td>333 ± 106</td>
</tr>
<tr>
<td>8 MVC flexion (Nm)</td>
<td>204 ± 41</td>
<td>205 ± 47</td>
</tr>
<tr>
<td>9 Age (years)</td>
<td>21 (18 - 29)</td>
<td>21 (19 - 32)</td>
</tr>
</tbody>
</table>

Table 6.1 Physical characteristics of the two matched groups (\( \bar{x} \) ± SD).
6.3 Results

6.3.1 Exercise intensity
Mean heart rate was similar between matched groups (Figure 6.1), no statistical differences were found between groups before exercise, or during each exercise block ($F_{1,14} = 0.264; P=0.616$). There were no detectable differences in HR over time during the 90 min of exercise ($F_{4,52} = 0.529; P=0.480$). No statistical differences in mean sprint performance was found between matched groups, no bias was detected either between groups ($F_{1,14} = 0.804; P=0.386$) or over time ($F_{5,70} = 3.467; P=0.02$)(Figure 6.2). The subjective measure of exercise intensity administered (RPE) suggests that perceived exertion was similar between groups ($F_{1,14} = 0.493; P=0.495$), with RPE at the end of the 6th block of exercise rated at 16 ± 2 and 17 ± 2 in the respective groups.

6.3.2 Hydration Status
There was no difference between matched groups in the osmolality of the first urine of the day (701 ± 194 mOsmol·kg$^{-1}$ vs. 759 ± 228 mOsmol·kg$^{-1}$); all subjects' urine was < 900 mOsmol·kg$^{-1}$ on arrival at the laboratory. The total BM lost following the exercise protocol was 0.39 (± 0.45) vs. 0.34 (0.86) kg. This equated to a percentage change in BM of 0.55 ± 0.58 % and 0.43 ± 1.07 % respectively, there were no differences found between groups in this variable.

6.3.3 Systematic bias
An analysis of variance with repeated measures did not detect any systematic bias in $T_{int}$ between the groups of matched pairs during the exercise protocol ($F_{1,14} = 0.014; P=0.909$). The paired students t-test reported in Table 6.2 highlights significant differences between groups in during the 1st and final blocks of exercise. An overall comparison of $T_{int}$ (see Σ row) shows no significant bias between groups ($t_{(602)} = -2.98, P<0.01$). Basal temperature was 37.4 ± 0.2 °C and 37.3 ± 0.3 °C in the two groups, at the termination of exercise this was 38.4 ± 0.6 °C and 38.5 ± 0.6 °C in these two groups respectively.

6.3.4 Relative reliability
Pearson's product-moment correlation co-efficients calculated from logarithmically transformed data show a high correlation between repeated trials in each block of exercise (Table 6.2). The agreement between all measurements combined is also
strong \((r = 0.930, P<0.01)\). Intraclass correlation co-efficients indicate excellent reliability, the ICC for all comparisons combined was 0.96.

6.3.5 Absolute reliability
The overall standard error of measurement was \(\pm 0.11^\circ C\). Heteroscedacity was detected in these comparisons so the value of this measure may be questionable. The coefficient of variation for each approximately 15 min block of exercise is shown in Table 6.2, the overall CV for all measurements was 0.32%. Antilogs taken from the differences between LOA show that in 95% of cases intestinal temperature differs between repeated trials by \(\pm 1.1\) %. Limits of agreement between subjects are represented graphically in Figure 6.4. All measures of absolute reliability exhibit a trend toward increased reliability with exercise duration. This trend can be seen graphically as a contraction of the LOA when comparing plots representing separate blocks of exercise (Figure 6.5).

6.3.6 Environmental conditions
Mean environmental temperature \((18.7 \pm 0.8\ ^\circ C)\) and relative humidity \((62.6 \pm 7.8\ %)\) remained constant and no differences were detected over time or between experimental pairs (separate mornings of testing) in these parameters.

6.3.7 Habitual dietary intake
There was no difference within experimental pairs in energy intake over the 48 h period prior to exercise. Mean energy intake of the two groups was 12.8 \(\pm\) 2.7 MJ·day\(^{-1}\) and 13.1 \(\pm\) 3.1 MJ·day\(^{-1}\). By applying linear regression analysis to the findings of Harris et al. (1991), the mean oroecal transit time of the temperature sensor can be approximated to 88 \(\pm\) 31 min and 86 \(\pm\) 35 min in the two groups respectively.
Figure 6.1 Mean heart rate ($\bar{x} \pm nCl$; beats·min$^{-1}$) for matched groups during the 90 min LIST protocol.

Figure 6.2 Mean 15 m sprint times ($\bar{x} \pm nCl$; s) of matched groups during the 90 min LIST protocol.
Figure 6.3  Mean intestinal temperature ($\bar{x} \pm SD$; °C) of matched groups during the 90 min LIST protocol.

Figure 6.4  A Bland-Altman plot representing comparisons of intestinal temperature (°C) between matched pairs during the 90 min LIST trial. Bias and random error lines (95% limits of agreement) are included.
Figure 6.5  Bland-Altman plots representing comparisons of intestinal temperature (°C), between matched pairs during the LIST protocol. The 6 blocks of exercise are presented on separate ordinates to the same scale. Bias and random error lines (95% limits of agreement) are included.
### Statistical Variable

<table>
<thead>
<tr>
<th>Block</th>
<th>( t ) (°C)</th>
<th>( r ) (ln)</th>
<th>ICC</th>
<th>SEM (°C)</th>
<th>CV (%)</th>
<th>LOA</th>
<th>RLOA (ln)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Block 1</td>
<td>( t (164) = -4.28^{**} )</td>
<td>( r = 0.843^{**} )</td>
<td>0.91</td>
<td>± 0.13</td>
<td>0.40</td>
<td>0.08 ± 0.50</td>
<td>1.002 ± 1.013</td>
</tr>
<tr>
<td>Block 2</td>
<td>( t (164) = -1.74 )</td>
<td>( r = 0.892^{**} )</td>
<td>0.94</td>
<td>± 0.13</td>
<td>0.37</td>
<td>0.04 ± 0.50</td>
<td>1.001 ± 1.013</td>
</tr>
<tr>
<td>Block 3</td>
<td>( t (164) = 1.492 )</td>
<td>( r = 0.916^{**} )</td>
<td>0.95</td>
<td>± 0.11</td>
<td>0.32</td>
<td>-0.02 ± 0.41</td>
<td>0.999 ± 1.011</td>
</tr>
<tr>
<td>Block 4</td>
<td>( t (164) = 0.51 )</td>
<td>( r = 0.926^{**} )</td>
<td>0.96</td>
<td>± 0.01</td>
<td>0.29</td>
<td>-0.01 ± 0.38</td>
<td>1.000 ± 1.010</td>
</tr>
<tr>
<td>Block 5</td>
<td>( t (164) = -0.04 )</td>
<td>( r = 0.955^{**} )</td>
<td>0.98</td>
<td>± 0.08</td>
<td>0.23</td>
<td>0.00 ± 0.30</td>
<td>1.000 ± 1.008</td>
</tr>
<tr>
<td>Block 6</td>
<td>( t (179) = 3.33^{*} )</td>
<td>( r = 0.952^{**} )</td>
<td>0.97</td>
<td>± 0.09</td>
<td>0.27</td>
<td>-0.04 ± 0.33</td>
<td>0.999 ± 1.009</td>
</tr>
<tr>
<td>( \Sigma )</td>
<td>( t (1004) = -0.64 )</td>
<td>( r = 0.930^{**} )</td>
<td>0.96</td>
<td>± 0.11</td>
<td>0.32</td>
<td>0.00 ± 0.42</td>
<td>1.000 ± 1.011</td>
</tr>
</tbody>
</table>

Table 6.2 Results of statistical comparisons of intestinal temperature (°C) between repeated trials, during each approximately 15 min block of the LIST. Table reports two-way paired t-test statistic (t), Pearson's product-moment correlation (r), intraclass correlation coefficient (ICC), standard error of measurement (SEM), coefficient of variation (CV), Bland-Altman's absolute limits of agreement (LOA) and ratio limits of agreement (RLOA). (ln) denotes tests have been performed on logarithmically transformed data. \( \Sigma \) row indicates data from the entire test. * denotes \( P < 0.05 \); ** denotes \( P < 0.01 \).
6.4 Discussion

There were no differences detected in heart rate or sprint performance between groups suggesting that the exercise intensity was controlled to an acceptable degree. There were also no differences in the subjective rating of exertion. The overall lack of any significant bias in $T_{\text{int}}$ between groups supports previous work that has proposed thermoregulatory changes occur in response to the relative intensity of the exercise (Gant et al., 2004; Greenhaff, 1989; Saltin and Hermansen, 1966). Subjects also lost comparable amounts of fluid during the protocol.

The degree of error present between subjects over the time course of the experiment follows a similar pattern to Chapter 5. There is a marked contraction of the LOA which is particularly prominent during the first three blocks. The absolute reliability of comparison increases over time possibly as a result of this. This may potentially be due to the differences in basal temperatures which become similar during exercise, or this pattern could suggest that the sensor reaches a similar location or comparable degree of splanchnic blood flow is achieved during exercise.

For obvious reasons the LOA are considerably larger in the present study than the within-subject comparisons. Nevertheless these findings do show that the motility and temperature within the GI tract responds to exercise in a similar manner between trained individuals. It appears that the plasticity and sensitivity of gut motility is similar in sportmen that are of comparable dimensions and fitness.

A considerable proportion of the differences in $T_{\text{int}}$ between groups will be due to factors other than those related to the measurement technique itself. Havenith (2001) examined the relative influence of a number of inter-individual factors on the response to heat stress during low-intensity cycling exercise. After quantifying the between-subject differences in $T_{\text{rec}}$ and $T_{\text{oes}}$ that were due to anthropometry, $\dot{V}O_{2}\text{max}$ and acclimation, a substantial amount of residual variation remained. It appears that even when cohorts are closely matched for the variables that predominantly influence heat production and storage during exercise, a large degree of unaccountable variation remains in this type of study design.
Nevertheless, the amount of random error observed between groups in this study suggests that reliable enough results may be obtained from $T_{int}$ for use in sporting and occupational setting. However, these comparisons may not be robust enough to draw clinically accurate conclusions in between-subjects study designs.
CHAPTER 7

THE INFLUENCE OF INGESTING A CARBOHYDRATE-ELECTROLYTE SOLUTION AND WATER DURING THE LIST IN 30°C

7.1 Introduction

A number of studies have demonstrated that a suitable CES can enhance exercise capacity (Carter et al., 2003; Galloway and Maughan, 2000) and performance (Below et al., 1995; Davis et al., 1988b; Fritzche et al., 2000; Millard-Stafford et al., 1997; 1992; 2005; Murray et al., 1987) during cycling and treadmill running in the heat. However, this is by no means a consistent finding and it is currently unclear if any benefits can be derived from the ingestion of a CES over water alone during prolonged, high-intensity exercise in the heat. The debate regarding the efficacy of CHO ingestion during strenuous exercise in the heat centres around the fact that the fatigue process under these circumstances is often a consequence of dehydration and hyperthermia rather than carbohydrate availability (Maughan, 1991; Montain and Coyle, 1992b). Therefore the volume of drink that is ingested and absorbed is considered more important than CHO intake.

Recently it has been shown during variable-intensity shuttle running (Morris et al., 2003) and cycling (Fritzsche et al., 2000) in the heat, that the rise of T_{core} may be more rapid when a CES is compared with a carbohydrate-free FW. There are two obvious possibilities why a CHO drink could promote an increase in the rate of heat storage in the body. The first is that the carbohydrate component of the drink slowed gastric emptying sufficiently to limit water absorption and thereby constrain thermoregulation (González-Alonso et al., 1997; Montain and Coyle, 1992b). The second is that exogenous carbohydrate allowed the subjects to exercise at a slightly higher intensity, resulting in greater heat production and hence storage (Nielsen, 1970). The findings of Fritzche (2000) support this second suggestion as an increase in maximal power output was evident during periods of sprinting when ingesting the CES. But this did not result in any change in \dot{\text{VO}}_2, so whether these periods were sufficient to impose a discernibly faster rate of heat storage is arguable. In the study of Morris and co-workers (2003) exercise intensity was considered to have been the
same in all trials because no statistical differences were detected in heart rate, sprint times, total distance run, total exercise duration or ratings of perceived exertion. However, because there was a trial order effect in this study, it is possible that differences in exercise intensity promoted by consumption of the CES drink may have been obscured.

The purpose of the present study is to re-examine the findings of the studies above by investigating the thermoregulatory, metabolic and performance responses to the ingestion of a 6% CES and FW during the LIST protocol at an environmental temperature of 30°C. The method of measuring $T_{int}$ that has previously been shown to accurately represent $T_{core}$ at air temperatures of $\sim 15°C$ (Chapters 4, 5 and 6), was employed in order to increase the $T_{core}$ sampling frequency used by Morris et al. (2003) and Fritzche et al (2000).

The exercise protocol in the present study is essentially the same as that used in the previous chapters and a cohort of subjects with comparable physiological characteristics to previous studies took part. Despite these similarities this measurement technique has not been validated during exercise in an environmental temperature of 30°C, or under circumstances when $T_{core}$ exceeds 38.7 °C during exercise. It is possible that the mobility of the temperature sensor may be significantly altered under these conditions, however, hyperthermia *per se* has been shown to have no effect on gut motility (Harris et al., 1990). It is also possible that substantial changes in splanchnic blood flow may occur relative to the previous studies in response to the increased physiological stress of performing the LIST in 30°C. Increased splanchnic ischemia may potentially lead to a variation in the temperature of the GI tract relative to other sites or measurement. Baring these potential limitations in mind, $T_{int}$ was considered a suitable method of detecting substantial differences in $T_{core}$ between trials ($\sim 0.5 °C$) during the LIST.

A fixed duration test that the cohort of subjects were capable of completing was utilised in order to reduce the degree of subject drop out seen by Morris and colleagues toward the later stages of their protocol.
7.2 Methods

7.2.1 Subjects
Six competitive male games players and five endurance athletes aged 23 (22 - 26) years with a mean body mass of 75 ± 11 kg and predicted $\dot{V}O_2\text{max}$ values of 58 ± 4 ml·kg$^{-1}$·min$^{-1}$ took part in this study. Subjects habitually spent 224 min·week$^{-1}$ (± 142) engaged in training activities applicable to the exercise protocol.

7.2.2 Experimental Design
Following the preliminary tests described in Chapter 3.3.3 three blocks of the LIST protocol were completed under experimental conditions at an ambient temperature of ~15°C, with the blood sampling procedures omitted. This familiarisation session was conducted in order to accustom subjects with the demands and activity patterns of the protocol, sampling procedures and instrumentation. The above preliminary tests were performed 1 – 2 weeks before main experimental trials. The duration of the LIST activity cycle for the cohort of subjects was 80.5 ± 1.1 s (range (79 – 82 s)

Two main experimental trials were performed which were separated by 14 days. Repeated trials occurred on the same weekday and all trials were conducted at 08:00 between Tuesday and Friday. Treatments were assigned in a random crossover design and on each occasion subjects consumed either the CES or FW solutions described in Chapter 3.3.8.

Subjects arrived at the laboratory following an overnight fast (10 h) and initial $T_{\text{int}}$ measurements were made to establish temperature sensors were working correctly and to confirm that $T_{\text{core}}$ was in a steady state and similar before both trials. If necessary subjects sat quietly in an environmental temperature of 25°C in order for this to be achieved. An indwelling cannula was then inserted into an antecubital vein, all subjects were cannulated no later than 20 min before exercise. Subjects then voided prior to the measurement of nude body mass and remained in a standing position whilst being instrumented with heart rate monitoring equipment and a forehead temperature thermistor (EUS-VS-0, Grant instruments Ltd, Cambridge, UK). Thermistors were calibrated as described in Chapter 4.2.2. Subjective rating scales were then administered and an 11 ml resting venous blood sample was drawn and treated as described in Chapter 3.4. Microvascular blood flow was then determined.
via laser Doppler flowmetry (using the basic principles described by Shepherd and Oberg, 1990). This was achieved using a laser Doppler blood perfusion monitor (MBF 3D, Moor instruments, Axminster, UK) operating at a wavelength of 780 nm and calibrated according to the manufacturer's instructions. A receptacle for the optical probe was attached to a predetermined position on the dorsal skin of the left arm, this remained in position throughout the protocol. Erythrocyte flux was calculated over a 1 minute period whilst the subject remained motionless. Temperature of the forehead thermistor was then measured via a data logger (SQ800, Grant instruments Ltd, Cambridge, UK) and heart rate monitoring commenced at 5 s sampling intervals.

Subjects were escorted into the sports hall adjacent to the laboratory (3 min before exercise) and given a volume of appropriate test solution equivalent to 6.5 ml·kg BM⁻¹, which was consumed within 180 s. During this period, light, static stretches of the lower limbs were permitted if subjects wished. The exercise protocol consisting of 4 blocks of LIST activity (outlined in Chapter 3.3.4) then commenced. Intestinal temperature was measured during each walking phase which totalled 11 measurements per exercise block. Throughout the exercise protocol environmental variables were monitored and adjusted accordingly as described in Chapter 3.3.1.2. Subjective rating scales (Appendix G) were administered during the final walking phase of each exercise block and a mean heart rate obtained for the exercise period. Immediately after each block of activity a venous blood sample was drawn, microvascular blood flow and forehead temperature were measured and a test drink with a volume equivalent to 3.5 ml·kg BM⁻¹ was then ingested within 150s.

At the end of the exercise test a venous blood sample was drawn and subjects were moved from the hall into a 15°C room where towel-dried body mass was obtained. For safety reasons an experimenter escorted each subject and monitored T_int for a 15 min period after exercise. Figure 7.1 illustrates a schematic representation of the experimental design.

7.2.3 Statistical Analyses
Statistical analyses were performed as described in Chapter 3.5. Statistics are based on a population of 11 except for serum electrolyte concentrations which are based on a sample population of 8. An effect size analysis based on a changes seen during the
pilot study (Appendix J), estimates that a sample size of eleven has 88% power to
detect a difference in $T_{\text{core}}$ of 0.22°C, assuming a SD of differences of approx 0.32 °C,
using a one sided significance test.
Figure 7.1 A schematic representation of the experimental protocol. The relative time scale shown is equivalent to a $\text{VO}_2\text{max}$ value of 60 ml·kg$^{-1}$·min$^{-1}$. The four blocks of exercise each total approximately 15 min and are comprised of 11 repeated cycles of the activity pattern described in Chapter 3.3.4.
7.3 Results

7.3.1 Deep body temperature

Intestinal temperature at the start of the protocol was 37.3 ± 0.4 °C in the CES trial and 37.1 ± 0.4 °C in the FW trial. There were no differences in basal $T_{int}$ between fluid conditions ($t_{(10)} = 1.360$, $P=0.211$). During the 60 minutes of exercise significant differences in $T_{int}$ were detected between fluid conditions ($F_{1,10} = 6.600$; $P<0.033$; Figure 7.2). Analysis of the incremental area under the $T_{int}$ curve (°C·15 min$^{-1}$), reveals that differences between trials reach statistical significance during the 2nd block of exercise and remain so for the remainder of exercise. Intestinal temperature at the end of exercise was 39.5 ± 0.6 °C and 38.9 ± 0.9 °C in the CES and FW trials respectively. Figure 7.3 shows the changes in $T_{int}$ with respect to resting temperature, there were no statistical differences detected in mean rate of rise in $T_{int}$ over the entire exercise protocol. The rate of rise was 2.2 °C·h$^{-1}$ in the CES trial and 1.8 °C·h$^{-1}$ in the FW trial.

7.3.2 Forehead temperature and forearm skin blood flow

Forehead temperature (Figure 7.4), and microvascular blood flow were determined pre-exercise, post-exercise and during each rest period. Forehead temperature increased throughout exercise ($F_{4,40} = 21.811$; $P<0.001$) and temperature after the final block of exercise was higher than after the first exercise block. There were no differences in this variable between trials. Red blood cell flux was similar at rest in both trials, equating to 12.8 ± 11.5 arbitrary perfusion units in the CES trial and 12.1 ± 10.3 units in the FW trial. Perfusion increased similarly throughout exercise in both trials ($F_{4,40} = 14.633$; $P=0.003$) and was 42.8 ± 12 and 40.2 ± 30 units at the end of exercise in the CES and FW trials respectively.

7.3.3 Sprint performance

Mean 15 m sprint times declined throughout the hour of exercise irrespective of treatment (Figure 7.10). Sprint times during the 3rd and 4th blocks of exercise were significantly slower than during the first 30 min of the LIST. Differences in sprint performance were detected between trials, with sprint performance maintained more effectively under the CES fluid condition (CES vs. FW, $F_{1,10} = 5.090$; $P=0.044$). Cumulatively subjects spent a greater amount of time sprinting during the exercise...
protocol in the FW trial (104.3 ± 4 s) than during the CES trial (103.4 ± 4.1s) (t (8) = -2.453, P=0.040).

### 7.3.3 Heart rate and blood lactate

Heart rate increased during the 4 blocks of the exercise protocol ($F_{3,30} = 108.487; P<0.001$; Figure 7.5), and was significantly higher during the 4th block of exercise compared with the 1st block. Heart rate at the end of the exercise protocol was 184 ± 11 beats·min$^{-1}$ in the CES trial and 179 ± 10 beats·min$^{-1}$ in the FW trial. Mean HR appears to be higher in the CES trial throughout exercise, however, there is no statistical support for this trend.

There were no differences in blood lactate concentration during exercise between fluid conditions or over time (Figure 7.6). A main effect of time was present in these data ($F_{4,40} = 7.25; P=0.003$), blood lactate concentrations were significantly elevated above resting values by the exercise protocol. Immediately after the exercise period blood lactate concentrations were 3.86 ± 2.94 mmol·l$^{-1}$ in the CES trial and 3.59 ± 1.65 mmol·l$^{-1}$ in the FW trial. A coefficient of variation of 1.7% was calculated for the determination of blood lactate concentration via this method.

