Fluid movement and availability following ingestion of glucose solutions at rest and after exercise

by

Gethin H. Evans, BSc (Aberdeen)

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List of Abbreviations

ANOVA	analysis of variance
bpm	beats per minute
CHO	carbohydrate
Cl^-	chloride
CV	coefficient of variation
g	grams
h	hour
K^+	potassium
K_2EDTA	potassium ethylenediamine tetra acetic acid
kg	kilogram
km	kilometre
L	litre
m	metre
M	mole
mg	milligram
ml	millilitre
mmol	millimole
mosm	milliosmole
min	minute
Na^+	sodium
NaCl	sodium chloride
NaOH	sodium hydroxide
$NaHCO_3$	sodium bicarbonate
pmol	pecomole
s	second
SD	standard deviation
$\dot{V}O_{2max}$	maximum rate of oxygen uptake
$\dot{V}O_{2peak}$	peak rate of oxygen uptake
W	watts
y	years
°C	degrees Celsius

^{2}H	deuterium
$^{2}H_20$	deuterium oxide

Abstract

The consequences of ingesting different carbohydrate solutions on fluid movement and availability have not been systematically examined. In addition, the role of carbohydrate in the post-exercise rehydration period has received little attention despite the need for substrate replenishment following exercise and the role of carbohydrates in stimulating water absorption in the intestine. The aims of this thesis were to assess fluid absorption characteristics and availability of solutions containing increasing concentrations of glucose and to evaluate their role in the restoration and maintenance of fluid balance following a period of exercise-induced dehydration.

The ingestion of a single bolus of a commercially available hypertonic 18% carbohydrate solution (chapter 3) and a hypertonic 10% glucose solution (chapter 4) resulted in reductions in plasma volume that are most likely due to acute net secretion of water into the intestinal lumen. When investigating recovery of whole body hydration status after sweat loss, a hypertonic 10% glucose-electrolyte solution maintained whole body fluid balance for a longer period than a hypotonic 2% glucose-electrolyte solution and an electrolyte only solution when a fixed volume of fluid was consumed during a rehydration period of one hour following cycle exercise in the heat (chapter 5). When fluid was consumed *ad libitum* over a two hour period following similar cycle exercise in the heat, a hypertonic 10% glucose-electrolyte solution was as effective in restoring and maintaining fluid balance as a 2% hypotonic glucose-electrolyte solution and an electrolyte only solution (chapter 6). The reduced rate of gastric emptying that accompanies the ingestion of high carbohydrate solutions was likely to be the primary cause for the difference in urine production reported between the trials during this study (chapter 7).

In conclusion, ingestion of hypertonic carbohydrate solutions results in a reduction in extracellular fluid volume that is most likely due to secretion of water into the intestinal lumen and the carbohydrate content of an ingested solution is of importance in the post-exercise rehydration period.

Keywords: Dehydration, Rehydration, Gastric Emptying, Intestinal Absorption, Carbohydrate, Fluid Movement, Fluid Availability.

Chapter 1

General Introduction

Body water

Water accounts for approximately 50-60% of body mass in the normal human body. This equates to approximately 35-42 L for an average 70 kg male. Water is separated into intracellular and extracellular compartments with intracellular fluid accounting for approximately two thirds of total body water and extracellular fluid accounting for the remaining third. The extracellular fluid can be further separated into the interstitial fluid (15% total body mass) and the plasma (5% total body mass) (Sawka, 1990).

Despite the large volume of water present in the human body, it is necessary to maintain this level within relatively narrow limits (Maughan and Burke, 2002). There is a likelihood of collapse following a reduction in body water of approximately 7% (Greenleaf, 1992) while excessive water consumption, in the absence of an appropriate diuresis, can also lead to health consequences such as hyponatremia (Maughan and Burke, 2002). Individuals are said to be in a state of euhydration when water intake equals water losses and in a state of hypohydration or hyperhydration when body water is decreased or increased respectively. In temperate conditions, euhydration is maintained within ± 0.22% of body weight whereas in the heat and during exercise this is increased to ± 0.48% of body weight (Greenleaf, 1992).

Water balance is achieved by water intake matching water losses. The main avenues of water loss are through urine and sweat losses with relatively small volumes of water being lost in expired air and faeces. During exercise, the main avenue for water loss is normally sweat production due to the need to dissipate metabolic heat (Maughan and Burke, 2002).

Water intake is largely controlled by the sensation of thirst but this seems to be a relatively poor indicator of hydration status following acute deviations in levels of body water (Greenleaf, 1992). The mechanisms behind the sensation of thirst and subsequent fluid consumption are complex. Brain centres in the pons and medulla respond to deviations in plasma osmolality of as little as 2-3% as well as changes in blood volume and pressure leading to changes in the subjective feeling of thirst. Consequently, the thirst response is regulated by similar mechanisms to those that

regulate water and electrolyte re-absorption (Greenleaf, 1992; Maughan and Shirreffs, 2003).

Adolph *et al.* (1954) described a multiple-factor theory of thirst that involves numerous factors, including changes in serum osmolality and changes in plasma volume, acting in concert to stimulate thirst and subsequent fluid intake. It has been shown that following a 37 hour period of fluid restriction, subjects ingested a volume of fluid in the following 20 minutes that was sufficient to restore only 37 ± 16% of their body mass losses when given unlimited access to a variety of drinks (Shirreffs *et al.* 2004). In addition, Rolls *et al.* 1980 observed that 65% of subjects total fluid intake during a 20 minute period following 24 hours of fluid restriction was consumed during the first 2 and a half minutes and that subjective feelings of thirst were alleviated before a significant amount of fluid absorption occurred. From these results, it would seem that the sensation of thirst is not always a sufficient stimulus to replenish fluid losses following deviations in body water content.

Following deviations in body water content, the renal system acts quickly to maintain body water levels by increasing or decreasing urine production as required. Arginine vasopressin (AVP) is a hormone secreted from the posterior pituitary that acts on the collecting ducts of the kidney. An increase in circulating concentrations of AVP, as is seen in situations of hypohydration, results in relatively low urine volumes due to increased water retention.

A close correlation between plasma osmolality and plasma AVP exists. Nose *et al.* (1988a) observed that ingestion of a large volume of plain water resulted in rapid acute decreases in plasma osmolality and, consequently, an acute decrease in circulating AVP concentrations that stimulated urine production. Linear regression analysis of data obtained using radioimmunoassay measurements of plasma AVP concentrations have shown that a change in plasma osmolality of 1 mosm kg^{-1} results in an average change in plasma AVP concentration of 0.41 pmol L^{-1} (Baylis, 1987). Similarly, Robertson (1974) reported that a change in plasma osmolality of approximately 3 mosm kg^{-1} results in a change in plasma AVP concentration of approximately 1 pg ml^{-1} and that this magnitude of change in plasma AVP results in a change in urine osmolality of approximately 250 mosm kg^{-1}. AVP secretion seems to

be blunted at plasma osmolalities of approximately 280 mosm kg^{-1} (Baylis, 1987; Robertson, 1974) and may be the reason why excessive fluid consumption can lead to water intoxication and/or hyponatremia (Noakes *et al.* 1985).

The extent of hypovolemia and/or hypotension appears to have an effect on circulating levels of AVP with changes in blood volume of 8-10% resulting in changes in secretion of AVP of approximately 1-2 pmol L^{-1} (Baylis, 1987). It would seem that plasma osmolality is a more potent stimulator of AVP secretion than hypovolemia (Robertson and Athar, 1976) and that the avoidance of large fluctuations in plasma osmolality and, therefore, circulating AVP concentrations are essential for the maintenance of body water content.

Aldosterone is a steroid hormone that is synthesised in the zona glomerulosa cells and released from the adrenal cortex (Pocock and Richards, 1999). Its main function is the reabsorption of sodium and, in association with AVP, water in the distal segment of the nephron. The release of aldosterone is governed by a number of factors including the renin-angiotensin system and a reduction in extracellular fluid volume (Briggs *et al.* 1990). Aldosterone has also been shown to increase sodium reabsorption in the sweat gland (Ladell and Shephard, 1961; Sato and Dobson, 1970). Aldosterone synthesis and release is linearly related to the degree of hypohydration induced (Francesconi *et al.* 1985) and the exercise intensity undertaken (Freund *et al.* 1991). It is also clear that hypohydration and the exercise intensity have an additive effect on aldosterone release (Montain *et al.* 1997).

