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Carbohydrate versus caffeinated-carbohydrate beverages: effects on exercise performance and hydration status at rest and during exercise.

Mary Beades

Thesis submitted for the award of PhD. to the Department of Physiology,

Trinity College Dublin

May 2007

Abstract

Carbohydrate versus caffeinated-carbohydrate beverages: effects on exercise performance and hydration status at rest and during exercise.

The consumption of sports drinks to enhance athletic performance had been the subject of extensive research for many years. Critical areas of interest are the volume and timing of fluid ingestion and the source and concentration of carbohydrate (Coombes and Hamilton, 2000). In addition, the use of caffeine to enhance exercise performance has been widely researched (Graham and Spriet, 1995; Cox *et al.*, 2002; Kovacs *et al.*, 1998). However, the use of a rehydrating beverage containing caffeine is not recommended because of the proposed diuresis associated with caffeine. Scientific research investigating commercially available sports drinks and possible benefits associated with the addition of caffeine to carbohydrate drinks is limited. Therefore the principal aims of the research described in this dissertation were to investigate the effect of carbohydrate and caffeinated carbohydrate ingestion on exercise performance at various intensities and on rehydration following exercise induced hypohydration and voluntary fluid restriction.

In the research described in Chapter 3 of this dissertation subjects ingested either Lucozade Sport, Red Bull or water (9.37 $ml.kg^{-1}$, equivalent to 3 $mg.kg^{-1}$ caffeine) prior to an endurance cycling trial to failure; 60 min at a load equivalent to 65% $\dot{V}O_2max$, 30 min at a load equivalent to 85% $\dot{V}O_2max$ followed by intermittent sprints to failure. To assess rehydration following exercise a further 50% of the pre-exercise volume was ingested 30 min post-failure and subjects were monitored over a 60 min rehydration phase. The principal finding of this study was that exercise time to failure was similar across all three trials; in addition plasma volume changes and plasma osmolality were similar following rehydration across all three drinks, suggesting that Red Bull despite the presence of caffeine did not negatively affect rehydration.

The second study (Chapter 4) investigated the effect of Lucozade Sport, isoRB (Red bull diluted 60:40 with water) and water ingested 20 min pre-exercise (4.3 $ml.kg^{-1}$) and every 10 min (1.4 $ml.kg^{-1}$) during intermittent exercise on cycling time to failure. Intermittent exercise performance was significantly improved following Lucozade Sport (26%) and isoRB (29%) ingestion compared to water. However, no significant difference in exercise performance was recorded comparing Lucozade Sport and isoRB (72.8±6.4 vs. 76.7±9.4 min).

Previous research, examining the effect of caffeine on exercise performance has predominately administered caffeine in capsular form. In order to allow for a direct comparison of the two exercise studies detailed above with available scientific research the

pharmacokinetics of caffeine when ingested at rest in capsular or liquid form was investigated (Chapter 5). The results of this study suggest that the rate of appearance of caffeine is faster when ingested in liquid compared to capsular form. In addition, the concentration of non-esterified fatty acids differed significantly between the two caffeine (3 $mg.kg^{-1}$) administration regimes. When isoRB was ingested the concentration of NEFA was significantly lower compared with the capsular trial, possibly due to inhibition of lipolysis associated with the presence of carbohydrate in the isoRB formulation.

Finally the area of rehydration following 30 hr of voluntary fluid restriction inducing a mild hypohydrated state was investigated. Lucozade Sport, isoRB and water were ingested in a volume equivalent to 150% body mass loss, mean body mass loss was approximately 1.7%. Hydration status was assessed by multiple markers and had returned to euhydrated levels within 2 hr. Restoration of euhydration was similar across all 3 trials, suggesting that caffeine ($3mg.kg^{-1}$) does not negatively affect hydration status in hypohydrated individuals at rest. Instead isoRB ingestion appeared to aid rehydration in a manner similar to both Lucozade Sport.

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I declare that this thesis is all my own work and has/will not be submitted to this or any other university as part of any other post graduate programme. I grant permission to the Trinity College librarian to lend or copy this thesis in part on request.

Signature Mary Beade May 2007.

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ABBREVIATIONS

µg	Microgram
ACOD	Acyl-CoA oxidase
ADH	Anti Diuretic Hormone
ADP	Adenosine Diphosphate
ANOVA	Analysis of Variance
ATP	Adenosine Triphosphate
BIA	Bioelectrical Impedance Analysis
Bla	Blood Lactate
BMI	Body Mass Index
C	Carbon
Ca^{2+}	Calcium
cAMP	cyclic Adenosine Monophosphate
CES	Carbohydrate Electrolyte Solution
CHO	Carbohydrate
Cl^-	Chloride
CO_2	Carbon dioxide
DW	Dry Weight
ECF	Extracellular fluid
ECG	Electrocardiogram
ECW	Extracellular water
Ex	Exhaustion
$FABP_{pm}$	Plasma membrane Fatty Acid Binding Protein
FAD	Flavin Adenine Dinucletide
FER	Forced Expiratory Rate
FEV_1	Forced Expiratory Volume in one second
FFA	Free Fatty Acid
FVC	Forced Vital Capacity
g	Gram
GFR	Glomerular Filtration Rate
GLUT	Glucose Transporter
H^+	Hydrogen ion
Hct	Haematocrit
Hb	Haemoglobin
hr	Hour
ICF	Intracellular Fluid
ICW	Intracellular water
IOC	International Olympic Committee
IR	Insulin Receptor
IRS	Insulin Receptor Substrate
isoRB	Isocaloric Red Bull (Red Bull diluted 60:40 with water)
K^+	Potassium ion
kg	Kilogram
kHz	Kilo Hertz
kJ	Kilojoule
l	Litre
LCFA	Long Chain Fatty Acid
LS	Lucozade Sport
LVEDV	Left Ventricular End Diastolic Volume
LVESV	Left Ventricular End Systolic Volume
m	Metre

MCFA	Medium chain fatty acid
mg	Milligram
Mg^{2+}	Magnesium ion
min	Minute
mL	Millilitre
mmol	Millimole
mOsm	Milliosmol
Na^{+}	Sodium ion
NAD	Nictinamide Adenine Dinucleotide
NEFA	Non Esterified Fatty Acid
ng	Nanogram
nm	Nanomertre
O_2	Oxygen
P	Probability
PC	Phosphocreatine
PF	Peak Expiratory Flow
Pi	Inorganic Phosphate
PL	Placebo
POD	Peroxidase
PV	Plasma Volume
QC	Quality Control
RB	Red Bull®
RBC	Red Blood Cell Count
RER	Respiratory Exchange Ratio
RH	Rehydration
s	Second
SD	Standard Deviation
SEM	Standard Error of the Mean
TBW	Total body Water
TCA	Tricarboxylic Acid
TG	Triglycerol
TTE	Time to Exhaustion
Usg	Urine specific gravity
Uosm	Urine osmolality
V_A	Diastolic Inflow Velocity
$\dot{V}_E$	Minute Ventilation
$\dot{V}_{O_2}$	Oxygen uptake
$\dot{V}_{O_2}max$	Maximal Oxygen uptake
W	Watt
WBC	White Blood Cell Count
yr	Year

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Chapter 1

Introduction

1.1 The metabolic system

There are 3 physiological systems that operate in the body to provide energy for all cellular functions both at rest and during exercise. Two of these systems, namely the ATP-Phosphogen system and the glycolytic energy system, function without the presence of oxygen and so are termed anaerobic energy systems. On the other hand the third energy system, oxidative phosphorylation, encompasses aerobic pathways.

The principle outcome of all 3 systems is the production of Adenosine Triphosphate (ATP), the energy component that governs all cellular functions. The ATP molecule contains 3 inorganic phosphate groups (Pi) that are attached to an adenosine molecule, formed by the phosphorylation of ADP (adenosine diphosphate). When a phosphate group is removed from this ATP molecule through hydrolysis by the action of the ATPase enzyme, a large amount of energy is released as well as the by-products, ADP and Pi which are then usually recycled in one of the 3 energy systems.

The 3 energy systems will now be described briefly with later reference to their importance during exercise of varying intensity and duration.

1.1.1 ATP-Phosphogen system

This anaerobic system can also be termed the ATP-Phosphocreatine (PC) system. ATP and PC are stored within muscle and as such are available for immediate use. However, only a limited amount of ATP and PC are available within muscle. PC contains a phosphate group attached to a creatine molecule by a high energy bond. When a phosphate group is removed from the creatine molecule, through the actions of creatine kinase, it can then combine with ADP to form ATP.

Role of the ATP- Phosphogen system during exercise

The ATP-PC system has a large power capacity (9 mmol $ATP.kg^{-1}.s^{-1}$), providing a large amount of energy quickly. This short time is due to the limited ATP and PC muscle stores. Therefore, this system is ideal for the supply of energy during short duration high intensity exercise such as sprinting or track-cycling. The ATP-PC system only has the capacity to generate sufficient ATP to sustain maximal exercise for 10 to 30 seconds (Hoffmann, 2002). When the levels of PC decline during maximal exercise the rate of anaerobic energy production cannot be sustained and this contributes to the decline in power output observed during all-out exercise (Hargreaves, 2006).

1.1.2 Glycolytic Energy system

Glycolysis is the process whereby glucose is converted to pyruvic acid within the cytosol, resulting in the net production of 2 or 3 ATP molecules depending on the origin of the

glucose molecule. Similar to the ATP-Phosphogen system glycolysis is an anaerobic process. Glucose metabolism during glycolysis can be sourced from the circulation or from glycogen stores in liver or muscle.

Glycogen can be converted to glucose by gluconeogenesis. Glycogen is metabolised into glucose-1-phosphate in the presence of phosphorlyase and then further broken down to glucose-6-phosphate. When glucose is phosphorylated it does not have the ability to diffuse from the liver or muscle cells into the circulation. However an enzyme, phosphatase, present only in the liver dephosphorylates glucose allowing the glucose molecule be transported into the circulation and to where it is needed as a fuel source.

Glycolysis is a multi-step process (see Figure 1.1), the details of which are beyond the scope of this review. In summary, 3 ATP molecules are formed when stored glycogen is used as a substrate whereas 2 ATP molecules are produced when glucose from the circulation is used. Pyruvic acid formed is reduced to lactic acid at the end stage of this process.

Role of the glycolytic energy system during exercise

Glycolysis is capable or producing a greater amount of energy (190-300 mmol ATP.kg^{-1}dry weight) compared to ATP-PC system (55-95 mmol ATP.kg^{-1}dry weight) but cannot produce as much energy per unit time (4.5 mmol ATP.kg.s^{-1}). Therefore anaerobic glycolysis is the primary energy source for high intensity exercise lasting 1-3 min. During high intensity exercise where glycolysis is the principle energy provider the concentration of lactic acid increases and muscle pH decreases.

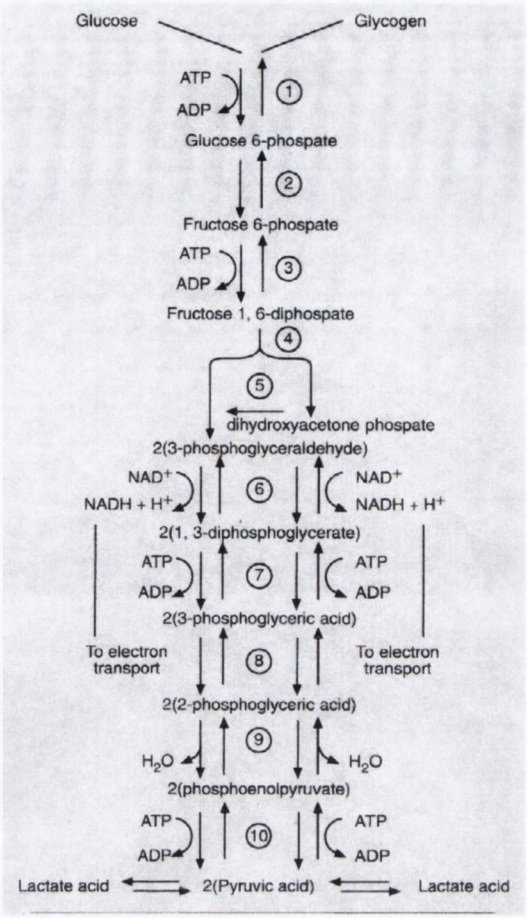


Figure 1.1: Stages of glycolysis (adapted from Physiological Aspects of Sport Training and Performance, Hoffman, 2002).

1.1.3 Oxidative phosphorylation

Oxidative phosphorylation begins with glycolysis producing pyruvic acid from the breakdown of glucose. Pyruvic acid produced in the cytosol is then converted into Acetyl Co-enzyme A and transported into the mitochondria. This molecule then enters the Tricarboxylic Acid Cycle (TCA) and electron transport chain. Carbon dioxide (expired later by the lungs) and H^+ is produced during the TCA cycle. The hydrogen combines with co-enzymes nicotinamide adenine dinucleotide (NAD) and flavin adenine dinucleotide (FAD) and these 2 molecules are then transported from the cell cytoplasm to the mitochondria where they enter the electron transport chain. The hydrogen atoms involved in the electron transport chain are split into protons and electrons. The protons combine with oxygen to form water and the electrons pass through a series of reactions that phosphorylate ADP to form ATP in oxidative phosphorylation (Hoffman, 2002). Carbohydrates, fat and proteins may all act as substrates for oxidative metabolism. One molecule of glycogen produces a net gain of approximately 33 ATP during oxidative metabolism.

Role of the glycolytic energy system during exercise

During the process of glycolysis ATP is produced in much larger quantities but at a slower rate than the previously described anaerobic systems. It also differs from the phosphogen system and anaerobic glycolysis because energy is produced in the presence of oxygen within the mitochondria of cells. The oxidative production of ATP cannot provide energy at a rate sufficient to sustain high intensity exercise but can provide sufficient energy for prolonged sub-maximal exercise, making it the principle energy system for long duration aerobic activities.

1.1.4 Carbohydrate substrates for exercise metabolism/ muscle glucose uptake

As noted above muscle glycogen plays an important role as a substrate in exercise metabolism. Muscle glycogen levels decline as exercise intensity and duration increase. It is at this point that blood glucose becomes the prominent carbohydrate source. Blood glucose can be derived from the liver or from exogenous carbohydrate stores following ingestion. The uptake of glucose into the exercising muscle is exercise intensity and duration dependent. Muscle glucose uptake is increased due to increased sarcolemmal glucose transport as a result of:

- translocation of GLUT 4 glucose transporters to the plasma membrane
- activation of the metabolic pathways responsible for glucose metabolism
- enhanced glucose delivery due to increased muscle blood flow (Burke and Deakin, 2006).

The transport of glucose across the sarcolema is thought to be the rate limiting step in the process of glucose uptake (Richter *et al.*, 2001). While the GLUT category of transporters contains 6 isoforms, GLUT 4 is thought to be the principle transporter that is influenced by exercise (Tremblay *et al.*, 2003). The rate of glucose transport can be increased by the recruitment of GLUT 4 from intracellular storage vesicles during exercise (Tremblay *et al.*, 2003). The movement of glucose via these transporters into the muscle cells is by a process of facilitated diffusion. The detailed biochemical pathways involved in the translocation of GLUT 4 transporters are beyond the scope of this report, however it is important to point out that insulin does play a role in glucose uptake. Insulin is a hormone produced in the pancreas and is increased in response to carbohydrate feeding. It stimulates the uptake of glucose into muscle and adipose tissue and together with glucagon is responsible for glucose homeostasis (Akiba *et al.*, 2004). It is unclear whether or not exercise and insulin similarly activate the intracellular stores of the GLUT 4 transporter.

The role of insulin during glucose uptake will be revisited later in relation to caffeine and the proposed inhibitory effects of caffeine on insulin-stimulated glucose uptake (see section 1.6.4).

As the rate of muscle glucose uptake increases during exercise the rate of liver glucose output also increases in an effort to maintain blood glucose levels at or slightly above resting levels. Fatigue during prolonged exercise has been associated with muscle glycogen depletion and or hypoglycaemia- the term used to describe the situation which results when liver glucose output is less than muscle glucose uptake (Coggan and Coyle, 1988). Hypoglycaemia will be detailed later in this chapter.

1.1.5 Muscle glycogen and blood glucose utilisation: exercise and fatigue

Saltin and Karlsson (1971) investigated the rate of muscle glycogen usage at different intensities, namely 50, 70 and 100% $\dot{V}O_2$max. They reported that the rate of muscle glycogen usage increased with increasing exercise intensity, and was 0.7, 1.4 and 3.4 $mmol.kg^{-1}.min^{-1}$ during exercise intensities of 50, 70 and 100% $\dot{V}O_2$max, respectively. Glycogenolysis, the process of glycogen breakdown, was reported to be dependent on the muscle glycogen content. If the muscle glycogen concentration was high at the onset of exercise then the rate of glycogenolysis was faster than if muscle glycogen was low. Therefore, as the concentration decreased with increasing exercise intensity or duration, so did the rate of glycogenolysis. In terms of blood glucose uptake; as exercise intensity and duration increased there was an exponential increase in glucose uptake (Sherman *et al.*, 1981). It has been suggested in a review by Ivy in 1999, that the increase in blood glucose uptake into the muscle at high exercise intensities compensated for the decrease in glycogenolysis. In this way, the carbohydrate requirements for exercise at high intensities could be maintained by slowly shifting from a reliance on muscle glycogen to blood glucose.

Coggan and Coyle (1988) hypothesised that fatigue during prolonged exercise was often due to an inadequate rate of carbohydrate oxidation occurring from insufficient availability of muscle glycogen and/or plasma glucose. Glucose can maintain the carbohydrate needs of the muscle at exercise intensities between 60 and 75% $\dot{V}O_2$max so long as the blood glucose concentration is maintained above 2.5 –3.5 $mmol.l^{-1}$, a range set following studies by Coggan and Coyle in 1987, 1988 and 1989. When the blood glucose concentration fell below this point fatigue occurred shortly afterwards. At higher exercise intensities typically 75- 85% $\dot{V}O_2$max, fatigue was associated with muscle glycogen depletion as blood glucose uptake was too slow to support the carbohydrate needs of the muscle (Coggan and

Coyle, 1991) while accumulation of lactic acid was the principal cause of fatigue at intensities above 85% $\dot{V}O_2max$.

In conclusion, the carbohydrate substrate used during exercise is dependent on the intensity and duration of exercise and the pre-exercise endogenous stores. Following a brief discussion on fat metabolism and a review of body fluid regulation the area of carbohydrate supplementation at rest and in relation to exercise will be reviewed.

1.2 Fat Metabolism

Fat or triglycerides (TG) are stored mainly in adipose tissue and are recognised as one of the main energy sources at rest as well as during low intensity sub-maximal exercise up to 70-75% $\dot{V}O_2max$. Adipose tissue represents the largest energy store in the body, containing 400 MJ of energy compared to only 4-5 MJ as carbohydrate in liver and muscle stores in the average 70 kg male (Bulow, 2004). TG are also stored in the liver but are not mobilized or utilised during exercise (Bulow, 2004).

TG contain 3 free fatty acid (FFA) molecules and 1 molecule of glycerol. To obtain energy from FFA they must firstly be released from TG within adipocytes. This is achieved through lipolysis in the presence of lipo-protein lipase. The free fatty acids are bound to albumin in the circulation and utilised at tissue sites such as the heart, liver or skeletal muscle, where the demands for energy are greatest (Ranallo and Rhodes, 1998). When used by muscle, FFA enter the mitochondria of muscle cells and undergo the process of β-oxidation, which results in the formation of acetyl Co-A. Each Acetyl Co A molecule enters the TCA cycle (similar to oxidative phosphorylation) ultimately producing ATP. The metabolism of 1 FFA molecule produces on average 130 molecules of ATP, considerably more than that available from the oxidation of carbohydrate (Guyton and Hall, 1996).

The role of Fats during exercise

A review of fats by Turcotte in 1999 outlined the contribution of circulating plasma TG and FFA and muscle TG during exercise. The contribution of fats as a fuel source during exercise is dependent on exercise intensity, duration and training and dietary status. The rate of adipose tissue lipolysis increases at the onset of low to moderate intensity exercise, and appears to increase with increasing exercise intensity. However, if exercise intensity continues to rise the rate of lipolysis begins to decline (Wolfe *et al.*, 1990).

During prolonged exercise of moderate intensity there is a progressive change from carbohydrate metabolism to lipid metabolism. The rate of appearance and concentration of FFA are lower during exercise at an intensity of 85% $\dot{V}O_2max$ compared to 65% $\dot{V}O_2max$ (Romijn *et al.*, 1993). The use of FFA by muscle during exercise is regulated in part by the exercise-induced increase in plasma FFA availability. As exercise intensity increases from moderate to high the rate of FFA mobilization from adipose tissue decreases resulting in a reduced delivery to the exercising muscle and a shift to a reliance on carbohydrate metabolism. Together with plasma FFA availability, FFA transport from the plasma to the mitochondria and intracellular metabolism is also critical to the rate of lipid metabolism in muscle during exercise (Turcotte, 1999). The details and hormonal regulation of these 2

stages in lipid utilisation will not be discussed in detail, however, it is important to mention that lipid metabolism can be affected by at least 7 hormones. Adrenaline and noradrenaline, released by the adrenal medulla during heavy exercise directly activate lipoprotein lipase increasing the rate of lipolysis in adipose tissue. Insulin is a potent inhibitor of lipolysis. Insulin levels are increased in the presence of carbohydrate promoting glucose uptake and increasing fat storage. However, in the absence of carbohydrate the subsequent decrease in insulin decreases fat storage and increases lipolysis. Ingestion of carbohydrate prior to exercise has been reported to decrease lipid oxidation (Horwitz *et al.*, 1997).

1.3 Body composition

Body composition refers to the proportion of the body mass that is fat and the proportion that is lean tissue (Hoffman, 2002). Methods for assessing body composition include hydrostatic weighing, skinfold measurements and bioelectrical impedance analysis.

1.3.1 Fluid compartments and body fluid regulation

Approximately 63.3% of the body mass of an average male (70 kg) is water and is a critical component of the body as it is used to facilitate all chemical reactions and dissipate heat, transport nutrients and remove metabolic waste. Total body water (TBW, approximately 42 l) is divided into 2 compartments; extracellular fluid (ECF) and intracellular fluid (ICF) in an approximate ratio of one-third to two thirds, respectively. The ECF, all fluid outside of cells including interstitial fluid and blood plasma comprises about 24.9% of body mass (Armstrong, 2005). The ECF contains sodium (140 mM), chloride (110 mM) and bicarbonate (26 mM) ions, sodium is readily exchangeable between the interstitial fluid and the plasma. While all fluids contain a certain level of oxygen and carbon dioxide, the plasma contains oxygen to be transported to all necessary organs, glucose, fatty acids and amino acids. Carbon dioxide being transported from the cells back to the lungs and other cellular products transported to the kidneys for excretion or reabsorption are also present in the plasma. The ICF is the fluid within the tissue cells which makes up about 38.4% of body mass and contains many ions, the principal ones being potassium, magnesium and phosphate ions (Armstrong, 2005).

The transport of the ECF through all parts of the body occurs in 2 stages:

1. The constant movement of blood through the circulatory system
2. The movement of fluid between capillaries and cells.

As blood passes through capillaries a continual exchange of fluid occurs across the permeable membrane by a process of diffusion mediated by blood pressure changes. As a result the ECF everywhere in the body, that of the plasma and interstitial fluid, is continuously being mixed thereby maintaining almost complete homogeneity throughout the body.

Maintaining a constant volume and stable composition of the extra- and intracellular fluid compartments is essential for homeostasis and the kidneys play a major role in this area, this will be discussed in greater detail later in this section. Even at rest fluid intake can be very variable and intake must be carefully matched with output to maintain homeostatic conditions.

1.3.2 Movement between ECF and ICF

Substances that can readily pass across the capillary endothelium are exchanged between the plasma and the interstitial fluid. The capillary endothelium allows most solutes (excluding proteins) of the ECF to pass between individual endothelial cells by diffusion, bulk flow or active transport. Therefore the composition of interstitial fluid and plasma is similar but with a greater protein concentration in the plasma. The distribution of ECF between the plasma and interstitial fluid is controlled by hydrostatic and colloid osmotic forces (oncotic pressure) across the permeable capillary membrane. Oncotic pressure occurs because of the presence of dissolved protein in greater concentrations in the plasma compared to the interstitial fluid thereby exerting osmotic pressure at the cell membrane. In plasma, the oncotic pressure is only about 0.5% of the total osmotic pressure. This may be a small percent but oncotic pressure is extremely important in transcapillary fluid dynamics. The ICF is separated from the ECF by a selective cell membrane, highly permeable to water but not to electrolytes. The distribution of water between these 2 compartments is determined by the osmotic effect of sodium chloride and other electrolytes that are not evenly distributed between these compartments. Osmosis is the movement of water from an area of low solute concentration (high water concentration) to an area of high solute concentration (low water concentration).

1.3.3 Osmotic equilibrium between the ICF and ECF

The movement of fluids across the cell membrane can occur very rapidly and any differences in the osmolality of the compartments can be corrected very quickly. However, whole body equilibrium may take 30 min to occur following water ingestion because the fluid enters the intestine to be transported by the blood to all the tissues.

- An isotonic fluid is described as one that neither shrinks nor swells a cell. Similar to the cell itself the osmolality of an isotonic fluid is approximately 280 $mOsm.kg^{-1}$ and so water movement is not necessary to achieve equilibrium.
- A hypotonic fluid (a solution with a lower concentration of solute compared to the cell) will result in the movement of water from the ECF into the cell causing it to swell.
- In contrast the presence of a hypertonic solution, with a higher concentration of solute compared to the cell will cause the loss of water from the ICF to the ECF, diluting the hypertonic solution and causing the cell to shrink.

Tonicity will be referred to later in this thesis with reference to commercial sports drinks.

1.3.4 Fluid intake and loss

The principle routes for water intake are food (approximately 30%) and fluid (approximately 60%) ingestion, while water produced during metabolism contributes minimally to the restoration of fluid balance. In contrast water can be lost from the body in many ways (see Table 1.1):

1. Insensible water loss: Water is continuously lost by evaporation from the respiratory tract and the skin. This loss of fluid, can be almost 700 $ml.day^{-1}$ under normal resting conditions, and is termed insensible water loss.
2. Sweating: the rate of sweating can vary greatly across individuals and is largely dependent on physical activity and environmental factors.
3. Loss in faeces: only a small volume of fluid (100 $ml.day^{-1}$) is lost in faeces; however in cases of severe diarrhoea this can be significantly increased.
4. Kidneys: the majority of fluid loss occurs daily through urine excretion. Multiple mechanisms control urine excretion as discussed later. The kidneys must continuously adjust the rate of water and electrolyte excretion to maintain long term fluid balance.

Origins of fluid loss	**Volume ($ml.day^{-1}$)**
Insensible fluid loss	700
Faeces	100
Kidneys	1500
Sweating	Varies depending on activity and environmental factors

Table 1.1: Summary of approximate daily fluid losses.

1.3.5 Factors affecting fluid intake

There are many factors that affect fluid intake; including thirst, hormonal and neurological factors, hypovolemia and cellular dehydration. The temperature, flavour, palatability and carbonation of a beverage can also influence ingestion (Passe *et al.*, 2004). Thirst and hormonal mechanisms, principally involving antidiuretic hormone are responsible for keeping TBW within a narrow range (Bossingham *et al.*, 2005). Thirst is one of the first cues to the body for the need to rehydrate initiating the desire to drink (Casa *et al.*, 2000). Thirst is stimulated by an increase in plasma osmolality, a decrease in plasma volume or a decrease in blood pressure. Fluid regulating hormones are released in response to exercise and increases in plasma osmolality. These hormones include renin, angiotensin II, aldosterone, atrial natriuretic hormone (ANP) and antidiuretic hormone (ADH) and promote the absorption of water and the active uptake of solutes by the kidneys (Ray *et al.*, 1998).

It has been reported that following consumption of a beverage the initial alleviation of thirst occurs prior to the fluid entering the digestive organs and being absorbed. Therefore, it is thought that other factors may influence the volume of fluid intake along with increasing osmolality and decreasing extracellular volume. Shirreffs and Maughan (2000) in a review of fluid balance during recovery suggested that receptors in the mouth, oesophagus and stomach may play a role in the regulation of fluid volume ingested. In addition, Carter *et al.* (2004) reported a significant improvement in exercise performance following administration of a carbohydrate mouth rinse when compared to a water placebo rinse. While the area of carbohydrate receptors in the mouth warrants further investigation the authors suggested that the increase in performance may be the result of an increase in central drive or motivation rather that a metabolic cause.

1.3.6 Osmolality and the thirst mechanism

Normal serum osmolality is in the range 285-290 $mOsm.kg^{-1}$. This narrow range is maintained through the regulation of water excretion by the kidneys, which is principally achieved by ADH and the sensation of thirst. The principal thirst control centres are in the hypothalamus where a precursor molecule for ADH is synthesised, packaged into granules and transported to the posterior pituitary gland (Lord, 1999). As the precursor molecule is being transported along the axons it is cleaved into 3 peptides, one of which is ADH. An increase in osmolality is detected by osmoreceptors located in the hypothalamus, stimulating the secretion of ADH. A 1% change in osmolality will result in the release of ADH. When plasma osmolality decreases to 280 $mOsm.kg^{-1}$ ADH release is suppressed.

The site of action for ADH is the renal nephrons, where it binds to V2 receptors in the collecting ducts. This binding promotes the insertion of vesicles containing aquaporin 2 into the luminal membrane and therefore increases the permeability of the membrane to water. The resulting increased water absorption by the kidney lowers plasma osmolality. Marked hypovolemia can also result in the increased release of ADH (Lord, 1999).

1.3.7 The importance of the kidneys in the regulation of body fluids.

The excretion of water and electrolytes by the kidneys is generally matched to intake. There are 3 stages in the formation of urine;

1. glomerular filtration
2. tubular reabsorption
3. tubular secretion.

- Glomerular filtration

Glomerular filtration is the first step in the process of urine formation by the kidney. Most substances in the plasma except proteins filter from the glomerular capillaries into the Bowman's capsule. As this filtered fluid moves from the Bowman's capsule to the proximal tubule of the nephron, reabsorption of water and other solutes, primarily sodium occurs. Also secretion of substances from the peritubular capillaries into the tubules can occur.

The glomerular filtration rate (GFR) is defined as the volume of filtrate produced by the kidneys per minute.

Factors affecting GFR:

Vasoconstriction or dilation of the afferent arterioles supplying the millions of nephrons in the kidneys affects blood flow and therefore GFR. The 2 main regulatory mechanisms are sympathetic nerve innervation and renal autoregulation of renal blood flow and therefore GFR.

Renal excretion of water and solutes is therefore controlled by autoregulation. Sympathetic nerve activity that occurs during stress or exercise results in constriction of afferent arterioles delivering blood to the Bowman's capsule. As a result blood volume is preserved and may be diverted to skeletal muscle and heart. GFR is reduced as is the rate of urine formation. Changes in blood pressure can cause local changes at the site of the afferent arterioles to regulate urine output. If blood pressure decreases the afferent arterioles dilate whereas an increase in blood pressure will cause the arterioles to constrict. In this way GFR can remain relatively constant. Autoregulation also occurs through a negative feedback loop involving the afferent arterioles and the volume of fluid in the filtrate. The

afferent arterioles have the ability to constrict when the flow of filtrate is increased, thereby lowering GFR and decreasing the volume of filtrate.

- Reabsorption

A large proportion (65%) of the salt and water that is filtered by the kidney is reabsorbed in the proximal tubule. Reabsorption of salt and water also occurs through the descending limb of the loop of Henle regardless of the hydration status of the individual. Therefore, approximately 85% of the water and salt in the original filtrate is reabsorbed independent of any endocrine influence. At the distal convoluted tubule a large volume of filtrate still remains and is reabsorbed in accordance with the body's hydration state. It is at this stage that endocrine factors play a role in the regulation of urine formation, the details of which are summarised in Table 1.2.

- Secretion

Secretion occurs when unwanted materials in the peritubular capillaries are transferred to the renal tubular lumen and excreted from the body in the urine. Tubular secretion occurs mainly by active transport. These substances are present in great excess, or are natural poisons.

Hormone	Stimulus for release	Action
Antidiuretic hormone (ADH)	Increased plasma osmolality	Increased water reabsorption
Aldosterone	Decreased blood volume	Increased Na^+ reabsorption and K^+ secretion
Atrial Natriuretic peptide (ANP)	Increased blood pressure	Increased Na^+ and water secretion

Table 1.2: Hormones involved in urine formation.

Renin-Angiotensin system (RAS)

An increase in plasma [K^+] directly results in the secretion of aldosterone, but low blood [Na^+] acts indirectly via the RAS to stimulate aldosterone secretion and the resulting reabsorption of Na^+. In summary renin, secreted from the afferent arterioles in response to a decrease in blood volume and pressure, catalyses the conversion of angiotensinogen to angiotensin I. Angiotensin I is converted into the polypeptide angiotensin II which stimulates the adrenal cortex to secrete aldosterone. Through a negative feedback system an increase in aldosterone secretion results in less Na^+ excreted in the urine.

1.3.8 The role of the digestive system in fluid and electrolyte absorption

A large proportion of the fluid and electrolytes entering the gastrointestinal tract are absorbed by the small intestine. Approximately 100 $ml.day^{-1}$ is excreted in the faeces. Water is absorbed passively in the intestine via the osmotic gradient set up by the active transport of electrolytes.

The importance of these mechanisms in achieving homeostasis and fluid balance will be further highlighted later in this chapter during the review of rehydration.

1.4 Carbohydrate Supplementation

Fluid balance and optimal nutrition before, during and after exercise has been an area of extensive scientific interest for many years. Multiple associations have published recommendations on nutritional preparation for training and competition, including the American College of Sports Medicine and the American Dietetic Association who published a joint position statement on nutrition and athletic performance in 2000. In this document it was recommended to ingest 400-600 ml of fluid 2-3 hr before exercise to optimise hydration status prior to exercise. Despite these recommendations debate exists in the literature with respect to the timing and quantity of carbohydrate supplementation before exercise, as every research group and sports drink manufacturer works to produce the beverage most suitable for athletes.

1.4.1 Hypoglycemia

Carbohydrate consumption during the hours immediately before exercise increases circulating plasma glucose and insulin concentrations approximately 30 min after ingestion (Moseley *et al.*, 2003). At rest insulin increases glucose uptake into muscle. When exercise begins insulin production is reduced yet blood glucose uptake into muscle is increased. This may seem contradictory with respect to the role of insulin in glucose uptake but during exercise while the rate of insulin production is reduced the efficiency of uptake is increased. Due to the exercise-induced increase in glucose uptake into the exercising muscles and the presence of circulating insulin, the rate of disappearance of blood glucose exceeds the rate of appearance and a fall in blood glucose concentration is observed. Sometimes the decline may temporarily result in blood glucose falling below 3.5 $mmol.l^{-1}$ before returning to normal euglycaemic levels, this condition is referred to as transient or rebound hypoglycaemia (De Marco *et al.*, 1999). Also when plasma insulin is high the concentration of plasma free fatty acids declines due to insulin induced inhibition of lipolysis resulting in a greater reliance on muscle glycogen compared to exercising when fasted (Coyle *et al.*, 1985). A study by Costill *et al.* (1977) investigated the effects of glucose ingestion (75 g) 30-45 min prior to exercise (70-75% $\dot{V}O_2max$) on plasma glucose and insulin concentrations. Both variables were reported to be elevated at the start of exercise resulting in a faster rate of muscle glycogen usage during exercise. The aim of carbohydrate feeding is to spare muscle glycogen, so with the opposite occurring in this study performance was decreased. Most subjects participating experienced hypoglycaemia during exercise in that study although it is no longer thought to be so common (see below). The quantitative definition of hypoglycaemia in the literature varies slightly, in some cases

a blood glucose concentration of less than 2.5 mmol.l^{-1} is used while in others a higher concentration of 3.5 mmol.l^{-1} is used (Jentjens *et al.*, 2003). Recent research by Jentjens *et al.*, Moseley *et al.*, Achten and Jeukendrup (all 2003) has defined rebound hypoglycaemia as plasma glucose concentration below 3.5 mmol.l^{-1} and this same cut off value will be used in relation to the research performed as part of this dissertation.

1.4.2 Carbohydrate supplementation prior to prolonged exercise

Studies cited by Coombes and Hamilton (2000) and more recent work performed by Jeukendrup and colleagues in the University of Birmingham (Jentjens *et al.*, 2003, Moseley *et al.*, 2003, Achten and Jeukendrup, 2003) have shown that responses to pre-exercise glucose feeding can be very variable with not all subjects showing a hypoglycaemic response early in exercise. In addition, the majority of studies reported no change or an improvement in exercise performance following carbohydrate feeding pre-exercise.

The 5 factors listed below adapted from a review of sports drinks by Coombes and Hamilton (2000) are likely to influence the hyperinsulaemic response to pre-exercise carbohydrate ingestion;

(i) The timing of carbohydrate ingestion

(ii) The amount of carbohydrate consumed

(iii) The extent of muscle glycogen synthesis following consumption

(iv) Individual variations with respect to the hyperinsulaemic response

(v) The change in the rate of muscle glycogen depletion during exercise.

In an effort to clarify the area of rebound hypoglycaemia, pre-exercise carbohydrate feeding and the influence of some of the above listed variables, Moseley *et al.* (2003) investigated the effect of carbohydrate feeding (75 g glucose) ingested either 15, 45 or 75 min prior to 20 min cycling exercise at 65% max power output on metabolism and subsequent time trial performance. Immediately pre-exercise blood glucose was significantly higher when carbohydrate was ingested 15 min pre-exercise compared with 45 and 75 min. Plasma insulin concentrations were also significantly higher when carbohydrate was ingested 15 min before exercise compared to 75 min, this difference had disappeared after 10 min of steady state exercise. Out of the 8 subjects participating in the study, 2 subjects became hypoglycaemic during the trials when exercise commenced 15 min after carbohydrate ingestion where 3 and 5 subjects were transiently hypoglycaemic in the 45 and 75 min trials, respectively. Group mean data for each trial did not report blood glucose concentration below 3.5 mmol.l^{-1}. The authors suggested that for some individuals

there may be a threshold of insulin exposure and above this threshold the likelihood of the occurrence of hypoglycaemia is increased. Interestingly, in the time trial following the 20 min sub-maximal exercise no significant improvement in performance was observed across these trials and those individuals who had experienced rebound hypoglycaemia did not perform any differently to those who had not. Therefore, this study reported that the timing of ingestion pre-exercise may not affect performance.

Jentjens *et al.* (2003) investigated the effects of altering the amount of carbohydrate ingested (0, 25, 75, 200 g) 45 min before the same exercise protocol as above on metabolism and performance. Following glucose ingestion pre-exercise (25, 75 and 200 g) rebound hypoglycaemia was observed in 6 subjects during the steady state exercise. There was no significant improvement in subsequent time trial performance during any of the 4 trials. The ingestion of 75 g of carbohydrate 45 min before exercise did not result in a further decline in blood glucose concentration compared to the ingestion of 25 g of carbohydrate.

Finally, Achten and Jeukendrup (2003) investigated the effect of exercise intensity on the glycemic and insulinaemic response to carbohydrate feeding pre-exercise. Subjects ingested 75 g of carbohydrate 45 min prior to 20 min of exercise at power outputs equivalent to 55, 77 and 90% $\dot{V}O_2$max. Blood glucose concentration decreased after 5 min of exercise and then stabilised in all trials but was not affected by exercise intensity.

A study by Sherman *et al.* (1989) reported that ingestion of 312 g of carbohydrate 4 hours before 95 min of interval cycling significantly improved performance compared to ingestion of 45 or 156 g of carbohydrate. Wright *et al.* (1991) investigated the effect of carbohydrate feeding before exercise, during exercise or a combination of both on endurance cycling performance at 70% $\dot{V}O_2$max to exhaustion. The greatest increase (44%) in time to exhaustion was observed when carbohydrate was consumed both before- and during-exercise. Time to exhaustion following a single carbohydrate bolus prior to exercise was increased by 17%, with a 19% increase in total work output. In this study a 5 $g.kg^{-1}$ body mass carbohydrate solution was ingested 180 min prior to exercise.

In summary, the above research has reported no link between hypoglycaemia and the timing of ingestion, the quantity of carbohydrate ingested or the exercise intensity. In addition none of the interventions affected time trial performance. The results reported by Moseley *et al.* (2003), Jentjens *et al.* (2003) and Achten and Jeukendrup (2003) are somewhat contradictory to earlier research reporting a positive effect of carbohydrate ingestion pre-exercise on endurance performance (Sherman *et al.*, 1989, Wright *et al.*, 1991). It must also be noted that the amount of carbohydrate ingested during the trial

performed by Sherman *et al.* (1989) was much greater that that consumed during the more recent studies.

It is evident from the above review that the area of carbohydrate ingestion before endurance exercise and its effects on performance is still a topical issue. Some researchers suggest that athletes should avoid carbohydrate intake within the hour before exercise (Jentjens *et al.*, 2003), while others have reported positive effects of pre-exercise ingestion.

1.4.3 Carbohydrate supplementation during prolonged and intermittent exercise

A clear review of the literature on carbohydrate supplementation during exercise is very difficult because of the many variations that exist across the research. It is generally accepted that the use of carbohydrate drinks during exercise is beneficial when the duration of exercise exceeds 2 hr (Jeukendrup, 2004). In addition, studies have observed improvements in performance during exercise at moderate to high intensities (75% $\dot{V}O_2$max) (Jeukendrup *et al.*, 1997, Below *et al.*, 1995, el-Sayed *et al.*, 1997, Coggan and Coyle, 1988). The use of solid or liquid forms of carbohydrate during exercise appears to benefit performance to a similar degree (Lugo *et al.*, 1993). Hargreaves *et al.* (1984) reported a 46% improvement in sprint performance after 4 hr of prolonged cycling following the ingestion of a candy bar (43 g carbohydrate, 9 g fat and 3 g protein). However, during running liquid ingestion of carbohydrate is mostly preferred due to the ease at which the drink can be ingested and tolerated by the digestive system. The volume of fluid ingested voluntarily by an athlete is determined principally by palatability; however, in the majority of cases fluid intake does not match fluid losses when athletes are allowed to drink *ad libitum* (Coombes and Hamilton, 2000). A carbohydrate intake as low as 16 $g.hr^{-1}$ has been reported to improve exercise performance by 14% compared to water (Maughan *et al.*, 1996). In contrast, ingesting carbohydrate above 75 $g.hr^{-1}$ has been reported to be no more effective in improving exercise performance compared to lower dosages (40 to 75 $g.hr^{-1}$). One possible reason for this is that ingestion of carbohydrate in quantities of 40-75 $g.hr^{-1}$ results in optimal availability of carbohydrate and so ingestion of carbohydrate at a higher rate will have no added benefit (Coggan and Swanson, 1992). In addition, at higher rates of ingestion, saturation of the membrane receptors for glucose may occur. Jeukendrup and colleagues have been to the forefront of recent research in the area of oxidation rates of carbohydrate when glucose is ingested in combination with fructose or glucose polymers. Glucose is oxidised at relatively high rates (1 $g.min^{-1}$) (Jeukendrup, 2004). Jentjens and Jeukendrup (2005) reported that the combined ingestion of fructose and glucose results in higher exogenous carbohydrate oxidation rates compared with glucose

ingestion alone (1.14 $g.min^{-1}$). Many of the earlier studies investigating carbohydrate ingestion during exercise were performed on subjects following an overnight fast, more recently however studies have investigated the non-fasted state. Exercising in the fasted state results in a reduction in the availability of endogenous carbohydrate and so it seems logical that the outcomes of such studies would suggest that ingestion of carbohydrate would improve performance because the presence of exogenous carbohydrate would compensate for the reduced endogenous stores. In the 2 exercise studies performed for this thesis overnight fasting was not required, as it was felt that in the majority of cases athletes rarely compete in the fasted state with reduced endogenous carbohydrate stores. Therefore, performing a study with athletes in a fasted state would not be reflective of normal training and competitive behaviour.

Scientific research has primarily focussed on constant cycling and running effort, and only a few studies have investigated the use of carbohydrate feedings during intermittent exercise (Nicholas *et al.*, 1999; Nicholas *et al.*, 1995; Murray e*t al.*, 1987, Winnick *et al.*, 2005). Murray *et al.* (1987) investigated the effects of fluid and carbohydrate feedings during intermittent cycling exercise. Each of the 13 male subjects completed 4 trials, ingesting a different beverage on each occasion; flavoured placebo (WP), 5.0% glucose polymer (GP), 6.0% carbohydrate solution (4% glucose, 2% sucrose) and electrolytes (SG), and a 7.0% carbohydrate solution (5.0% glucose polymer, 2% fructose) with electrolytes (PF). The exercise involved repeated sub-maximal cycling exercise bouts at various intensities from 55-95% $\dot{V}O_2max$ interspersed with rest periods ranging from 3 to 15 min. Two short high-intensity performance rides were also included. Exercise performance was measured according to the time taken to complete the high intensity rides (240 and 480 revolution exercise bouts), but did not involve exercise to exhaustion. In addition, the subjects used were not trained cyclists unlike those used in many other studies, making comparison with other studies difficult.

Winnick *et al.* (2005) investigated the use of carbohydrate feeding during intermittent exercise. The exercise protocol simulated a match situation, for example basketball where subjects ingested the test drinks pre-exercise, after the first and third quarters and during half-time. Following carbohydrate ingestion significant improvements were recorded in 20 m sprint time and average jump height when a battery of tests were performed during the half time period. It was suggested by the authors that carbohydrate feedings during high intensity exercise benefited both peripheral and central nervous system function late in exercise.

There have been many proposed mechanisms for the observed improvements in exercise performance following carbohydrate ingestion such as maintenance of blood glucose, increased carbohydrate oxidation, sparing of endogenous glycogen and the synthesis of glycogen during low intensity exercise in situations where intermittent exercise occurs (Jeukendrup, 2004).

1.5 COMMERCIAL SPORTS DRINKS

Many studies have examined the effects of different sports drinks on performance, recovery and other physiological variables. However, before reviewing this area, the physiological benefits for the use of these sports beverages in many sports will be discussed.

1.5.1 Rationale for using sports drinks

In a review by Coombes and Hamilton (2000) sports drinks were described as having 5 main objectives:

(i) prevent dehydration

(ii) supply carbohydrate to augment available energy

(iii) provide electrolytes to replace losses due to perspiration and assist absorption of fluid and electrolytes

(iv) conform to requirements imposed by regulatory authorities

(v) to be highly palatable.

Low concentration carbohydrate drinks are primarily used before and during exercise training or competition, while high carbohydrate drinks are used during carbohydrate loading pre-competition and post competition (Coombes and Hamilton, 2000). Both the carbohydrate and electrolyte concentrations are important considerations during the manufacture of sports drinks. The majority of sports drinks contain between 6 and 8% carbohydrate composed mainly of glucose and fructose monomers, sucrose and maltodextrins. (Coombes and Hamilton, 2000). Maltodextrins are also known as glucose polymers and have become more popular in recent years as they allow for increased availability of carbohydrate without a subsequent increase in osmolality. Electrolytes are added to sports drinks to improve palatability and to help maintain fluid/electrolyte balance (Coombes and Hamilton, 2000). The concentration of electrolytes such as sodium, potassium and chloride can vary considerably across commercially available sports drinks. The importance of electrolytes in sports beverages is discussed in more detail in Section 1.9.3.

1.5.2 Variables to be considered when choosing a sports drink

So why do we use sports drinks? How do we benefit from their consumption? The answers to these questions depend on a number of variables;

(i) Quantity of the beverage ingested

(ii) The rate of gastric emptying

(iii) The rate of intestinal absorption

(iv) Whether or not the drink attenuates endogenous carbohydrate oxidation.

These 4 topics have been areas of extensive research in recent years.

Volume ingested

The volume of beverage consumed voluntarily by the athlete is influenced primarily by palatability and habituation to ingesting fluids before, during and after exercise. Therefore, in the majority of cases where *ad libitum* ingestion takes place fluid ingestion does not meet sweat loss (Pugh *et al.*, 1967). If fluid ingestion was adequate this would decrease cardiovascular drift, limit any increases in core temperature and decreases in plasma volume (Montain and Coyle, 1992). The American National Athletic Trainer's Association published a position statement on fluid replacement for athletes, similar to that of the American College of Sports Medicine, where they recommended that athletes consume approximately 500 ml of fluid 2-3 hr prior to exercise and a further 200-300 ml approximately 20 min before exercise (Casa *et al.*, 2000). In contrast to these recommendations it has been reported that elite Kenyan endurance runners and also elite runners of other ethnicity consume less fluid than recommended but yet perform excellently (Fudge *et al.*, 2006). It was suggested that elite runners have the ability to avoid carrying excess body mass during completion that may increase energy costs by only consuming water as dictated by thirst.

Gastric emptying

The rate of gastric emptying is a very important factor in assessing the benefit of sports beverages. The volume of fluid ingested and the carbohydrate content are 2 important factors affecting gastric emptying (Coombes and Hamilton, 2000). Gastric emptying rate is fastest when gastric volume is greatest (Murray, 1987). Initially pressure receptors in the wall of the stomach respond to increased distension by increasing the rate of movement from the stomach to the duodenum (Minami and Mc Callum, 1984). This rate of emptying following fluid ingestion then returns to a slower rate that is dependent on the nutritional content of the fluid (Murray, 1987).

Multiple studies have been carried out investigating the effect of caloric content on the rate of gastric emptying. The result in brief of all these studies is that an increase in the caloric content of the fluid ingested causes a decrease in gastric emptying rate (Murray, 1987), as illustrated in Figure 1.2.

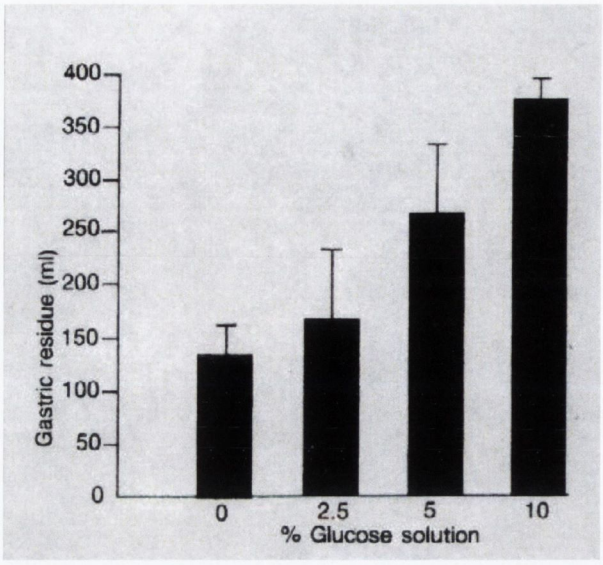


Figure 1.2: Effect of ingested glucose content upon gastric emptying. Gastric residue aspirated 15 min after ingestion of 400 ml of test solution. (adapted from Costill and Saltin, 1974).

By increasing the caloric content of the sports drink a decrease in the availability of water ensues. Therefore, a study by Brener *et al.* (1983) recommended that the carbohydrate content should be below 10% to allow for the adequate availability of water. Brener *et al.* (1983) also reported that the average gastric emptying rate for fluids containing calories ranged from 5 to 20 $ml.min^{-1}$ at rest.

The type of carbohydrate present in the sports drink may also play a role in determining the rate of gastric emptying. Brouns *et al.* (1995) investigated the effects of twelve different drinks on the rate of gastric emptying. Six of the drinks were of equal osmolality, but contained different concentrations of carbohydrate increasing from 45 to 90 $g.l^{-1}$. The other 6 drinks contained 60 $g.l^{-1}$ of carbohydrate, but the osmolality of the drinks ranged from 243 to 374 $mOsm.kg^{-1}$. In all trials 8 $ml.kg^{-1}$ of the test drink was ingested on an empty stomach. The results of this study showed that increasing the carbohydrate content with uniform osmolality had a negative effect on the rate of gastric emptying. The 6 drinks with a constant carbohydrate content, but different osmolalities, showed no changes in the rate of gastric emptying from the stomach. However, it appears that fructose solutions had a faster rate of gastric emptying when compared to equimolar glucose solutions (Sole and Noakes, 1989). While osmolality may be seen as an important factor in determining the rate of gastric emptying, the volume and caloric content of the fluids entering the stomach influence the rate of gastric emptying to a greater extent.

It is now common practice for sports drinks to contain combinations of monosaccharides, disaccharides and glucose polymers. Glucose polymers or maltodextrins have a lower

osmolality than solutions of glucose and a study by Foster *et al.* (1979) reported that a 5% glucose polymer solution had a significantly faster rate of gastric emptying than a 5% glucose solution following ingestion of 400 ml of the test solution.

Intestinal Absorption

The absorption of water, electrolytes and carbohydrates occurs principally in the duodenum and jejunum (Coombes and Hamilton, 2000). Large variations exist in the absorptive properties of carbohydrates namely; glucose, sucrose, fructose and maltodextrins and the conditions required for their absorption.

In a review by Murray (1987) it was suggested that the effectiveness of any carbohydrate-electrolyte beverage on exercise performance is based predominately on the stimulatory effect of glucose on water absorption in the small intestine. Figure 1.3 as cited by Murray (1987) illustrates the range of glucose concentrations required for maximal water absorption. From this graph it can be interpreted that water absorption is greatest when intestinal lumen glucose content is 60 to 160 mmol.l^{-1} (1 to 3% glucose).

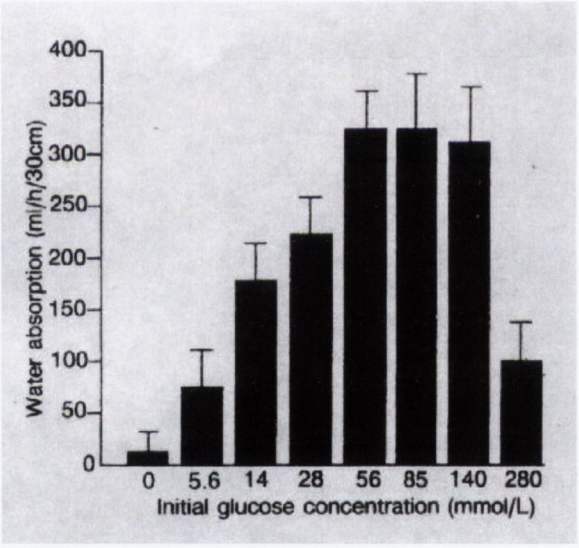


Figure 1.3: Water absorption from isotonic saline and isotonic saline-glucose mixtures. Adapted from Sladen and Dawson (1969).

The summary below (Table 1.3), detailing the conditions required for optimal absorption of different types of carbohydrate, is adapted from the review paper by Coombes and Hamilton (2000) and is based on evidence from studies cited in their review.

- The dimer sucrose cannot be absorbed, but is broken down to monomers glucose and fructose.
- Glucose polymers need to be broken down to glucose monomers for absorption.
- Glucose absorption, an active process, requires Na^+ co-transport carrier proteins.
- Fructose absorption is via facilitated diffusion and is independent of sodium.
- When ingested in large amounts (>10%) fructose can produce gastrointestinal distress and decreased water absorption.
- When fructose is ingested in equal amounts with glucose or consumed as sucrose, the absorption of it is dose dependent.
- Sucrose and maltodextrins have the advantage of providing a high carbohydrate load with minimal effect on osmolality.

Table 1.3: Conditions required for the absorption of different types of carbohydrates (Adapted from Coombes and Hamilton, 2000).

1.5.3 Importance of Osmolality

The significance of the osmolality of sports drinks has been addressed briefly in previous sections. Achieving the correct balance between carbohydrate content and fluid delivery is important. Osmolality is affected by the concentration and type of carbohydrate and the electrolyte concentration in the sports drinks. The use of simple sugars in sports drinks increases their osmolality making them hypertonic with respect to the plasma. However, since the 1980's the use of maltodextrins in sports beverages allows for the addition of more carbohydrate without the subsequent increase in osmolality. An increase in the osmolality of a drink has a negative effect on water absorption, drawing water from the cells, increasing secretion into the gastrointestinal lumen, potentially resulting in dehydration (Maughan and Noakes, 1991). In contrast, hypotonic and isotonic solutions promote water absorption. While osmolality plays a major role on the rate of intestinal absorption it has been reported that the osmolality is only of secondary importance to carbohydrate type when determining the rate of gastric emptying because the osmolality of fluid entering and leaving the stomach is almost the same (Vist and Maughan, 1995).

1.5.4 Commercial sports drinks and exercise performance

The ingredients as they appear on the packaging are quoted below (Table 1.4) for the 2 sports beverages investigated in the research studies contained in this report; Lucozade Sport and Red Bull®.

Lucozade Sport per 500 ml	Red Bull® per 500 ml
Carbohydrate: 32 g (Dextrose and maltodextrins)	Carbohydrate: 56 g (sucrose and glucose)
Calories: 140 kcal	Calories: 225 kcal
Sodium: 250 mg	Sodium: 200 mg
Potassium: 44 mg	Potassium: trace
Colour (Beta Carotene).	Taurine: 2000 mg
	Glucuronolactone: 1200 mg
	Caffeine: 160 mg

Table 1.4 Principal ingredients of Lucozade Sport and Red Bull®

Nicholas *et al.* (1995 and 1999) investigated the effects the ingestion of a commercial sports drink during intermittent running exercise. In the 1995 study, 9 male games players participated in 2 separate trials. The 2 test beverages under investigation were Lucozade Sport (6.9% carbohydrate-electrolyte beverage) and a non-carbohydrate placebo. The exercise protocol was performed in a gymnasium over a 20 m distance. Fluid (5 $ml.kg^{-1}$) was ingested immediately prior to commencement of a 75 min intermittent exercise test and an additional 2 $ml.kg^{-1}$ was ingested every 15 min during the exercise trial. Using pre-determined $\dot{V}O_2max$ data, by means of a progressive shuttle run test, subjects ran at velocities corresponding to 55 and 95% of their $\dot{V}O_2max$ alternating every 20 m until fatigue following the 75min of intermittent exercise. Time to fatigue was significantly longer (33%) following ingestion of the carbohydrate electrolyte solution compared to placebo ($P<0.05$). This equated to a running time of 2.2 min longer for the carbohydrate electrolyte trial.