### 7.3.4 Plasma glucose

At rest plasma glucose concentration was similar between trials and glucose concentrations were higher at each exercise time point compared with rest in both trials. The concentration of plasma glucose was significantly elevated at each exercise time point in the CES trial compared with FW ($F_{1,9} = 21.347; P=0.006$; Figure 7.7). The coefficient of variation for this biochemical assay was 1.2%.

### 7.3.5 Plasma FFA and glycerol

Plasma FFA concentration increased with time ($F_{4,40} = 21.811; P<0.001$), there appears to be a trend toward higher concentrations during the FW trial (Figure 7.8), however, no statistical differences were detected between fluid conditions. There were also no differences between treatments in plasma glycerol concentration (Figure 7.9). There was a main effect of time ($F_{4,40} = 15.478; P<0.001$), glycerol concentrations at the end of exercise showed a marked increase from the pre-exercise sample. The coefficients of variation for these assays were 2.0% and 5.3% for plasma FFA and glycerol respectively.
7.3.6 Serum osmolality and electrolytes
Serum osmolality reported in Table 7.1 was similar between solutions and no significant changes were detected over time. During the CES trial serum Na⁺ concentration was significantly increased above corresponding values in the FW trial \(F_{1,7} = 16.924; P = 0.006\). The concentration of serum Cl⁻ measured by coulometric titration was varied compared with the expected physiological response and showed only a weak correlation to the concentration of serum Na⁺ \((r=0.589, P<0.01)\). There were no differences in serum K⁺ concentration between fluid conditions. Potassium concentrations were higher after the protocol than before exercise.

7.3.7 Plasma volume and body mass
No changes in plasma volume were detected between trials (Table 7.1). Percentage changes in plasma volume at fatigue compared with rest were -4.5 % (± 1.5) in the CES trial and -4.4 % (± 1.2) in the FW trial. There were no differences in adjusted post-exercise body mass between trials; the mean body mass lost as sweat was 2.38 kg (± 0.49) and 2.42 kg (± 0.31) in the CES and FW trials respectively. Assuming all ingested fluid was emptied into the small intestine and absorbed, the fluid deficit expressed as a mean percentage change in body mass was CES: -1.3% (± 0.3) and FW: -1.4% (±0.2).

7.3.8 Subjective responses
There were no significant differences between fluid conditions in any of the subjective measures presented in Table 7.2. Individuals’ rating of perceived exertion was significantly higher during the last block of exercise compared with the first block \(F_{3,30} = 25.221; P=0.008\). Subjective feeling of thermal comfort and perceived thirst were elevated during exercise compared to the resting measures. Perceived thermal stress was greater during the final block of exercise compared with all other timepoints.

7.3.9 Environmental conditions and trial order effects
Environmental temperature \((30.6 ± 0.1 °C)\) and relative humidity \((31.8 ± 0.1 %)\) remained constant and no differences were detected between trials or over time in either of these parameters. There were no statistical differences detected in the trial order of any parameters reported in this study.
7.3.10 Habitual food intake

Statistical analysis revealed that dietary and physical activity records were replicated successfully before repeated trials and no differences were found in energy intake or consumption of CHO, fat or protein. Mean energy intake over the 48 h period prior to exercise was 12.7 ± 4.3 MJ·day$^{-1}$. The mean mass of CHO, Fat and protein consumed during the recording period was 7.3 ± 3.0 g·kg BM$^{-1}$·day$^{-1}$, 0.8 ± 0.4 g·kg BM$^{-1}$·day$^{-1}$ and 1.4 ± 0.5 g·kg BM$^{-1}$·day$^{-1}$ respectively. The average amount of caffeine ingested in the diet was 7.9 ± 3.1 mg·day$^{-1}$. By applying linear regression analysis to the findings of Harris et al. (1991), the orocecal transit time of the temperature sensor can be approximated to 87 ± 34 min at the energy intake recorded by this cohort of subjects.
Figure 7.2 Intestinal temperature ($\bar{x} \pm nC; ^\circ C$) during the LIST test, under the carbohydrate (CES) and flavoured water (FW) fluid conditions. Main effect $P<0.05$ CES vs. FW.

Figure 7.3 Change in intestinal temperature ($\bar{x} \pm nC; ^\circ C$) with respect to baseline (0 min), in the carbohydrate (CES) and flavoured water (FW) trials. Main effect $P<0.05$ CES vs. FW.
Figure 7.4 Forehead temperature (\(\bar{x} \pm nCI; ^\circ C\)) whilst resting pre, post and during each 3 min rest period of the LIST protocol; separate lines indicate the CES and FW test drinks. \(t P<0.05\); Rest 1 vs. Post.

Figure 7.5 Mean heart rate (\(\bar{x} \pm nCI; \text{beats} \cdot \text{min}^{-1}\)) during each 15 min exercise block for the carbohydrate (CES) and flavoured water (FW) trials during the LIST. \(\dagger P<0.05\); Block 4 vs. Block 1.
Figure 7.6 Blood lactate concentration ($\bar{x} \pm nCI; \text{mmol}\cdot\text{l}^{-1}$) during the LIST protocol for the carbohydrate (CES) and flavoured water (FW) trials.

Figure 7.7 Plasma glucose concentrations ($\bar{x} \pm nCI; \text{mmol}\cdot\text{l}^{-1}$) for the carbohydrate (CES) and flavoured water (FW) trials, during the LIST test. * $P<0.05$ CES vs. FW; † $P<0.05$ rest vs. exercise.
Figure 7.8 Concentration of plasma free fatty acids (\( \bar{x} \pm nCI; \text{mmol}\cdot\text{L}^{-1} \)) during the LIST for the carbohydrate (CES) and flavoured water (FW) trials.

Figure 7.9 Plasma glycerol concentrations (\( \bar{x} \pm nCI; \text{mmol}\cdot\text{L}^{-1} \)) during the LIST under the carbohydrate (CES) and flavoured water (FW) fluid conditions. † \( P<0.05 \) post exercise vs. rest.
<table>
<thead>
<tr>
<th>Block Number</th>
<th>Rest</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Na⁺</strong> (mmol·l⁻¹; n=8)</td>
<td>CES</td>
<td>141 ± 2</td>
<td>144 ± 3</td>
<td>143 ± 2</td>
<td>143 ± 1</td>
</tr>
<tr>
<td></td>
<td>FW</td>
<td>139 ± 2</td>
<td>140 ± 2</td>
<td>140 ± 2</td>
<td>141 ± 3</td>
</tr>
<tr>
<td><strong>K⁺</strong> (mmol·l⁻¹; n=8)</td>
<td>CES</td>
<td>4.6 ± 0.3</td>
<td>4.7 ± 0.3</td>
<td>4.8 ± 0.4</td>
<td>5.0 ± 0.4</td>
</tr>
<tr>
<td></td>
<td>FW</td>
<td>4.5 ± 0.2</td>
<td>4.5 ± 0.4</td>
<td>4.6 ± 0.4</td>
<td>4.9 ± 0.5</td>
</tr>
<tr>
<td><strong>Cl⁻</strong> (mmol·l⁻¹; n=8)</td>
<td>CES</td>
<td>106 ± 6</td>
<td>106 ± 7</td>
<td>105 ± 7</td>
<td>100 ± 8</td>
</tr>
<tr>
<td></td>
<td>FW</td>
<td>109 ± 3</td>
<td>103 ± 5</td>
<td>102 ± 7</td>
<td>101 ± 6</td>
</tr>
<tr>
<td><strong>Serum Osmolality (mOsmol·kg⁻¹)</strong></td>
<td>CES</td>
<td>290 ± 4</td>
<td>291 ± 6</td>
<td>293 ± 8</td>
<td>293 ± 6</td>
</tr>
<tr>
<td></td>
<td>FW</td>
<td>288 ± 6</td>
<td>289 ± 7</td>
<td>292 ± 4</td>
<td>293 ± 5</td>
</tr>
<tr>
<td><strong>Δ Plasma Volume (%)</strong></td>
<td>CES</td>
<td>------</td>
<td>-1.4 ± 1.5</td>
<td>-2.5 ± 1.3</td>
<td>-3.4 ± 1.4</td>
</tr>
<tr>
<td></td>
<td>FW</td>
<td>------</td>
<td>-1.2 ± 1.8</td>
<td>-1.9 ± 1.3</td>
<td>-4.0 ± 1.0</td>
</tr>
</tbody>
</table>

Table 7.1 Concentration of serum electrolytes (mmol·l⁻¹), osmolality (mOsmol·kg⁻¹) and percentage change plasma volume (PV; %) relative to the resting time point. Table reports mean ± SD. P<0.05: †, exercise blocks vs. rest; §, block 4 vs. rest; *, treatment effect CES vs. FW.

Figure 7.10 Mean 15 m sprint times (x ± nCI; s) for the carbohydrate (CES) and flavoured water (FW) fluid conditions during the 60 min LIST protocol. † P<0.05; vs. block 1.
<table>
<thead>
<tr>
<th></th>
<th>CES</th>
<th>FW</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>RPE</td>
<td>11.8 ± 1.8</td>
<td>13.7 ± 1.7</td>
<td>14.8 ± 2.3</td>
<td>14.9 ± 2.7</td>
<td></td>
</tr>
<tr>
<td></td>
<td>11.3 ± 1.7</td>
<td>12.7 ± 1.8</td>
<td>14.6 ± 2.6</td>
<td>15.3 ± 2.9</td>
<td></td>
</tr>
<tr>
<td>Thermal Sensation</td>
<td>0.0 ± 0.8</td>
<td>3.3 ± 4.8</td>
<td>4.8 ± 1.2</td>
<td>5.8 ± 1.1</td>
<td>5.7 ± 1.4</td>
</tr>
<tr>
<td></td>
<td>0.8 ± 1.0</td>
<td>3.4 ± 1.8</td>
<td>4.7 ± 1.9</td>
<td>5.0 ± 1.7</td>
<td>5.5 ± 1.7</td>
</tr>
<tr>
<td>Thirst</td>
<td>7.9 ± 0.6</td>
<td>12.6 ± 2.3</td>
<td>14.3 ± 1.1</td>
<td>14.0 ± 2.5</td>
<td>14.7 ± 1.7</td>
</tr>
<tr>
<td></td>
<td>8.4 ± 2.0</td>
<td>11.9 ± 1.6</td>
<td>13.4 ± 2.0</td>
<td>14.7 ± 2.2</td>
<td>14.0 ± 2.9</td>
</tr>
<tr>
<td>Gut Fullness</td>
<td>7.5 ± 1.1</td>
<td>7.9 ± 0.9</td>
<td>8.9 ± 2.3</td>
<td>9.9 ± 2.5</td>
<td>10.6 ± 2.7</td>
</tr>
<tr>
<td></td>
<td>7.4 ± 0.9</td>
<td>7.7 ± 1.1</td>
<td>9.1 ± 1.4</td>
<td>9.5 ± 2.1</td>
<td>10.4 ± 3.5</td>
</tr>
</tbody>
</table>

Table 7.2 Ratings of perceived exertion (RPE), thermal sensation, thirst and gut fullness (x ± SD) during each LIST exercise block (arbitrary units). † P<0.05; vs. all other time points. § P<0.05; block 4 vs. block 1.

7.4 Discussion

The main findings of this study were that the ingestion of a 6.4% CES allowed better maintenance of sprint performance compared with a FW during 60 min of intermittent high-intensity shuttle running in 30°C. An elevated deep body temperature in the CES trial was detectable from the second block of exercise onward, suggesting that participants were under greater thermal strain in this trial compared with the FW. This may have been the result of an increase in energy expended whilst sprinting or another factor related to the ingestion of CHO.

A decline in sprint performance occurred over the course of exercise protocol in both trials. This was accompanied by a parallel fall in blood lactate concentration, which together suggest that a reduction in exercise intensity during the sprinting phase of the LIST occurred. One possibility is that this decline in performance was the result of an inability to generate sufficient muscular force. It is well reported that the depletion of glycogen within the active muscle leads to an inability to sustain force production during high-intensity activity and it is has been shown that under certain circumstances the rate of glycogenolysis is elevated when the increase in Tcore and
duration of exercise are sufficient (Febbraio et al., 1994a; Febbraio et al., 1994b; Morris et al., 2005; Parkin et al., 1999). In the present study the duration of exercise is somewhat less than that previously shown to deplete muscle glycogen stores in well-nourished subjects. Jacobs (1981) suggests that a critical concentration 175 glucosyl units·kg⁻¹ DM exists, below which anaerobic performance will be restricted. Following 100 min of a similar protocol to that used this chapter Morris et al. (2005) calculated a mean post-exercise glycogen concentration of 200 mmol glucosyl units·kg⁻¹ DM in the m. vastus lateralis. Their subjects also had a considerably lower daily intake of CHO and energy over the days before the experimental protocol than the present subjects. Therefore the endogenous CHO stores within the cohort of subjects used in the present study would probably be sufficient to maintain performance during the 44 sprints attempted. The blood-borne availability of substrate also appears to be satisfactory in the present study under both fluid conditions, with plasma glucose concentrations remaining above 5 mmol·l⁻¹ for the duration of exercise. Blood glucose concentration may not be a suitable reflection of muscle glucose uptake during exercise that induces hyperthermia as this has been shown to be restricted during exercise in the heat (Jentjens et al., 2002). Nevertheless it seems unlikely that the decline of supramaximal sprinting is due to substrate availability in this protocol.

An alternative explanation for the decline in performance seen in both trials involves change within the CNS. The majority of the individuals that took part in the study reached a Tcore similar to levels shown previously to induce fatigue through altered serotonergic neurotransmission or reduced ability to voluntarily recruit a maximal number of motor units (>39°C). It is plausible that the decrements seen in physical work during the sprinting phases of the LIST are the outcome of an anticipatory response mediated by the CNS, reducing muscle recruitment in order to curtail heat production.

The cardiovascular capacity of the subjects used in this investigation is likely to be sufficient to adequately respond to the levels of hyperthermia experienced during the protocol. However the additional influence of net fluid losses in excess of 1% of BM may be one of the causes of the elevated HR seen during the last 15 minutes of exercise. The combination of hyperthermia and dehydration have been shown to
compromise \( \dot{Q} \) and performance at levels that independently would have little effect (González-Alonso, 1998; 1997). Core temperatures at a lower level to those seen at the end of exercise in the present study have been observed at the point of thermoregulatory fatigue when individuals were substantially dehydrated (Adams et al., 1975; Sawka et al., 1992). The circulatory limitations imposed by hypohydration combined with an intolerable rate of heat storage seems to be the most likely explanation for the decrement in performance and increase in HR seen over time in the present study.

During the exercise protocol an increased rate of heat storage occurs in the CES trial during the early stages of exercise, regardless of the slight temperature differences that exist at the start of exercise. This difference between solutions reaches statistical significance during the second block of exercise (15 - 30 min of exercise) and remains higher for the remainder of the protocol when the rate of rise in \( T_{\text{int}} \) begins to plateau. This elevation of \( T_{\text{int}} \) provides some support for previous studies that have shown participants exhibit a higher \( T_{\text{core}} \) when ingesting a CES (Fritzsche et al., 2000; Hall et al., 2005; Millard-Stafford et al., 2005; Morris et al., 2003). The magnitude of the difference after 60 min of exercise (0.5°C) is similar to that observed by Millard-Stafford and colleagues (0.6°C) during high-intensity running exercise. One suggestion made by Morris et al. (2003) is that an increase in \( T_{\text{core}} \) when ingesting a CES may be the result of a slower rate of gastric emptying and fluid delivery. In the present study similar changes in body mass, plasma volume and serum osmolality were recorded in both trials and the indicators of heat loss to the environment (skin temperature, blood perfusion and sweat rate) were also comparable. The increased rate of fluid absorption shown to occur from solutions containing CHO (Gisolfi et al., 1992; Rehrer et al., 1992) may to some degree compensate for a slower rate of emptying caused by the CHO. Nevertheless the possibility remains that differences in gastric emptying or blood flow to the visceral organs between solutions may alter the dynamics of the thermoregulatory process. A higher gastric volume is also associated with increased gastrointestinal distress which may curtail performance during maximal sprinting. However this was not detected by the subjective rating scales administered during the present study, which show no differences in perceived gut fullness between fluid conditions.
A small but significant increase in serum sodium concentration was detected during the exercise protocol when the CES was ingested. Sodium absorbed from a CES may aid the maintenance of plasma osmolality, sustain thirst drive and preserve fluid gains for an extended period. None of these advantages were apparent over the FW variables measured during the current protocol, but it is likely that over a more prolonged period of exercise and in the time following exercise these benefits would become evident.

A statistical difference in 15 m sprint performance was found between trials and cumulatively subjects spent less time sprinting when ingesting the CES. This finding is in agreement with previous studies performed in temperate environmental conditions, these have shown that individuals are able to sprint at higher intensities in temperate environments when a 6% CES is compared with a similar volume of water (Ali et al., 2002; MaClaren and Close, 2000; Shirreffs and Merson, 2003; Welsh et al., 2002). It is possible that the elevated plasma glucose concentration caused by the ingestion of the CES provides a significant contribution to the total CHO oxidation rate and thus allows greater force production during the sprinting exercise. This is in agreement with the suggestions of Fritzche and co-workers (2000), but an increase in CHO oxidation is by no means synonymous with the ingestion of CHO in the heat. The majority of published work does not support this phenomenon and similar RER values between CES and placebo solutions are regularly reported during exercise in the heat. An alternative ergogenic effect of CES may be centred around an influence on the CNS. Recently it has been proposed that brain metabolism during exercise may be augmented by CHO supplementation (Nybo, 2003). It has been shown that following prolonged exercise during which CHO was ingested MVC of the leg extensors can be achieved that is closer to that evoked electrically than without CHO ingestion (Nybo, 2003). In the present investigation the exogenous supply of CHO may have induced an increased cerebral energy turnover or increased thermal tolerance preventing the central causes of decreased sprint performance. It is worth bearing in mind that the decreased cerebral energy turnover rates reported in the previous studies were recorded at plasma glucose concentrations of around 3 mmol·l⁻¹, considerably lower than at any point during the present protocol. Aside from the concentration of glucose in the blood it has been postulated that oropharyngeal receptors may elicit performance enhancing biochemical changes in the brain when
CHO is detected in the mouth or elsewhere (Carter et al., 2004a; Liu et al., 2000). Although these suggestions await further investigation they support a previous correlation seen between subjective affective valance and sprint performance when CHO was ingested during the LIST in cool conditions (Ali, 2002).

Given that the subjects were probably expending more energy when ingesting the CES, the additional work performed may be responsible for some or all of the extra heat gained. This seems the most likely source of the increase in $T_{\text{int}}$ on the CES trial, but in the present study there are no reliable means of quantifying the amount of energy stored or expended during the protocol. Calculating a measure of total heat content using only forehead temperature may be unwise without other sites of measurement. It has also been suggested that this site provides a closer approximation of core than the overall skin temperature, as vasoconstriction in the forehead is limited (Parsons, 2003).

The findings of the present study show that well trained games players and endurance athletes are capable of maintaining sprint performance more effectively during 1 hour of the LIST in 30°C when a CES is ingested rather than water. This increase in performance and presumably energy expenditure seems to occur at the cost of an elevated $T_{\text{core}}$, that appears to have no detrimental effect on fluid balance or any subjective measures of stress over 1 hour of exercise. Whether or not some of the additional heat gain is due to a delayed rate of gastric emptying and hence fluid delivery remains to be established, as does if the higher $T_{\text{core}}$ will ultimately lead to a more rapid onset of fatigue when ingesting the CES over a more prolonged period of exercise.
CHAPTER 8

GASTRIC EMPTYING OF FLUIDS DURING THE LIST IN 30°C

8.1 Introduction

During field sports such as soccer, rugby and hockey the taste and availability of drinks are probably the main factors that determine the volume that is ingested during match-play. However when an optimal amount of drink is ingested the rates of gastric emptying and intestinal absorption are the key factors regulating the availability of the drink for use by the body (Leiper, 2001; Maughan, 1991).

It is commonly assumed that the rate of gastric emptying is the major limiting factor determining the availability of an ingested drink for use during exercise (Coombes and Hamilton, 2000; Maughan, 1991). It has been shown that intermittent, high-intensity exercise delays the gastric emptying of fluids compared with an equivalent energy expenditure during constant load exercise (Leiper et al., 2001a) or low-intensity walking (Leiper et al., 2005; Leiper et al., 2001b). Solutions containing 4% or more carbohydrate have also been shown to empty more slowly from the stomach at rest than an equivalent volume of water (Vist and Maughan, 1994). It is therefore possible that the drinks with a CHO concentration of around 6% used in the study of Morris et al. (2003) and in Chapter 7 were emptied from the stomach more slowly than the carbohydrate-free FW during the LIST in the heat. If this was the case then it was probably dehydration and hypovolaemia that led to the faster rate of rise in core temperature during the CES trials in these studies. On the other hand no differences were detected in the gastric emptying rates of a 6.4% CES drink and of a carbohydrate-free solution during 30 min of the LIST carried out under relatively cool (15-17 °C) environmental conditions (Leiper et al., 2005). In that study, the exercise intensity of the LIST delayed the gastric emptying of both the CES and the placebo drink to an equivalent extent compared with similar volumes of the same solutions consumed during low-intensity walking. It remains to be determined if a longer period of intermittent exercise or the hyperthermia experienced in the heat influences the rate of gastric emptying of these solutions.
The main purpose of this study was to investigate whether gastric emptying of a 6% CES is slowed during 60 min of the LIST in the heat compared with a carbohydrate-free solution with a lower osmolality. This was achieved by incorporating periods of gastric sampling into the exercise protocol described in Chapter 7. The other aims of the investigation were to determine if the increased sprint performance observed in the previous chapter is a repeatable finding and to quantify the rate of heat storage by calculating the total body heat content in additional to $T_{int}$.

8.2 Methods

8.2.1 Subjects

Nine healthy male soccer players were selected on the basis of their $\dot{V}O_2_{max}$, training status and ability to be successfully intubated with an oro-gastric aspiration tube. Subjects were of a semi-professional, or ex-professional soccer standard and were from a range of outfield playing positions. Their median age, mean mass, height and $\dot{V}O_2_{max}$ were 22 (18 - 31) years, 80.8 ± 8.5 kg, 1.80 ± 0.08 m and 55.5 ± 4.2 ml·kg$^{-1}$·min$^{-1}$, respectively. At the time of the investigation subjects were unaccustomed to exercise in a warm environment.