Atrial natriuretic peptide (ANP) is secreted by atrial myocytes in response to high cardiac filling pressures and, therefore, secretion is increased during the transition from rest to exercise and is proportional to exercise intensity (Freund *et al.* 1991). ANP acts in contrast to aldosterone by increasing renal excretion of water and salt (Pocock and Richards, 1999). During exercise, the combined action of AVP and aldosterone ensure that a diuresis does not occur despite observed increases in ANP (Freund *et al.* 1991).

Effect of dehydration on exercise performance

The loss of body water that occurs during prolonged exercise is at least partly responsible for the progressive development of fatigue that eventually leads to cessation of exercise in the heat.

Astrand & Saltin (1964) observed that cross country skiers dehydrated by 5.5% of their initial body mass following an 85km race showed a marked decrease in total work output after the race. In addition, Saltin (1964) showed that dehydration amounting to 5.2% of initial body mass resulted in a decrease in stroke volume and increase in heart rate, relative to the euhydrated state, during submaximal exercise. Although there was no change in circulatory response after dehydration during a maximal exercise test, there was a marked decrease in work time. However, although these studies will have induced a significant degree of dehydration, the observations can not solely be attributed to reductions in body water content as muscle glycogen will have been depleted and will have contributed to the reduction in physical capacity (McConell *et al.* 1999).

Investigations that have examined the effect of dehydration on exercise performance have used a number of techniques to induce a reduction in body water content. These techniques include exercise (Gonzalez-Alonso *et al.* 1997; Gonzalez-Alonso *et al.* 1998; Buono and Wall, 2000), diuretics (Armstrong *et al.* 1985; Roy *et al.* 2000), heat exposure (Buskirk *et al.* 1958; Saltin, 1964) and food and/or fluid restriction (Oliver *et al.* 2007). Caldwell *et al.* (1984) examined the physiological effects of sauna, diuretic and exercise induced dehydration on maximal and submaximal work capacity and concluded that the method of weight loss may have an effect on physical performance.

Armstrong *et al* (1985) examined the effect of dehydration, induced by administration of a diuretic (40 mg of furosemide), on 1500, 5000 and 10000 m running performance. Dehydration amounted to a level of 1.9, 1.6 and 2.1% for each of the distances examined. Running time significantly increased by 0.16, 1.31 and 2.62 minutes respectively following dehydration compared to euhydrated performance.

This equates to a decrease in running velocity of 3.1, 6.7 and 3.1 % respectively with dehydration.

Gonzalez-Alonso *et al* (1997) observed that superimposing dehydration amounting to 4% body mass on 15 hyperthermic cyclists (core temperature = 39.3 °C) in the heat resulted in a reduced ability to maintain cardiac output and blood pressure by reducing stroke volume to a greater extent than hyperthermia or dehydration alone. Similarly, Roy *et al.* (2000) reported that diuretic induced dehydration resulted in a reduced cardiac output and stroke volume during exercise when compared with a control trial. These effects of dehydration on cardiovascular function are likely to be due to a reduction in plasma volume that has been repeatedly observed following dehydration protocols (Saltin, 1964; Armstrong *et al.*, 1985; Nielsen *et al.*, 1986; Gonzalez-Alonso *et al.*, 1997; Roy *et al.* 2000).

Gonzalez-Alonso *et al.* (1998) reported that dehydration, induced by exercise in the heat, resulted in a reduction in blood flow to the exercising muscles while also decreasing blood flow to the forearm. Similarly, Buono and Wall (2000) observed that dehydration amounting to 5% of initial body mass resulted in an increase in core temperature during subsequent exercise in ambient temperatures of 23 °C and 33 °C. In addition, sweat rate and forearm blood flow were significantly reduced during exercise in the heat compared to temperate conditions. The results of these studies suggest that dehydration causes an impairment in thermoregulation by reducing heat transfer to the periphery and that this is exacerbated during exercise in hot conditions. Gonzalez-Alonso *et al.* (1999) reported that time to exhaustion during exercise in the heat was inversely related to initial rectal temperature.

The avoidance of a significant reduction in body water is, therefore, sometimes critical during exercise however, as reductions in exercise capacity are unlikely to occur until a reduction in body mass of approximately 2% of body mass is achieved (Cheuvront *et al.* 2003; Coyle, 2004) reductions in body water content may, in some cases, be beneficial. Ebert *et al.* (2007) observed that reducing body mass by approximately 2% resulted in a reduction in the power output required to perform a given speed however, time to exhaustion during an uphill cycle exercise task was significantly reduced when dehydrated. Further research is required to establish

whether the reduction in power output required to perform a given speed following reductions in body water content may be of benefit in both short duration high intensity and prolonged endurance exercise.

The restoration and maintenance of fluid balance following cessation of exercise is also necessary, particularly when a further bout of exercise is to be performed. As little absorption of fluid and nutrients occurs while in the stomach (Maughan, 1991), the rate of gastric emptying and the rate of intestinal absorption are important considerations when formulating rehydration solutions.

Anatomy of the digestive tract

The digestive tract is essentially a long tube running from the mouth to the anus and consists of the oral cavity, pharynx, oesophagus, stomach, small intestine, large intestine, rectum, anal canal and the anus. The gastrointestinal tract refers to the stomach and the intestines (Pocock and Richards, 1999).

The main purpose of the stomach is the mixing and digestion of foods. Very little absorption of food or fluid occurs within the stomach. The oesophagus opens into the stomach at the oesophageal opening. The three main structures in the stomach are the fundus, the body and the pyloric region. The stomach opens into the small intestine at the pyloric opening and secretion into the small intestine is dependent on the opening and closing of the pyloric sphincter (Pocock and Richards, 1999).

The small intestine consists of the duodenum, jejunum and the ileum. The duodenum is approximately 25 cm long and joins the jejunum at the duodenojejunal flexure. The majority of absorption occurs in the small intestine however, any unabsorbed material is passed into the large intestine and most will be excreted.

Gastric emptying

Measurement of gastric emptying

A number of techniques are available for measuring total volume of fluid in the stomach and, therefore, the rate at which fluid is emptied from the stomach into the small intestine. These techniques include gastric aspiration, scintigraphy, ultrasound, magnetic resonance imaging, epigastric impedance, and stable isotopic tracers such as ^{13}C (Leiper and Maughan, 1988; Maughan and Leiper, 1990).

Scintigraphy involves the use of radioisotopes and subsequent recording of the distribution of radioactivity using images collected using a gamma camera (Harris *et al.* 1987). Similarly, ultrasound (Bateman and Whittingham, 1982) and magnetic resonance imaging (Schwizer *et al.* 1992) involve collecting images of the stomach and the rate of gastric emptying can be estimated as a result. Epigastric impedance involves the administration of a low current through the abdomen. A decline in the impedance of the current follows as fluid is emptied from the stomach (McClelland and Sutton, 1985). Administration of ^{13}C and subsequent time to ^{13}C enrichment has also been used as a measure of the rate of gastric emptying (van Nieuwenhoven *et al.* 2000). These techniques are not truly quantitative as they do not allow measurement of gastric secretions (Maughan and Leiper, 1990). In addition, they require technical expertise and can be costly procedures.

Most investigations that have examined the rate of gastric emptying of fluids have used a gastric aspiration technique that involves the positioning of a thin flexible tube at the base of the stomach before aspiration of the residual stomach contents. After administration of the test solution, the volume of fluid remaining in the stomach at a given time point can be determined using the serial test meal technique of Hunt and Spurrell (1951) or the double sampling technique of George (1968).

The serial test meal technique involves aspiration of the stomach contents at a fixed time following ingestion of the test solution. The volume of fluid emptied into the intestine over the time period is taken as the difference between the volume of test meal administered and the volume of fluid aspirated at the end of the trial. The

procedure is repeated on subsequent days with different time intervals so that the pattern of gastric emptying of a given solution can be determined.

The double sampling technique involves aspiration of a small volume of the gastric contents (~5 ml) before addition of a known volume and concentration of a non-absorbable dye. The dye is then mixed with the gastric contents and a further sample obtained. The total volume present in the stomach at this time point is then calculated from changes in the concentration of the dye in the aspirated gastric samples. Beckers *et al.* (1988) outlined a simple calculation that determines the volume of gastric secretions added to the original test meal. The double sampling technique appears to be a reliable measure of total gastric and secretion volumes (Beckers *et al.* 1991).