In the second study Nicholas *et al.* (1999) used the Loughborough intermittent shuttle test on 6 trained games players using equivalent beverages to those used in their previous study. In this study muscle glycogen utilisation was assessed using muscle biopsies taken pre and post-exercise. A 22 % reduction in muscle glycogen utilisation was recorded following ingestion of the carbohydrate electrolyte beverage compared to placebo (245 ± 2.9

mmol.kg^{-1} dry mass compared to 192.5 ± 26.3 mmol.kg^{-1} dry mass, respectively). In discussing the rationale behind the decreased muscle glycogen utilisation the authors suggested that the ingested carbohydrate may have been utilised by the muscle, thereby sparing muscle glycogen stores. Serum insulin concentrations recorded after 30 min of exercise were higher following carbohydrate ingestion, facilitating glucose uptake by the active muscle and therefore sparing endogenous glycogen stores. Unfortunately, exercise time to fatigue was not recorded in this study, but sprint times were similar across both trials.

1.6 Caffeine

1.6.1. Introduction

Caffeine (1,3,7–trimethylxanthine) was first discovered over 1000 years ago. It is most commonly found in tea, coffee and chocolate, and as such it is one of the most widely consumed substances in the world with 82-92% of North American adults estimated to consume caffeine on a daily basis (Armstrong, 2002). In addition caffeine is now a common component to the cocktail of substances found in some sports beverages.

Until late 2003 caffeine was listed as a banned substance by the International Olympic Committee (IOC), where a urine concentration above 12 $\mu g.ml^{-1}$ was deemed a positive doping offence, as a result caffeine consumption is now not deemed a doping offence.

Despite this and with some conflicting evidence, caffeine has long been considered an ergogenic aid and therefore was and still is consumed in large amounts by many athletes. The ergogenic potential of caffeine surrounds its effect on a number of biochemical pathways including:

1. increased production of catecholamines
2. central nervous system adenosine receptor antagonism (Fredholm *et al.* 1999)
3. mobilization of calcium stores in the sacroplasmic reticulum
4. increased cAMP production via phosphodiesterase inhibition (Nehlig and Derby, 1994)

These pathways will be discussed in detail later in this section. However, it is important to note that the normal daily consumption of 0.25 – 4 $mg.l^{-1}$ (1-20 μM) produces peak circulating concentrations of caffeine where the only effect of which appears to be via antagonistic action on the adenosine receptor (Figure 1.4) (Fredholm *et al.*, 1980, 1999).

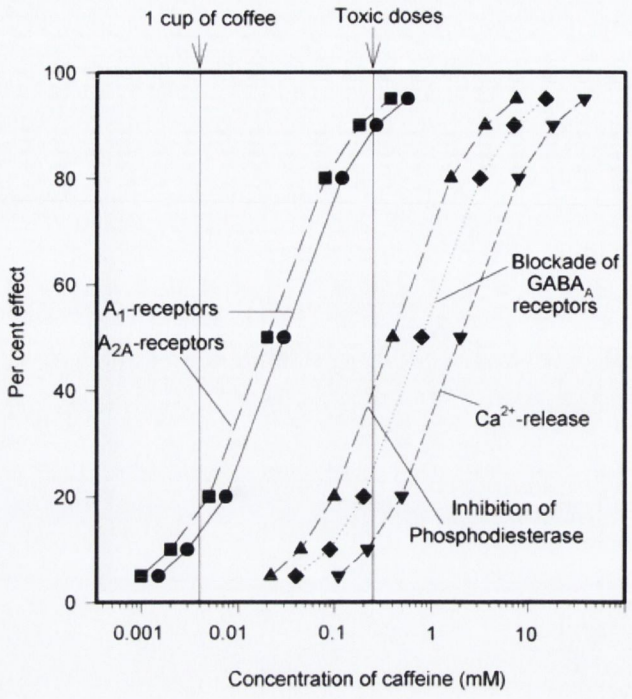


Figure 1.4: Effect of caffeine on different biochemical targets in relation to its levels in humans.

As depicted in Figure 1.4 caffeine is able to significantly block adenosine effects on A_{2A} (most potent) and A_1 receptors at the low concentrations achieved after a single cup of coffee. To inhibit cyclic nucleotide breakdown via inhibition of phosphodiesterase, 20 times higher concentrations are required; to block $GABA_A$ receptors, 40 times higher concentrations; and to mobilize intracellular calcium depots, concentrations of 100 times higher are needed. These latter levels are unlikely to be attained in humans by any form of normal usage of caffeine-containing beverages (modified from Fredholm, 1980).

1.6.2 Caffeine absorption, metabolism and pharmacokinetics

Caffeine administered orally is completely absorbed by the stomach and small intestine within 45 min of ingestion (Mc Lean and Graham, 2002).

Caffeine metabolism takes place principally in the liver through the cytochrome p-450 enzyme system. However, the brain and kidney may also metabolise small amounts of caffeine (Mc Lean and Graham, 2002). Caffeine is metabolised by processes of dimethylation and oxidation. In the metabolic pathway in humans paraxanthine is formed (1,7- dimethylxanthine) leading to the principal urinary metabolites, 1-methylxanthine, 1-methyluric acid and an acetylated uracil derivative. Peak plasma caffeine concentrations are reached between 15 and 120 min following caffeine ingestion. Ingestion of a caffeine dose of 5 to 8 $mg.kg^{-1}$ can yield plasma concentrations of approximately 8 to 10 $mg.l^{-1}$ (Fredholm *et al.*, 1999). A single cup of coffee contains approximately 0.4 to 2.5 $mg.kg^{-1}$ caffeine, yielding an estimated peak plasma concentration of 0.25 to 2 $mg.l^{-1}$ or 1 to 10 μM (Fredholm *et al.*, 1999). In urine the mean time to reach peak caffeine concentrations is 2.2 hr (1-3 hr range) (Armstrong, 2002).

The pharmacokinetics of caffeine are similar when administered orally or intravenously, and due to the hydrophobic properties of caffeine it readily passes through all biological membranes. (Fredholm *et al.*, 1999) When ingested in doses below 10 $mg.kg^{-1}$ the half-life of caffeine can range from 2.5 to 4.5 hr in humans. The rate of clearance of caffeine is low in 1-month-old infants (31 $ml.kg^{-1}.hr^{-1}$) and increases to 155 $ml.kg^{-1}.hr^{-1}$ in adult humans. The rate of elimination of methylxanthines is influenced by genetic and environmental factors, such as acute and chronic exercise, smoking, obesity and dietary factors. (Mc Lean and Graham, 2002) In adult male smokers the half-life of caffeine is reduced by 30 to 50% compared to non-smokers. Collomp *et al.* (1991) reported that moderate exercise (30% $\dot{V}O_2max$), in the first hour of an 8 hour investigation period raised the maximal plasma caffeine level and decreased the plasma half-life. On the other hand, Schlaeffer *et al.* (1984) reported an increase in the half-life of caffeine following exercise of low and moderate intensity in both normal and hot environments.

Some of the metabolites of caffeine, 1,3-dimethylxanthine (theopylline) and 1,7 dimethylxanthine (paraxathine) display marked pharmacological activity and therefore the actions of these metabolites should be considered if possible when examining the biological actions of caffeine containing beverages (Fredholm *et al.*, 1999).

1.6.3 Caffeine and catecholamines

As previously mentioned the production of catecholamines, namely adrenaline and noradrenaline is increased in the presence of caffeine.

It is widely accepted that caffeine has 2 possible modes of action in relation to catecholamine release:

1. activation of the sympathetic nervous system releasing noradrenaline
2. direct activation of the adrenal medulla stimulating adrenaline release. (Thong and Graham, 2002)

Under normal conditions noradrenaline and adrenaline are released from the adrenal medulla into the blood or as neurotransmitters from noradrenergic and adrenergic neurons in the sympathetic nervous system, respectively. Catecholamine release is stimulated under many conditions; hypolycemia, physical or mental stress, circulatory failure, exercise and illness to list some examples (Hoffman, 2002).

Plasma adrenaline concentrations are increased following the ingestion of caffeine, which may or may not be associated with an increase in noradrenaline suggesting that caffeine may specifically stimulate the adrenal medulla and not the sympathetic nervous system (Armstrong, 2002). Le Blanc *et al.* (1985) described caffeine as a potentiator of catecholamine action on β-receptors. When released these catecholamines bind to β-receptors, thereby enhancing the activity of the enzyme adenylate cyclase. This enzyme is the catalyst in the formation of cAMP from ATP. It has been reported that phosphodiesterase, the enzyme that inactivates cAMP is blocked by caffeine resulting in a prolonged elevation of cAMP from ATP.

Graham and Spriet (1995) investigated the metabolic, catecholamine and exercise performance responses to various doses of caffeine (3, 6 and 9 $mg.kg^{-1}$). They reported that caffeine ingestion stimulated adrenaline release, with significantly higher concentrations recorded 1 hr post-ingestion of the 6 and 9 $mg.kg^{-1}$ doses of caffeine. There was no change in plasma adrenaline levels compared to placebo after ingestion of the low dose of caffeine (3 $mg.kg^{-1}$). The exercise that followed in this study caused a further increase in adrenaline, reaching statistical significance at the higher doses at exhaustion. It is interesting to note that while significant increases in plasma adrenaline were recorded following the ingestion of 9 $mg.kg^{-1}$ of caffeine this was the only dose where an improvement in performance was not recorded above placebo treatment, making interpretation of their results difficult and possibly suggesting that the effect of caffeine on endurance exercise and adrenaline are not directly dependent on each other.

1.6.4 Caffeine and insulin

Along with the link to catecholamines, caffeine has also been associated with alterations in insulin resistance. Insulin has been discussed in detail earlier, in section 1.1.4. Thong *et al.* (2002) investigated the effects of caffeine ingestion during a 100 min euglycemic-hyperinsulinemic clamp on glucose uptake. Caffeine (5 mg kg^{-1}) or placebo was ingested 1 hour before the clamp. They reported that caffeine impaired insulin stimulated glucose uptake and glycogen synthase activity in rested and exercised human skeletal muscle. However, exercise did reduce the detrimental effects of caffeine on insulin action in muscle.

Two mechanisms have been proposed for the inhibition of insulin by caffeine.

1. Protein Kinase B (Akt) inhibition leading to a reduction in GLUT4 translocation in muscle (Akiba *et al.* 2004).
2. Adenosine receptor antagonism has also been linked to the impairment of insulin actions in skeletal muscle (Thong and Graham, 2002).

1.6.5 Adenosine receptor antagonism

Caffeine mediates some it its neurochemical affects through adenosine receptor antagonism. Adenosine is an endogenous neuromediator that inhibits neuronal activity, the release of neurotransmitters, especially catecholamines, and disrupts synaptic transmission. Caffeine however, has the opposite effect to adenosine by increasing release of catecholamines. By blocking adenosine receptors caffeine causes an increase in circulating catecholamines and therefore an increase in muscle activity and increased alertness. Thong and Graham (2002) investigated whether the caffeine-induced impairment of insulin was mediated by an increase in adrenaline release or by adenosine receptor antagonism by administering caffeine in the presence or absence of the non-selective β-adrenergic receptor blocker propranolol. Propranolol blocks the action of adrenaline on β1 and β2 adrenergic receptors. Using this protocol the authors were able demonstrate that the antagonistic effects of caffeine on insulin occurred as a result of elevated adrenaline and not peripheral adenosine receptor antagonism. Keijers *et al.* (2002) have also made similar conclusions during their investigation into the effects of caffeine on insulin activity.

1.6.6 Caffeine and exercise

The use of caffeine before and during sporting events has become more popular since the arrival of caffeinated carbohydrate foods and beverages on the market. Athletes are also

substituting carbohydrate drinks in the later stages of races with caffeinated drinks such as defizzed Coca-Cola, believing it to have an ergogenic effect. (Cox *et al.*, 2002) The vast majority of scientific research into the area of exercise and the role of caffeine has suggested that caffeine enhances endurance performance, however, some studies have reported conflicting results.

Principally caffeine is said to improve endurance exercise performance greater than 1 hr duration (Armstrong, 2002). However, there are a few studies to the contrary, suggesting that caffeine may induce different effects for different types of exercises (Anderson *et al.*, 2000 and Bruce *et al.*, 2000, Bridge and Jones, 2006). Time recorded during 2000 m rowing time trials have been reported to be improved in female rowers following the ingestion of caffeine (6-9 $mg.kg^{-1}$) 1 hr prior to this short duration exercise (6-8 min) (Anderson *et al.*, 2000 and Bruce *et al.*, 2000). Recently 8 km running performance in a field setting was reported to be significantly improved following the ingestion of 3 $mg.kg^{-1}$ caffeine by 8 male distance runners (Bridge and Jones, 2006).

Theories of ergogenicity:

1. a direct effect on the central nervous system altering the perception of effort.
2. a direct effect on skeletal muscle performance possibly involving effects on regulating enzymes controlling glycogenolysis.
3. increased catabolism of muscle triglycerides and reduced muscle glycogenolysis. (Graham and Spriet, 1996)

This section will now review some of the relevant literature investigating caffeine ingestion and the proposed theories of ergogenicity by the authors involved.

Graham and Spriet (1995) investigated the effects of various doses (3, 6 or 9 $mg.kg^{-1}$) of caffeine on metabolic, catecholamine and exercise performance during sustained prolonged exercise on a treadmill, using 8 male subjects. Plasma caffeine concentrations increased in a dose related manner, as did plasma paraxanthine data during the 3 and 6 $mg.kg^{-1}$ trials, with no further increase observed with the 9 $mg.kg^{-1}$ dose. Plasma noradrenaline was greater at exhaustion in all 3 caffeine trials compared with placebo. Time to exhaustion increased compared to placebo in the 3 and 6 $mg.kg^{-1}$ caffeine trials, however, endurance performance with 9 $mg.kg^{-1}$ of caffeine was not significantly different from placebo treatment. Time to exhaustion with placebo was 49.4 ± 4.2 min, whereas increases of 22.0 ± 9.0 and $21.9 \pm 7.2\%$ were reported for the 3 and 6 $mg.kg^{-1}$ trials, respectively.

Cytochrome p450 1A2 is the enzyme involved in the production of paraxanthines in a process of dimethylation. Kotake *et al.* (1982) and Denaro *et al.* (1990) have shown that

with doses of between 3 and 5 $mg.kg^{-1}$ this enzyme pathway can become saturated with caffeine. This information correlated sufficiently with the plasma paraxanthine response observed by Graham and Spriet (1998), where no further significant dose dependent increase in paraxanthine was observed at 9 $mg.kg^{-1}$. Graham and Spriet (1995) also recorded changes in blood-borne metabolites during the 4 trials. Following the ingestion of the largest caffeine dose (9 $mg.kg^{-1}$) there were significantly higher resting FFA concentrations, and glycerol was increased throughout the trial when compared to placebo, suggesting mobilization of stored triglycerides. However, no increase in endurance performance was reported during the trial with the largest caffeine dose.

Kovacs *et al.* (1998) reported significantly faster cycling time trial performances following the ingestion of a carbohydrate solution (CES) containing either 225 $g.l^{-1}$ or 320 $g.l^{-1}$ of caffeine compared to the other 3 trials (58.9 ± 1.0 and 58.9 ± 1.2 min for CES-225 and CES-320, respectively, compared to 62.5 ± 0.13, 61.5 ± 1.1 and 60.4 ± 1.0 min for placebo-water, placebo CES and CES-150, respectively). Kovacs *et al.* (1998) administered caffeine in concentrations of 2.1, 3.2 and 4.5 $mg.kg^{-1}$. Therefore the study findings were consistent with the findings of Kotake *et al.* (1982) and Denaro *et al.* (1990), because no improvement in performance was recorded following ingestion of 2.1 $mg.kg^{-1}$ of caffeine, yet improvements were observed during the other 2 trials using higher doses. Cox *et al.* (2002) observed a significant improvement in time trial performance undertaken at the end of 120 min of prolonged exercise following ingestion of 6 $mg.kg^{-1}$caffeine. The increased improvement was recorded independent of the timing of ingestion. Graham *et al.* (1998) investigated the metabolic and exercise endurance effects of coffee and caffeine ingestion. 9 subjects participated in 5 treadmill-running trials. In terms of endurance performance the ingestion of caffeine capsules (4.45 $mg.kg^{-1}$ of caffeine) resulted in an increase in endurance time (7-10 min) when compared to the other 4 trials; namely, decaffeinated coffee, decaffeinated coffee and caffeine, placebo capsules and regular coffee. All 4 trials involving the consumption of coffee showed similar concentrations of methylxanthines, but only one trial (caffeine capsules) showed enhanced performance. It was therefore suggested by the authors that some components in coffee antagonised the normal ergogenic responses of caffeine.

In the exercise studies contained in this dissertation the effect of caffeine ingestion (3 $mg.kg^{-1}$), as a component of a sports drink, is investigated on both prolonged and intermittent exercise.

1.6.7 Caffeine and diuresis

Caffeine has been shown to exhibit some diuretic effects at rest and therefore it seems reasonable to suggest that caffeine should not be added to any beverage aimed at replacing fluids during prolonged exercise. Any diuresis during exercise would further increase exercise induced dehydration and lead to a greater reduction in plasma volume, stroke volume and cardiac output and an increase in core temperature (Wemple *et al.,* 1997). Wemple *et al.* (1997) investigated the diuretic effects of caffeine during exercise and demonstrated that caffeine ingested prior to moderate endurance exercise did not compromise hydration, even though a weak diuresis was evident at rest. Urine flow rate at rest was significantly greater ($P<0.01$) during the caffeine trial compared to placebo, however, exercise reduced urine production compared to rest. Wemple *et al.* (1997) suggested that the diuretic effects observed at rest may be overridden by alteration in renal function during exercise, thereby overruling the conventional wisdom that caffeine would accelerate dehydration during exercise. In the study described earlier by Kovacs *et al.* (1998) no significant increase in urine volume following ingestion of the 3 different caffeine concentrations compared to the non-caffeinated beverages at rest or at the end of the experiment were reported (see Table 1.5).

Treatment	Urine Volume (ml) At Rest	Urine Volume (ml) End of experiment
Placebo – Water	85 ± 21	322 ± 65
Placebo – CES	85 ± 20	199 ± 57
Placebo – CES 150	87 ± 24	216 ± 49
Placebo – CES 225	121 ± 35	290 ± 58
Placebo – CES 320	94 ± 16	215 ± 48

Table 1.5: Mean urine volume (ml) recorded at rest and at the end of the experiment across 5 drinks treatments (Kovacs *et al.*, 1998).

The authors suggested that the diuretic effect of caffeine was counteracted by the presence of catecholamines during exercise reducing glomerular filtration rate, a theory originally proposed by Clausen and Trap-Jensen (1974). In 1980 Bello-Reuss reported that catecholamines may increase Na^+ and Cl^- reabsorption in both the proximal and distal tubules by affecting aldosterone and/or antidiuretic hormone, thereby resulting in water conservation.

More recently McLean and Graham (2002) investigated the effects of exercise (1 hr at 65% $\dot{V}O_2max$) on caffeine pharmacokinetics, they reported no effect on urine levels in

men with caffeine concentrations ranging from 4.95 to 9.16 $\mu g.ml^{-1}$, and no subject recorded urine concentrations above the previously set IOC limit of 12 $\mu g.ml^{-1}$. The dosage used by McLean and Graham (6 $mg.kg^{-1}$) was higher than that used in a previous study by Kovacs *et al.* (1998) that reported similar findings. Kovacs *et al.* (1998) measured post-exercise urine caffeine concentrations to investigate if they were within the doping limit of 12 $\mu g.ml^{-1}$ set by the IOC at that time. All 3 trials yielded urinary caffeine concentrations below the IOC doping limit, the highest caffeine dosage of 4.5 $mg.kg^{-1}$ resulted in a mean urinary caffeine concentration of 2.5 $\mu g.ml^{-1}$. It was suggested that at higher concentrations the rate of caffeine clearance is prolonged due to the saturation of the metabolic pathways.

1.6.8 Tolerance, habituation and withdrawal of caffeine under normal circumstances

Tolerance to caffeine may develop in only 4 or 5 days in adults who regularly consume caffeine. Caffeine is then required in greater quantities to produce a typical physiological response. In 1928 Eddy and Downs as cited in a review by Armstrong (2002) determined that the mean minimal effective dose of caffeine was 0.48 mg kg^{-1} body weight in subjects who abstained from caffeine for 2 months. Following habituation to caffeine the minimal effective dose was 1.12 $mg.kg^{-1}$, determined by the dosage that caused an obvious diuretic effect. In resting individuals, habituation to caffeine results in a decreased effect of caffeine on plasma and urinary catecholamines (Robertson *et al.*, 1981).

Withdrawal symptoms include weariness, apathy, weakness, drowsiness, headaches, increased heart rate and muscle tension and occasionally individuals may experience nausea, vomiting and flu like symptoms. (Fredholm *et al.*, 1999) Withdrawal symptoms generally begin about 12 to 24 hr after ceasing caffeine ingestion and reach a peak after 20 to 48 hr. In some cases however, withdrawal symptoms may appear after only 3 hr and can last for up to 1 week.

In designing research studies to investigate the effect of caffeine on exercise performance the prior use of caffeine may be an important factor but is rarely strictly controlled. Inconsistency in results regarding the effect of caffeine on exercise performance may stem from the lack of control over prior caffeine use in participating subjects.

1.6.9 Tolerance, habituation and withdrawal of caffeine in relation to exercise

Nehlig and Derby (1994) in their review suggested that habituation to caffeine may attenuate the effect of caffeine on exercise performance as consumers developed tolerance to the effects of caffeine. This observation was made during steady state exercise, but not during maximal performance. In contrast, time trials to exhaustion have shown that tolerance to caffeine does not alter the ergogenic effects of caffeine (Tarnoplsky, 1989). In this instance habituation to caffeine did not alter the ergogenic effect of large doses (6 $mg.kg^{-1}$) and did not show a significant effect on any variables ($\dot{V}O_2$, heart rate, respiratory exchange ratio, plasma glucose, adrenaline, noradrenaline) that may be ergogenic to endurance performance. However, this issue remains inconclusive, as the experimental designs employed by the investigators were not similar.

Following withdrawal periods of 2 to 4 days no effect has been observed for time to exhaustion (Van Soeren and Graham, 1998). However, acute caffeine administration did result in a significantly prolonged time to exhaustion, yet this occurred without any

changes in noradrenaline concentration and respiratory exchange ratio data. From these findings Van Soeren and Graham (1998) concluded that the mechanism of action of caffeine as an ergogenic aid may be independent of catecholamines or metabolic substrates and went further to suggest that caffeine may act directly on muscle.

1.7 Taurine

1.7.1 Introduction

Taurine otherwise known as 2-aminoethane sulfonic acid is an amino acid that differs from most others because it is a sulfonic rather than a carboxylic amino acid. An average 70 kg male contains 70 g of taurine (Huxtable, 1992). The highest concentrations of taurine are located in the mammalian heart or brain with a large proportion also found in the musculature. The majority of taurine consumed is excreted unchanged in the urine (Huxtable, 1992). Taurine has many physiological functions in both the cardiovascular and central nervous systems (see Table 1.6), although most of these are beyond the scope of this review and will not be discussed in detail. However, it is clear that taurine has many complex roles in the body. Taurine is an active ingredient in Red Bull and some studies investigating the effects of this energy drink have concentrated in detail on the role played by taurine. One such study by Geiss *et al.* (1994) investigated the effects of Red Bull on endurance performance. The details of this study are described later, but in concluding the authors cited taurine as being crucial to the improved performance observed in the study.

1.7.2 Cardiovascular effect of Taurine

Taurine comprises over 50 percent of the total free amino acid pool of the heart (Huxtable, 1992). Taurine has been used in a clinical and experimental environment in the treatment of various cardiovascular diseases, namely hypertension, congestive heart failure and diabetes mellitus (Militante, 2001). The concentration gradient of taurine across the plasma membrane of the heart is bigger than that of all other amino acids, and is believed to be an essential feature for the protection of the heart in a variety of different pathological conditions (Militante, 2001). Franconi *et al.* (1983) reported that while taurine decreased noradrenaline levels in the blood, it also minimized the binding of catecholamines on heart muscle cells. In this way taurine played a protective role on the heart during periods of high stress induced by the high release of catecholamines. This protective effect was similar to the lower heart rates and improved performance observed by Geiss *et al.* (1994).

	Action
Cardiovascular	• Antiarrthymic properties • Hypotensive actions • Modulation of calcium channels • Increased resistance of platelets to aggregation • Protection during calcium overload
Central Nervous System	• Anticonvulsant • Modulator of neuronal excitability • Maintenance of cerebellar function • Thermoregulation • Anti aggressive action • Altered learning • Regulation of cardio respiratory responses • Suppression of eating and drinking • Alteration of sleeping duration
Liver	Bile salt synthesis
Reproductive System	Sperm motility factor
Muscle	Muscle membrane stabiliser
Others	• Antioxidation • Regulation of phosphorlyation • Stimulation of glycolysis and glycogenesis • Modulator of neurotransmitter and hormone release • Osmoregulation • Lowers cholesterol • Xenobiotic conjugation

Table 1.6: Biological actions of Taurine (Adapted from Huxtable, 1992).

1.7.3 Other actions of Taurine

Calcium movements may be modulated by taurine (Geiss *et al.,* 1994). Huxtable (1992) in his review of taurine listed the biochemical effects of taurine on calcium movements including increased calcium (Ca^{2+}) storage capacity of the sarcoplasm reticulum and stimulation of the pumping rate of Ca^{2+} activated ATPase pumps.

In the brain taurine blocks the cysteine sulfinate induced elevation in cAMP concentration in brain slices and the stimulatory effects of noradrenaline, adenosine and histamine on cAMP concentration in the hippocampus. (Baba *et al.,* 1982). It has also been reported that taurine acts on the insulin receptor (Kudo, 1988). Taurine has therefore been hypothesised to affect carbohydrate metabolism; by increasing glucose uptake, potentiating the actions of insulin itself, enhancing glycogenesis, glycolysis and glucose oxidation.

On searching the literature relating to taurine and its effects there are no data to my knowledge to suggest that taurine is harmful. Studies investigating the effects of taurine on exercise performance are based on using whole energy drinks compared to a variety of other drinks compositions but without taurine, and not on taurine supplementation alone.

The next section will review Red Bull and the available literature regarding the proposed performance-enhancing role played by this popular commercial beverage during exercise.

1.8 Red Bull

1.8.1 Introduction

It is becoming increasingly popular for athletes to consume a caffeinated sports drink in the final stages of an endurance competition (Cox *et al.,* 2002). Research has investigated the use of coca cola towards the end of endurance exercise and the effects of the caffeine present on exercise performance (Cox *et al.,* 2002).

Red Bull is another drink that is used frequently in a similar context and will be used in all 4 studies contained in this report. It is a very popular beverage among college students who believe it improves memory and performance at exam time (Bichler *et al.*, 2006). In the US alone 1.5 billion cans of Red Bull were sold in 2004 (Bichler *et al.*, 2006). The ingredients of Red Bull include; carbohydrate, caffeine, taurine and glucuronolactone, see Table 1.4. The following sections will outline the scientific research available in relation to Red Bull and the effects on reaction time, mood, mental performance and exercise performance.

1.8.2 Red Bull effects on reaction time and memory

A lot of marketing and advertising of Red Bull has been aimed at those spending long periods of time driving, such as taxi and truck drivers (Official Red Bull website). Some scientific investigations have therefore been based on the theory that Red Bull will help the driver stay alert. In one such study, ingestion of 500 ml of Red Bull prior to a two and half hour test using an interactive real-car driving simulator showed significant reductions in both lane drifting and reaction times when compared to placebo (Horne and Reyner 2001). Similarly, reaction time was reduced 1hr following Red Bull ingestion when assessed using EEG imaged brain motor pathways and an "attention capacity test" (D2 test) (Seidl *et al.* 2000). When compared with other trials involving no drink, ingestion of carbonated water and flavouring, or plain water, Red Bull had a positive effect on standard tests of choice reaction times, subjective alertness, number cancellation and immediate memory recall. Authors have suggested that a combination of the active ingredients in Red Bull is likely to be responsible for the improvements observed in mental capacity or processing (Seidl *et al.*, 2000, Alford *et al.,* 1999). Recently, Bichler *et al.* (2006) reported that short-term memory was not altered following the ingestion of caffeine (100 mg) and taurine (100 mg) in capsule form in concentrations similar to those contained in 250 ml of Red Bull. However, this study did report that heart rate was decreased and mean arterial pressure was increased 45 min post ingestion compared to placebo. These cardiovascular changes were linked to pressure-induced reflex bradycardia associated with caffeine consumption and may be enhanced by the presence of taurine (Bichler *et al.*, 2006).

1.8.3 Red Bull and exercise performance

Scientific research in the area of Red Bull and exercise performance is limited. To my knowledge only 3 studies have investigated the effects of Red Bull on exercise performance parameters (Geiss *et al.*, 1994, Alford *et al.*, 1999, Baum and Weiss, 2001). Geiss *et al.* (1994) designed a study using trained athletes to investigate whether 500 ml of Red Bull would produce an increase in performance above that reported using the carbohydrate equivalent of the drink.

The 3 test drinks contained the following:

1) Red Bull, without taurine and glucuronolactone, but containing caffeine and carbohydrate ("U1")
2) Red Bull without taurine, glucuronolactone and caffeine, but containing carbohydrate ("U2")
3) Red Bull original drink, containing taurine, glucuronolactone, caffeine and carbohydrate. ("U3")

Geiss *et al.* (1994) reported a significant increase in time to exhaustion following ingestion of Red Bull (U3) (858 ± 236 s) compared to the other 2 test drinks (792 ± 189 and 689 ± 92 s for trials U2 and U1, respectively), endurance time in U2 was also significantly greater than U1. In this case RB was ingested following 30 min of sub-maximal exercise. In addition, a second bout of exercise was performed 24 hours after ingesting the drinks and endurance capacity with Red Bull (U3) was significantly greater than endurance capacity with the drink without taurine and glucuronolactone (U1). No significant differences were recorded for blood glucose, blood lactate, plasma insulin and heart rate data across all 3 trials at exhaustion. Catecholamine levels were significantly lower with Red Bull than with the drinks without taurine, a result that prompted Geiss *et al.* (1994) to review the effects of taurine in their report.

While Geiss *et al.* (1994) reported the improvements in performance to be related to the effects of taurine, a more recent study by Baum and Weiss (2001) suggested a combined effect of taurine and caffeine. In their study the effects of Red Bull were investigated on cardiac parameters, before and after exercise, measured by echocardiography. The 3 drinks used in their study were identical to those used by Geiss *et al.* (1994). 500 ml boluses were administered in a double-blind crossover design study. Echocardiographic examinations were performed prior to the ingestion of the test drinks, immediately prior to exercise and during the recovery period when the heart rate was approximately 70 beats.min^{-1}. The results of this study showed a significant increase in stroke volume following Red Bull

ingestion compared to the other drinks. Baum and Weiss (2001) concluded that taurine alone or in combination with caffeine was responsible for the cardiovascular changes observed in their study. More concise details of these 2 studies will be eluded to in the course of the discussion of this dissertation where needed.

1.8.4 Red Bull and Diuresis

Riesenhuber *et al.* (2006) reported no additive effect on the diuretic effects of caffeine when ingested in combination with taurine in the form of Red Bull (750 ml). This study concluded that the diuretic potential of Red Bull does not significantly differ from other caffeine containing beverages.

1.9 Rehydration

1.9.1 Introduction

Rehydration is the process of fluid and electrolyte replenishment lost through dehydration. The principle routes for water loss from the body are the urinary system, skin, gastrointestinal tract and the respiratory system, while water is restored through both food and fluid ingestion, see section 1.3.4. During exercise water loss occurs primarily through sweating. Water produced during metabolism contributes minimally to the restoration of fluid balance. For athletes and coaches it is very important to be able to recognise the first symptoms of dehydration. The early signs and symptoms include thirst and general discomfort. With time these symptoms are followed by flushed skin, weariness, cramps and apathy. If fluid losses persist the athlete may suffer from dizziness, headaches, vomiting, nausea and decreased exercise performance (Casa *et al*, 2000). A 2% loss in body mass primarily via dehydration has been associated with a decrease in high intensity cycling time (Walsh *et al.*, 1994). The ability to identify the early signs of dehydration can help minimise the onset of more adverse effects for the athlete.

The restoration of fluid balance in the studies in this thesis focuses on 2 methods of dehydration; firstly dehydration following exercise and secondly dehydration as a result of fluid restriction. Scientific literature relating to voluntary fluid restriction is very limited with research in this area primarily focussing on patients suffering from specific pathologies. However, Shirreffs *et al.* (2004) investigated the effects of voluntary fluid restriction on physiological and subjective feelings in humans. They reported feelings of headache, decreased alertness and a decrease in the ability to concentrate when body mass was reduced by 2.7% following fluid restriction. The details of that study will be alluded to at a later stage in comparison with findings from the final study in this dissertation.

Increases in ambient temperature play a major role in fluid loss and dehydration in athletes but because it is not investigated in the experiments contained in this report it will not be reviewed in detail here.

The composition of the body's fluid compartments (ECF and ICF) have been described in detail in section 1.3 alongside the discussion of how the body maintains a strict equilibrium between these compartments and the importance of electrolytes in maintaining homeostasis under normal and stressed conditions.

During exercise muscular heat production is increased significantly from rest. The body needs to balance endogenous heat production with exogenous heat accumulation and this is achieved by dissipating heat via conduction, convection, evaporation and radiation (Casa *et al.*, 2000). When the ambient temperature exceeds skin temperature evaporation of sweat

from the skin surface is the only method of heat loss. If fluids are not consumed to replace those lost through sweating progressive dehydration will occur. Body mass losses close to 8% have been recorded for marathon runners competing in warmer climates. In cooler environments during high intensity exercise sweat rates may also be high (Shirreffs and Maughan, 2000). Water movement between the ECF and ICF occurs due to hydrostatic pressure and osmotic gradients. Sweat is hypotonic relative to body water and so an increase in extracellular tonicity results in the movement of water from the intracellular to extracellular compartments. As a result all water compartments contribute to the water deficit associated with dehydration (Casa *et al.*, 2000). There is compelling evidence to suggest that dehydration will result in a decrease in performance (reviewed by Casa *et al.*, 2000) and therefore to combat this water deficit it is essential that athletes adopt a hydration strategy to minimise fluid losses. Athletes should consider fluid consumption in 3 stages; before exercise, during exercise and after exercise, and each stage has different requirements according to the volume, composition and timing of the fluid to be ingested. Many exercise and sports science associations have published recommendations for fluid consumption and fluid balance. For example the position stand published by the American College of Sports Science (1996) on exercise and fluid replacement recommends that athletes ingest approximately 500 ml of fluid 2 hr before exercise and begin to drink early and at regular intervals during exercise to attempt to replace all water lost through sweating.

1.9.2 Rehydration beverages

The National Athletic Trainers Association (USA) published a position statement in 2000 on "fluid replacement for athletes" and recommended the following:

1.Post exercise hydration should be completed within 2 hr.

2.The beverage should contain water to restore hydration status, carbohydrates to replenish glycogen stores and electrolytes to speed rehydration and assist fluid retention (Casa *et al.*, 2000).

Every manufacturer of a commercial sports beverage faces a challenge to optimise the flavour system in order to encourage voluntary fluid intake. Palatability includes flavour, taste, mouthfeel and temperature. During and shortly after exercise, subjects demonstrate a lower acceptability for fluids that are highly sweetened and have strong flavours (Horswill, 1998). When fluid ingestion was permitted *ad libitum*, palatability played an important role in the volume of fluid ingested. A study by Maughan and Leiper (1993) examined 4 different drinks where subjects were allowed to drink as much as they wanted. Subjects consumed the sports drink and the orange/lemonade mixture in greater volumes compared to plain water or a glucose electrolyte solution. The volumes consumed in all cases were sufficient to restore fluid balance. While the taste of the fluid had a profound effect on the volume consumed, the electrolyte content determined if that fluid was retained in the body. Any benefits observed from the higher intake of the more palatable drinks were abolished due to the associated higher urine output.

1.9.3 Composition of rehydration beverages

The ingestion of plain water as a rehydration beverage is not recommended as an effective method of achieving euhydration. Ingestion of large volumes of electrolyte free drinks results in a decline in plasma osmolality that in turn suppresses the drive to drink and also stimulates urine production (Maughan and Leiper 1995, Ray *et al*, 1998, Casa *et al.*, 2000). While the thirst signals described above work to restore fluid loss some research suggests that the sensation of thirst is suppressed before fluid volume has been completely restored. The most important electrolyte involved in the restoration of fluid balance is sodium, followed by potassium. Sodium plays 4 main roles when present in a rehydration beverage:

1. Maintains serum osmolality (Nose *et al.*, 1988). The presence of sodium in a rehydration beverage maintains serum osmolality despite plasma volume being replenished. Therefore the athlete consumes more fluid and comes closer to meeting their fluid requirements compared to a situation where Na^+ is not present.
2. Maintains body fluid balance

3. Restores Na^+ electrolytes lost in sweat
4. Stimulates fluid and carbohydrate uptake in the gut. (Horswill, 1998)

The presence of potassium alone in concentrations much greater than those present in commercial drinks has been reported to contribute to euhydration (Maughan *et al.*, 1994). When K^+ is combined with Na^+ in a beverage there appears to be no added benefits in terms of body fluid balance. It was recommended by Casa and colleagues in a review of the literature that the rate of Na^+ ingestion should be approximately 0.3 – 0.7 $g.hr^{-1}$ when contained in a rehydration beverage (Casa *et al.*, 2000). The Na^+ content of the 2 drinks investigated in this thesis is 600 $mg.l^{-1}$ for Lucosade Sport and 800 $mg.l^{-1}$ for Red Bull.

The presence of carbohydrate in a rehydration solution enhances the rate of intestinal absorption of Na^+ and water and aids the replenishment of glycogen stores depleted during exercise (Maughan *et al.*, 1996). The co-transport of glucose and Na^+ creates an osmotic gradient, promoting net water absorption and therefore the rate of rehydration is greater in the presence of combined glucose –Na^+ beverages compared with plain water (Shirreffs and Maughan, 2000).

1.9.4 Volume of fluid ingested

The volume of fluid to be ingested post exercise has been a topic of much research. It is generally accepted that following exercise induced sweating the volume of fluid consumed must be greater than the volume of sweat lost to achieve positive fluid balance (Shirreffs and Maughan, 2000). The efficacy of rehydration beverages in volumes equivalent to 50, 100, 150 and 200% sweat loss was investigated by Shirreffs *et al.* (1996). It was reported that the urine volume produced was proportional to the volume of fluid ingested. The smallest volumes were produced when 50% of the sweat loss was consumed and the greatest when 200% of the loss was consumed. Following ingestion of a volume equivalent to 150% sweat loss with a low Na^+ content subjects were still slightly hypohydrated 6 hr post ingestion. When the same volume was fluid was ingested containing a high Na^+ concentration subjects reached a state of hyperhydration, outlining the importance of the inclusion of Na^+ in rehydration beverages.

1.10 Markers of Hydration Status

The restoration of hydration status can be monitored in many ways and the accuracy and reliability of these methods can vary considerably. The next section will look at the markers of hydration status that will be used in the studies contained in this thesis.

1.10.1 Bioelectrical Impedance Analysis

Bioelectrical impedance analysis (BIA) has been used as a tool for assessing hydration status for many years (Armstrong, 2005). Prior to 1990 only single frequency impedance was used to estimate fat mass, fat free mass and TBW in children, adults and those with various illnesses (Armstrong, 2005). However, bio impedance can now be measured across a range of frequencies. Low frequency currents pass through the extracellular tissues but at higher frequencies the current penetrates the cell membranes and therefore passes through both intra- and extracellular tissues.

Bioelectrical impedance is a safe, fast and non-invasive method that takes approximately 5 minutes to perform and requires the placement of 2 electrodes on the surface of both the hand and the foot.

The gold standard method for determining TBW is isotope dilution. Studies comparing BIA to this method have reported a standard error in the estimation of TBW ranging from 1.5 to 2.9l. If measurement protocols are not strictly adhered to the error can be much greater. Dehydration, fluid ingestion and posture changes may affect the accuracy of BIA. Shanholtzer and Patterson (2003) assessed the test-retest reproducibility for BIA measures of extracellular water (ECW), TBW and intracellular water (ICW) in normal individuals and those categorised as being hypohydrated or hyperhydrated over a 1 week period. The use of BIA as a measure of hydration status in 100 collegiate students aged 18-30 yr was reported to be highly reliable ($r = 0.99$ for TBW, ECW and ICW). This study also reported that BIA can be used to classify individuals as being either chronically hypohydrated or hyperhydrated across time.

When hydration status is compromised the electrolyte balance is altered and so the ratio of intra- to extracellular water is affected. It has been reported in several studies reviewed by O'Brien *et al.* (2002) that this will in turn affect TBW measurements by BIA. Therefore, the use of BIA alone to monitor acute changes in hydration status has been questioned and in this thesis will be used alongside other markers of hydration status.

1.10.2 Body mass

The use of body mass changes as a marker of hydration status is quick, easy and inexpensive and represents a safe technique to assess changes in fluid balance with or without exercise. When an athlete is in caloric balance their body mass loss equals water loss because no other body constituent is lost at a similar rate. The body mass loss must be corrected for food and fluid intake, urine and faecal losses and sweat by evaporation (Armstrong, 2005). In order to use the change in body mass as an accurate measure of hydration status it is important that the measurement protocol be standardised. Athletes must be weighed nude or minimally clothed, should not be wearing sweat soaked clothes and should towel off excess sweat prior to weighing (Oppliger and Bartok, 2002). Importantly, the ingestion of fluids do not necessarily equate to the equilibration of water in the ICF and ECF and so this is one limitation when using the change in body mass as a tool to assess hydration. It has been shown that the ingestion of fluid matched to body mass loss does not return plasma osmolality to euhydrated levels (Popowski *et al.*, 2001).

1.10.3 Urine volume, specific gravity and osmolality

As described earlier, renal water and electrolyte excretion is under the control of ADH. Depending on whether the body is dehydrated or not has an effect on urinary indices, for example volume, specific gravity and osmolality.

Under normal conditions, urine volumes range between 1.5 and 2.5 $l.day^{-1}$ (Oppliger and Bartok, 2002). The use of urine volume as a measure of hydration status requires a high degree of compliance from the athlete but otherwise is quick and non-invasive.

Urine specific gravity (Usg) is described as the density of a urine sample compared with the density of water (Oppliger and Bartok, 2002). There are many methods of measuring Usg including hydrometry, reagent strips and refractometry. Refractometry is used in the studies contained in this report. It involves the passing of a beam of light through a urine sample and measuring the degree of refraction. Popowski *et al.* (2001) reported that those presenting with a Usg reading of 1.020 would be at the upper range of euhydration. During dehydration Usg exceeds 1.030. When excess water exists Usg values range from 1.001 to 1.012 (Armstrong, 2005). In the final study of this thesis a Usg reading above 1.020 was considered indicative of dehydration.

Urine osmolality (Uosm) is a measure of the total urine solute content and is affected by all the dissolved particles (urea, glucose, protein) in a known volume of fluid. According to a review by Armstrong (2005) osmolality provides the most accurate measurement of total solute concentration and therefore is the best measure of the kidney's concentrating ability.

The ingestion of large boluses of fluid following dehydration will produce diluted urine despite the individual remaining dehydrated and therefore yielding inaccurate readings of Uosm and Usg. In this case the kidneys respond to the immediate fluid overload by producing large quantities of dilute urine before the body equilibrates the water in the ICF and ECF (Oppliger and Bartok, 2002). When fluid is ingested gradually, the ECF equilibrates with the ICF slowly. Therefore, there may be differences in urinary indices from individual samples or composite urine samples collected over time (Armstrong *et al.*, 1998) and as a consequence it is recommended to collect a number of urine samples over time following fluid ingestion. Armstrong *et al.* (1998) carried out a study to investigate the relationship between Usg and Uosm as indices of hydration status. They reported a strong correlation between Uosm and Usg (r=0.98) and as a result recommended their use interchangeably to assess hydration status during periods of large fluid turnover.

1.10.4 Plasma Osmolality

The use of plasma osmolality, as a marker of hydration status is the most widely used technique because important fluid regulating mechanisms, such as water reabsorption are influenced by extracellular fluid osmolality (Armstrong, 2005) The concentration of antidiuretic hormone can be increased following an increase of only 1% in osmolality thereby stimulating water reabsorption in the kidneys. The hormonal regulation of osmolality is very tight and therefore under normal hydrated conditions serum osmolality will only deviate by 1-2% from the normal value of 287 $mOsm.kg^{-1}$. Plasma osmolality in these studies will be measured using the freezing point depression technique.

Chapter 2

Materials and Methods

2.1 Anthropometric Measurements

Height and body mass were recorded using a counter balancing weighing scales and stadiometer (Seca Ltd., Germany). The weighing scales was calibrated regularly using multiple weights of known mass (25 kg). During the cycling studies (Chapters 3 and 4) the subjects were weighed in their cycling clothing having removed their footwear. For all other studies subjects were weighed in their regular clothing having removed their footwear. Body mass index (BMI) was then calculated using the following equation from Mc Ardle *et al.* (1994):

$$BMI\ (kg.m^{-2}) = Body\ Mass\ (kg)/Height^2\ (m^2)$$

2.2 Percentage Body Fat

A Harpenden skin fold calliper (Baty Ltd., England) was used to assess percentage body fat, see Figure 2.1. Measurements were taken from 4 sites: triceps, biceps, subscapular and suprailiac, all on the dominant side. The skinfold measurement was assessed with the subject standing on a level surface. Using the left hand a fold of skin and subcutaneous tissue was picked up, the plates of the calliper held in the right hand were allowed to exert full pressure below the position of the left hand, before recording to the nearest 0.1 millimetre (mm). The average of 3 measurements was recorded at each site.

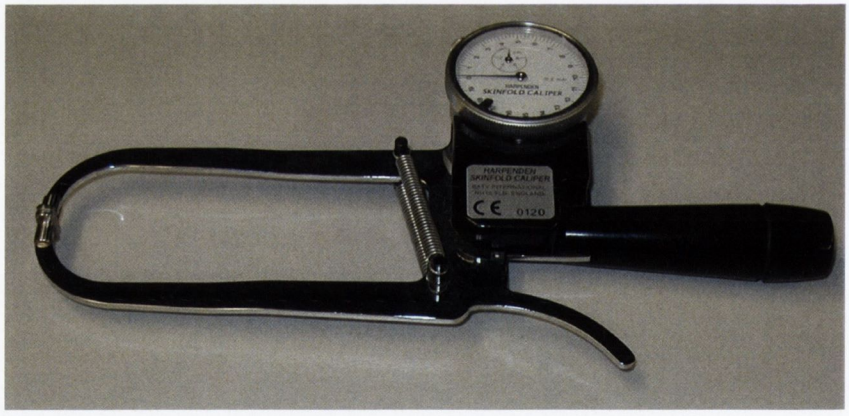


Figure 2.1 Harpenden skinfold calliper.

1. Triceps: The fold was picked up at the midpoint of a line connecting the acromion and the olecranon process while the arm was hanging loosely with the elbow extended.
2. Biceps: The skinfold was measured directly above the centre of the cubital fossa at the same level at which the triceps reading was taken.

3. Subscapular: the subscapular skinfold was picked up just beneath the inferior angle of the scapula in a direction obliquely downwards and outwards at 45° angle.
4. Supra-iliac: the fold was picked up 5-7 cm above the Anterior Superior Iliac Spine (ASIS) at an angle of 45° above the horizontal

The sum of the 4 skin folds was noted and percentage body fat calculated using the equivalent fat content tables (Durnin and Wormersly, 1974), grouped by age and gender.

2.3 Lung function measurements

Lung function data were assessed as part of the medical screening for all studies and also after the incremental test in studies described in chapters 3 and 4. The "Microlab" microspirometer (Micro Medical Ltd., England) was used for this assessment, see Figure 2.2. A disposable mouthpiece was attached to the spirometer. The procedure was verbally explained to each subject and was then carried out on 2 or more occasions to achieve results with less than 2% variation. Wearing a nose clip, to exclude nasal breathing, the subject inhaled maximally from the room and then, having placed the mouthpiece in their mouth, exhaled maximally for as long as possible.

The following 4 measurements were recorded:

Forced Vital Capacity (FVC) in litre (l)

Forced Expiratory Volume in 1 second (FEV_1)

Forced Expiratory Rate (FER) in %

Peak Expiratory Flow (PF) in litre per second ($l.s^{-1}$)

Figure 2.2: "Microlab" microspirometer.

2.4 Haematological Analysis

Single blood samples were taken from the medial cubital vein in the antecubital fossa of the left arm using the vacutainer system; this system involved the use of a 21G 1.5 inch needle (Precision Glide^{TM}, UK) and a 5 ml EDTA tube (Vacutainer Ltd., UK). The blood sample was analysed in the automated cell counter (Coulter Counter System, Model Act Diff, Coulter Electronics, England). Data for the variables haemoglobin (Hb) in $g.dl^{-1}$, haematocrit (Hct) in %, red blood cell count (x $10^{12}.l^{-1}$) and white blood cell count (x $10^{9}.l^{-1}$) were recorded from these blood samples. Where necessary blood samples were also centrifuged at 50,000 rpm, plasma separated and analysed fresh or stored for later analysis of specific variables.

2.5 Heart Rate analysis

Heart rate (HR) was recorded by radiotelemetry, using a Cardiosport heart rate monitor (Cardiosport Ltd., Taiwan), see Figure 2.3. A small amount of ultrasound transmission gel (Aquasonic 100, Parker Ltd., USA) was placed on the transmitter electrodes to allow for more stable readings. During the exercise tests contained in this dissertation the heart rate monitor was positioned on the cyclists back attached to the belt out of the view of the cyclist.

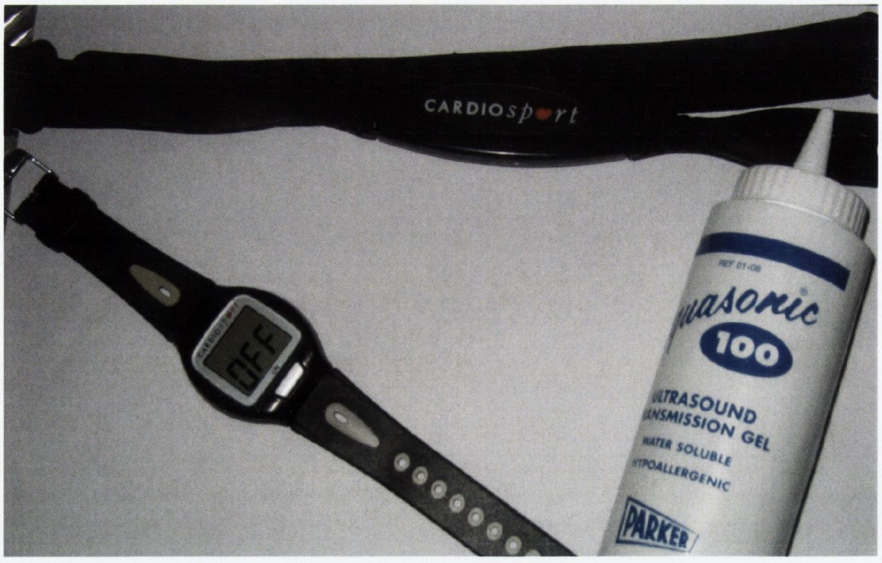


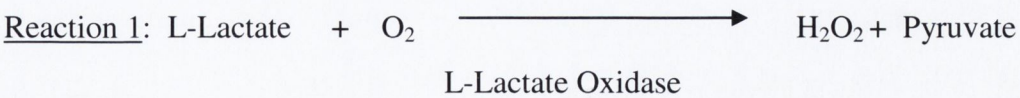
Figure 2.3: Cardiosport Heart Rate monitor and transmitter with ultrasound transmission gel.

2.6 Blood Lactate Analysis

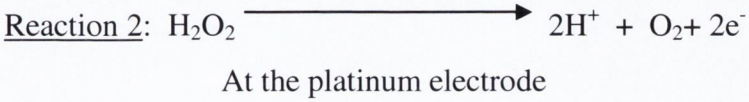
Blood lactate concentrations were measured using the YSI 1500 Sport Lactate analyser (Yellow Springs Instruments Ltd., USA), see Figure 2.4.

Figure 2.4: YSI 1500 Sport Lactate analyser.

The measurement of blood lactate concentration [BLa] utilises a series of chemical reactions within the sensor probe of the analyser. The probe consists of a silver cathode and platinum anode, covered by a three-layered membrane consisting of polycarbonate, immobilized L-Lactate oxidase and cellulose acetate. When a blood sample is injected into the reaction chamber using the 25 µl syringepet, some of the sample diffuses through the membrane. The outer polycarbonate membrane is porous and resists diffusion of enzymes but is large enough to allow the passage of O_2, H_2O, H_2O_2 (hydrogen peroxide), NaCl and lactate. The inside membrane of cellulose acetate is permeable to H_2O_2, but impermeable to ascorbic acid and other substances with a molecular weight greater than 200. When lactate in the blood sample diffuses through the outer polycarbonate membrane and comes into contact with the L-Lactate oxidase membrane, the lactate is rapidly oxidised producing H_2O_2 and pyruvate according to the reaction below.



The H_2O_2 produced passes through the inner membrane of cellulose acetate and is oxidised at the platinum anode producing electrons.



The circuit is completed at the silver reference cathode where the following reaction occurrs:

Reaction 3: $2AgCl + 2e^- \longrightarrow 2Ag + 2Cl^-$

The current produced is linearly proportional to the lactate concentration in the sample chamber. The analyser calculates the lactate concentration of the sample by comparing the current produced by the sample with the reference sample of a known lactate concentration.

Calibration Procedure:

Fresh buffer (500 ml water and 6.35 ± 0.35 g YSI 2357 Buffer (YSI Ltd., USA)) and reference solution (250 ml buffer solution and 2 ml of 30 $mmol.l^{-1}$ lactate standard) were placed in the machine prior to each test. To calibrate the analyser 25 µl of a 5 $mmol.l^{-1}$ lactate standard solution was passed into the reaction chamber using the syringe pipette (YSI Ltd., USA) when prompted by the software. To proceed with testing it was necessary for the 5 $mmol.l^{-1}$ solution to yield a lactate concentration in the range 4.95 to 5.05 $mmol.l^{-1}$.

Testing Procedure:

For the incremental tests, samples for blood lactate analysis were collected using the fingertip capillary method. The subject's fingertip was wiped clean of any sweat or old blood clots using paper towelling and then lanced with a sterile lancet (Softclix®, Germany). The blood sample was collected in a heparinised capillary tube (Brand Ltd., Germany) by holding the tube horizontal and in contact with the sample. The fingertip was then wiped again to remove any excess blood. 25 µl of blood was drawn from the capillary tube using the syringe pipette. The sample was passed into the reaction chamber following the prompt by the software and following measurement the resulting lactate concentration was printed on the LCD display. All lancets and capillary tubes were discarded into a "sharps box".

For any subsequent endurance trials blood samples were collected in 4.5 ml EDTA tubes and aliquots withdrawn to assess blood lactate concentration in a similar manner.

2.7 Blood Glucose analysis

A YSI model 2300 Glucose and Lactate analyser (Yellow Springs Instruments, USA) was used to measure glucose concentration from blood samples taken, see Figure 2.5. Samples were drawn from the 4.5 ml vacutainer using a sipper contained in the analyser before being instilled into a buffer filled chamber. Glucose within the sample then diffuses across a polycarbonate membrane along with oxygen and other molecules. When the glucose comes into contact with L-glucose oxidase the following reaction occurs:

$$\text{L-Glucose} + O_2 \xrightarrow[\text{L-glucose oxidase}]{} \text{Pyruvate and } H_2O_2$$

The H_2O_2 produced diffuses across a thin cellulose acetate membrane, which allows only molecules with a molecular weight of less than 200 to cross, thereby reducing the risk of contamination. The H_2O_2 produced is then in contact with a platinum anode which catalyses the following reaction;

$$H_2O_2 \longrightarrow 2H^+ + O_2 + 2e^-$$

The subsequent electron flow is therefore linearly proportional to the steady state glucose concentration. A buffer solution is used to wash out the chamber after each sample analysis, and if the baseline current is found to be unstable (comparing initial and final baseline currents) the buffer pump ran a second flush cycle. The buffer and 10 $mmol.l^{-1}$ glucose standard are automatically passed from their reservoir bottles (within the machine) into the reaction chamber upon entering the run mode. Auto-calibration is carried out under software control. The glucose reference standard used had a concentration of 10 $mmol.l^{-1}$.

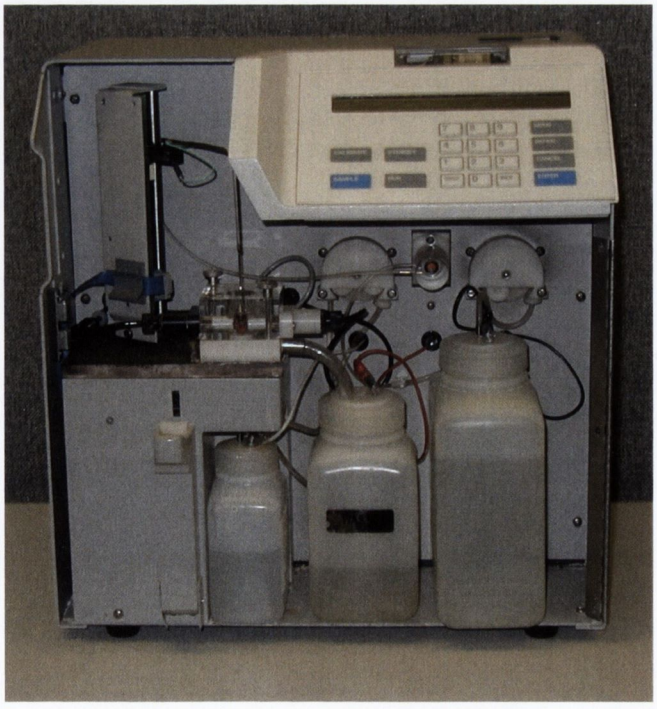


Figure 2.5: YSI model 2300 Glucose and Lactate analyser.