8.2.2 Experimental Design

The experimental protocol was designed in order to be as similar as possible to that employed in Chapter 7, modifications were introduced to incorporate gastric sampling procedures.

All potential subjects were screened in order to determine those who could be successfully intubated with the oro-gastric tube both at rest and after a 15 min period of the exercise protocol. Subjects were each provided with an individually marked gastro-duodenal feeding tube (French Levine, 14 gauge: Vygon Ltd., Ecouen, France) which was sterilised between trials. The tube was slowly passed down the oesophagus until the tip was ~0.5-0.6 m from the mouth dependent upon the height of the subject. The residual stomach contents were then aspirated as fully as possible and a modified water recovery test (Vist and Maughan, 1994) was conducted to ascertain that the tube tip was appropriately positioned and to wash the stomach. If necessary the tube was
relocated and additional tests were performed until the tip was appropriately located. The required tube depth was recorded and marked on each oro-gastric tube. Subjects then completed 1 block of the LIST protocol and were immediately intubated and 100 ml of water was instilled and aspirated. Subjects who could tolerate this process, without feeling anxious or nauseous were invited back to participate in the study.

On a separate visit to the laboratory 3 blocks of the LIST protocol were completed under experimental conditions at an ambient temperature of ~15°C, the blood sampling procedures and gastric sampling process were omitted. Preliminary tests were performed 1 – 2 weeks before main experimental trials and the duration of each LIST activity cycle for the cohort of subjects was 81 ± 1 s (range (78 – 83 s)).

Two main experimental trials were performed separated by 7 days. Repeated trials occurred on the same weekday and all trials were conducted at 08:00 between Wednesday and Friday. On each occasion subjects consumed either the CES or FW solutions described in Chapter 3.3.8. with the addition of 23 ± 3 mg·l⁻¹ of the non-absorbable, water-soluble dye, phenol red (PR) (BDH, Poole, UK).

Subjects arrived at the laboratory following an overnight fast (10 h) and initial T_int measurements were made to establish that temperature sensors were working correctly and that T_core was in a steady state and similar before both trials. If necessary subjects sat quietly in an environmental temperature of 25°C in order for this to be achieved. Subjects voided their bladders and nude body mass was obtained. The body was then instrumented with four skin thermistors (409B, YSI, Ohio, USA) positioned on the chest, upper arm, thigh, and calf at the locations outlined by Mitchell and Wyndham (1969). Thermistors were calibrated as described in Chapter 4.2.2. Thermistors and cable were secured with medical tape (Transpore, 3M, Loughborough, UK) and sites of attachment were marked for the subsequent trial by the removal of body hair. Skin thermistors were connected to a light-weight (25g), portable data logger (ML2002, Mini-Mitter Inc, Oregon, USA) which was tightly secured to the torso in a neoprene waist pouch. Subjects were then instrumented with heart rate monitoring equipment and subjective rating scales were administered.
Subjects’ immersed their left hand in warm water (40-42 °C) for 5 minutes (Nauck et al., 1992) prior to piercing of the thumb with a lancet (Softclix Accu-chek, Roche Diagnostics, Mannheim, Germany). Duplicate 20 μl volumes of blood were collected in non-heparinised micro-pipettes (Brand GMBH, Wertheim, Germany) and triplicate 50 μl samples in heparinised pipettes (Brand GMBH, Wertheim, Germany). Arterialized blood samples were analysed to determine haemoglobin concentration and packed cell volume as described in Chapter 3.4.

Subjects were escorted into the sports hall adjacent to the laboratory 5 min before exercise in order to carry out the gastric sampling procedure. They were immediately seated and swallowed the gastric aspiration tube (French Levine, 14 gauge: Vygon Ltd., France), positioning the tip in their stomach to the pre-determined length established during the familiarisation stage. The fasting gastric contents were emptied from the stomach via the tube using a 50 ml catheter tipped syringe (Becton Dickinson, Drogheda, Ireland), and the stomach was washed by rapidly infusing and aspirating 100 ml of distilled water. A recovery test was then carried out to ensure that the aspiration tube was correctly positioned (Hassan and Hobsley, 1970). Subjects then orally ingested (121 ± 38 s) a volume of the appropriate test solution equivalent to 6.5 ml·kg BM⁻¹, containing 23 ± 3 mg·l⁻¹ of PR dye. The stomach contents were thoroughly mixed by repeated aspiration and rapid re-injection using a 50 ml syringe. A 2.5 ml aliquot of gastric contents was removed for determination of residual fluid volume. The gastric tube was then removed, HR and Tsk logging commenced at 5 s intervals and subjects then began the exercise protocol. Intestinal temperature was measured during each walking phase which totalled 11 measurements per 15 min exercise block. Throughout the exercise protocol environmental temperature and humidity were monitored and adjusted accordingly as described in Chapter 3.3.1. Subjective rating scales (Appendix G) were administered during the final walking phase of each exercise block and a mean heart rate obtained for each exercise period. Immediately after each 15 min period of exercise subjects sat down and repositioned the gastric tube. The stomach contents were mixed as before and a 2.5 ml aliquot of gastric contents was collected. One ml of phenol red at a concentration of 1000 mg·l⁻¹ was instilled directly into the stomach, and the contents mixed again before a second 2.5 ml sample was collected. The gastric tube was removed and the subjects then
rapidly ingested (188 ± 88 s) a volume adjusted for body mass (3.5 ml·kg\(^{-1}\) body mass) of the appropriate test drink containing 23 ± 3 ml\(^{-1}\) phenol red.

After the final block of exercise the gastric contents were mixed once more and a 2.5 ml aliquot collected, 1 ml of phenol red at a concentration of 1000 mg\(^{-1}\) was instilled into the stomach, and the contents mixed again before a second 2.5 ml sample was collected. One hundred ml of distilled water was introduced into the stomach, mixed with the gastric contents and the total fluid volume of the stomach was emptied as completely as possible by aspiration. This volume, minus the 100 ml of distilled water, was recorded and used to compare whether the total gastric volume at the end of each trial was similar to that calculated by the method of Beckers et al. (1988), and from the dilution of the phenol red concentration of the stomach contents by the 100 ml of distilled water wash.

Following the gastric sampling procedure, subjects' immersed their hand in warm water prior to a second collection of arterialised capillary blood. Subjects were then removed from the hall into a cool laboratory where towel-dried body mass was obtained. An experimenter escorted each subject and monitored T\(_{\text{int}}\) for a 15 min period after exercise. Figure 8.1 illustrates a schematic representation of the experimental design.

8.2.3 Analysis of samples
The volume of fluid in the stomach was calculated using the dye dilution technique of George (1968) and calculations of Beckers et al. (1988). The concentration of phenol red in test solutions and gastric samples was determined by adding 1 ml of a buffer (250 mmol\(^{-1}\) NaOH, 500 mmol\(^{-1}\) NaHCO\(_3\)) to 0.1 ml of the gastric aspirate. Absorbance was then analysed by spectrophotometry (1240, Shimadzu, Kyoto, Japan) at a wavelength of 560 nm. As phenol red dye is poorly absorbed by the stomach (Bloom et al., 1967), the difference in concentration of the dye in the original test drink and the collected samples can be used to calculate the total volume in the stomach and the volume of test drink remaining in the stomach at the specific time points (Rehrer et al., 1990b). The difference between the total gastric volume and the test drink volume is the volume of secretions and swallowed saliva that have entered the stomach lumen over that time period.
8.2.4 Calculations and Statistical Analyses

Weighted mean skin temperature ($\bar{T}_{sk}$) and total body heat content (TBHC) were calculated using the method described by Ramanathan (1964) and Bittel (1987). Changes in body mass during exercise and the heat debt imposed by test drinks (Nadel and Horvath, 1969) were considered when calculating TBHC. Power analysis using data from a previous similar study (Leiper et al., 2001b) suggested that with 9 subjects there was an 87% chance of detecting a real difference in gastric emptying of 20% in the test drink volumes emptied in the present investigation. Statistical analyses were performed as described in Chapter 3.5. Inferential statistics are based on a population of 11, except for $\bar{T}_{sk}$ and TBHC because skin thermistors became dislodged during the LIST protocol resulting in a sample population of 5 for these measures.
Figure 8.1 A schematic representation of the experimental protocol. The relative time scale shown is equivalent to a $\dot{V}O_2_{\text{max}}$ value of 60 ml·kg$^{-1}$·min$^{-1}$. The four blocks of exercise lasting approximately 15 min comprise of 11 repeated cycles of the activity pattern described in Section 3.3.4.
8.3 Results

8.3.1 Residual gastric volume
Following ingestion of the initial bolus the total volume of fluid in the stomach was similar \((P = 0.66)\) on both the CES (555 (59) ml) and FW (542 (62) ml) trials. The volume of test drink ingested immediately before the start of the first bout of exercise was 525 (55) ml. Therefore it is clear that the residual gastric volume remaining in the stomach after the initial aspiration of the fasting contents and washing procedure was relatively small and similar in both trials.

8.3.2 Timing of drinks, time intervals
The interval between starting drinking the initial bolus of test drink and commencing the first exercise block was similar \((P = 0.94)\) for both the CES (362 ± 164 s) and FW (355 ± 218 s) trials, and this period was essentially the same as that subsequently taken between exercise blocks on the CES (277 ± 53 s; \(P = 0.17\)) and FW trials (330 ± 81 s; \(P = 0.96\)). The time interval between the end of the fourth exercise block and the completion of the gastric emptying measurement at the end of the experiment was similar \((P = 0.94)\) on both the CES (220 ± 128 s) and FW trials (225 ± 122 s). The time taken to ingest the test drinks was similar throughout the CES trial (112 ± 41 s; \(P = 0.41\)) and FW trial (123 ± 40 s; \(P = 0.69\)) with no significant difference between trials \((P = 0.24)\).

8.3.3 Total volume in stomach
There was a tendency for greater total volume of fluid to be retained in the stomach in the CES trial than in the FW placebo trial (Figure 8.4) but no significant differences were detected \((P = 0.054)\). The total fluid volume in the stomach was less \((P = 0.045)\) before the start of the initial exercise block (555 ± 59 ml) in the CES trial compared with that at the start of the last exercise block (769 ± 216 ml), but similar to that at the start of the other two exercise blocks (652 ± 156 ml and 636 ± 141 ml, respectively). On the FW trial, the total gastric volume was similar at the start of the four exercise blocks (599 ± 156 ml; \(P = 0.28\)).

The total volume of fluid emptied every 15 min was similar in the CES trial (224 ± 195 ml; \(P = 0.24\)). On the FW trial, the total fluid volumes emptied during the first (232 ± 146 ml), second (363 ± 139 ml) and fourth (311 ± 129 ml) exercise blocks
were essentially the same \((P = 0.17)\), but less volume was emptied during the third
\((150 \pm 104 \text{ ml}; P = 0.032)\) exercise block. No differences were detected in the total
fluid volumes emptied during each exercise block between trials \((P = 0.42)\), nor in the
cumulative total fluid volume emptied between trials \((P = 0.39)\).
The total gastric fluid volume measured at the end of the last exercise bout on the
CES trial was the same \((P = 0.90)\) whether estimated using the formula of Beckers et
al. (1988), or from dilution of the phenol red dye by the 100 ml of distilled water wash
or by aspiration of the stomach contents (Table 8.2). Similarly, the final total gastric
volume measurement was similar \((P = 0.98)\) on the FW trial as estimated by the three
methods.

8.3.4 Test drink volume in stomach
The volume of test drink retained in the stomach was similar between the two trials
\((P = 0.20)\). The drink volume in the stomach was similar at the start of all exercise
blocks in the CES trial \((595 \pm 147 \text{ ml}; P = 0.10)\) and FW trial \((565 \pm 143 \text{ ml}; P = 0.46)\),
and no differences were found between trials \((P = 0.38)(\text{Figure 8.2})\). The volume of
test drink emptied over each 15 min period of exercise on the CES trial was
essentially the same \((P = 0.23)\). This amounted to an average of \(15.6 \pm 10.7 \text{ g}\) of
carbohydrate being delivered to the duodenum during each exercise block. On the FW
trial, the test drink volumes emptied during the first, second and fourth exercise
blocks were essentially the same \((P = 0.22)\), but less volume was emptied during the
third (Figure.8.3; \(P = 0.032\) exercise block. No differences were detected in the test
drink volumes emptied during each exercise block between trials \((P = 0.41)\), nor in the
cumulative fluid volume emptied between trials over the entire LIST \((P = 0.51)\).

8.3.5 Carbohydrate delivered to the small intestine
Total carbohydrate intake over the 60 minutes of exercise when ingesting the CES
solution was \(87.9 \pm 9.3 \text{ g}\) of which \(62.4 \pm 11.9 \text{ g}\) was emptied from the stomach into
the duodenum (Table 8.1). The average amount of carbohydrate emptied every 15 min
was \(15.6 \text{ g} (\pm 4.1)\). The total carbohydrate intake from the FW solution was \(1.37 \pm
0.14 \text{ g}\) over the entire experimental protocol.
8.3.6 Volume of gastric secretion
A small but significantly greater volume of gastric secretions ($F_{1,6} = 14.239; P=0.009$) were present in the stomach throughout the CES trial ($71 \pm 22 \text{ ml·15 min}^{-1}$) compared with that on the FW trial ($41 \pm 23 \text{ ml·15 min}^{-1}$) (Figure 8.5).

8.3.7 Deep body temperature
The basal intestinal temperature measured after the initial intubation procedure was similar in the CES ($37.15 \pm 0.25 \degree C$) and FW ($37.04 \pm 0.22\degree C$) trials. Intestinal temperature during the hour of exercise was different between the two fluid conditions ($F_{1,8} = 16.447; P=0.004$) and was higher during the CES trial (Figure 8.6). Post-hoc analysis of the incremental area under the intestinal temperature curve ($\degree C$·15 min$^{-1}$), shows differences between trials that reach statistical significance during the 4th block of exercise. This difference is also apparent in the rate of rise in $T_{\text{int}}$ during the exercise protocol ($F_{1,8} = 8.991; P=0.017$). At the end of exercise $T_{\text{int}}$ was $39.63 \degree C (\pm 0.68)$ and $39.23 \degree C (\pm 0.21)$ in the CES and FW trials respectively ($t(8) = 5.819, P<0.001$). The mean rate of rise in $T_{\text{int}}$ during the LIST test was $2.57 \degree C\cdot h^{-1}$ in the CES trial and $2.35 \degree C\cdot h^{-1}$ in the FW trial ($t(8) = -2.889, P=0.017$).

8.3.8 Skin temperature and total body heat content
Immediately before exercise $\bar{T}_{\text{sk}}$ was similar in the CES ($33.9 \pm 0.42 \degree C$) and FW ($33.66 \pm 0.39\degree C$) trials. Weighted mean skin temperature increased over time in both fluid conditions ($F_{43,86} = 4.457; P=0.075$). The change over time was not significantly different between trials but subjects appeared to exhibit a higher $\bar{T}_{\text{sk}}$ during the last block of the CES when compared with the FW trial. (Figure 8.7). A post-hoc effect size test showed that the sample size ($n=5$) was not sufficient to detect any significant difference given the reported mean distributions and SD. Post-exercise $\bar{T}_{\text{sk}}$ was $35.42 \degree C (\pm 1.96)$ and $34.98 \degree C (\pm 0.92)$ in the CES and FW trials respectively. Mean TBHC showed a marked increase during the protocol ($F_{43,86} =19.778; P=0.031$; Table 8.3), there were no differences at rest or as a change over time during exercise between fluid conditions. At the end of exercise TBHC was $10.37 \text{ MJ (±0.55)}$ in the CES trial and $10.24 \text{ MJ (±0.73)}$ in the FW trial, a gain of 654 KJ and 541 KJ in the CES and FW trials respectively.
8.3.9 Heart rate and sprint performance

There were no differences in heart rate between trials (Figure 8.8). A time effect was detected during the exercise test and showed a tendency to increase ($F_{2,24} = 108.487; P<0.001$). Mean heart rate was significantly higher during the 4th block of exercise compared with the first three blocks. Heart rate during the first block of exercise was lower than the remainder of the protocol. No differences could be detected between trials in mean sprint times for each exercise block ($P = 0.48$). However, when expressed as cumulative sprint time ($s\cdot h^{-1}$) subjects spent a greater total amount of time sprinting on the FW (103.6 (97.4-111.6) s) than on the CES trial (102.4 (94.9-108.8) s) ($z = 2.090, P=0.037$). Sprint performance did not appear to decline over time during either treatment ($F_{3,24} = 1.316; P=0.295$), but there was a tendency for sprinting speed to be slower during the first block of exercise. This decrease appears to be predominately influenced by the first two sprints attempted ($\bar{x}_{CES} 2.61 \pm 0.09 s$ vs. FW $2.67 \pm 0.09 s$). During these sprints a number of subjects reported discomfort from the initial gastric intubation protocol, which possibly decreased their exertion during these sprints.

8.3.10 Plasma volume, body mass and urine osmolality

Plasma volume exhibited a marked reduction following the 60 min of exercise in both fluid conditions, with percentage decreases of $2.2 \pm 0.7 \%$ in the CES trial and $2.5 \pm 1\%$ in the FW trial. There was no statistical difference in this change in percentage plasma volume between fluid conditions ($t_{(8)} = -0.214, P=0.839$).

The volume of fluid lost as sweat during the protocol was $2.1 \pm 0.5 l$ and $1.4 \pm 0.7 l$ in the CES and FW trials respectively. These fluid losses were significantly different between trials ($t_{(8)} = -3.323, P=0.01$), but the overall percentage of body mass lost was not significantly different between trials ($t_{(8)} = -1.966 P=0.09$) and was $1.5 \pm 0.7 \%$ BM in the CES and $0.9 \pm 0.7 \%$ BM in the FW trial, assuming that all fluid emptied into the small intestine was absorbed.

The osmolality of the urine collected on arrival at the laboratory was similar before exercise on both the CES ($790 \pm 480 mOsmol\cdot kg^{-1}$) and FW trial ($790 \pm 610 mOsmol\cdot kg^{-1}$) ($P = 0.536$).
8.3.11 Subjective responses

No significant differences were apparent between fluid conditions in any of the subjective measures taken (Table 8.4). However, differences were found over time in the rating of perceived exertion with less exertion perceived during the first block of exercise compared with the remainder of exercise ($F = 3.24 14.511; P < 0.001$). Subjective reports of thermal stress were lower during the first 15 minutes of exercise compared with remainder of the protocol ($F = 3.24 3.244; P = 0.011$). Individuals also reported greater feelings of gastric fullness during the first block of exercise compared with the remaining three blocks of exercise ($F = 3.24 4.109; P = 0.032$).

8.3.12 Environmental conditions and trial order effects

Environmental temperature ($30.4 \pm 0.1 \, ^\circ C$) and relative humidity ($31.8 \pm 0.6 \, %$) remained essentially constant and no differences were detected between trials or over time in these parameters. There were no statistical differences detected as an effect of trial order between the physiological parameters reported in this study.

8.3.13 Habitual food intake

There were no differences between repeated trials in habitual energy intake or consumption of CHO, fat or protein. Mean energy intake over the 48 h period prior to exercise was $14.8 \pm 7.5 \, MJ\cdot day^{-1}$. The mean mass of CHO, Fat and protein consumed during the recording period was $8.4 \pm 4.3 \, g\cdot kg \, BM^{-1}\cdot day^{-1}$, $0.7 \pm 0.3 \, g\cdot kg \, BM^{-1}\cdot day^{-1}$ and $1.6 \pm 0.9 \, g\cdot kg \, BM^{-1}\cdot day^{-1}$ respectively. By applying linear regression analysis to the findings of Harris et al. (1991), the orocecal transit time of the temperature sensor can be approximated to $75 \pm 51 \, min$ at the energy intake recorded by this cohort of subjects. No caffeine was detected in the diets over the monitoring period.
Figure 8.2  Total volume of ingested drink present in the stomach ($\bar{x} \pm nCI; \text{ml}$) during each approximately 15 min LIST exercise block, in the CES and FW trials.

Figure 8.3  The volume ($\bar{x} \pm nCI; \text{ml}$) of ingested drink emptied from the stomach during each 15 min LIST exercise block, when ingesting the CES and FW test drinks.
Figure 8.4  Total fluid volume ($\bar{x} \pm nCI; \text{ml}$) within the stomach during each 15 min LIST exercise block, under the CES and FW fluid conditions.

Figure 8.5  Total volume of gastric secretion (ml) present in the stomach ($\bar{x} \pm nCI$) during each 15 min LIST exercise block, when ingesting the CES and FW test drinks. *$P<0.05$ CES vs. FW.
**Table 8.1** Mass of carbohydrate (CHO) ingested (g) and calculated mass delivered to the duodenum (g·15 min\(^{-1}\)), during each LIST exercise block and in total (\(\bar{x} \pm SD\)).

<table>
<thead>
<tr>
<th></th>
<th>LIST Block Number</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>CHO Ingested (g)</td>
<td>33.61</td>
</tr>
<tr>
<td>± 3.54</td>
<td>± 1.91</td>
</tr>
<tr>
<td>Total (g)</td>
<td>87.90</td>
</tr>
<tr>
<td>CHO Emptying (g·15 min(^{-1}))</td>
<td>13.67</td>
</tr>
<tr>
<td>± 6.95</td>
<td>± 13.60</td>
</tr>
<tr>
<td>Total (g)</td>
<td>62.37</td>
</tr>
</tbody>
</table>

**Table 8.2** Comparison of total volume (\(\bar{x} \pm SD\); ml) remaining in the stomach at the end of each trial measured by the dye dilution method of Beckers et al. (1988), by the dilution of the dye by the wash volume of distilled water, and by direct aspiration.