Hunt and Spurrell (1951) noted the effect that stress and anxiety may have had on their observed rates of gastric emptying and, as such, it is important to realise that the invasive nature of the double sampling technique may alter gastric emptying patterns even though it is the recommended method of measurement of the rate of gastric emptying (Maughan and Leiper, 1999).

Factors affecting the rate of gastric emptying

A number of factors have been shown to influence the rate of fluid delivery to the small intestine. Of these, stomach volume, energy density, solution osmolality and exercise have been studied extensively. Other factors such as solution temperature and caffeine (Costill, 1990; Rehrer *et al.* 1990a) have also been investigated but will not be covered here as their influence on the rate of gastric emptying appears to be relatively small.

Volume of fluid in stomach

A number of investigators have observed an exponential decrease in the volume of fluid present in the stomach following ingestion of a test solution (Murray, 1987; Vist and Maughan, 1994; Vist and Maughan, 1995). Rehrer *et al.* (1989) observed that a constant percentage of a test solution was emptied at any given time interval

following ingestion of a single bolus during exercise at approximately 70% $\dot{V}O_2$max. This suggests that stomach volume plays a major role in the emptying of fluids into the small intestine.

An increase in stomach volume seems to have an impact on the rate of gastric emptying due to an elevated intragastric pressure that relaxes the gastric musculature which aids gastric motility (Costill and Saltin, 1974).

Rehrer *et al.* (1990b) observed that repeated ingestion of a 180 g L^{-1} carbohydrate solution during exercise resulted in a reduced fluid delivery to the intestine 40 minutes after the ingestion of an initial bolus of the test solution when gastric volumes were maintained between 600 and 250ml. In addition, Mitchell and Voss (1990) observed that repeated ingestion of large volumes of a 75 g L^{-1} carbohydrate solution during cycle exercise caused an increased prevalence of gastric discomfort. This suggests that the rate of gastric emptying is slowed in the presence of very large stomach volumes as well as the characteristics of the solution ingested.

Solution energy density

The provision of both fluid and carbohydrate during exercise has been shown to delay the onset of fatigue and result in an improvement in performance (Below *et al.* 1995). As such, the effect of high energy density solutions on the rate of gastric emptying has been the subject of a number of investigations.

Vist and Maughan (1994) investigated the rate of gastric emptying following ingestion of 600 ml of water, 20 g L^{-1}, 40 g L^{-1} and 60 g L^{-1} glucose solutions using a double sampling measurement technique. The addition of 20 g L^{-1} of glucose had no effect on the rate of gastric emptying when compared to water however after the first ten minutes, addition of 40 g L^{-1} and 60 g L^{-1} resulted in a delay in the rate of gastric emptying. This reduction in the rate of gastric emptying with an increase in energy content of an ingested solution is observed elsewhere (Costill and Saltin, 1974; Murray *et al.* 1999).

Simpson *et al.* (2001) examined the effect of ingesting 495 ml of water, a 20 g L^{-1} hypotonic carbohydrate solution, a 60 g L^{-1} hypotonic carbohydrate solution and a 60 g L^{-1} isotonic carbohydrate solution on the rate of gastric emptying. No difference in the rate of water delivery to the small intestine was observed following ingestion of the 20 g L^{-1} hypotonic carbohydrate solution when compared to water however the rate of gastric emptying was reduced during both 60 g L^{-1} carbohydrate trials. In addition, carbohydrate delivery to the small intestine was not different between the two 60 g L^{-1} carbohydrate solutions.

Solution osmolality

An increase in both the carbohydrate content and osmolality of a solution has been shown to result in a decrease in the rate of gastric emptying of a solution (Vist and Maughan. 1994; Costill and Saltin, 1974; Murray *et al.* 1999; Simpson *et al.* 2001). As solution osmolality generally increases with an increase in carbohydrate content, early investigations were unable to determine the relative contribution of solution energy density or solution osmolality to the reduction in the rate of gastric emptying.

Hunt and Pathak (1960) reported that a relationship existed between the osmolality of a test solution and the rate at which it empties from the stomach and that the relationship changed depending on the type of solute investigated. To explain these results, the authors proposed the existence of an osmoreceptor within the intestine that increases and decreases in size in response to hypotonic and hypertonic solutions respectively. Subsequent feedback from the osmoreceptor acts to regulate the rate of gastric emptying of the solution.

Vist and Maughan (1995) investigated the effect of ingesting 600 ml of a 40 g L^{-1} glucose monomer solution (osmolality of 230 ± 3 mosm kg^{-1}), a 40 g L^{-1} glucose polymer solution (osmolality of 42 ± 1 mosm kg^{-1}), a 188 g L^{-1} glucose monomer solution (osmolality of 1300 ± 4 mosm kg^{-1}) and a 188 g L^{-1} glucose polymer solution (osmolality of 237 ± 3 mosm kg^{-1}) on the rate of gastric emptying. Solutions were chosen so that the two 40 g L^{-1} were isoenergetic as were the two 188 g L^{-1} solutions. The half emptying time of the 188 g L^{-1} solution with an osmolality of 237 ± 3 mosm kg^{-1} was less than the half emptying of the 188 g L^{-1} solution with an osmolality of

1300 ± 4 mosm kg^{-1} and both were slower than the two 40 g L^{-1} solutions. The results of this study suggested that, although solution osmolality did have an effect on the rate of gastric emptying, the greater energy density of the 188 g L^{-1} solutions was the most important factor that contributed to the reduction in the rate of gastric emptying.

Brouns *et al.* (1995) observed that ingesting six carbohydrate solutions with the same energy content (60g L^{-1}) but a range of osmolarities from 243 – 374 mosm kg^{-1} did not result in any differences in the rate of gastric emptying. In the same study, a significant negative relationship between solution energy density and the rate of gastric emptying was observed following ingestion of six carbohydrate solutions with the same osmolarity but different carbohydrate concentrations. The finding that solution osmolality has little effect on the rate of gastric emptying is supported by the results of Simpson *et al.* (2002) who observed that the gastric emptying rates of four 64 g L^{-1} carbohydrate monomer and/or polymer solutions with osmolalities ranging from 25 – 390 mosm kg^{-1} was the same.

Exercise

As the rate of gastric emptying of a solution will have a major impact on the rate at which fluid is absorbed into the body and therefore the avoidance of dehydration during exercise, the effect of exercise on gastric emptying characteristics has been investigated frequently.

Costill and Saltin (1974) observed that the rate of gastric emptying of a 100 g L^{-1} glucose solution was the same during cycle exercise at 40-70% of maximal aerobic power however, the rate of gastric emptying of the same solution was reduced when exercise intensity exceeded 70% of maximal aerobic power.

Rehrer *et al.* (1989) examined the effect of exercise intensities of 50 and 70% $\dot{V}O_{2max}$ on the rate of gastric emptying of sweetened water, a 150 g L^{-1} glucose solution, a 150 g L^{-1} maltodextrin solution with 30 g L^{-1} fructose and a 70 g L^{-1} sucrose solution. Although a trend towards a reduced rate of gastric emptying of the carbohydrate solutions in the initial stages of both exercise intensities was observed compared to

rest, no significant differences in the rate of gastric emptying of any solution compared to a resting trial was reported.

Leiper *et al.* (2001) examined the effect of intermittent high intensity exercise at an average power output of 75% $\dot{V}O_{2max}$ and the effect of steady state exercise at an average power output of 66% $\dot{V}O_{2max}$ on the gastric emptying rate of a 60 g L^{-1} carbohydrate-electrolyte solution. No difference in the rate of gastric emptying of the solution was observed between rest and exercise at 66% $\dot{V}O_{2max}$ however, the rate of gastric emptying was significantly reduced during exercise at 75% $\dot{V}O_{2max}$ compared to exercise at 66% $\dot{V}O_{2max}$ and rest.

The results of these studies suggest that exercise does not significantly alter the gastric emptying characteristics of ingested solutions until an intensity of 70-75% $\dot{V}O_{2max}$ is reached.