2.8 Metabolic data analysis

Metabolic variables were recorded using the Quark B^2 Cardiopulmonary exercise system (Cosmed Ltd, Italy) on a breath-by-breath basis (Figure 2.6). The system consists of a flowmeter, a zirconia oxygen analyser and an infra-red carbon dioxide analyser. The flowmeter uses a bi-directional digital turbine, air passing through the helical conveyors, takes a spiral motion that causes rotation of the turbine rotor. The rotating blade interrupts an infrared light beam emitted by the 3 diodes of the optoelectronic reader. Every interruption represents a 16.6% turn of the rotor; this allows measurement of the number of turn in a fixed time. There is a constant ratio between air passing through the turbine and the number of turns allowing for the accurate measurement of volume and flow rate. The zirconia oxygen analyser has a range of 1-100% Oxygen (O_2), a response time of <120 ms and an accuracy of <0.05% O_2. The fast carbon dioxide analyser measures carbon dioxide CO_2 concentrations by infra-red radiation absorption (response time <150 ms). The CO_2 that passes through the sensor cell absorbs a certain amount of radiation; the absorption is proportional to the quantity of carbon dioxide in the sample line. The variables of importance measured using this system were:

- Oxygen consumption ($\dot{V}O_2$) in $l.min^{-1}$ or when corrected for body mass, $\dot{V}O_2$ measured in $ml.kg^{-1}.min^{-1}$.
- Minute ventilation ($\dot{V}E$) in $l.min^{-1}$, the volume of air that passes through the lungs in 1 minute.
- Respiratory frequency in $breath.min^{-1}$
- Respiratory exchange ratio (RER= ($\dot{V}CO_2/\dot{V}O_2$). This is the ratio of the volume of CO_2 produced to the volume of O_2 consumed.

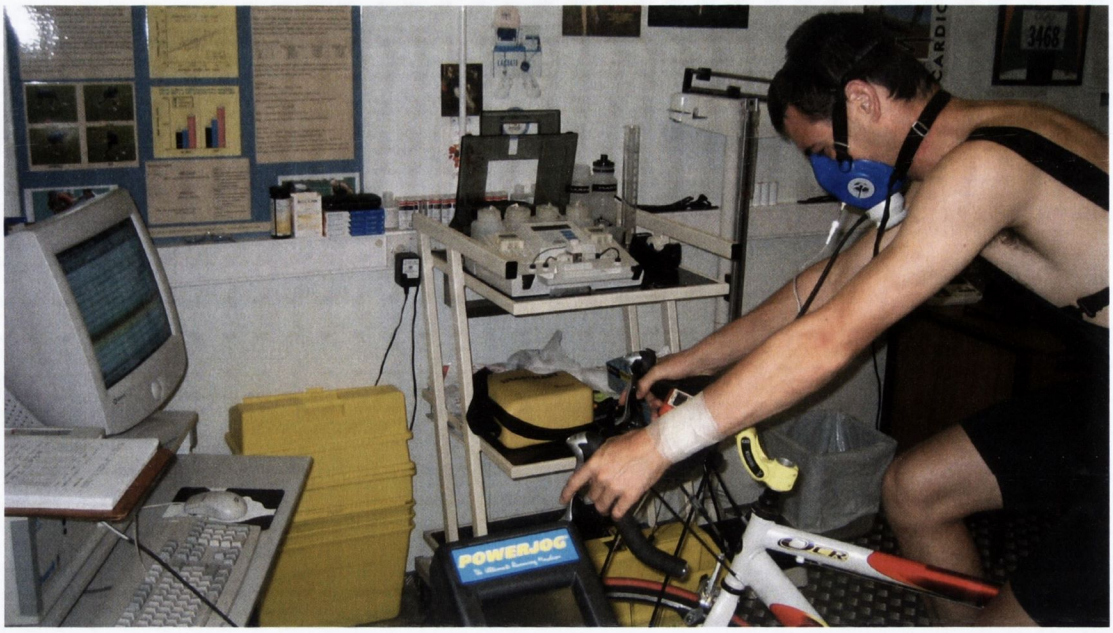


Figure 2.6: Cyclist with face mask attached to the metabolic analyser, positioned on cycle ergometer to begin test.

Calibration procedure:

The Quark B^2 system was powered on 30 min before calibration prior to every test to allow the system to warm up. To perform the gas sensor calibration it was necessary to have a certified cylinder filled with a known mixture of gas, containing 5% CO_2, 16% O_2 and N_2 for balance. Having connected the sampling line into the sampling socket on the front of the metabolic unit the gas sensor was easily calibrated using the software commands of the system. Both room air and a certified standard mixture of gases were calibrated twice. A 3-litre calibration syringe (Cosmed Ltd., Italy) was used to calibrate the flowmeter. The syringe was connected to the optoelectronic reader. Following a prompt from the software the syringe piston was moved in and out for 5 inspiratory strokes and 5 expiratory strokes before the first value appeared on the screen. The syringe piston was then moved through a further 10 inspiratory and expiratory strokes. At the end of the calibration, the percentage errors for inspiration and expiration were displayed and the new calibration factors stored. The volume calibration was also repeated.

The subject wore a facemask (Hans Ruldolph Ltd, USA), which was connected to the metabolic system. Air inspired from the room was expired and analysed for volume and O_2 and CO_2 content by the computerised metabolic cart. It was necessary to attach the mask as tightly as possible with adjustable straps to avoid any volume losses during the test.

2.9 Electrocardiogram (ECG) analysis

ECG analysis was performed automatically by the electrocardiograph (Cardiofax, ECG-8420K, Japan). The electrode pads (Red Dot™, Germany) were attached to the subject at the specified sites (4 limb, 6 chest) and the leads connected as illustrated in Figure 2.7. The rhythm was maintained continuously in the real-time analysis mode for the observation of the recording. An analysis was executed on the 10-second data immediately before the stop time. The EMG filter option was used to reduce the influence of EMG activity on the recorded waveform. Following data capture the ECG was auto-analysed by the software and the printed record viewed by the attending medical physician for clearance for the individual to participate in the subsequent study.

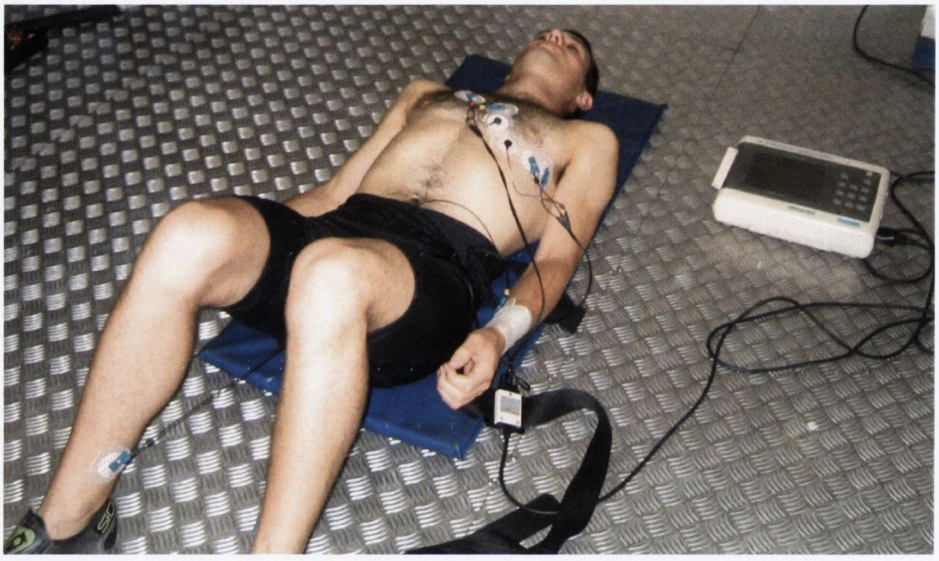


Figure 2.7: The positions of the recording electrodes for ECG analysis.

2.10 Insertion of the IV catheter

Therefore to reduce the discomfort of repetitive blood sampling during the course of the research contained in this dissertation an indwelling catheter was inserted into a forearm vein. The intravenous catheter (20GA 1.1 x 30 mm, Insyte®, Spain) was inserted by a medical practitioner. The area of insertion was thoroughly wiped with an isopropyl alcohol pre-injection swab (Seton Healthcare Ltd., UK). Having inserted the catheter into the forearm vein of the arm a Posiflow extension set (B-D PosiFlow$_{TM}$, Mexico) was attached. A yellow "bung" with injection membrane (Vygon Ltd., Germany) was attached to the end of the extension set for use during sampling (Figure 2.8). The catheter was held secured using albupore taping (Red Dot™, Germany) and a sample drawn immediately using a vacutainer system similar to that described earlier. After each sample was collected the catheter was flushed with a heparinised saline flush containing 10 ml sodium chloride injection BP 0.9%w/v and 2.5 µl heparin injection BP (Antigen Pharmaceuticals Ltd., Ireland) and the clamp closed on the posiflow extension set.

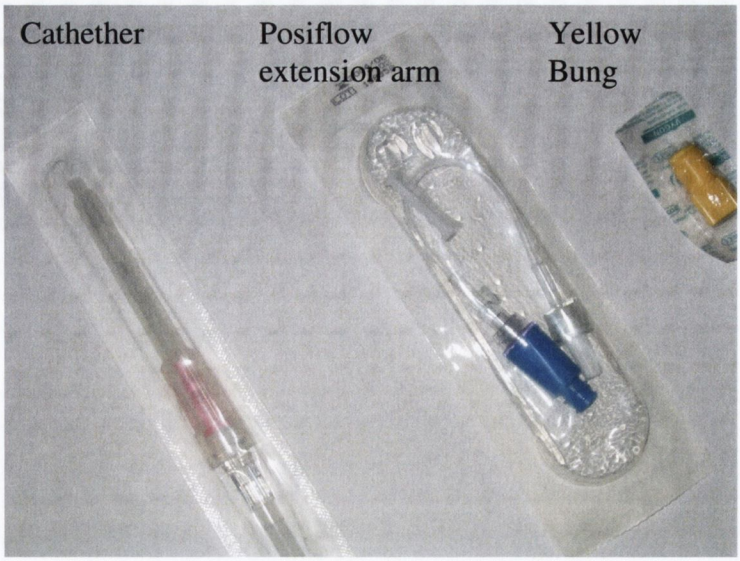


Figure 2.8: IV cathether, posiflow extension arm and yellow bung.

2.11 Bicycle ergometer

During the research described in Chapters 3 and 4, the cyclists used a Giant OCR road-racing bicycle (Giant Europe BV/Nederland, The Netherlands), see Figure 2.9. A SRM training monitor was attached to the handlebars giving continuous digital readouts of power output, cadence and heart rate, see Figure 2.10. The SRM power cranks measure power output by calculating the deformation of patented components within the drive wheel. A strain gauge strip measures the bend of these components, and these characteristics are converted to power, proportionate to the pedalling force. The power is measured from the sum of the tangential forces of the angular velocity of the crank as the cyclists perform each revolution.

Figure 2.9: Giant OCR road racing bicycle (Giant Europe BV/Nederland, the Netherlands).

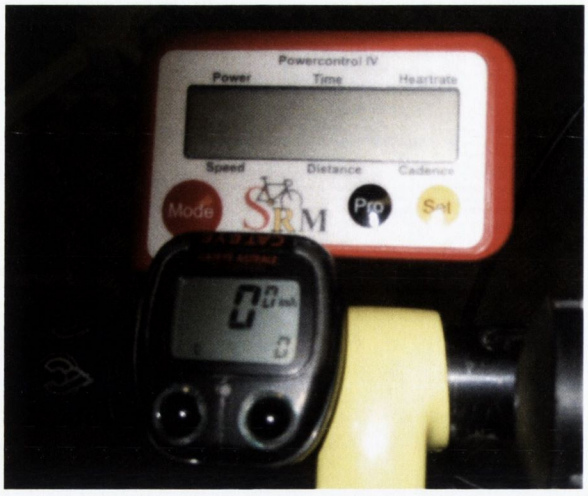


Figure 2.10: SRM training monitor and Cateye speedometer.

2.12 Osmometry

Plasma osmolality was recorded using a micro-osmometer (Model 3300, Advanced Instruments, USA), see Figure 2.11. Osmometers are used for the determination of the concentration of solutions by means of freezing point measurement. By measuring the freezing point, the concentration of an aqueous solution can be easily determined. High precision thermometers are used to measure the freezing point of a sample, determining differences of ± 1 $mOsm.kg^{-1}$ H_2O. The osmometer operates by supercooling the solution to several degrees below freezing point thereby inducing crystallization. Upon reheating the heat of fusion liberated causes the sample temperature to rise and a liquid/solid equilibrium is reached. This equilibrium temperature is the freezing point of the solution. The osmometer contains an operating cradle, sample port and a high precision thermistor probe. The operating cradle guides and introduces the sampler into the freezing chamber. The sampler contains a disposable plastic tip that is replaced after each test. A thermoelectric module controls the cooling temperature, while the thermistor sample probe measures the dynamic temperature of a sample. Test results are based on the freezing point of the sample and are automatically displayed on the LCD screen.

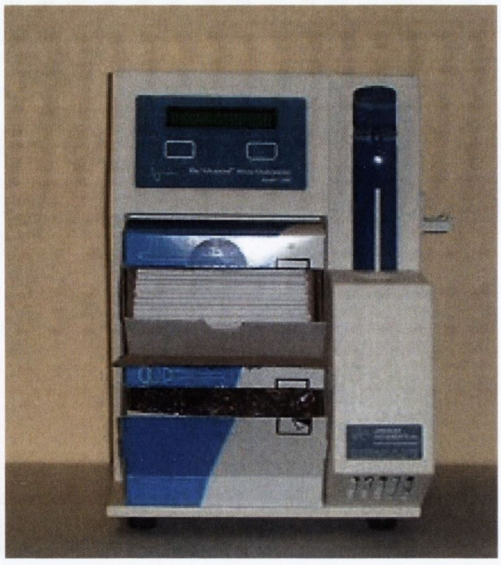


Figure 2.11: Advanced Micro-osmometer, Model 3300.

Calibration procedure:

Prior to sample testing Quality Control (QC) tests were carried out using 50, 290 and 850 $mOsm.kg^{-1}$ standards to ensure repeatability (Advanced Instruments Inc, USA). The calibration procedure was only performed following prompts from the osmometer calibration program. Having entered the calibration program a 50 $mOsm.kg^{-1}$ standard was processed in duplicate similar to the normal test procedure. Following the 2 tests there was a prompt to process a 850 $mOsm.kg^{-1}$ standard. The program reported the sample result and requested another 850 $mOsm.kg^{-1}$ standard until the instrument display reported "calibration complete". The calibration was subsequently verified by running a Clinitrol 290 reference solution prior to testing unknown samples.

Test Procedure:

Plasma and urine osmolality was assessed similarly. Following centrifugation, coded samples were stored at -20°C for subsequent batch analysis. As the osmolality assessment procedure was based on the freezing point depression of the sample it was first necessary to completely defrost the plasma samples prior to osmolality assessment. However having consulted recent literature prior to the start of the final study of this dissertation it was decided to analyse plasma osmolality immediately following plasma separation. It had been reported in a review by Armstrong (2005) that as storage time increases in mild to cool environments plasma osmolality may decrease. A plastic sample tip (Advanced Instruments, Inc., USA) was placed on the sampler and snapped into place. The plunger of the sampler was depressed and the sample tip inserted into the sample to be tested. The plunger was gently released to unload a 20 µl sample ensuring no air bubbles entered the sample tip. It was necessary to ensure that there were no clinging droplets on the tip or fluid protruding from the tip. If so the tip was gently blotted with paper tissue. The

sampler was then placed in the operating cradle beneath the cradle top. To begin the test the entire cradle was pushed downwards until it stopped. The result of the test appeared on the display after approximately 1 minute. Having recorded the result the operating cradle was raised and the sampler removed. A clean dry chamber cleaner (Advanced Instruments, Inc., USA) was then inserted in the sample port an rotated 4 or 5 times in the sample port, removed and inserted again using the opposite end. The sample tip was removed from the plunger by pressing firmly on the plunger and was discarded in a sharps container.

2.13 Non-Esterified Fatty Acid Assaying

An optimised enzymatic colourmetric assay method (Roche Diagnostics Ltd, Germany) was used to determine plasma NEFA concentration. One tablet containing adenosine-5-triphosphate (ATP), acyl-CoA synthetase (Acyl CS), peroxidase, ascorbate oxidase, 4-aminoantipyrine and stabilisers, was added to a bottle of potassium phosphate buffer using a forceps to produce reaction mix A. The tablet was fully dissolved in the buffer before decanting into cuvettes (VWR Instruments Ltd., UK). Into each cuvette 1.00 ml of reaction mix A was added to 0.05 ml of plasma. A blank sample consisting of 1.00 ml of reaction mix A and 0.05 ml of double distilled water was also prepared. The samples were mixed before being placed into a water bath maintained at a temperature of 25°C for 10 min to allow the reaction to occur. In the presence of Acyl CS, non-esterified fatty acids are converted into acyl-coenzyme A (acyl-CoA) by ATP and coenzyme A (CoA), which results in adenosine-5-monophosphate (AMP) and pyrophosphate being produced in the following reaction:

$$\text{NEFA} + \text{CoA} + \text{ATP} \xrightarrow[\text{Acyl CS}]{} \text{acyl-CoA} + \text{AMP} + \text{pyrophosphate}$$

After this 10 min period, the samples were removed from the water bath and to each sample, including the blank sample; 0.05 ml of N-ethyl-maleinimide-solution was added. Excess moisture was removed from the cuvettes with a lint free cloth to prevent diffraction of the light beam and each sample was mixed gently before the absorption of the samples after reaction A (A_1) was measured using a photo spectrometer (Cecil CE1011, Cecil Ltd., UK). Absorption was measured in the visible range at a wavelength of 546 nm. The presence of N-ethyl-maleinimide in the test is necessary for the removal of an existing surplus of CoA before the oxidation of the activated fatty acids by acyl-CoA oxidase (ACOD)

For reaction B, a tablet containing ACOD and stabilisers was added and dissolved in approximately 0.05 ml of ACOD dilution solution containing stabilisers to create reaction mix B. To each cuvette, 0.05 ml of reaction mix B was added before the solutions in the cuvettes were mixed and again placed in the water bath for a further 15 min period. During this time Acyl-CoA reacts with oxygen (O_2) in the presence of ACOD to form 2,3-enoyl-coenzyme A (enoyl-CoA) and hydrogen peroxide (H_2O_2), in the following reaction:

$$\text{Acyl-CoA} + O_2 \xrightarrow[\text{(ACOD)}]{} \text{enoyl-CoA} + H_2O_2$$

The resulting H_2O_2 converts 2,4,6-tribromo-3-hydroxy-benzoic acid (TBHB) and 4-aminoantipyrine (4-AA) to a red dye in the presence of peroxidase (POD), in the following reaction:

$$H_2O_2 + \text{4-AA} + \text{TBHB} \xrightarrow[\text{(POD)}]{} \text{red dye} + 2\,H_2O + HBr$$

After this 15 min period the cuvettes were removed from the water bath, dried with a lint free cloth and the absorption of the samples after reaction B was measured (A_2). The red dye produced by reaction B was measured in the visible range at 546 nm. The absorbance differences ($A_2 - A_1$) for both blank and samples were calculated to provide absorbance difference for the blank sample (ΔA_b) and the absorbance difference of the sample (ΔA_s). For every 10 samples a blank was assessed. ΔA_b was subtracted from ΔA_s to provide the absorbance difference (ΔA) as follows:

$$\Delta A = \Delta A_s - \Delta A_b$$

To calculate NEFA concentration the following equation was used:

$$c = (V/\epsilon \times d \times v) \times \Delta A \ (\text{mmol.l}^{-1})$$

where, V = final volume of each sample solution in the cuvette,

v = sample volume in ml

d = light path in cm

ϵ = absorption coefficient of the dyestuff at 546 nm (19.3 [l mmol l^{-1} cm^{-1}]), depended upon the type of buffer, the pH of the assay system and the purity of TBHB. Under the conditions stated above, it varied from 19.1 to 19.5 l mmol.l^{-1} cm^{-1}.

Substituting for constants in the above equation yields an NEFA concentration in mmol.l^{-1}:

$$c = (1.15/19.3 \times 1 \times 0.05) \times \Delta A \longrightarrow 1.192 \times \Delta A \text{ (mmol } l^{-1})$$

2.14 Caffeine concentration analysis by ELISA

A commercially available ELISA test kit (Neogen Ltd., USA) was used to detect and assay caffeine in plasma samples. The kit operates on the principle of competition between the caffeine in the sample and the drug enzyme conjugate for a limited number of antibody binding sites.

Before proceeding with the test on plasma samples it was necessary to determine a standard curve using known caffeine concentrations. The concentrations of caffeine used were 200, 150, 100, 50, 25, 12.5 and 6.25 ng.ml^{-1} and water as a control. The standard curve was determined by running the ELISA test using these standards. 8 aliquots of each standard were used together with 4 positive and 4 negative control samples supplied with the kit as qualitative controls. By assaying each standard sample 8 times on 1 plate it was possible to assess the reproducibility and reliability of the kit and the measurement technique. When analysing the plasma samples each sample was assayed in duplicate on each plate. Also each of the standards were assayed in duplicate and 4 positive 4 and negative controls were also assayed on each plate.

Test Procedure

1 µl of drug enzyme conjugate was diluted in 180 µl of EIA buffer for each well. The solution was prepared for 100 wells to account for any losses incurred when using a multi channel pipette. 20 µl of the standard caffeine solutions (200, 150, 100, 50, 25, 12.5 and 6.24 ng.ml^{-1}) and water were added in duplicate to the wells. Four 20 µl samples of positive and negative controls (Neogen Ltd., USA) were also added to the plate. 10 µl of the plasma samples were added in duplicate to the plate and 190 µl of drug enzyme conjugate was added to each well containing a plasma sample, while 180 µl was added to all other wells (standards and positive and negative controls).

Preliminary assaying indicated that caffeine concentrations in the plasma samples were deemed to be outside the detectable range of the kit, to overcome this problem the volume of sample added to each well used for plasma samples was halved from 20 µl to 10 µl and the volume of drug enzyme conjugate added to the wells was increased from 180 µl to 190

µl. This dilution factor was accounted for in the subsequent calculations of individual caffeine concentrations.

The plate was shaken gently, covered and incubated at room temperature for 45 min. Concentrated wash buffer was diluted 10 fold with ultra-pure double distilled water, (180 ml of water and 20 ml of concentrated wash buffer). When the incubation period was complete the liquid was discarded from the plate and tapped gently on a paper towel to remove and remaining liquid. The plate was washed 3 times with 300 µl of wash buffer per well, inverted and tapped dry between each wash. 150 µl of K-blue substrate (Neogen Ltd., USA) was then added to each well and the plate was gently shaken and incubated for 30 min at room temperature. 50 µl of red stop solution (Neogen Ltd., USA) was then added to each well to terminate the enzyme reaction. The manufacturers recommended that the absorbance was recorded at a wavelength of 650 nm, however 630 nm was the highest wavelength possible on the microplate reader (Fluoroskan Ascent FL, Medical supply co. Ltd., Ireland) in the laboratory and was deemed sufficient.

Testing Principle

During the 45 min incubation period after the sample and drug enzyme conjugate have been added to the well, competition between the drug in the sample and the drug enzyme conjugate for binding to specific sites on antibodies that are immobilized in the wells occurs. Washing the plate 3 times removes any unbound sample or drug enzyme conjugate. The addition of K-blue substrate recognises the presence of the bound drug enzyme conjugate. 30 min after substrate addition the reaction is halted with the addition of red stop solution. The absorbance is measured at 630 nm. The extent of colour development is inversely proportional to the amount of caffeine in the sample or control, therefore the presence of caffeine results in decreased or no colour development.

Data analysis

Due to the exponential nature of the standard curve for optical density versus caffeine concentration, a log-log transformation of optical density against concentration was computed and linear regression analysis yielded correlation coefficient data. Following manipulation the equations were applied to determine the concentration of caffeine present in the samples analysed.

The sample results obtained were expressed in $ng.ml^{-1}$. To account for the 50% dilution factor applied during the assay all results for plasma concentrations obtained were multiplied by a factor of 2 to yield the corrected plasma caffeine concentration.

2.15 Bioelectrical Impedance Analysis (BIA)

A BodyStat (DualScan 2005) dual frequency analyser was used to assess impedance, see Figure 2.12. The principles and theory behind the use of BIA have been outlined earlier; see section 1.10.1. In summary, bio-impedance instruments apply a low amperage current that passes between source and detector, normally placed on the wrist and ankle, see Figure 2.13. Ions in aqueous solution transmit the current and therefore conductivity is proportional to the concentration of ions. The DualScan unit applies 2 currents; one at a low frequency of 5 kHz passing mainly through extracellular tissues and the second at 200 kHz penetrating cell membranes and therefore passing through the intra- and extracellular tissues. The unit then uses predictive regression equations at those frequencies to estimate ECW and TBW. ICW is then calculated by subtraction. Total body mass is necessary for the use of the predictive equations.

Calibration:

The DualScan 2005 unit automatically calibrates before each measurement. Also a separate calibrator is supplied to independently ensure the unit is in calibration at all times. The calibrator was used regularly before bio impedance measurements.

The calibrator has 2 terminals. One pair of red and black leads was attached to each terminal of the calibrator. The DualScan unit was powered on and the default displays accepted on the LCD screen. After "measuring" is displayed it is possible to scroll through the results until the impedance values are reached. The results of 5 kHz and 200 kHz should read in the range 496 to 503 to ensure accurate measurements in subsequent tests.

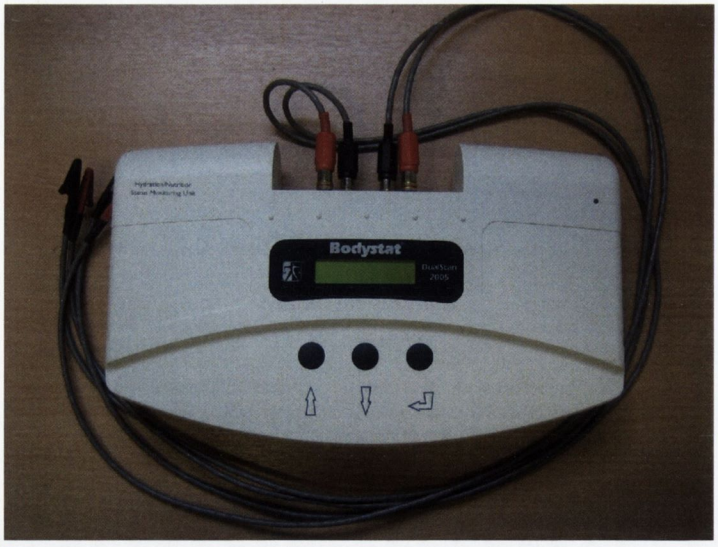


Figure 2.12: Bodystat (DualScan 2005)

Testing Procedure:

The subjects' height and body mass were recorded and entered on the unit appropriately. It was also necessary to enter the subjects' age. Having removed the right shoe and sock the

subject was required to lie flat with arms and legs spread slightly so that no parts of the body were touching one another. The self-adhesive disposable electrodes were attached to the right foot and right hand. The black leads were connected to the electrodes on the right wrist and right ankle. The red leads were attached behind the toes on the foot and behind the knuckles on the hand, see Figure 2.13. The subject remained lying flat for 4-5 min prior to any measurement and 2 measurements were performed at each time point to ensure reproducibility. The following recordings were noted from the LCD screen on the unit ECW, ICW, TBW and impedance at 5 and 200 kHz.

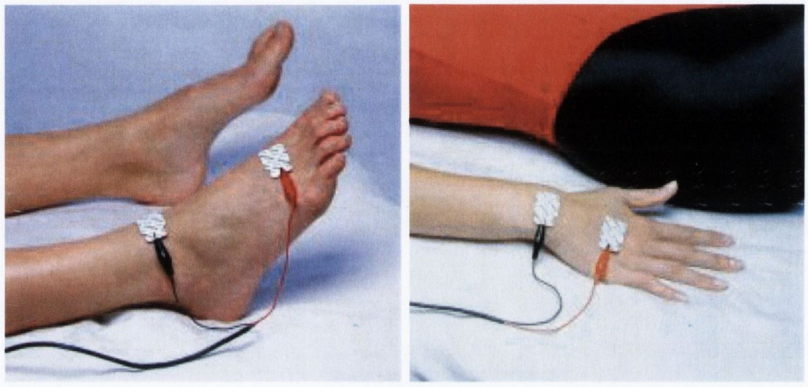


Figure 2.13: Positioning of electrodes for BIA.

2.16 Urine specific gravity analysis

Specific gravity was assessed on urine samples using a handheld refractometer (Eclipse, Bellingham and Stanley. United Kingdom), see Figure 2.14.

Testing Principle:

Refractometry is the method of assessing the composition or purity of substances by measuring their refractive index. The refractive index of a substance is strongly influenced by temperature and the wavelength of light used to measure it. The refractometer utilizes lenses and prisms to projects a shadow line onto a small glass reticle inside the instrument, viewed by the user through a magnifying eyepiece. In the case of using the refractometer to measure urine specific gravity a sample of urine is sandwiched between a measuring prism and a cover plate. Light travelling through the sample is either passed through to the reticle or internally reflected. The result is that a shadow line is formed between the illuminated area and the dark area. It is at the point that this shadow line crosses the scale that a reading is taken.

Procedure:

A small sample of urine (approx 1 drop) was drawn using a pipette from the urine sample to be analysed. With the flap in the upward position the sample was allowed drip onto the

prism and the flap closed. The refractometer was held up to the light and by looking through the eyepiece it was possible to read the urine specific gravity. The scale ranges from 1.00 to 1.04 and the reading was recorded to an accuracy of 0.001.

Following the reading of each sample the prism and flap were washed immediately with water and dried with a clean cloth.

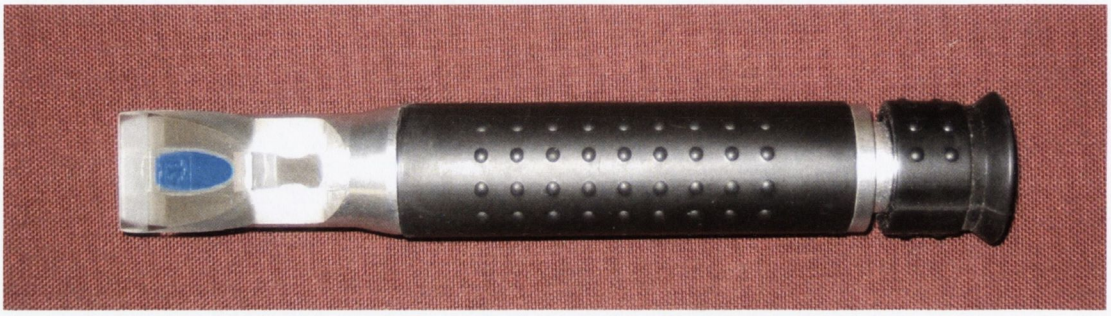


Figure 2.14: Handheld refractometer (Eclipse, Bellingham and Stanley, United Kingdom).

2.17: Serum and urinary electrolytes

A Corning 400 flame photometer (Corning Ltd., USA) was used to measure serum and urinary electrolytes of interest from samples collected during the research described in Chapter 6, see Figure 2.15. Flame photometry is a common, relatively inexpensive method used in the detection of metal salts principally Na, K, Li, Ca and Ba. Solutions containing these metal salts are brought in contact with a hot flame that evaporates the solvent, atomises the metal and excites a valence electron in the metal salt to a higher energy state. Light is emitted at characteristic wavelengths for each metal as the excited electron returns to the basal ground state. By comparing the emission intensities of the samples to standard solutions it is possible to determine the quantity of metal in the analysed samples.

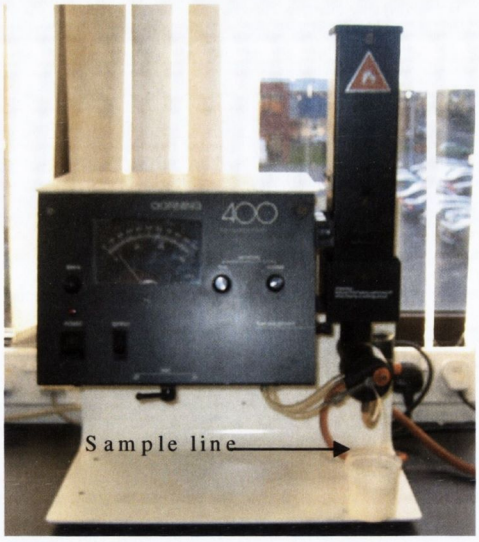


Figure 2.15: Flame Photometer (Corning Ltd., USA).

Procedure:

The following standard solutions were accurately prepared in triplicate using KCl and NaCl salts:

5 mmol.l^{-1} K^+

150 mmol.l^{-1} K^+

150 mmol.l^{-1} Na^+

The samples were prepared in a 1:100 dilution with distilled water.

To calibrate the flame photometer the display readout was adjusted to zero by placing the sample line in contact with distilled water. Then using the known standard the peak reading was adjusted. This was achieved by placing the sample line into the tube containing the known standard and setting the photometer reading on the readout to 100. To check for accuracy and repeatability the standards were measured several times using all the prepared triplicates of the same standard. The study samples to be analysed were fully defrosted prior to testing. They were prepared by pipetting 80 µl of sample into 2 separate clean plastic tubes and diluting them with 7.92 ml of ultra-pure deionised distilled water using an automated diluter, this resulted in a 1:100 dilution. To analyse the collected urine samples for urinary [Na^+] and [K^+] they were compared to the known 150 mmol.l^{-1} Na^+ and K^+ standards. To analyse the serum samples for the presence of Na^+ and K^+ the 150 Na^+ and 5 mmol.l^{-1} K^+ standards, respectively, were used. The sample line was aspirated with deionised water between each sample to clean out the sample line and the aspirator. During the batch sample analysis the known standards were regularly passed through the flame photometer to ensure accurate and reproducible readings of the samples. The standard solutions were diluted by the same factor as the samples being analysed and

so the concentration of the electrolytes under investigation could be read directly from the flame photometer.

2.18: Composition of test drinks

	Lucozade Sport (bottle)	Lucozade Sport (powder)	Red Bull	isoRB	Water
Carbohydrate ($g.l^{-1}$)	64	64	112	70	Nil
Caffeine ($mg.l^{-1}$)	Nil	Nil	320	200	Nil
Osmolality ($mOsm.kg^{-1}$)	286.3 ± 2.5	270.6 ± 2.6	646.2 ± 10	403.3 ± 5.8	8.5 ± .8
Sodium ($mg.l^{-1}$)	470	470	800	500	25
Potassium ($mg.l^{-1}$)	9.6	9.6	Nil	10.1	27

Table 2.1 Composition of all test drinks used during the research contained in this dissertation.

2.19: Protocol for the incremental test to failure

For study 1 (Chapter 3) and study 2 (Chapter 4) each subject performed a maximal incremental test to volitional exhaustion to determine loads equivalent to % $\dot{V}O_2max$ to be used on the 3 subsequent drinks trials. On arrival at the laboratory each subject completed a detailed medical questionnaire and underwent a detailed medical examination to rule out any contraindications to maximal exercise. Cardiovascular and respiratory variables were assessed; in addition the attending physician examined the throat and lymph glands. Lung function data were measured using spirometry. Skinfold thickness was measured using a skinfold calliper at 4 sites; biceps, triceps, sub-scapular and supra-iliac to estimate percentage body fat. Height and body mass were also recorded. A blood sample was taken from the cubital vein and a full haematological assessment carried out using the Coulter counter. The subject was then required to adjust the bicycle to best suit them before completing a 10 min warm up at a nominal workload of 120 W. Following a period of stretching, the incremental test began with a 3 min rest period, during which time the cyclist was in his normal cycling position. During this time and throughout the incremental test the subject wore a heart rate transmitter and a facemask connected to the Quark B^2 metabolic system. Following the 3 min rest element, exercise began at 120 W for 3 min. The workload was increased by 40 W every 3 min thereafter until volitional exhaustion. Immediately after the test a warm down period of 10 min was completed at 120 W. Post-exercise lung function data were assessed out to rule out exercise-induced broncho-constriction.

Figure 2.16 illustrates the time lines for data collection during the incremental test.

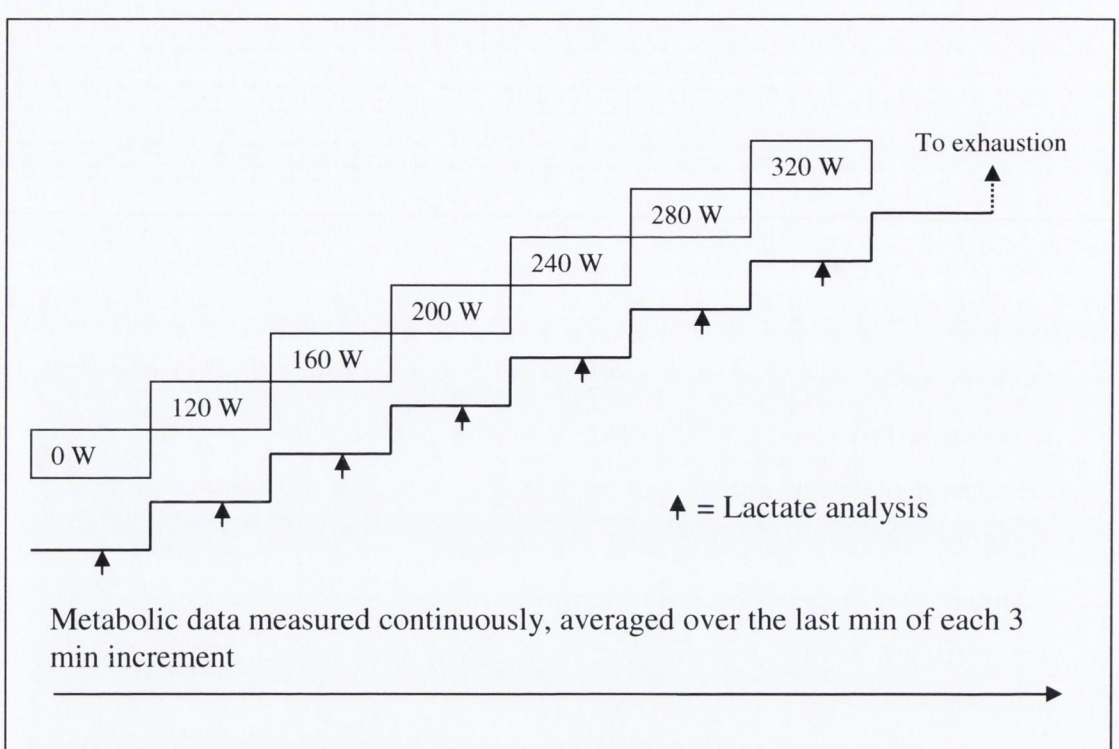


Figure 2.16: Schematic of data collection during the incremental test to exhaustion.

Chapter 3

An investigation of pre- and post-exercise ingestion of commercially available drinks on exercise performance and rehydration in male cyclists.

3.1 Introduction

Fluid and carbohydrate ingestion before, during and after exercise is common practice among the vast majority of athletes today. Research performed in the 1970's investigating the effect of carbohydrate supplementation on exercise performance did not report a statistically significant benefit of carbohydrate ingestion on exercise performance except in subjects suffering from neurogluopenia (Coggan and Coyle, 1991). However as interest in this area escalated in the 1980's and 1990's much of the research reported significant improvements in exercise performance following carbohydrate ingestion and principally during endurance exercise (Coyle *et al.*, 1986; Wright *et al.*, 1991; Okanu *et al.*, 1988). In all of the above studies the carbohydrate supplement was ingested prior to endurance exercise; however the timing of supplement ingestion differed across the cited studies. The increased use of commercial sports drinks containing carbohydrate and in some cases carbohydrate and caffeine has prompted additional research in this area. However, the protocols used in the research vary considerably which therefore makes it difficult to establish a clear understanding of the effects of carbohydrate supplementation on exercise performance.

With the arrival of Red Bull onto the market the effects of caffeine ingestion on exercise performance have become a regular feature in scientific journals (Armstrong, 2002; Hunter *et al.*, 2002; Graham *et al.*, 1998). There are also specific studies investigating Red Bull itself (Geiss *et al.*, 1994; Baum and Weiss 2001; Bichler *et al.*, 2006). While there is a substantial amount of research in the areas of carbohydrate and caffeine ingestion there is little evidence comparing the effects of commercially available sports drinks on exercise performance in scenarios reflective of common cycling practice and therefore the present study was completed in this light.

The aim of this study was to investigate the possible ergogenic effect of a carbohydrate or a caffeinated carbohydrate beverage compared to water during constant load endurance cycling exercise in non-overnight fasted racing cyclists.

The objective of this study was to assess any additive effects that caffeine may have on exercise performance compared to a carbohydrate beverage. Following exercise the test drinks were again ingested to assess any differences that may exist during a monitored post-exercise rehydration phase.

3.2 Methods

3.2.1 Study design

This study was un-blinded and randomised involving 4 visits in total to the Human Performance Laboratory, Trinity College Dublin. Ethics approval was granted by the Faculty of Health Sciences ethics committee. The first visit to the laboratory involved a standard incremental cycling test to volitional exhaustion followed by 3 subsequent visits, separated by approximately seven days, where subjects performed a cycling exercise trial to failure. For each exercise trial subjects received a different test drink detailed below. It was not possible to perform a blinded study because the distinctive taste of some of the drinks did not allow such a scenario.

3.2.2 Details of the drinks

In this study Lucozade Sport (LS, bottle), Red Bull (RB) and Water were the 3 test drinks. They were ingested iso-volumetrically, with the volume ingested calculated to be equivalent to a caffeine load of 3 $mg.kg^{-1}$ during the Red Bull trial. Graham and Spriet (1995) had previously reported a statistically significant ($P<0.05$) improvement in performance (22%) using this dosage in male endurance runners. Two thirds of the test drink was ingested 60 min pre-exercise and one-third 30 min post-exercise. For example a 74.4 kg subject received 700 ml pre- and 350 ml post-exercise. This computation resulted in consumed volumes equating to 9.37 $ml.kg^{-1}$ of the test drinks under investigation 60 min pre-exercise and a supplemental post-exercise bolus of 4.68 $ml.kg^{-1}$ administered at 30 min into the rehydration phase. These volumes equated to 43.8 and 21.9 g of carbohydrate for LS and 60.2 and 30.1 g carbohydrate for RB being consumed pre and post-exercise, respectively. The composition of LS, RB and W are described in Table 2.1.

3.2.3 Subject recruitment

10 males were recruited to participate in this study. They were recruited from the official website www.irishcycling.com and from cycling clubs in the greater Dublin area through contacts established by other members of the Human Performance Laboratory. All subjects were racing cyclists competing in category A, B or C, the senior racing grades in Ireland. All subjects received an information sheet and completed a consent form prior to beginning the test. The study protocol was clearly explained to each individual verbally and in the supplied written information sheet.

3.2.4 Inclusion and exclusion criteria

All subjects were male aged between 18 and 37 yr. Any volunteer displaying any respiratory, cardiac or systemic disorder at the time of medical screening that may have been affected by maximal exercise was excluded from the study. Also those with biomechanical difficulties or caffeine abstainers/caffeine naïve subjects were excluded.

3.2.5 Medical screening

A full medical examination, carried out by a registered medical practitioner specializing in sports and exercise medicine was performed on each subject prior to the maximal incremental test. Blood pressure, lymph nodes, heart and respiratory sounds and chest expansion were examined. Percentage body fat was estimated using skinfold measurements from four sites as described in section 2.2. Lung function was assessed to rule out any respiratory disorders, and again after the incremental test to rule out any exercise induced respiratory obstruction. The use of the micro-spirometer is described in section 2.3. Blood samples were collected from the medial cubital vein in the antecubital fossa of the left arm during medical screening to record red blood cell count (RBC), white blood cell count (WBC), haemoglobin (Hgb) and haematocrit (Hct).

3.2.6 Anthropometric measurements

Height and body mass were measured during medical screening at visit 1. At the beginning of each drinks trial body mass was measured post urine collection. The volume of test drink to be ingested was determined from this body mass recording.

3.2.7 ECG analysis

An ECG was performed on each subject as part requirement of the medical examination to rule out any cardiac problems that may be affected by caffeine ingestion during the study, see section 2.9.

3.2.8 Incremental test to exhaustion

The protocol used for the incremental cycling test to volitional exhaustion is detailed in section 2.19. Using the data collected from the incremental test, a graph was plotted for each subject for HR, blood lactate and $\dot{V}O_2$ against workload, and loads equivalent to 65 and 85% $\dot{V}O_2$ max were calculated for each subject. These individually calculated workloads were used in the 3 subsequent time to failure trials.

3.2.9 Time to failure trial

Each subject completed 3 endurance trials of approximately five hours duration during which time one of 3 drinks under investigation was ingested: RB, LS or Water, the volume determined from pre-test body mass measurements. The subject ingested the test drink at the beginning of a 60 min pre-exercise period. The volume ingested was equivalent to that which would provide 3 $mg.kg^{-1}$ of caffeine during the RB ingestion trial. Also 30 min post-exercise a rehydration bolus was ingested in a volume equivalent to 50% of that ingested pre-exercise.

During the 60 min pre-exercise period HR data were noted every 5 min. After 40 min rest subjects completed a 20 min warm-up (10 min @120 W) and stretching period (10 min) until the beginning of exercise. The first 60 min of exercise was completed at a workload equivalent to 65% $\dot{V}O_2$max determined from the incremental test (see Section 2.19 and Figure 2.16). This period was followed immediately by 30 min of cycling at a load equivalent to 85% $\dot{V}O_2$max. On completion of this endurance element the cyclist then began a series of sprints of 1 min duration with 1 min active rest between each sprint. The sprints were completed at a load equivalent to 95% $\dot{V}O_2$max, while the active rest was at a load equivalent to 65% $\dot{V}O_2$max. Time to failure was defined as the point when the subject was unable to maintain the workload of that particular section of the trial. A subsequent 10 min warm down period ensued before body mass was re-measured. The rehydration and recovery phases were monitored over the following 90 min period. Following a 30 min post-exercise period where no fluid was ingested, 50% of the individual pre-exercise volume of the test drink was consumed as a rehydration bolus and the subject was monitored for a further 60 min. Schematic 1 depicts the order of data collection during the drinks trials.

3.2.10 Haematological, cardio-respiratory and urinary analysis

An indwelling venous catheter was placed in the forearm at the beginning of the trial to allow for regular blood sampling during the trial. The materials and techniques necessary for the insertion and use of the canula are described in section 2.10. The timing of blood sampling is outlined in Schematic 1. In summary, blood samples were used to analyse blood lactate and glucose concentrations, plasma volume changes, plasma osmolality and plasma caffeine concentrations.

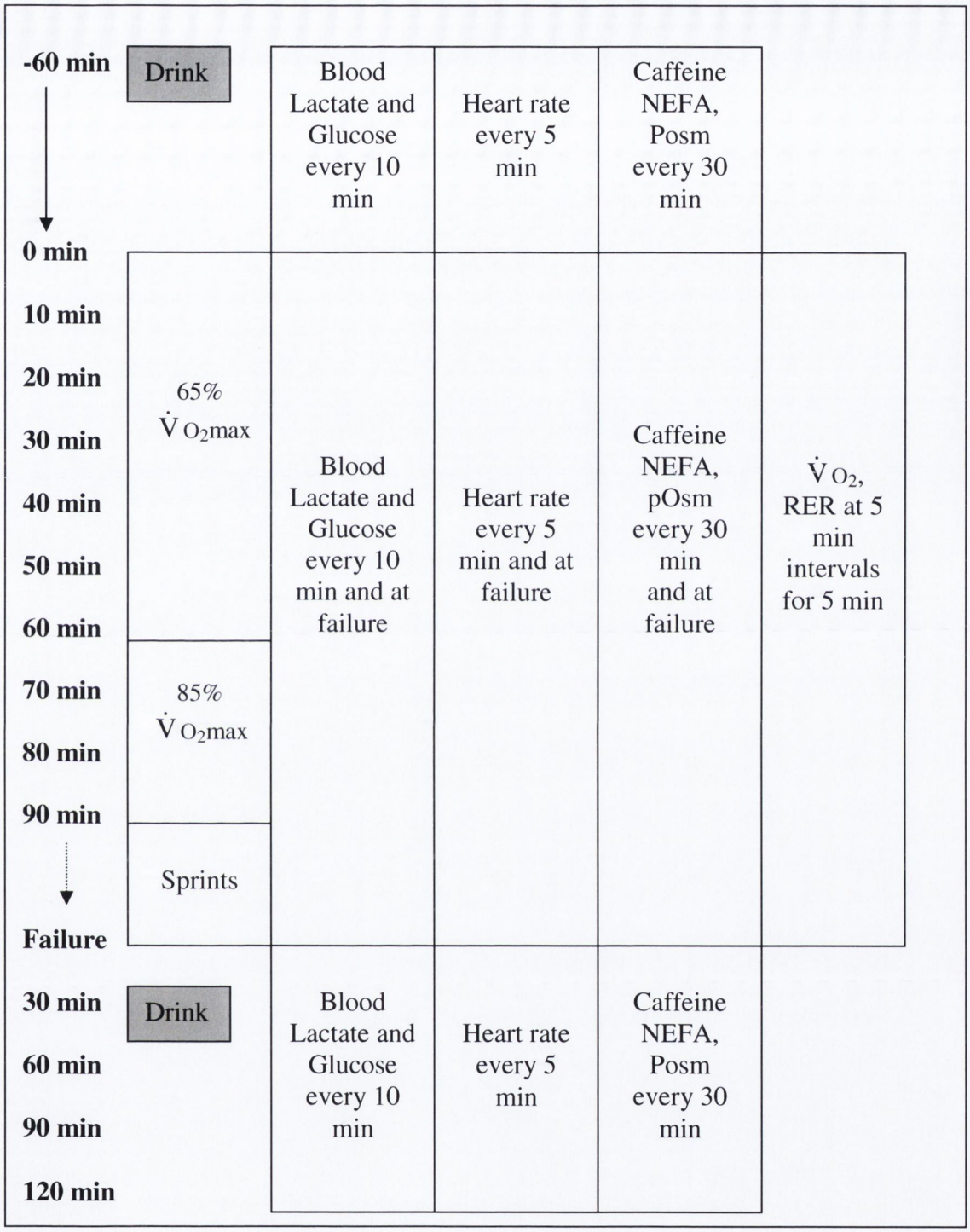
Blood samples collected in EDTA tubes were analysed for Hgb (Coulter Counter) and Hct (centrifugation in triplicate) for the calculation of plasma volume changes according to the equations described by Dill and Costill (1974). These blood samples were also used to measure blood lactate and glucose concentrations. In addition, blood samples collected in clean dry tubes were spun down, the plasma separated and frozen for further analysis of plasma caffeine concentrations (See section 2.14). Plasma osmolality was calculated from blood samples collected pre-drink, pre-exercise, after 30 and 60 min of exercise, at failure and every 30 min during the rehydration phase, see Schematic 1. The micro-osmometer used is described in detail in section 2.12. Heart rate data were recorded at 5 min intervals during the pre-exercise phase, throughout the first 90 min of exercise and during the post-exercise phase. Heart rate was also recorded at the end of each sprint, and at failure.

3.2.11 Metabolic analysis

During the drinks trials metabolic data were recorded at specific time points outlined in Schematic 1. $\dot{V}O_2$ and RER were recorded in 5 min blocks during the first 90 min of exercise and continuously thereafter until failure. $\dot{V}O_2$ data were also recorded at rest. No metabolic data were collected during the post-exercise phase.

3.2.12 Statistical analysis

All subject physical characteristics are presented in table format as mean ± standard deviation (SD) and all graphical data as mean ± standard error of the mean (SEM). A two-way repeated measures ANOVA (time by drink) was used to assess the effects of the 3 drinks across time on measured variables, with *post-hoc* Bonferroni tests used to quantify any significant differences detected using Sigma-stat. A one-way repeated measures ANOVA was used to assess the effects of the 3 drinks on performance time. For all statistical tests values of $P<0.05$ were considered statistically significant.



Schematic 1: Order of data collection during the drinks trials.

3.3 Results

3.3.1 Physical Characteristics

Anthropometric data:

The mean (± SD) physical characteristics of the subjects are presented in Table 3.1. The physiological profile of the group based on $\dot{V}O_2max$ and maximum workload compared favourably to that of normal racing cyclists throughout the country (in- house laboratory database). Therefore, the physiological responses to the 3 test drinks observed in this study should reflect that experienced by amateur Irish cyclists. Cyclists in this study had been competing for 10 ± 2 yr.

	Mean ± SD
Age (yr)	27 ± 6
Body mass (kg)	74.2 ± 5.2
Height (m)	1.8 ± 0.1
BMI ($kg.m^{-2}$)	22.5 ± 1.6
Body fat (%)	11.4 ± 2.2
VO_2max ($ml.kg^{-1}.min^{-1}$)	70.5 ± 7.5
Max workload (W)	368 ± 32

Table 3.1: Mean physical (± SD) characteristics, n=10.

Haematological and pulmonary data

Haematological and pulmonary function test data were normal, with no signs of sub-clinical infection, or obstructive or restrictive airway disorders (see Table 3.2).

Haematological data	**Mean ± SD**	**Pulmonary data**	**Mean ± SD**
Hb ($g.dl^{-1}$)	14.7 ± 1.0	**FEV_1 (l)**	4.6 ± 0.5
Hct (%)	42.9 ± 2.7	**FVC (l)**	5.5 ± 0.4
RBC ($x10^{12}.l^{-1}$)	4.6 ± 0.3	**PF ($l.min^{-1}$)**	638 ± 85
WBC ($x10^{9}.l^{-1}$)	5.4 ± 1.2	**FEV_1/FVC (%)**	85.2 ± 8.2

Table 3.2: Mean (± SD) haematological and pulmonary data at rest, n=10.

3.3.2 Time to failure

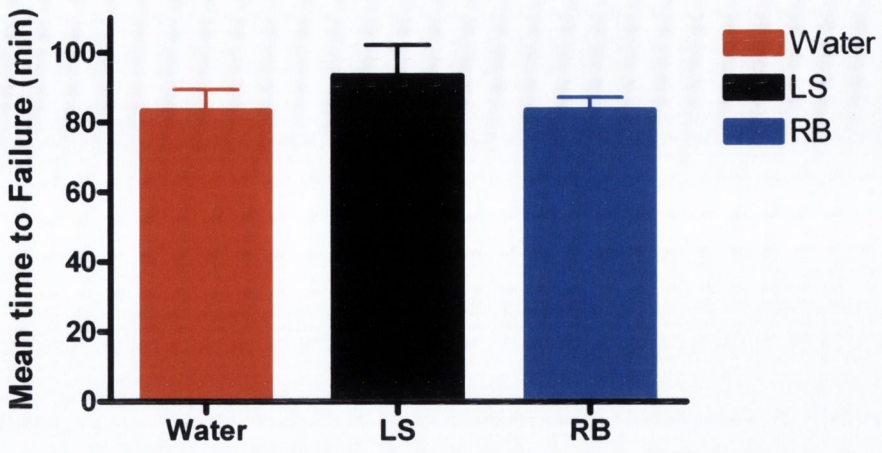


Figure 3.1: Mean (± SEM) time to failure across trial, n=10.

There were no significant differences observed in time to failure (repeated measures ANOVA, $P<0.05$) across the 3 trials. Mean time to failure data recorded for the 3 drinks were 83.2 ± 6.1, 93.3 ± 8.8 and 83.5 ± 3.6 min for Water, LS and RB, respectively.

3.3.3 Heart Rate

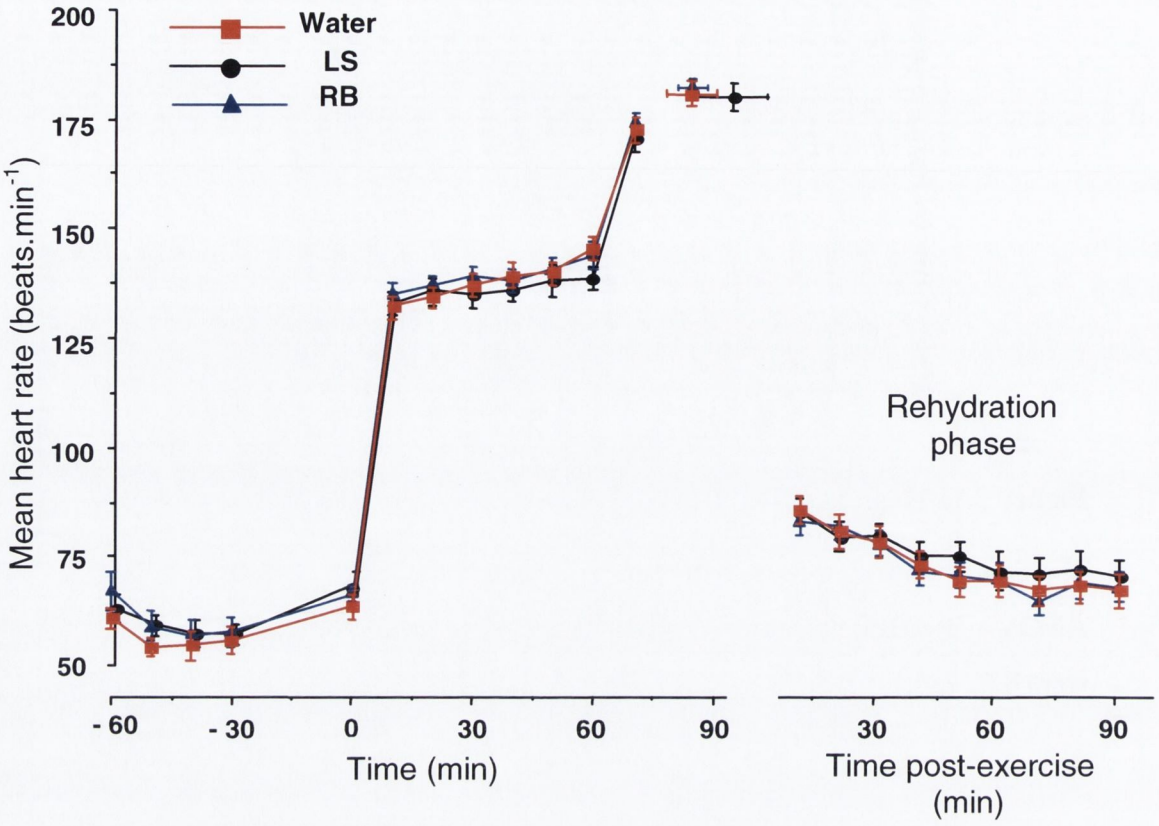


Figure 3.2: Mean (± SEM) heart rate across time and trial, n=10.