<table>
<thead>
<tr>
<th></th>
<th>Trial</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CES</td>
<td>FW</td>
</tr>
<tr>
<td>Calculated gastric volume (ml) (Beckers et al., 1988)</td>
<td>470</td>
<td>371</td>
</tr>
<tr>
<td>± 131</td>
<td>± 220</td>
<td></td>
</tr>
<tr>
<td>Calculated gastric volume (ml) (dilution by water wash volume)</td>
<td>476</td>
<td>357</td>
</tr>
<tr>
<td>± 140</td>
<td>± 210</td>
<td></td>
</tr>
<tr>
<td>Aspirated gastric volume (ml) (minus the wash out volume)</td>
<td>447</td>
<td>354</td>
</tr>
<tr>
<td>± 136</td>
<td>± 201</td>
<td></td>
</tr>
</tbody>
</table>
Figure 8.6  Intestinal temperature (\(\bar{x} \pm nCI; ^\circ C\)) during the LIST protocol, under the carbohydrate (CES) and flavoured water (FW) fluid conditions. Main effect \(P<0.05\) CES vs. FW.

Figure 8.7  Weighted mean skin temperature (\(\bar{x} \pm nCI; ^\circ C\)) during the LIST protocol, under the carbohydrate (CES) and flavoured water (FW) fluid conditions (\(n=5\)).
Table 8.3  Total body heat content (TBHC) (\( \bar{x} \pm SD; MJ \)) and \( \Delta TBHC \) (\( \bar{x} \pm SD; KJ \)) with respect to rest, immediately after each exercise block of the LIST for the carbohydrate (CES) and flavoured water (FW) test drinks. \( \dagger P<0.05; \) vs. all other time points (n=5).

<table>
<thead>
<tr>
<th>Block Number</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>TBHC (MJ)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CES</td>
<td>9.72</td>
<td>10.00</td>
<td>10.09</td>
<td>10.26</td>
</tr>
<tr>
<td></td>
<td>( \pm 0.59 )</td>
<td>( \pm 0.57 )</td>
<td>( \pm 0.49 )</td>
<td>( \pm 0.54 )</td>
</tr>
<tr>
<td>FW</td>
<td>9.70</td>
<td>9.96</td>
<td>10.11</td>
<td>10.20</td>
</tr>
<tr>
<td></td>
<td>( \pm 0.69 )</td>
<td>( \pm 0.74 )</td>
<td>( \pm 0.70 )</td>
<td>( \pm 0.75 )</td>
</tr>
<tr>
<td>( \Delta TBHC ) (KJ)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CES</td>
<td>284</td>
<td>371</td>
<td>545</td>
<td>654</td>
</tr>
<tr>
<td>FW</td>
<td>255</td>
<td>410</td>
<td>497</td>
<td>541</td>
</tr>
</tbody>
</table>

Figure 8.8  Mean heart rate (\( \bar{x} \pm nCI; \) beats\( \cdot \)min\(^{-1} \)) for the carbohydrate (CES) and flavoured water (FW) trials during each 15 min LIST block. \( \dagger P<0.05; \) at fatigue vs. first 45 min. \( \dagger P<0.05; \) at 15 min vs. remaining 45 min.
Figure 8.9  Mean 15 m sprint times (\( \bar{x} \pm nCI; s \)) for the carbohydrate (CES) and flavoured water (FW) fluid conditions during each block of the LIST.

<table>
<thead>
<tr>
<th>LIST Block Number</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>RPE</td>
<td>CES</td>
<td>12.6 ± 1.0</td>
<td>14.8 ± 1.9</td>
<td>15.1 ± 2.6</td>
</tr>
<tr>
<td></td>
<td>FW</td>
<td>11.9 ± 1.3</td>
<td>13.8 ± 1.4</td>
<td>15.4 ± 1.7</td>
</tr>
<tr>
<td>Thermal Sensation</td>
<td>CES</td>
<td>4.0 ± 1.1</td>
<td>5.6 ± 1.2</td>
<td>6.2 ± 1.1</td>
</tr>
<tr>
<td></td>
<td>FW</td>
<td>4.0 ± 0.9</td>
<td>5.1 ± 0.6</td>
<td>6.1 ± 1.9</td>
</tr>
<tr>
<td>Thirst</td>
<td>CES</td>
<td>11.4 ± 2.6</td>
<td>12.8 ± 4.6</td>
<td>13.4 ± 3.9</td>
</tr>
<tr>
<td></td>
<td>FW</td>
<td>12.8 ± 2.7</td>
<td>12.2 ± 3.1</td>
<td>15.4 ± 3.6</td>
</tr>
<tr>
<td>Gut Fullness</td>
<td>CES</td>
<td>10.2 ± 1.9</td>
<td>13.4 ± 2.6</td>
<td>13.8 ± 2.9</td>
</tr>
<tr>
<td></td>
<td>FW</td>
<td>11.0 ± 2.7</td>
<td>11.5 ± 4.1</td>
<td>13.6 ± 2.6</td>
</tr>
</tbody>
</table>

Table 8.4  Ratings of perceived exertion (RPE), thermal sensation, thirst and gut fullness (\( \bar{x} \pm SD \)) during each block of the LIST for the carbohydrate (CES) and flavoured water (FW) test drinks. † P<0.05; vs. all other time points.
8.4 Discussion

The main finding of this study was that the volume of fluid emptied from the stomach during the LIST in 30°C was no different between a 6.2% CES and a FW. The ingestion of the CES resulted in an improved cumulative sprint performance and a higher core temperature when compared with FW during the 60 min of the exercise.

Deep body temperature was significantly higher during the CES trial during this protocol, which is in agreement with findings of Chapter 7, with previous work (Fritzsche et al., 2000; Millard-Stafford et al., 2005) and with the faster rate of rise in rectal temperature observed by Morris and colleagues (2003). Therefore individuals appear to accumulating more heat when ingesting a CES than when drinking equivalent amounts of water while undertaking a LIST trial in 30°C. The nature of this response is somewhat different to that observed during Chapter 7, with the differences between trials not becoming significant until later on in exercise during the present study. Nevertheless $T_{int}$ at the end of the protocol and the magnitude of this difference is comparable the Chapter 7.

The purpose of the present study was to ascertain whether this additional heat storage was due to either the separate or synergistic effect of (a) dehydration, caused by delayed gastric emptying as a consequence of the energy content of the sports drink. Or (b) an increase in metabolic heat production occurring as a result of individuals working at a higher exercise intensity supported by the additional carbohydrate within the CES.

The total volume of fluid, which includes test drink, saliva and gastric secretions, emptied into the intestine during each of the 15 min blocks of the exercise protocol in the present study was similar in both the CES and FW trials. There was however a strong trend for a slightly larger total volume of fluid to be retained in the stomach when subjects ingested the CES ($P=0.054$). Previous investigations have shown that solutions with carbohydrate concentrations of 6% or greater empty more slowly than energy-free drinks at rest (Maughan, 1991; Vist and Maughan, 1994) or during low-intensity exercise (Leiper et al., 2005), on the other hand the total fluid volume emptied following ingestion of either a CES or a carbohydrate-free placebo drink was similar during 30 min of the LIST protocol carried out in temperate conditions (Leiper
et al., 2005). In the present study, the difference in the total volume emptied between trials amounted to an average of approximately 100 ml of fluid over the 60 min of exercise. Even if the difference was statistically significant this is not a large volume relative to the total fluid intake, but may potentially be sufficient to induce a marked increase in central blood volume. Although there was no significant difference in the volume of test drinks emptied over this period, the volume of secretions present in the stomach was slightly higher on the CES trial than on the FW trial. This approximated to an additional 120 ml of secretions being produced during the whole of the CES trial compared with that on the FW trial, which in all probability accounts for the tendency for a larger gastric fluid volume on the CES trial.

The trend toward a reduction in the rate of gastric emptying in the present study could have resulted in decreased availability of water and hence reduced water uptake. It is possible that ingesting the carbohydrate beverage resulted in a small but marked decrease in water replacement compared with the FW drink due to fluid movement into the GI tract. If this did occur then dehydration and hypovolaemia may be responsible for a proportion of the faster rise in core temperature observed in the CES trial. However, a divergence in the rate of temperature increase is evident after 15 min of exercise at a time when the difference in the total volume emptied between trials was about 50 ml, and over the 60 min of exercise the difference in fluid volume emptied totalled only about 100 ml. Clearly of the 1.4 ± 0.2 l of test drink ingested in each trial, the majority of the volume ingested was emptied during the exercise in the heat during the CES (69.2 ± 10.7 %) and FW (74.3 ± 14.5 %) trials. The volume of sweat lost in the CES trial was in excess of that measured during the FW trial, suggesting that fluid availability was not limiting sweat production in subjects in the CES trial. In addition, at the end of the 60 min of the LIST the change in plasma volume was similar in both trials so dehydration levels were comparable. These results suggest that there was no significant difference in the rates of fluid delivery and absorption between carbohydrate-electrolyte and energy-free placebo solutions during the LIST trial in the heat.

During the 60 min of exercise subjects sprinted 11 times during each of the 15 min periods of exercise. The total time spent sprinting during the hour of exercise in the present study was less when subjects ingested the CES. This suggests that the overall
sprint performance was improved when the CES was ingested compared with flavoured water alone. This finding supports previous work that has demonstrated that carbohydrate ingestion during intermittent high-intensity exercise in temperate environmental conditions enhances sprint performance and offsets the decline in sprinting normally observed over time in (Ali et al., 2002; MaClaren and Close, 2000; Shirreffs and Merson, 2003; Welsh et al., 2002). The magnitude of the improvement in sprint performance in the CES trial was similar to that recorded during Chapter 7. However the significant decline over time seen in the previous chapter was not evident in this study. This is possibly due to the cohort of subjects used in the present study, who were all soccer players and presumably multiple-sprint trained.

Total carbohydrate intake over the 60 min of exercise on the CES trial was on average 87.9 g of which 62.4 g was emptied from the stomach into the duodenum. During the first 15 min of the CES trial approximately 13.7 g of carbohydrate was emptied into the small intestine and the average amount of carbohydrate emptied every 15 min was 15.6 g. The present findings suggest that the availability of this exogenous supply of carbohydrate which is emptied at rate in excess of 1 g·min⁻¹ (and is likely to be absorbed at an equivalent rate), may have enabled subjects to perform at higher exercise intensities during the sprint phase of each exercise cycle. The small additional amount of work performed whilst sprinting seems likely to have contributed to the higher core temperatures observed during the CES trial. The more rapid sweat rate during this trial may be a reflection of the extra evaporative cooling initiated to dissipate the extra heat. It also seems possible that the mean skin temperature was also elevated during this period in order to increase heat flux, but the impracticalities of measuring this variable during the LIST reduced the statistical power of this trend. The additional energy expended cannot be quantified with the available data recorded in the present study; however, it is possible to estimate differences from the amount of energy gained as heat during the protocol. There was a discrepancy of 130 KJ in the amount of heat gained between trials, if all of the additional heat stored during the CES trial was the result of on increased energy expenditure this would support the increases in sprint performance observed.

In the present study, there was a tendency for deep body temperature to be higher on the CES than on the FW trial by the end of the first 15 min block of exercise.
Although no statistical difference could be found in sprint speeds during this initial exercise block, of the 11 sprints undertaken in this period, mean sprint times were numerically shorter on 8 occasions during the CES than with the FW trial. This occurred over a time period when energy availability from muscle glycogen is likely to be similar between the two trials and therefore it would not normally be considered that exercise intensity would be different (Nicholas et al., 1995; Tsintzas and Williams, 1998). However in the LIST study carried out by Ali and colleagues (2002), energy expenditure was greater over the initial 15 min block of exercise and throughout the duration of the exercise test when a carbohydrate drink was consumed compared with ingestion of a carbohydrate-free beverage. It is possible that these early enhancements in performance may occur independently of the appearance of glucose in the blood. If so, this provides some support for the presence of central pathways that are possibly stimulated through oropharyngeal CHO receptors (Carter et al., 2004a; Liu et al., 2000).

No changes were found in subjective perception of exercise intensity between fluid conditions, even though performance data suggests that subjects were exercising at a slightly higher intensity when ingesting the CES solution. It may be that the perception scales used are not sensitive enough to highlight the minor changes in exercise intensity or that CES ingestion allows subjects to enhance sprint performance without invoking a negative perception of the additional exercise load.

Over the relatively short duration of exercise employed in the present study, the additional heat stored appears to have been of no detriment to performance. During more prolonged exercise it is possible that this additional heat production may ultimately lead to a decrease in exercise capacity due to changes within the CNS associated with the attainment of a critically high core temperature (González-Alonso et al., 1999b; Nielsen et al., 1993).

It seems most likely that the faster rise in core temperature when ingesting the CES observed here, in the previous chapter and in the studies of Morris et al. (2003) and Fritzsche et al. (2000) is due mainly to a small increase in energy expenditure during the sprint phases of these protocols. Because gastric emptying and hydration status were not different between the CES and placebo trial in the present study, it is
improbable that the faster rate of heat storage when carbohydrates are ingested is mainly a result of increased levels of dehydration. It remains a possibility that an increase in the volume of blood required to support the visceral organs during the postprandial period of the CES trial altered the dynamics of the thermoregulatory process.
CHAPTER 9

THE INFLUENCE OF INGESTING A CARBOHYDRATE-ELECTROLYTE SOLUTION AND WATER ON ENDURANCE CAPACITY DURING THE LIST IN 30°C

9.1 Introduction

In temperate ambient conditions a large number of studies have shown that ingesting carbohydrate drinks during intermittent exercise improves exercise capacity and performance. But these measures of enhancement are normally seen near the end of a prolonged period of physical activity and are attributed to attenuation of the fatigue process by sparing muscle glycogen and maintaining CHO oxidation rates (Shi and Gisolfi, 1998; Tsintzas and Williams, 1998). In hot environments that are uncompensable there is no clear consensus as to whether endurance capacity is either improved (Carter et al., 2003) or not (Febbraio et al., 1996b; Morris et al., 2003) when a CES is compared with FW. In the heat the aetiology of fatigue is likely to related to an intolerable rate of heat storage and hence the attainment of a capacity limiting state of hyperthermia, which will occur before the muscle is in a state of energy crisis (González-Alonso et al., 1999b; Parkin et al., 1999). If this is the case an exogenous source of CHO may be of little benefit, in fact the findings of Chapter 7, 8 and previous published research (Fritzsche et al., 2000; Morris et al., 2003) report physiological variables that suggest an increased thermal strain is induced when ingesting a 6% CES. If the rate of rise of Tcore is more rapid when ingesting a CES, players may reach a critical Tcore more rapidly and hence endurance capacity will be reduced. This has been previously investigated during study of Morris and co-workers (2003) using the LIST. However, their subjects were withdrawn from the test when their core temperatures reached 39.5°C in order to comply with local Ethical Committee recommendations. These individuals were not necessarily exhausted at this deep body temperature and in addition a trial order effect was detected in Tcore and HR in their results. Therefore the effects of a 6% CES and FW on endurance capacity remains to be successfully addressed during a protocol that simulates the demands of intermittent exercise.
Chapters 7 and 8 report consistent improvements in 15 m sprinting performance during the LIST protocol when a 6% CES was ingested. One possible cause of the elevated rate of heat storage in these investigations could be an increase in energy expended during the sprinting phases of the protocol. Sprinting performances recorded in the previous investigations provide no means of relating small differences in velocity to tangible alterations in energy expenditure or substrate metabolism. Fritzsche and colleagues (2000) failed to note any differences in \( \dot{V}O_2 \) whilst sprint performance was significantly enhanced by a CES during cycling exercise in the heat. However, in a LIST exercise study carried out in temperate conditions over 90 min, energy expenditure was found to be significantly greater when a 6.4% CES (67.7 kJ·min\(^{-1}\)) was consumed compared with a carbohydrate-free placebo drink (65.4 kJ·min\(^{-1}\)) (Ali et al., 2002). Therefore estimating energy expenditure using indirect calorimetry during the LIST protocol appears to be sensitive enough to detect changes in sprint performance.

The aim of the present study was to determine the influences of ingesting a CES and FW on energy expenditure and endurance running capacity during an exhaustive LIST protocol in 30°C.

9.2 Methods

9.2.1 Subjects
Nine competitive male games players aged 23 (21 - 27) years with a mean body mass of 77 ± 7 kg, a height of 1.76 ± 0.04 m and predicted \( \dot{V}O_2 \)\(_{max} \) values of 60 ± 4 ml·kg\(^{-1} \)·min\(^{-1} \) took part in this study. Subjects were well trained, habitually spent 283 min·week\(^{-1} \) (± 94) engaged in training activities applicable to the exercise protocol and were not involved in physical activities in a warm environment at the time of the study. All subjects had previously (within 6 months) completed an exhaustive protocol using the LIST test and hence were familiar with demands of undertaking this protocol until the point of exhaustion. They had also previously completed an exercise protocol(s) in a temperature of 30°C.
9.2.2 Experimental Design

Subjects completed The Multistage Fitness Test (Ramsbottom et al., 1988) as previously described in Chapter 3.3.3 in order to estimate \( \dot{V}O_2 \text{max} \). This value was used to calculate shuttle speeds corresponding to 55% and 95% of \( \dot{V}O_2 \text{max} \) for appropriate phases of the LIST protocol and to group subjects into experimental pairs. The duration of the LIST activity cycle for the cohort of subjects was \( 80 \pm 1 \) s (range \( 78 - 82 \) s). On a separate visit to the laboratory 3 blocks of the LIST protocol were completed under experimental conditions at an ambient temperature of \( \sim 15^\circ \text{C} \). Expired air samples were collected as described for main trials and blood sampling procedures were omitted. This familiarisation session was conducted in order to accustom subjects with the demands and activity patterns of the protocol, sampling procedures and instrumentation. The above preliminary tests were performed 1 – 2 weeks before main experimental trials.

Two main experimental trials were performed which were separated by 14 days. Repeated trials occurred on the same weekday and all trials were conducted at 08:00 between Tuesday and Friday. Treatments were assigned in a random crossover design and on each occasion subjects consumed either the CES or FW solutions described in Chapter 3.3.8.

Subjects arrived at the laboratory following an overnight fast (10 h) and initial T\text{int} measurements were made to establish temperature sensors were working correctly and to confirm that T\text{core} was in a steady state and similar before both trials. If necessary subjects sat quietly in an environmental temperature of \( 25^\circ \text{C} \) in order for this to be achieved. An indwelling cannula was then inserted into an antecubital vein, all subjects were cannulated no later than 20 min before exercise.

An expired air sample was then collected in a 150 l Douglas bag whilst subjects were in a standing position (as described by Åstrand et al., 2003). A noseclip, mouthpiece, low resistance two-way valve and tubing assembly (Harvard Apparatus Ltd, Edenbridge, UK) was given to the subject 30 s prior to gas collection. This enabled evacuation of dead space with expired air. The assembly was then connected to the Douglas bag (Harvard Apparatus Ltd, Edenbridge, UK) via a two-way tap (Harvard...
Apparatus Ltd, Edenbridge, UK) and a 5 min sample of expired air was collected. Analysis of the collected expired air samples is described in Section 9.9.3. Subjects then voided and nude body mass was measured before heart rate monitoring equipment was attached. Subjective rating scales were then administered and an 11 ml resting venous blood sample was drawn and treated as described in Chapter 3.4.

Subjects were escorted into the sports hall adjacent to the laboratory (3 min before exercise) and given a volume of appropriate test solution equivalent to 6.5 ml·kg BM$^{-1}$, which was consumed within 180 s. During this period, light, static stretches of the lower limbs were permitted if required. The open-ended exercise protocol represented in Figure 9.2 then commenced, consisting of repeated 15 min blocks of the exercise and rest periods until exhaustion. The point of exhaustion was either determined volitionally by subjects, at a time they considered they could no longer continue, or by the following criteria: Failure to maintain the intensity dictated by audio signals on 2 consecutive shuttles. A decline in sprint performance below 90% of predetermined maximum performance, that did not improve within 2 consecutive cycles after a verbal warning. Repeated T$_{int}$ measurements $\geq$40.5 °C. The time at which the test was terminated was recorded as the measure of exercise capacity.

During the walking phase in the 9th cycle of each block, subjects donned a modified Douglas bag apparatus (Figure 9.1). This consisted of the equipment assembly described for resting samples, attached to a lightweight aluminium frame and webbing yoke (PLCE, Wyvern, UK), a total mass of 1.4 kg. The equipment and protocol was modified from that used by Ali et al. (2002), Foskett et al. (2004; 2003b) and De Groot et al. (1983). Subjects completed 1 exercise cycle wearing the equipment in order to expel dead space with expired air. Expired air was then collected for one complete cycle (cycle number 10) and the exact duration of gas collection was recorded. The modified Douglas bag assembly was then removed during the walk phase of cycle 11.

Intestinal temperature was measured during the walking phase of each cycle which totalled 11 measurements per 15 min exercise block and at the point of fatigue. Throughout the exercise protocol environmental temperature and humidity were monitored and adjusted accordingly as described in Section 7.3.1.2. Subjective rating
scales (Appendix G) were administered during the final walking phase of each exercise block and at the point of fatigue. Immediately after each block of activity and within 20 s after the point of fatigue a venous blood sample was drawn. Test drinks equivalent to 3.5 ml·kg BM$^{-1}$ were ingested within 150 s during each rest period separating 15 min exercise blocks until the point of fatigue.

Following the final venous blood sample subjects moved or were carried from the hall into a private 15°C room containing an examination couch and shower cubical. Intestinal temperature was monitored closely and appropriate measures were taken to increase heat loss. As soon as it was practical (~ 5 min) a towel-dried body mass was obtained.

9.2.3 Expired Air analysis

Expired air was analysed for O$_2$ and CO$_2$ by drawing the gas through a combined paramagnetic O$_2$ analyser and infra-red CO$_2$ analyser (Servomax, 1440C, Crowborough, UK). The concentration of gases was expressed as a percentage accurate to ±0.01%. The analysers were calibrated against the appropriate certified reference gases of known concentrations (BOC Gases, Surrey, UK) prior to each series on analyses. A digital dry gas meter (Harvard Apparatus Ltd, Edenbridge, UK) that was regularly calibrated (3 l calibration syringe, 5530, Hans Rudolph Inc,
Missouri, USA) was used to determine gas volumes. Temperature of expired air was determined during evacuation using a thermistor probe (2984, Edale Instruments, Cambridge, UK).