Neufer *et al.* (1986) observed that the rate of gastric emptying was enhanced during running when compared to rest due to the increased mechanical movement of fluid. However, Rehrer *et al* (1990b) and Houmard *et al.* (1991) both observed no differences in the rate of gastric emptying of an ingested solution between running and cycling. Further investigation is necessary to establish whether the mode of exercise does influence the rate of gastric emptying as comparisons between studies that have employed different modes of exercise may be limited.

Hypohydration

van Nieuwenhoven *et al.* (2000) examined the effect of dehydration amounting to 3% body mass induced by heat exposure on the rate of gastric emptying of a 6.88 g L^{-1} carbohydrate-electrolyte solution during exercise at 70% W_{max}. The results of this study showed that the rate of gastric emptying was reduced when subjects were dehydrated compared to when they were euhydrated, as time to peak ^{13}C enrichment in a breath sample was approximately 6.5 minutes longer following heat exposure. In contrast, Ryan *et al.* (1998) reported that the rate of gastric emptying of a water placebo, 60 g L^{-1} carbohydrate-electrolyte solution and a 90 g L^{-1} carbohydrate-

electrolyte solution ingested during cycle exercise at 65% $\dot{V}O_{2max}$ was unaffected by dehydration amounting to approximately 3% body mass induced by low intensity exercise in the heat. These studies differed not only in the exercise intensity but also the method of dehydration employed which is a possible reason for the conflicting results reported.

Rehrer *et al.* (1990) observed that dehydration amounting to 4% body mass resulted in a reduction in the rate of gastric emptying of a 70 g L^{-1} carbohydrate-electrolyte solution compared to when euhydrated. Similarly, Neufer *et al.* (1989) reported that dehydration resulting in a reduction in body mass of approximately 5% reduced the rate of gastric emptying of water during treadmill exercise in relatively warm conditions. The results of these studies would seem to suggest that the extent of dehydration has a major impact on the gastric emptying characteristics of an ingested solution.

In addition, the results of the investigation by Neufer *et al.* (1989) showed a correlation between the rate of gastric emptying and the increase in rectal temperature observed during treadmill exercise in an ambient temperature of 35°C. Bowen and Shirreffs (2001) reported that an increase in rectal temperature of approximately 0.6°C induced by immersion in water heated to approximately 42°C had no effect on the rate of gastric emptying of a 60 g L^{-1} carbohydrate-electrolyte solution when compared to immersion in cold water at a temperature of approximately 24°C that induced a reduction in rectal temperature of approximately 0.5°C. The results of these studies suggest that not only the extent of dehydration but also the extent of heat stress affects the gastric emptying characteristics of an ingested solution.

Intestinal absorption

Measurement of intestinal absorption

Most investigations that have measured intestinal absorption have used perfusion techniques. This involves subjects being intubated with a multi-lumen perfusion set in the proximal small intestine. Test solutions, containing a poorly absorbed marker, are

then perfused at a fixed rate and the difference between this amount and the amount of test solution recovered further along the intestine is used to calculate net absorption. At a steady state, the differences in the concentration of the marker that is present in the test solution and the intestinal lumen can be used to calculate the net transport of water from the test solution (Leiper and Maughan, 1988; Leiper, 1998). Studies that involve subjects being intubated in the duodenojejunum generally involve an infusion port being positioned 5-10 cm beyond the pyloric sphincter, a proximal sampling site 10 cm distal to the infusion port which is, therefore, still in the duodenum and a distal sampling site in the jejunum (Gisolfi *et al.* 1990).

Although perfusion techniques provide relatively accurate means of measuring the rate of intestinal absorption of test solutions, the pattern of absorption is likely to be different if the test solutions are ingested orally. Perfusion of a solution only gives a measure of fluid absorption within the intestine and does not take into account the rate of gastric emptying of a test solution or any changes in the characteristics of a test solution that may occur before delivery to the small intestine, both of which will have an impact on the rate of total fluid uptake. In addition, measurements of water absorption when using a perfusion set depend on the location of the set and the length of the test segment that is examined (Shi, 1994).

The addition of deuterium oxide (D_2O) to orally ingested fluids and the subsequent accumulation rate of deuterium (^2H) in the blood has been proposed as an alternative measure of total fluid uptake of a test solution. This has the advantages of being non-invasive as well as giving a measure of the combined effects of the rate of gastric emptying and intestinal absorption on the fate of an ingested solution.

To examine whether the accumulation of ^2H provided an accurate measurement of intestinal fluid uptake compared to intestinal perfusion techniques, Gisolfi *et al.* (1990) examined the accumulation rate of ^2H in the plasma following perfusion of labelled distilled water, a 60 g L^{-1} carbohydrate-electrolyte solution and a 100 g L^{-1} glucose solution directly into the duedonojejenum. Perfusion of distilled water and the 60 g L^{-1} carbohydrate-electrolyte solution resulted in net water uptake whereas perfusion of the 100 g L^{-1} glucose solution resulted in net water secretion. The ^2H accumulation rate was independent of the drink tested suggesting that D_2O labelled

solutions are not indicative of net fluid movement within the intestine as intestinal absorption is a bi-directional process. However, Davis *et al.* (1987) reported that the accumulation rate of ^{2}H following ingestion of five labelled solutions was as expected given their known gastric emptying and intestinal absorption characteristics. Gisolfi *et al.* (1992a) reported that drinks labelled with D_2O resulted in similar results in terms of net water flux as did perfusion into the small intestine. This suggests that the use of D_2O as an estimation of the total fluid uptake of an ingested solution is a valid technique however, it does not give any accurate measurement of the reason behind any differences observed between ingested solutions.

Factors affecting the rate of intestinal absorption

The movement of water within the small intestine is a passive bi-directional process that depends largely on the osmotic gradient that is established between the extracellular fluid and the contents of the intestinal lumen. As such, the characteristics of a test solution play a major role in the movement of water between the small intestine and the extracellular fluid. These factors include the type of carbohydrate, the osmolality and the sodium content of a test solution.

Type of carbohydrate

Carbohydrates are almost invariably absorbed as monosaccharides, therefore, a number of investigations have been carried out to examine whether any difference in the rate of water absorption can be observed following infusion of solutions consisting of different types of carbohydrate.

Both glucose and fructose are transported into the extracellular fluid by carrier-mediated processes located in the brush border membrane. However, fructose is transported by a different carrier to that of glucose. It has been shown that glucose stimulates a greater amount of water and electrolyte uptake than fructose (Fordtran, 1975) and sucrose (Wheeler *et al.* 1986). However, it has been postulated that a mixture of carbohydrates in a solution may enhance the overall rate of water absorption due to the stimulation of different carrier mechanisms within the brush border membrane.

Gisolfi *et al.* (1992b) observed that altering the form of carbohydrate by the addition of maltodextrins, fructose and sucrose to a test solution, had little effect on the absorption of water when perfused directly into the duodenojejenum up to a carbohydrate concentration of 60 g L^{-1}. However, an 80 g L^{-1} carbohydrate solution containing a maltodextrin or sucrose resulted in an increased rate of water absorption when compared to an 80 g L^{-1} carbohydrate solution consisting of glucose or corn syrup solid. Similarly, Shi *et al.* (1995) observed that solutions consisting of a mixture of substrates resulted in greater water absorption than a solution consisting of only one substrate. This was attributed to the stimulation of more transport mechanisms with the addition of more than one transportable substrate.

However, in contrast, Leiper *et al.* (1996) reported that altering the type of carbohydrate present in moderately hypotonic carbohydrate-electrolyte solutions by the addition of sucrose and fructose to a test solution had little effect on the absorption of water following perfusion into the jejunum.

The results of these studies suggest that the type of carbohydrate present in a test solution does have an effect on the extent of water uptake in the intestine, especially at high overall carbohydrate concentrations.

Solution osmolality

Plasma osmolality is normally 280 – 290 mosm kg^{-1} (Geigy Scientific Tables, 1962). As intestinal absorption is a passive process that is governed by local osmotic gradients. If the contents of the intestinal lumen are < 280 mosm kg^{-1} this will lead to net water absorption. Similarly, if the contents of the intestinal lumen are greater than that of the plasma, net water secretion will occur.