There were no significant differences in heart rate data across the 3 trials when the data were analysed at pre- exercise, every 10 min during the first 80 min of exercise, at failure and every 10 min post-exercise for 90 min. Mean heart rate data at resting time points (-60-0 min and 0-90 min post-exercise) were significantly lower ($P<0.001$) than all other time points analysed for all 3 drinks treatments. Increasing exercise intensity from a load equivalent to 65% $\dot{V}O_2max$ to a load equivalent to 85% $\dot{V}O_2max$ after 60 min of exercise also resulted in significantly higher ($P<0.001$) heart rate data for all 3 trials. No significant differences were recorded in mean heart rate data at 60 and 90 min post-exercise compared to resting pre-test data (-60 min) illustrating that the heart rate had returned to normal resting levels prior to the completion of the reyhdration phase. The mean (± SEM) heart rate data at failure were 181 ± 3, 180 ± 3 and 182 ± 2 beats.min^{-1} for Water, LS and RB, respectively, with no significant differences recorded at any time point between the 3 drinks. Mean data across time and trial are presented in Appendix 3.

3.3.4 Blood Lactate

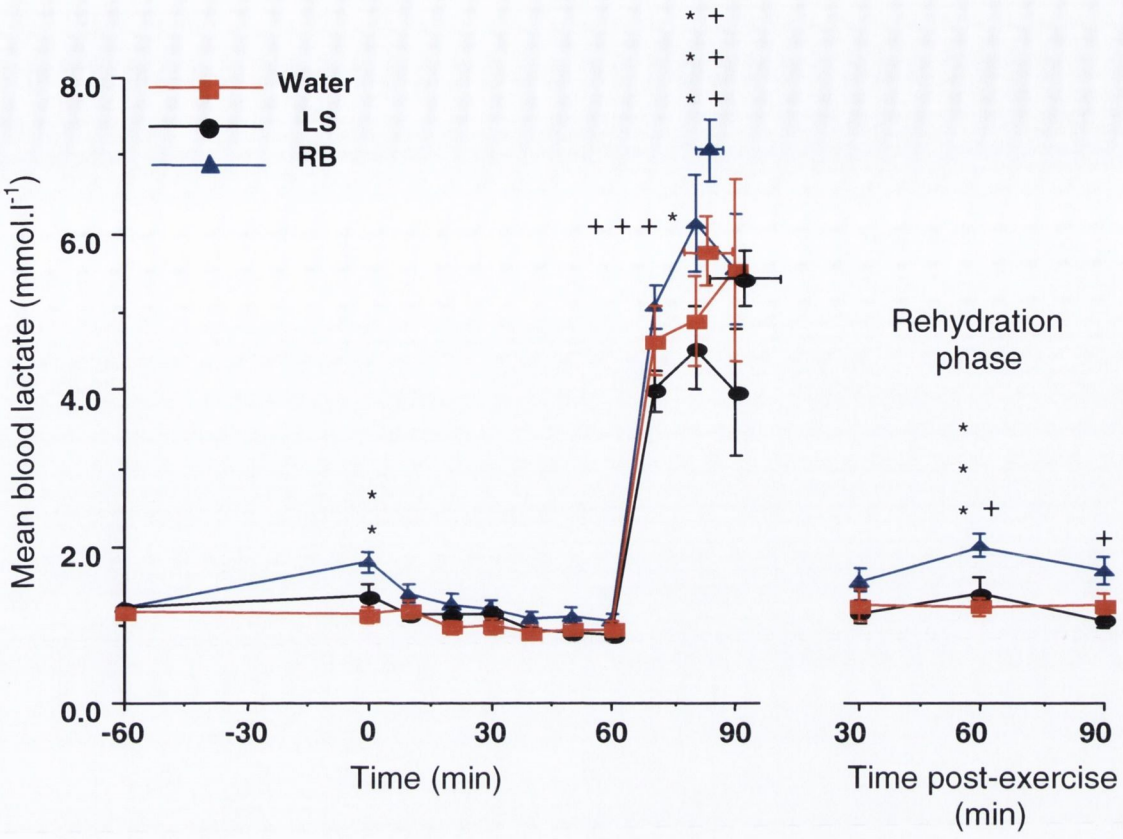


Figure 3.3: Mean (± SEM) blood lactate across time and trial, n=10.

+++ and +	Implies significantly higher data (P<0.001 and <0.05) for RB compared to LS.
*** and ** and *	Implies significantly higher data (P<0.001, <0.01 and <0.05) for RB compared to Water.

Blood lactate data recorded prior to fluid ingestion (-60) were similar across all 3 trials, with significant increases observed in all 3 trials with increasing exercise intensity, Figure 3.3. Mean blood lactate data at 70 min and at failure were significantly higher (P<0.001) than data recorded at all other time points during all 3 trials. Significantly higher (P<0.001) blood lactate concentrations were observed for all 3 drinks at failure compared to all other time points during the trial. Blood lactate data recorded at 0 min during the RB trial were significantly higher (P<0.05) than data recorded following 60 min of exercise at a load equivalent to 65% V_{O_2}max. Each subject ingested half of the pre-exercise volume of the test drink 30 min post-failure and was monitored for a further 60 min. During the Water and LS trials there was no change in blood lactate data observed following the ingestion of the reyhdration beverage. However, following exercise in the RB trial blood lactate data recorded 30 min post-ingestion (60 min post-exercise) were significantly higher than data recorded pre-test (-60 min) and at 30, 40, 50 and 60 min of exercise. Pre-exercise blood lactate data (0) following RB ingestion (1.8 ± 0.1 mmol.l^{-1}) were significantly higher

compared with Water (1.1 ± 0.1 mmol.l^{-1}) at the same time point. Blood lactate data were significantly higher ($P<0.001$) following RB ingestion at failure compared with the LS and Water ingestion. At 60 min during the rehydration phase blood lactate data during the RB trial were significantly higher compared with data recorded during the Water and LS trials ($P<0.001$ and <0.05, respectively) and again 90 min post-exercise blood lactate data were significantly higher following RB ingestion compared to LS ingestion ($P<0.05$). Mean data across time and trial are presented in Appendix 3.

3.3.5 Blood Glucose

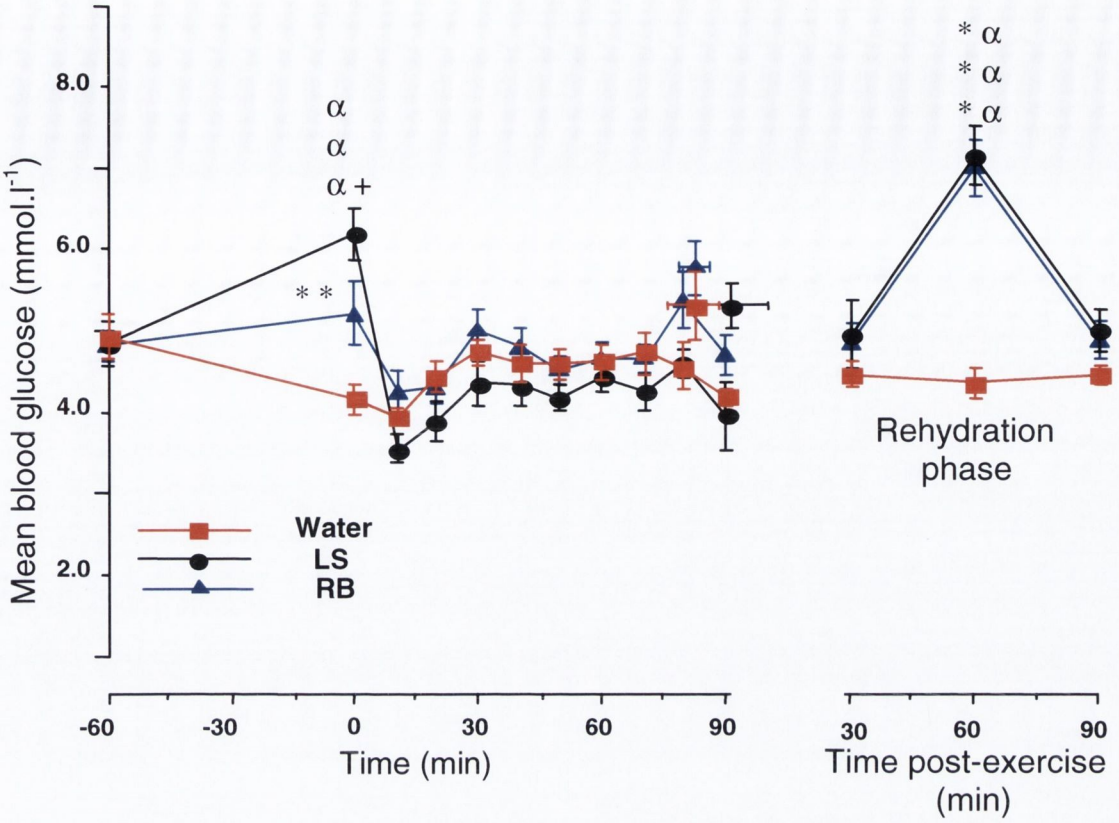


Figure 3.4: Mean (± SEM) blood glucose across time and trial, n=10.

**	Implies significantly higher (P<0.01) data for RB compared with Water.
+	Implies significantly higher (P<0.05) data for LS compared with RB.
α α α	Implies significantly higher (P<0.001) data for LS compared with Water.

Blood glucose data recorded prior to fluid ingestion (-60) was similar across all 3 trials, see Figure 3.4. During the Water trial blood glucose data remained relatively unchanged throughout until failure, however, a significant increase in blood glucose data relative to 0 (P<0.01) and 10 min (P<0.001) were recorded at failure. Following LS ingestion blood glucose data increased significantly (P<0.01) at 0 min compared to pre-test (-60 min) data. Blood glucose data recorded at 0 min during the LS trial were significantly higher than any other time points analysed except failure and 60 min post- exercise. This observation of elevated blood glucose concentrations pre-exercise compared to other time points was not observed for either RB or Water, despite the presence of carbohydrate in RB. The ingestion of LS and RB (at –60 min) resulted in significantly higher blood glucose concentrations at time 0 relative to Water (P<0.001 and <0.01, respectively). Blood glucose data during the LS trial were also significantly higher compared to the RB trial (P<0.05) at t=0. Following the ingestion of the reyhdration bolus significantly higher (P<0.001) blood glucose data were recorded during both the LS (7.1 ± 0.4 mmol.l^{-1}) and RB (6.9 ± 0.4 mmol.l^{-1}) trials

compared to Water (4.30 ± 0.2 $mmol.l^{-1}$) at 60 min post-exercise. Mean data across time and trial are presented in Appendix 3.

3.3.6 Oxygen Uptake

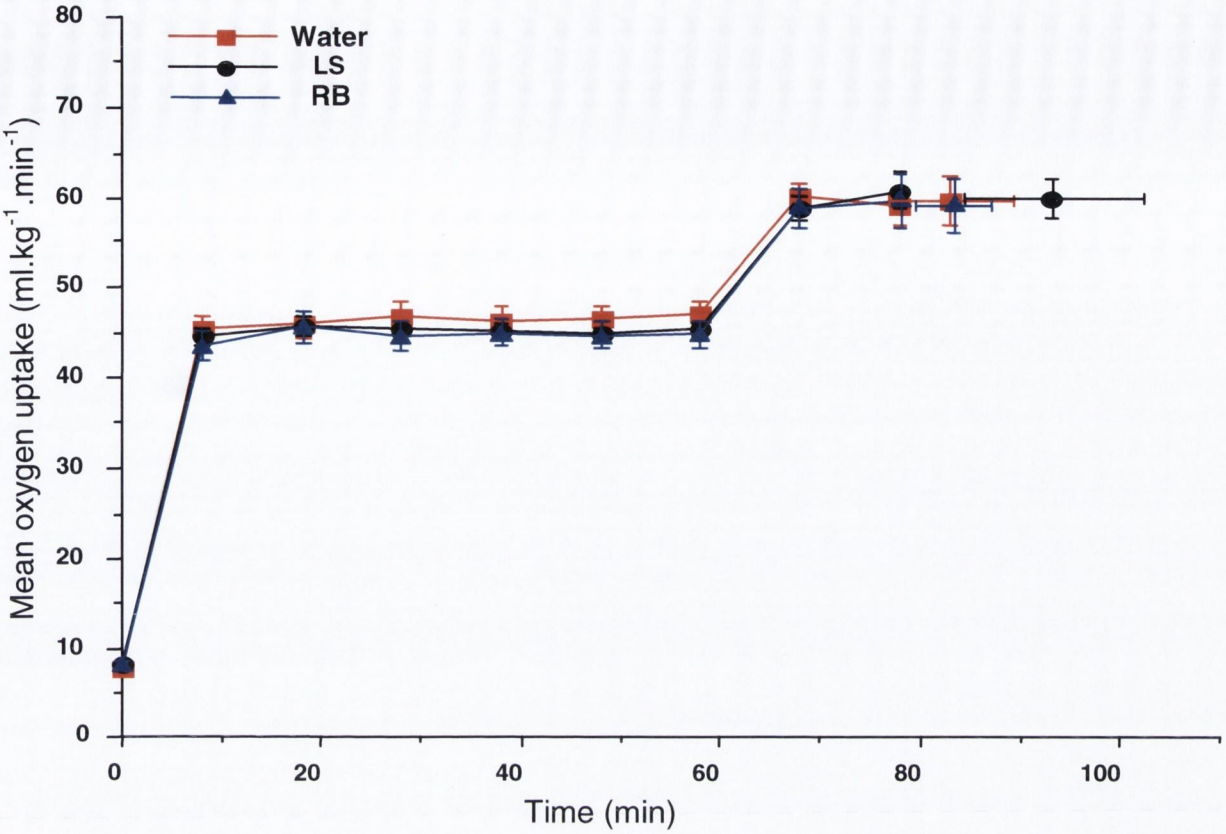


Figure 3.5: Mean (± SEM) oxygen uptake across time and trial, n=10.

Mean oxygen uptake data were analysed at rest, every 10 min during exercise (beginning at 8 min) and at failure. There was, as expected, a significant increase ($P<0.001$) in oxygen uptake when the exercise intensity was increased from a load equivalent to 65% $\dot{V}O_2$max to 85% $\dot{V}O_2$max, evident on Figure 3.5 by the increase in oxygen uptake data between 58 and 68 min. Data recorded at 68 and 78 min and at failure were significantly greater ($P<0.001$) compared to all other time points. There were no significant differences in oxygen uptake data across the 3 treatment arms at any time point. The mean absolute percentages of $\dot{V}O_2$max data that were recorded during both exercise intensities are presented in Table 3.3. The percentage of $\dot{V}O_2$max that subjects were exercising at during both bouts of exercise was almost identical to those targeted in the protocol (65% and 85% $\dot{V}O_2$max) in all 3 trials. Mean data across time and trial are presented in Appendix 3.

Time (min)	Exercise Intensity	LS	RB	Water
8	65% $\dot{V}O_2max$	63.6 ± 2.2	62.1 ± 2.1	63.47 ± 1.0
18		65.1 ± 1.7	64.8 ± 1.9	64.02 ± 1.3
28		64.8 ± 1.8	63.3 ± 1.7	65.4 ± 1.6
38		65.5 ± 2.0	63.9 ± 1.8	64.6 ± 1.5
48		64.2 ± 2.2	63.6 ± 1.8	66.1 ± 1.4
58		64.8 ± 2.1	63.7 ± 1.6	67.0 ± 1.6
68	85% $\dot{V}O_2max$	84.0 ± 2.2	84.0 ± 2.9	87.2 ± 3.5 (n=9)
78		85 ± 3.3 (n=8)	85.4 ± 4.2 (n=8)	83.2 ± 4.0 (n=5)

Table 3.3: Mean (± SEM) % maximal oxygen uptake at loads equivalent to 65 and 85% $\dot{V}O_2max$, n=10, unless stated otherwise.

3.3.7 Respiratory Exchange Ratio

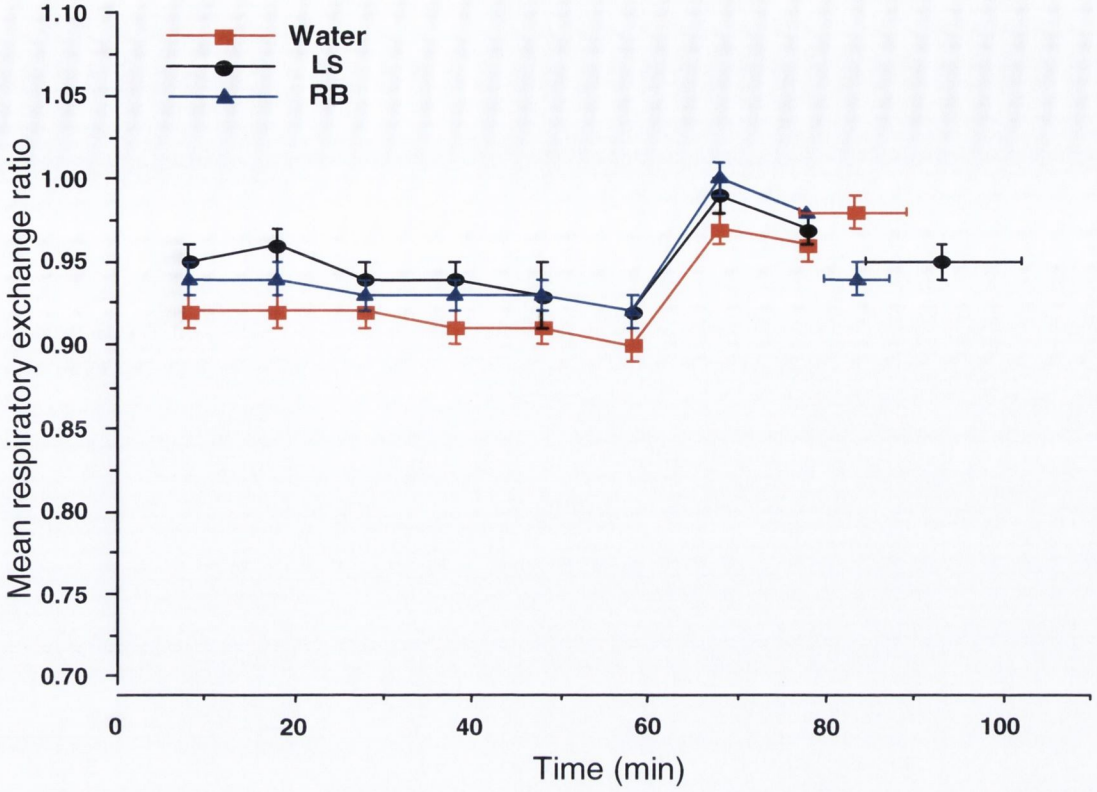


Figure 3.6: Mean (± SEM) RER across time and trial, n=10.

Similar to the oxygen uptake data, mean RER data increased significantly with increasing exercise intensity, see Figure 3.6. During the LS trial mean RER increased significantly from 0.92 ± 0.01 to 0.99 ± 0.01 as the exercise intensity increased (from 58 to 68 min, respectively) and similarly following ingestion of RB and Water. No significant differences were recorded between the 3 trials at any time point. Mean data across time and trial are presented in Appendix 3.

3.3.8 Plasma Osmolality

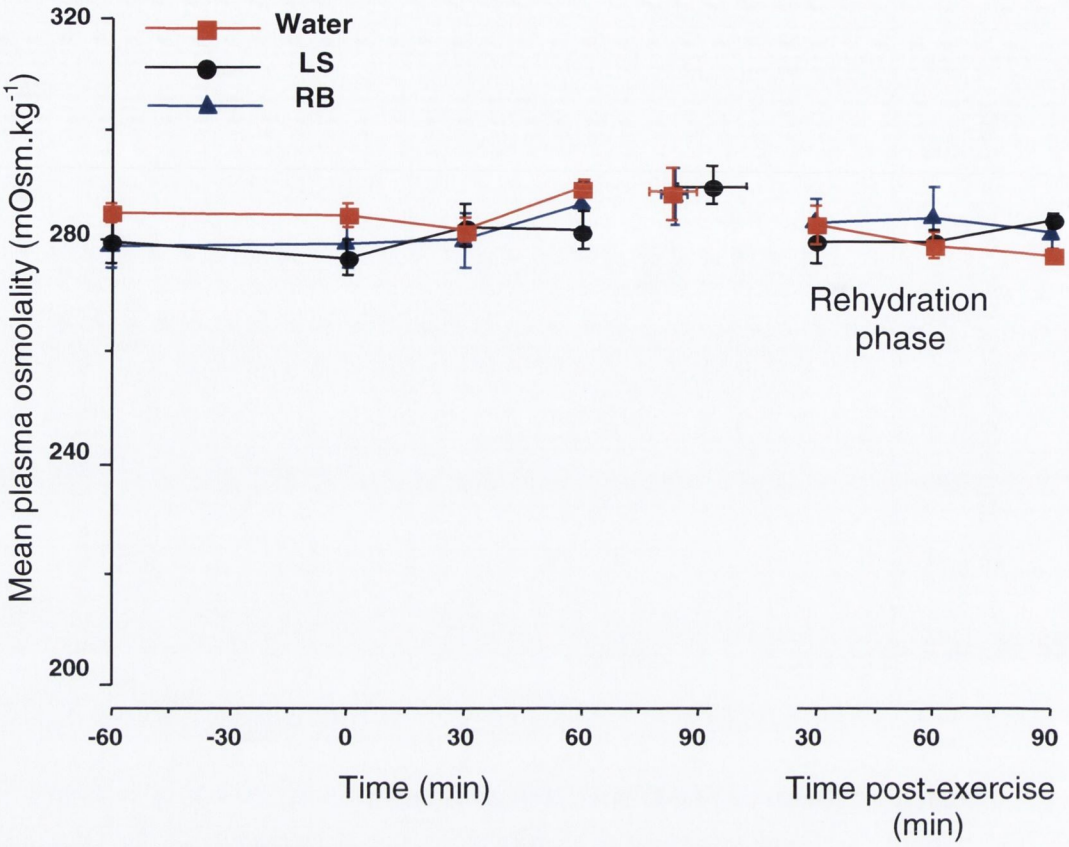


Figure 3.7: Mean (± SEM) plasma osmolality across time and trial, n=10.

Mean (± SEM) plasma osmolality data are presented in Figure 3.7. No significant differences were recorded in plasma osmolality across the 3 drinks at any time point during the trials. Mean plasma osmolality data remained between 275 and 290 mOsm.kg^{-1} throughout all 3 drinks trials, similar to normal plasma osmolality cited in the literature as 287 mOsm.kg^{-1} (Armstrong *et al.*, 2005). Mean data across time and trial are presented in Appendix 3.

3.3.9 Plasma caffeine concentration

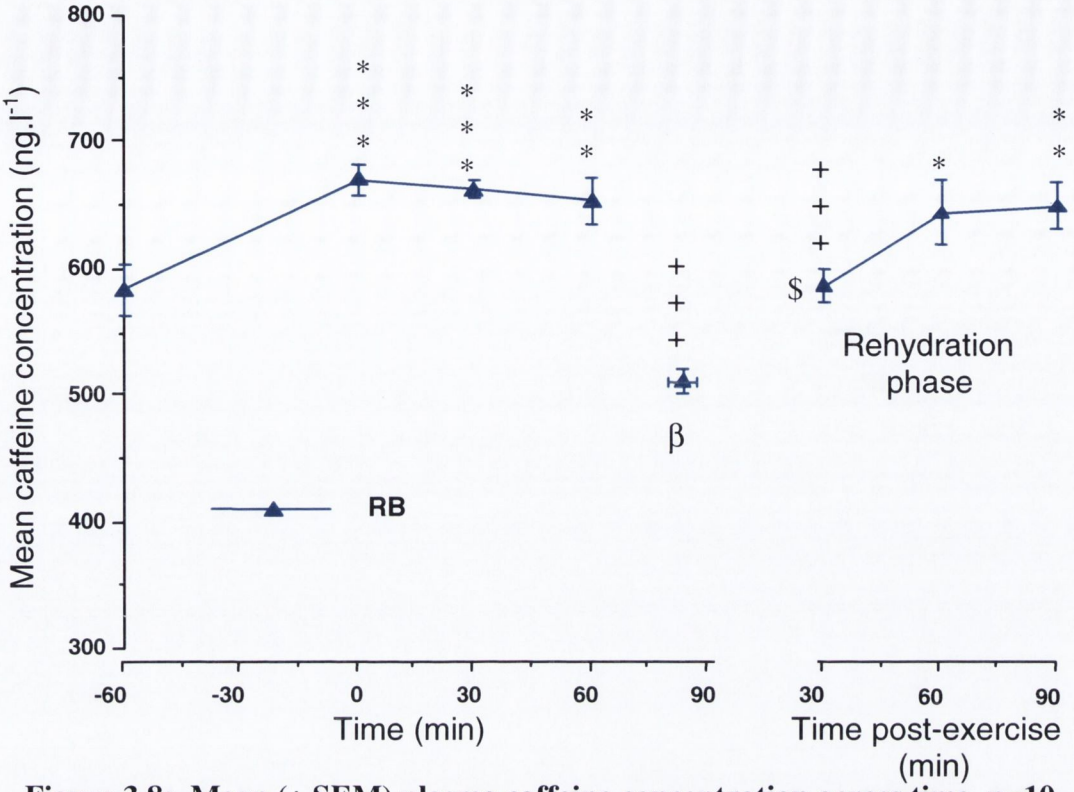


Figure 3.8: Mean (± SEM) plasma caffeine concentration across time, n=10.

+++	Implies significantly lower (P<0.001) data compared to 0, 30 and 60 min.
***, ** and *	Implies significantly higher (P<0.001, 0.01 and 0.05) data compared to -60.
β	Implies significantly lower (<0.05) than –60 and 30, 60 and 90 min of the reyhdration phase.
$	Implies significantly lower (P<0.05) data compared to 0, 30, 60 min of the exercise phase and 60 and 90 min of the reyhdration phase.

The mean ± SEM caffeine concentration from all 10 subjects during the RB trial at each time point is illustrated in Figure 3.8. The graph illustrates that 60 min following Red Bull ingestion (0) the plasma caffeine concentration was elevated and remained so for the subsequent 60 min of cycling exercise at a load equivalent to 65% $\dot{V}O_2$max. The mean plasma caffeine concentration data at failure were significantly lower than all other time points analysed. A second bolus of Red Bull was ingested 30 min after failure (30 min post-exercise) and 30 and 60 post-ingestion (60 and 90 min post-exercise) plasma caffeine concentrations were significantly higher than those immediately prior to fluid ingestion. Post ingestion of the second bolus of RB the plasma caffeine concentration increased towards levels similar to those recorded pre-exercise (0) and during the first 60 min of exercise. Mean data across time and trial are presented in Appendix 3.

3.3.10 Plasma Volume

Changes in plasma volume were calculated using the equations proposed by Dill and Costill (1974) from haemoglobin and haematocrit data recorded during the different phases of the exercise and rehydration trials. Table 3.4 presents these data as mean ± SEM. Only a fraction of the subjects completed the first 90 min of exercise in each of the 3 trials. The number of subjects is displayed in brackets in Table 3.4 at 90 min. For this reason the plasma volume changes calculated for the time period 0 –90 (*) were not included in the statistical analysis.

Time points	Red Bull ΔPV (%)	Lucozade Sport ΔPV (%)	Water ΔPV (%)
-60 – 0	-1.7 ± 1.1	1.4 ± 1.8	0.2 ± 1.3
0– 30	-3.1 ± 1.2	-5.8 ± 1.3	-6.7 ± 1.4
0 – 60	-3.7 ± 1.5	-6.6 ± 1.2	-6.7 ± 1.7
0 – 90 *	-7.7 ± 1.9 (n=3)	-9.7 ± 0.1 (n=3)	-10.3 ± 3.3 (n=2)
0 – Failure	-9.6 ± 1.6	-12.5 ± 1.3	-12.2 ± 1.6
Failure – 30 post-ex	9.8 ± 1.8 ###	13.7 ± 1.7 ###	14.0 ± 1.7 ###
30 post-ex – 60 post-ex	1.4 ± 1.3	2.0 ± 1.3	-1.8 ± 0.9
30 post-ex – 90 post-ex	1.7 ± 2.4	-0.9 ± 0.9	-2.4 ± 1.4
-60– 90 post-ex	-0.7 ± 2.3	-0.4 ± 1.3	-2.4 ± 1.5

Table 3.4: Mean plasma volume changes (± SEM), n=10 unless stated.

(post-ex implies post – exercise)

Implies significantly higher (P<0.001) data compared with all other time points for all 3 drinks.

There were no significant differences in plasma volume changes across the 3 treatment arms when analysed for the effect of the ingestion of the drinks, the effect of exercise, the effect of exercise cessation or the effect of ingestion of the rehydration bolus. However, analysis of the data showed that the changes in plasma volume observed in this study within each treatment arm followed a similar trend to those expected during hydration, exercise and rehydration. Plasma volume changes during the 30 min non-hydrated

recovery period post-exercise (Failure-30 post-ex) were significantly higher ($P<0.001$) for all 3 drinks compared to changes observed at all other time points. Table 3.4 also illustrates that plasma volume was restored to pre-test levels 60 min post-ingestion (-60 – 90 post-ex) of the rehydration bolus for both LS (-0.4 ± 1.3 %) and RB (-0.7 ± 2.3 %), with a mild dehydrating effect observed with Water ingestion which failed to reach statistical significance.

3.4 Discussion

This study compared the effects of 3 drinks, Lucozade Sport, Red Bull® and water on endurance exercise performance and post-exercise rehydration in 10 male cyclists. The 3 test drinks were matched iso-volumetrically to allow for accurate assessment of their effects on reyhdration. The preliminary findings were that pre-exercise ingestion of the drinks did not have any significant effect on exercise performance or rehydration, see Figure 3.1. Despite the presence of a higher quantity of carbohydrate in RB (43.8 vs 60.2 g for LS and RB) when ingested pre-exercise there was no difference in exercise performance recorded between LS and RB. Moreover, there was no difference in exercise performance recorded for Water when compared to either LS or RB. There is evidence to support and contradict the findings of this study in the literature. Costill *et al.* (1977) reported a significant decline in exercise capacity following ingestion of a glucose solution 25 min before sub-maximal running. In another study cycling time to exhaustion was reduced by 19% following the ingestion of a 25% glucose solution 30 min before exercise (Foster *et al.*, 1979). Transient hypoglycaemia was observed during this trial accompanied by an acceleration of muscle glycogenolysis and glucose oxidation. Following carbohydrate ingestion hyperinsulaemia ensures increasing glucose uptake by the exercising muscles, resulting in hypoglycaemia, decreasing lipolysis and free fatty acid availability at the start of exercise. It was therefore recommended by Foster *et al.* (1979) that carbohydrate should not be ingested during the hour before exercise. The ingestion of a 25% glucose solution 30 min before exercise did not alter endurance running capacity (Chryssanthopoulos *et al.*, 1994). In contrast Gleeson *et al.* (1986) reported significant increases of 7.4% in exercise performance following the ingestion of 75 g carbohydrate 45 min prior to exercise. Exercise performance was enhanced in this case despite elevated insulin concentration at the start of exercise and a decrease in blood glucose early in exercise. Sherman *et al.* (1991) (cited by el-Sayed *et al.* 1997) reported significant improvements in exercise performance when 75 g of carbohydrate was consumed 60 min prior to 90 min of exercise. The quantity of carbohydrate consumed during the trials by Gleeson *et al.* (1986) and Sherman *et al.* (1991) was higher than those ingested in the present study.

Like all scientific research, differences exist in the protocols applied that may account for the different outcomes reported. In the present study the test drinks were ingested 60 min prior to exercise. However, significant improvements in exercise performance have previously been reported following carbohydrate ingestion (5 $g.kg^{-1}$) 180 min prior to exercise (Wright *et al.*, 1991).

Research suggests that caffeine ingestion exerts a positive effect on exercise performance during prolonged events even at dosages as low as 3 $mg.kg^{-1}$ (Graham and Spriet, 1995, Kovacs *et al.*, 1998, Cox *et al.*, 2002). Geiss *et al.* (1994) investigated the effects of the ingestion of Red Bull on endurance performance in male cyclists. Red Bull® was ingested (500 ml, equivalent to 2.28 $mg.kg^{-1}$ caffeine) 30 min into a 60 min cycling element at a load equivalent to 70% $\dot{V}O_2max$. This exercise element was followed by an incremental cycle test to exhaustion with workloads increasing by 50 W every 3 min. Exercise performance was significantly improved following ingestion of Red Bull® compared with a Red Bull supplement that did not contain taurine, glucuronolactone or caffeine. Endurance performance was also increased following ingestion of Red Bull® compared with a beverage containing only carbohydrate and caffeine but again no glucuronolactone or taurine. Therefore Geiss and co-workers concluded that the presence of caffeine or taurine, or a combination of caffeine and taurine may be responsible for the improvements observed in endurance exercise performance. The concentrations of caffeine ingested during the trials by Geiss *et al.* (1994) were lower than the concentration used in the present study ($3mg.kg^{-1}$) and no improvement in performance capacity was recorded, and similarly lower than that used by Graham and Spriet (1995) where a significant increase in exercise time to exhaustion was noted. Therefore, this makes interpretation of these results difficult.

Heart rate data remained unchanged throughout the trials in this study with no significant differences recorded across the 3 drinks, Figure 3.2. Blood lactate data were significantly increased following RB ingestion when compared to Water at 60 min post-ingestion (0 min). Also at failure blood lactate data were significantly higher following RB ingestion when compared to Water and LS.

Following LS ingestion the blood glucose concentration decreased and remained significantly lower than pre-test (0 min) at all time points during exercise except at failure. Significantly higher blood glucose data were recorded following LS ingestion when compared to both RB and Water immediately prior to exercise. Following RB ingestion significantly higher blood glucose data were also recoded at 0 min when compared to Water, however despite the presence of carbohydrate the same decline in blood glucose recorded during the LS was not observed following RB ingestion. Figure 3.4 illustrates a transient hypoglycaemia from 0-30 min of exercise with the blood glucose concentration decreasing within 10 min of exercise and then increasing in the following 20 min before reaching a plateau that was still significantly lower than data at 0 min. The mean blood

glucose concentration recorded at 10 min was 3.5 ± 0.2 compared to 6.1 ± 0.3 mmol.l^{-1} at 0 min.

Plasma volume data decreased significantly during exercise. However during the 30 min post-exercise period prior to fluid ingestion there was a marked increase in plasma volume indicative of a redistribution of fluids within the extracellular space.

Following the ingestion of LS, RB or water 30 min post-exercise a similar effect on plasma osmolality (Figure 3.7) and plasma volume (Table 3.4) over the following 60 min was observed. This contradicts reports that caffeine acts as a diuretic at rest (Armstrong, 2002). The results of the present study on rehydration following exercise-induced hypohydration would suggest that RB does not negatively affect athletes in an already hypohydrated state but restores plasma volume in a manner similar to both LS and Water. This area will be addressed further in another study described in Chapter 6.

Chapter 4

The effect of two isocaloric drinks, one containing caffeine on intermittent exercise performance in elite male cyclists.

4.1 Introduction

The research described in Chapter 3 involved the ingestion of test drinks before endurance exercise and no improvement in exercise performance was reported. The ingestion of fluid before and during exercise has also been an important area of scientific research. Several studies have reported significant improvements in exercise capacity when carbohydrate is ingested before and during exercise (Coggan and Coyle, 1989; Mitchell *et al.*, 1988; Jeukendrup *et al.*, 1997). The improvement in exercise performance has been attributed to the ability of the athlete to maintain a given exercise intensity for a longer duration. It has also been suggested that when carbohydrate is ingested during exercise it is possible for the athlete to exercise at a high intensity during the later stages of prolonged exercise (Coggan and Swanson, 1992). Constant load endurance exercise is not commonplace among many sports especially games sports namely, soccer, rugby, hockey; even during some cycling events the intensity of exercise can vary throughout the different stages of a race. While the previous study investigated the role of carbohydrate feeding prior to endurance exercise and reported no improvement in exercise performance, there appears to be an ergogenic potential for carbohydrate ingestion during shorter duration high intensity exercise (Jeukendrup, 2004). Nicholas *et al.* (1995, 1999) previously reported a significant improvement in performance when a carbohydrate-electrolyte drink was ingested before and during intermittent running exercise. The role of caffeine ingestion during shorter duration intermittent exercise is less conclusive with positive effects being reported during rowing and running (Anderson *et al.*, 2000; Stuart *et al.*, 2005, respectively). A review by Armstrong in 2000 suggests that caffeine does not enhance performance in events lasting between 8 and 22 min or in events lasting less than 90 s.

In an attempt to further investigate the use of commercial sports drinks and their use during intermittent exercise the following study was designed.

The aim of the study was to investigate the effect of ingestion 2 isocaloric drinks, 1 of which contains caffeine on intermittent cycling exercise performance when ingested before and during exercise. By matching the drinks calorically it was hoped to assess any additive effects of caffeine on exercise performance compared to carbohydrate, for control purposes water was consumed as a zero calorie placebo.

4.2 Methods

4.2.1 Study design

This study was un-blinded and randomised involving 4 visits to the Human Performance Laboratory, Trinity College Dublin. Ethics approval was granted by the Faculty of Health Sciences ethics committee. On the first visit subjects completed an incremental cycling test to volitional exhaustion. This was then followed by 3 drinks trials during which subjects performed intermittent exercise at loads equivalent to 60 and 90% $\dot{V}O_2max$ until failure. The test drinks were ingested both before and during exercise. It was not possible to perform a blinded study because the distinctive taste of some of the drinks did not allow such a scenario.

4.2.2 Details of the drinks

In this study Lucozade Sport (LS, powder), a diluted form of Red Bull (isoRB) and Water were the 3 test drinks. IsoRB was diluted (60:40) with water to make a beverage that was isocaloric to LS. Therefore, the volume ingested and the concentration of carbohydrate in each of the 2 drinks was identical. The subjects ingested the test drink 20 min before exercise, every 10 min during exercise and at failure. For example a 70 kg subject received 300 ml pre-exercise and 100 ml every 10 min during exercise, and at failure. This volume equated to a caffeine load of 2.5 pre- and 1.7 $mg.kg^{-1}.hr^{-1}$ during exercise. In the isoRB trial the carbohydrate ingestion rates were 0.75 and 0.60 $g.kg^{-1}.hr^{-1}$ pre-exercise and during exercise, respectively, compared to 0.71 and 0.57 $g.kg^{-1}.hr^{-1}$ in the LS trial. Carbohydrate was present in the form of glucose and maltodextrins in LS and in the form of glucose and sucrose in isoRB. The composition of LS, isoRB and W are described in Table 2.1.

4.2.3 Subject recruitment

9 male cyclists were recruited to participate in this study. They were recruited from the official website www.irishcycling.com, from cycling clubs in the greater Dublin area and also through contacts established by other members of the Human Performance Laboratory. All subjects were trained racing cyclists competing as category A or B cyclists. Each subject signed a consent form and received an information sheet outlining all details of the study. The protocol was also explained verbally to each subject. Some of those who had participated in the research study described in Chapter 3 were also recruited for this study. This was not deemed to be a limitation as all subjects were familiar with the laboratory procedures from previous service testing.

4.2.4 Inclusion and exclusion criteria

All subjects were male aged between 20 and 30 yr. Any volunteer displaying any respiratory, cardiac or systemic disorder or any contraindication to maximal exercise at the time of the medical screening was excluded from the study. Also caffeine abstainers/caffeine naïve subjects were excluded.

4.2.4 Medical screening and anthropometric measurements

Height and body mass were recorded during the medical screening on visit 1. To calculate the volume of fluid to be ingested, body mass was reassessed before each drinks trial to provide the subject with the test drink in a volume dependant on their body mass. Body mass was also recorded at the end of each trial to assess body mass changes during the trials. As part of the medical examination percentage body fat was estimated from four sites as described in section 2.2. Lung function data were collected prior to the maximal incremental test to rule out any restrictive or obstructive disorders that may prevent the subject for exercising maximally. Spirometry was also performed after exercise to rule out exercise-induced bronchoconstriction. The use of the micro-spirometer is described in section 2.3. Blood samples were collected from the medial cubital vein in the antecubital fossa of the left arm during medical screening to record red blood cell count (RBC), white blood cell count (WBC), haemoglobin (Hgb) and haematocrit (Hct).

4.2.6 ECG analysis

An ECG was performed on each subject during the medical examination to rule out any cardiac abnormalities that may be negatively affected following caffeine ingestion during the drinks trials. The position of the leads and details of the electrocardiogram used are described in section 2.9.

4.2.7 Incremental test to exhaustion

The protocol used for the incremental cycling test to volitional exhaustion is detailed in section 2.19. Using the data collected from the incremental test, a graph was plotted for each subject for HR, blood lactate and $\dot{V}O_2$ against workload, and loads equivalent to 60 and 90% $\dot{V}O_2$ max were calculated for each subject. These individually calculated workloads were used in the 3 subsequent time to failure trials.

4.2.8 Time to exhaustion trial

Each subject completed 3 intermittent exercise trials, each trial involving the ingestion of 1 of the following randomised drinks: Red Bull, Lucozade Sport or Water. Red Bull was diluted with water (60-40) to achieve an isocaloric solution compared to Lucozade Sport. This formulation is referred to as isoRB throughout the results and discussion relating to this study. An indwelling venous catheter was inserted and pre-test body mass was measured. All urine voided during the trial was collected and the volume noted for comparison across all 3 trials. A pre-test blood sample was collected immediately prior to a 20 min preparation period. During this 20 min the subjects firstly ingested the test drink, warmed up for 10 min (120 W), stretched and had resting metabolic data collected. Another blood sample was taken immediately before commencing the exercise trial. Lucozade Sport and isoRB were ingested in an isovolumetric and isocaloric fashion.

A cyclist weighing 70 kg would receive 300 ml pre-exercise and 100 ml every 10 min during the trial and at failure. The preparatory 20 min period was followed immediately by intermittent cycling to failure. Exercise began with 3 min at a load equivalent to 60% $\dot{V}O_2max$ followed by 2 min cycling at a load equivalent to 90% $\dot{V}O_2max$ and these intervals were alternated until failure. The load equivalent to these intensities was calculated using the metabolic data collected during the incremental test (section 2.19). Failure was defined as the point when the subject was unable to maintain the workload of that particular interval. A 10 min warm down period ensued before body mass was re-measured and the indwelling canula removed.

4.2.9 Haematological cardio-respiratory and urinary analysis

During the incremental test blood lactate was assessed using the finger tip capillary method. An indwelling forearm canula was inserted (section 2.10) at the beginning of the each of the drinks trials to allow for multiple blood samples to be collected during the trial with minimal discomfort to the subjects. The timing of blood sampling during the incremental test and drinks trials is detailed on Figure 2.16 and Schematic 2, respectively. Blood samples collected in EDTA tubes were analysed for Hgb (Coulter Counter) and Hct (centrifugation in triplicate) for the calculation of plasma volume changes according to the equations described by Dill and Costill (1974). These blood samples were also used to measure blood lactate and glucose concentrations. Blood lactate and glucose was assessed pre-test, pre-exercise, every 10 min during exercise and at failure. Also blood samples collected every 30 min during the drinks trials were spun down and the plasma separated and frozen for later batch analysis of plasma osmolality and plasma caffeine and non-

esterified fatty acid concentration. Heart rate data was recorded at rest, every minute during exercise and at failure.

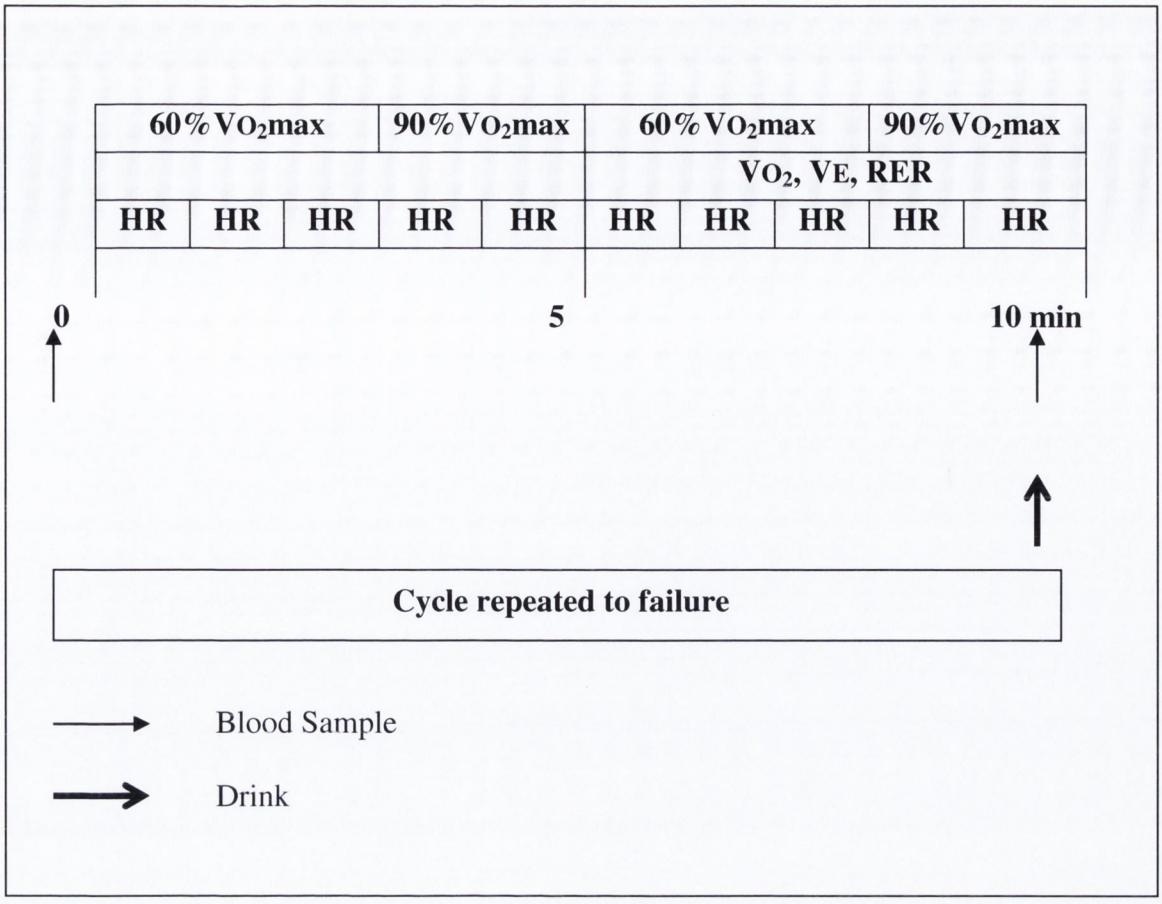
Urine volumes were recorded and used in the comparison of urine output across the 3 trials.

4.2.10 Metabolic Analysis

Metabolic data was collected throughout the incremental test and averaged over the last minute and a half of each increment, see Figure 2.16. During the drinks trials $\dot{V}O_2$ and RER were recorded at rest and in alternate 5 min blocks during the exercise protocol. Therefore, during each block metabolic data were recorded while the subject exercised at loads equivalent to 60 and 90% $\dot{V}O_2$max, see Schematic 2.

4.2.11 Statistical Analysis

All subject physical characteristics are presented in table format as mean ± standard deviation (SD) and all graphical data as mean ± standard error of the mean (SEM). A two-way repeated measures ANOVA (time by drink) was utilised to assess the effects of the 3 drinks across time on measured variables, with *post-hoc* Bonferroni tests used to quantify any significant differences detected using Sigma stat. A one-way repeated measures ANOVA was used to assess the effects of the 3 drinks on performance time. For all statistical tests values of $P<0.05$ were considered statistically significant.



Schematic 2: Order of data collection during the drinks trials.

4.3 RESULTS

4.3.1 Physical Characteristics

Anthropometric data:

	Mean ± SD
Age (yr)	26 ± 4
Body mass (kg)	75.3 ± 6.6
Height (m)	1.8 ± 0.1
BMI ($kg.m^{-2}$)	22.8 ± 2.3
Body fat (%)	12.0 ± 2.5
VO_2max ($ml.kg^{-1}.min^{-1}$)	68.9 ± 6.5
Max. workload (W)	373 ± 28

Table 4.1: Mean (± SD) physical characteristics, n=9 at time of incremental test.

Haematological and pulmonary data:

Haematological and pulmonary function test data were normal, with no signs of sub-clinical infection, or obstructive or restrictive airway disorders (see Table 4.2).

HAEMATOLOGICAL DATA	Mean ± SD	PULMONARY DATA	Mean ± SD
Hb ($g.dl^{-1}$)	14.5 ± 0.5	**FEV_1 (l)**	5.0 ± 0.5
Hct (%)	42.3 ± 1.3	**FVC (l)**	5.8 ± 0.7
RBC ($x10^{12}.l^{-1}$)	4.5 ± 0.2	**PF ($l.min^{-1}$)**	636 ± 68
WBC ($x10^{9}.l^{-1}$)	5.4 ± 1.2	**FEV_1/FVC (%)**	86.6 ± 5.4

Table 4.2: Mean ± SD haematological and pulmonary data, n=9 at time of incremental test.

The physiological profiles of the subjects detailed above (Table 4.1) were similar to those who participated in the study described in Chapter 3 (Table 3.1) and are reflective of the general population of amateur Irish cyclists competing at A and B category. The results of the present study would potentially be reflective of the responses experienced by cyclists to exercise and the effect of pre-exercise and intermittent sports drinks' consumption on their exercise performance.

4.3.2 Time to Failure

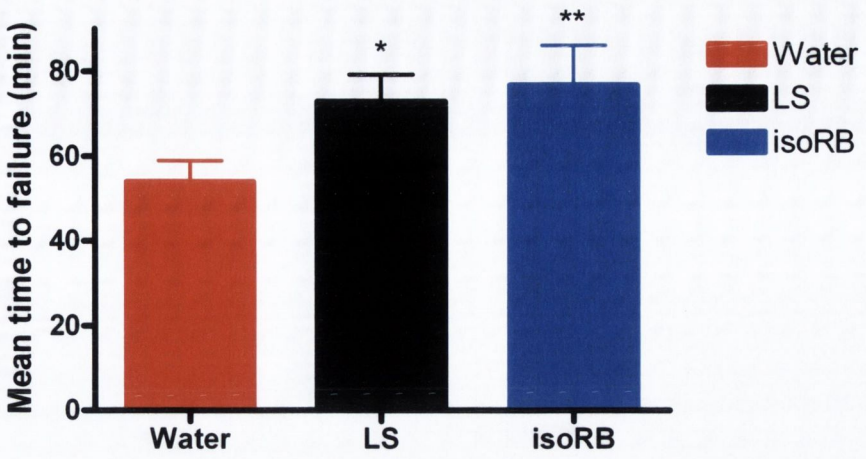


Figure 4.1: Mean (± SEM) time to failure across trials, n=9.

** and * Implies significantly higher (P<0.01 and <0.05) data compared to Water.

There were no significant differences observed in time to failure between isoRB and LS. However, time to failure was significantly higher for isoRB (76.7±9.4 min) compared with Water (53.9±5.0 min, P<0.01) and for LS (72.8±6.4 min, P<0.05) compared with Water. These changes represented a 29% increase in mean exercise performance time following isoRB ingestion and a 26% improvement in mean exercise performance time following LS ingestion pre- and during exercise when compared with Water.

Note: For the remaining Figures in this chapter the data for each variable are plotted against time for each trial. Due to the variation in time to failure both within and across trials statistical analysis was not performed on data plotted for the later stages of some of the trials. The reason for this is because the statistical package used does not allow analysis to be performed if data are missing for some subjects at any time point.

4.3.3 Heart Rate

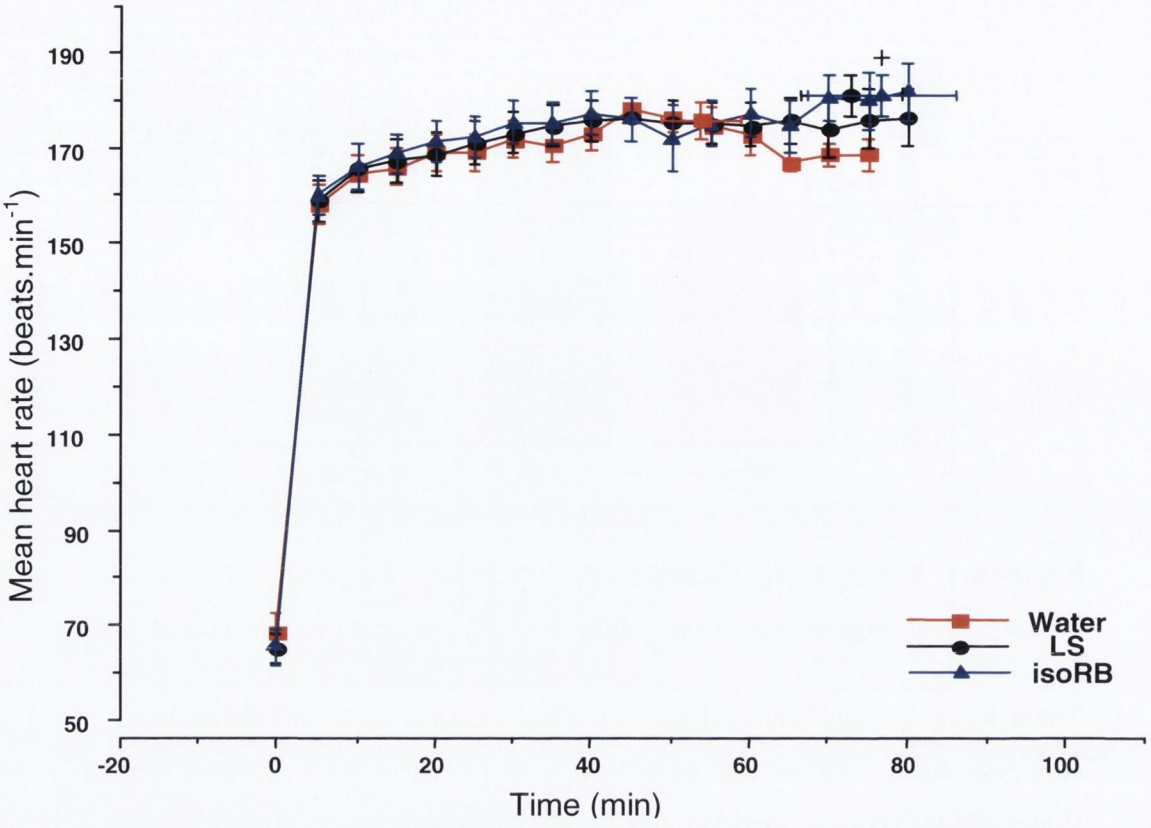


Figure 4.2: Mean (± SEM) heart rate across time and trials.

\+ Implies significantly higher (P<0.05) data for isoRB compared with Water at failure.

Heart rate data are presented in graphical format at rest, every 5 min for 80 min of exercise and at failure, see Figure 4.2. However, due to the decreasing number of subjects exercising after 60 min, statistical analysis was only performed on data collected at rest, every 5 min for the first 60 min of exercise and at failure.

It can be observed from Figure 4.2 that the heart rate increased rapidly at the beginning of exercise and slowly increased during the following 60 min of intermittent exercise. During exercise after 60 min the sample size is decreasing and possibly explaining why the slow elevation in heart rate data evident in the first 60 min of exercise is not observed thereafter. Heart rate data recorded at failure were significantly greater than data recorded at 5, 10 and 15 min for all 3 trials.

At failure mean heart data during the isoRB trial (181.1±4.3 beats.min^{-1}) were significantly higher compared to Water (175±3.7 beats.min^{-1}; P<0.05). Mean data across time and trial are presented in Appendix 4.

4.3.4 Blood Lactate

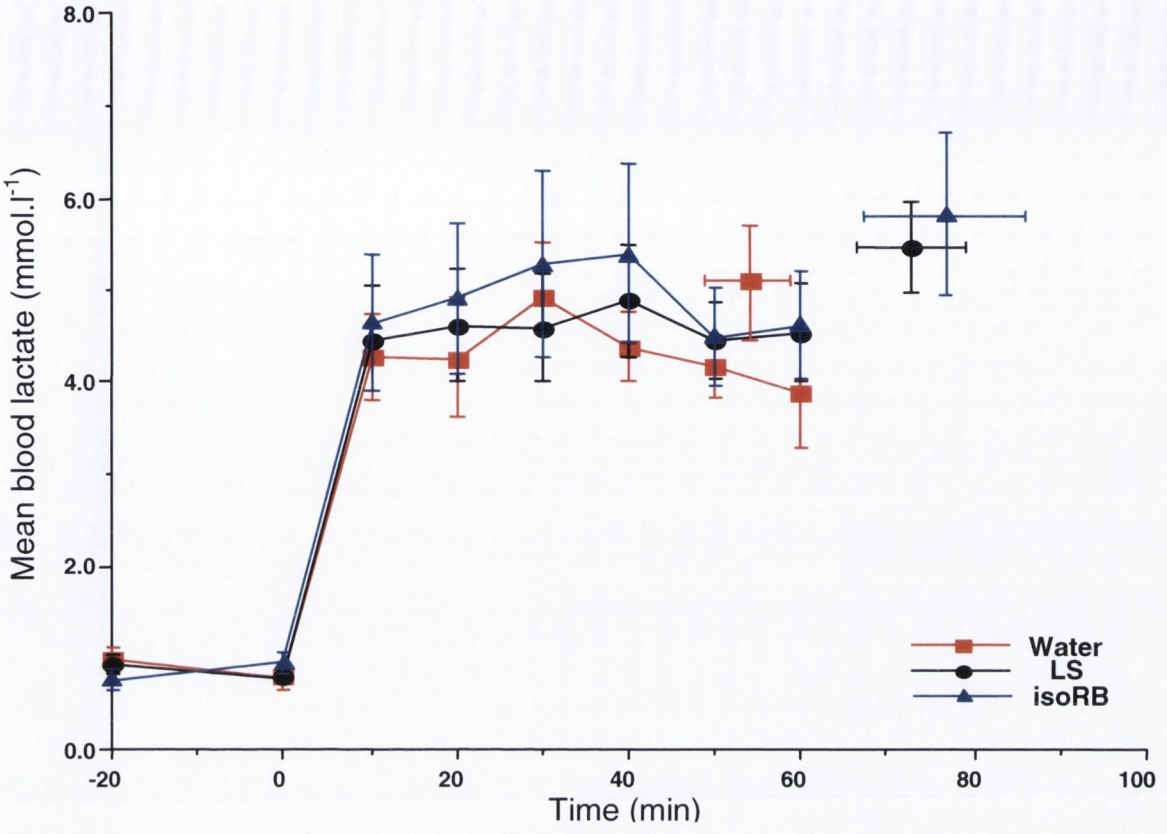


Figure 4.3: Mean (±SEM) blood lactate at rest and at 10 min intervals during exercise across time and trials.