All gas volumes were corrected to standard temperature and pressure of a dry gas and oxygen uptake ($\dot{V}O_2$), carbon dioxide production ($\dot{V}CO_2$) and respiratory exchange ratio (RER) were calculated by indirect calorimetry using the Haldane transformation (Consolazio et al., 1963). Energy expenditure and rates of substrate oxidation were calculated according to the non-protein respiratory quotients of Péronnet and Massicotte (1991).

9.2.4 Statistical Analyses
Statistical analyses were performed as described in Chapter 3.5. Statistics are based on a population of 9. An effect size analyses based on a similar previous study (Morris et al., 2003), estimates that a sample size of nine has 95% power to detect a difference in run times of 10 min, assuming a SD of differences of approx 7.5 min, using a one sided significance test.
Figure 9.2 A schematic representation of the experimental protocol. The relative time scale shown is equivalent to a $\dot{V}O_2_{\text{max}}$ value of 60 ml·kg$^{-1}$·min$^{-1}$. Blocks of exercise are approximately 15 min in duration and are comprised of 11 repeated cycles of the activity pattern described in Chapter 3.3A. Indicates repetition of the protocol until the point of fatigue.
9.3 Results

9.3.1 Deep body temperature and exercise capacity
Basal intestinal temperature was similar between the CES (37.09 ± 0.70 °C) and FW (37.11 ± 0.63°C) trials. Although there appears to be a trend toward increased $T_{\text{int}}$ in the later stages of the CES trial, there were no significant differences in $T_{\text{int}}$ between fluid conditions during the exercise protocol. As shown in Figure 9.3 temperature increased significantly with exercise duration ($F_{4,32} = 0.295; P<0.001$). No differences were detected in $T_{\text{int}}$ at the point of fatigue; mean intestinal temperature at fatigue was $39.50 ± 0.50^\circ C$ (38.9 – 40.2°C) and $39.47 ± 0.40^\circ C$ (38.9 – 40.0°C) in the CES and FW trials respectively. There was no difference between treatments in the rate of rise in $T_{\text{int}}$, however, there was a strong trend toward a difference in the rate of rise over the first 4 blocks of exercise ($F_{4,1,205} = 3.188; P=0.059$). During the first 4 blocks of exercise the mean rate of rise in $T_{\text{int}}$ was $2.78 ^\circ C \cdot h^{-1} (±0.79)$ and $2.58 ^\circ C \cdot h^{-1} (± 0.74)$ in the CES and FW trials respectively. The time taken to reach fatigue was not different between trials, with subjects running for 86.95 min (± 19.89) in the CES trial and 94.05 (± 28.22) in the FW trial. This equates to a total distance of 12.73 km (± 2.95) and 13.93 km (± 4.18) respectively, a 9.4% discrepancy in total distance covered between test drinks. Five subjects ran for a longer duration when ingesting the CES and four subjects ran longer when ingesting the FW.

All participants maintained sprint performance within 90% of their pre-determined maximum until the termination of exercise. No participant was withdrawn from the protocol due to an unacceptable decline in sprint performance or the attainment of a $T_{\text{int}} ≥ 40.5^\circ C$. Eight of the subjects voluntarily withdrew from the test on both trials and 1 subject suffered syncope at a $T_{\text{int}}$ of 40.3°C and 39.8°C in the CES and FW trials respectively.

9.3.2 Sprint performance and exercise intensity
Mean 15 m sprint times were similar between fluid conditions during the LIST protocol. There was a trend toward better maintenance of mean sprint performance under the CES fluid condition (CES vs. FW, $F_{1,8} = 4.947; P=0.057$) shown in Figure 9.5. Cumulatively subjects spent a greater amount of time sprinting during the FW trial than during the CES trial ($t_{(8)} = -2.453, P=0.040$). Sprint performance decreased
over time irrespective of treatment ($F_{4,32} = 4.546; P= 0.007$) and sprint times at fatigue were significantly slower than during the first 30 min of the LIST.

Exercise intensity ($\% \dot{V}O_2_{\text{max}}$) decreased throughout the first 60 min of the LIST ($F_{5,32} = 151.227; P<0.001$), in both trials (Figure 9.6). After 60 minutes of the LIST the mean exercise intensity was significantly lower (Δ-13% $\dot{V}O_2_{\text{max}}$) than during the first 15 minutes of exercise.

### 9.3.3 Oxygen uptake, carbon dioxide production and RER

There were no differences between the trials during the first four blocks of the LIST in $\dot{V}O_2$ or $\dot{V}CO_2$. During the fourth block of exercise $\dot{V}O_2$ and $\dot{V}CO_2$ were significantly lower than the first block of exercise ($P<0.05$; Table 9.3). There was a trend toward a higher RER during exercise when the CES was ingested ($F = 1,8 17.094; P=0.054$), no effect of exercise duration was found in RER during the LIST protocol.

### 9.3.4 Energy Expenditure, CHO and fat oxidation

During each LIST block energy expenditure was similar in both trials and there were no significant differences between treatments or over time (Table 9.3). However, total energy expenditure during the 1st hour of exercise was significantly different between trials ($t (34) = 2.495, P= 0.018$), mean total energy expenditures were 3.7 MJ (± 0.3) and 3.4 MJ (± 0.4) in the CES and FW trials respectively. The rate of carbohydrate oxidation during the first hour of exercise was higher in the CES trial (202 ± 22 g·h$^{-1}$) compared with the FW trial (168 ± 25 g·h$^{-1}$) ($t (8) = 1.869, P= 0.04$). Fat metabolism during the first hour of exercise also varied between treatments with 7.4 ± 6.3 g·h$^{-1}$ oxidised during the CES trial compared with 23.2 ± 5.6 g·h$^{-1}$ oxidised during the FW trial ($t (8) = 4.421, P= 0.02$).

### 9.3.5 Heart rate and blood lactate

There were no differences in heart rates during the two trials (Figure 9.4) and heart rate was significantly higher at the point of fatigue compared with all other time points ($P<0.05$). No differences in blood lactate concentration were detected during exercise between trials (Figure 9.12). There was a main effect of time ($F = 5,30 8.004;$
indicating that blood lactate concentrations during exercise were significantly higher than at rest. The coefficient of variation for this assay was 1.3%.

**9.3.6 Plasma glucose and serum insulin**

Plasma glucose concentrations were significantly higher during the CES trial compared with FW ($F = 1.8, 29.635; P=0.012$; Figure 9.8). Plasma glucose concentrations were significantly higher at all time points during the exercise protocol compared with pre-exercise values. At the point of fatigue concentrations were $8.06 \pm 0.84 \text{ mmol}\cdot\text{l}^{-1}$ and $6.06 \pm 0.72 \text{ mmol}\cdot\text{l}^{-1}$ in the CES and FW trials respectively. There was a trend toward a lower serum insulin concentration when the FW was ingested, but no statistical differences exist between treatments or time (Figure 9.9). The coefficients of variation were 1.2% and 6.6% for the plasma glucose and serum insulin assays respectively.

**9.3.7 Plasma FFA and glycerol**

Plasma FFA concentrations were higher during the FW trial (Figure 9.10). An interaction effect of time $\times$ treatment ($F = 5, 30 6.195; P=0.034$), suggests these differences between trials occur at fatigue (CES $0.21 \pm 0.14 \text{ mmol}\cdot\text{l}^{-1}$ vs. FW $0.59 \pm 0.29 \text{ mmol}\cdot\text{l}^{-1}$). There was no main effect of treatment on plasma glycerol concentration (Figure 9.11). However, there was a main effect of time ($F = 5, 30 12.630; P=0.004$), suggesting that glycerol concentrations were higher during exercise vs. rest and at fatigue vs. the first 30 minutes of exercise. Coefficients of variation equivalent to 0.5% and 1.0% were calculated for the FFA and glycerol assays respectively.

**9.3.8 Plasma volume and serum osmolality**

Changes in plasma volume were not different between trials (Table 9.2). Plasma volume was significantly lower during exercise than at rest in both trials ($P<0.05$), but no significant differences were detected as exercise progressed. Percentage change in plasma volumes at fatigue compared with rest were $-4.4 \% \ (\pm 1.8)$ in the CES; trial and $-4.1 \% \ (\pm 2.5)$ in the FW trial. No significant changes were detected in serum osmolality either between fluid conditions or over time (Table 9.2).
9.3.9 Body mass and urine osmolality
There were no significant differences in adjusted body mass reduction between trials; the mean body mass lost as sweat was 1.85 kg (± 0.75) and 1.52 kg (± 0.52) in the CES and FW trials respectively. If all ingested fluid was emptied from the stomach and absorbed, this equates to a mean percentage change in body mass of CES; -1.07% (±0.48) and FW; -0.76% (±0.36). Pre-exercise (-30 min) urine osmolality was similar between trials, mean osmolality was 446 ± 220 mOsmol kg⁻¹ in the CES trial and 546 ± 325 mOsmol kg⁻¹ in the FW trial.

9.3.10 Subjective responses
There were no significant differences between trials in any of the subjective measures recorded. The subjective rating of perceived exertion increased throughout exercise in a linear fashion and was significantly higher in both trials immediately prior to fatigue than at any other time point (F = 4,32 24.494; P< 0.001; Figure 9.7). Differences were found over time in perceived thermal sensation (F = 4,32 31.015; P<0.001), participants reported greater thermal stress at fatigue compared with the first 45 minutes of exercise (Table 9.1). At the point of fatigue the rating of thermal sensation was 8 and 7 in the CES and FW trials respectively, these correspond to subjective ratings between “very hot, uncomfortable” and “extremely hot, close to limit” (Appendix G). Perceived thermal stress higher during the later stages of exercise when compared with the first 15 minutes. A greater thirst drive was reported at fatigue compared to the preceding LIST block (F = 4,32 10.671; P= 0.001). Subjects also reported greater feeling of fullness in the gut at fatigue compared with the first three blocks of exercise (F =4,32 14.376; P< 0.001).

9.3.11 Environmental conditions and trial order effects
The environmental temperature (30.4 ± 0.7 °C) and relative humidity (35.3 ± 2.5 %) remained constant throughout each experimental trial. There were no statistical differences in these variables between repeated trials, nor was there a trial order effect in any of the primary measures reported in this chapter.

9.3.12 Habitual food intake
Dietary and lifestyle habits were recorded in the two days before the first experimental trial and repeated prior to the second. Mean energy intake over the 48 h
period prior to exercise was 13.4 ± 3.4 MJ·day\(^{-1}\). The mean mass of CHO, Fat and protein consumed during the recording period was 8.3 ± 2.0 g·kg\(\text{BM}^{-1}\)·day\(^{-1}\), 0.7 ± 2.0 g·kg\(\text{BM}^{-1}\)·day\(^{-1}\) and 1.6 ± 3.4 g·kg\(\text{BM}^{-1}\)·day\(^{-1}\) respectively. Dietary analysis revealed no caffeine was ingested during the period recorded. By applying linear regression analysis to the findings of Harris et al. (1991), the orocecal transit time of the temperature sensor can be approximated to 84 ± 23 min at the energy intake recorded by this cohort of subjects.
Figure 9.3 Intestinal temperature ($\bar{x} \pm nCI; ^\circ C$) during the LIST to fatigue, under the carbohydrate (CES) and flavoured water (FW) fluid conditions.

Figure 9.4 Mean heart rate ($\bar{x} \pm nCI; \text{beats.min}^{-1}$) for the carbohydrate (CES) and flavoured water (FW) trials during the LIST to fatigue. † $P<0.05$; at fatigue vs. 15 min in the CES trial.
Figure 9.5  Mean 15 m sprint times (x ± nCI; s) during the first 4 blocks of the LIST protocol and the final sprint prior to fatigue. † P<0.05; at fatigue vs. first 30 min in the CES trial.

Figure 9.6  Exercise intensity (x ± nCI; %VO₂ max) during the first 60 min of the LIST for the carbohydrate (CES) and flavoured water (FW) treatments. † P<0.05; at 66 min vs. 12 min.
Figure 9.7  Rating of perceived exertion ($\bar{x} \pm \text{SD}$) during the LIST to fatigue for the carbohydrate (CES) and flavoured water (FW) test drinks. $\dagger$ $P<0.05$; at fatigue vs. first 45 min of the LIST.

Table 9.1  Ratings of perceived exertion (RPE), thermal sensation, thirst and gut fullness ($\bar{x} \pm \text{SD}$) during each LIST exercise block. $\dagger$ $P<0.05$; vs. all other exercise time points. Values correspond to subjective descriptors included in Appendix G.
Figure 9.8  Plasma glucose concentrations ($\bar{x} \pm n\text{CI}$; mmol·l$^{-1}$) during the carbohydrate (CES) and flavoured water (FW) trials. * $P<0.05$ CES vs. FW; † $P<0.05$ at rest vs. exercise time points.

Figure 9.9  Serum insulin concentrations ($\bar{x} \pm n\text{CI}$; pmol·l$^{-1}$) during the LIST to fatigue for the carbohydrate (CES) and flavoured water (FW) trials.
Figure 9.10 Concentration of plasma free fatty acids (\( \bar{x} \pm \text{nCl} \); mmol·l\(^{-1}\)) during the LIST to fatigue for the carbohydrate (CES) and flavoured water (FW) trials. * \( P<0.05 \); FW vs. CES.

Figure 9.11 Plasma glycerol concentrations (\( \bar{x} \pm \text{nCl} \); mmol·l\(^{-1}\)) during the LIST to fatigue under the carbohydrate (CES) and flavoured water (FW) fluid conditions. † \( P<0.05 \), rest vs. LIST time points; § \( P<0.05 \), fatigue vs. first 30 min exercise.
Figure 9.12  Blood lactate concentrations ($\bar{x} \pm nCI; \text{mmol} \cdot \text{l}^{-1}$) during the LIST to fatigue for the carbohydrate (CES) and flavoured water (FW) trials. † $P<0.05$; rest vs. LIST time points.

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<td>Δ Plasma Volume (%)</td>
<td>CES</td>
<td>------</td>
<td>-0.3 ±</td>
<td>-3.0 ±</td>
<td>-5.8 ±</td>
<td>-4.3 ±</td>
<td>-4.4 ±</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>3.63</td>
<td>1.35</td>
<td>1.90</td>
<td>1.24</td>
<td>1.82</td>
</tr>
<tr>
<td></td>
<td>FW</td>
<td>------</td>
<td>0.7 ±</td>
<td>-2.2 ±</td>
<td>-4.6 ±</td>
<td>-3.2 ±</td>
<td>-4.1 ±</td>
</tr>
<tr>
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<td></td>
<td>1.61</td>
<td>1.28</td>
<td>0.85</td>
<td>3.00</td>
<td>2.51</td>
</tr>
</tbody>
</table>

Table 9.2  Serum osmolality (mOsmol·kg⁻¹) and percentage change in plasma volume (PV; %) relative to the resting time point. Table reports mean ± SD.
Table 9.3  Oxygen uptake ($\dot{V}O_2$), carbon dioxide production ($\dot{V}CO_2$), respiratory exchange ratio (RER) and energy expenditure at rest and during each 15 min block of the LIST. Table reports mean ± SD in the carbohydrate (CES) and flavoured water (FW) trials. † denotes a significant difference ($P<0.05$) vs. LIST block number 1. * denotes significant difference ($P<0.05$) between fluid conditions (CES vs. FW).
9.4 Discussion

The main findings of this study were that an increased exogenous supply of CHO and electrolytes did not alter endurance capacity. Fatigue appears to occur at a similar, high core temperature between subjects, at a time when carbohydrate availability is unlikely to be restricted. The ingestion of the CES enabled subjects to perform at higher energy expenditures during the sprint phases of the LIST and supported a higher rate of CHO oxidation, with no detectable differences in the amount of heat stored. A trend was seen toward a higher $T_{int}$ in the CES trial, but in contrast to previous chapters this difference was not significant.

The point of fatigue during the LIST protocol occurred at a similar $T_{int}$ under both fluid conditions and this critical temperature can be extrapolated with some accuracy from the rate of rise in intestinal temperature seen in each individual. This suggests that an uncompensable rate of heat storage was the major contributing factor in the fatigue process. The progressive elevation in heart rate seen in both trials may also be indicative of the cardiovascular instability associated with uncompensable environments. The intestinal temperatures recorded at the cessation of exercise are similar to those seen during previous LIST protocols conducted in 30°C (39.4°C: Morris et al., 1998; 39.5°C: Morris et al., 2003) and support the suggestion that the point of fatigue within the same individuals will routinely occur at similar critical level of hyperthermia (González-Alonso et al., 1999b; Nielsen et al., 1993; Walters et al., 2000). The subjective measures administered show that the perception of workload increased in a linear fashion throughout the exercise period, occurring in line with the increases in thermal sensation. These findings are consistent with the suggestion that RPE increases in parallel with $T_{core}$ (Nielsen et al., 2001; Nybo and Nielsen, 2001b, 2001a) and are symptomatic of the biochemical alterations occurring at brain level that eventually result in the volitional cessation of exercise.

Interestingly if the mean point of fatigue in the present study was applied to a multiple sprint sport in similar environmental conditions, players would barely be capable of completing 90 min of match-play. Taken that the demands of the LIST protocol are similar to those seen in sports such as soccer, rugby and hockey it must follow that the workrate of players decreases during the later stages of competition in order to curtail the rate of heat storage.
Exercise intensity ($\% \dot{V}O_2_{\text{max}}$) and sprint performance declined in an inverse fashion to the increases in $T_{\text{int}}$ and subjective ratings. It seems unlikely over the first hour of this experimental protocol that a reduced availability of substrate would be reason for the decrements of around 13% in relative exercise intensity (-12.3 ml·kg$^{-1}$·min$^{-1}$) seen in both trials. Indeed at the point of fatigue during a similar protocol (100 min), Morris and colleagues (2005) calculated muscle glycogen concentration in the $m.\ vastus\ lateralis$ to be $207 \pm 34$ mmol glucosyl units·kg$^{-1}$ DM when subjects were in a fasted state. The blood-born markers of metabolic activity sampled in the present study also concur with this suggestion, with blood glucose concentration remaining above pre-exercise values and suggesting there would be reduced reliance on endogenous stores within the muscle.

Under both fluid conditions it appears that the onset of fatigue was not or only partly a metabolic phenomenon. The voluntary termination of exercise by all participants was likely to be caused by alterations in mind-state due to change or dysfunction within the CNS that is thought to occur during severe hyperthermia. However, a discussion of these biochemical alterations within the brain are beyond the scope of this thesis (see Nielsen and Nybo, 2003).

In the present study there was no detectable effect of the test solutions on exercise capacity. An overall bias was evident indicating an increase of 9.4% in the total distance covered when ingesting the FW, but this trend is only present in 40% of the subjects. The inconsistency in distance covered between trials is considerably less than the 19% tendency ($P=0.08$) to cover a greater distance when ingesting a FW reported by Morris et al. (2003). The significantly increased rate of heat storage when ingesting the CES observed by these authors and the elevated $T_{\text{int}}$ observed in Chapters 7 and 8 is also absent in the present study. This is inconsistent with previous investigations within this thesis and is a possible reason why no marked differences in $T_{\text{int}}$ at the point of fatigue were detectable. The lack of any differences in $T_{\text{int}}$ at the point of fatigue does not agree with the recent suggestion that the CNS exhibits an improved tolerance to a raised $T_{\text{core}}$ when CHO solutions are ingested (Carter et al., 2003).
The reason why no significant differences in $T_{core}$ were detected between solutions in the present study, despite similar increases in sprint performance to Chapters 7 and 8, is unknown. The $T_{core}$ response to the protocol is similar to that observed during Chapter 8 and a tendency toward elevated $T_{int}$ in the CES trial appears to be evident. But the number of subjects exhibiting this response was less in the current investigation, which perhaps provides an explanation for the decreased bias and statistical power separating trials.

Subjects exhibited an overall enhancement in sprint performance when drinking the CES. Cumulatively the 15 m sprint times during the protocol were significantly faster when the CES was ingested. This finding is in agreement with the previous chapters and improvements are of a similar magnitude to those reported by (Ali et al., 2002) during the LIST in temperate ambient conditions. The total energy expenditure during the first hour of exercise was higher during the CES trial compared with the FW, this demonstrates that the small differences seen in sprint performance can be detected as an overall increase in energy expenditure. It is probable that a fraction of this increase will be due to the metabolic processes associated with handling the CHO, however this is likely to be small in comparison with the total increase recorded.

A shift toward increased CHO metabolism and decreased fat oxidation accompanied the increases in energy expenditure when the CES was ingested. An increased rate of CHO oxidation was calculated over the first hour of exercise, but these changes were not powerful enough to be reflected in other serial measurements such as $\dot{V}O_2$ and relative exercise intensity, which only show a tendency to be better maintained with ingestion of the CES. It has been previously shown during cycling exercise that the uptake and rate of oxidation of glucose at a whole body level and in the active musculature is unaffected by the ingestion of CHO when the heat stress is severe (Angus et al., 2001; Jentjens et al., 2002). One suggestion that may explain this is that hyperthermia-induced hepatic glucose production results in a degree of hyperglycaemia, to which the addition of exogenous CHO will have no further effect on glucose uptake (Febbraio, 2001). An alternative theory is that peripheral distribution of blood away from the organs of the abdominal cavity restricts the absorption of CHO and fluids and hence results in an increased rate of glycogenolysis. However, the findings of Chapter 8 show that an adequate amount of CES is emptied
from the stomach during the LIST exercise protocol, and the marked elevation in plasma glucose and tendency toward an increased serum insulin concentration in the CHO trial vs. FW of this study suggests that the absorption of the fluid and glucose occurred at an adequate rate. In the present investigation the systemic concentration of glucose was considerably higher than values reported by Jentjens et al. (2002) and peak plasma glucose concentration was comparable to the majority of other studies that have noted performance enhancements with the ingestion of carbohydrate-electrolyte solutions in the heat (Davis et al., 1988a; Millard-Stafford et al., 1997; 1992; Murray et al., 1987). It may be that the augmentation of CHO metabolism in the heat only occurs at doses higher than those shown to have an ergogenic effect in cooler conditions.