Wapnir and Lifshitz (1985) studied a range of solution osmolalities and their effects on intestinal water uptake in rats. A significant negative correlation was observed between luminal osmolality and the rate of intestinal absorption. The same investigation suggested that solutions with an osmolality of 190 – 200 mosm kg^{-1} resulted in the fastest rate of water absorption.

A study in resting human subjects by Leiper and Maughan (1986) showed that perfusion of a solution with an osmolality of 488 ± 53 mosm kg^{-1} in the jejunum resulted in net water and electrolyte secretion into the intestinal lumen whereas an isotonic carbohydrate-electrolyte solution and water resulted in net water absorption. Similarly, Shi *et al.* (1994) observed that perfusion of a hypotonic solution resulted in a greater amount of fluid absorption than a hypertonic solution.

Merson *et al.* (2002) observed that ingestion of a hypertonic 120 g L^{-1} glucose solution resulted in a lower plasma volume, calculated from haemoglobin concentration and haematocrit values, when compared to a hypotonic 20 g L^{-1} glucose solution. This suggests that the net secretion of water into the intestinal lumen that occurs following ingestion of hypertonic solutions may have a measurable effect on circulating plasma and extracellular fluid volume.

Sodium

The addition of sodium to a rehydration solution has been considered to enhance the rate of intestinal absorbance due to its role in active co-transport with carbohydrates (Schedl and Clifton, 1963). However, a study by Gisolfi *et al.* (2001) observed that ingestion of a hypotonic 60 g L^{-1} carbohydrate solution containing 50 mmol L^{-1} sodium during exercise did not enhance the rate of water absorption compared to an isotonic 60 g L^{-1} carbohydrate solution or a hypotonic carbohydrate solution containing no sodium. In contrast, it has been shown using deuterium tracer techniques that net rate of fluid absorption is greater following ingestion of a glucose-sodium solution compared to a glucose-sodium free solution and a fructose-sodium solution (Leiper, 1998). The conflicting observations between these studies may be due to differences in experimental design but, currently, the role of sodium in promoting water absorption within the intestine is unclear.

Restoration of fluid balance

Strategies to restore and maintain whole body fluid balance following a period of dehydration have received much attention in recent years. Most studies have

investigated the volume and composition of a solution and their effectiveness on restoring and maintaining whole body fluid balance.

Drink volume

Following a period of exercise-induced dehydration, water continues to be lost from the body due to the need for obligatory urine losses to eliminate waste products as well as continued sweat loss and water lost during respiration. In order to effectively return to a positive state of whole body fluid balance following exercise-induced dehydration, a drink volume greater than sweat loss during exercise should be ingested. Shirreffs *et al.* (1996) dehydrated subjects via intermittent exercise in the heat to a level of approximately 2% body mass before subjects consumed a drink volume that amounted to 50, 100, 150 or 200% of the sweat loss during exercise. A state of euhydration was only maintained when a drink volume amounting to greater than the volume of sweat loss during exercise was consumed. In the same study, a drink with a high sodium content (61 mmol L^{-1}) ensured that subjects remained in a state of euhydration for the entire six hour recovery period whereas when a drink with a low sodium content (23 mmol L^{-1}) was consumed subjects were hypohydrated by the end of the recovery period. The results of this study demonstrate the need for a drink volume greater than sweat loss during exercise and an interaction between drink volume, drink sodium content and whole body rehydration. Mitchell *et al.* (1994) also observed that the extent of rehydration was improved as a result of drinking a volume equivalent to 150% body mass lost compared to a drink volume equivalent to 100% body mass during a three hour rehydration period following exercise that resulted in a reduction in body mass of approximately 2.9%.

The current American College of Sports Medicine (ACSM) (2007) position stand states that if rapid fluid replacement is required following cessation of exercise then an individual should consume approximately 1.5 L of fluid for each kilogram of body mass lost during exercise.

Rate of fluid ingestion

The rate of fluid intake during the rehydration period is an important factor when considering post-exercise rehydration. Archer & Shirreffs (2001) dehydrated subjects via intermittent exercise in the heat to a level of approximately 2% body mass before subjects consumed a volume of sports drink equivalent to 150% body mass lost during exercise over a period of either 30 minutes or 90 minutes. Cumulative urine output was significantly greater when the volume was ingested over a period of 30 minutes relative to when the same volume was consumed over a period of 90 minutes. This resulted in subjects being more hypohydrated at the end of a four hour recovery period during the fast rate of ingestion trial relative to the slow rate of ingestion. Kovacs *et al.* (2002) also observed that urine output during the initial two hours of a recovery period was greater following ingestion of a drink volume amounting to 120% body mass lost during exercise when a fast rate of fluid ingestion was employed compared to a low rate of fluid ingestion.

Ad libitum fluid ingestion

Most studies that have been performed in the area of post-exercise rehydration have employed a drink volume equivalent to a percentage of body mass lost during exercise due to the highly variable nature of voluntary fluid ingestion. Consequently, there is little evidence available on *ad libitum* fluid ingestion during the post-exercise rehydration period.

Wemple *et al.* (1997) investigated the effects of *ad libitum* ingestion of artificially sweetened water, a flavoured 60 g L^{-1} sucrose drink containing 25 mmol L^{-1} NaCl and a flavoured 60 g L^{-1} sucrose drink containing 50 mmol L^{-1} NaCl over a three hour period preceded by 90 minutes treadmill exercise in the heat. Total fluid intake amounted to 123 ± 16, 130 ± 19 and 105 ± 15 % body mass lost for the flavoured water, low sodium and high sodium drinks respectively. The low sodium drink was more effective in restoring whole body water levels than both the sweetened water and high sodium drinks. Brouns *et al.* (1996) observed that subjects ingested a significantly greater volume of an isotonic carbohydrate electrolyte solutions with high sodium content (32 mmol L^{-1}) than mineral water and that this resulted in greater

restoration of estimated plasma volume and whole body fluid balance. The volume of a caffeinated soft drink ingested during this investigation was not different from either of the other solutions and did not result in any difference in whole body fluid balance relative to the isotonic carbohydrate electrolyte solution or the high sodium solution. Maughan and Leiper (1993) observed that subjects ingested significantly greater volumes of a sports drink and an orange/lemonade drink than a 16.2 g L^{-1} glucose solution and a high sodium and potassium solution during a two hour *ad libitum* fluid ingestion period following exercise induced dehydration. Despite the greater total fluid intake during the sports drink and orange/lemonade trials, there was no difference in whole body fluid balance 12 hours after the recovery. The taste of the sports drink and orange/lemonade drink was perceived to be better than the glucose and electrolyte drinks. These studies highlight the importance of taste preference and electrolyte content of solutions in situations of *ad libitum* fluid ingestion.

Drink composition

It has been well established that plain water is not the most effective rehydration solution as its ingestion results in large volumes of urine being produced leading to subjects becoming hypohydrated after a relatively short period of time (Costill & Sparks, 1973; Nielsen *et al.* 1986; Gonzalez-Alonso *et al.* 1992). Ingestion of large volumes of water results in a rapid decrease in plasma osmolality and sodium concentrations (Nose *et al.* 1988a; Nose *et al.* 1988b) and, consequently, a return to pre-dehydration concentrations of circulating arginine vasopressin and aldosterone (Nose *et al.* 1988b). This results in a reduced dipsogenic stimulus and increased urine formation. Both of these consequences are considered undesirable during the post-exercise rehydration period. Many of the studies performed in the area of post-exercise rehydration have focused on the electrolyte content of a rehydration solution and, to a lesser extent, the carbohydrate content.

Electrolyte content

The addition of sodium to a rehydration solution is justified due to its role in maintaining plasma osmolality and plasma sodium concentrations (Nose *et al.* 1988a). In addition, sodium may increase the net rate of water absorption in the intestine

(Schedl and Clifton, 1963; Leiper, 1998) via active co-transport but the exact role of sodium on intestinal water absorption is currently unclear (Gisolfi *et al.* 2001).

Sodium is the main ion in the extracellular fluid and is, therefore, the main electrolyte lost in sweat. It has been proposed that rehydration solutions should have a sodium content similar to that of the sweat produced during exercise (Maughan, 1991) however, the electrolyte content of sweat is highly variable and can range from 20 – 60 mmol L^{-1} (Verde, 1982) meaning it is unlikely that a rehydration solution could be produced that would meet the needs of every individual.