Figure 4.3 illustrates mean blood lactate data recorded pre-drink (t =-20), pre-exercise (t=0), at 10 min intervals for the first 60 min and at failure. Data recorded at 10 min intervals after 60 min of exercise were not included because the sample size was decreasing as subjects reached failure. Mean blood lactate data at –20 min and 0 min were significantly lower ($P<0.001$) than all time points during exercise, no significant differences in blood lactate data were observed between –20 and 0 for all 3 drinks trials. There were no significant differences in blood lactate data recorded at any time point during exercise across all 3 drinks. Mean data across time and trial are presented in Appendix 4.

4.3.5 Blood Glucose

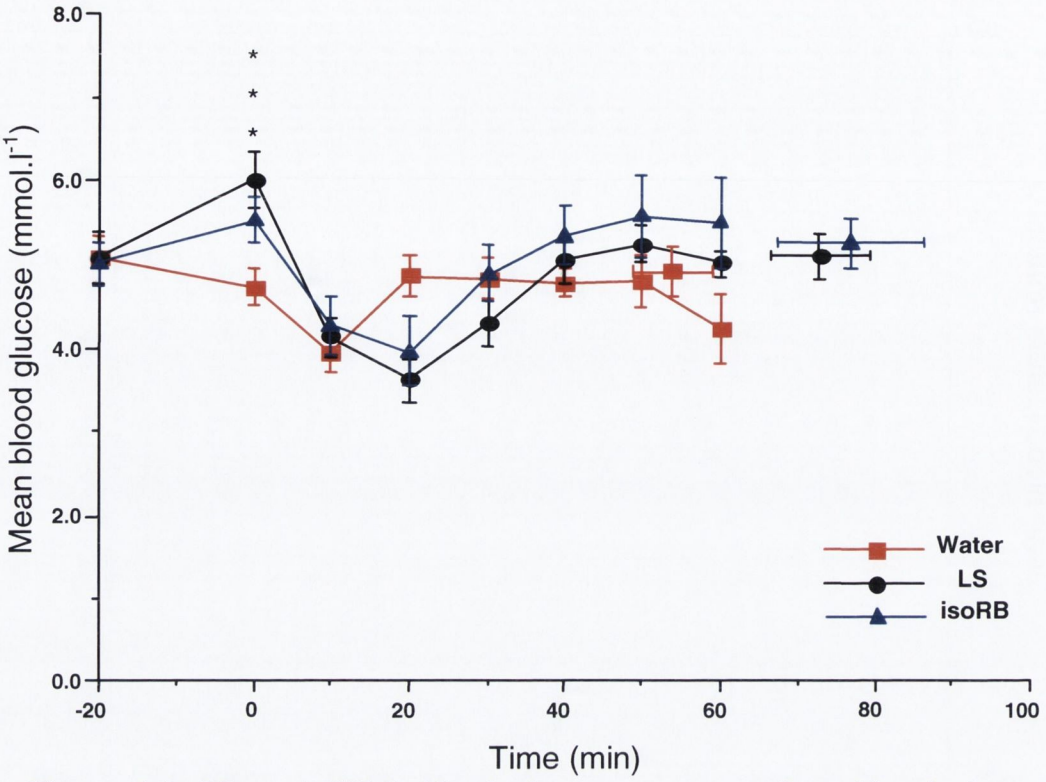


Figure 4.4: Mean (±SEM) blood glucose at rest and at 10 min intervals during exercise across time and trials.

*** Implies significantly higher blood glucose data (P<0.001) for LS compared to Water.

Mean glucose data collected for 1 subject were excluded from the statistical analysis as data were not collected during the Water trial due to an operational problem with the glucose analyser. Following 10 min of exercise blood glucose data during the Water trial were significantly lower (P<0.05) than data recorded at –20 and at 40 and 50 min during exercise. Across time significant drinks differences were also detected following ingestion of LS and isoRB. During the LS trial blood glucose data declined significantly at 10, 20, 30 and 40 min of exercise and at failure when compared to 0 min. A rebound hypoglycaemic effect was observed as blood glucose data were significantly greater (P<0.001) following 40, 50 and 60 min and at failure compared to 20 min. Similarly, following isoRB supplementation, blood glucose data at 10 and 20 min were significantly lower (P<0.05) compared with –20, 0, 40, 50 and 60 min. However, at failure, blood glucose data had increased significantly (P<0.001) compared to 20 min. Within time points, significantly higher blood glucose data were recorded for LS (P<0.001) compared to Water at t=0, see Figure 4.4. Mean data across time and trial are presented in Appendix 4.

4.3.6 Urine Volume

Urine volume/flow rate	Water	LS	isoRB
	Mean (±SEM)		
Pre-exercise (ml)	145 ± 27 (n=8)	52 ± 21 (n=6)	125 ± 40 (n=6)
Pre-exercise (ml.hr^{-1})	387±81 (n=8)	112 ± 63 (n=6)	249 ± 122 (n=6)
Failure (ml)	120 ± 15	89.78 ± 20	118 ± 12
Failure (ml.hr^{-1})	143 ± 22	80 ± 17	104 ± 16
Total (ml)	249 ± 38	127 ± 12 *	201 ± 40
Total (ml.hr^{-1})	213 ± 37	83 ± 8 **	139 ± 33

Table 4.3: Mean (± SEM) urine volume (ml) and urine flow rate (ml.hr^{-1}) across trials, (n=9 unless stated otherwise).

** and * Implies significantly lower data (P<0.01, <0.05) for LS compared to Water.

Urine volumes voided were recorded on arrival at the laboratory, immediately pre-exercise (t=0) and at failure. However, not all subjects provided a sample at each time point and when no sample was provided at discrete time points that subject was removed from the analysis at that time point, see Table 4.3. The total volume of urine voided and the urine flow rate per hour were calculated pre-exercise (20 min), at failure and in total (pre-exercise + time to failure) taking into consideration the exercise time to failure for each individual. Significantly lower volumes were recorded during the LS trial compared to Water in terms of total urine output (ml; P<0.05) and urine flow rate (ml.hr^{-1}; P<0.01).

4.3.7 Oxygen Uptake

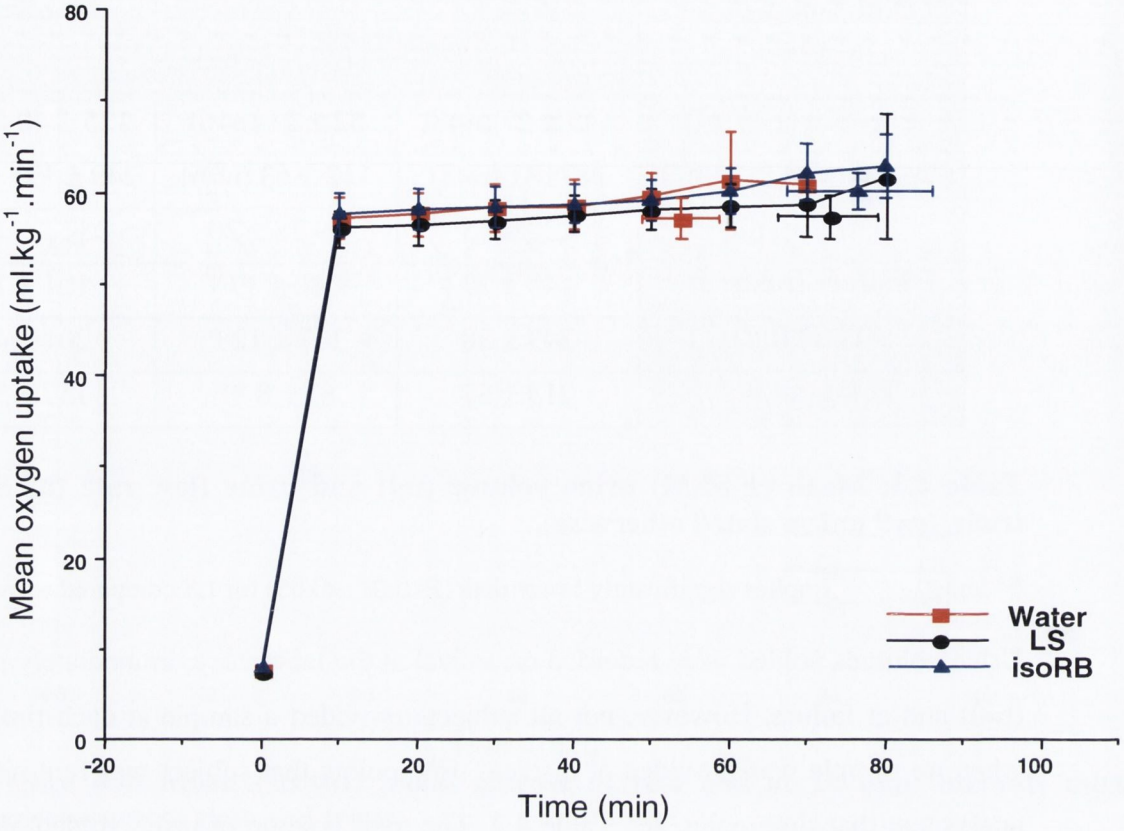


Figure 4.5: Mean (±SEM) oxygen uptake at rest and at 10 min intervals during high intensity exercise element across time and trials.

Oxygen uptake is presented in graphical format at rest, every 10 min for 80 min of exercise and at failure, see Figure 4.5. However, due to the decreasing number of subjects exercising after 60 min, statistical analysis was only performed on data collected at rest, every 10 min for the first 60 min of exercise and at failure. Mean oxygen uptake data did not change during exercise and no significant differences were recorded at any time comparing across the 3 drinks.

The mean absolute percentages of $\dot{V}O_2max$ recorded at 10 min intervals during the first 50 min for all 3 drinks trials are tabulated in Table 4.4. From Table 4.4 it is clear that subjects were exercising at a slightly higher intensity than 60% $\dot{V}O_2max$ (63-65% approximately) and slightly lower than 90% $\dot{V}O_2max$ (81-85% approximately) during the respective time intervals. Mean data across time and trial are presented in Appendix 4.

	10 min	20 min	30 min	40 min	50 min
% $\dot{V}O_2$max during exercise at a load equivalent to 60% $\dot{V}O_2$max					
Water	64.8±1.6	63.5±1.9	62.3±2.1	63.1±1.3 (n=8)	64.2±2.0 (n=6)
LS	63.3±2.0	63.8±2.0	64.6±1.6	64.6± 2.0	63.7±1.4 (n=8)
isoRB	63.7±1.0	65.0±1.7	64.8±1.5	65.9±1.5	66.4±2.0 (n=7)
% $\dot{V}O_2$max during exercise at a load equivalent to 90% $\dot{V}O_2$max					
Water	82.8±1.7	80.8±1.9	84.6±2.2	84.6±2.0 (n=8)	84.3±2.6 (n=6)
LS	81.3±1.7	81.8±1.7	82.6±1.7	83.6±1.5	84.1±1.8 (n=8)
isoRB	83.6±1.6	82.3±1.5	84.8±1.8	85.3±1.7	84.7±2.6 (n=7)

Table 4.4: Mean ± SEM % maximal oxygen uptake at loads equivalent to 60 and 90% $\dot{V}O_2$max across trials, n=9 unless stated.

4.3.8 Respiratory Exchange Ratio

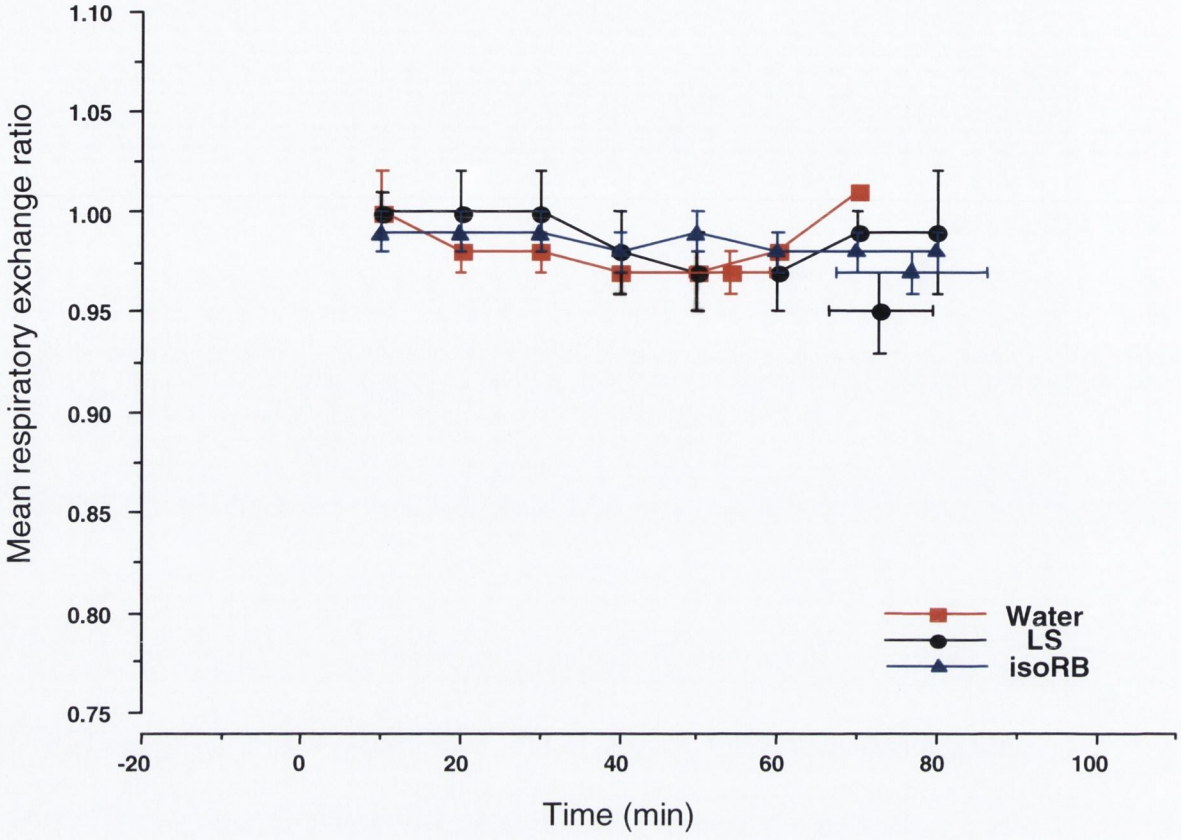


Figure 4.6: Mean (±SEM) Respiratory Exchange Ratio during high intensity exercise element across time and trials.

Similar to the oxygen uptake data in Figure 4.5, statistical analysis was only performed on RER data collected during the first 60 min of exercise and at failure. No significant time effect was reported during the isoRB trial. However, following Water ingestion, the mean RER increased significantly at 40 min and at failure compared to 10 min. In contrast, mean RER data at failure (0.97±0.02) following LS ingestion were significantly lower than data recorded at 10 (1.00±0.01), 20 (1.00±0.02) and 30 (1.00±0.02) min. There were no significant differences recorded in RER data across all 3 drinks at any of the time points analysed, see Figure 4.6. Mean data across time and trial are presented in Appendix 4.

4.3.9 Plasma Osmolality

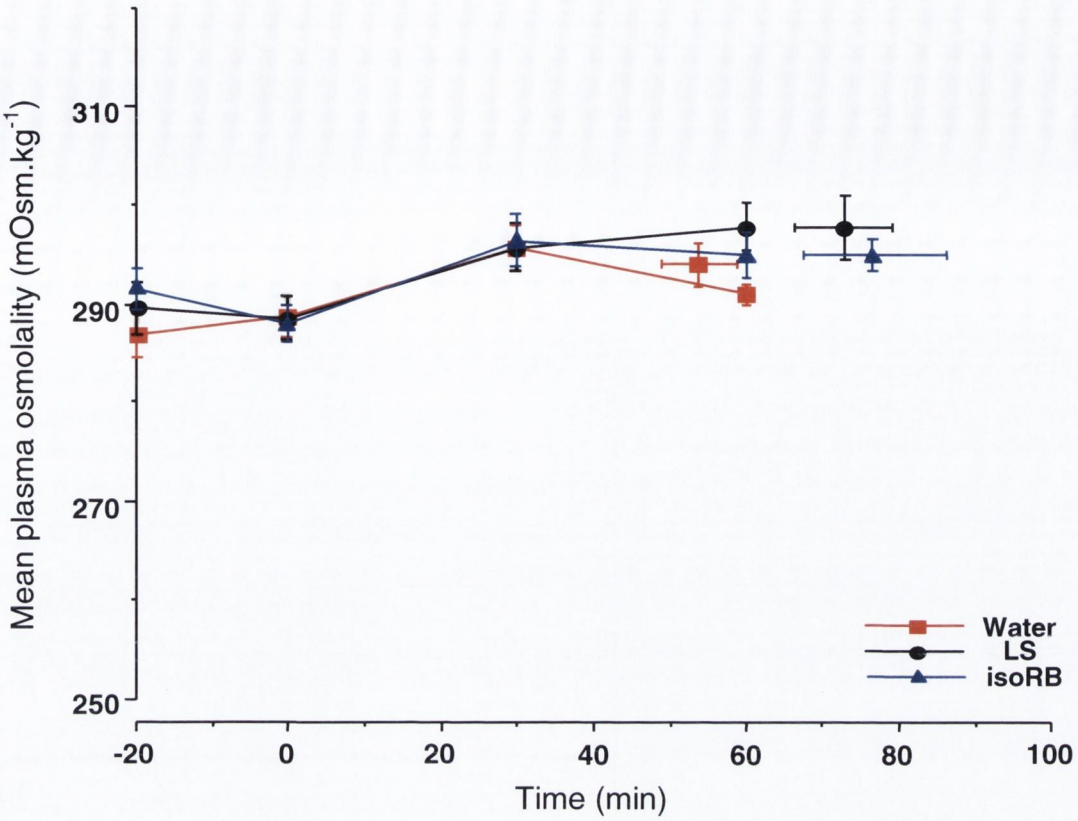


Figure 4.7: Mean (±SEM) plasma osmolality at rest and during exercise across time and trials, n=9 (at 60 min n=3 Water, n=7 LS and isoRB).

Blood samples were collected for analysis of plasma osmolality pre-test (-20), pre-exercise (0), at 30 and 60 min during exercise and at failure, Figure 4.7. Statistical analysis was not performed on data collected at 60 min due to the reduced samples size because some subjects had already failed before 60 min. Exercise did have a significant effect on plasma osmolality during all trials. Mean plasma osmolality following LS ingestion was significantly higher at failure (297.7±3.2 $mOsm.kg^{-1}$) compared to 0 (288.6±2.2 $mOsm.kg^{-1}$, $P<0.01$) and –20 min (289.6±2.7 $mOsm.kg^{-1}$, $P<0.05$). At 30 min, mean plasma osmolality data during the Water and isoRB trials were significantly greater than –20 and 0, respectively, ($P<0.05$). Plasma osmolality data stayed within the physiological range throughout all tests. Mean plasma osmolality data throughout the 3 trials were similar to the normally cited value of 287 $mOsm.kg^{-1}$ (Armstrong *et al.*, 2005). No significant differences were recorded across the 3 drinks at any time point. Mean data across time and trial are presented in Appendix 4.

4.3.10 Non-esterified fatty acids

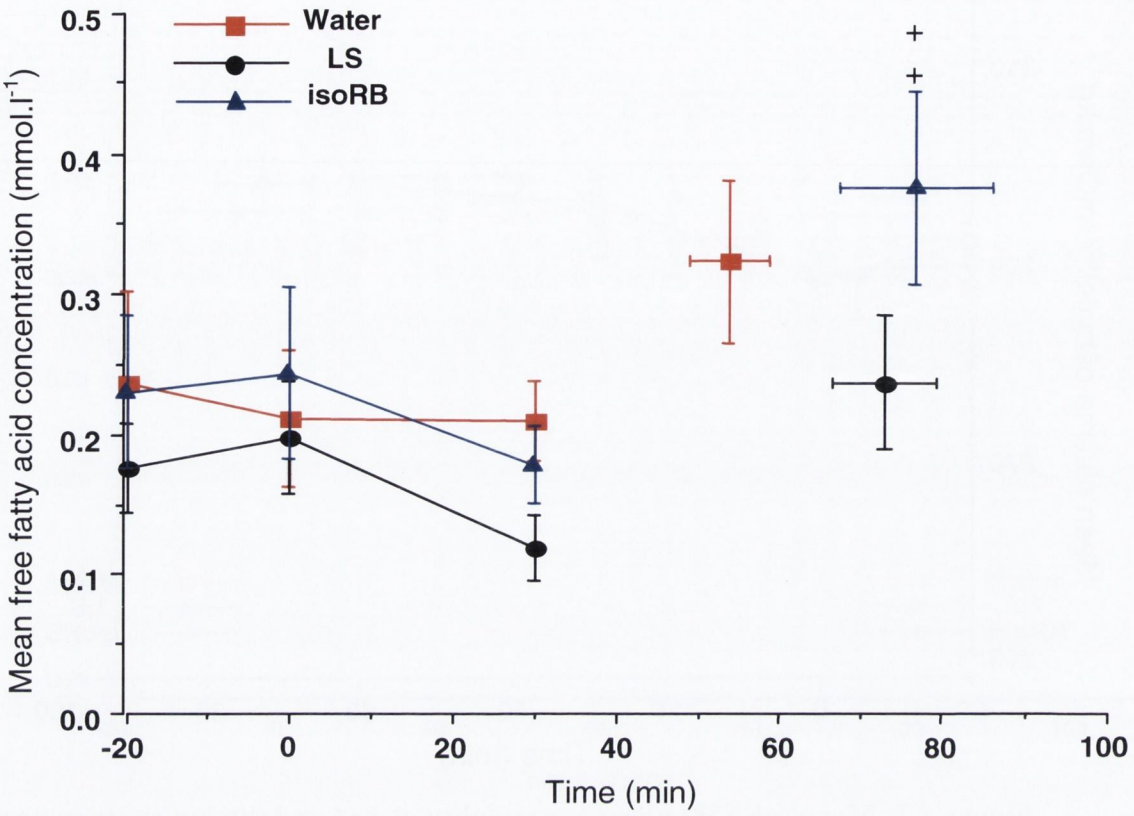


Figure 4.8: Mean (±SEM) non-esterified free fatty acid concentration at rest and during exercise across time and trials, n=9.

++ Implies significantly greater (P<0.01) for isoRB compared to LS.

The mean NEFA concentration recorded at failure (0.376±0.07 mmol.l^{-1}) during the isoRB trial was significantly greater (P<0.05) than data recorded at all other time points during the isoRB trial, Figure 4.8. No significant differences were recorded across time during the Water or LS trials. The mean concentration of NEFA recorded at exhaustion during the isoRB trial was significantly higher (P<0.01) than LS (0.0237±0.05 mmol.l^{-1}). However, no differences were recorded across the 3 drinks at any other time point during the trials. Mean data across time and trial are presented in Appendix 4.

4.3.11 Plasma caffeine concentration

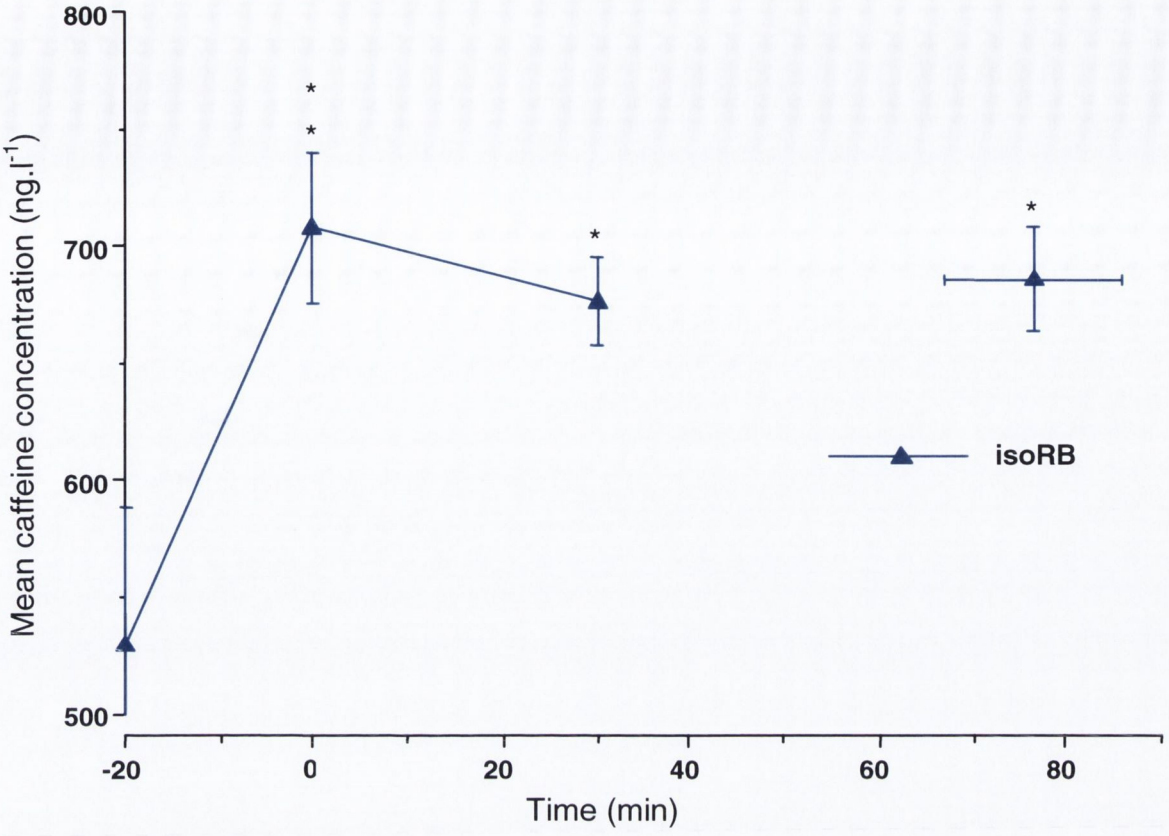


Figure 4.9: Mean (±SEM) plasma caffeine concentrations at rest and during exercise for the isoRB trial, n=9.

* * and * Implies significantly higher (P<0.05 and <0.05) data compared with -20.

The mean plasma caffeine concentration from all 9 subjects at each time point is illustrated in Figure 4.9. The graph illustrates that there was a sustained elevated increase in plasma caffeine concentration compared to –20 min data. Despite the ingestion of isoRB every 10 min during exercise there was no further increase in caffeine concentration following the initial increase 20 min after ingestion of the first bolus (t=0). The mean caffeine concentration recorded at –20 appears to be very high, as subjects had been requested to abstain from caffeine intake from midnight prior to the trial. The ELISA technique for plasma caffeine estimation was repeated on 3 occasions, each time yielding the same results. Mean data across time and trial are presented in Appendix 4.

4.3.12 Plasma Volume

Changes in plasma volume were calculated within specific time points, using the equations proposed by Dill and Costill (1974). Table 4.5 presents the mean ± SEM plasma volume changes across time and drink. Not all subjects completed 60 min of intermittent exercise; the numbers in brackets in Table 4.5 at 60 min represents the number of subjects included in the data analysis. For this reason the plasma volume changes calculated for the time period 0–60 min were not included in the statistical analysis. There were no significant differences in plasma volume changes within the time points analysed across the 3 drinks. However, analysis of the data showed that the changes in plasma volume from the beginning of the trials to the start of exercise (-20-0) were, as expected, significantly lower than at all other time points analysed ($P<0.001$). This trend was observed for all 3 treatments. It can be observed from the Table 4.5 that exercise had a dehydrating effect, but to a similar degree in all trials, -5.9±0.9, -5.3±1.2 and –5.2±1.0% for LS, isoRB and Water, respectively, even though subjects exercised for a significantly longer duration following the ingestion of isoRB and LS.

Time points (min)	Red Bull ΔPV (%)	Lucozade Sport ΔPV (%)	Water ΔPV (%)
-20 – 0	- 0.5±0.8***	-0.1±0.6***	0.8±0.5 (n=8)***
0 – 30	-5.7±1.0	-6.2±0.6	-6.5±0.7
0 – 60	-4.4±0.6 (n=7)	-4.7±0.8 (n=7)	-4.2 ± 0.7 (n=3)
0 – Failure	-5.4±0.9	-5.5±0.9	-5.9±0.6
-20 – Failure	-5.9±0.9	-5.3±1.2	-5.2±1.0 (n=8)

Table 4.5: Mean (± SEM) plasma volume changes across trials, n=9 unless otherwise stated.

*** Implies significantly lower $P<0.001$) plasma volume changes compared with all other time points analysed for all 3 drinks trials.

4.3.13 Body mass losses

Mass (kg)	Water	LS	isoRB
Pre-test body mass	74.7 ± 2.2	74.8 ± 2.1	74.5 ± 2.2
Net gain/loss	0.773 ± .07	1.039 ± 0.06	1.048 ± .105
Pre-exercise body mass	75.5 ± 2.2	75.8 ± 2.1	75.8 ± 2.1
Body mass at failure	74.1 ± 2.2	74.0 ± 2.1	74.1 ± 2.2
Body mass loss	1.35 ± 0.15	1.85 ± 0.20 ++	1.67 ± 0.15

Table 4.6: Mean (±SEM) body mass losses across trials, n=9.

++ Implies significantly higher ($P<0.01$) body mass loss for LS compared with Water.

Table 4.6 illustrates mean (± SEM) body mass data recorded at the beginning of each trial, prior to exercise and at failure. Together with these data and the net fluid gain/loss; calculated by subtracting the volume of urine voided from the volume of fluid ingested, it was possible to calculate body mass changes. The total body mass loss for Water was significantly lower than that for LS ($P<0.01$). This finding was most likely due to the duration of exercise completed as body mass losses per minute of exercise were calculated at 0.03±0.003, 0.03±0.002 and 0.02±0.002 $kg.min^{-1}$ for Water, LS and isoRB respectively and no significant differences were recorded between trials.

4.4 Discussion

This study investigated the effects of 3 drinks: Lucozade Sport (LS), Red Bull® (isoRB) and Water on intermittent exercise performance in 9 male cyclists. In this study Red Bull was diluted (60-40) with water to make it isocaloric to Lucozade Sport. The test drinks were ingested 20 min before and at 10 min intervals during exercise of alternating intensity. The principal findings of the study were significant increases in time to failure for LS (26%) and isoRB (29%) when compared with Water, with no differences in performance times detected between the 2 iso-calorically matched drinks despite the presence of caffeine and taurine in isoRB. The mean times to failure (± SEM) for isoRB (76.7±9.4 min, $P<0.01$) and LS (72.8±6.4 min, $P<0.05$) were significantly greater than for Water (53.9±5.0 min), see Figure 4.1.

In this study, caffeine was ingested at a rate of 2.5 and 1.7 $mg.kg^{-1}.hr^{-1}$ pre-test and every 10 min during exercise, respectively, during the isoRB trial. Previously Graham and Spriet (1995) had reported a significant improvement in endurance running performance following ingestion of 3 and 6 mg kg^{-1} caffeine in the form of capsules ingested 60 min pre-exercise compared with placebo. In the study by Graham and Spriet (1995) caffeine was ingested prior to a period of warm-up and stretching and no additional caffeine ingestion took place during exercise. The timing of ingestion in the present study was different (20 min pre-exercise) to that used by Graham and Spriet (1995) and also the test drink was ingested during exercise. Both studies however recorded significant improvements in exercise performance regardless of the form of caffeine used. Exercise time to exhaustion, running at a velocity equivalent to 85% $\dot{V}O_2max$, was approximately 60 min following the ingestion of 3 and 6 $mg.kg^{-1}$ of caffeine compared with 49 min during their placebo trial (Graham and Spriet, 1995). The mean time to failure recorded following the ingestion of caffeine in this study (isoRB, 76.7 min) was greater than that recorded by Graham and Spriet (1995) possibly due to the intermittent nature of the exercise protocol used.

The principal difference between isoRB and LS in this study was the presence of caffeine and taurine in isoRB. No significant differences in time to failure were observed between LS and isoRB, see Figure 4.1, therefore it may be possible that the mechanism responsible for the differences observed between LS and isoRB compared with Water relied heavily on the carbohydrate component of the 2 drinks. In addition, the supply of exogenous carbohydrate every 10 min during exercise in the LS and isoRB trials may be one reason why significant increases in time to failure were reported in this study and not in the previous study (Chapter 3).

To my knowledge there are no previously published data comparing the effects of 2 presently available sports drinks on intermittent exercise performance. Much of the earlier scientific research investigating carbohydrate ingestion during exercise has primarily focused on low to moderate intensity prolonged endurance type exercise (Coyle *et al.*, 1986; Coyle *et al.*, 1983). An increase in performance during intermittent exercise following the ingestion of a carbohydrate electrolyte beverage compared with placebo had previously been reported by Nicholas *et al.* (1995) in trained games players during a running test. Maximal oxygen uptake was estimated during a progressive shuttle test and running velocities corresponding to 55% and 95% $\dot{V}O_2max$ were employed for the 2 exercise trials. Intermittent running time to fatigue (55 and 95% $\dot{V}O_2max$) after a 75 min period of intermittent exercise (walking, jogging, running and sprinting) was significantly longer (33%) following ingestion of Lucozade Sport compared with a non-carbohydrate placebo ($P<0.05$). This improvement of 33% in endurance running capacity corresponds to an increase in running time of only 2.2 min, yet this study still forms the basis for the vast majority of the marketing and advertising of Lucozade Sport in the media to the present day. The distance covered during the run to fatigue for the carbohydrate electrolyte trial was 400 m longer than for the placebo trial. In this present study the differences in cycling time to failure during the LS and isoRB trials compared to Water were 22.8 (29%) and 18.9 min (26%), respectively. These results represent a much greater difference in exercise time and therefore a significantly greater distance was cycled during the LS and isoRB trials compared to Water. In the study by Nicholas *et al.* (1995) a volume equivalent to 5 $ml.kg^{-1}$ of the test drink was ingested immediately before the start of exercise and 2 $ml.kg^{-1}$ every 15 min during exercise. This was in comparison to 4.29 $ml.kg^{-1}$ 20 min pre-exercise and 1.43 $ml.kg^{-1}$ every 10 min during exercise in this study. Welsh *et al.* (2002) investigated the effects of carbohydrate ingestion on physical and mental performance during intermittent exercise to fatigue. Ten games players (5 male, 5 female) participated in their shuttle running test, the test was divided into 5 sections, 4 of 15 min duration followed by a shuttle run to fatigue. During the 15 min quarters, intermittent exercise was performed at various percentages of $\dot{V}O_2max$ (walking, jogging and sprinting). Carbohydrate or placebo was ingested pre-exercise and following each of the four quarters. Performance time in the shuttle run to fatigue following carbohydrate ingestion was improved by 37% (97 s) compared with placebo. Coggan and Coyle (1988) investigated the effect of carbohydrate feeding (1 $g.kg^{-1}$ at 10 min after starting exercise and 0.6 $g.kg^{-1}$ every 30 min thereafter) during high intensity exercise and reported an 18% improvement in time to fatigue, corresponding to 31 min of exercise, following ingestion of the

carbohydrate solution compared with placebo during intermittent cycling exercise alternating between 60 and 85% $\dot{V}O_2$max. The intervals of exercise (15 min) used by Coggan and Coyle (1988) were longer than those in the present study to allow for recordings of steady state metabolic responses. Wright *et al.* (1991) reported a 17-44% increase in time to exhaustion following the ingestion of carbohydrate pre-exercise, during exercise or in combination, compared to a placebo treatment 3 hr before exercise. When carbohydrate was ingested before and during exercise the performance time was significantly greater by 88.6 min. Increases in exercise time of 65.1 and 35.7 min were recorded when carbohydrate was ingested pre-exercise or during exercise, respectively. In contrast, Hargreaves *et al.* (1987) investigating the effects of carbohydrate ingestion 75 min prior to cycling exercise reported no significant difference in time to exhaustion when compared to a flavoured placebo or water.

In their review of carbohydrate ingestion and prolonged exercise, Coggan and Coyle (1991) concluded that exercise performance was improved when carbohydrate was ingested at a rate of 25 – 60 g hr^{-1}. In this study during the isoRB trial, carbohydrate was ingested at a rate equivalent to 52.5 and 42 $g.hr^{-1}$ at 20 min pre- and every 10 min during exercise, respectively compared with 49.7 and 39.9 $g.hr^{-1}$ during the LS trial. There were no significant differences in mean blood lactate data recorded across the 3 drinks treatments in this study, see Figure 4.2, and the present findings are consistent with results reported by Nicholas *et al.* (1995), investigating Lucozade Sport and Geiss *et al.* (1994), investigating Red Bull. Analysis of mean blood lactate data at 10 min intervals, see Figure 4.3, for the first 60 min of exercise reported no significant differences across the 3 trials, with the expected increases occurring early in exercise compared to pre-exercise data and remaining elevated throughout exercise until failure. The low intensity interval (3 min at a load equivalent to 60% $\dot{V}O_2$max) in the protocol may have prevented any time related increase in blood lactate during exercise by allowing a reduction in blood lactate to occur prior to the next high intensity interval (2 min at a load equivalent to 90% $\dot{V}O_2$max).

The urine flow rates recorded during the LS trial were significantly greater compared to water when calculated for each trial taking into consideration the pre-exercise time period and each individual's time to failure. No diuretic effect of caffeine was observed. Wemple *et al.* (1997) suggested that the diuretic effects of caffeine observed at rest might be overridden by alterations in renal function during exercise, thereby overruling the conventional wisdom that caffeine would accelerate dehydration during exercise.

One of the proposed effects of caffeine on endurance exercise is associated with increased catabolism of muscle triglycerides and reduced muscle glycogenolysis. An increase in

lipolysis would potentially result in the increased availability of free fatty acids promoting an enhancement of fat metabolism and a sparing of carbohydrate metabolism, increasing exercise capacity. There were no changes in blood NEFA or epinephrine reported by Graham and Spriet (1995) following ingestion of 3 or 6 mg kg^{-1}, but significant differences were observed in exercise performance during these trials. As a result the authors concluded that the effects of caffeine on exercise, NEFA and epinephrine were not dependent on each other, possibly indicating different actions of caffeine on different tissues with different concentrations. Analysis of data in the present study following the ingestion of isoRB revealed that the NEFA concentration was significantly higher at failure compared with all other time points during that trial. The concentration of NEFA were also significantly higher at failure following the ingestion of isoRB when compared with LS, see Figure 4.8. The results for plasma caffeine concentration and the NEFA data may illustrate some association between the presence of caffeine and an increase in the availability of NEFA, but no significant difference in exercise time to failure was detected between LS and isoRB trials.

When reviewing the literature with reference to the effects of caffeine on exercise performance it was noted that ingestion of caffeine was principally administered in capsular form. Supplementation of caffeine in a liquid form via RB and isoRB in the 2 studies described so far may have resulted in altered pharmacokinetics of caffeine when compared with capsular administration making a like-for-like comparison with results reported in the literature difficult. With this in mind the research described in Chapter 5 was designed.

Chapter 5

Pharmacokinetics of caffeine following ingestion in capsular or liquid format in healthy males.

5.1 Introduction

The studies detailed in Chapters 3 and 4 in this dissertation investigated the effects of ingestion of either a carbohydrate (LS) or caffeinated carbohydrate (RB and isoRB, respectively) beverage on exercise performance compared to a water placebo. Significant improvements following the ingestion of LS and isoRB were reported in Chapter 4 when compared with Water, while no differences in exercise performance were reported in Chapter 3 when LS and RB were administered pre-exercise only. When comparing the data from these 2 studies with those in the literature it was noted that when investigating the effect of caffeine on exercise performance, previous research predominately administered caffeine in capsular form. The absorptive, metabolic and pharmacokinetic properties of caffeine are detailed in Section 1.6.2 and it has been reported that caffeine administered orally is completely absorbed and metabolised within 45 min following ingestion (Mc Lean and Graham, 2002). In Chapter 3, the caffeine concentration was noted to increase significantly 60 min post-ingestion of RB (Figure 3.8) and within 20 min following the ingestion of isoRB in Chapter 4 (Figure 4.9). In order to compare the findings of the research in this dissertation with those available in the literature the following study was designed to compare the pharmacokinetics of caffeine (3 $mg.kg^{-1}$) in liquid (isoRB) and capsular form (CAFF). The isoRB formulation was chosen as it had previously been reported to significantly improve intermittent exercise performance (Chapter 4). This study was performed under resting conditions to eliminate the effect of exercise on the rate of gastric emptying and intestinal absorption during the isoRB trial due to the presence of carbohydrate.

5.2 Methods

5.2.1 Study Design

This study was an un-blinded randomised study involving 3 separate visits to the Human Performance Laboratory, Trinity College Dublin of approximately 3 hr duration. It was not possible to perform a blinded study due to the distinctive taste of Red Bull but during the other 2 trials the subjects were not aware if caffeine or placebo was in the test capsule. Ethics approval was granted by the Faculty of Health Sciences Ethics Committee.

5.2.2 Details of the test supplements

The 3 supplements under investigation were caffeine in capsular form (CAFF), isoRB and Placebo (PL). Each subject received the equivalent of 3 $mg.kg^{-1}$ of caffeine in capsule (CAFF) or liquid form (isoRB). To prepare the beverage termed isoRB commercially available Red Bull was diluted with water (60:40) similar to the beverage used the studies described in chapters 4 and 6 of this dissertation. In the placebo trial each subject received 2 capsules, each containing 150 mg lactose, along with a volume of water equivalent to the volume of isoRB ingested. During the CAFF trial a caffeine dose equivalent to $3mg.kg^{-1}$ was prepared, divided into 2 gelatin capsules and ingested with water, isovolumetric to isoRB. The supplements were allocated in a randomised order and blinded for the CAFF and PL trials.

5.2.3 Subject Recruitment

9 male subjects volunteered to participate in the study. They were recruited through colleagues in the Human Performance Laboratory and some fellow students in Trinity College Dublin. There was no exercise element in the test protocol and therefore it was possible to recruit both active and sedentary individuals.

5.2.4 Inclusion and exclusion criteria

All subjects were male, non-smokers aged between 18 and 30 yr. Any volunteer displaying cardiac abnormalities at the time of the medical and ECG examination were excluded from participation. Caffeine abstainers/caffeine naïve individuals were also excluded.

5.2.5 Medical Screening and anthropometric data

On arrival at the laboratory for the first trial each subject completed a detailed medical questionnaire (Appendix 2) and underwent a medical examination. Cardiovascular and

respiratory variables were assessed; in addition the attending physician examined the throat and lymph glands. Following bladder emptying, height and body mass were recorded using a counterbalance weighing scales and stadiometer (Seca Ltd., Germany), pulmonary function was assessed using the "Microlab" microspirometer (Micro Medical Ltd., England), see section 2.3. Percent body fat was estimated using Harpenden Skinfold callipers at four sites.

5.2.6 ECG analysis

A 12 lead electrocardiogram was performed on each subject and examined by the physician for any cardiac abnormalities that would be affected by caffeine ingestion during the trial.

5.2.7 Study protocol

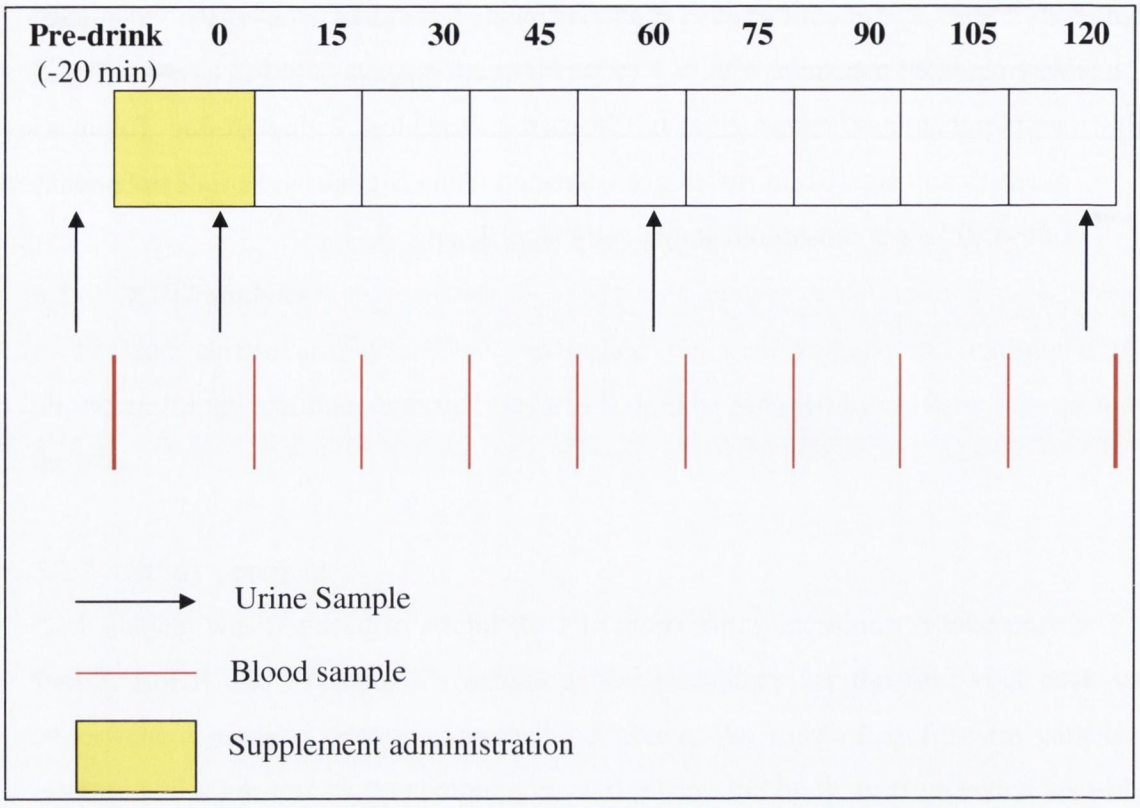
Each subject was required to attend the laboratory on 3 occasions to take part in 3 trials: CAFF, isoRB and Placebo. On arrival at the laboratory for the first visit each subject underwent a medical examination detailed above. An indwelling forearm catheter was inserted and a pre-test blood sample collected. Using the body mass recorded post-bladder emptying the volume of fluid to be ingested was prepared. The subject was then required to ingest all of the test drink/capsule in the subsequent 20 min period. After this 20 min period the subject was monitored for a further 2 hr with regular blood sampling (every 15 min) and urine sampling (every 60 min), see Schematic 3. The canula was removed before the subject left the laboratory.

5.2.8 Haematological and urinary analysis

An indwelling forearm canula was inserted prior to each trial to reduce the discomfort for the subject as a result of multiple blood sampling over the course of each trial. The first blood sample collected from the indwelling catheter was analysed in the automated cell counter (Coulter Counter system, Model Act Diff, Coulter Electronics, England) for the variables haemoglobin (Hgb in $g.dl^{-1}$), haematocrit (Hct) in %, red blood cell count (x $10^{12}.l^{-1}$) and white cell count ($x10^{9}.l^{-1}$). Subsequent samples collected at set time points, see Schematic 3, were analysed for plasma volume changes (Dill and Costill, 1974), glucose, caffeine and NEFA concentrations. Plasma osmolality was also assessed using the micro-osmometer, see section 2.12. Urine volumes were recorded pre and post fluid ingestion and every hour during the 2 hr monitoring period.

5.2.9 Statistical Analysis

All subject physical characteristics are presented in Table format as mean ± standard deviation (SD) and all graphical data as mean ± standard error of the mean (SEM). A two-way repeated measures ANOVA (time by treatment) was used to assess the effects of the 3 supplementations across time on measured variables, with *post-hoc* Tukey tests used to quantify any significant differences detected using Sigmastat. For all statistical tests values of $P<0.05$ were considered statistically significant.



Schematic 3: Order of data collection during the supplement trials.

5.3 Results

5.3.1 Physical Characteristics

	Mean ± SD
Age (yr)	23 ± 4
Body mass (kg)	84.0 ± 11.6
Height (m)	1.80 ± 0.06
BMI ($kg.m^{-2}$)	26.0 ± 3.6
Body fat (%)	16.1 ± 6.8

Table 5.1: Mean (± SD) physical characteristics, n=9.

Haematological data	Mean ± SD	Pulmonary data	Mean ± SD
Hb ($g.dl^{-1}$)	14.9 ± 0.5	**FEV_1 (l)**	4.2 ± 0.5
Hct (%)	42.2 ± 1.5	**FVC (l)**	5.7 ± 0.7
RBC ($x10^{12}.l^{-1}$)	4.7 ± 0.3	**PF ($l.min^{-1}$)**	577 ± 19
WBC ($x10^{9}.l^{-1}$)	6.4 ± 1.7	**FEV_1/FVC (%)**	76 ± 12

Table 5.2: Mean (± SD) haematological and pulmonary data, n=9.

All subjects displayed normal haematological and pulmonary variables, see Table 5.2. There were no indications of obstructive or restrictive airway disorders.

5.3.2 Caffeine concentration

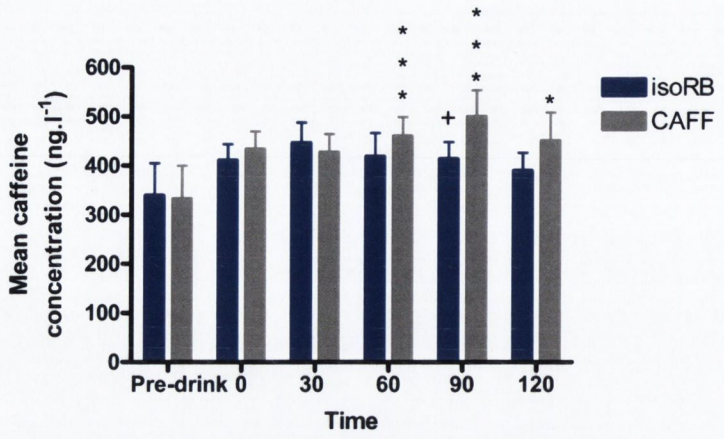


Figure 5.1: Mean (± SEM) caffeine concentration across time and trials, n=9.

*** and * Implies significantly higher (P<0.001, <0.05) data compared to pre-drink.
\+ Implies significantly lower data (P<0.05) compared to CAFF at t=90.

The serum caffeine concentration of samples collected pre-drink, immediately post-drink (0) and every 30 min during the 2 hr monitoring period were analysed using an ELISA technique for both the CAFF and isoRB trials. No significant changes across time were recorded during the PL and isoRB trials, however, during the CAFF trial significant differences were recorded at 60 (460 ± 39, P<0.001), 90 (499 ± 54, P<0.001), and 120 (450 ± 58, P<0.05) min compared to pre-drink (332 ± 68 $ng.l^{-1}$) data, see Figure 5.1. A significant treatment effect was recorded across the 2 supplements at 90 min; the caffeine concentration during the CAFF trial was significantly higher (P<0.05) than isoRB.

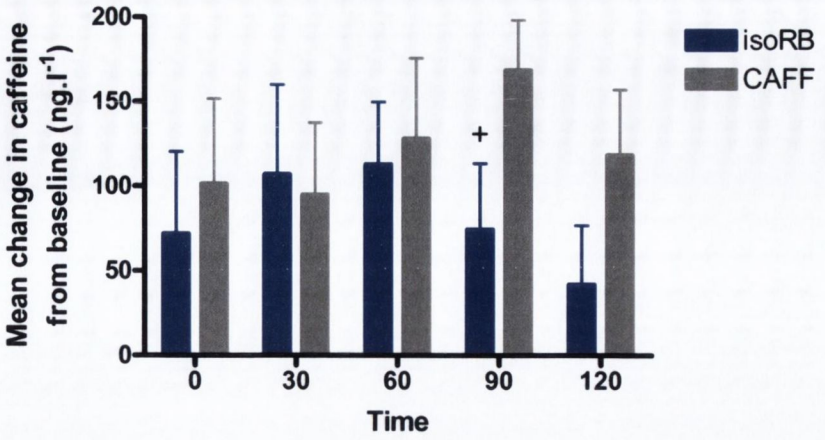


Figure 5.2: Mean (± SEM) change in caffeine concentration from baseline (pre-drink), n=9.

\+ Implies significantly lower ($P<0.05$) data compared to CAFF.

The data were also analysed for changes across time relative to baseline (pre-drink) data to eliminate any effect of variations in each individual's pre drink data across the trials, see Figure 5.2.

5.3.3 NEFA

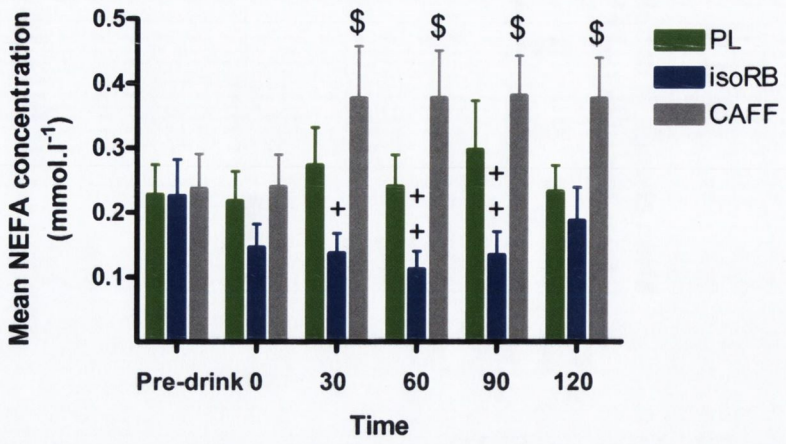


Figure 5.3: Mean (± SEM) NEFA concentration across time and trials n=9.

+ and ++	Implies significantly lower (P<0.05 and <0.01) compared to CAFF.
$	Implies significantly higher (P<0.05) compared to pre-drink and t=0.

The mean (± SEM) concentration of non-esterified fatty acids was analysed for all 3 trials pre- and post-drink (0) and at 30, 60, 90 and 120 min, the results are presented in Figure 5.3. No significant changes were recorded across time during the PL trial. NEFA concentration recorded at 30, 60, 90 and 120 min were significantly greater (P<0.05) than pre-drink and 0 during the CAFF trial. At 30, 60 and 90 min NEFA concentration was significantly higher in the CAFF trial compared to the isoRB trial at the same time points.

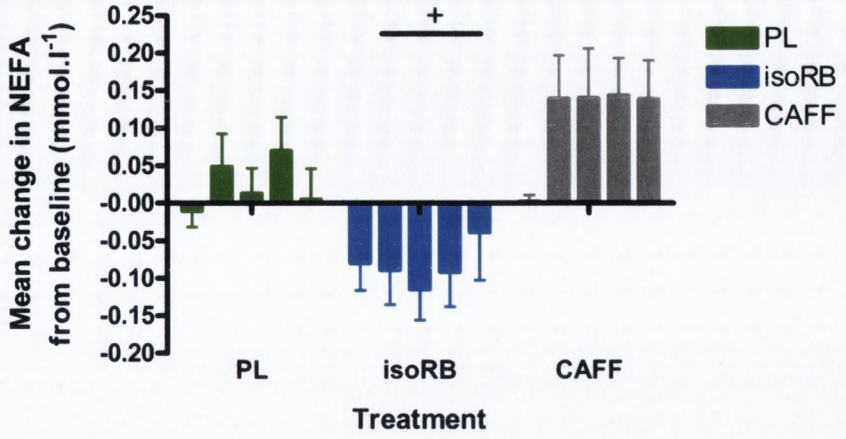


Figure 5.4: Mean (± SEM) change in NEFA from baseline (pre-drink), n=9.

+ Implies significantly different to CAFF.

The different changes in NEFA concentration between isoRB and CAFF were also analysed relative to baseline (pre-drink) data, Figure 5.4. Relative to baseline data, NEFA concentration during the isoRB trial decreased, with increases recorded during the CAFF trial. Therefore significant differences ($P<0.001$) were recorded between these 2 trials after 30, 60, 90 and 120 min relative to baseline.

5.3.4 Serum glucose

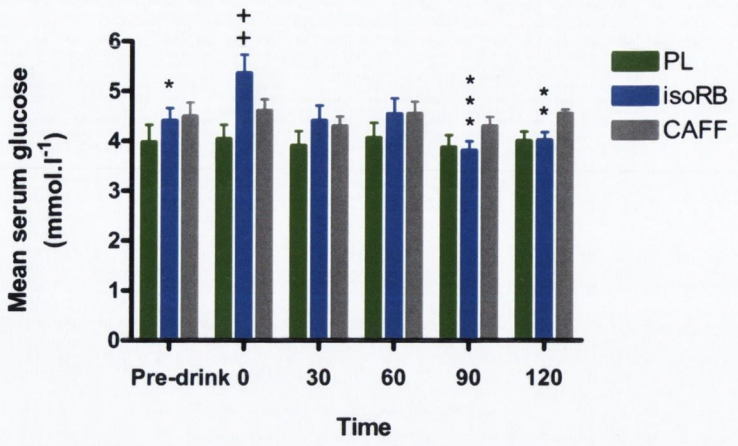


Figure 5.5: Mean (± SEM) serum glucose concentration across time and trials, n=9.

++	Implies significantly higher (P<0.05) compared to PL.
*** and ** and *	Implies significantly lower (P<0.001 and P<0.01 and P<0.05) compared to t=0.

Serum glucose concentration (mean ± SEM) during all 3 trials is illustrated in Figure 5.5. As expected no significant changes were recorded in serum glucose data throughout the CAFF and PL trials. During the isoRB trial significantly lower data were recorded at 90 (3.8±0.2, P<0.001) and 120 (4.0±0.2, P<0.01) min compared to t=0 (5.4±0.4 $mmol.l^{-1}$) data. Immediately after the 20 min ingestion period (t=0) the serum glucose concentration during the isoRB trial was significantly higher (P<0.01) compared to PL.

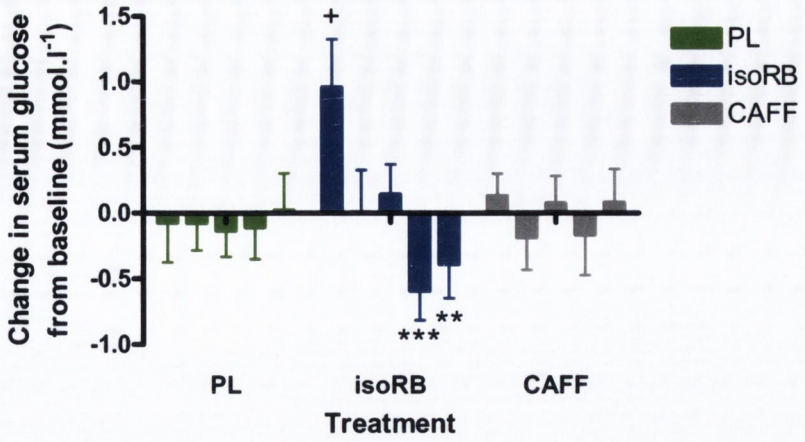


Figure 5.6: Mean (± SEM) change in serum glucose concentration from baseline (pre-drink), n=9.