The RER values used to measure CHO oxidation rates during this protocol should be interpreted with caution. The transient increases in lactate that occur when intermittent high-intensity exercise is performed can reduce the HCO$_3^-$ pool leading to a positive shift in the RER (Christmass et al., 1999b) and hence the overestimation of CHO oxidation an underestimation of fat oxidation. However Christmass and colleagues (1999a; 2001) support the use of indirect calorimetry and showed that HCO$_3^-$ concentration was relatively stable during repeated high-intensity intermittent exercise.

In summary the results of the present investigation show that during rapid exercise-induced heat storage and moderate levels of dehydration (-0.9% BM), the beneficial effects of exogenous CHO oxidation are noticeable. The elevated plasma glucose concentration maintained throughout the duration of exercise may have supported a higher intracellular glucose concentration and hence allowed greater force generation during the sprinting phase of the protocol. However, whether the additional CHO that was oxidised originated from the sports drink, the liver, or muscle stores cannot be confirmed without the use of a tracer technique. Improvements in performance during prolonged high-intensity, intermittent exercise have been previously observed when CHO solutions are ingested in cool conditions, the findings of the present study allow these observations to be extended to exercise in the heat. Regardless of performance it seems that in uncompensable environmental conditions the cessation of this form of exercise will occur at a critical level of hyperthermia.
CHAPTER 10

THE ACUTE INFLUENCES OF CARBOHYDRATE FEEDING ON THERMOGENESIS AND INTESTINAL TEMPERATURE

10.1 Introduction

Postprandial heat production is a well reported acute phenomenon that follows the ingestion of foods. The metabolic processes underpinning this response are not fully understood but it is thought to occur as a consequence of the energy-requiring processes of intestinal absorption and the initial stages of nutrient metabolism and storage. Aside from the metabolic cost of handling nutrients another component of this thermogenesis, possibly mediated by the sympathetic nervous system, is thought to be central in energy balance and obesity resistance (for a review see Westerterp, 2000).

During the postprandial period a transient increase in metabolic rate is detectable, the magnitude of which is largely determined by the energy content and macronutrient composition of the meal (Hill et al., 1984). In a study conducted at rest Sharief and Macdonald (1982) noted a 1.4 KJ·min⁻¹ difference in thermogenesis between a drink containing 5 g·kg BM⁻¹ of sucrose and water. Similarly Hill and associates (1984) report a 1.2 KJ·min⁻¹ thermic effect of ingesting a solution containing 4.8 MJ of energy (50% of which was CHO) in well trained men. Feeding results in marked increases in the metabolic rate of visceral brown adipose tissue (Cannon and Nedergaard, 2004) and elevations in blood flow to the splanchnic organs (Norryd et al., 1975; Waaler and Eriksen, 1992). Also during submaximal exercise the redistribution of blood appears to have little effect on this digestion-induced vasodilation of the major vessels that supply the intestinal tract (Perko et al., 1998).

When $T_{core}$ is measured from within the intestinal tract, local metabolic and circulatory alterations to feeding may confound the interpretation of $T_{int}$. During the postprandial period it is possible that local tissue temperature within this region may not accurately reflect the typical core temperature. Indeed Shlygin et al. (1991) detected a distinct rise in tissue temperature of some organs following the ingestion
921 KJ of food by using a radiothermometry technique. More specifically a rise in
temperature of liver, intestinal and brown fatty tissues (0.5-0.9°C) was noted 3-10 min
after feeding. These observations provide a potential explanation for the higher $T_{core}$
temperatures recorded during the CES feeding trials in a number of the previous
investigations within this thesis.

Therefore the purpose of the current study was to examine the suitability of using $T_{int}$
as a measure of $T_{core}$ during the postprandial period. This would determine whether
elevations in $T_{int}$ reported in previous studies following the ingestion of a CES are due
to dietary induced thermogenesis. Additionally quantifying the amount of energy
required to process the CHO in a CES may show what proportion of the $\sim$3 KJ-min$^{-1}$
difference in energy expenditure recorded between solutions in Chapter 9 is due to
differences in physical work and what fraction is due to thermogenesis. The feeding
schedule described in previous chapters was administered whilst the affects on
metabolism and deep body temperature ($T_{rec}$ and $T_{int}$) were monitored.

10.2 Methods

10.2.1 Subjects

Six healthy males subjects aged 26 (22 - 38) years with a body mass of 77 ± 15 kg and
height of 1.74 ± 0.04 m took part in this study. Skinfold thickness was measured at
four sites as previously described (Chapter 6.2.1) and the mean sum of these skinfolds
was 36 mm (±15).

10.2.2 Experimental design

Experimental trials were conducted at 08:00 between the days of Tuesday and Friday.
Subjects arrived at the laboratory following an overnight fast (10 h) and after
ingesting a temperature sensor (as described in Chapter 3.3.6.2). They then changed
into a long sleeved upper garment and ankle-length trousers. Subjects were then
instrumented with a rectal thermistor as described in Chapter 4.2.2 and four skin
thermistors using the method outlined in Chapter 8.2.2. All wired thermistors were
connected to a data logger (SQ800, Grant Instruments Ltd, Cambridge, U.K.) and a
lightweight $T_{int}$ data recorder was secured to the torso in a neoprene waist pouch.
Subjects were then seated in a small air-conditioned environmental chamber (~4 m$^3$),
maintained at 25°C and 46 %RH. After 30 minutes of resting quietly data loggers
were activated simultaneously with a sampling interval of 5 s and resolutions of 0.01°C and 0.05°C for the Tint and SQ800 loggers respectively. After a further 10 min had elapsed an expired air sample was collected for a 5 min period using the technique described in Chapter 9.2.3. Four subsequent expired air samples were collected at 20 min intervals. After 50 min a volume of the test drink equivalent to 6.5 ml·kg BM\(^{-1}\) was subsequently rapidly ingested (<180 s), further drinks with volumes equivalent to 3.5 ml·kg BM\(^{-1}\) were also rapidly ingested (<150 s) at 18 min intervals. This fluid intake regime was administered in order to repeat the same feeding schedule as described in the previous experimental chapters. After 130 min of this protocol the data loggers were stopped and subjects retired to a private area to remove the rectal thermistor.

10.2.3 Statistical analyses
Statistical analyses were performed as described in Chapter 3.5 and all inferential statistics are based on a population of 6. Automatically logged data were treated using the method outlined in Chapter 4.2.3
10.3 Results

10.3.1 Deep body temperature

Figure 10.1 graphically represents $T_{core}$ throughout the experimental protocol at both sites of measurement and during both trials. Basal intestinal temperature was similar between the CES ($36.71 \pm 0.16^\circ C$) and FW ($36.72 \pm 0.18^\circ C$) trials and rectal temperature was also the same (CES $36.53 \pm 0.16^\circ C$ vs. FW $36.54 \pm 0.26^\circ C$). There was however a marked difference between $T_{int}$ and $T_{rec}$ at the start of both trials that remained throughout the protocol (CES $F_{1,5} = 18.847$, $P=0.02$; FW $F_{1,5} = 15.069$, $P=0.03$). The mean bias between the two measurement techniques was $0.18 \pm 0.12^\circ C$ and $0.14 \pm 0.06^\circ C$ (bias $\pm 1.96SD$) in the CES and FW trials respectively. A close association existed between changes in $T_{rec}$ and $T_{int}$ during each exercise trial (CES $r = 0.898$, $P<0.01$; FW $r = 0.978$, $P<0.01$). There were no differences between trials (CES vs. FW) in either $T_{int}$ ($F = 1,5 0.015$; $P=0.910$) or $T_{rec}$ ($F = 1,5 0.256$; $P=0.654$). A weak trend toward a higher $T_{int}$ during the CES trial ($0.15 \pm 0.05^\circ C$ (bias $\pm 1.96SD$)) was evident from the 100 min timepoint onward. An $a posteriori$ effect size test suggested that the current sample size had sufficient power to detect any significant differences in these variables, given the reported mean distributions and SD. A significant decrease in temperature at both $T_{int}$ and $T_{rec}$ was determined over time in both trials ($T_{int} F_{20,100} = 12.806, P<0.001$; $T_{rec} F_{20,100} = 20.546, P=0.007$).

10.3.2 Skin temperature and total body heat content

Weighted mean skin temperature was significantly different between fluid conditions at the onset of the protocol (CES $32.9 \pm 1.1^\circ C$; FW $32.6 \pm 1.2^\circ C$) ($F = 1,5 21.297$; $P=0.019$). However, there were no differences in the rate of change of this variable between trials ($F = 1,5 0.372$; $P=0.585$). Weighted mean skin temperature decreased by 0.22 $\pm 0.37^\circ C$ and 0.27 $\pm 0.36^\circ C$ for the CES and FW respectively during the trial, final $T_{sk}$ was $32.6 \pm 1.1 ^\circ C$ and $32.1 \pm 0.9 ^\circ C$. At the start of the protocol TBHC (calculated using $T_{rec}$) was similar between fluid conditions (CES $9.48 \pm 1.94$ MJ; FW $9.45 \pm 1.96$ MJ). There were no differences between trials in this variable during the protocol and at the end of the test TBHC was $9.40 \pm 1.93$ MJ and $9.37 \pm 1.95$ MJ in the CES and FW trials respectively. Figure 10.3 shows the change in TBHC during this period, a loss of $75 \pm 31$ KJ occurred over the course of the CES trial and a decrease of $85 \pm 28$ KJ was imposed by drinks during the FW trial ($F_{20,100} = 39.676$; $P<0.01$).
10.3.3 Oxygen uptake, carbon dioxide production and RER

Oxygen uptake increased during the postprandial period with significant differences seen in both trials during at 40 min vs. baseline ($F_{4,16} = 4.863$, $P=0.015$) (Table 10.1). Carbon dioxide production showed a similar trend that failed to reach statistical significance ($F_{4,16} = 5.820$, $P=0.08$). These variables were also significantly different between trials at the final three sampling points, with both $\mathrm{VO}_2$ ($F_{1,5} = 21.768$, $P=0.019$) and $\mathrm{VCO}_2$ ($F_{1,5} = 31.415$, $P=0.011$) elevated during the CES trial compared with the FW trial. There were no statistical differences in the RER during the experimental protocol, but a strong trend toward a higher RER was evident in the postprandial period of the CES trial ($F_{1,5} = 7.536$, $P=0.071$).

10.3.4 Energy Expenditure, CHO and fat oxidation

The estimated amount energy expended during the protocol increased following the ingestion of both solutions ($F_{4,16} = 5.996$, $P=0.007$), this difference reached statistical significance from the 80 min sampling point onward. At the 80 min and 120 min sampling points estimated energy expenditure was higher in the CES trial ($F_{1,5} = 27.927$, $P=0.013$; Figure 10.4). The estimated CHO oxidation rate increased significantly following the ingestion of the CES, this response was not seen in the FW trial and CHO oxidation rates were significantly different between fluid conditions during the last 20 min of the protocol ($F_{1,5} = 30.313$, $P=0.012$). The oxidation rate of fat appears to decline over time during the CES trial and remains relatively constant during the FW trial. At the final sampling point (120 min) there was a tendency toward a lower fat oxidation rate in the CES trial versus the FW trial ($F_{1,5} = 6.818$, $P=0.08$).

10.3.5 Environmental conditions and trial order effects

The environmental temperature ($25.4 \pm 0.1 \, ^\circ\text{C}$) and relative humidity ($46 \pm 6 \, \%$) remained constant throughout each experimental trial. No statistical differences were detected between trials and no trial order effects were found in any of the primary measures reported in this chapter.
10.3.6 Habitual food intake and body composition

Dietary and lifestyle habits were recorded in the two days before the first experimental trial and repeated prior to the second. Mean energy intake over the 48 h period prior to each trial was $7.2 \pm 3.6 \text{ MJ} \cdot \text{day}^{-1}$. The mean mass of CHO, Fat and protein consumed during the recording period was $2.8 \pm 0.5 \text{ g} \cdot \text{kg BM}^{-1} \cdot \text{day}^{-1}$, $1.5 \pm 1.3 \text{ g} \cdot \text{kg BM}^{-1} \cdot \text{day}^{-1}$ and $0.8 \pm 0.1 \text{ g} \cdot \text{kg BM}^{-1} \cdot \text{day}^{-1}$ respectively. Dietary analysis revealed no caffeine was ingested during the period recorded. By applying linear regression analysis to the findings of Harris et al. (1991), the orocecal transit time of the temperature sensor can be approximated to $126 \pm 24 \text{ min}$ at the energy intake recorded by this cohort of subjects.

The mean BMI was $25 \pm 4$, when combined with the average sum of skinfolds ($36 \pm 15 \text{ mm}$) this suggests that potential obesity-induced suppression of dietary thermogenesis will have little impact on this group of individuals.
Figure 10.1  Mean rectal and intestinal temperature (°C) under the carbohydrate (CES) and flavoured water (FW) fluid conditions. For clarity a graphical representation of dispersion from the mean has been omitted.

Figure 10.2  Mean intestinal temperature (\(\bar{x} \pm n\sigma\); °C) under the carbohydrate (CES) and flavoured water (FW) fluid conditions. Main effect CES vs. FW, \(P=0.910\).
Figure 10.3 Mean change in total body heat content (\(\bar{x} \pm nCl; \text{KJ}\)) during the carbohydrate (CES) and flavoured water (FW) trials versus baseline (30 min).

Figure 10.4 Estimated energy expenditure (\(\bar{x} \pm nCl; \text{KJ.min}^{-1}\)) during the carbohydrate (CES) and flavoured water (FW) trials. * \(P<0.05\) CES vs. FW. † \(P<0.05\) vs. Baseline (40 min).
<table>
<thead>
<tr>
<th></th>
<th>Baseline 40 min</th>
<th>60 min</th>
<th>80 min</th>
<th>100 min</th>
<th>120 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \dot{V}O_2 ) (ml·kg(^{-1})·min(^{-1}))</td>
<td>CES</td>
<td>3.26 ± 0.32</td>
<td>3.42 ± 0.34</td>
<td>3.59 ± 0.42</td>
<td>( ^\dagger )</td>
</tr>
<tr>
<td></td>
<td>FW</td>
<td>3.18 ± 0.20</td>
<td>3.26 ± 0.31</td>
<td>3.41 ± 0.27</td>
<td>( ^\ddagger )</td>
</tr>
<tr>
<td>( \dot{V}CO_2 ) (ml·kg(^{-1})·min(^{-1}))</td>
<td>CES</td>
<td>2.95 ± 0.40</td>
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<td>( ^\dagger )</td>
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<tr>
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<td>FW</td>
<td>2.83 ± 0.23</td>
<td>2.92 ± 0.26</td>
<td>3.10 ± 0.33</td>
<td>( ^\dagger )</td>
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<tr>
<td>RER</td>
<td>CES</td>
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<tr>
<td>CHO oxidation rate (g·min(^{-1}))</td>
<td>CES</td>
<td>0.20 ± 0.06</td>
<td>0.20 ± 0.03</td>
<td>0.36 ± 0.04</td>
<td>( ^\dagger )</td>
</tr>
<tr>
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<td>FW</td>
<td>0.17 ± 0.06</td>
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<td>Fat oxidation rate (g·min(^{-1}))</td>
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<td>0.04 ± 0.03</td>
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<td>0.03 ± 0.02</td>
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<td>0.05 ± 0.03</td>
<td>0.05 ± 0.01</td>
<td>0.04 ± 0.03</td>
<td>0.05 ± 0.02</td>
</tr>
<tr>
<td>Energy expenditure (KJ·min(^{-1}))</td>
<td>CES</td>
<td>5.13 ± 0.66</td>
<td>5.37 ± 0.65</td>
<td>5.63 ± 0.55</td>
<td>( ^\dagger )</td>
</tr>
<tr>
<td></td>
<td>FW</td>
<td>5.04 ± 0.85</td>
<td>5.13 ± 0.63</td>
<td>5.37 ± 0.70</td>
<td>( ^\dagger )</td>
</tr>
</tbody>
</table>

Table 10.1 Oxygen uptake (\( \dot{V}O_2 \)), carbon dioxide production (\( \dot{V}CO_2 \)), respiratory exchange ratio (RER), energy expenditure, CHO and fat oxidation rates, at baseline (40 min) and subsequently every 20 min during the postprandial period. Table reports mean ± SD for the carbohydrate (CES) and flavoured water (FW) trials. * denotes significant difference (\( P<0.05 \)) between fluid conditions (CES vs. FW). \( ^\dagger \) denotes a significant time effect (\( P<0.05 \)) vs. baseline (40 min).
10.4 Discussion

The main finding of this investigation was that the ingestion of a 6% CES did not result in any notable differences in T_{int} when compared with a FW. A systematic bias existed between T_{int} and T_{rec} throughout the protocol, this was similar in magnitude to that reported during exercise in Chapter 4. The ingestion of these fluids at 4°C in equal volumes to that administered during the LIST resulted in a significant reduction in T_{core} and TBHC.

The discrepancy in temperature between T_{int} and T_{rec} remained at a relatively constant magnitude throughout each trial and this difference was similar to that observed during the LIST protocol described in Chapter 4. This difference between sites of T_{core} measurement is comparable to the bias reported by Edwards et al. (2002) during normal daily activities. The mean bias calculated during the FW trial in the present study (T_{int}-T_{rec} 0.14°C) was identical to that observed when ingesting the same solution over the LIST protocol (Chapter 4). This suggests that during exercise induced temperature changes, the relative blood flow and thermal inertia of these two sites is similar to temperature changes that occur when at rest. The relative reliability of comparing T_{int} with T_{rec} during this study is also equal to that seen when ingesting a FW during LIST (Chapter 4, r=0.976, (P<0.01); Chapter 10, r=0.978 (P<0.01)). This demonstrates an excellent consistency of comparison between these sites of T_{core} measurement both during free running exercise and whilst seated at rest. The consistent bias seen in the present findings also confirms that the shift in bias seen over time in Chapter 4 was brought about by the physiological changes induced by the LIST exercise protocol and not the drinks consumed.

No differences were detectable in T_{int} or T_{rec} between repeated trials (CES vs. FW), demonstrating that the ingestion of 1.09 g·kg BM\(^{-1}\) of CHO in this time period has no measurable effect on T_{core}. There was however a weak trend toward an increase in T_{int} from 100 min onward that was not mirrored in T_{rec}, but the magnitude and statistical power of this difference is unlikely to confound the interpretation of T_{int} during the postprandial period. A shift in substrate metabolism is evident after the ingestion of the CES, with increases in the CHO oxidation rate and strong trends toward an elevated RER. This is accompanied by an increased energy expenditure, with the energy expenditure at 120 min estimated to be 0.5 KJ·min\(^{-1}\) higher in the CES trial.
compared with the FW. This increase in metabolic rate between solutions is likely to be due to energy-requiring process of digestion stimulated during the postprandial period. The magnitude of this increase is comparable to previous work (Hill et al., 1984; Sharief and Macdonald, 1982) and it is considered that the extent of this response ranges from 2% to 7% of total ingested energy (Glickman et al., 1947; op cit. Hill et al., 1984). This difference is small in comparison to the discrepancy in estimated energy expenditure measured between solutions in Chapter 9. The same relative volume of CES ingested during the LIST produced mean increases in energy expenditure that are 10 times greater than those recorded in this chapter. This confirms that these changes are caused by increases in work done during sprinting rather than the thermic effect of feeding.

The calculated energy required to heat the ingested beverages from 4°C to $T_{core}$ in this cohort of subjects was $179 \pm 34$ KJ. From the first of the serial feedings until the end of the trial TBHC declined by an average of $80 \pm 30$ KJ. A thermoneutral laboratory environment was used, therefore these data suggest that body cooling occurred as a consequence of decreased intragastric temperature, with the stomach acting as a heat sink. The rate of fall in $T_{core}$ and TBHC was less toward the later half of the protocol, this indicates that the appropriate thermoregulatory reflexes occurred in response to this challenge. An increase in energy expenditure and a reduction in $\bar{T}_{sk}$ recorded during this period suggests that peripheral vasoconstriction and an increased metabolic rate played the major role in this response. This relatively minor perturbation to thermal homeostasis imposed by these solutions is not likely to have a lasting influence during intense exercise in the heat, when peripheral heat generation is likely to rapidly eradicate the heat sink produced by the beverages (Lee et al., 2004, 2005; Wimer et al., 1997). Nevertheless the 2.3 KJ·kg BM$^{-1}$·h$^{-1}$ cooling effect of these solutions is likely to have a significant impact on heat storage. Considering that the average TBHC gained during 60 min of the LIST (Chapter 8) was 598 KJ, participants will benefit from the heat dissipating effects of ingesting 17 ml·kg BM$^{-1}$·h$^{-1}$ of these solutions during prolonged high-intensity, intermittent exercise.

It appears that the acute thermogenic effect of ingesting a 6% CES can be detected by indirect calorimetry. However, this process occurs without notably influencing deep body temperature when measured as either $T_{int}$ or $T_{rec}$. The influence of this thermic
effect on energy expenditure is minor compared with changes measured between solutions during exercise in Chapter 9. What remains to be established is whether or not increased blood flow to the intestine after CHO feeding is of sufficient magnitude to alter the dynamics of heat loss during exercise in the heat.
CHAPTER 11

GENERAL DISCUSSION

11.1 Introduction
It is clear from previous research and a pilot investigation that ingesting a dilute carbohydrate-electrolyte solution in the heat may influence temperature regulation and exercise performance in a different manner to drinking water alone. This programme of research firstly set out to determine a reliable method of measuring deep body temperature frequently during unconstrained high-intensity intermittent exercise. Thereafter the subsequent studies used this methodology to examine the influence of ingesting a CES and FW on the physiological responses to prolonged high-intensity intermittent exercise in the heat. The main findings are summarised below:

• Chapters 4, 5 and 6 demonstrate that ingestible temperature sensor capsules are a valid, reliable and a repeatable method of measuring core temperature during prolonged, high-intensity intermittent exercise in 15°C that involves the consumption of fluids. The findings reported in Chapter 10 also support the validity of measuring $T_{int}$ at rest in 22°C and confirm that ingesting a 6% CES has no influence on $T_{int}$ during the postprandial period when compared with consuming water.