Shirreffs & Maughan (1998a) dehydrated subjects to a level of approximately 2% body mass by intermittent exercise in the heat before subjects consumed a volume of fluid amounting to 150% body mass lost containing approximately 0, 25, 50 or 100 mmol L^{-1} sodium. Six hours after the end of the rehydration period, subjects were still in a state of euhydration during the 100 mmol L^{-1} sodium trial but had returned to a state of hypohydration in all other trials. However, subjects excreted large quantities of potassium during the 100 mmol L^{-1} sodium trial despite relatively small potassium ingestion during the rehydration period, meaning that although the high sodium drink resulted in significantly greater water retention than the other trials its effect on body potassium levels was undesirable for long term situations. Mitchell *et al.* (2000) also studied the role of sodium during post-exercise rehydration following exercise in the heat that elicited a level of dehydration equivalent to 2.9% body mass. Subjects were then given a high (50 mmol L^{-1}) or low (25 mmol L^{-1}) sodium drink with a drink volume equivalent to either 100 or 150% body mass lost over a period of 180 minutes. A greater level of rehydration was achieved when the greater drink volume was consumed but no differences in rehydration were seen when a higher sodium content was added to either drink volumes. However, this is likely to be due to both the rate of fluid ingestion as well as the duration of the recovery period. The length of time that the subjects were observed in this study may not have been sufficient to ascertain the extent of urine production during each trial.

The current American College of Sports Medicine (ACSM) (2007) position stand on fluid replacement after exercise suggests that drinks or snacks should contain sodium due to its role in fluid retention and the stimulation of thirst.

Potassium is the major ion in the intracellular fluid and it has been suggested that the addition of potassium to a solution may aid rehydration by increasing the amount of fluid retained in the intracellular space. Yawata *et al.* (1990) compared the effectiveness of *ad libitum* ingestion of a potassium solution against a sodium solution and tap water in rats thermally dehydrated to a level of 9% body mass. The volume of fluid ingested was greater during the sodium trial compared to the potassium trial and urine output was only slightly greater in the sodium trial, resulting in the sodium solution being the most effective rehydration solution.

Maughan *et al.* 1994 investigated the effectiveness of a glucose drink (16.2 g L^{-1}), a sodium drink (60 mmol L^{-1} NaCl) and potassium drink (25 mmol L^{-1} KCl) and a drink containing all three constituents following a period of exercise that resulted in a level of approximately 2% dehydration in human subjects. All drinks were consumed in a volume equivalent to 100% body mass lost. Total urine production was less when electrolytes were added to the drink compared with the electrolyte free drink but the fraction of ingested fluid retained after six hours was not different between any of the electrolyte trials. As the volume of fluid ingested was equal to body mass lost during exercise, subjects will have been hypohydrated throughout all the trials and this will have affected the volume of urine produced during the later stages of the trials. Shirreffs *et al.* (2007) examined the effectiveness of a sports drink (6 ± 1 mmol L^{-1} potassium), a carbonated water/apple juice mixture (30 ± 1 mmol L^{-1} potassium) and two different brands of mineral water on rehydration after exercise that reduced body mass by approximately 2%. Drink volume was equivalent to 150% body mass lost during exercise. Four hours after the rehydration period subjects were in negative fluid balance during the carbonated water/apple juice mixture and the mineral water trials but were essentially euhydrated during the sports drink trial. The results of this study suggest that drinks with high potassium content do not result in a greater amount of water retention.

Carbohydrate content

The primary aim of a rehydration solution is to restore body water content however, a secondary aim may be to restore muscle glycogen levels that will have been depleted during prolonged exercise. This has led to the hypothesis that rehydration solutions

should be moderately hypotonic and contain relatively small amounts of carbohydrate (Galloway, 1999; Leiper, 1998).

Gonzalez-Alonso *et al.* (1992) observed that a 60 g L^{-1} carbohydrate-electrolyte beverage was more effective than diet cola or water in terms of restoration of whole body fluid balance following a 2 hour rehydration period during which time 100% body mass lost was consumed. Nielsen *et al.* (1986) reported that ingestion of 2700 ml of a commercially available sports drink with an osmolality of approximately 465 mosm kg^{-1} over a period of 2 hours resulted in a similar volume of urine being produced at the end of the rehydration period as when a high sodium drink, a high potassium drink and a control drink were consumed.

Maughan *et al.* (1994) observed that ingestion of a 16.2 g L^{-1} glucose drink with an osmolality of approximately 120 mosm kg^{-1} resulted in significantly greater urine production than a 60 mmol L^{-1} sodium solution, a 25 mmol L^{-1} potassium solution and a solution containing all three constituents when a drink volume equal to body mass lost during exercise was consumed over a period of one hour.

It has been hypothesised that the secretion of water into the intestinal lumen that occurs if the contents of the lumen are more concentrated than the extracellular fluid (Leiper and Maughan, 1986) renders hypertonic solutions ineffective rehydration beverages (Leiper, 1998). During exercise, plasma volume expansion is likely to be advantageous due to its role in maintaining cardiovascular function and effective thermoregulation however, in the post-exercise rehydration period excessive plasma volume expansion may be a disadvantage as this will result in reductions in circulating levels of arginine vasopressin (Baylis, 1987) and cause water loss due to an increased production of urine.

Costill and Sparks (1973) observed that ingestion of a 100 g L^{-1} glucose solution with an osmolality of 444 mosm kg^{-1} was more effective than de-mineralized water in restoring whole body fluid balance following a reduction in body mass of approximately 4% via heat exposure although neither solution returned subjects to pre-dehydration levels as a drink volume equal to body mass lost was employed. Similarly, Lambert *et al.* (1992) reported that a 100 g L^{-1} glucose-fructose solution

with an osmolality of 648 mosm kg^{-1} was as effective as non-carbohydrate solutions in restoring fluid balance following exercise that induced a reduction in body mass of approximately 4%. Drink volume in this study again amounted to that of body mass lost during exercise and was ingested over a period of four hours which may explain the lack of significant differences observed between trials.

The role of carbohydrate during post-exercise rehydration has not been extensively studied and further research needs to be performed to enhance knowledge of this area.

Protein

Seifert *et al.* (2006) examined the effectiveness of adding 15 g L^{-1} protein to a 60 g L^{-1} carbohydrate-electrolyte solution on restoration of fluid balance following exercise that reduced body mass by approximately 2.5%. It was reported that the addition of protein to a carbohydrate drink resulted in improved fluid retention over a subsequent three hour period than a carbohydrate-electrolyte drink or water. The reason given for the improved water retention were an enhancement of water and electrolyte absorption in the presence of protein due to amino acids being absorbed by multiple transport systems. However, in this study a relatively large volume of the test drink (100% body mass lost during exercise) was consumed over a relatively short period (20 minutes). Further research needs to be performed to establish whether the addition of protein to a rehydration solution does enhance fluid retention and the mechanisms behind why this may occur.

Aims of this thesis

The aims of this thesis were to assess fluid movement and availability following ingestion of different glucose solutions and, consequently, the contribution of these solutions towards restoring and maintaining fluid balance following a period of exercise-induced dehydration.

Chapter 2

General Methods

Ethical Approval

All studies described in this thesis involved healthy human volunteers. Ethical approval was obtained from the Loughborough University Ethical Advisory Committee prior to the start of each of the experiments described in this thesis (e-mails received from the ethics committee confirming approval are included in Appendix A). Subjects were made aware of the procedures to be undertaken during each investigation and their right to withdraw from the study, verbally and in writing, before signed informed consent was obtained.

All subjects were healthy adults between the ages of 18 and 35 years.

Standardisation prior to experimental trials

Unless otherwise stated, subjects were asked to prepare for experimental trials in the same manner for all studies reported in this thesis. Trials commenced in the morning after an overnight fast and subjects were instructed to follow similar nutritional and physical activity patterns during the 24 hours prior to the start of each experimental trial. To ensure that subjects were adequately hydrated at the start of each experimental trial, 500 ml of water was ingested approximately 2 hours before the start of the trial with the exception of the study reported in chapter 7 which involved subjects undertaking a 13 hour period of fluid restriction prior to arrival at the laboratory.

Blood sampling and analysis

Blood samples were collected during all studies reported in this thesis. Specific information regarding the times, manner and volume of blood samples collected are described in the materials and methods section of each experimental chapter. Analytical procedures and the chapters that they apply to are detailed below.