+ Implies significantly different (P<0.05) compared to PL at the same time point.
*** and ** Implies significantly different (P<0.001 and <0.01) compared to t=0 data.

Figure 5.6 illustrates the change in serum glucose from baseline across all 3 trials. The ingestion of a large volume of glucose during the isoRB trial resulted in a significantly (P<0.05) elevated serum glucose concentration relative to pre-drink data at t=0 that was not observed during the PL trial, see Figure 5.6. The significant decline in serum glucose data at 90 and 120 min compared to t=0 during the isoRB trial is also evident in Figure 5.6.

5.3.5 Urine flow rate

Urine flow rate ($ml.hr^{-1}$)	PL	isoRB	CAFF
Pre-drink – 0	342 ± 103	347 ± 124	199 ± 17 $$
0 - 60 min	659 ± 79 **	736 ± 60 ***	738 ± 86 ***
60 - 120 min	396 ± 47 +	402 ± 31 +++	504 ± 55 +
Total	501 ± 51	537 ± 29	560 ± 58

Table 5.3: Mean (± SEM) mean urine flow rate during the three trials, n=9.

*** and ** Implies significantly greater (P<0.001, <0.01) compared to pre-drink–0.
+++ and + Implies significantly lower (P<0.001, <0.05) compared to 0-60 min.
$$ Implies significantly lower (P<0.01) compared to 60-120 and Total.

Urine volume was recorded immediately after the 20 min ingestion period and at 60 and 120 min during the 2 hr monitoring period and standardised with time to depict urine flow rate, see Table 5.3. No diuretic effect of caffeine was observed during the isoRB and CAFF trials because no significant treatment effect was observed at any stage in the trial comparing across all 3 supplements. During all 3 trials the urine flow rate during the first hour of the 2 hr monitoring period (0-60 min) was significantly higher (P<0.05) compared to the period of fluid ingestion (pre-drink-0) and the second hour of the trial (60-120 min). The total urine flow rate and the urine flow rate in the last hour (60-120 min) of the CAFF trial were significantly higher (P<0.001, <0.01, respectively) compared to the fluid ingestion period (pre-drink-0).

5.4 Discussion

This study examined the pharmacokinetics of caffeine (3 $mg.kg^{-1}$) ingested in capsular (CAFF) and liquid (isoRB) form at rest relative to a lactose placebo. Despite the caffeine concentration remaining similar to baseline levels throughout the isoRB trial, there appears to be a trend for the caffeine concentration to increase between 0 (410 ± 33 $ng.l^{-1}$) and 30 min (446 ± 41 $ng.l^{-1}$) and to slowly decline over the remaining 2 hours (Figure 5.1). In contrast during the CAFF trial, caffeine concentration did not become significantly elevated above baseline values until 60 min, but this elevation compared to pre-drink data was maintained for the remaining hour of the trial, see Figure 5.1. From the data it appears that the pharmacokinetics of caffeine are different when administered in a liquid form compared with a capsular form. The rate of appearance of caffeine is faster when ingested in liquid form, perhaps more of a result of a delay in processing caffeine when ingested in a gelatine capsule that must first dissolve before caffeine can be absorbed across the gastrointestinal tract.

In a review by Graham and Spriet (1996) 3 theories were proposed by which caffeine may provide an ergogenic effect or improve performance (see Section 1.6.6). One of these theories involved the increased catabolism of muscle triglycerides, increasing the intracellular availability of NEFA, and subsequently reducing muscle glycogenolysis. This increase in NEFA concentration along with increased glycerol has been reported during exercise following the ingestion of caffeine at a dosage of 9 $mg.kg^{-1}$ (Graham and Spriet, 1995). However, in their study no significant improvement in exercise performance was reported following the ingestion of 9 $mg.kg^{-1}$caffeine dose, but significant improvements were reported following ingestion of 3 and 6 $mg.kg^{-1}$. Significantly higher NEFA data were recorded at failure following isoRB ingestion when compared to LS in the research described in Chapter 4, see Figure 5.3. However, no significant improvement in exercise performance (time to failure) was recorded comparing these 2 isovolumetric and isocalorically matched drinks. Similarly the present study reports higher plasma NEFA data at 30, 60 and 90 min following ingestion of capsular caffeine compared to isoRB, (see Figure 5.3) even though the caffeine concentration administered in both formulations was identical (3$mg.kg^{-1}$). Also, across time there was a significant decrease in plasma NEFA concentration following isoRB ingestion relative to pre-ingestion. This may be as a result of the carbohydrate contained in Red Bull inducing a hyperinsulemic response and a subsequent inhibition of lipolysis.

There is very little scientific research reported in the literature investigating the pharmacokinetics of different forms of caffeine administration. One group of researchers

have investigated the effects of caffeine ingested in the form of chewing gum compared to capsular form on the ability to maintain alertness and performance in sleep deprived participants. Kamimori *et al.* (2002) investigated the rate of absorption and relative bioavailability of caffeine at rest when ingested as chewing gum or capsules in different concentrations (50, 100, 200 mg) in a single dose compared to placebo. Twelve healthy non-smoking males were recruited for each of the seven groups in this study. The authors suggested that there may be an earlier onset of the pharmacological effects of caffeine when in the gum formulation and that this may be advantageous where the rapid reversal of alertness is the desired response. A second study applied a multiple dose regime and reported positive effects in terms of the subjects' ability to remain alert despite being sleep deprived (Syed *et al.*, 2005).

At rest caffeine is reported to have diuretic properties whereby caffeine acts to increase sodium and chloride excretion at the proximal and distal renal tubules, that in turn causes an increase in urinary water loss (Armstrong, 2002). However, in the present study no diuretic effect was observed following ingestion of either capsular caffeine or isoRB compared to placebo, using an isovolumetric study design (see Table 5.3). Unfortunately analysis of electrolytes was not performed in the present study and in addition, no diuretic effect of isoRB ingestion was observed before, during or after exercise as described in Chapter 4. Therefore, it was decided to revisit the area of rehydration with sports drinks including those containing caffeine and to investigate in more detail especially the impact of LS, isoRB and Water on hydration status. This study will be described in Chapter 6.

Chapter 6

The effects of carbohydrate and caffeine beverages on hydration status following voluntary fluid restriction.

6.1 Introduction

Rehydration with LS and RB following exercise-induced hypohydration was investigated in this dissertation in Chapter 3. No significant differences in terms of plasma volume changes or plasma osmolality were reported across the 3 drinks tested; Water, LS and RB during the 90 min non-exercise rehydration period. Therefore despite the presence of caffeine in RB the plasma osmolality and volume had returned to pre-test data at the end of the trial. Unfortunately timed urine collection was not strictly adhered to in that study and so it was not possible to accurately calculate urine flow rates to investigate any diuretic effects of caffeine. In Chapter 4 caffeine did not display diuretic properties when ingested in the form of isoRB either before or during exercise. Similarly, in Chapter 5, no diuretic effect of caffeine ($3mg.kg^{-1}$) was observed following liquid (isoRB) or capsular (CAFF) administration when compared to Placebo.

It has been recommended that post-exercise rehydration should be complete within 2 hr and that the rehydration beverage should contain water, carbohydrate and electrolytes (Casa *et al.*, 2000), all of which were present in the consumed beverages during the LS and RB trials detailed in Chapter 3. Sports science associations regularly publish position statements on a wide range of topics, including rehydration post-exercise, in an effort to make the most up to date information available to both coaches and athletes. It has been recommended that the volume of fluid ingested as a rehydration beverage should be equivalent to 150% of the body mass lost during exercise or dehydration procedures to ensure that a state of euhydration is rapidly attained (<2 hr). In calculating fluid losses, sweat and obligatory urine production must be accounted for (Joint position statement: ACSM, American Dietetics Association and Dieticians of Canada, 2000).

Hypohydration following voluntary fluid restriction has received little attention in the literature, particularly in relation to healthy individuals. One study reported the physiological responses and subjective feelings of individuals following 37 hr of fluid restriction (Shirreffs *et al.*, 2004). In their study a relatively small degree of hypohydration was induced ($2.7 \pm 0.2\%$) with subjects reporting feelings of headache, reduced alertness and a greater difficulty concentrating.

The authors concluded that it is important to acknowledge the subjective feelings of hypohydrated individuals as well as the physiological effects incurred even at low levels of hypohydration.

With this in mind and the need to investigate the area of rehydration using commercially available sports drinks in more detail than that addressed in Chapter 3 the following study was carried out. Ten subjects were recruited to voluntary fluid restrict over a period of 30

hr after which time they received 150% of the body mass lost as either Water, LS or isoRB on 3 separate occasions. The latter 2 beverages (LS and isoRB) contain carbohydrate and electrolytes, widely recommended components of a rehydration beverage (Shirreffs and Maughan, 2000). Multiple markers of hydration status were measured, (section 1.10). The isoRB formulation was again used in this study as it has previously been reported in this dissertation to increase exercise performance with no observable diuretic effect detected. It was hypothesised that the ingestion of 150% body mass loss would be sufficient to rehydrate the subjects and that isoRB would not limit rehydration or have a negative effect on mildly hypohydrated individuals.

6.2 Methods

6.2.1 Study design

This study was un-blinded and randomised involving 9 visits in total to the Human Performance Laboratory. The study consisted of 3 discrete trials and each trial involved 3 separate visits to the laboratory over a period of 30 hr. The study required participants to voluntarily fluid restrict for 30 hr before returning to the laboratory for a period of monitored rehydration using one of 3 different test drinks for each trial. It was not possible to perform a blinded study because the distinctive taste of some of the drinks did not allow such a scenario. Ethics approval for this study was granted by the Faculty of Health Sciences ethics committee.

6.2.2 Details of the drinks

In this study Lucozade Sport (LS, powder), a diluted form of Red Bull (isoRB) and Water were the 3 test drinks investigated. IsoRB was diluted (60:40) with water to formulate a beverage that was isocaloric to LS. Therefore, the volume ingested and the concentration of carbohydrate in each of these 2 drinks (LS and isoRB) was identical. The test drinks were ingested at a volume equivalent to 150% of the body mass lost during the 30 hr period of fluid restriction. The concentration of caffeine ingested during the isoRB trial was 3 $mg.kg^{-1}$ body mass. When the volume of the rehydration beverage to be ingested during the isoRB and LS trials exceeded 1litre, subjects received the test drink in a volume equivalent to receiving a caffeine load equivalent to 3 $mg.kg^{-1}$ and the remaining volume was made up with water.

For example: 70 kg male; 2 kg body mass loss; volume to be ingested = 3000 ml

Volume of isoRB = 1050 ml, Volume of water = 1950 ml

This was to try and ensure that the sodium content of the test drinks remained relatively similar and that subjects would not be ingesting large volumes of caffeine in the form of isoRB. The composition of LS, isoRB and W are described in Table 2.1.

6.2.3 Subject recruitment

10 males were recruited to participate in this study. They were recruited through college media advertising and through contacts established by other members of the Human Performance Laboratory. As there was no exercise component to this study, it was not necessary to target trained individuals to participate. All subjects received an information sheet and a consent form prior to commencing the study. The study protocol was clearly explained to each individual verbally and in the written information sheet.

6.2.4 Inclusion and exclusion criteria

All subjects were male aged between 18 and 32 yr. Any volunteer displaying any respiratory, cardiac or systemic disorder at the time of the medical screening was excluded from the study. Also caffeine abstainers/caffeine naïve subjects were excluded.

6.2.5 Medical screening and anthropometric measurements

On arrival at the laboratory on the first day of trial 1 each subject completed a detailed medical questionnaire (Appendix 2). Following bladder emptying, height and body mass were assessed using a stadiometer (Seca Ltd., Germany) and counter balance weighing scales, pulmonary function was recorded using the "Microlab" microspirometer (Micro Medical Ltd., England). Percent body fat was estimated using Harpenden skinfold callipers at 4 sites (see section 2.2). Body mass was assessed regularly throughout the trials, always following bladder emptying because body mass changes over time were used as one measure of hydration status in this study.

6.2.6 ECG analysis

A 12 lead electrocardiogram was performed on each subject and examined by the physician for any cardiac abnormalities that would be affected by caffeine ingestion during the trial. The details of the electrocardiograph used are described in Section 2.9

6.2.7 Haematological and urinary analysis

On Day 1 of the trial a blood sample was taken from the medial cubital vein in the antecubital fossa to assess red blood cell count (RBC), white blood cell count (WBC), haemoglobin (Hb) and haematocrit (Hct) again as part of the medical screening. This blood sample was analysed for the above variables using the Coulter Counter (see section 2.4). Throughout the 3 trials blood samples were collected by venopuncture from the medial cubital vein at t = 0 and t = 24 hr and by forearm cannulation during the rehydration phase of each trial. Samples collected in EDTA tubes were analysed for Hb (Coulter counter) and Hct (centrifugation in triplicate) to calculate plasma volume changes using equation proposed by Dill and Costill (1974). The blood samples were then spun down and the plasma separated to assess plasma osmolality. Samples collected in Z serum tubes were also centrifuged, the serum separated and frozen for batch analysis of serum sodium and potassium concentrations. Urine volumes were recorded throughout the 30 hr period of fluid restriction and during the rehydration period. A sample of all urine voided during the

laboratory visits was frozen for batch analysis of urine osmolality and electrolyte concentration.

6.2.8 Insertion of the IV catheter

At t = 30 hr of each trial an indwelling forearm canula was inserted to allow for regular blood sampling during the rehydration phase. The materials used for the canula are detailed in section 2.10.

6.2.9 Bioelectrical Impedance Analysis

BIA was performed on each subject at t=0, 24 and 30 hr and at regular intervals during the rehydration phase of each trial, see Schematic 4. The BodyStat dual frequency BIA analyser and the procedure for testing are described in detail in section 2.15. This methodology was previously used by other researchers in the Human Performance Laboratory.

6.2.10 Study protocol

In preparation for the trials each subject was required to complete a dietary record on 3 non-consecutive days to allow for estimation of their normal fluid intake. On 1 of those 3 days each subject was also required to collect and measure total urine volume voided throughout the day and therefore it was possible to calculate approximate fluid turnover under euhydrated conditions for each participating subject.

Day 1, t=0

Each trial involved 3 visits to the laboratory over a 30 hr period, 2 morning visits of approximately 40 min and 1 afternoon session of approximately 3 hr. For the first visit (t=0) each subject reported to the laboratory following an overnight fast. 90 min prior to this visit each subject was advised to ingest 500 ml of water to ensure a state of euhydration at t=0. Subsequently each subject completed a detailed medical questionnaire and height, body mass and resting blood pressure were recorded following urine collection. Bioelectrical impedance analysis (BIA) was also performed on each subject. A blood sample was collected from the cubital vein. All the above measurements were recorded in a fasted euhydrated state. The following markers of hydration status were assessed following the above measurements: body mass, urine volume, urine osmolality, urine specific gravity, plasma osmolality, plasma volume, electrolyte concentration, bioelectrical impedance.

The subjects then received a standardised breakfast, see Table 6.1 and 6.2 and commenced a 30 hr period of voluntary fluid restriction. During this 30 hr fluid restriction phase each subject was required to record food and fluid intake and to measure all urine voided. An information leaflet outlining suitable foods with a low water content was given to each subject (Appendix 2).

Day 2, t=24 hr

Following 24 hours of fluid restriction subjects returned to the laboratory, again in a fasted state. All measurements recorded on Day 1 were repeated to assess hydration status and subjects again received a standardised breakfast. In the time period between t=24 and 30 hr each subject was requested to only consume the two 37 g cereals bars (Nutrigrain, Kelloggs, Ireland) provided, to ensure that body mass would not be excessively increased as a result of eating a large lunch prior to the monitored rehydration phase.

Day 2, t=30 hr

Subjects returned to the laboratory later on Day 2 having completed the 30 hr period of fluid restriction to commence a period of monitored rehydration.

All variables measured during the previous 2 visits, t=0 and 24 hr were again repeated at the beginning of the rehydration phase and at distinct time-points throughout. Following initial measurements the subjects remained seated for a pre-hydration period of 45 min. Subjects were then requested to ingest the test drink over the following 30 min. This was followed by a 2 hr monitoring period. Schematic 4 illustrates the time-points during the rehydration phase when the assessed variables were measured. The drinks ingested were Lucozade Sport (LS), Red Bull (diluted 60:40 with water, isoRB) and water (W) in a volume equivalent to 150% body mass loss according to recommendations published by the ACSM.

Food	Quantity
Cornflakes	50 g
Full milk	200 ml
Orange Juice	100 ml
Brown bread	2 slices
Butter and jam	On request

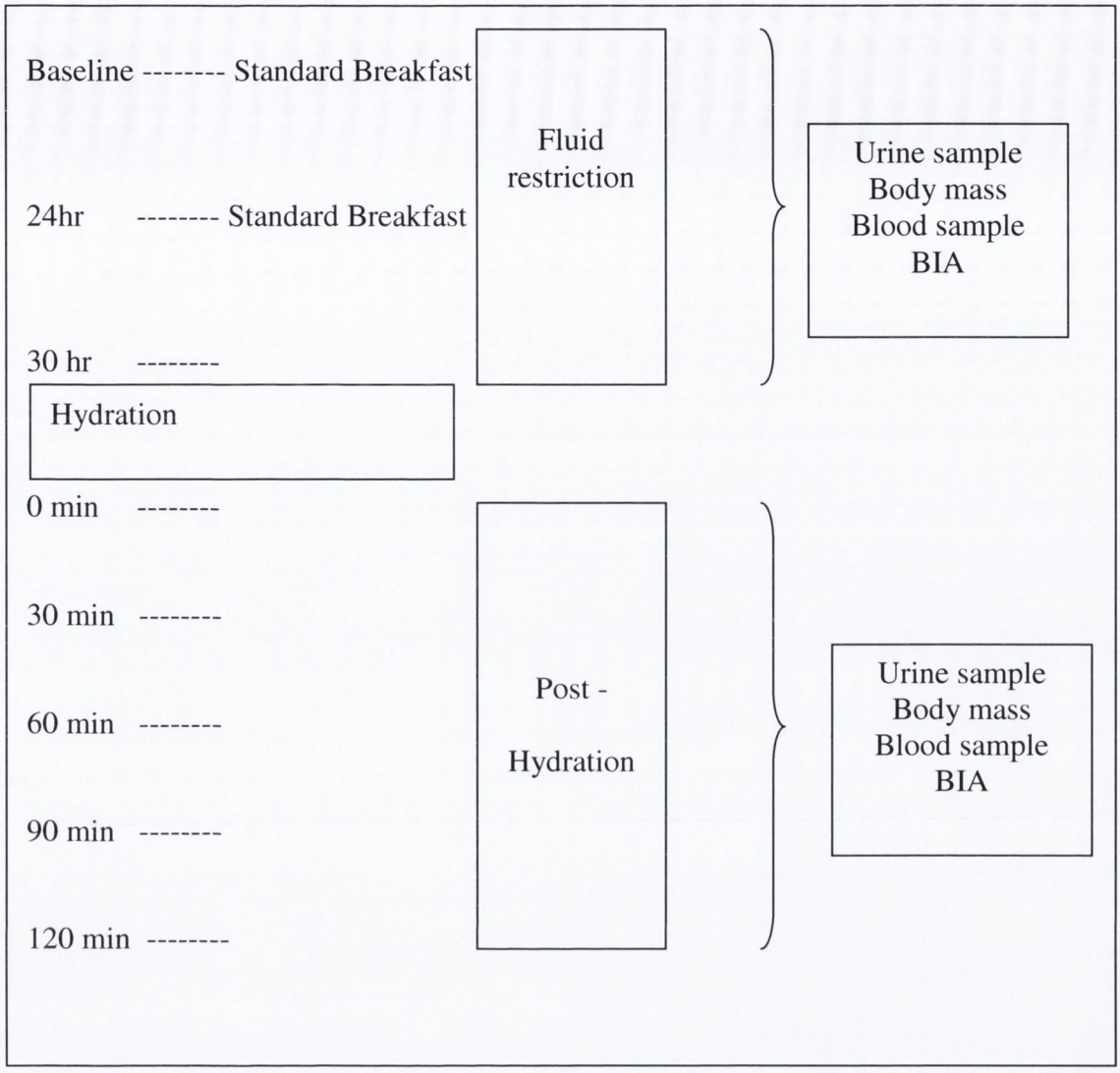
Table.6.1 Details of the standardised breakfast received by each subject at t=0 and t=24 hr.

	Quantity
Energy (kCal)	739
Protein (g)	18.4
Carbohydrate (g)	126.9
Fat (g)	21.1
Fibre (g)	2.5

Table 6.2: Nutritional analysis of the standardised breakfast received by each subject prior to and following 24 hr of fluid restriction.

6.2.11 Statistical Analysis

All subject physical characteristics are presented in table format as mean ± standard deviation (SD) and all graphical data as mean ± standard error of the mean (SEM). Two-way repeated measures ANOVA (time by drink) was used to assess the effects of the 3 drinks across time on measured variables, with *post-hoc* Bonferroni tests used to quantify any significant differences detected using Sigma stat. For all statistical tests, values of $P<0.05$ were considered statistically significant.



Schematic 4: Order of data collection during fluid restriction, hydration and post-hydration periods.

6.3 Results

6.3.1 Physical Characteristics

Anthropometric data:

	Mean ± SD
Age (yr)	27 ± 3
Body mass (kg)	84.1 ± 11.7
Height (m)	1.81 ± 0.07
BMI ($kg.m^{-2}$)	25.2 ± 3.7
Body fat (%)	18.4 ± 3.3

Table 6.3: Mean ± SD physical characteristics, n=10 at trial 1.

Haematological and pulmonary data:

Haematological and pulmonary function test data were normal, with no signs of sub-clinical infection, or obstructive or restrictive airway disorders (see Table 6.3).

HAEMATOLOGICAL DATA	Mean ± SD	PULMONARY DATA	Mean ± SD
Hb ($g.dl^{-1}$)	15.7 ± 1.0	**FEV_1 (l)**	4.5 ± 0.5
Hct (%)	44.2 ± 2.5	**FVC (l)**	5.2 ± 0.8
RBC ($x10^{12}.l^{-1}$)	4.90 ± 0.32	**PF ($l.min^{-1}$)**	617 ± 82
WBC ($x10^{9}.l^{-1}$)	5.82 ± 0.96	**FEV_1/FVC (%)**	80.3 ± 6.2

Table 6.4: Mean ± SD haematological and pulmonary data, n=10 at trial 1.

6.3.2 Total Body Water

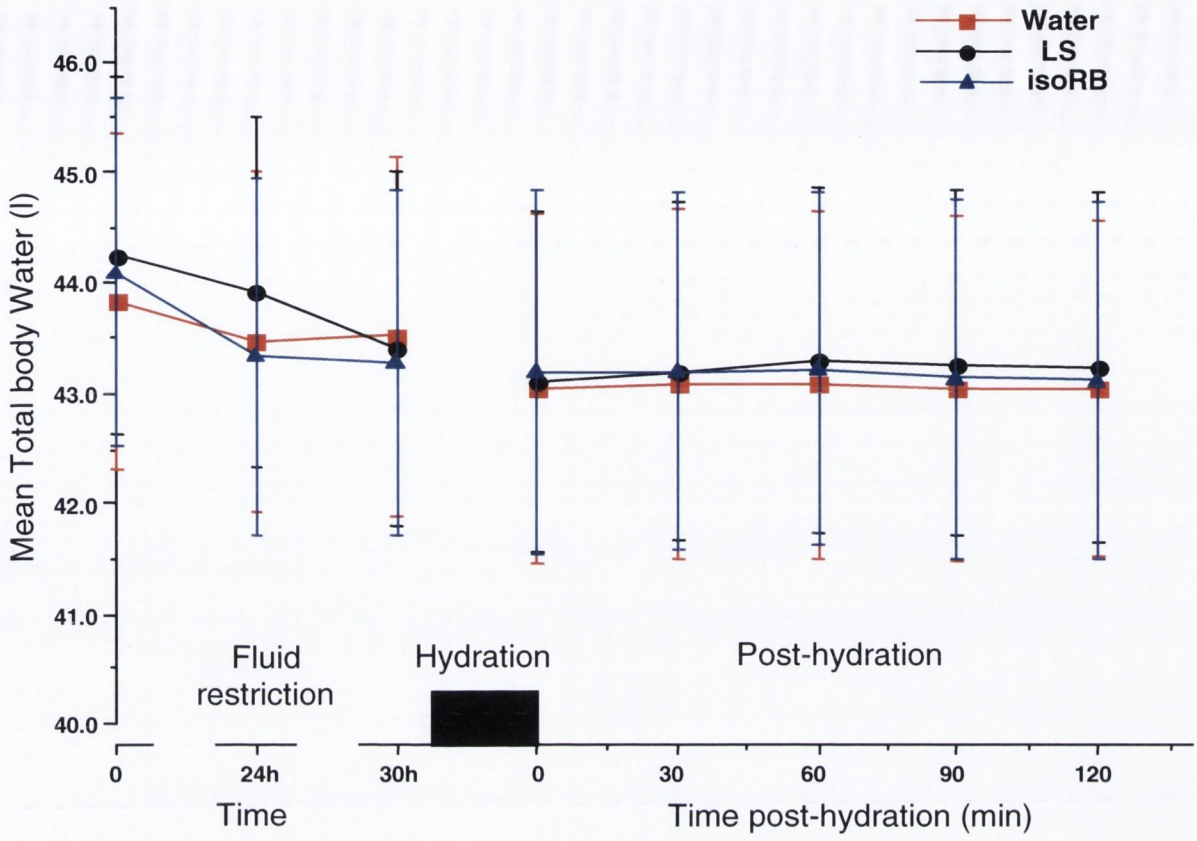


Figure 6.1: Mean (± SEM) total body water (l) during fluid restriction, hydration and post-hydration across time and trials, n=10.

Note: In describing the results observed in this study the time point t=0 at the beginning of the trial will be referred to as baseline. The second time point labelled "0" on all graphs indicates the beginning of the post-hydration period.

Figure 6.1 illustrates the mean TBW content assessed using BIA during fluid restriction, hydration and post-hydration for each drinks trial. No significant differences were recorded across time for any of the 3 drinks. Also no significant drinks effect was reported at any time point during the trials. Mean data across time and trial are presented in Appendix 6.

6.3.3 Extracellular Water volume

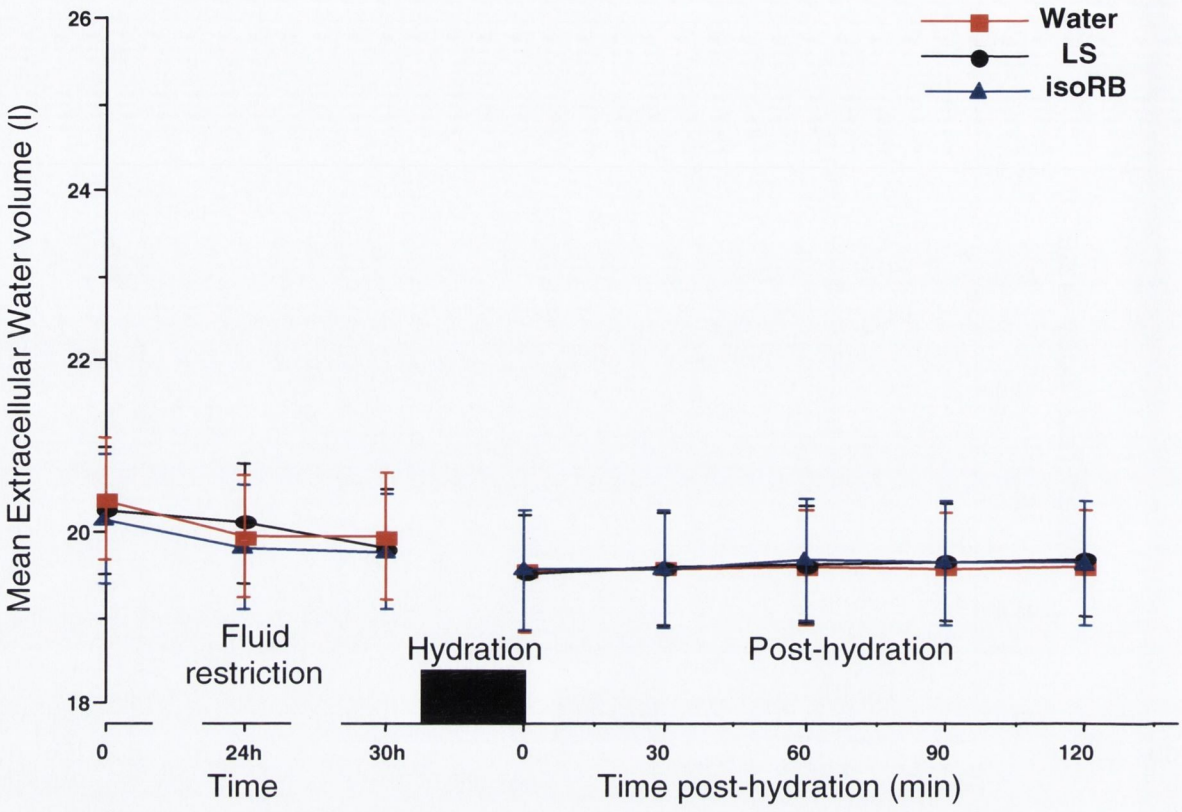


Figure 6.2: Mean (± SEM) extracellular water content (l) during fluid restriction, hydration and post-hydration across time and trials, n=10.

Following fluid ingestion, the mean extracellular water volume was significantly reduced at all time points post-hydration (0, 30, 60, 90 and 120 min) compared to baseline for all 3 drinks trials. During the LS trial significant differences were also recorded for all time points in the post-hydration phase compared to 24 hr ($P<0.05$). No significant drinks effect was recorded at any time point throughout the 3 drinks trials, see Figure 6.2. The mean ± SEM extracellular water volume following 30 hr of fluid restriction during the 3 drinks trials were 20.0±0.7, 19.8±0.7 and 19.8±0.7 l for water, LS and isoRB, respectively. Mean data across time and trial are presented in Appendix 6.

6.3.4 Intracellular Water volume

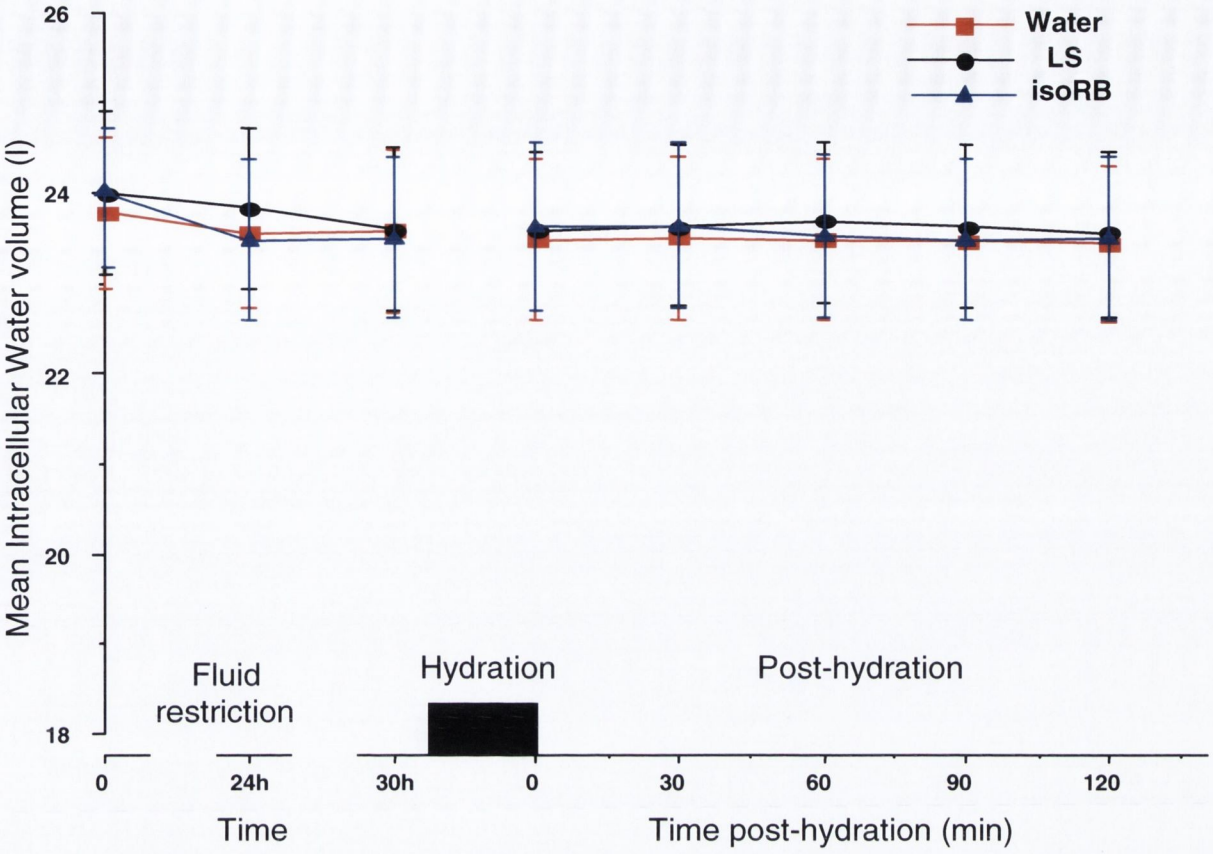


Figure 6.3: Mean (± SEM) intracellular water content (l) during fluid restriction, hydration and post-hydration across time and trials, n=10.

There were no significant differences across time reported during the Water trial. Following LS ingestion the mean intracellular water volume was significantly lower ($P<0.05$) at 120 min compared to baseline. Following 24 and 30 hr of fluid restriction, during the isoRB trial, there was a significant decline ($P<0.05$) in intracellular water volume compared to baseline. In addition, at 90 and 120 min post-hydration in the isoRB trial significantly lower ($P<0.05$) data were also recorded compared to baseline.

There was no significant difference comparing across the 3 drinks at any time point during the trials, see Figure 6.3. Mean data across time and trial are presented in Appendix 6.

6.3.5 Plasma Osmolality

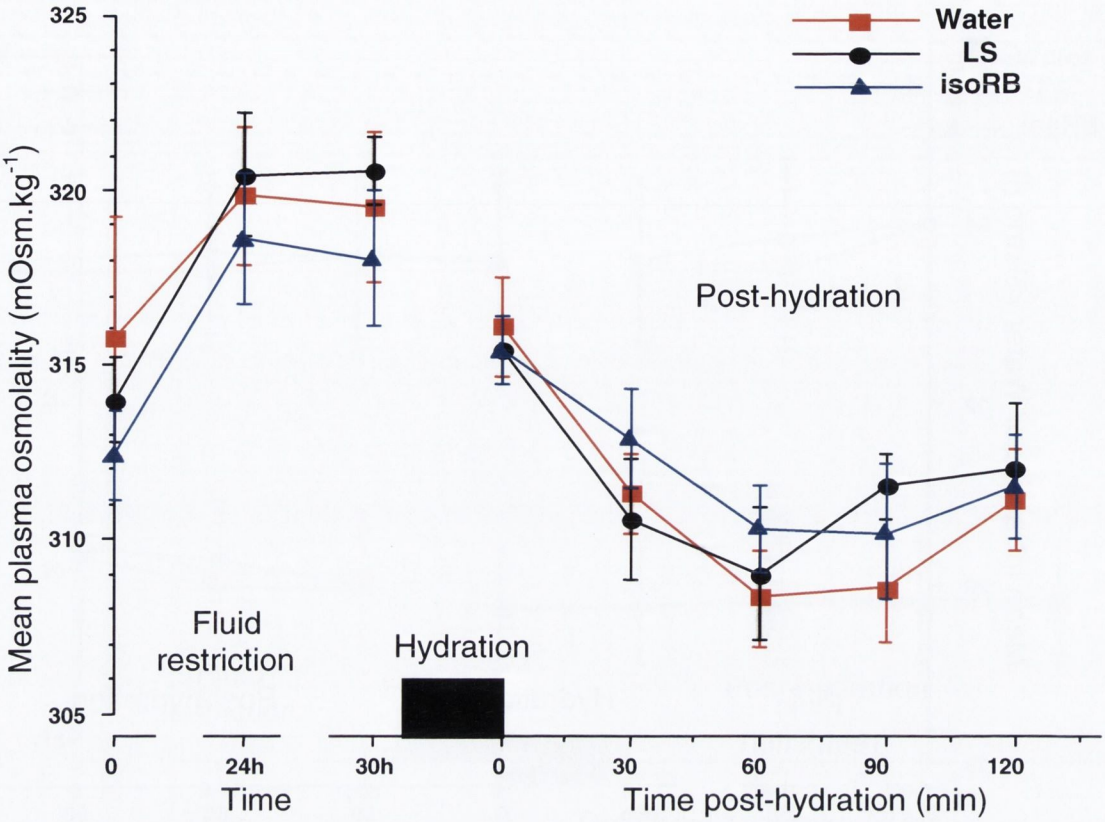


Figure 6.4: Mean (± SEM) plasma osmolality (mOsm.kg^{-1}) during fluid restriction, hydration and post-hydration across time and trials, n=10.

Mean plasma osmolality increased significantly compared to baseline during the first 24 hr of fluid restriction in the LS ($P<0.05$) and isoRB ($P<0.01$) trials but not in the Water trial. Following 30 hr of fluid restriction, significantly higher data compared to baseline were only reported during the LS trial. Following the ingestion of the test drinks plasma osmolality declined significantly compared to 24 hr at all time points (30, 60, 90 and 120 min) during the post-hydration period for all 3 trials. During the post-hydration period decreases in plasma osmolality were recorded compared to t=0 min, the time immediately post-ingestion of the rehydration beverage. At 60 min post-ingestion plasma osmolality data were significantly lower ($P<0.05$) compared to t=0 in the Water and LS trials, in addition at 90 min post-hydration plasma osmolality data were significantly lower compared to baseline during the same trials, indicating a mild hypotonic effect compared to baseline. There were no significant drinks effects recorded at any time points throughout the 3 trials. The mean (± SEM) plasma osmolality data recorded 120 min post fluid ingestion were 311.2±1.5, 312.0±1.9 and 311.6±1.5 mOsm.kg^{-1} during the Water, LS and isoRB trials, respectively. Mean data across time and trial are presented in Appendix 6.

6.3.6 Serum [Na^+]

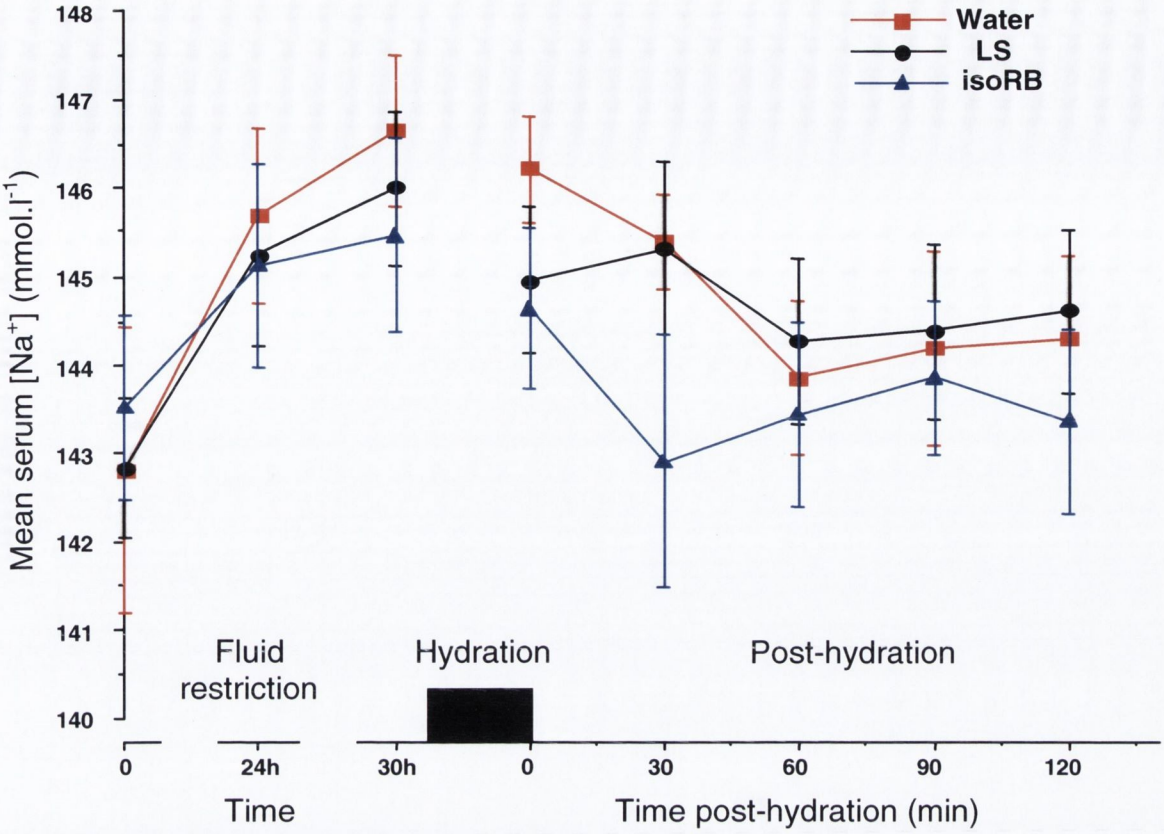


Figure 6.5: Mean (± SEM) serum [Na^+] ($mmol.l^{-1}$) during fluid restriction, hydration and post-hydration across time and trials, n=10.

Following 30 hr of fluid restriction, mean serum sodium concentration data significantly increased ($P<0.05$) compared to baseline during the Water and LS trials. Following the ingestion of the rehydration beverage serum [Na^+] was unchanged during all 3 trials over the 2 hr monitoring period. Following 1 hr of the 2 hr post-hydration phase (t=60 min) the serum sodium concentration was similar to that recorded prior to commencing fluid restriction (baseline), 144.3±0.9, 144.6±0.9 and 143.4±1.0 $mmol.l^{-1}$ for Water, LS and isoRB, respectively.

No significant differences were recorded comparing across the 3 drinks trials at any time point. Mean data across time and trial are presented in Appendix 6.

6.3.7 Serum [K^+]

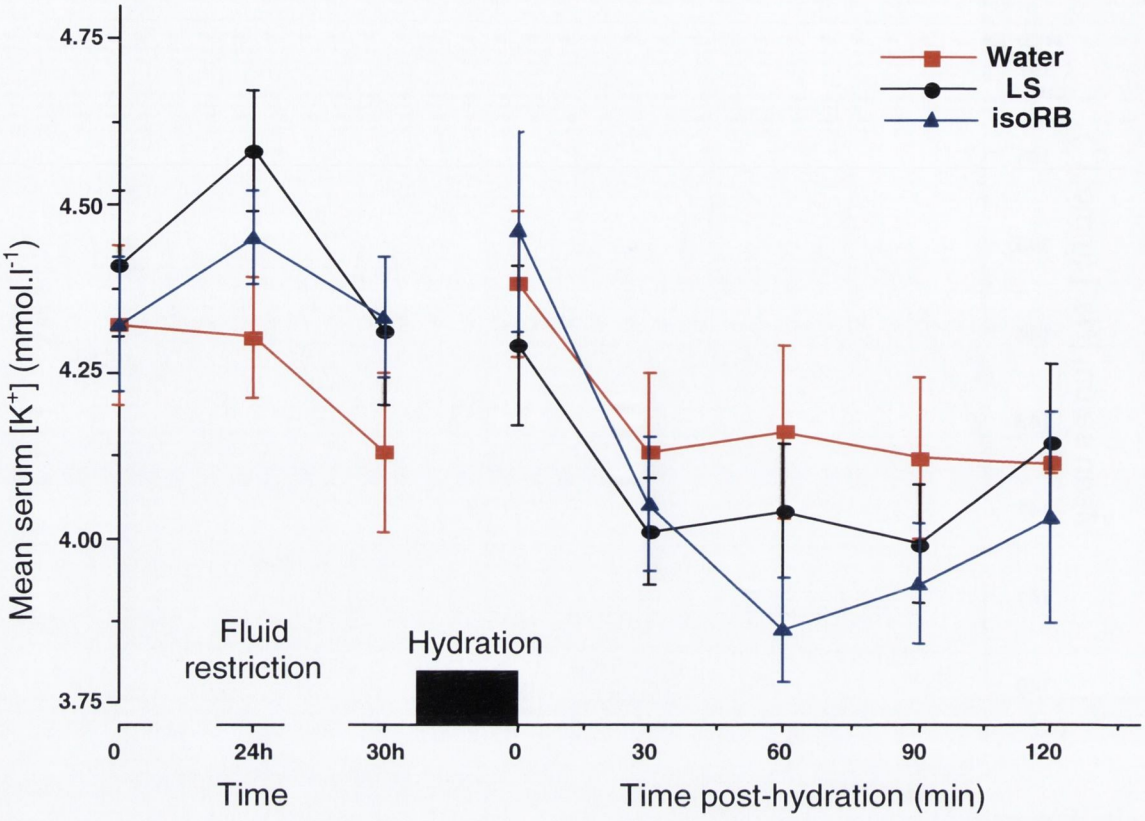


Figure 6.6: Mean serum [K^+] concentration (mmol.l^{-1}) during fluid restriction, hydration and post-hydration across time and trials, n=10.

Figure 6.6 illustrates the mean serum [K^+] concentration recorded during all 3 trials. No significant differences were recorded throughout the Water trial. However, following LS and isoRB ingestion the serum [K^+] declined significantly ($P<0.01$) compared to 24 hr at all time points during the post-hydration phase (30, 60, 90 and 120 min). During the isoRB trial significantly lower ($P<0.01$) data were recorded at 60 and 90 min post –hydration compared to 30 hr. In addition, during the isoRB trial serum [K^+] data were significantly lower ($P<0.01$) at 30, 60, 90 and 120 min compared to 0.

No significant drinks effect was recorded at any time point comparing across the 3 drinks trials. Mean data across time and trial are presented in Appendix 6.

6.3.8 Urine [Na^+]

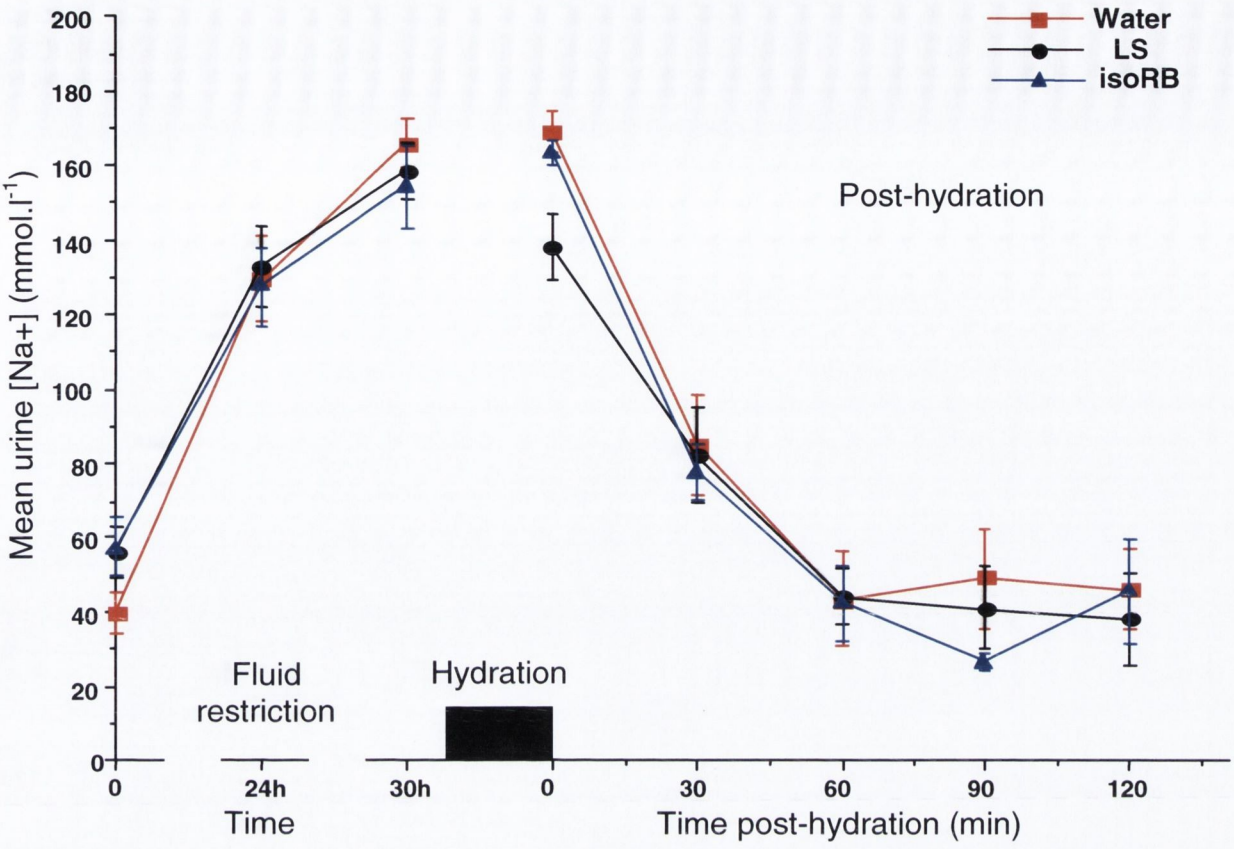


Figure 6.7: Mean (± SEM) urine Na^+] ($mmol.l^{-1}$) during fluid restriction, hydration and post-hydration across time and trials, n=10.

During all 3 trials urine [Na^+] increased significantly ($P<0.001$) after 24 and 30 hr of fluid restriction compared to baseline data, Figure 6.7. Following fluid ingestion there was a similar significant ($P<0.01$) decline in urine [Na^+] across all 3 drinks at 30, 60, 90 and 120 min compared to 24 and 30 hr. During the LS trial the mean urinary [Na^+] was significantly lower ($P<0.05$) at 60, 90 and 120 min following fluid ingestion compared to 30 min post-hydration. A similar decrease in urinary [Na^+] data compared to 30 min was observed at 60 and 120 min following water ingestion and 90 min post-hydration following isoRB ingestion. Mean urinary [Na^+] data had returned to baseline within 60 min post-hydration during all 3 trials.

No significant difference in urinary [Na^+] was recorded between the 3 drinks at any time point comparing across the trials. Mean data across time and trial are presented in Appendix 6.

6.3.9 Urine [K^+]

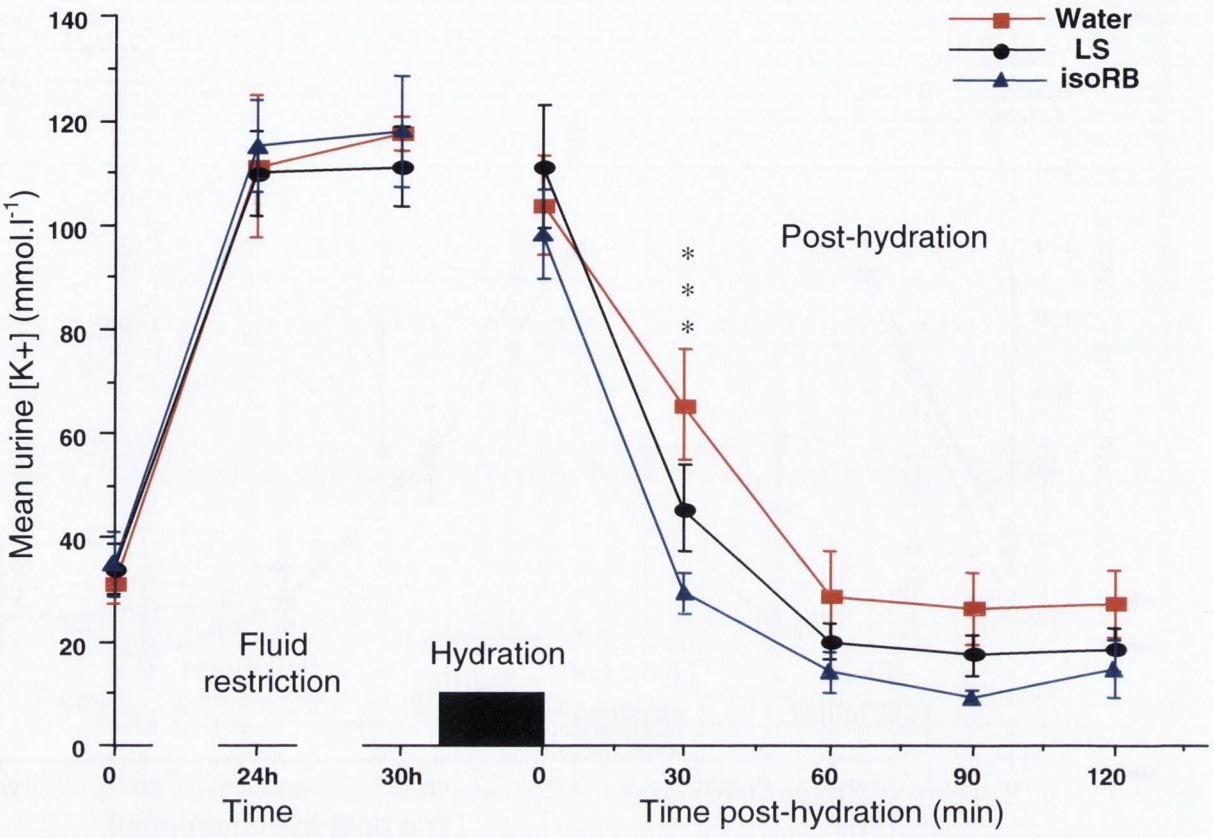


Figure 6.8: Mean (± SEM) urine [K^+] (mmol.l^{-1}) during fluid restriction, hydration and post-hydration across time and trials, n=10.

*** Implies significantly greater (P<0.001) data for Water compared to isoRB.

Similar to the urine [Na^+] data, mean urine [K^+] data increased significantly (P<0.001) following 24 and 30 hr of voluntary fluid restriction compared to baseline data, Figure 6.8. At all time points during the post-hydration period the mean urine [K^+] data were significantly lower (P<0.01) than those recorded immediately after fluid ingestion (t=0 min) and 24 and 30 hr. During the Water trial, urine [K^+] data recorded 30 min post-hydration were significantly greater (P<0.01) than 60, 90 and 120 min post-hydration. The urine [K^+] appeared to have reached a plateau at 60 min post-hydration with no further decline recorded during any of the 3 trials. In addition, 30 min post-hydration urine [K^+] data were significantly greater (P<0.001) following Water (65.7±10.9 mmol.l^{-1}) compared to isoRB (29.6±3.3 mmol.l^{-1}) ingestion. Mean data across time and trial are presented in Appendix 6.

6.3.10 Urine osmolality

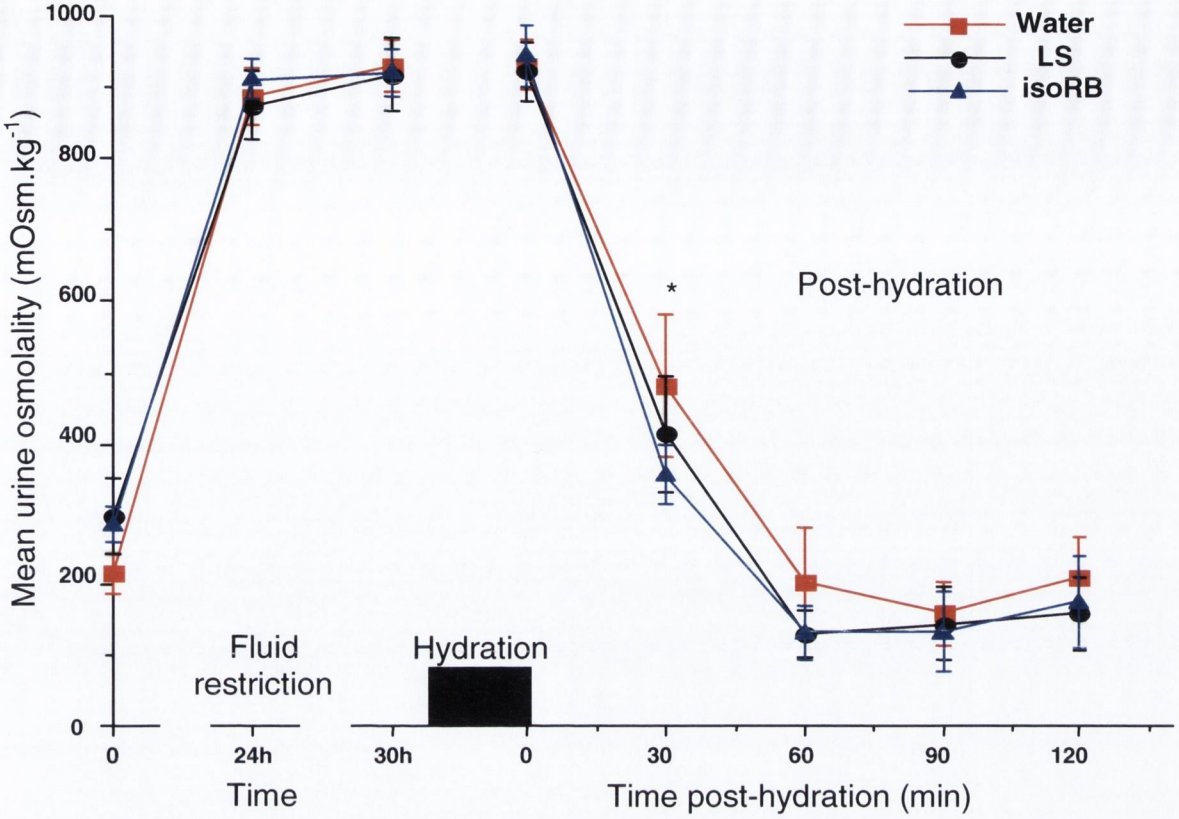


Figure 6.9: Mean (± SEM) urine osmolality (mOsm.kg^{-1}) during fluid restriction, hydration and post-hydration across time and trials, n=10.

* Implies significantly greater (P<0.05) data for Water compared to isoRB.