• The findings of Chapters 7, 8 and 9 suggest that sprinting performance is improved during 60 min of high-intensity intermittent exercise when ingesting a 6% CES compared with flavoured water. Data from Chapter 9 demonstrate that it is possible to detect increases in energy expenditure during the LIST supporting the numerical increases in sprint performance.

• In two of the studies in the heat (Chapters 7 and 8) core temperature was higher in the CES trial than the FW trial. This appears to be caused by increases in energy expended during sprinting and does not curtail sweat loss, skin blood flow or the ability to perform any other phase of the LIST.
• The rate of gastric emptying of a 6% CES is similar to that of FW during prolonged high-intensity intermittent exercise in the heat (Chapter 8). The rate of gastric emptying is not reduced to an extent that restricts fluid uptake, neither does the CES restrict heat dissipation via the variables measured ($\bar{T}_{sk}$, skin blood flow and sweat loss).

• Chapter 9 demonstrates that the point of fatigue during prolonged intermittent high-intensity exercise in 30°C occurs at a $T_{core}$ of approximately 39.5°C and hyperthermia is the limiting factor for exercise capacity. Under these conditions the inclusion of CHO and electrolytes in the rehydration solution seem to have little influence on the time taken to reach the point of exhaustion.

11.2 The benefits of ingesting a CES versus FW

The three investigations within this thesis that were conducted at an environmental temperature of 30°C consistently found improvements in sprint performance following the ingestion of a 6% CES compared with a FW. Deep body temperature also responded in a different manner during two of these studies. The following section will outline the differing responses elicited by these test drinks and discuss the potential mechanisms underpinning them.

11.2.1 Performance

An improvement in 15 m sprint performance appears to be the major quantifiable advantage of ingesting a CES versus plain water during 1 hour of the LIST test in 30°C. A reduction in the total time spent sprinting is evident in each feeding study within this thesis, this is a novel finding that has not been detected previously during this protocol in the heat. Sprint performance was measured during a similar protocol by Morris and colleagues (2003), this study found no differences in performance between solutions, but a trial order effect evident in several primary variables suggests that their results may have been influenced by an acclimation process or learning effect. In cooler ambient conditions Ali et al. (2002) have previously shown that a 6% CES will improve cumulative 15 m sprint time by 1.9 s over 90 minutes of the LIST protocol. The magnitude of this difference over the first 60 minutes of exercise in that study is comparable to the current findings (0.9 s) over the same time period.
difference in energy expenditure calculated between a 6% CES and a placebo solution by Ali and co-workers (2002) is also similar to that reported in Chapter 9. Figure 11.1 shows the cumulative sprint times for each of the feeding studies reported within this thesis. There is a notable increase in the total time spent sprinting in Chapter 9 relative to Chapters 7 and 8. This is most likely to be due to the movement restrictions imposed by the Douglas bag assembly, this apparatus was worn during 8 of the 44 sprinting phases in Chapter 9 and resulted in notably slower mean running velocities during these sprints in both trials. It is clear from the chart that the performance improvement afforded by ingesting the CES over water alone is a constant and repeatable finding, which results in a total improvement of ~0.77 s over 60 min of exercise. This difference between solutions appears small considering the number of sprints attempted, however, in a match situation even a fraction of a second can be critical in successfully performing a crucial pass, tackle or shot. The dispersion of the bias within these studies is also skewed and has a tendency to be larger toward the later stages of exercise, a time period during match-play when the greatest number of goals are scored or conceded (Abt et al., 2002).
11.2.2 Energy Metabolism

Previous investigators have found it difficult to explain any metabolic advantage of ingesting CHO during exercise in the heat when the duration of exercise is relatively short (≤60 min). In this timeframe muscle glycogen stores are not thought to be limiting in well-nourished individuals and it has been shown that no additional uptake of exogenous glucose by the muscle occurs when CHO is infused during cycling exercise (Jentjens et al., 2002). However, the results reported in Chapter 9 suggest there is indeed an increased rate of CHO oxidation when a CES is ingested during the LIST, which appears to occur from the first 15 min of exercise.

The data presented in Chapter 8 demonstrate that around 16 g·15 min⁻¹ of CHO is emptied from the stomach and hence becomes available for intestinal absorption. The speed of gastric emptying is considered the rate limiting factor for the provision of substrate during high-intensity intermittent exercise in temperate conditions (Leiper et al., 2001a; 2005; 2001b). If that is also the case in the investigations within this thesis this could amount to the appearance of glucose in the blood at a rate of around 1 g·min⁻¹, close to the maximum achievable absorption rate of glucose monomers 1.3 g·min⁻¹ (Duchman et al., 1997). However, the effect of hyperthermia on intestinal absorption when exercising in a fed state is currently unknown. It is possible that feeding could either increase blood flow to this region (Perko et al., 1998), or result in mesenteric ischemia that would limit the absorption and appearance of ingested glucose beyond the rate of gastric emptying (Rowell, 1974; Rowell et al., 1968). This untested suggestion forms one of the arguments for why CHO ingestion is often considered to be ineffective in these circumstances. On the other hand, the rapid and sustained elevation of plasma glucose seen in the CES trials of Chapters 7 & 9 shows that the appearance in the blood of glucose from the GI tract is not notably suppressed. In these trials the relatively high plasma concentration of glucose throughout exercise seems disproportionate to CHO ingestion and may be the result of a failure of the liver to regulate systemic glucose concentration. An increase in hepatic glucose production in the heat is a well reported phenomenon and it seems that liver glycogenesis, which is known to balance the perturbations caused by the release of large amounts of CHO from the gut, dysfunctions or is absent (Febbraio, 2001). But regardless of exogenous CHO availability the uptake of glucose into the muscle has been shown to be significantly constrained during high-intensity cycling exercise in
the heat (Jentjens et al., 2002). This does not appear to have happened notably in the present series of studies, because the trend toward higher RER values during the early stages of the protocol suggest that the ingested CHO could indeed support an increased rate of muscle glucose uptake and oxidation.

In previous work marked increases in CHO oxidation rates when an exogenous supply is provided tend only to occur when subjects' endogenous CHO stores are depleted. Prior to the present series of experimental trials the dietary and physical activity regime adopted by participants would suggest they do not undertake the protocol in a glycogen-depleted state. On the other hand a more rapid rate of glycogenolysis, which has been previously reported under thermal stress, may accelerate the depletion of CHO stores compared to cooler conditions. These suggestions are not supported by the high muscle glycogen concentrations measured following the LIST in 30°C (Morris et al., 2005). Previous work examining glucose kinetics during exercise has predominately focused on continuous forms of exercise performed at moderately high intensities. It may be possible that during the LIST protocol the low-intensity phases of exercise (walking) allow periods in which glucose derived from the blood can be oxidised by muscle.

A number of studies have noted some kind of exercise performance enhancement when a dilute CES is ingested in uncompensable environments (Table 2.2) and several have reported no benefits (Table 2.3). One explanation for these contrasting findings may be related to the dose and timing of the CHO administered. In the studies of Davis et al. (1988a) and Millard-Stafford et al. (1990), in which no performance improvements were found, subjects were provided with only moderate amounts of CHO that were administered some time after the onset of exercise. The studies that report increases in performance typically provided a substantial bolus of CHO before exercise, followed by regular serial feedings during exercise. In these studies and in the results of Chapters 7 and 9 of this thesis, this feeding results in a sustained elevation in plasma glucose that is evident from the early stages of exercise. It is possible that during high-intensity intermittent exercise in the heat, when the glucose sensitivity of muscle is potentially restricted, a relatively high systemic glucose concentration is required to support higher CHO oxidation rates.
The metabolic benefits of ingesting a 6% CES during intermittent exercise in the heat may be a combined effect comprising of the sparing of muscle glycogen in type II muscle fibres and an increased oxidation rate of CHO. Although the former is of little advantage when the cause of fatigue is related to hyperthermia it may become more important in situations when repeated prolonged efforts are required, such as during tournament play.

11.2.3 Temperature regulation
During the studies of Chapters 7, 8 and in the pilot investigation accompanying this thesis a significantly elevated Tcore developed when subjects drank a CES throughout exercise. This is not a novel finding and has been reported previously in the heat during high-intensity intermittent shuttle running (Morris et al., 2003), cycling with periods of sprinting (Fritzsche et al., 2000) and running (Millard-Stafford et al., 2005). Fritzsche and colleagues attribute this rise to an increase in maximal power output and higher CHO oxidation rate when the 6% CES was ingested. This also seems to be the most likely explanation for the additional heat gain during the protocols within this thesis. Sprint performance was increased as a result of ingesting the CES in each of the feeding studies included in this thesis (Figure 11.1). This additional energy expenditure is likely to result in an increased rate of heat production and hence storage. The difference in cumulative sprint time between trials appears small in comparison to the amount of extra heat generated, however, when the differences in energy expenditure over an hour of the protocol estimated during Chapter 9 (290 KJ) are compared with the differences in TBHC calculated in Chapter 8 (130 KJ), it seems reasonable to suggest that an increase in energy expenditure could account for the extra heat generated and stored. This elevation in heat storage does not appear to have a detrimental effect on any aspect of the LIST over 60 min of exercise. It also seems that this difference may not influence the time taken to reach exhaustion, however, the investigation that addressed endurance capacity did not detect any clear bias in Tcore between trials.

Morris and co-workers (2003) postulate that a decreased rate of gastric emptying when ingesting a CES may result in reduced fluid availability and hence a more rapid rise in Tcore. Chapter 8 addressed this question and demonstrated that there were no significant differences in the volume of a 6% CES and FW emptied over 60 min of exercise.
the LIST in the heat. Furthermore it seems likely that the isotonic CHO solution would elicit a faster rate of fluid transport from the small intestine than water alone.

The possibility that the elevation in $T_{core}$ when ingesting a 6% CES was the consequence of the thermogenic effect of transporting and processing nutrients has been ruled out by the investigation that formed Chapter 10. In this investigation no differences in $T_{core}$ when measured as both $T_{rec}$ and $T_{int}$ were detected between solutions during the postprandial period. All of the investigations within this thesis used a time interval after pill ingestion that is likely to place the sensor in an advanced region of the large intestine, that is presumably free from the thermal influences of digestion. Furthermore, hyperthermia has been shown to have no effect on gut motility (Harris et al., 1990), so the rate at which the sensor progresses through the GI tract during the LIST protocols conducted in the heat is likely to be similar to that in Chapters 4, 5 and 6. The increase in energy expenditure arising during the postprandial period of the CES trial in Chapter 10 accounts for around only 7% of the total increase in energy expenditure recorded between trials during the LIST protocol in Chapter 9. Although the thermogenic effect of CHO feeding has no detectable effects on $T_{core}$, the fraction of blood needed to support these postprandial processes may have been of a large enough volume to have significantly impacted the total blood volume available for thermoregulatory processes during exercise. This could potentially explain the increases in $T_{core}$ observed when subjects were in a fed state, but was not evident in the measures of peripheral blood availability recorded during the studies within this thesis ($\bar{T}_{sk}$ or skin blood flow). This suggestion is supported by evidence that has shown a reduction in vasoconstriction of the mesenteric artery during exercise in the postprandial period compared with a fasted state (Perko et al., 1998).

One factor that has not been explored within this programme of research is the separate effects of the electrolyte component of the CES. The beneficial effects for fluid restoration when electrolytes are administered in similar quantities to those used in this thesis are extensively documented. However, the beneficial effects of electrolyte replacement on thermoregulation and performance during relatively short exercise protocols is less well understood. Recently Ruby et al. (2005) found no differences in $T_{core}$ or $T_{sk}$ when a water-electrolyte solution was compared with water
alone during several hours of moderately intense work in the heat. Similarly no significant treatment effect in $T_{\text{core}}$ was seen by Vrijens and Rehrer (1999) when distilled water was compared with a sodium containing sports drink over a 3 h exercise period in the heat. The serum electrolyte concentrations and osmolalities observed within the experimental chapters of this thesis would suggest that the omission of electrolytes from the water had little influence on the processes of thermoregulation or circulatory function. Nevertheless caution must be exercised when attributing all physiological and performance differences observed between trials to solely the CHO component of the CES.

The confidence by which conclusions regarding temperature regulation can be drawn from the present series of investigations is restricted by the degree to which the thermoregulatory responses in each study reflect one another. The absolute differences in $T_{\text{int}}$ between trials at the end of exercise are comparable in Chapters 7 and 8. However, the bias in $T_{\text{int}}$ between trials is somewhat less at similar time points in Chapter 9 with no statistical significance detectable in this difference. This occurred despite similar improvements in sprint performance during the CES trial, which seems the most likely source of the additional heat gain. It has been shown in Chapter 5 that the $T_{\text{int}}$ response to exercise can be satisfactorily repeated between LIST trials in the same individuals. But Chapter 6 showed that the level of agreement in the $T_{\text{int}}$ response between similar sportsmen is not precise enough to make accurate inferences between groups for small temperature changes. This may provide one explanation for why three different cohorts of subjects produced notably different thermoregulatory responses when ingesting the same solution, during a protocol that is predominantly compromised of exercise at relative intensities.

11.2.4 Central factors

The ingestion of a 6% CES during cycling exercise has been shown to increase the cerebral uptake of glucose and $O_2$ (Nybo et al., 2003) and offset neuromuscular fatigue (Nybo, 2003). It is possible that an enhancement of brain metabolism was supported by the ingestion of the CES in the present series of investigations, and hence may have contributed to a delaying of the fatigue process. The level at which hypoglycaemia has been shown to limit cerebral energy turnover is considerably lower than the plasma glucose concentrations reported during exercise and rest in
Chapters 7 and 9 (~3 mmol\textsuperscript{1}l\textsuperscript{-1}). Nevertheless the dose effect of this relationship is not known during exercise when humans are hyperthermic. Carbohydrate ingestion also blunts the rise in brain serotonin in animals and the markers of serotonergic activity in humans. This is thought to occur through a reduction in lyposis during CHO feeding, which can alter the concentration of free tryptophan. The trend toward a lower FFA availability during the fed state reported in Chapters 7 & 9, may support this mechanism, and it is plausible that decreased central activation may be responsible for the differences in sprint performance between solutions.

When blood glucose and muscle glycogen availability are unlikely to be limiting, the differences in performance between trials could potentially be explained by the recently published theory that proposes a central controller governs physical work in response to net CHO status (Lambert et al., 2005; Noakes et al., 2005b; St Clair Gibson et al., 2005). In this highly complex model afferent signals from the liver, muscle and other tissue provide information on overall CHO status, from which efferent signals bring about changes in activity levels or exercise intensity. During rapid increases in body temperature, when the regulation of hepatic glucose production is not effective, the interpreter of net CHO status may compute a decline in total available stores. This homeostatic mechanism could then play a role in reducing exercise intensity, sprint performance in the case of the FW trials during the LIST, to avoid catastrophe. The influences of CHO ingestion upon the CNS have also been shown to occur independently of glucose appearance in the blood. Rinsing a CHO solution in the mouth has been shown to increase performance, possibly through the modulation of central pathways via receptors in the oral cavity (Carter et al., 2004a). Increased cerebral activation has also been noted after CHO ingestion at a time when the solution would not have left the stomach (Liu et al., 2000). These findings suggest that neural control mechanisms may also have means of quantifying anticipated CHO status some time before actual glucose appearance in the blood.

The results within this thesis may also provide evidence that the potential metabolic central control mechanism discussed above acts independently of neural mechanisms that provide anticipatory regulation of behaviour to avoid hyperthermia (at moderate core temperatures). The catastrophe theory proposed by Marino (2004) suggests that exercise intensity in the heat is regulated in order to provide a pre-determined rate of
rise in $T_{\text{core}}$ from the early stages of exercise. The increases in performance seen when subjects ingest a 6% CES, if due to enhanced CHO availability, allow this set-point of $T_{\text{core}}$ to reach a higher level depending on energy status. Carbohydrate mediated changes within the CNS during exercise remain speculative and theoretical models of central neural control are complex and in their infancy. Nevertheless it appears that the brain may have effective mechanisms in place for controlling energy expenditure in accordance with the body's energy state or even the anticipated delivery of ingested substrate. In previous research central factors are often implicated when no clear metabolic bases for CHO supplementation are apparent. In the current program of research it seems likely that the increased CHO oxidation rate and energy expenditure, supported by the ingestion of the CES, are primarily responsible for the majority of the performance enhancement. In addition, the lack of any difference in $T_{\text{int}}$ at the point of fatigue does not support the suggestion that CHO ingestion improves central tolerance to hyperthermia.

11.3 Summary
The performance of high-intensity sprinting deteriorates during the LIST protocol in 30°C and it appears that exhaustion under these conditions occurs as a result of an intolerable rate of heat storage. The ingestion of a 6% CES was not found to prolong exercise capacity in these circumstances, but before a critical level of hyperthermia is reached drinking a suitable volume enhances sprinting performance. In answer to the question posed at the beginning this programme of research, the ingestion of a dilute CHO solution with added electrolytes appears to be more advantageous than plain water during prolonged intermittent exercise, even in circumstances where the volume of fluid replaced is the main concern. To date there remains no well controlled study showing that individuals perform better during exercise when drinking water than when consuming a well formulated sports drink. The potential causes of fatigue during prolonged, intermittent high-intensity exercise in the heat are summarised in Figure 11.2 along with the possible advantages of ingesting either a 6% CES or FW.

In practical terms, games players and coaches who currently avoid using CHO-electrolyte drinks in the heat, or dilute their 6% CES thorough fear of impaired rehydration, may be wiser to continue using the same formulation that they would drink in cooler conditions. As well as performance gains this may also help lower the
risk of players developing hyponatraemia over long periods of competition and fluid consumption.

In addition to the main findings of this thesis ingestible temperature sensors have been demonstrated to be a suitable and reliable means of determining $T_{core}$ during high-intensity intermittent exercise, in both temperate and hot environmental temperatures.

11.4 Future directions

- Examine the contribution of exogenous and endogenous CHO to total oxidation using labelled isotopes.
- Determine the influence of a CES when participants ingest a low CHO diet or undertake the protocol in a glycogen depleted state.
- Quantify cerebral function by measuring blood borne markers of serotonergic activity, or calculate changes in brain activity using EEG or fMRI techniques.
- Measure splanchnic blood flow during the LIST in a fasted and fed state, in order to quantify the differences in blood distribution during exercise in the postprandial period.
- Study the validity of administering intestinal temperature sensors as suppositories when the time gap needed between oral ingestion is not practical.
Mechanism of fatigue

Decreased motivational drive with increased perception of work and thermal load. Inability to voluntarily maintain neuromuscular function.

Restricted rate of gastric emptying and fluid delivery to the small intestine.

Limited rate of intestinal absorption due to mesenteric ischemia.

Depletion of muscle glycogen stores and reduced glucose uptake.

Reduced central blood volume, $Q$ and blood supply to the active musculature.

Potential advantage of drink

<table>
<thead>
<tr>
<th>CES</th>
<th>FW</th>
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<tbody>
<tr>
<td>Enhancement of cerebral energy turnover. Alteration of central neurotransmitter activity through suppressed lipogenesis. Speculated neural activation via oropharyngeal or gastric CHO receptors.</td>
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<tr>
<td>Similar rate of gastric emptying</td>
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<tr>
<td>Increased active and passive transport of fluid with CHO.</td>
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</tr>
<tr>
<td>Improved systemic glucose availability and greater reliance on exogenous CHO. Increased total CHO oxidation rate.</td>
<td></td>
</tr>
<tr>
<td>Prevention of haemodilution by electrolytes resulting in better maintenance of plasma osmolality and volume.</td>
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<tr>
<td>Smaller fraction of total blood volume required to support the visceral organs during the postprandial period.</td>
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</table>

Figure 11.2 Potential mechanisms of fatigue during prolonged high-intensity intermittent exercise in 30°C and the respective advantages of ingesting a 6% carbohydrate-electrolyte solution (CES) versus a flavoured water (FW).
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APPENDIX A

Statement of Informed Consent

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

Signed ___________________ Witnessed by ___________________

Date / / 

Daily Health Questionnaire

Please complete the following brief questions to confirm your fitness to participate in Today's experiment:

At present do you have any health problems for which you are:

1) On medication, prescribed or otherwise
   Yes □ No □

2) Attending your general practitioner
   Yes □ No □

Have you any symptoms of ill health, such as those associated with a cold or other common infection?
   Yes □ No □

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

...........................................................................................................................................

...........................................................................................................................................

Do you want to take part in today's experiments? Yes □ No □

Signature: ___________________ Date / /

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APPENDIX B

Health Screen for Study Volunteers

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

Please complete this brief questionnaire to confirm fitness to participate:

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

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

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

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

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

Health Screen for Ingestible Temperature Sensors

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

Please complete this brief questionnaire to confirm fitness to participate:

1. At present, or in the past have you had any of the following health problems:

   (a) Any known or suspected obstructive disease of the gastrointestinal tract, including but not limited to diverticulitis and inflammatory bowel disease.

   Yes □ No □

   (b) Any inflammatory bowel disease.

   Yes □ No □

   (c) A history of disorders or impairment of the gag reflex.

   Yes □ No □

   (d) Any previous gastrointestinal surgery.

   Yes □ No □

   (e) Are you or might you undergo Nuclear Magnetic Resonance (NMR) scanning during the period that the disposable temperature sensor is within the body.

   Yes □ No □

   (f) Any hypomotility disorders of the gastrointestinal tract including but not limited to Illus.