Haemoglobin concentration, haematocrit and blood glucose concentration

Whole blood samples were collected in a syringe before being dispensed into a tube containing K_2EDTA before analysis for haemoglobin concentration and haematocrit. Haemoglobin concentration was determined by the cyanmethaemoglobin method and haematocrit was determined by microcentrifugation (Hawksley micro-haematocrit centrifuge, UK). 100 µl of whole blood was deproteinised by being added to 1000 µl of ice cold 0.3 M perchloric acid in duplicate. This was then used to determine blood glucose concentration by the GOD-PAP method (Randox). Haemoglobin and blood glucose concentrations were made in duplicate while haematocrit was measured in triplicate.

Percentage changes in blood volume, red cell volume and plasma volume were calculated using the formulae described by Dill and Costill (1974).

The analysis described was used in all experimental chapters reported in this thesis.

Serum osmolality and electrolyte concentrations

An aliquot of a whole blood sample was collected in a plain tube before centrifugation at 1500 g for 15 minutes at 4°C (ALC multispeed refrigerated centrifuge, UK). Serum samples were stored in the refrigerator at approximately 10 °C until analyzed. All analysis was performed within 14 days of the sample being collected.

Serum osmolality was measured by freezing point depression (Gonotec Osmomat 030 Cryoscopic Osmometer; Gonotec, Berlin, Germany), sodium and potassium concentration by flame photometry (Corning Clinical Flame Photometry 410C; Corning Ltd., Halstead, Essex, UK) and chloride concentration by coulometric titration (Jenway Chloride Meter; Jenway Ltd., Dunmow, Essex, UK). All analysis was carried out in duplicate.

Serum osmolality and sodium concentration were analysed during the studies reported in chapters 4, 5, 6 and 7, serum potassium during the studies reported in chapters 4, 5

and 6 and serum chloride concentration during the studies reported in chapters 5 and 6.

Urine collection and analysis

Specific details regarding the timing of urine sample collection are provided in the materials and methods section of each experimental chapter. Subjects were instructed to empty their bladder as completely as possible and collect all urine produced into a container that was provided. The total volume of urine produced was measured and recorded before a 5 ml sample was retained for analysis of osmolality by freezing point depression (Gonotec Osmomat 030 Cryoscopic Osmometer; Gonotec, Berlin, Germany). Urine osmolality was analysed in duplicate during all studies reported in this thesis.

Urinary electrolytes were measured during the rehydration studies described in chapters 5 and 6. Sodium and potassium concentration was analysed by flame photometry (Corning Clinical Flame Photometry 410C; Corning Ltd., Halstead, Essex, UK) and chloride concentration by coulometric titration (Jenway Chloride Meter; Jenway Ltd., Dunmow, Essex, UK). All analysis was carried out in duplicate.

Sweat collection and analysis

Sweat samples were collected during the rehydration study described in chapter 6 of this thesis. An absorbent patch was placed on the back of the shoulder to collect the sweat sample. Following completion of exercise, the patch was removed and the sweat was aspirated into an epindorph tube. Sweat samples were analysed for electrolyte content. Sodium and potassium concentration was analysed by flame photometry (Corning Clinical Flame Photometry 410C; Corning Ltd., Halstead, Essex, UK) and chloride concentration by coulometric titration (Jenway Chloride Meter; Jenway Ltd., Dunmow, Essex, UK). All analysis was carried out in duplicate.

Measurement of peak oxygen uptake ($\dot{V}O_{2peak}$)

Peak oxygen uptake was measured during the rehydration studies reported in chapters 5 and 6 of this thesis. Subjects performed a discontinuous, incremental test protocol on an electronically braked cycle ergometer (Lode, Groningen, Netherlands) that involved cycling for 5 minutes during an initial stage at 100 W and 3 minutes during subsequent stages. Workloads during subsequent stages were determined using heart rate and rating of perceived exertion data collected 30 seconds before the end of each stage. Expired air samples were collected in a Douglas bag during the final 2 minutes of the initial stage and the final 1 minute of the subsequent stages before determination of oxygen and carbon dioxide content (Servomex 1400, Crawley, East Sussex, United Kingdom), gas volume (Harvard Dry Gas Meter, Harvard Apparatus Ltd, Kent, United Kingdom) and gas temperature (Edale digital thermometer).

Calculations used during rehydration studies described in chapters 5 and 6

The efficacy of a rehydration solution can be ascertained by calculating the fraction of ingested fluid retained during a recovery period as well as monitoring the effect a solution has on whole body net fluid balance.

The fraction of ingested fluid retained was calculated at each time point by subtracting cumulative urine output produced from the hour following rehydration up to a given time point from total drink volume and was expressed as a percentage of total drink volume.

Whole body net fluid balance was calculated using data obtained for sweat loss, drink volume and urine output. Subjects were assumed to be euhydrated, based on urine osmolality data, and have a net fluid balance of zero before beginning the dehydration phase of each study. Sweat loss and urine production move an individual towards negative fluid balance and drink volume towards positive fluid balance.

Coefficient of variation for analytical procedures

Coefficient of variation (CV) for the analytical procedures described is shown in table 2.1. CV was calculated as the standard deviation of the difference between duplicates and expressed as a percentage of the mean value obtained for samples produced throughout this thesis.

Table 2.1 Mean, SD and Coefficient of variation (%) of duplicates obtained for analytical procedures conducted throughout this thesis.

Assay	n	Mean	SD	CV
Haemoglobin concentration (g 100 ml^{-1})	30	14.2	2.6	1.3
Haematocrit (%)	30	44	3	0.7
Serum osmolality (mosm kg^{-1})	30	281	5	0.7
Serum Na^+ concentration (mmol L^{-1})	30	141	2	1.7
Serum K^+ concentration (mmol L^{-1})	30	4.9	0.6	3.1
Serum Cl^- concentration (mmol L^{-1})	30	104	2	1.7
Urine osmolality (mosm kg^{-1})	30	430	253	0.6
Urine Na^+ concentration (mmol L^{-1})	30	88	48	2.6
Urine K^+ concentration (mmol L^{-1})	30	103	53	2.2
Urine Cl^- concentration (mmol L^{-1})	30	74	43	2.4

Statistical procedures

Specific details regarding the statistical procedures undertaken during each investigation are reported in the materials and methods section of each experimental chapter.

In general, data was tested for normal distribution before being subjected to a two factor repeated measures analysis of variance. Main effects of time and trial are

reported however, appropriate post-hoc tests were employed only when an interaction effect was observed. Significance level was set at $P < 0.05$.

Chapter 3

The acute effects of ingesting commercially available solutions on estimated changes in blood and plasma volume

Introduction

The osmolality of an ingested solution determines the osmotic gradient that is the driving force behind the movement of water across the intestinal wall and is, therefore, an important factor determining the direction and rate of movement of water in the small intestine.

The rate of intestinal water absorption is enhanced in the presence of solutes, such as carbohydrates and sodium, due to active transport of solutes from the lumen into the mucosa (Gisolfi *et al.* 1992; Schedl and Clifton, 1963). Rat models have suggested that intestinal absorption is negatively correlated with luminal osmolality and that hypotonic solutions (190-200 mosm kg^{-1}) result in the greatest rate of water absorption when compared to a range of solution osmolalities (Wapnir and Lifshitz, 1985) following delivery to the small intestine.

In man, Shi *et al.* (1994) investigated the effect of a range of solution osmolalities (186-403 mosm kg^{-1}) on intestinal absorption during rest by perfusing the test solutions, via a triple-lumen tube, into the duodenojejenum. Perfusion of the hypotonic solution (osmolality = 186 mosm kg^{-1}) resulted in a 17% greater fluid absorption relative to a hypertonic solution (osmolality = 403 mosm kg^{-1}) and a more rapid increase in plasma volume. This finding is supported by other segmental perfusion studies (Gisolfi *et al.* 1992; Hunt *et al.* 1992; Leiper and Maughan, 1986; Shi *et al.* 1995). It has been shown that dehydration leading to a reduction in body mass of approximately 4% resulted in a decrease in both muscle and forearm blood flow (Gonzalez-Alonso *et al.* 1998), suggesting that reductions in circulating blood volume may impair the ability of the cardiovascular system to provide oxygen for exercising muscles and effective thermoregulation. The ingestion of hypotonic solutions and the fast rate of fluid absorption in the small intestine that their ingestion provides is, therefore, likely to be of benefit during exercise.