Mean (± SEM) urine osmolality data are illustrated on Figure 6.9. Urine osmolality increased significantly (P<0.001) at 24 and 30 hr compared to baseline data. Following the ingestion of the rehydration beverage, mean urine osmolality data declined significantly (P<0.001) within 30 min and remained reduced for the remainder of the post-hydration period (60, 90 and 120 min) compared to 24 and 30 hr and 0 min during all 3 trials. During the Water and LS trials data recorded at 30 min were significantly greater (P<0.01) than 60, 90 and 120 min, following isoRB ingestion significant differences (P<0.05) were only recorded at 60 and 90 min post-hydration compared to 30 min. Urine osmolality had returned to baseline at 60 min post-hydration, with no significant differences recorded for any of the drinks compared to baseline data for the remainder of the trial. Significantly greater (P<0.05) data were recorded at 30 min during the Water trial (483±100 mOsm.kg^{-1}) compared to isoRB (357±41 mOsm.kg^{-1}). Mean data across time and trial are presented in Appendix 6.

6.3.11 Urine Specific Gravity

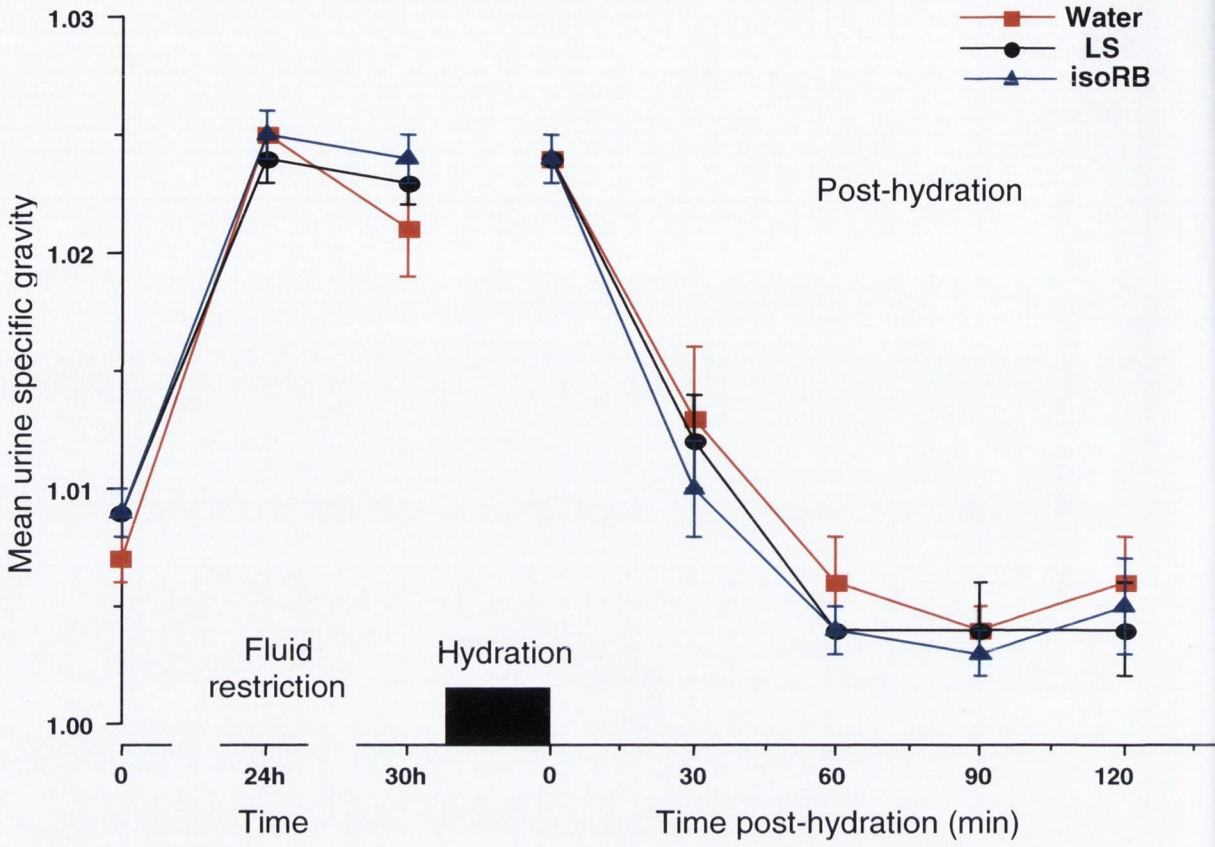


Figure 6.10: Mean (± SEM) urine specific gravity during fluid restriction, hydration and post-hydration across time and trials, n=10.

Mean (± SEM) urine specific gravity data measured by refractometry are illustrated in Figure 6.10. As expected, following fluid restriction, mean Usg increased significantly ($P<0.001$) at 24 and 30 hr compared to baseline. Following 30 hr of fluid restriction mean Usg data were 1.021±0.001, 1.023±0.001 and 1.024±0.001 for Water, LS and isoRB, respectively. Fluid ingestion resulted in a rapid and significant ($P<0.001$) decline in Usg recorded within 30 min post-hydration and remained reduced compared to 24 and 30 hr, and 0 min for the remainder of the post-hydration period. At 30 min, Usg data were significantly greater than data recorded at 60, 90 and 120 min during the Water and LS trials and at 90 min during the isoRB trial ($P<0.01$). It can be observed from Figure 6.10 that the mean Usg data had returned towards baseline within 60 min post-hydration with no further decreases recorded over the remainder of the post-hydration period.

No significant drinks effect was recorded at any time comparing across the 3 trials. Mean data across time and trial are presented in Appendix 6.

6.3.12 Plasma volume changes

Change in PV (%)	Water	LS	isoRB
	Mean ± SEM		
Baseline-30 hr	-2.72 ± 1.3	-2.00 ± 0.7	-2.62 ± 0.9
0-30 min	-0.09 ± 0.6	1.94 ± 0.6	1.30 ± 0.7
0-60 min	0.71 ± 0.9	2.41 ± 0.5	0.55 ± 0.6
0-90 min	0.92 ± 0.8	2.56 ± 0.7	0.30 ± 0.9
0-120 min	0.44 ± 0.8	1.25 ± 0.8	-1.20 ± 0.9

Table 6.5: Mean (± SEM) plasma volume changes across trials, n=10.

Changes in plasma volume were calculated using the equation proposed by Dill and Costill (1974) from haemoglobin and haematocrit data recorded throughout the trial and are presented in Table 6.5. It can be observed from Table 6.5 that there was only a small decrease in plasma volume during the fluid restriction period (baseline-30 hr). No significant differences were detected comparing across the 3 drinks during the post-hydration period

6.3.13 Body mass loss

	Water	LS	isoRB
	Mean ± SEM		
Baseline (kg)	84.4 ± 3.9	84.7 ± 4.0	84.5 ± 4.1
24 hr (kg)	83.5 ± 3.8	83.6 ± 3.9	83.3 ± 4.1
30 hr (kg)	83.2 ± 3.8	80.2 ± 3.9	83.1 ± 4.1
Body mass loss (kg)	1.3 ± 0.2	1.5 ± 0.2	1.5 ± 0.2
% Body mass loss	1.5 ± 0.2	1.8 ± 0.2	1.7 ± 0.2
120 min (kg)	84.0 ± 3.9	83.9 ± 3.9	83.7 ± 4.0

Table 6.6: Mean (± SEM) body mass and percent body mass losses during each trial, n=10.

Table 6.6 presents mean (± SEM) body mass (kg) data from baseline, following 24 and 30 hr of fluid restriction and 120 min post-hydration during all 3 trials. The mean body mass loss and percent body mass loss are also tabulated. There were no significant differences in terms of body mass loss and percent body mass loss across the 3 trials.

6.3.14 Fluid volume ingested and retained

	Water	LS	isoRB
	Mean ± SEM		
Volume of test drink ingested (ml)	1875 ± 295	2220 ± 246	2190 ± 235
Volume of fluid retained (%)	58 ± 5	55 ± 5	46 ± 6

Table 6.7: Mean (± SEM) fluid volumes ingested during the hydration phase and percent retained during the post-hydration period across trials, n=10.

The volume of fluid ingested during each trial was equivalent to 150% of the body mass loss recorded following 30 hr of fluid restriction. There were no significant differences in fluid volume ingested during the hydration period across the 3 test drinks. In addition there was no significant difference in the percent of the ingested volume that was retained, having taken into consideration the volume of urine voided throughout the 2hr post-hydration period.

The percent of fluid retained during each trial was calculated as follows:

(Volume of fluid ingested – urine volume / volume ingested) x100

6.3.15 Urine flow rate

Urine flow rate ($ml.hr^{-1}$)	Water	LS	isoRB
During 30 hr fluid restriction	43 ± 5	44 ± 5	49 ± 3
Post-hydration	296 ± 39	405 ± 60	467 ± 48 **

Table 6.8: Mean (± SEM) Σ urine volume, during fluid restriction and rehydration across trials, n=10.

** Implies significantly greater ($P<0.01$) urine volume compared to Water

Mean urine flow rates were calculated for 2 distinct periods of each trial and are presented in Table 6.8. Firstly the 30 hr fluid restriction period, where urine volumes recorded in the laboratory at 24 and 30 hr were summed with those recorded by the subject during the 30 hr period of fluid restriction. Secondly all urine voided immediately post hydration (t=0 min) and every 30 min for the subsequent 2 hr post-hydration period were summed and presented as "post-hydration" data in Table 6.8. The urine flow rate during the post-

hydration period of the isoRB trial was significantly greater ($P<0.01$) compared to the Water trial.

However it is interesting to note that the significantly greater urine volume recorded post-hydration during the isoRB trial compared to Water (Table 6.8) did not result in a significant difference in the percent of fluid retained across the 2 trials (Table 6.7).

6.3.16 Fluid restriction calculated from food diaries.

	% Fluid in diet		
Normal days	81.8 ± 0.9		
	Water	**LS**	**isoRB**
During 30 hr of fluid restriction	60.7 ± 1.4	61.6 ± 1.6	62.0 ± 1.8

Table 6.9: Mean (±SEM) percent of daily food and fluid intake that was fluid during normal and fluid restricted periods, n=10.

Prior to completing the 3 trials each subject was requested to record all food and fluid intake on 3 non-consecutive "normal" days. Using this information it was possible to calculate the normal percentage of fluid ingested by each subject. During the drinks trials the subjects were again requested to record all food and fluid intake throughout the 30 hr of fluid restriction. Table 6.9 presents the mean percent of the subject's diet that was fluid during the "normal" days and during the 30 hr of fluid restriction. No significant differences were recorded across the 3 trials. However, it is evident that subjects did significantly reduce their fluid intake during the 30 hr fluid restriction period by approximately 20%.

6.4 Discussion

This study investigated the effects of Water, LS and isoRB ingestion on multiple markers of hydration status following 30 hr of voluntary fluid restriction. The principal findings were that hydration status returned to baseline within 2 hr post-hydration following the ingestion of all 3 drinks. Body mass decreased by 1.5±0.2, 1.8±0.2 and 1.7±0.2 % during the 30 hr fluid restriction period prior to the ingestion of Water, LS and isoRB, respectively, see Table 6.6 and there was no significant difference in terms of body mass loss across the 3 trials. Previously body mass losses of 2.7±0.2% were recorded following 37 hr of voluntary fluid restriction (Shirreffs *et al.*, 2004).

In the present study each subject was required to record all food and fluid intake during 3 non-consecutive days prior to participation in the trial. The percentage of fluid in their diets on the normal days and again during the fluid restriction trials was calculated and is presented in Table 6.9. Analysis of dietary data indicated that fluid intake was significantly restricted during the fluid restriction trials and that the body mass loss recorded were as a result of hypohydration and not food restriction *per se*. Following the ingestion of the test drinks in a volume equivalent to 150% body mass loss their body mass had returned to baseline levels at the end of the 2hr post-hydration period, see Table 6.6.

During the 30 hr fluid restriction period all makers of hydration status; BIA, plasma osmolality, serum $[Na^+]$ and $[K^+]$, urine $[Na^+]$ and $[K^+]$, urine osmolality, urine specific gravity and plasma volume were recorded at baseline, 24 and 30 hr. With the exception of the BIA data all other variables reported a significant changes during the 30 hr fluid restriction period. Following the ingestion of the test drinks a significant decline was observed in all urinary markers and plasma osmolality. However, serum electrolyte concentrations ($[Na^+]$ and $[K^+]$) were not affected to the same extent following fluid ingestion. Serum $[K^+]$ declined significantly during the LS and isoRB trials at all time points post-hydration compared to 24 hr, however, no changes were recorded during the water trial. There is no K^+ present in the commercially available beverage Red Bull, but when diluted (60:40) with Water isoRB contains 10.1 $mg.l^{-1}$ compared to 9.6 $mg.l^{-1}$ in LS, because the Water used throughout the research in this dissertation contained 27 $mg.l^{-1}$ of K^+. However serum $[Na^+]$ did not change significantly over the 2 hr post-hydration period when compared to 24 or 30 hr data for any of the 3 drinks, despite the presence of Na^+ in both LS (470 $mg.l^{-1}$) and isoRB (500 $mg.l^{-1}$). Previous research has reported that the ingestion of a beverage containing 61 $mmol.l^{-1}$ Na^+ in a volume equivalent to 150% body mass loss post-exercise was more effective in achieving net fluid balance when compared to the same volume of fluid containing only 23 $mmol.l^{-1}$ Na^+ (Shirreffs *et al.*, 1996).

Table 6.7 presents the volume of fluid retained as a percent of that ingested, taking into consideration the volume of urine collected during the 2 hr post-hydration period. No significant differences were recorded across the 3 drinks. This result suggests that the significantly higher urine flow rates observed following isoRB ingestion when compared to Water (Table 6.8) did not significantly affect the rehydration process as no differences were subsequently recorded in terms of the fluid retention.

In general, similar responses to fluid ingestion were recorded across the 3 drinks. Significant treatment effects were recorded at 30 min post-hydration during the water and isoRB trials for urine [K^+] (Figure 6.8) and urine osmolality (Figure 6.9), suggesting that in the early stages of the rehydration process urine [K^+] and osmolality decline quicker following isoRB ingestion. However, by the end of the monitored 2 hr post-hydration period no significant differences were recorded between these 2 drinks. It has been reported that the volume of urine produced during rehydration is directly proportional to the volume of fluid ingested (Shirreffs and Maughan, 2000). Shirreffs *et al.* (1996) reported that the ingestion of fluid in a volume equivalent to 150% body mass loss with a high concentration of Na^+ (61 $mmol.l^{-1}$) was more effective in achieving a state of euhydration compared to the same volume of fluid with a low concentration of Na^+ (23 $mmol.l^{-1}$), again indicating the importance of the presence of electrolytes in rehydration beverages.

Previous research has recommended against using water as a rehydration beverage. The ingestion of water, an electrolyte free beverage, results in a decline in plasma osmolality that may suppress the need to drink and may also stimulate urine production (Maughan and Leiper, 1995; Ray *et al.*, 1998; Casa *et al.*, 2000). However this theory was not supported in this study. At this point however it is important to note that 2 subjects had to repeat the water trial, 1 suffered diarrhoea and the other reported feelings of severe nausea immediately following the ingestion of Water. The mean volume of water ingested was 1875±295 ml, a large volume to be ingesting over a 30 min period. These 2 subjects had to ingest large volumes (3150 and 2700 ml) of water as their % body mass loss was at the upper end of the scale. In addition, 5 other subjects expressed feelings of fullness and discomfort following Water ingestion. The presence of Na^+ in a rehydration beverage has been reported to stimulate fluid and carbohydrate uptake in the gut (Horswill, 1998). This may explain why the ingestion of LS and isoRB, both containing Na^+ did not result in any adverse feelings being reported in the LS and isoRB trials.

O'Brien *et al.* (2002) in a review of bioelectrical impedance stressed that changes in hydration status rarely occur without concomitant alterations in intra- and extracellular

fluid and electrolyte content. These changes in ECW and ICW in turn affect TBW. In this study ECW was significantly reduced following fluid ingestion at 30, 60, 90 and 120 min post-hydration when compared to baseline (see Figure 6.2), however there was a delay in the decline reported in the ICW during the LS and isoRB trials, and no changes were observed during the Water trial. Following LS and isoRB ingestion significantly lower ICW data compared to baseline were observed at 120 min, and 90 and 120 min, respectively. In addition, no significant changes in TBW were observed across time during all 3 drinks trials in the present study and therefore no interaction between ICW, ECW and TBW was observed. It may be possible that the hypohydration induced during the 30 hr fluid restriction period was too small for changes in volume distribution to be detected by BIA.

Armstrong *et al.* (1998) reported a strong correlation between Usg and Uosm (r=0.98) and recommended their use interchangeably. In this present study urine specific gravity (Figure 6.10) and urine osmolality (Figure 6.9) followed similar patterns during the fluid restriction, hydration and post-hydration periods.

On reflection of all the results of this study it can be recommended that following voluntary hypohydration, euhydration was achieved over a similar time scale in all 3 trials, suggesting that caffeine ingestion in the supplement isoRB does not negatively affect hydration status in those already hypohydrated. Instead isoRB containing the supplement caffeine appears to aid rehydration in a manner similar to either Water or LS.

Chapter 7

Overall Discussion

This chapter will focus more closely on the integrated findings of the 4 studies contained in this dissertation. In general intermittent exercise performance was enhanced following the ingestion of either LS and isoRB compared to Water (Chapter 4), with no improvements in endurance exercise performance observed when Water, LS and RB were ingested 60 min prior to exercise (Chapter 3). There appears to be a delay in the kinetics of caffeine ($3mg.kg^{-1}$) when ingested in capsular form compared to liquid form (isoRB). Also the presence of carbohydrate in isoRB may inhibit increases in NEFA normally observed following caffeine ingestion (Chapter 5). Rehydration using Water, LS or isoRB in a volume equivalent to 150% body mass loss by voluntary dehydration does not differ greatly across the 3 drinks.

One of the principal objectives of this research was to investigate if caffeinated-carbohydrate drinks are more beneficial or detrimental to exercise and rehydration compared to beverages containing carbohydrate alone. Red Bull was chosen as the caffeinated carbohydrate beverage because of the popularity of this commercially available beverage among athletes. Lucozade Sport was the chosen carbohydrate beverage because some research studies have previously reported significant performance enhancing effects of this drink compared with placebo (Nicholas *et al.*, 1995 and 1999) but not in direct comparison with a caffeinated carbohydrate beverage. The findings of each study have been discussed earlier but the important areas will be addressed in more detail now.

The use of exogenous carbohydrate during exercise results in a delay in the onset of fatigue through the process of muscle glycogen sparing. Hargreaves *et al.* (1984) observed a 20% reduction in muscle glycogen utilisation compared to placebo when 43 g of solid carbohydrate (in 400 ml of water) were ingested prior to and every hour during 4 hr of intermittent exercise (30 min intervals; 20 min at 50% $\dot{V}O_2max$ and 10 min of intermittent exercise). Nicholas *et al.* (1995) concluded that the increase of 33% in endurance capacity recorded following carbohydrate ingestion was due to reduced muscle glycogen utilisation during the first 75 min of intermittent exercise in their carbohydrate trial (75 min intermittent exercise; walking, running and sprinting followed by a run time to fatigue alternating between 55 and 95% $\dot{V}O_2max$ every 20 m). Coggan and Coyle (1988) reported that blood glucose oxidation could not support exercise at intensities >75% $\dot{V}O_2max$ late in exercise, therefore without adequate muscle glycogen stores the exogenous carbohydrate would be unlikely to improve exercise time to fatigue at these high intensities. Bosch *et al.* (1994) and Jeukendrup *et al.* (1999) have reported a sparing of liver glycogen when carbohydrate was administered during exercise. Yaspelkis *et al.* (1993) investigated the effects of ingestion of solid or liquid carbohydrate during intermittent (45 and 75%

$\dot{V}O_2$max) exercise. Muscle biopsies showed that muscle glycogen concentrations were significantly greater following liquid carbohydrate supplementation compared with placebo and this corresponded to a significant increase in time to fatigue (31 min) in the liquid carbohydrate trial compared with water. Time to fatigue was also significantly greater (21.5 min) following ingestion of solid carbohydrate compared with water. During low intensity intervals it has been suggested that the ingested carbohydrate was used to synthesise muscle glycogen (Jeukendrup, 2004).

Significantly higher heart rate data have been observed following caffeine ingestion (Bridge and Jones, 2006; Kovacs *et al.*, 1998). These higher heart rates have been attributed to the direct effect of caffeine as a stimulant or due to caffeine's effects upon central perceptions of effort (Graham, 2001). In the study described in Chapter 4 no significant increase in heart rate data was recorded during exercise following isoRB ingestion, but at failure significantly higher heart rate data (181±4 beats.min^{-1}) were recorded comparing to Water (175±4 beats.min^{-1}, $P<0.05$). In addition, no significant difference in heart rate data was recorded between LS and isoRB at failure. In Chapter 3 no significant differences in heart rate data were recorded across the 3 drinks at any stage throughout the exercise trial, see Figure 3.2.

Geiss *et al.* (1994) reported significantly lower heart rates following the ingestion of their taurine containing beverages compared with those without taurine. The research in this dissertation does not support this observation; as the data recorded during exercise in both studies revealed no significant differences in heart rate data across the 3 drinks, except at failure in Chapter 4, suggesting that the exercise protocol may also influence the heart rate response. In study 2, the mean volume of isoRB ingested during the intermittent exercise trial was 1159.6 ± 85.6 ml, corresponding to a mean intake of approximately 2782 mg of taurine. Mean heart rate data recorded during exercise at 70% $\dot{V}O_2$max were below 120 beats.min^{-1} during all 3 exercise trials in the Geiss *et al.* (1994) study. These heart rate data appear to be very low for the exercise intensity at which the subjects were required to be exercising. Maximal heart rate data at the end of the trials were approximately 187-190 beats.min^{-1}, only marginally higher when compared with the maximal heart rate data recorded in Chapters 3 and 4 following RB (182±2 beats.min^{-1}) and isoRB (181±4 beats.min^{-1}) ingestion, respectively. In Chapter 4 of the present report the mean heart rate data recorded during exercise at a load equivalent to 60% $\dot{V}O_2$max were between 146 and 156 beats.min^{-1} (80 – 86% HR max) during all 3 supplementation regimes towards the end of the trials. In the Nicholas *et al.* (1995) study the heart rate data recorded at 15 min intervals during the 75 min period of intermittent exercise were between 90 and 95% of

maximal heart rate. In contrast, during trials in the Geiss *et al.* (1994) study at an exercise intensity reported to be equivalent to 70% $\dot{V}O_2$max the mean heart rate data recorded were below 120 beats.min^{-1} (< 64 % HR max), and only approximately 30 beats.min^{-1} greater than the heart rate recorded at rest. These heart rate data are very inconsistent with the basic physiological principals of exercise, when heart rate would be expected to be approximately 80% of maximal heart rate at an exercise intensity equivalent to 70% $\dot{V}O_2$max and therefore makes it difficult to interpret the other findings in their study.

The maintenance of euglycemic conditions during exercise is 1 mechanism by which carbohydrate feeding during exercise may improve endurance exercise performance (Jeukendrup, 2004). In Chapter 3 blood glucose data recorded immediately before exercise (t=0 min) were significantly higher compared with –60 min data and also data recorded during exercise at loads equivalent to 65 and 85% $\dot{V}O_2$max following LS ingestion. This trend was not evident following the ingestion of RB, despite the presence of carbohydrate in the form of glucose and sucrose, see Figure 3.4. The test drinks were consumed 60 min prior to the start of exercise and this resulted in a significant increase in circulating blood glucose immediately pre-exercise. In Chapter 4 no significant differences ($P>0.05$) were recorded in blood glucose data, in all 3 trials, when compared at -20 and 0 min, see Figure 4.4. There was a 20 min time period for a low intensity warm-up (120 W) and stretching between these time points, possibly not long enough to produce a significant increase in circulating blood glucose. The mean blood glucose data for the LS trial at t=0 were almost identical in both studies (6.1 ± 0.3 in Chapter 3 and 6.0 ± 0.4 mmol.l^{-1} in Chapter 4). Similar to blood glucose levels, plasma insulin levels peak approximately 30-60 min following the ingestion of carbohydrate (Coggan and Swanson, 1992). If exercise begins during this time the active muscle immediately requires a source of energy and blood glucose levels quickly fall below normal due to the increased rate of glucose uptake that results from the synergistic effect of insulin and muscle contractions. For this reason a significant decline in blood glucose was recorded early in exercise in Chapter 4, see Figure 4.4. In the research described in Chapter 4 the blood samples were collected every 10 min immediately after the high intensity exercise element at a load equivalent to 90% $\dot{V}O_2$max. Figure 4.4 illustrates that blood glucose concentrations had returned towards pre-test (-20 min) concentrations with no significant differences across the 3 drinks at failure. This sequence of events is known as transient hypoglycaemia as the decline in blood glucose may remain suppressed, return to normal or even exceed the pre-exercise level. Analysis of the mean blood glucose concentrations at 10 min intervals during exercise reported a significant hypoglycaemic response after 10, 20, 30 and 40 min of

exercise compared to 0 min following LS ingestion. In the isoRB trial blood glucose concentrations at 10 and 20 min were significantly lower than 0, 40 50 and 60 min. In Chapter 3, transient hypoglycaemia was also observed following LS ingestion, Figure 3.4. Coggan and Swanson (1992) in their review of nutritional manipulations and exercise performance referred to the timing of ingestion as being crucial to the subsequent effects on performance. During the 20 min time period prior to commencing exercise, subjects ingested the test beverage and then completed a 15 min warm-up (120 W for 10 min, 10 min and stretching) before pre-exercise blood and metabolic data were recorded. Nicholas *et al.* (1995) administered their drink immediately prior to exercise with resting mean blood glucose data approximately 4.2 $mmol.l^{-1}$, similar to resting blood glucose data recorded by the same author in 1999. Blood glucose data were recorded every 15 min during exercise and no decline was recorded early in exercise. After 30 min of exercise blood glucose concentrations following the ingestion of LS were significantly higher when compared with their non-carbohydrate placebo. Arkinstall *et al.* (2001) investigated the effect of carbohydrate ingestion on metabolism during both running and cycling (n=7) and reported significant increases in blood glucose 10 min after the ingestion of a carbohydrate beverage when compared with water. The timing of fluid ingestion and also the protocol used for data collection therefore potentially play a key role in any observed changes in blood glucose data. In the exercise described in Chapter 4 as exercise proceeded, carbohydrate was ingested every 10 min during the LS and isoRB trials. This supply of exogenous carbohydrate regularly during exercise, coupled with hepatic gluconeogenesis allowed for blood glucose concentrations to return to their normal levels later in exercise. No subject in the present study experienced any effects of transient hypoglycaemia severe enough to cause cessation of exercise. This may be due to the fact that the decrease in blood glucose was too small or too brief to have a serious effect, or possibly the intermittent nature of the protocol prevented it. The ingestion of caffeine has been shown to inhibit insulin stimulated glucose uptake (Akiba *et al.*, 2004). No significant differences were observed in blood glucose concentrations during exercise comparing between the LS and RB/isoRB trials despite the presence of caffeine in both RB and isoRB. The caffeine concentration administered during these trials may not have been large enough to illicit the proposed block on insulin stimulated glucose uptake or possibly the exercise intensity performed by the subjects increased translocation of GLUT4 transporters sufficiently to sustain cellular glucose uptake. Fatigue during exercise has been related to a decline in plasma glucose late in exercise and previous research has concluded that regular feeding during exercise can delay fatigue (Coyle *et al.*, 1986; Coyle *et al.*, 1983). To reverse this

fatigue late in exercise Coggan and Coyle (1987) administered carbohydrate orally or intravenously following exercise to fatigue at 70% $\dot{V}O_2max$ to restore euglycemia before a second bout of exercise commenced. They reported that the decline in carbohydrate oxidation observed during the first bout of exercise could be reversed and exercise continued for a further 45 min when euglycemia was restored by intravenous infusion of glucose. However the practicalities for the use of this method of carbohydrate feeding by athletes on a regular basis is questionable.

Little scientific research has been carried out to date to compare the effects of commercially available sports drinks on exercise performance. A study by Johnson *et al.* (1988) compared 3 commercial drinks, no longer available, on performance in 6 male subjects during 4 hr of intermittent running exercise at 35°C. They reported that time to exhaustion assessed by a maximal treadmill test at the end of the exercise bout was similar across all 3 drinks. The exercise protocol and environmental factors used by Johnson *et al.* (1988) would not be reflective of the activity patterns of a wide range of athletes in comparison with the studies in this report, where the protocols and duration of exercise would be similar to that for both games players and cyclists.

It has previously been proposed that any possible ergogenic effect of caffeine may be mediated by enhancing fat and decreasing carbohydrate oxidation in the active muscle (Graham *et al.*, 2000), suggesting a decrease in RER following caffeine ingestion. However, this was not observed in both exercise studies contained in this report, and likewise in studies reported by Graham and Spriet (1991) and Spriet *et al.* (1992), where exercise performance was enhanced following caffeine ingestion. The effect of caffeine on fat/carbohydrate metabolism in active muscle is uncertain, as many research studies (Spriet *et al.*, 1992; Graham and Spriet 1991) have relied on pulmonary measurements and metabolite concentrations in the blood, rather than assessing muscle metabolism directly. For this reason Graham *et al.* (2000) examined the effect of caffeine ingestion (6 $mg.kg^{-1}$) on muscle carbohydrate and fat metabolism during 1 hr of exercise at 70% $\dot{V}O_2max$ compared with placebo. Muscle biopsies and direct Fick measurements were used to assess leg muscle metabolism. The results of the study reported that fat and carbohydrate oxidation was unaltered in the leg while circulating adrenaline concentration as expected was significantly higher at rest and during exercise following caffeine ingestion, as were blood glucose and lactate concentration. It was concluded by Graham *et al.* (2000) that the changes in glucose, lactate and free fatty acid concentration recorded must be under the control of tissues other than the exercising muscles.

While much of the research involving caffeine ingestion had focussed on endurance exercise, more recently the effect of caffeine ingestion on shorter duration high intensity exercise has been a topic for investigation.

Caffeine ($3mg.kg^{-1}$) ingested in capsular form 60 min before exercise has been reported to improve 8 km running performance under valid competitive circumstances (Bridge and Jones, 2006). The authors of that study strongly questioned the mechanism by which caffeine acted to improve exercise performance. It has been regularly suggested that performance improvements associated with caffeine ingestion relate to the effect of caffeine on lipolysis and the subsequent availability of free fatty acids, and glycogen sparing. However, caffeine has been reported to improve high intensity short duration exercise where glycogen sparing is not the limiting factor (Anderson *et al.*, 2000; Bruce *et al*, 2000; Bridge and Jones, 2006). Therefore there may be other mechanisms or a combination of different mechanisms (metabolic, central nervous system, cardiovascular and skeletal muscle effects) contributing to performance improvements following caffeine ingestion.

Caffeine has also been reported to enhance prolonged intermittent sprint ability in team sport athletes (Schneiker *et al.*, 2006). The intermittent protocol in this research involved two 36 min periods with 10 min rest between each period. Each half was divided into two 18 min blocks and each block consisted of a 4 s sprint followed by 100 s active recover (35% $\dot{V}O_2max$) and 20 s of passive recovery. More sprint work was performed in each half (8.5% in the first half and 7.6% in the second) of the intermittent sprint test following caffeine ingestion ($6mg.kg^{-1}$).

Different types of carbohydrate are associated with different osmolalities, affecting gastric emptying and intestinal absorption, and so the effect of the test drinks on plasma osmolality was assessed in both studies. Osmolality data for the test drinks used in both studies are presented in Table 2.1. The osmolality of RB was 642 ± 10 $mOsm.kg^{-1}$, considerably hypertonic compared to LS (286 ± 3 $mOsm.kg^{-1}$), Water (9 ± 2 $mOsm.kg^{-1}$) and plasma (287 $mOsm.kg^{-1}$, Armstrong, 2002). Table 2.1 illustrates that despite the fact that LS and isoRB were iso-calorically matched in Chapter 4, the osmolality of isoRB was higher than LS possibly due to the different types of carbohydrate present in both drinks. Figure 3.7 and 4.7 presents the mean plasma osmolality data recorded at fixed time points during the studies detailed in Chapters 3 and 4, respectively. Plasma osmolality data were significantly higher ($P<0.05$) following ingestion of LS at failure compared with 0 and –20 min data in study 2, see Figure 4.7. In addition, plasma osmolality increased significantly at 30 min compared to 0 min following isoRB ingestion. The osmolality of

isoRB while hypotonic with respect to the RB used in Chapter 3 (due to dilution with water) was hypertonic compared to body fluids, indicating possibly that less water absorption would be stimulated resulting in a potentially dehydrating effect following isoRB ingestion. However, analyses of plasma volume changes, body mass losses and urine output do not support this because no differences were observed across the 3 drinks for any of these parameters. In Chapter 3, plasma osmolality data during the LS trial were significantly higher ($P<0.05$) at exhaustion compared with pre-exercise data and no significant differences were recorded across the 3 drinks at any time point.

In Chapter 4 plasma volume changes were similar across all 3 trials, with exercise resulting in a significant decrease in plasma volume (Table 4.5). Similarly no significant differences were observed in plasma volume changes in Chapter 3 across the 3 trials, see Table 3.4. Exercise resulted in dehydration in all 3 trials with plasma volume being restored towards normal levels following rehydration, indicative of a redistribution of fluids to the extracellular space.

A similar finding was reported by Morris *et al.* (2003) when investigating the effects of ingestion of a carbohydrate electrolyte beverage compared to flavoured water and placebo during intermittent running, they recorded a decline in plasma volume of the order of 3.6% at the end of the trials compared with pre-test data. Similarly, Murray *et al.* (1987) reported that changes in plasma volume were influenced by exercise but "did not change among subjects in response to beverage treatments". Both studies by Morris *et al.* (2003) and Murray *et al.* (1987) were performed in a warm climate (30 and 33°C, respectively). Nicholas *et al.* (1995, 1999) also reported no significant differences in plasma volume changes across the 2 drinks under investigation in both of their studies.

Increased blood lactate concentrations have been observed in previous studies post-exercise following the ingestion of caffeine (Bridge and Jones, 2006; Greer *et al.*, 2000; Ryu *et al.*, 2001). However higher resting blood lactate concentrations have not been reported following caffeine ingestion. In Chapter 3 significantly higher blood lactate concentrations were recorded at rest 60 min post-ingestion of RB when compared to Water. At failure blood lactate concentrations were significantly higher in the RB trial when compared to both LS and Water. Following isoRB ingestion (Chapter 4), no significant difference in blood lactate was reported when compared to LS and Water at any time point. Bridge and Jones (2006) reported significantly higher blood lactate concentrations 3 min post-exercise following caffeine ingestion and related this increase to substrate metabolism during exercise. Unfortunately lactate was not recorded immediately at failure to all direct comparison to the results reported in the Chapter 4.

In Chapter 4 the ingestion of isoRB resulted in significantly higher NEFA concentrations at failure when compared to all other time points within that trial. This time related difference was not reported during the Water and LS trials. In the same study plasma caffeine concentrations were increased at all time points during the isoRB trial when compared to –20 min. The results for plasma caffeine concentrations and NEFA data illustrate some association between the presence of caffeine and an increase in the availability of free fatty acids. In Chapter 5 NEFA data were analysed during trials where isoRB, caffeine capsules (CAFF) or placebo was consumed. NEFA data at rest were significantly decreased at 30, 60 and 90 min following isoRB supplementation when compared to caffeine in capsular form. Caffeine is known to inhibit insulin-stimulated glucose uptake, section 1.6.4. However, in the presence of carbohydrate, as isoRB at rest, insulin is released thereby inhibiting lipolysis, possibly explaining why NEFA concentrations were decreased following isoRB ingestions despite the presence of caffeine. Caffeine has long been associated with diuresis at rest (Armstrong, 2002). Previous research has reported that urine production following exercise-induced hypohydration is increased following the ingestion of a caffeinated diet cola or plain water when compared to a 6% carbohydrate electrolyte beverage (Ganzalez-Alonso *et al.*, 1992) . The results of this study suggest that the low solute content in diet cola and plain water and the presence of caffeine accounted for these differences in urine production. This finding is in contrast to that reported in Chapter 4 where following ingestion of Water, a caffeinated carbohydrate beverage (isoRB) and a carbohydrate electrolyte beverage (LS), no significant differences in urine flow rates were recorded during the isoRB trial when compared to LS, but significantly greater urine flow rates were reported during the Water trial when compared to LS, Table 4.3. In Chapter 5 no diuretic effect of caffeine was reported when comparing across the 3 supplements: PL, CAFF and isoRB. Recently Riesenhuber *et al.* (2006) reported that the diuretic potential of Red Bull does not differ significantly from other caffeine containing beverages. Throughout all the research performed in this dissertation caffeine ingestion as RB, isoRB or capsules did not display any diuretic properties when ingested at rest or during exercise. In Chapter 6 where isoRB was ingested following 30 hr of fluid restriction, the urine flow rates were significantly higher during a 2 hr monitoring period compared to Water (Table 6.8). However the percentage of the ingested fluid volume retained during this monitored 2 hour post-hydration period was the same across all 3 trials (Table 6.7). Therefore the increase in urine flow rates observed during the isoRB trial may simply reflect the slightly higher

volume of fluid ingested (2190±235 ml) compared to Water (1875±295 ml) and not any effect of caffeine on urine production.

Armstrong *et al.* (2005) questioned the notion that caffeine consumption acts as a diuretic. This study involved 4 different trials. During the first 6 days of the trial all subjects ingested 3 $mg.kg^{-1}$caffeine, on days 7-11 subjects were divided into 3 separate groups: placebo (no caffeine), 3 $mg.kg^{-1}$ or 6 $mg.kg^{-1}$. It was reported that body mass, urine osmolality, urine specific gravity, 24 hr urine volume, 24 hr urinary Na^+ and K^+ concentration, urine color, 24 hr creatine, blood urea nitrogen, serum Na and K concentration, serum osmolality, hematocrit and total plasma protein were all within normal clinical ranges on days 1,3 6, 9 and 11. This study therefore supported other recent reviews that have suggested that moderate caffeine ingestion does not induce detrimental fluid-electrolyte imbalances in active individuals (Armstrong, 2002). The research in Chapter 6 of this study also supports this theory, as hydration status during the 2hr post-hydration period did not differ across the 3 trials despite the ingestion of caffeine in the form of isoRB.

In Chapter 6 hydration status was assessed using multiple markers: BIA, osmolality, serum and urine electrolytes. Bioelectrical impedance data appeared to be affected least by changes in hydration status throughout the trials, TBW remained unchanged throughout, ECW decreased significantly following fluid ingestion and ICW declined significantly late in 2hr monitoring period following LS and isoRB ingestion (see Figures 6.1, 6.2 and 6.3). It may be possible that, considering the small levels of hypohydration induced, there is minimal change in BIA data due to the sensitivity of this non-invasive technique. Research performed in the Human Performance Laboratory in Trinity College (unpublished data) reported that BIA was not significantly sensitive to detect modest heat-induced hypohydration (1.7% body mass loss), no significant differences in TBW, ECW and ICW volumes were recorded over time. One of the suggestions for these findings was that the prediction equations used in the development of the DualScan may have been based on a subject population not reflective of the normal population, but more so those with underlying conditions that would affect fluid homeostasis, namely individuals with chronic kidney disease (Ryan, 2001). Plasma volume decreased as expected during the 30 hr fluid restriction period but the percent decline and the subsequent increase following fluid ingestion were small, Table 6.5. The changes in plasma volume observed in Chapter 6 were not as marked compared to those reported in Chapter 3 possibly because of the exercise protocol involved in the latter study. With exercise plasma volume was markedly decreased, however 30 min post-exercise plasma volume had returned to normal pre-

exercise levels before supplementation with the post-exercise test beverages, Table 3.4. Shirreffs *et al.* (2004) also recorded decreases in plasma volume after 37 hr of fluid restriction but the variation across subjects was quite large (mean ± SD -6.2 ± 5.1 %). Previous research has reported significant increases in urine osmolality following hypohydration (Shirreffs *et al.*, 2004). Following 37 hr of fluid restriction urine osmolality increased from 432 ± 88 (mean ± SD) to approximately 1000 $mOsm.kg^{-1}$, no significant differences were recorded in urine osmolality in subjects during the euhydration trial. Similarly in the research described in Chapter 6, urine osmolality increased significantly during the 24 hr fluid restriction period in all 3 trials, in addition, no differences were recorded across the 3 drinks during the post-hydration period, Figure 6.9. Plasma osmolality is the most widely used haematological index of hydration status because fluid regulatory mechanisms are stimulated by extracellular fluid osmolality and some investigators consider it to be the only valid mechanism. An increase of only 1% in osmolality can initiate the sensation of thirst (Armstrong, 2002). In the research described in Chapter 6 plasma osmolality increased significantly during the first 24 hr of fluid restriction in the LS ($P<0.05$) and isoRB ($P<0.01$) trials but not in the Water trial and declined significantly in all trials following the ingestion of the test drinks, Figure 6.4. No significant differences were reported across all 3 drinks at any time point in the trials. Armstrong *et al.* (1998) reported that Usg and Uosm were valid markers of hydration status and that the sensitivity of these markers was not compromised under conditions of marked dehydration, exercise and hydration, and similar observations were made in Chapter 6 in relation to mild hypohydration and rehydration.

In conclusion, the research in this dissertation does support a solid argument for the use of carbohydrate during exercise, especially exercise of an intermittent nature. The added benefit of caffeine with carbohydrate is questionable, as no significant differences were recorded in exercise performance following RB or isoRB ingestion when compared to LS. However, if athletes are going to use caffeinated beverages before, during or after exercise it appears that diuresis may not be an issue. In addition, the presence of caffeine in RB and isoRB did not negatively affect the process of rehydration in mildly hypohydrated individuals.

Limitations and further research:

The research described in this dissertation did not involved blinded studies due to the distinctive taste of both Red Bull and Lucozade Sport. Also in designing the studies it was hoped that the formulation used would be easily reproduced by any athlete willing to use them based on the findings of the research. Subjects who participated in the research were not matched in relation to normal daily caffeine consumption. This may have been a limitation to the studies but all subjects did consume caffeine on a regular basis prior to the studies.

As a follow on to this dissertation it is may be beneficial to repeat both exercise studies documented in this thesis and include a trial where caffeine is administered in capsular form. This may clarify if there is a true difference in kinetics between liquid and capsular forms of caffeine and whether or not this difference affects exercise performance. Diuresis was not observed following the ingestion of relatively low concentrations of caffeine (3 $mg.kg^{-1}$) but it may be possible that caffeine would increase urine production, resulting in a diuretic response, when ingested in higher concentrations. Therefore the study described in Chapter 4 could be repeated with the addition of another trial where the concentration of caffeine is increased but the caloric content of the carbohydrate containing beverages is maintained. The area of rehydration could also be revisited in the study described in Chapter 3 by monitoring the post-exercise rehydration period for 2 hours and assessing many of the markers of hydration status used in Chapter 6, namely plasma osmolality, serum and urinary electrolyte concentrations, urine volume and urine specific gravity and osmolality as these parameters reflect even modest changes in hydration status as reported in Chapter 6.

References

Achten J & Jeukendrup AE (2003). Effects of pre-exercise ingestion of carbohydrate on glycemic and insulinaemic responses during subsequent exercise at differing intensities. *European Journal of Applied Physiology* **88**, 466-471.

Akiba T, Yaguchi K, Tsutusmi K, Nishioka T, Koyama I, Nomura M, Yokogawa K, Moritani S & Miyamoto K (2004). Inhibitory mechanism of caffeine on insulin-stimulated glucose uptake in adipose cells. *Biochemical Pharmacology* **68**, 1929-1937.

Alford C, Cox H & Wescott R (1999). "Red Bull" energy drink significantly improves concentration, reaction time, memory, mood, aerobic and anaerobic endurance. *Amino Acids* **17**, 94-95.

Anderson ME, Bruce CR, Fraser SF, Stepto NK, Klein R, Hopkins WG & Hawley JA (2000). Improved 2000-meter rowing performance in competitive oarsmen after caffeine ingestion. *International Journal of Sports Nutrition and Exercise Metabolism* **10**, 464-475.

Arkinstall MJ, Bruce CR, Nikolopoulos V, Garnham AP & Hawley JA (2001). Effect of carbohydrate ingestion on metabolism during running and cycling. *Journal of Applied Physiology* **91**, 2125-2134.

Armstrong LA (2002). Caffeine, body fluid-electrolyte balance, and exercise performance. *International Journal of Sports Nutrition and Exercise Metabolism* **12,** 189-206.

Armstrong LE (2005). Hydration assessment techniques. *Nutrition Reviews* **63**, S40-54.

Armstrong LE, Herrera Soto JA, Hacker FT, Casa DJ, Kavouras SA & Maresh CM, (1998). Urinary indices during dehydration exercise and rehydration. *International Journal of Sports Nutrition* **8**, 345-355.

Baba A, Lee E, Tatsuno T & Iwata H (1982). Cysteine sulfonic acid in the central nervous system: antagonistic effect of taurine on cysteine sulfonic acid-stimulated formation of cyclic AMP in guinea pig hippocampal slices. *Journal of Neurochemistry* **38**, 1280-1285.

Baum M & Weiss M (2001). The influence of a taurine containing drink on cardiac parameters before and after exercise measured by echocardiography. *Amino Acids* **20,** 75-82.

Bello-Reuss E (1980). Effect of catecholamines in fluid reabsorption by the isolated proximal convoluted tubule. *American Journal of Physiology* **238**, F347-F352.

Below PR, Rodriguez-Mora R, Gonzalez Alonso J and Coyle EF (1995).Fluid and carbohydrate ingestion independently improve performance during 1 h of intense exercise. *Medicine and Science in Sports and Exercise* **27**, 200-210.

Bichler A, Swenson A & Harris MA (2006). A combination of caffeine and taurine has no effect on short term memory but induces changes in heart rate and mean arterial pressure. *Amino Acids* **31**, 471-476.

Bosch AN, Dennis SC & Noakes TD (1994). Influence of carbohydrate ingestion on fuel substrate turnover and oxidation during prolonged exercise. *Journal of Applied Physiology* **76**, 2364-2372.

Bossingham MJ, Carnell NS & Campbell WW (2005). Water balance, hydration status and fat-free mass hydration in younger and older adults. *American Journal of Clinical Nutrition* **81**, 1342-1350.

Brener W, Hendrix TR & Mc Hugh PR (1983). Regulation of the gastric emptying of glucose. *Gastroenterology* **85**, 76-82.

Bridge CA & Jones MA (2006). The effect of caffeine ingestion on 8km run performance in a field setting. *Journal of Sports Science* **24**, 433-439.

Brouns F, Senden J, Beckers EJ & Saris WH (1995). Osmolarity does not affect the gastric emptying rate of oral rehydration solutions. *Journal of Parenteral Enteral Nutrition* **19,** 403-406.

Bruce CR, Anderson ME, Fraser SF, Stepto NK, Klein R, Hopkins WG & Hawley JA (2000). Enhancement of 2000-m rowing performance after caffeine ingestion. *Medicine and Science in Sports and Exercise* **32**, 1958-1963.

Bulow J (2004). Lipid metabolism and utilisation. *Medicine and Sport Science* **46,** 227-278.

Carter JM, Jeukendrup AE, Jones DA (2004). The effect of Carbohydrate mouth rinse on 1hr cycle time trial performance. *Medicine and Science in Sport and Exercise* **36**, 2107-2111.

Casa DL, Armstrong LE, Hillman SK, Montain SJ, Reiff RV, Rich BSE, Roberts WO & Stone JA (2000). National Athletic Trainers Association Position Statement: Fluid replacement for athletes. *Journal of Athletic Training* **35**, 212-224.

Chryssanthopoulos C, Hennessy LCM & Williams C (1994). The influence of pre-exercise glucose ingestion on endurance running capacity. *British Journal of Sports Medicine* **28**, 105-109.

Clausen JP & Trap-Jensen J (1974). Arteriohepatic venous oxygen difference and heart rate during initial phases of exercise. *Journal of Applied Physiology* **37**, 716-719.

Coggan AR & Coyle EF (1987). Reversal of fatigue during prolonged exercise by carbohydrate infusion or ingestion. *Journal of Applied Physiology* **63**, 2388-2395.

Coggan AR & Coyle EF (1988). Effect of carbohydrate feedings during high intensity exercise. *Journal of Applied Physiology* **65**, 1703-1709.

Coggan AR & Coyle EF (1989). Metabolism and performance following carbohydrate ingestion late in exercise. *Medicine and Science in Sports and Exercise* **21**, 59-65.

Coggan AR & Coyle EF (1991). Carbohydrate ingestion during prolonged exercise: effects on metabolism and performance. *Exercise and Sports Science Reviews* **19,** 1-40.

Coggan AR & Swanson SC (1992) Nutritional manipulations before and during endurance exercise: effects on performance. *Medicine and Science in Sports and Exercise* **24,** S331-S335.

Collomp K, Anselme F, Audran M, Gay JP, Chanal JL & Prefaut C (1991). Effects of moderate exercise on the pharmacokinetics of caffeine. *European Journal of Clinical Pharmacology* **40,** 279-282.

Coombes JS & Hamilton KL (2000). The effectiveness of commercially available sports drinks. *Sports Medicine* **29**, 181-209.

Costill DL & Saltin B (1974). Factors limiting gastric emptying during rest and exercise. *Journal of Applied Physiology* **37**, 679-683.

Costill DL, Coyle E, Dalsky G, Evans W, Fink W & Hoopes D (1977). Effects of elevated plasma FFA and insulin on muscle glycogen usage during exercise. *Journal of Applied Physiology* **43**, 695-699.

Cox GR, Desbrow B, Montgomery PG, Anderson ME, Bruce CR, Marcides TA, Martin DT, Moquin A, Roberts A, Hawley JA & Burke LM (2002). Effect of different protocols of caffeine intake on metabolism and endurance performance. *Journal of Applied Physiology* **93**, 990-999.

Coyle EF, Coggan AR, Hemmert MK & Ivy JL (1986). Muscle glycogen utilization during prolonged strenuous exercise when fed carbohydrates. *Journal of Applied Physiology* **61**, 165-172.

Coyle EF, Coggan AR, Hemmert MK, Lowe RC & Walter TJ (1985). Substrate usage during prolonged exercise following a pre-exercise meal. *Journal of Applied Physiology* **59,** 429-433.

Coyle EF, Hagberg JM Hurley BF, Martin WH, Ehsani AA & Holloszy JO (1983). Carbohydrate feeding during prolonged strenuous exercise can delay fatigue. *Journal of Applied Physiology: Respiratory and Environmental Exercise Physiology* **55**, 230-235.

DeMarco HM, Sucher KP, Cisar CJ & Butterfield GE (1999). Pre-exercise carbohydrate meals: application of glycemic index. *Medicine and Science in Sports and Exercise* **31**, 164-170.

Denaro CP, Brown CR, Wilson M, Jacob P & Benowitz NL (1990). Dose dependency of caffeine metabolism with repeated dosing. *Clinical Pharmaceutical Therapeutics* **48,** 277-285.

Dill DB & Costill DL (1974). Calculation of percentage changes in volume of blood plasma, red cells in dehydration. *Journal of Applied Physiology* **37,** 247-248.

Durnin JVGA and Wormersley J (1974). Body fat assessed from total body density and its estimations from skinfold thickness: measurement on 481 men and women aged from 16-72 years. *British Journal of Nutrition* **32**, 77-97.

El-Sayed MS, MacLaren D & Rattu AJ (1997). Exogenous carbohydrate utilisation: effects on metabolism and exercise performance. *Comparative Biochemical Physiology* **118**, 789-803.

Foster C, Costill DL & Fink WJ (1979) Effects of pre-exercise feedings on endurance performance. *Medicine and Science in Sports and Exercise* **11**, 1-5.

Franconi F, Stendardi I, Gailli P, Matucci R, Bennardini F, Matucci R, Manzini S & Giotti A (1983). Taurine antagonises the alpha-adrenergic positive ionotropic effect of phenylephrine. *In Sulfur Amino Acids: Biochemical and Clinical Aspects*, edited by K Kuriyama, RJ Huxtable and H Iwata, New York Press. pp 51-59.

Fredholm BB, Batig K, Holmen J, Nehlig A & Zvartau EE (1999). Actions of caffeine in the brain with specific reference to factors that contribute to its widespread use. *Pharmacological Reviews* **51**, 83-133.

Fredholm BB (1980). Theophylline actions on adenosine receptors. *European Journal of Respiratory Disease.* Suppl.**109**, 29-36.

Fudge B, Pitsiladia YP, Kingsmore D, Noakes TD & Kayser B (2006). Outstanding performance despite low fluid intake: the Kenyan running experience. In, *East African excellence in distance running- nature or nurture?* Ed. Pitsiladis Y, Bale J, Sharp C & Noakes TD. pp 2-11. Routledge, London Great Britain.

Geiss KR, Jester I, Falke W, Hamm M & Waag KL (1994). The effect of a taurine containing drink on performance in 10 endurance athletes. *Amino Acids* **7,** 45-56.

Gleeson M, Maughan RJ and Greenhaff PL (1986) Comparison of the effects of pre-exercise feeding of glucose, glycerol and placebo on endurance and fuel homeostasis in man. *Europoean Journal of Applied Physiology* **55**, 645-653.

Gonzalez-Alonso J, Heaps CL and Coyle EF (1992). Rehydration after exercise with common beverages and water. *International Journal of Sports Medicine* **13**, 399-406.

Graham TE & Spriet LL (1991) Performance and metabolic responses to a high caffeine dose during prolonged exercise. *Journal of Applied Physiology* **71**, 2292-2298.

Graham TE & Spriet LL (1995). Metabolic, catecholamine, and exercise performance responses to various doses of caffeine. *Journal of Applied Physiology* **78**, 867-874.

Graham TE & Spriet LL (1996). Caffeine and exercise performance. *Sports Science Exchange* **9**, no. 1

Graham TE (2001) Caffeine and exercise metabolism, endurance and performance. *Sports Medicine* **31**, 785-807.

Graham TE, Hibbert E & Sathasivam P (1998). Metabolic and exercise endurance effects of coffee and caffeine ingestion. *Journal of Applied Physiology* **85**, 883-889.

Graham TE, Welge JW, Maclean DA, Kiens B & Richter EA (2000). Caffeine ingestion does not alter carbohydrate or fat metabolism in human skeletal muscle during exercise. *Journal of Physiology* **529**, 837-847.

Greer F, Friars D and Graham (2000). Comparison of caffeine and theophylline ingestion; exercise metabolism and endurance. *Journal of Applied Physiology* **89**, 1837-1844.

Guyton AC & Hall JE (1996). Lipid metabolism. In, *Textbook of Medical Physiology.* 9th Edition. pp 865-875. Saunders, Ph.

Hargreaves M (2006). Exercise physiology and metabolism. In, *Clinical Sports Nutrition*, ed. Burke L & Deakin V. pp 1-11. Mc Graw Hill, NJ.

Hargreaves M, Costill DL, Coggan A, Fink WJ & Nishibata I (1984). Effect of carbohydrate feedings on muscle glycogen utilization and exercise performance. *Medicine and Science in Sports and Exericse* **16**, 219-222.

Hargreaves M, Costill DL, Fink WJ, King DS & Fielding RA (1987). Effect of pre-exercise carbohydrate feeding on endurance cycling performance. *Medicine and Science in Sports and Exercise* **19,** 33-36.

Hoffman J (2002).Metabolic system and exercise. In, *Physiological Aspects of Sport Training and Performance*, ed. Bahrke MS, Norris PA & Alexander L. pp.28-37. Human Kinetics, Champaign, Il.

Horne JA & Reyner LA (2001). Beneficial effects of an "energy drink" given to sleepy drivers. *Amino Acids* **20**, 83-89.

Horowitz JF, Mora-Rodriguez R Byerley LO & Coyle EF (1997). Lipolytic suppression following carbohydrate ingestion limits fat oxidation during exercise. *American Journal of Physiology* **36**, E768-775.

Horswill CA (1998). Effective fluid replacement. *International Journal of Sports nutrition* **8**, 175-195.

Hunter AM, Gibson A, Collins M, Lambert M & Noakes TD (2002). Caffeine ingestion does not alter performance during a 100km cycling time trial performance. *International Journal of Sports Nutrition and Exercise Performance* **12**, 438-452.

Huxtable RJ (1992). Physiological actions of taurine. *Physiological Reviews* **72,** 101-163.

Ivy JL (1999). Role of carbohydrate in physical activity. *Clinics in Sports Medicine* **18,** 469-484.

Jentjens RLPG & Jeukendrup AE (2005). High rates of exogenous carbohydrate oxidation from a mixture of glucose and fructose ingested during prolonged exercise. *British Journal of Nutrition* **93**, 485-492.

Jentjens RLPG, Cale C, Gutch C & Jeukendrup AE (2003). Effects of pre-exercise ingestion of differing amounts of carbohydrate on subsequent metabolism and cycling performance. *European Journal of Applied Physiology* **88**, 444-452.

Jeukendrup AE (2004). Carbohydrate intake during exercise and performance. *Nutrition* **20**, 669-677.

Jeukendrup AE, Brouns F, Wagenmakers AJ & Saris WH (1997). Carbohydrate-electrolyte feedings improve 1hr trial cycling performance. *International Journal Sports Medicine* **18**, 125-129.

Jeukendrup AE, Wagenmakers AJ, Stegen JH, Gijsen AP, Brouns F & Saris WH (1999). Carbohydrate ingestion can completely suppress endogenous glucose production during exercise. *American Journal of Physiology* **276**, E672-683.

Johnson HL, Nelson RA & Consolazio CF (1988). Effects of electrolyte and nutrient solutions on performance and metabolic balance. *Medicine and Science in Sports and Exercise* **20,** 26-33.

Joint position statement: American College of Sports Medicine, American Dietetic Association and Dieticians of Canada (2000). Nutrition and athletic performance *Medicine and Science in Sports and Exercise* **32**, 2130- 2145.

Kamimori GH, Karyekar CS, Otterstetter R, Cox DS, Balkin TJ, Belenky GL & Eddington ND (2002). The rate of absorption and relative bioavailability of caffeine administered in

chewing gum versus capsules to normal healthy volunteers. *International Journal of Pharmaceutics* **234**, 159-167.

Keijers GB, De Galan BE, Tack CJ & Smits P (2002). Caffeine can decrease insulin sensitivity in humans. *Diabetes Care* **25,** 364-369.