   Yes □ No □
<table>
<thead>
<tr>
<th>Physical Activity Questionnaire for Soccer Players</th>
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<tr>
<td><strong>Current Level</strong></td>
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<tr>
<td>(e.g. university 1st team, recreational, national etc.)</td>
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<tr>
<td><strong>Current Fitness/Training Status</strong></td>
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<td>(e.g. fully/half match fit, recovering etc.)</td>
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<tr>
<td><strong>How many training sessions a week do you usually participate in?</strong></td>
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<td><strong>Do you practice Skills Training?</strong></td>
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<tr>
<td>Yes / No</td>
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<td>If yes, how many sessions per week?</td>
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<td>How many minutes does each session last?</td>
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<tr>
<td><strong>Do you practice Interval Training?</strong></td>
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<td>Yes / No</td>
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<td>If yes, how many sessions per week?</td>
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<td>How many minutes does each session last?</td>
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</tr>
<tr>
<td><strong>Do you practice Resistance Exercise (weights)?</strong></td>
</tr>
<tr>
<td>Yes / No</td>
</tr>
<tr>
<td>If yes, how many sessions per week?</td>
</tr>
<tr>
<td>____ per week</td>
</tr>
<tr>
<td>How many minutes does each session last?</td>
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<tr>
<td>____ min</td>
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<tr>
<td><strong>Do you practice Endurance Running?</strong></td>
</tr>
<tr>
<td>Yes / No</td>
</tr>
<tr>
<td>If yes, how many runs per week?</td>
</tr>
<tr>
<td>____ per week</td>
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<tr>
<td>How many minutes does each run last?</td>
</tr>
<tr>
<td>____ min</td>
</tr>
<tr>
<td>What is your weekly running mileage?</td>
</tr>
<tr>
<td>____ miles per week</td>
</tr>
</tbody>
</table>
APPENDIX E

Body Temperature Measurement Pill

PLEASE READ BEFORE SWALLOWING THE PILL

You will have already completed a health screen questionnaire confirming your suitability to use the temperature sensor pill. You must not swallow the pill if any of the following applies to you:

- Any presence of any known or suspected obstructive disease of the gastrointestinal tract, including but not limited to diverticulitis and inflammatory bowel disease.
- Have or suspected to have any inflammatory bowel disease.
- Exhibit or having a history of disorders or impairment of the gag reflex.
- Previous gastrointestinal surgery.
- Will be undergoing Nuclear Magnetic Resonance (NMR) scanning during the period that the disposable temperature sensor is within the body (up to 72 hours).
- Hypomotility disorders of the gastrointestinal tract including but not limited to Illus.

Instructions

Before swallowing the pill please handle with care, replacements for lost or damaged pills are costly and must be imported from the US.

Your temperature sensor pill has been placed within a screw-top plastic tube to prevent accidental damage. Remove the sensor from the tube and take it out of the plastic bag it is sealed within. The pill is wrapped in an orange label to indicate it is clean, has not been tampered with and is calibrated correctly. Attached to the outside of this wrapper is a small magnet. This magnet holds a magnetic switch closed within the pill that stops the battery and hence the pill from working. Remove this magnet and the label before swallowing the pill. This will start the pill operating; from then on it has a battery life of 72 hours. Please place the magnet and orange label back inside the plastic bag and bring them along with you on the morning of the trial, so that we can confirm you have done this.

Swallow the sensor pill before you go to bed or before midnight (whichever is first) on the evening before the experimental trial. You should find no difficulty in swallowing the pill as it is coated in silicone which becomes very slippery when wet. Swallow in the same way as you would swallow a normal pill whilst standing or sitting, you may wish to swallow it with a glass of water. If you experience difficulty swallowing the pill, place it on the back of your tongue, tilt your head back and swallow with water. If you experience nausea and/or vomiting then stop immediately. If you have problems or queries contact Nick Gant on ####### or ####### (24 hour number).

On arrival to the laboratory the pill will be located with a receiver to check that it is functioning correctly and to ascertain its position in the body. After you finish the main trial, the pill will pass through your gastrointestinal tract at a rate dependent on your motility and appear in your faeces. The pill is designed to safely flush down the toilet with your faeces and cause no harm to the environment.
## APPENDIX F

### Diet and Physical Activity Diary

**Day 1**

<table>
<thead>
<tr>
<th>Time</th>
<th>Brand Name of Item (except fresh food etc.)</th>
<th>Full description of each item*</th>
<th>Weight served (g)</th>
<th>Weight leftover (g)</th>
</tr>
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<tbody>
<tr>
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<td></td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

**General Comments:**

* Include - fresh, frozen, dried, canned etc. - cooked, boiled, grilled, fried (including type of fat), roasted.

<table>
<thead>
<tr>
<th>Time</th>
<th>Activity (Irregular to your normal daily routine)</th>
<th>Duration (min)</th>
<th>General Comments</th>
</tr>
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<tbody>
<tr>
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**Wake-Up Time**

**Bed Time**

**General Comments:**

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### APPENDIX G

<table>
<thead>
<tr>
<th>Rating of Gut Fullness</th>
<th>Rating of Thermal Sensation</th>
<th>Rating of Perceived Exertion</th>
<th>Rating of Thirst</th>
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<tr>
<td>6</td>
<td>6</td>
<td>6</td>
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<td>7</td>
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<td>19</td>
<td>8</td>
<td>19</td>
<td>19</td>
</tr>
<tr>
<td>20</td>
<td>9</td>
<td>20</td>
<td>Maximal</td>
</tr>
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</table>

- **Most areas of body feel cold**
- **Some areas of body feel cold**
- **Neutral**
- **Some areas of body feel warm**
- **Most areas of body feel hot**
- **Very hot, uncomfortable**
- **Extremely hot, close to limit**
- **Heat impossible to bear**
Chapter 4  Bland-Altman plots representing absolute comparisons between rectal and intestinal temperature (°C), for individual subjects during the 4 block LIST protocol. Individual subjects are presented on separate ordinates to the same scale. Bias and random error lines (95% limits of agreement) are included.
Chapter 5  Bland-Altman plots representing absolute comparisons in temperature (°C) between repeated trials, for individual subjects during the 6 block LIST protocol. Individual subjects are presented on separate ordinates to the same scale. Bias and random error lines (95% limits of agreement) are included.
**APPENDIX I**

Data contained within the figures of Chapter 7

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
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<th>3</th>
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<td>7.1</td>
<td>Intestinal temperature (°C)</td>
<td>CES</td>
<td>37.3 ± 0.4</td>
<td>38.5 ± 0.3</td>
<td>39.2 ± 0.3</td>
<td>39.4 ± 0.5</td>
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<td>37.1 ± 0.3</td>
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<td>Forehead temperature (°C)</td>
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<td>35.4 ± 1.0</td>
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<td>7.3</td>
<td>Heart rate (beats·min⁻¹)</td>
<td>CES</td>
<td>95 ± 20</td>
<td>167 ± 11</td>
<td>175 ± 11</td>
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<td>Sprint performance (s)</td>
<td>CES</td>
<td>------</td>
<td>2.55 ± 0.09</td>
<td>2.57 ± 0.11</td>
<td>2.61 ± 0.12</td>
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<tr>
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<td></td>
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<td>------</td>
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<td>2.65 ± 0.13</td>
</tr>
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<td>Plasma glucose (mmol·L⁻¹)</td>
<td>CES</td>
<td>4.43 ± 0.75</td>
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<td>8.12 ± 0.95</td>
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<td>FW</td>
<td>4.68 ± 0.87</td>
<td>5.76 ± 0.67</td>
<td>5.91 ± 0.83</td>
<td>5.88 ± 1.09</td>
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<td>Plasma FFA (mmol·L⁻¹)</td>
<td>CES</td>
<td>0.25 ± 0.13</td>
<td>0.12 ± 0.06</td>
<td>0.13 ± 0.07</td>
<td>0.17 ± 0.11</td>
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<td>0.27 ± 0.10</td>
<td>0.26 ± 0.11</td>
<td>0.28 ± 0.14</td>
<td>0.26 ± 0.21</td>
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<tr>
<td>7.8</td>
<td>Plasma glycerol (mmol·L⁻¹)</td>
<td>CES</td>
<td>0.05 ± 0.02</td>
<td>0.06 ± 0.04</td>
<td>0.10 ± 0.05</td>
<td>0.12 ± 0.03</td>
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<td>FW</td>
<td>0.05 ± 0.03</td>
<td>0.08 ± 0.01</td>
<td>0.09 ± 0.05</td>
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<td>7.4</td>
<td>Blood lactate (mmol·L⁻¹)</td>
<td>CES</td>
<td>1.44 ± 0.75</td>
<td>5.50 ± 4.42</td>
<td>4.40 ± 3.63</td>
<td>3.96 ± 3.09</td>
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<td>FW</td>
<td>1.61 ± 0.49</td>
<td>5.13 ± 3.13</td>
<td>4.01 ± 2.11</td>
<td>3.86 ± 2.11</td>
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</table>

Figure 7.1–7.9 Mean intestinal temperature and forehead temperature (°C), mean heart rate (beats·min⁻¹), mean sprint time (s), concentrations of blood metabolites (mmol·L⁻¹) at rest and during each 15 min block of the LIST. Data reported as $\bar{x} \pm SD$. 

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APPENDIX I

Data contained within the figures of Chapter 8

<table>
<thead>
<tr>
<th>Figure 8.6</th>
<th>Intestinal temperature (°C)</th>
<th>Figure 8.7</th>
<th>Weighted mean skin temperature (°C)</th>
<th>Figure 8.8</th>
<th>Heart rate (HR)</th>
<th>Figure 8.9</th>
<th>Sprint time (s)</th>
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<td>CES</td>
<td>37.2 ± 0.2</td>
<td>38.1 ± 0.4</td>
<td>38.9 ± 0.4</td>
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<td>± 0.4</td>
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<td>33.7 ± 0.4</td>
<td>35.0 ± 0.4</td>
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<td></td>
<td>± 0.2</td>
<td>± 0.3</td>
<td>± 0.3</td>
<td>± 0.5</td>
<td>± 0.6</td>
<td>± 0.8</td>
<td>± 0.9</td>
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<td>FW</td>
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<td>± 0.4</td>
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<td>± 1.5</td>
<td>± 1.7</td>
<td>± 2.0</td>
<td>± 0.4</td>
<td>164 ± 9</td>
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<td>35.0</td>
<td>85 ± 14</td>
<td>166 ± 11</td>
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<td>± 0.4</td>
<td>± 0.8</td>
<td>± 0.9</td>
<td>± 0.7</td>
<td>± 0.9</td>
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<td>± 1.7</td>
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<td>± 0.7</td>
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<td>± 1.0</td>
<td>± 1.1</td>
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</table>

Figure 8.6 – 8.9 Mean intestinal temperature, weighted mean skin temperature (°C), mean heart rate (beats·min⁻¹) and mean sprint time (s), at rest and during each 15 min block of the LIST. Data reported as $\bar{x} \pm SD$.

<table>
<thead>
<tr>
<th>Fig 8.2</th>
<th>Total drink volume (ml)</th>
<th>Fig 8.3</th>
<th>Drink volume emptied (ml)</th>
<th>Fig 8.4</th>
<th>Total volume in stomach (ml)</th>
<th>Fig 8.5</th>
<th>Volume of secretions (ml)</th>
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<tbody>
<tr>
<td>Block 1</td>
<td>Pre</td>
<td>Post</td>
<td>Pre</td>
<td>Post</td>
<td>Pre</td>
<td>Post</td>
<td>Pre</td>
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<tr>
<td>CES</td>
<td>525.2 ± 55.4</td>
<td>311.7 ± 122.7</td>
<td>594.7 ± 139.3</td>
<td>288.2 ± 132.2</td>
<td>571.2 ± 129.2</td>
<td>407.3 ± 191.1</td>
<td>690.3 ± 196.5</td>
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<td>FW</td>
<td>525.2 ± 55.4</td>
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<td>570.8 ± 182.8</td>
<td>234.1 ± 105.8</td>
<td>536.9 ± 127.5</td>
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<td>630.6 ± 173.9</td>
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<td>CES</td>
<td>213.6 ± 108.5</td>
<td>306.4 ± 212.5</td>
<td>163.9 ± 127.5</td>
<td>88.8 ± 174.4</td>
<td>290.7 ± 211.4</td>
<td>309.7 ± 216.4</td>
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<td>359.8 ± 124.7</td>
<td>174.3 ± 147.4</td>
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<td>CES</td>
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<td>369.4 ± 138.4</td>
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<td>486.4 ± 211.7</td>
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<td>605.8 ± 198.3</td>
<td>268.9 ± 117.5</td>
<td>571.6 ± 139.4</td>
<td>383.6 ± 156.3</td>
<td>683.9 ± 178.4</td>
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<td>CES</td>
<td>29.33 ± 27.89</td>
<td>57.78 ± 30.76</td>
<td>65      ± 20.49</td>
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Figure 8.2 – 8.5 Gastric emptying data (ml), calculated from intubation measurements before and after each block of the LIST. Data reported as $\bar{x} \pm SD$.
**APPENDIX I**

Data contained within the figures of Chapter 9

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<td>±0.7</td>
<td>±0.6</td>
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<td>175</td>
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<td>FW</td>
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<td>174</td>
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<td>Sprint performance (s)</td>
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<td>9.10</td>
<td>Plasma FFA (mmol(^{-1}))</td>
<td>CES</td>
<td>0.32</td>
<td>0.24</td>
</tr>
<tr>
<td></td>
<td>FW</td>
<td>0.42</td>
<td>0.29</td>
<td>0.29</td>
</tr>
<tr>
<td>9.11</td>
<td>Plasma glycerol (mmol(^{-1}))</td>
<td>CES</td>
<td>0.04</td>
<td>0.07</td>
</tr>
<tr>
<td></td>
<td>FW</td>
<td>0.05</td>
<td>0.08</td>
<td>0.13</td>
</tr>
<tr>
<td>9.12</td>
<td>Blood lactate (mmol(^{-1}))</td>
<td>CES</td>
<td>1.26</td>
<td>3.94</td>
</tr>
<tr>
<td></td>
<td>FW</td>
<td>1.27</td>
<td>3.30</td>
<td>3.54</td>
</tr>
</tbody>
</table>

Figure 9.3-9.12 Mean intestinal temperature (°C), mean heart rate (beats·min\(^{-1}\)), mean sprint time (s), exercise intensity (% \(\dot{V}O_2\) max), concentrations of blood metabolites (mmol\(^{-1}\)) and hormones (pmol\(^{-1}\)) at rest and during each 15 min block of the LIST. Data reported as \(\bar{x} \pm SD\).
| Figure 10.1 | Rectal Temperature (°C) | CES   | 36.5   | 36.5   | 36.5   | 36.3   | 36.2   | 36.2   | 36.1   | 36.1   | 36.1   | 36.1   |
|             |                        | FW    | 36.6   | 36.5   | 36.5   | 36.4   | 36.2   | 36.1   | 36.2   | 36.2   | 36.2   | 36.2   |
| Figure 10.1 & 10.2 | Intestinal Temperature (°C) | CES   | 36.7   | 36.6   | 36.6   | 36.3   | 36.3   | 36.4   | 36.4   | 36.5   | 36.4   | 36.5   |
|                |                        | FW    | 36.7   | 36.7   | 36.7   | 36.5   | 36.4   | 36.3   | 36.3   | 36.3   | 36.3   | 36.3   |
| Figure 10.3 | ΔTBHC (J) | CES   | ------ | -3.36  | -4.51  | -32.53 | -56.62 | -65.71 | -79.14 | -73.26 | -83.80 | -78.10 |
|              |                        | FW    | ------ | 2.69   | 2.62   | -29.9  | -56.83 | -71.04 | -71.70 | -83.62 | 86.76  | -92.84 |

Figure 10.1 – 10.3 Mean rectal temperature (°C), Mean intestinal temperature (°C), and total body heat content (TBHC; J) in 10 min intervals during the experimental protocol. Data reported as $\bar{x} \pm SD$. 
APPENDIX J

CARBOHYDRATE AND FLUID INGESTION DURING VARIABLE-INTENSITY TREADMILL RUNNING IN 30°C

Introduction

The aim of this pilot investigation was to ascertain if any differences can be detected in the thermoregulatory responses, subjective ratings or substrate utilisation between a 6% carbohydrate-electrolyte solution (CES) and a flavoured water (FW) during a variable-intensity treadmill running protocol. The additional purpose of the study was to assess the feasibility of using a motorised treadmill protocol to simulate the demands of prolonged high-intensity intermittent running.

Methods

Fifteen elite, competitive endurance runners aged 24 ± 1.4 years with a mean body mass of 68.2 ± 1.5 kg and a mean maximal oxygen uptake value of 70 ± 2 ml·kg⁻¹·min⁻¹ took part in the study. All subjects were fully informed of the demands and possible risks associated with participation in this study, and their right to withdraw their involvement at any time. This study was approved by the Loughborough University Ethical Advisory Committee.

Preliminary Measurements

Maximal oxygen uptake (\(\text{VO}_2\max\)) was determined using an uphill incremental running test to exhaustion (Williams et al., 1990). Subjects were familiarised with the treadmill activity pattern during 45 min of treadmill running in temperate ambient conditions. This preliminary run was also used to confirm and adjust the treadmill velocities in order to elicit the required oxygen uptakes.

Main Trials

The subjects reported to the laboratory on the morning of each trial following a 10-hour overnight fast. They were required to refrain from heavy exhaustive exercise.
over a 3 day period prior to each trial. Subjects voided and recorded nude body mass before a rectal probe was inserted (Grant instruments Ltd, Cambridge, U.K.). Subjects were then moved into the controlled environment where skin thermistors (Grant instruments Ltd, Cambridge, U.K.) were attached at four sites (Mitchell and Wyndham, 1969). Subjects then ingested 6.5 ml·kg⁻¹ of test drink and after standing for 10 min resting rectal and skin temperatures were measured and recorded and then logged every 30 s throughout the protocol (SQ800, Grant Instruments Ltd, Cambridge, U.K.).

The variable intensity treadmill running protocol involved running for two minute periods at alternating velocities equivalent to 40, 60 & 80 percent of $\dot{V}O_2 \max$. A 60 s period of exercise at 60% $\dot{V}O_2 \max$ was included in each 15 minute block of exercise in order to collect 60 second expired air samples using a Douglas bag (Williams et al., 1990). Every 15 min a rest period consisting of two minutes of walking (4 km·h⁻¹) was performed, at which time 3.5 ml·kg⁻¹ fluid was ingested. Ambient temperature and humidity were maintained using electric fan heaters and a high capacity dehumidifier, the environmental conditions remained essentially constant, mean environmental temperature was 30.3 ± 0.3 °C and relative humidity was 47.6 ± 2.9%.

Results

There was a trend toward a higher basal rectal temperature ($T_{rec}$) when the CES was ingested, however this did not reach statistical significance ($P=0.21$). A main effect of test drink was detected during exercise ($F_{1,14} = 8.253 \ (P=0.012)$), post-hoc analysis reveals that $T_{rec}$ during the CES trial was significantly elevated at 30 and 50 min compared with the FW (Figure 1). There were no statistically significant differences in weighted mean skin temperature between fluid conditions (Figure 2) and total body heat content at the end of exercise was 8.92 ± 0.75 MJ and 8.87 ± 0.84 MJ in the CES and FW trials respectively.

No bias was found between solutions in any of the measurements calculated via indirect calorimetry (Table 1). There was a time effect apparent in $\dot{V}O_2$, $\dot{V}CO_2$ and energy expenditure which all increased significantly during the protocol, but RER and
substrate oxidation rates remained constant throughout the protocol. Mean carbohydrate oxidation rate was 124 ± 39 g·h⁻¹ and 112 ± 25 g·h⁻¹ in the CES and FW trials respectively and the rate of fat oxidation was 28 ± 34 g·h⁻¹ and 34 ± 13 g·h⁻¹ for these trials.

There were no differences between trials in the heart rate response to exercise (P=0.477), which increased significantly over time in both instances (Figure 3). The volume of sweat lost during the protocol was also similar, 1.8 ± 0.1 l and 1.9 ± 0.2 l in the CES and FW trials respectively. The subjective ratings shown in Table 2 were alike between fluid conditions, a significant rise in RPE and thermal sensation was found over the final 45 min of exercise.
Figure 1  Rectal temperature (\( \bar{x} \pm SD; ^\circ C \)) during the 60 min treadmill running protocol, under the carbohydrate (CES) and flavoured water (FW) fluid conditions.

Figure 2  Weighted mean skin temperature (\( \bar{x} \pm SD; ^\circ C \)) for the carbohydrate (CES) and flavoured water (FW) trials during the exercise protocol.
Figure 3 Mean heart rate ($\bar{x} \pm SD$; beats·min$^{-1}$) during variable-intensity treadmill running for the CES and FW test drinks.

Table 1 Oxygen uptake ($\dot{\text{V}}\text{O}_2$), carbon dioxide production ($\dot{\text{V}}\text{CO}_2$), respiratory exchange ratio (RER) and energy expenditure samples every 15 min of exercise. Table reports mean ± SD, † denotes P<0.05; vs. first 30 min of exercise.
Table 2  Ratings of perceived exertion (RPE), thermal sensation, thirst and gut fullness every 15 min of exercise. † P<0.05; vs. 15 min time point.

Discussion

The main finding of this study is that $T_{rec}$ was significantly elevated during the variable-intensity treadmill running protocol when the consumption of a 6% CES was compared with a FW.

The differences in $T_{rec}$ between solutions are of a comparable magnitude to those reported by Hall et al. (2005) using a similar protocol with children. The discrepancy between solutions is most apparent during the transition phases between low and high intensity exercise. This bias reaches statistical significance after 30 minutes of exercise, at a time when fluid loss is likely to be only moderate. Nevertheless it is possible that the energy content of the CES resulted in a slower rate of gastric emptying during this period, or the splanchnic region received an increased fraction of total blood volume following the ingestion of the CES. If this was the case then the percentage of cardiac output available to dissipate heat would be less in the CES trial. However, there were no differences in skin temperature or the magnitude of fluid lost as sweat between trials to support these proposed differences in heat dissipation.
Although not significant there was a trend toward an elevation in $T_{rec}$ from the start of the exercise protocol. This bias remained relatively constant during the protocol and the rate of change in $T_{rec}$ was alike in both trials. It may be possible that in the 10 min postprandial period before exercise, the energy content of the CES induced a thermic effect of feeding that was detectable as a change in $T_{rec}$.

The amount of energy expended was also comparable between solutions and no differences were detected in substrate oxidation rates over the four gas collection periods. But it remains a possibility that during certain phases of the protocol subjects expended differing amounts of energy and hence exhibited a greater metabolic heat production when ingesting the CES.

The physiological variables measured during this study are comparable to mean values seen during prolonged high-intensity intermittent exercise in the heat. This protocol therefore appears to be a suitable laboratory simulation of these activities, but lacks the supra-maximal sprinting components or the frequent changes in direction seen in these sports.

References