Kotake AN, Schoeller DA, Lambert GH, Baker AL, Schaffer DD & Josephs H (1982). The caffeine CO_2 Breath test: Dose response and route on N-demethylation in smokers and non-smokers. *Clinical Pharmacology and Therapeutics* **32,** 261-269.

Kovacs, EMR, Stega JHCH & Brouns F (1998). Effect of caffeinated drinks on substrate metabolism, caffeine excretion and performance. *Journal of Applied Physiology* **85,** 709-715.

Kudo Y, Akiyoshi E & Akagi H (1988). Identification of two taurine receptor subtypes on the primary afferent terminal of frog spinal cord. *British Journal of Pharmacology* **94**, 1051-1056.

Le Blanc J, Jobin M, Cote J, Samson P & Labrie A (1985). Enhanced metabolic response to caffeine in exercise-trained human subjects. *Journal of Applied Physiology* **59**, 832-837.

Lord RCC (1999). Current concepts: osmosis, osmometry and osmoregulation. *Postgraduate medical journal* **75**: 67-73.

Lugo M, Sherman WM, Wimer GS & Garleb K (1994). Metabolic responses when different forms of carbohydrate energy are consumed during exercise. *International Journal of Sports Nutrition* **3**, 398-407.

Maughan RJ & Leiper JB (1993). Post-exercise rehydration in man: effects of voluntary intake of four different beverages. *Medicine and Science in Sports and Exercise* **25** suppl 2.

Maughan RJ & Leiper JB (1995). Sodium intake and post-exercise rehydration in man. *European Journal of Applied Physiology and Occupational Physiology* **71,** 311-319.

Maughan RJ & Noakes TD (1991). Fluid replacement and exercise stress. A brief review of studies on fluid replacement and some guidelines for the athlete. *Sports Medicine* **12**, 16-31.

Maughan RJ Owen JH, Shirreffs SM & Leiper JB (1994). Post-exercise rehydration in man: effects of electrolyte addition to ingested fluids. *European Journal of Applied Physiology* **69**, 209-215.

Maughan RJ, Bethell LR & Leiper JB (1996). Effects of ingested fluids on exercise capacity and on cardiovascular and metabolic repsonses to prolonged exercise in man. *Experimental Physiology* **81**, 847-860.

Mc Lean C & TE Graham (2002). Effects of exercise and thermal stress on caffeine pharmacokinetics in men and eumenorrheic women. *Journal of Applied Physiology* **93,** 1471-1478.

Militante JD & Lombardiniabm JB (2001). Increased cardiac levels of taurine in cardiomyopathy: the paradoxical benefits of oral taurine treatment. *Nutrition Research* **21,** 93-102.

Minami H. & Mc Callum RW (1984). The physiology and patho-physiology of gastric emptying in humans. *Gastroenterology* **86**, 1592-1610.

Mitchell JB, Costill DL, Houmard JA, Flynn MG, Fink WJ & Beltz JD (1988). Effects of carbohydrate ingestion on gastric emptying and exercise performance. *Medicine and Science in Sports and Exercise* **20**, 110-5.

Montain SJ & Coyle EF (1992). Fluid ingestion during exercise increases skin blood flow independent of increases in blood volume. *Journal of Applied Physiology* **73,** 903-910.

Morris JG, Nevill ME, Thompson D, Collie J & Williams C (2003). The influence of a 6.5% carbohydrate-electrolyte beverage solution on performance of prolonged intermittent high-intensity running at 30°C. *Journal of Sports Sciences* **21**, 371-381.

Moseley L, Lancaster GL & Jeukendrup AE (2003). Effects of timing of pre-exercise ingestion of carbohydrate on subsequent metabolism and cycling performance. *Eurpopean Journal of Applied Physiology* **88**, 453-458.

Murray R (1987). The effects of consuming carbohydrate-electrolyte beverages on gastric emptying and fluid absorption during and following exercise. *Sports Medicine* **4**, 322-351.

Murray R, Eddy DE, Murray TA, Seifert JG, Paul GL & Halaby GA (1987). The effect of fluid and carbohydrate feedings during intermittent cycling exercise. *Medicine and Science in Sports and Exercise* **19**, 597-604.

Nehlig A & Debry G (1994). Caffeine and sports activity: a review. *International Journal of Sports Medicine* **15**, 215-233.

Nicholas CW, Tzintzas K, Boobis L & Williams C (1999). Carbohydrate- electrolyte ingestion during intermittent high-intensity running. *Medicine and Science in Sports and Exercise* **31,** 1280-1286.

Nicholas CW, Williams C, Lakomy HKA, Phillips G & Nowitz A (1995). Influence of ingesting a carbohydrate-electrolyte solution on endurance capacity during intermittent, high-intensity shuttle running. *Journal of Sports Sciences* **13**, 283-290.

Nose H, Mack GW, Xiangrong S & Nadel ER (1988). Involvement of sodium retention hormones during rehydration in humans. *Journal of Applied Physiology* **65,** 332-336.

O'Brien C, Young AJ & Swaka MN (2002). Bioelectrical impedance to estimate changes in hydration status. *International Journal of Sports Medicine* **23**, 361-366.

Okano G, Takeda H, Morita I, Katoh M, Mu Z & Miyake S (1988). Effect of pre-exercise fructose ingestion on endurance performance in fed men. *Medicine and Science in Sports and Exercise* **20**, 105-109.

Oppliger RA & Bartok C (2002). Hydration testing for athletes. *Sports Medicine* **32**, 959-971.

Passe DH, Horn M, Stofan J & Murray R (2004). Palatability and voluntary intake of sports beverages, diluted orange juice, and water during exercise. *International Journal of Sports Nutrition and Exercise Metabolism* **14**, 266-278.

Popowski LA, Oppliger RA, Lambert PG, Johnson RF, Johnson AK & Gisolfi CV (2001). Blood and urinary measures of hydration status during progressive acute dehydration. *Medicine and Science in Sports and Exercise* **33**, 747-753.

Pugh LGCE, Corbett JL & Johnson RH (1957). Rectal temperatures, weight losses and sweat rates in marathon running. *Journal of Applied Physiology* **23**, 347-352.

Ranallo RF & Rhodes EC (1998) Lipid metabolism during exercise. *Sports Medicine* **26**, 49-42.

Ray Ml, Byran MW, Ruden TM, Baier SM, Sharp RL & King DS (1998). Effect of sodium in a rehydration beverage when consumed as a fluid or meal. *Journal of Applied Physiology* **85**, 1329-1336.

Richter EA, Wojtaszewski JF, Kristiansen S, Daugaard JR, Nielsen JN, Derave W & Kiens B (2001). Regulation of muscle glucose transport during exercise. *International Journal of Sports Nutrition and Exercise Metabolism* **11**, S71-77.

Riesenhuber A, Boehm M, Posch M & Aufricht (2006). Diuretic potential of energy drinks. *Amino Acids* **31**, 81-83.

Robertson D, Wade D, Workman R, Woosley RL & Oates JA (1981). Tolerance to the humoral and hemodynamic effects of caffeine in man. *Journal of Clinical Investigation* **67**, 1111-1117.

Romijn JA, Coyle EF, Siddossis LC, Gastaldelli A, Horwitz JF, Endert E & Wolfe RR (1993). Regulation of endogenous fat and carbohydrate metabolism in relation to exercise intensity and duration. *American Journal of Physiology* **265**, E380-E391.

Ryan W. (2001). The sensitivity of bio-impedance analysis (BIA) in detecting modest changes in hydrational status of young adult males. MSc. Thesis in Sports Medicine, Trinity College Dublin.

Ryu S, Choi SK, Joung SS, Suh H, Cha YS, Lee S & Lim K (2001). Caffeine as a lipolytic food component increases endurance performance in rats and athletes. *Journal of Nutrition Science Vitaminol* **47**, 139-146.

Saltin B & Karlsson J (1971). Muscle glycogen utilisation during work of different intensities. In: *Muscle Metabolism during Exercise*, ed Pernow P & Saltin B, pp.289. Plenum Press, New York.

Schlaeffer F, Engelberg I, Kaplanski J & Danon A (1984). Effects of exercise and environmental heat on theophylline kinetics. *Respiration* **45**, 438-442.

Schneiker KT, Bishop D, Dawson B & Hackett LP (2006). Effects of caffeine on prolonged intermittent-sprint ability in team-sport athletes. *Medicine and Science in Sports and Exercise* **38**, 578-585.

Seidl R, Peyrl A, Nicham R & Hauser E (2000). A taurine and caffeine containing drink stimulates cognitive performance and well-being. *Amino Acids* **19,** 635-642.

Shanholtzer BA & Patterson SM (2003). Use of bioelectrical impedance in hydration status assessment: reliability of a new tool in psychophysiology research. *International Journal of Psychophysiology* **29**, 217-226.

Sherman WM, Brodowicz G, Wright DA, Allen WK, Simonsen J & Dernbach A (1989). Effect of a 4h pre-exercise carbohydrate feedings on cycling performance. *Medicine and Science in Sports and Exercise* **21,** 598-604.

Sherman WM, Costill DL, Fink WJ & Millar JM (1981). Effect of exercise–diet manipulation on muscle glycogen and its subsequent utilization during performance. *International Journal of Sports Medicine* **2,** 114-118.

Sherman WM, Peden MC & Wright DA (1991). Carbohydrate feedings one hour before exercise improves cycling performance. *American Journal of Clinical Nutrition* **54**, 866-870.

Shirreffs AM & Maughan RJ (2000). Rehydration and recovery of fluid balance after exercise. *Exercise and Sport Science Reviews* **28**, 27-32.

Shirreffs AM, Merson SJ, Fraser SM & Archer DT (2004). The effects of fluid restriction on hydration status and subjective feelings in man. *British Journal of Nutrition* **91**, 951-958.

Shirreffs SM, Taylor AJ, Leiper JB & Maughan RJ (1996). Post-exercise rehydration in man: effects of volume consumed and drink sodium content. *Medicine in Science in Sports and Exercise* **28,** 1260-1271.

Sole CC & Noakes TD (1989). Faster gastric emptying for glucose-polymer and fructose solution than for glucose in humans. *European Journal of Applied Physiology* **58**, 605-612.

Spriet LL, MacLean DA, Dyck DJ Hultman E, Cederblad G & Graham TE (1992). Caffeine ingestion and muscle metabolism during prolonged exercise in humans. *American Journal of Physiology,* **262**, E891-898.

Stuart GR, Hopkins WG, Cook C & Cairns SP (2005). Multiple effects of caffeine on simulated high-intensity team-sport performance. *Medicine and Science in Sports and Exercise* **37**, 1998-2005.

Syed SA, Kamimori GH, Kelly W & Eddington ND (2005). Multiple dose pharmacokinetics of caffeine administered in chewing gum to normal healthy volunteers. *Biopharmaceutics and Drug Disposition* **26**, 403-409.

Tarnopolsky MA, Atkinson SA, MacDoughall JD, Sale DG & Sutton JR (1989). Physiological responses to caffeine during endurance running in habitual caffeine users. *Medicine and Science in Sports and Exercise* **21**, 418-424.

Thong FS, Derave W, Kiens B, Graham TE, Urso B, Wojtaszewski J, Hansen BF & Richter EA (2002). Caffeine-induced impairment of insulin action but not insulin signalling in human skeletal muscle is reduced by exercise. *Diabetes* **51**, 583-590.

Thong FSL & Graham TE (2002). Caffeine-induced impairment of glucose tolerance is abolished by β-adrenergic receptor blockade in humans. *Journal of Applied Physiology* **92**, 2347-2352.

Tremblay F, Dubois MJ & Marette A (2003). Regulation of GLUT4 traffic and function by insulin and contraction in skeletal muscle. *Frontiers in Bioscience* **1**, 1072-84.

Turcotte LP & Zavitz MD (1996). Training induced increase in $FABP_{PM}$ is associated with elevations in palmitate uptake and oxidation by contracting muscle. *Medicine and Science in Sports and Exercise* **28,** 77.

Turcotte LP (1999). Role of fats in exercise. *Clinics in Sports Medicine* **18,** 485-498.

Van Soeren MH & Graham TE (1998). Effect of caffeine on metabolism, exercise endurance and catecholamine responses after withdrawal. *Journal of Applied Physiology* **85**, 1493-1501.

Van Soeren MH, Sathasivam P, Spriet LL & Graham TE (1993). Caffeine metabolism and epinephrine responses during exercise in users and nonusers. *Journal of Applied Physiology* **75,** 805-812.

Vist GE & Maughan RJ (1995). The effect of osmolality and carbohydrate content on the rate of gastric emptying of liquid in man. *Journal of Physiology* **486**, 523-531.

Walsh RM, Noakes TD. Hawley JA & Dennis SC (1994). Impaired high-intensity cycling performance time at low levels of dehydration. *International Journal of Sports Medicine* **15**, 392-398.

Welsh RS, Davis JM, Burke JR & Williams HG (2002). Carbohydrates and physical/mental performance during intermittent exercise to fatigue. *Medicine and Science in Sports and Exercise* **34,** 723-731.

Wemple RD, Lamb DR & Mc Keever KH (1997). Caffeine versus caffeine-free sports drinks: effects on urine production at rest and during prolonged exercise. *International Journal of Sports Medicine* **18**, 40-46.

Winnick JJ, Davis JM, Welsh RS, Carmichael MD, Murphy EA, & Blackmon JA (2005). Carbohydrate feedings during team sport exercise preserve physical and CNS function. *Medicine and Science in Sports and Exercise* **37**, 306-315.

Wolfe RR, Klein S, Carraro F & Weber J (1990). Role of triglyceride fatty acid cycle in controlling fat metabolism in humans during and after exercise. *American Journal of Physiology* **258,** E382-E389.

Wright DA, Sherman WM & Dernbach AR (1991). Carbohydrate feedings before during or in combination improve cycling endurance performance. *Journal of Applied Physiology* **71,** 1082-1088.

Yaspelkis BB, Patterson JG, Anderla PA, Ding Z & Ivy JL (1993). Carbohydrate supplementation spares muscle glycogen during variable intensity exercise. *Journal of Applied Physiology* **75**, 1477-1485.

Zierler K (1999). Whole body glucose metabolism. *American Journal of Physiology* **276**, E409-426.

Appendix 1

Abstracts

An Investigation of pre- and post-exercise ingestion of commercially available drinks on exercise performance and rehydration in male cyclists.

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Introduction

Consumption of sports drinks to enhance athletic performance has been the subject of extensive research. Critical areas of interest are the volume and timing of fluid ingestion, and the source and concentration of carbohydrate (Coombes and Hamilton, 2000). However, little research data are available comparing commercially available sports drinks, and the combined ergogenic effects of carbohydrate and caffeine. This study investigated the effects of pre- and post-exercise ingestion of caffeinated and non-caffeinated commercially available sports drinks on exercise performance and rehydration in male cyclists.

Methods

Following local ethics approval,10 elite male cyclists (mean ± SD, age 27± 6.6yr, mass 74.2 ± 5.2kg, VO_2max 70.5 ± 7.5ml.kg-1.min-1) volunteered for this study. Initially, subjects undertook a maximal incremental test to exhaustion on a cycle ergometer to assess VO_2max and determine workloads equivalent to 65, 85 and 95% $\dot{V}O_2$max. The sports drinks investigated in subsequent trials, in randomised order, were Lucozade Sport (LS), Red Bull (RB), and water (W) was used as a control. On arrival, an indwelling forearm catheter was inserted and subjects then ingested the test drink. Volumes ingested were equivalent in all trials, and were calculated relative to body mass, while ensuring a caffeine intake of 3mg.kg-1 when consuming RB. Subjects undertook a 10min warm-up (120W) 30min post-ingestion. Subjects then cycled for 60min at 65% $\dot{V}O_2$max, followed by 30min at 85% $\dot{V}O_2$max. Subsequent responses to high intensity exercise were investigated using alternating 1min elements at 95 and 65% $\dot{V}O_2$max to exhaustion. Following a warm down at 120W, non-hydrated recovery was monitored for 30min, after which a rehydration bolus equivalent to half the initial pre-exercise volume of test drink was ingested, subjects were monitored for a further 60min. Blood samples were collected to measure blood glucose, lactate, and non-esterified fatty acids. Haemoglobin and haematocrit were measured to calculate plasma volume changes across time. Heart rate (by radio telemetry, Cardiosport

Ltd.) and breath-by-breath metabolic data ($\dot{V}O_2$, RER, Quark B2, Cosmed Ltd.) were recorded at regular intervals during the exercise phases. A repeated measures ANOVA and pairwise Tukey HSD *post-hoc* test was used to infer significant differences across drinks. For all statistical tests values of $P<0.05$ were considered significant.

Results

In all trials, subjects dehydrated to the same extent during the exercise period (~11% of plasma volume). Times to exhaustion data were similar for all test drinks (92.3±28.0, 83.5±11.3, 83.2±18.9min for LS, RB and W, respectively). Heart rate, metabolic data, plasma volume changes and urine output were not different across drinks. Pre-exercise blood glucose was significantly higher following ingestion of LS compared with W (6.1±1.0 and 4.1±0.5mmol.L^{-1}, respectively). Also, 60min post-ingestion of the rehydration bolus, blood glucose for LS (5.7±0.5mmol.L^{-1}) and RB (5.4±0.6mmol.L^{-1}) was significantly higher than that for W (4.2±0.5mmol.L^{-1}). Pre-exercise blood lactate was higher for RB (1.8±0.4mmol.L^{-1}) when compared to W (1.1±0.4mmol.L^{-1}). Following 60min of rehydration blood lactate was higher for RB (1.8±0.3mmol.L^{-1}) when compared with both LS and W (1.2±0.3 and 1.3 ±0.3mmol.L^{-1}, respectively).

Discussion

The results of this study revealed that pre-exercise ingestion of RB or LS relative to water did not limit dehydration at any time point during exercise, or indeed during rehydration following post-exercise ingestion. In addition exercise performance was unaffected by the composition of fluid ingestion prior to exercise in these male cyclists, and all markers of exercise metabolism showed little effect of the test drink throughout exercise and recovery. The available scientific data investigating the ergogenic effects of Red Bull have reported positive effects on exercise performance (Geiss *et al.*, 1994, Alford *et al.*, 1999). However, the present study failed to detect any significant ergogenic effect. Research data available regarding the effect of carbohydrate ingestion on exercise performance differ with respect to the exercise protocols used, and the timing and volume of fluid ingested. However, clear differences in performance are reported more consistently during constant load exercise to exhaustion. It appears likely, that any ergogenic effect of carbohydrate in the present study may be masked by the use of relatively intense workloads towards the end of the exercise trials. In conclusion, fluid ingestion prior to- and 30min post-exercise with LS, RB or W did not effect dehydration or rehydration, and thus performance and markers of metabolism were unchanged under these exercising conditions.

References

Coombes JS & Hamilton KL (2000). Sports Med 29: 181-209

Geiss KR et al (1994). Amino Acids 7: 45-56

Alford C et al (1999). Amino Acids 17: 94-95

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Carbohydrate, but not caffeine or other stimulants improves intermittent exercise performance in elite male cyclists.

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Introduction

It is generally accepted that carbohydrate drinks consumed during exercise are beneficial when the duration of low intensity exercise exceeds 2hr. The effect of carbohydrate ingestion during high intensity exercise of shorter duration has also been investigated (Jeukendrup, 2004). However, data on the effect of carbohydrate ingestion during intermittent exercise are limited. Nicholas *et al.* (1995) observed a 33% improvement in running time to exhaustion after a shuttle running test following ingestion of Lucozade Sport pre- and during exercise compared to placebo. Therefore, the aim of this study was to investigate the effect of ingestion of commercially available sports drinks on intermittent cycling performance.

Methods

Following local ethics approval, 9 elite male cyclists (age 26±3.8yr, mass 75±6.6kg, $\dot{V}O_2$max 68.9±6.5ml.kg^{-1}.min^{-1}, mean ± SD) volunteered. Subjects performed a maximal incremental cycling test to volitional exhaustion to assess $\dot{V}O_2$max and workloads equivalent to 60 and 90% $\dot{V}O_2$max. The drinks investigated in subsequent trials, in randomised order, were Lucozade Sport (LS); Red Bull (isoRB) made isocaloric to LS by a 60-40 dilution with water and Water (W) as placebo. Volumes of test drink ingested were 4.29ml.kg^{-1} and 1.43ml.kg^{-1} pre- and every 10min during exercise, equivalent to 2.5 and 1.7mg.kg^{-1}.hr^{-1} of caffeine in isoRB trials, respectively). Volumes ingested pre-exercise were equivalent in all trials, however, total volumes ingested were dependent on time to failure (TTF). Post-ingestion, a 20min warm up and stretching period was allowed followed by the intermittent exercise protocol consisting of alternate bouts at loads (%$\dot{V}O_2$max) equivalent to 60% for 3min and 90% for 2min, until failure. Blood samples were collected throughout the trial from an indwelling forearm catheter for analysis of blood glucose and lactate, and plasma non-esterified fatty acids (NEFA), osmolality and volume (δPV). Heart rate (by radio telemetry, Cardiosport Ltd.) and breath-by-breath metabolic data ($\dot{V}O_2$, RER, Quark B^2, Cosmed Ltd.) were recorded at regular intervals

during exercise. A two way repeated measures ANOVA and pairwise Tukey HSD *post-hoc* test were used to infer significant differences across time and drink, values of $P<0.05$ were considered statistically significant.

Results

A significant difference was observed in TTF following isoRB (76.7±9.4min, $P<0.01$) and LS (72.8±6.4min, $P<0.05$) ingestion, equating to a 29 and 26% increase in performance time relative to W. There were no significant differences in mean blood lactate data ($mmol.l^{-1}$) across drinks, however, pre-exercise blood glucose was significantly higher for LS ($P<0.001$) and isoRB ($P<0.05$) compared to W. Pre-exercise blood glucose (6.01±0.4$mmol.l^{-1}$) was significantly higher compared with data recorded at 20min (3.62±0.3$mmol.l^{-1}$), 30min (4.29±0.3$mmol.l^{-1}$) and 40min (5.04±0.3$mmol.l^{-1}$) in the LS trial, however this was not observed in the isoRB trial despite matched carbohydrate intake. No significant differences were observed in plasma osmolality across drinks, however, in the LS trial mean plasma osmolality ($mOsm.kg^{-1}$) was significantly higher at exhaustion (297±3) compared with pre-exercise (289±2, $P<0.01$) and pre-test (290±3, $P<0.05$). Plasma NEFA ($mmol.l^{-1}$) were significantly higher at exhaustion following ingestion of isoRB compared with LS (0.38±0.07 and 0.24±0.05, respectively, $P<0.01$). Mean RER data were similar at exhaustion across all three trials at both exercise intensities and subjects dehydrated to the same extent (δPV from -5.5 to -6%).

Discussion

The results of this study indicate that ingestion of LS or isoRB pre- and during intermittent exercise significantly improve performance relative to a placebo. No difference was observed in TTF between LS and isoRB despite the presence of caffeine in isoRB and the previously reported ergogenicity associated with a caffeine load of $3mg.kg^{-1}$ (Graham and Spriet, 1995). Transient hypoglycaemia was observed early in exercise, however blood glucose returned towards pre-test data with no significant differences across drinks at exhaustion. Fluid ingestion occurred 20min prior to exercise and previously Coggan and Swanson (1992) reported that commencement of exercise 30-60min post carbohydrate ingestion may result in a hypoglycaemic response similar to that observed in this study. IsoRB, matched calorically to LS, was slightly hypertonic to body fluids suggesting a possible dehydrating effect, but plasma volume changes were similar across drinks. Plasma NEFA were significantly higher at exhaustion in the isoRB trial compared with LS, although analysis of plasma caffeine pharmacokinetics may attribute a role for caffeine in this observation. In conclusion, these data suggest that the improvement in exercise

performance time following the ingestion of LS and isoRB is primarily determined by the presence of carbohydrate in both drinks.

References

Coggan A, Swanson S (1992) Med Sci Sports Exerc. 24: S331-5

Graham T, Spriet L (1995) J App Physiol. 78: 867-74

Jeukendrup A (2004) Nutrition 20: 669-77

Nicholas C et al (1995) J Sports Science 13: 283-90

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Pharmokinetics of caffeine following ingestion in capsular or liquid format in healthy males.

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Sport drinks containing caffeine are increasingly used by athletes. However, scientific evidence regarding the ergogenic potential of caffeine, and particularly its role in influencing fat metabolism is highly variable (Graham and Spriet 1995, Armstrong 2002). Some of these differences in performance may be explained by differences in the absorption and/or metabolism of caffeine when ingested in either liquid or capsule format.Therefore, the aim of this study was to compare the effects of caffeine ingestion in capsule and liquid format on the serum concentration of non-esterified fatty acids (NEFA's) and the pharmokinetics of caffeine, at rest.

Following ethics approval, 9 healthy male non-habitual caffeine users underwent a medical examination and ECG screening prior to participation in the study (age 23±4yr, mass 84±12kg and height 1.80±6.4m). Each subject completed three different, randomised ingestion trials consuming either a placebo (PL: 300mg lactose capsule), caffeine (CAFF: water plus caffeine capsule), or Red Bull (isoRB: diluted 60:40 with water). Fluid volume ingested determined by body mass was the same in all trials. The caffeine dose was $3mg.kg^{-1}$ for isoRB and CAFF. Ingestion was completed over a 20 min period, and venous blood samples were collected both pre- and post-ingestion, and every 30 min for 2 hr following ingestion. A two-way repeated measures ANOVA with *post-hoc* Bonferroni tests were used to infer differences across treatments. $P<0.05$ was considered statistically significant.

No changes in serum glucose ($mmol.l^{-1}$) were observed across time during PL and CAFF trials. IsoRB data were significantly lower at 90 (3.81±0.18) and 120 min (4.01±0.16) when compared to data immediately post-ingestion (5.36±0.37). In CAFF serum NEFA's increased steadily following ingestion, and were significantly higher at 90 and 120 min when compared to both pre-and post-ingestion. In contrast, in isoRB, NEFA's decreased compared to pre- ingestion, absolute changes relative to pre-ingestion were significantly different from CAFF ($P<0.01$) at 30, 60 and 90 min. A significant time effect was observed for serum caffeine concentration following ingestion ($P<0.05$). However, there was no

significant treatment effect observed in serum caffeine concentration comparing isoRB and CAFF at any time point.

The decrease in serum NEFA's relative to pre-ingestion in isoRB possibly resulted from the carbohydrate contained in Red Bull inducing an increase in insulin and subsequent inhibition of lipolysis. However, the main findings of this study show that the serum pharmokinetics of caffeine are similar in response to caffeine ingestion from either a liquid or a capsule.

References

Graham T, Spriet L (1995) J Appl Physiol. 78: 867-74

Armstrong L (2002) Int J Sports Nutr Ex Met. 12 189-206

Keywords: Caffeine, Capsule, Pharmokinetics

Appendix 2

SAMPLE SUBJECT CONSENT FORM

HUMAN PERFORMANCE LABORATORY, ANATOMY DEPARTMENT, TRINITY COLLEGE DUBLIN.

Project Title: Comparison of the pharmacokinetics of caffeine ingestion in capsule and liquid form.

Principal investigator: Mary Beades

DECLARATION:

I have read, or had read to me, this consent form. I have had the opportunity to ask questions and all my questions have been answered to my satisfaction. I freely and voluntarily agree to be part of this research study, though without prejudice to my legal and ethical rights. I understand that should any abnormalities be diagnosed during my medical examination that the details will be forwarded to my GP. I have received a copy of this agreement and I understand that, if there is a sponsoring company, a signed copy will be sent to that sponsor.

I understand I may withdraw from the study at any time.

(Name of sponsor:).....

PARTICIPANT'S NAME:.....

CONTACT DETAILS:.....

PARTICIPANT'S SIGNATURE:.....

Date:.....

Where the participant is incapable of comprehending the nature, significance and scope of the consent required, the form must be signed by a person competent to give consent to his or her participation in the research study (other than a person who applied to undertake or conduct the study). If the participant is a minor (under 18 years old) the signature of parent or guardian must be obtained: -

NAME OF CONSENTER, PARENT or GUARDIAN: .....

SIGNATURE: .....

RELATION TO PARTICIPANT: .....

Statement of investigator's responsibility: I have explained the nature and purpose of this research study, the procedures to be undertaken and any risks that may be involved. I have offered to answer any questions and fully answered such questions. I believe that the participant understands my explanation and has freely given informed consent.

INVESTIGATOR'S SIGNATURE:..... Date:.....

Human Performance Laboratory, Trinity College Dublin

Medical Questionnaire and Examination form Date:

Name: Age:
Date of birth: Occupation:
Address: Name and Address of GP:

Contact tel. no:
Email:

Personal and Family History

	Circle			*Circle*		*Details*
Smoking	Y	N	Heart Disease	Y	N	
Alcohol	Y	N	Strokes	Y	N	
Tea	Y	N	Diabetes	Y	N	
Coffee	Y	N	Asthma	Y	N	
Vegetarian	Y	N	Epilepsy	Y	N	

Caffeine intake per day:

Drug History

		Circle		*Details*
Medications		Y	N	
Vitamins		Y	N	
Over the counter	meds	Y	N	
Allergies		Y	N	

Current Medical Problem (last 3 months)

Recent Illnesses	Recent Injuries

Immunisations

DPT [ ] Flu [ ] Tetanus [ ] Booster [ ] Typhoid [ ] Hep A [ ] Hep B [ ] MMR [ ]
Recent Foreign Travel (details)

Last 7 days…	*Circle*		At any time	*Circle*	
F L S	Y	N	Hospital admissions	Y	N
Sore Throat	Y	N	Operations	Y	N
Cough	Y	N	Fractures	Y	N
Wheeze	Y	N	Sports Injuries	Y	N
Chest pains	Y	N	Diabetes	Y	N
Palpitations	Y	N	Asthma	Y	N
Nausea	Y	N	Epilepsy	Y	N
Diarrhoea	Y	N	Jaundice	Y	N
Vomiting	Y	N	Kidney problem	Y	N
Headaches	Y	N	Heart Murmur	Y	N
Fits or Faints	Y	N	Eye Problems	Y	N
Others	Y	N	Other Illness	Y	N

General Examination (Doctors use only)

Obs: Pulse beats.min^{-1} Reg./Irreg.
BP mmHg
Temp: °C
Head: Nose Throat
Neck: Nodes Tyroid
CVS: Apex beat Heart Sounds
RS: Exp^{n} Perc. / Ausc.
Others (ABDO/GU/Urinalysis):
FBC result:
Lung Function result:

Medical summary/Investigations/Recommendations to GP or Consultant

Fit for participation in trial Y N

Signature.....

Dietary Guidelines presented to each subject prior to participation in the fluid restriction trials, Chapter 6.

Adapted from those used by Shirreffs *et al.*, 2004.

In order to standardise water intake we would ask you to avoid consuming the following foods with a higher water content.
For example: instead of cereal with milk for breakfast, eat toast with butter or a spread. For lunch a cheese sandwich would be better then soup. For dinner rather than pasta with a sauce you could have a pizza.

Below are some suggestions for suitable meals.

Breakfasts	Lunches	Dinner
Toast	Sandwiches	Pizza
Butter/margarine	Sausage rolls or pastries	Fish and Chips
Spreads eg. Peanut butter	Pies	Chip
Jam	Quiche	Beef burgers
Croissants		Veggie burgers
Cereal Bars		

Snacks

Crisps	Chocolate
Biscuits	Sweets
Cheese	Cereal Bars
Scones	Pancakes
Muffins	Dried Fruit

Foods to avoid
Fruit
Vegetables
Soup
Rice
Pasta
Sauce based dishes
Yoghurts
Ice cream
Soft Cheeses
Cereals with milk
Porridge

Appendix 3

All data in this appendix relate to the study described in Chapter 3.

Time		Water	LS	RB
		Mean ± SEM (beats.min^{-1})		
-60	Pre-exercise	62 ± 2	63 ± 3	68± 4.6
-40		55 ± 2	59 ± 3	60 ± 3
-20		55 ± 3	57 ± 3	58 ± 3
-10		56 ± 3	57 ± 2	59 ± 3
0		64 ± 3	69 ± 3	67 ± 2
10	Exercise	133 ± 3	134 ± 3	136 ± 3
20		136 ± 2	137 ± 3	138 ± 2
30		138 ± 23	136 ± 3	140 ± 3
40		141 ± 3	137 ± 3	139 ± 3
50		141 ± 2	139 ± 4	142 ± 2
60		147 ± 3	140 ± 3	145 ± 3
70		174 ± 3 (n=8)	172 ± 3	176 ± 2
Failure		181 ± 3	180 ± 3	182 ± 2
10	Rehydration Period	86 ± 3	85 ± 3	83 ± 3
20		81 ± 4	79 ± 3	82 ± 3
30		78 ± 4	80 ± 2	78 ± 3
40		73 ± 3	75 ± 3	71 ± 3
50		69 ± 3	75 ± 3	70 ± 3
60		70 ± 4	71 ± 4	69 ± 4
70		67 ± 4	71 ± 4	64 ± 3
80		68 ± 4	72 ± 4	69 ± 3
90		67 ± 4.0	70 ± 4	68 ± 3

Mean (± SEM) heart rate across time and trial, n=10.

		Water	LS	RB
		Mean ± SEM ($mmol.l^{-1}$)		
-60	**Pre-exercise**	1.17 ± 0.07	1.21 ± 0.60	1.21 ± 0.06
0		1.13 ± 0.11	1.37 ± 0.13	1.80 ± 0.14
10	**Exercise**	1.19 ± 0.11	1.13 ± 0.09	1.41 ± 0.13
20		0.97 ± 1.00	1.13 ± 0.10	1.27 ± 0.12
30		0.99 ± 0.10	1.15 ± 0.09	1.20 ± 0.11
40		0.91 ± 0.07	0.94 ± 0.06	1.09 ± 0.09
50		0.94 ± 0.06	0.90 ± 0.07	1.11 ± 0.10
60		0.94 ± 0.10	0.89 ± 0.05	1.03 ± 0.09
70		4.65 ± 0.45 (n=8)	4.01 ± 0.26	5.08 ± 0.27
80		4.90 ± 0.58 (n=4)	4.54 ± 0.53 (n=6)	6.14 ± 0.63 (n=6)
90		5.54 ± 1.18 (n=3)	3.98 ± 0.83 (n=3)	5.52 ± 0.71 (n=3)
Failure		5.78 ± 0.43	5.43 ± 0.36	7.07 ± 0.39
30	**Rehydration period**	1.27 ± 0.20	1.16 ± 0.14	1.56 ± 0.15
60		1.23 ± 0.12	1.40 ± 0.19	2.01 ± 0.15
90		1.27 ± 0.13	1.05 ± 0.09	1.70 ± 0.16

Mean (± SEM) blood lactate across time and trial, n=10.

		Water	LS	RB
		Mean ± SEM ($mmol.l^{-1}$)		
-60	**Pre-exercise**	4.87 ± 0.28	4.79 ± 0.28	4.76 ± 0.20
0		4.10 ± 0.17	6.13 ± 0.32	5.15 ± 0.40
10	**Exercise**	3.89 ± 0.12	3.49± 0.18	4.20 ± 0.25
20		4.38 ± 0.19	3.83 ± 0.25	4.25 ± 0.19
30		4.68 ± 0.15	4.30 ± 0.26	4.96 ± 0.23
40		4.55 ± 0.21	4.27 ± 0.24	4.73 ± 0.24
50		4.54 ± 0.18	4.12 ± 0.16	4.45 ± 0.80
60		4.56 ± 0.20	4.38 ± 0.18	4.61 ± 0.19
70		4.67 ± 0.26 (n=8)	4.18 ± 0.22	4.60 ± 0.16
80		4.50 ± 0.28 (n=4)	4.55± 0.17 (n=6)	5.31 ± 0.34 (n=6)
90		4.13 ± 0.09 (n=3)	3.91 ± 0.42 (n=3)	4.65 ± 0.25 (n=3)
Failure		5.24 ± 0.41	5.25± 0.27	5.72 ± 0.33
30	**Rehydration period**	4.41 ± 0.17	4.91 ± 0.42	4.80 ± 0.24
60		4.30 ± 0.18	7.08 ± 0.37	6.93 ± 0.35
90		4.40 ± 0.13	4.94 ± 0.25	4.82 ± 0.23

Mean (± SEM) blood glucose across time and trial, n=10

Time (min)		Water	LS	RB
		Mean ± SEM ($ml.kg^{-1}.min^{-1}$)		
0	Pre-exercise			
8	Exercise	45.5 ± 1.3	44.5 ± 1.0	43.6 ± 1.6
18		45.9 ± 1.5	45.7 ± 1.2	45.6 ± 1.7
28		46.9 ± 1.5	45.3 ± 0.9	44.6 ± 1.7
38		46.3 ± 1.5	45.1 ± 0.8	44.9 ± 1.5
48		46.5 ± 1.4	44.9 ± 0.6	44.7 ± 1.5
58		47.1 ±1.4	45.3 ± 0.8	44.8 ± 1.5
68		60.2 ± 1.5 (n=8)	58.9 ± 1.4	60.0 ± 2.2
78		59.1 ± 2.0 (n=4)	60.9 ± 2.1 (n=6)	59.7 ± 3.0 (n=6)
Failure		59.8 ± 2.7	60. ± 2.2	59.1 ± 3.1

Mean (± SEM) oxygen uptake across time, n=10.

Time (min)		Water	LS	RB
		Mean ± SEM		
8	Exercise	0.92 ± 0.01	0.95 ± 0.01	0.94 ± 0.01
18		0.92 ± 0.01	0.96 ± 0.01	0.94 ± 0.01
28		0.92 ± 0.01	0.94 ± 0.01	0.93 ± 0.01
38		0.91 ± 0.01	0.94 ± 0.01	0.93 ± 0.01
48		0.91 ± 0.01	0.93 ± 0.02	0.93 ± 0.01
58		0.90 ± 0.01	0.92 ± 0.01	0.92 ± 0.01
68		0.97 ± 0.01 (n=8)	0.99 ± 0.01	1.00 ± 0.01
78		0.96 ± 0.01 (n=4)	0.97 ± 0.01 (n=6)	0.98 ± 0.00 (n=6)
Failure		0.98 ± 0.01	0.96 ± 0.01	0.96 ± 0.01

Mean (± SEM) RER across time, n=10.

		Water	LS	RB
		Mean ± SEM ($mOsm.kg^{-1}$)		
-60	**Pre-exercise**	285 ± 2	280 ± 4	279 ± 4
0		284 ± 2	277 ± 3	279 ± 4
30	**Exercise**	282 ± 2	283 ± 4	280 ± 5
60		290 ± 1	282 ± 4	286 ± 4
Failure		288 ± 5	290 ± 4	288 ± 5
30	**Rehydration period**	283 ± 3	280 ± 3	283 ± 4
60		279 ± 4	280 ± 2	284 ± 5
90		277 ± 4	284 ± 1	281 ± 4

Mean (± SEM) plasma osmolality across time, n=10.

Time (min)		RB
		Mean ± SEM ($ng.l^{-1}$)
-60	Pre-exercise	582 ± 21
0		670 ± 13
30	Exercise	663 ± 8
60		654 ± 18
Failure		511 ± 10
30	Rehydration period	586 ± 13
60		644 ± 26
90		649 ± 17

Mean (± SEM) plasma caffeine concentration across time, n=10.

Appendix 4

All data in this appendix relate to the study described in Chapter 4.

Time (min)	Water	LS	isoRB
	Mean ± SEM (beats.min^{-1})		
0	69 ± 4	65 ± 3	66 ± 4
5	158 ± 4	159 ± 4	160 ± 4
10	165 ± 4	166 ± 5	166 ± 5
15	166 ± 4	167 ± 4	169 ± 4
20	169 ± 4	169 ± 5	171 ± 5
25	169 ± 4	171 ± 4	172 ± 4
30	172 ± 4	173 ± 4	175 ± 4
35	171 ± 4	175 ± 4	175 ± 4
40	173 ± 4	176 ± 4	177 ± 5
45	178 ± 2	176 ± 5	176 ± 5
50	176 ± 3	176 ± 4	172 ± 7
55	175 ± 4 (n=5)	175 ± 4 (n=8)	175 ± 5 (n=7)
60	173 ± 4 (n=3)	175 ± 5 (n=7)	177 ± 5 (n=7)
65	169 ± 2 (n=2)	176 ± 5 (n=7)	175 ± 6 (n=6)
70	168 ± 2 (n=2)	174 ± 6 (n=5)	181 ± 5 (n=5)
75	169 ± 3 (n=2)	176 ± 6 (n=3)	180 ± 6 (n=4)
80	----------	176 ± 6 (n=3)	182 ± 6 (n=4)
Failure	176 ± 4	181 ± 4	181 ± 4

Mean (± SEM) heart rate across time and trials.

Time (min)	Water	LS	isoRB
	Mean ± SEM (mmol.l^{-1})		
-20	0.97 ± 0.13	0.93 ± 0.10	0.77 ± 0.10
0	0.78 ± 0.11	0.78 ± 0.05	0.94 ± 0.12
10	4.28 ± 0.49	4.47 ± 0.56	4.65 ± 0.74
20	4.26 ± 0.65	4.62 ± 0.61	4.91 ± 0.83
30	4.90 ± 0.62	4.59 ± 0.58	5.29 ± 1.00
40	4.39 ± 0.38	4.88 ± 0.61	5.34 ± 0.96
50	4.17 ± 0.33	4.45 ± 0.40	4.49 ± 0.53
60	3.89 ± 0.60 (n=3)	4.54 ± 0.53 (n=7)	4.62 ± 0.57 (n=7)
Failure	5.09 ± 0.62	5.46 ± 0.50	5.82 ± 0.88

Mean (±SEM) blood lactate at rest and at 10 min intervals during exercise across trials.

Time (min)	Water	LS	isoRB
	(mmol.l^{-1}) Mean ± SEM		
-20	5.1 ± 0.3	5.1 ± 0.3	5.0 ± 0.2
0	4.7 ± 0.2	6.0 ± 0.4	5.5 ± 0.3
10	3.9 ± 0.2	4.1 ± 0.2	4.3 ± 0.3
20	4.2 ± 0.2	3.6 ± 0.3	4.0 ± 0.4
30	4.8 ± 0.2	4.3 ± 0.3	4.9 ± 0.3
40	4.8 ± 0.2	5.0 ± 0.3	5.3 ± 0.3
50	4.8 ± 0.3	5.2 ± 0.2	5.6 ± 0.5
60	4.2 ± 0.4 (n=3)	5.0 ± 0.1 (n=7)	5.5 ± 0.6 (n=7)
Failure	4.9 ± 0.3	5.1 ± 0.3	5.3 ± 0.3

Mean (±SEM) blood glucose at rest and at 10 min intervals during exercise across trials.

Time (min)	Water	LS	isoRB
	(ml.kg^{-1}.kg^{-1}) Mean ± SEM		
0	7.6 ± 0.5	7.4 ± 0.5	7.99 ± 0.5
10	57.1 ± 2.2	56.0 ± 2.0	57.6 ± 2.3
20	57.6 ± 2.3	56.4 ± 2.2	58.1 ± 2.3
30	58.3 ± 2.7	56.9 ± 2.1	58.4 ± 2.2
40	58.6 ± 2.7	57.6 ± 1.9	58.7 ± 2.0
50	59.6 ± 2.6	58.1 ± 2.2	59.1 ± 2.3
60	61.3 ± 5.5 (n=3)	58.5 ± 2.5 (n=7)	60.2 ± 2.4 (n=7)
70	60.8 ± 0 (n=2)	58.7 ± 3.4 (n=5)	62.2 ± 3.1 (n=5)
80	----------	62.0 ± 6.8 (n=3)	62.8 ± 3.5 (n=4)
Failure	57.2 ± 2.1	57.3± 2.3	60.1 ± 2.0

Mean (±SEM) oxygen uptake at rest and at 10 min intervals during high intensity exercise element across trials, n=9.

Time (min)	Water	LS	isoRB
	Mean ± SEM		
10	1.00 ± 0.02	1.0 ± 0.01	0.99 ± 0.01
20	0.98 ± 0.01	1.0 ± 0.02	0.99 ± 0.01
30	0.98 ± 0.01	1.0 ± 0.02	0.99 ± 0.01
40	0.97 ± 0.01	0.98 ± 0.02	0.98 ± 0.01
50	0.97 ± 0.02	0.97 ± 0.02	0.99 ± 0.01
60	0.98 ± 0.01 (n=3)	0.97 ± 0.02 (n=7)	0.98 ± 0.01 (n=7)
70	1.01 ± 0.00 (n=2)	0.99 ± 0.01 (n=5)	0.98 ± 0.01 (n=5)
80	----------	0.99 ± 0.03 (n=3)	0.98 ± 0.01 (n=4)
Failure	0.97 ± 0.01	0.97 ± 0.01	0.97 ± 0.02

Mean (±SEM) Respiratory Exchange Ratio during high intensity exercise element across trials, n=9.

Time (min)	Water	LS	isoRB
	(mmol.l^{-1}) Mean ± SEM		
-20	287 ± 2	290 ± 3	292 ± 2
0	289 ± 2	289 ± 2	288 ± 2
30	296 ± 2	295 ± 1	296 ± 3
60	291 ± 1 (n=3)	299 ± 2 (n=7)	295 ± 2 (n=7)
Failure	294 ± 2	298 ± 3	295 ± 2

Mean (±SEM) plasma osmolality at rest and during exercise across trials, n=9.

Time (min)	Water	LS	isoRB
	Mean ± SEM (mmol.l^{-1})		
-20	0.238± 0.06	0.176 ± 0.03	0.230 ± 0.06
0	0.211 ± 0.05	0.198 ± 0.04	0.244 ± 0.06
30	0.211 ± 0.03	0.119 ± 0.02	0.178 ± 0.03
Failure	0.323 ± 0.06	0.237 ± 0.05	0.376 ± 0.69

Mean (±SEM) non-esterified free fatty acid concentration at rest and during exercise across trials, n=9.

Time (min)	isoRB
	mean ± SEM ($ng.l^{-1}$)
-20	529 ± 60
0	708 ± 33
30	676 ± 18
Failure	686 ± 22

Mean (±SEM) plasma caffeine concentrations at rest and during exercise for the isoRB trial, n=9.

Appendix 5

All data in this appendix relate to the study described in Chapter 5.

Time (min)	isoRB	CAFF
	Mean ± SEM ($ng.l^{-1}$)	
Pre-drink	339 ± 66	332 ± 68
0	411 ± 33	434 ± 36
30	446 ± 41	427 ± 37
60	419 ± 48	460 ± 39
90	414 ± 35	500 ± 54
120	390 ± 37	451 ± 58

Mean (± SEM) caffeine concentration, n=9.

Time (min)	isoRB	CAFF
	Mean ± SEM ($ng.l^{-1}$)	
0	72 ± 48	101 ± 50
30	107 ± 53	94 ± 42
60	113 ± 37	128 ± 48
90	74 ± 39	169 ± 30
120	42 ± 34	119 ± 39

Mean (± SEM) change in caffeine concentration from baseline (pre-drink), n=9.

Time (min)	isoRB	CAFF	PL
	Mean ± SEM ($mmol.l^{-1}$)		
Pre-drink	0.198 ± 0.06	0.237 ± 0.05	0.227 ± 0.05
0	0.145 ± 0.04	0.239 ± 0.05	0.217 ± 0.05
30	0.136 ± 0.03	0.377 ± 0.08	0.273 ± 0.06
60	0.112 ± 0.03	0.377 ± 0.07	0.240 ± 0.05
90	0.134 ± 0.04	0.381 ± 0.06	0.297 ± 0.08
120	0.187 ± 0.05	0.376 ± 0.06	0.232 ± 0.04

Mean (± SEM) NEFA concentration, n=9.

Time (min)	isoRB	CAFF	PL
	Mean ± SEM ($mmol.l^{-1}$)		
0	-0.08 ± 0.04	0.002 ± 0.01	-0.010 ± 0.02
30	-0.089± 0.05	0.139 ± 0.06	0.048 ± 0.04
60	-0.115 ± 0.04	0.139 ± 0.07	0.013 ± 0.03
90	-0.092 ± 0.05	0.143 ± 0.05	0.069 ± 0.05
120	-0.039 ± 0.064	0.139 ± 0.05	0.005 ± 0.04

Mean (± SEM) change in NEFA from baseline (pre-drink), n=9

Time (min)	isoRB	CAFF	PL
	Mean ± SEM ($mmol.l^{-1}$)		
Pre-drink	4.4 ± 0.3	4.5 ± 0.3	4.0 ± 0.4
0	5.4 ± 0.4	4.6 ± 0.2	4.0 ± 0.3
30	4.4 ± 0.3	4.3 ± 0.2	3.9 ± 0.3
60	4.5 ± 0.3	4.5 ± 0.2	4.1 ± 0.3
90	3.8 ± 0.2	4.3 ± 0.2	3.9 ± 0.3
120	4.0 ± 0.2	4.6 ± 0.1	4.0 ± 0.2

Mean (± SEM) serum glucose concentration, n=9.

Time (min)	isoRB	CAFF	PL
	Mean ± SEM ($mmol.l^{-1}$)		
0	0.96 ± 0.37	0.13 ± 0.17	-0.07 ± 0.30
30	0.00± 0.33	-0.18 ± 0.25	-0.07 ± 0.21
60	0.14 ± 0.23	0.07 ± 0.21	-0.13 ± 0.20
90	-0.59 ± 0.23	-0.16 ± 0.31	-0.11 ± 0.24
120	-0.39 ± 0.26	0.08 ± 0.26	0.02 ± 0.28

Mean (± SEM) change in serum glucose concentration from baseline (pre-drink), n=9.

Appendix 6

All data in this appendix relate to the study described in Chapter 6.

		Water	LS	isoRB
		Mean ± SEM (l)		
0	Fluid Restriction	43.8 ± 1.5	44.3 ± 1.6	44.1 ± 1.6
24 hr		43.5 ± 1.5	43.9 ± 1.6	43.3 ± 1.6
30 hr		43.5 ± 1.6	43.4 ± 1.6	43.3 ± 1.6
Hydration		43.0 ± 1.6	43.1 ± 1.5	43.2 ± 1.7
30 min	Post-Hydration	43.1 ± 1.6	43.2 ± 1.5	43.2 ± 1.6
60 min		43.1 ± 1.6	43.3 ± 1.6	43.2 ± 1.6
90 min		43.0 ± 1.6	43.3 ± 1.6	43.1 ± 1.6
120 min		43.0 ± 1.5	43.2 ± 1.6	43.2 ± 1.6

Mean (± SEM) total body water (l) during fluid restriction, hydration and post-hydration (n=10).

		Water	LS	isoRB
		Mean ± SEM (l)		
0	Fluid Restriction	20.0 ± 0.7	20.3 ± 0.7	20.2 ± 0.8
24 hr		20.0 ± 0.7	20.1 ± 0.7	19.8 ± 0.7
30 hr		20.0 ± 0.7	19.8 ± 0.7	19.8 ± 0.7
Hydration		19.6 ± 0.7	19.5 ± 0.7	19.6 ± 0.7
30 min	Post-Hydration	19.6 ± 0.7	19.6 ± 0.7	19.6 ± 0.7
60 min		19.6 ± 0.7	19.6 ±0.7	19.7 ± 0.7
90 min		19.6 ± 0.7	19.7 ± 0.7	19.7 ± 0.7
120 min		19.6 ± 0.7	19.7 ± 0.7	19.7 ± 0.7

Mean (± SEM) extracellular water content (l) during fluid restriction, hydration and post-hydration (n=10).

		Water	LS	isoRB
		Mean ± SEM (l)		
0	Fluid Restriction	24.0 ± 0.8	24.0 ± 0.9	24.0 ± 0.8
24 hr		23.4 ± 0.8	23.8 ± 0.9	23.5 ± 0.9
30 hr		23.6 ± 0.9	23.6 ± 0.9	23.5 ± 0.9
Hydration		23.5 ± 0.9	23.6 ± 0.9	23.6 ± 0.9
30 min	Post-Hydration	23.5 ± 0.9	23.6 ± 0.9	23.6 ± 0.9
60 min		23.5 ± 0.9	23.7 ± 0.9	23.5 ± 0.9
90 min		23.5 ± 0.9	23.6 ± 0.9	23.5 ± 0.9
120 min		23.4 ± 0.9	23.5 ± 0.9	23.5 ± 0.9

Mean (± SEM) intracellular water content (l) during fluid restriction, hydration and post-hydration (n=10).

		Water	LS	isoRB
		Mean ± SEM (mOsm.kg^{-1})		
0	Fluid Restriction	316 ± 3	314 ± 1	312 ± 1
24 hr		320 ± 2	320 ± 2	319 ± 2
30 hr		320 ± 2	321 ± 1	318 ± 2
Hydration		316 ± 1	315 ± 1	315 ± 1
30 min	Post-Hydration	311 ± 1	311 ± 2	313 ± 2
60 min		308 ± 1	306 ± 2	310 ± 1
90 min		309 ± 2	312 ± 1	310 ± 2
120 min		312 ± 2	312 ± 2	312 ± 2

Mean (± SEM) plasma osmolality (mOsm.kg^{-1}) during fluid restriction, hydration and post-hydration (n=10).

Time		Water	LS	isoRB
		Mean ± SEM (mmol.l^{-1})		
0	Fluid Restriction	142.8 ± 1.3	142.8 ± 0.8	143.5 ± 1.1
24 hr		145.7 ± 1.0	145.2 ± 1.0	145.3 ± 1.2
30 hr		146.7 ± 0.9	146.0 ± 0.9	145.5 ± 1.1
Hydration		146.2 ± 0.6	144.9 ± 0.8	144.6 ± 0.9
30 min	Post-Hydration	145.4 ± 0.5	145.3 ± 1.0	142.9 ± 1.4
60 min		143.9 ± 0.9	144.3 ± 0.9	143.4 ± 1.0
90 min		144.2 ± 1.1	144.4 ± 1.0	143.9 ± 0.9
120 min		144.3 ± 0.9	144.6 ± 0.9	143.4 ± 1.0

Mean (± SEM) serum [Na^+] (mmol.l^{-1}) during fluid restriction, hydration and post-hydration (n=10).

Time		Water	LS	isoRB
		Mean ± SEM (mmol.l^{-1})		
0	Fluid Restriction	4.3 ± 0.1	4.4 ± 0.1	4.3 ± 0.1
24 hr		4.3 ± 0.1	4.6 ± 0.1	4.5 ± 0.1
30 hr		4.1 ± 0.1	4.3 ± 0.1	4.3 ± 0.1
Hydration		4.4 ± 0.1	4.3 ± 0.1	4.5 ± 0.2
30 min	Post-Hydration	4.1 ± 0.1	4.0 ± 0.1	4.1 ± 0.1
60 min		4.2 ± 0.1	4.0 ± 0.1	3.9 ± 0.1
90 min		4.1 ± 0.1	4.0 ± 0.1	3.9 ± 0.1
120 min		4.1 ± 0.1	4.1± 0.1	4.0 ± 0.2

Mean serum [K^+] concentration (mmol.l^{-1}) during fluid restriction, hydration and post-hydration (n=10).

		Water	LS	isoRB
		Mean ± SEM ($mmol.l^{-1}$)		
0	Fluid Restriction	39.8 ± 5.7	56.1 ± 6.9	57.7 ± 7.4
24 hr		129.6 ± 11.5	133.2 ± 109	127.7 ± 10.3
30 hr		166.2 ± 6.4	158.7 ± 7.3	154.5 ± 11.0
Hydration		169.0 ± 5.2	168.4 ± 8.8	163.5 ± 9.1
30 min	Post-Hydration	85.2 ± 13.4	82.5 ± 12.5	78.0 ± 7.4
60 min		43.4 ± 12.9	44.2 ± 7.5	42.4 ± 9.9
90 min		49.2 ± 13.2	41.0 ± 11.2	26.6 ± 2.9
120 min		46.3 ± 11.0	38.2 ± 12.7	45.8 ± 13.9

Mean (± SEM) urine Na^+] ($mmol.l^{-1}$) during fluid restriction, hydration and post-hydration (n=10).

		Water	LS	isoRB
		Mean ± SEM ($mmol.l^{-1}$)		
0	Fluid Restriction	31.3 ± 4.0	34.2 ± 4.9	35.1 ± 6.3
24 hr		112.5 ± 13.4	110.2 ± 8.1	115.2 ± 8.6
30 hr		117.6 ± 3.2	111.2 ± 7.3	117.9 ± 10.5
Hydration		103.9 ± 9.6	112.3 ± 12.0	98.2 ± 8.7
30 min	Post-Hydration	65.7 ± 10.9	45.9 ± 8.3	29.6 ± 3.9
60 min		28.9 ± 8.8	20.3 ± 3.5	14.3 ± 3.9
90 min		26.55 ± 7.1	17.6 ± 4.0	9.5 ± 1.6
120 min		27.5 ± 6.5	18.8 ± 4.2	15.0 ± 5.6

Mean (± SEM) urine [K^+] ($mmol.l^{-1}$) during fluid restriction, hydration and post-hydration (n=10).

		Water	LS	isoRB
		Mean ± SEM ($mOsm.kg^{-1}$)		
0	Fluid Restriction	215 ± 29	301 ± 53	287 ± 26
24 hr		888 ± 39	875 ± 49	911 ± 28
30 hr		930 ± 37	919 ± 51	921 ± 34
Hydration		931 ± 36	923 ± 42	944 ± 44
30 min	Post-Hydration	483 ± 100	414 ± 81	357 ± 41
60 min		203 ± 79	129 ± 33	131 ± 39
90 min		159 ± 45	144 ± 52	133 ± 58
120 min		209 ± 60	158 ± 52	177 ± 66

Mean (± SEM) urine osmolality ($mOsm.kg^{-1}$) during fluid restriction, hydration and post-hydration (n=10).

		Water	LS	isoRB
		Mean ± SEM		
0	Fluid Restriction	1.007 ± 0.001	1.009 ± 0.001	1.009 ± 0.001
24 hr		1.025 ± 0.001	1.024 ± 0.001	1.025 ± 0.001
30 hr		1.021 ± 0.002	1.023 ± 0.001	1.024 ± 0.001
Hydration		1.024 ± 0.001	1.024 ± 0.001	1.024 ± 0.001
30 min	Post-Hydration	1.013 ± 0.003	1.012 ± 0.002	1.010 ± 0.002
60 min		1.006 ± 0.002	1.004 ± 0.001	1.004 ± 0.001
90 min		1.004 ± 0.001	1.004 ± 0.002	1.003 ± 0.001
120 min		1.006 ± 0.002	1.004 ± 0.000	1.005 ± 0.002

Mean (± SEM) urine specific gravity during fluid restriction, hydration and post-hydration (n=10).