The osmotic gradient that is established following the perfusion of hypotonic solutions promotes the movement of water from the lumen into the mucosa, resulting in fast rates of water absorption (Shi *et al.* 1994). However, hypertonic solutions establish an osmotic gradient that promotes secretion of water into the lumen. Leiper

& Maughan (1986) demonstrated that perfusion of a hypertonic solution with an osmolality of 488 ± 53 mosm kg^{-1} after transit through a "mixing" segment of a multilumen tube resulted in net secretion of water and electrolytes into the lumen, whereas perfusion of an initially isotonic glucose/electrolyte solution promoted uptake of water and electrolytes. It has been suggested that net water secretion following the ingestion of hypertonic solutions may result in a decrease in blood and plasma volume (Merson *et al.* 2002) which would not be beneficial during exercise due to the effect of fluid loss on the ability of the cardiovascular system to effectively thermoregulate and maintain muscle blood flow (Gonzalez-Alonso *et al.* 1998).

The aim of this study was to investigate the effect of ingesting commercially available carbohydrate solutions with different carbohydrate contents on estimated changes in blood and plasma volume.

Materials and Methods

Ten healthy male subjects with a mean age of 24 ± 3 years, height of 179 ± 6 cm and body mass of 74.1 ± 6.5 kg volunteered to participate in this investigation. Subjects reported to the laboratory having consumed no food for at least six hours and after having drunk approximately 500 ml of water two hours prior to their arrival at the laboratory. Experimental trials were separated by a period of at least seven days, took place at the same time of the morning and subjects followed similar physical activity and nutritional patterns for the 24 hours prior to the start of each trial.

Each subject participated in four experimental trials. The order of experimental trials was randomly assigned using a Latin square design. Solutions investigated were Evian water, Lucozade Sport (GlaxoSmithKline) Coca-Cola and Lucozade energy (GlaxoSmithKline) and contained approximately 0, 6, 11 and 18% carbohydrate respectively. The characteristics of the drinks are shown in table 3.1.

Table 3.1 Drink characteristics (Mean (SD)).

CHO content (%)	Na^+ concentration (mmol L^{-1})	K^+ concentration (mmol L^{-1})	Osmolality (mosm kg^{-1})
0	0.0 (0.0)	0.0 (0.0)	6 (1)
6	25.0 (0.0)	2.5 (0.1)	284 (5)
11	1.0 (1.0)	0.1 (0.0)	527 (5)
18	7.0 (2.0)	0.1 (0.0)	687 (16)

Upon arrival at the laboratory, subjects provided a urine sample before body mass was measured wearing minimal clothing on a beam balance (Marsdens type 150, Marsdens Weighing Machines, London, UK) to the nearest 10 g. A heart rate monitor (Polar Team System, Polar, USA) was positioned before the subject was seated for a period of ten minutes. A cannula, that remained in place for the duration of the trial and was kept patent between sample collection by flushing with heparinised isotonic saline, was inserted into a superficial forearm vein. Three resting 5 ml blood samples were obtained at intervals of ten minutes. Immediately after the third resting blood sample, 600 ml of the test solution was ingested over a period of five minutes. Blood samples (5ml) were collected at ten minutes intervals for 60 minutes following ingestion of the drink before a final urine sample was obtained. Subjects remained seated in an upright position for the duration of the trial to avoid the previously reported postural changes in plasma volume (Hagen *et al*, 1978; Shirreffs *et al.* 1994).

Sample analysis

Blood samples were analysed for haemoglobin concentration, haematocrit and blood glucose concentration. Haemoglobin concentrations and haematocrit were used to estimate changes in blood, red cell and plasma volumes as described by Dill and Costill (1974).

Pre- and post- trial urine volume was measured and a sample retained for analysis of osmolality.

All analysis was performed as described in the general methods section of this thesis.

Statistical analysis

All data sets were tested for normal distribution using the Kolmogorov-Smirnov test. Parametric data are presented as mean ± standard deviation.

Two-factor repeated measures ANOVA were used to evaluate differences between trials. One-factor ANOVA followed by Tukey or Dunnetts pairwise comparisons were used as parametric post tests, depending on whether a clear control time point was present. When appropriate, t-tests were used to locate differences.

Statistical analysis was performed using SPSS 12.0 for windows.

Results

Pre-trial data is shown in table 3.2. Baseline measures were within the expected normal range for subjects following a period of fasting (Geigy Scientific Tables, 1962) and the pre-trial urine osmolality data collected in this study suggested that subjects were adequately hydrated at the onset of each trial (Armstrong *et al.* 1994; Shirreffs and Maughan, 1998b).

Table 3.2 Pre-trial body mass, Hb concentration, haematocrit, blood glucose concentration and urine osmolality on all trials (Mean (SD)).

Measure	0% CHO	6% CHO	11% CHO	18% CHO	P-value
Body mass (kg)	73.8 (6.3)	74.1 (6.5)	74.1 (6.2)	74.3 (7.0)	0.999
Hb concentration ($g\ L^{-1}$)	156 (10)	151 (9)	152 (10)	155 (10)	0.723
Haematocrit (%)	43 (3)	43 (2)	42 (3)	43 (2)	0.770
Blood glucose concentration ($mmol\ L^{-1}$)	5.6 (0.1)	5.7 (0.7)	6.0 (0.6)	5.7 (1.0)	0.497
Urine osmolality ($mosm\ kg^{-1}$)	388 (235)	343 (177)	449 (230)	392 (241)	0.766

No difference in haemoglobin concentration (P = 0.530), haematocrit (P = 0.780) or blood glucose concentration (P = 0.508) was observed between the three resting blood samples (Table 3.3). As a result, the third sample was used as a baseline value as this was the sample taken immediately prior to drinking. The other two resting samples were not included in statistical analysis and are not shown in figures.

Table 3.3 Haemoglobin concentration (Hb), haematocrit (Hct) and blood glucose concentration of three resting blood samples. Values are mean (SD).

Resting sample	Hb ($g\ L^{-1}$)	Hct (%)	Blood glucose ($mmol\ L^{-1}$)
1	155 (10)	43 (2)	5.6 (0.5)
2	153 (9)	43 (2)	5.6 (0.6)
3	153 (10)	43 (2)	5.7 (0.6)
P-value	0.530	0.780	0.508

Drink electrolyte content

The sodium concentration of the 0% carbohydrate drink was lower than the 6% ($P < 0.001$) and the 18% ($P < 0.001$) carbohydrate drink. The sodium concentration of the 11% carbohydrate drink was lower than the 6% ($P < 0.001$) and 18% ($P < 0.001$) carbohydrate drinks and the sodium concentration of the 18% carbohydrate drink was lower than the 6% carbohydrate drink ($P < 0.001$) (Table 3.1).

The potassium concentration of the 6% carbohydrate drink was greater than the 0% ($P < 0.001$), the 11% ($P < 0.001$) and the 18% ($P < 0.001$) carbohydrate drinks and the potassium concentration of the 0% carbohydrate drink was lower than the 11% carbohydrate drink ($P = 0.003$) (Table 3.1).

Estimated changes in blood, red cell and plasma volumes

Two factor repeated measures ANOVA on blood volume data showed no main effect of trial ($P = 0.309$), a main effect of time ($P = 0.002$) but no interaction ($P = 0.063$) (Figure 3.1).

Two factor repeated measures ANOVA on plasma volume data showed no main effect of trial ($P = 0.219$), a main effect of time ($P < 0.001$) and an interaction ($P = 0.027$). No significant deviations ($P > 0.05$) from baseline values were detected however, plasma volume tended to be reduced ($P = 0.077$) following ingestion of the 18% carbohydrate drink (Figure 3.2).

Two factor repeated measures ANOVA on red cell volume data showed no main effect of trial ($P = 0.673$), a main effect of time ($P < 0.001$) but no interaction ($P = 0.372$) (Figure 3.3).

Figure 3.1 Estimated percentage changes in blood volume following ingestion of 0, 6, 11 and 18% carbohydrate solutions. Points are mean ± SD. Data is shown in Appendix C.

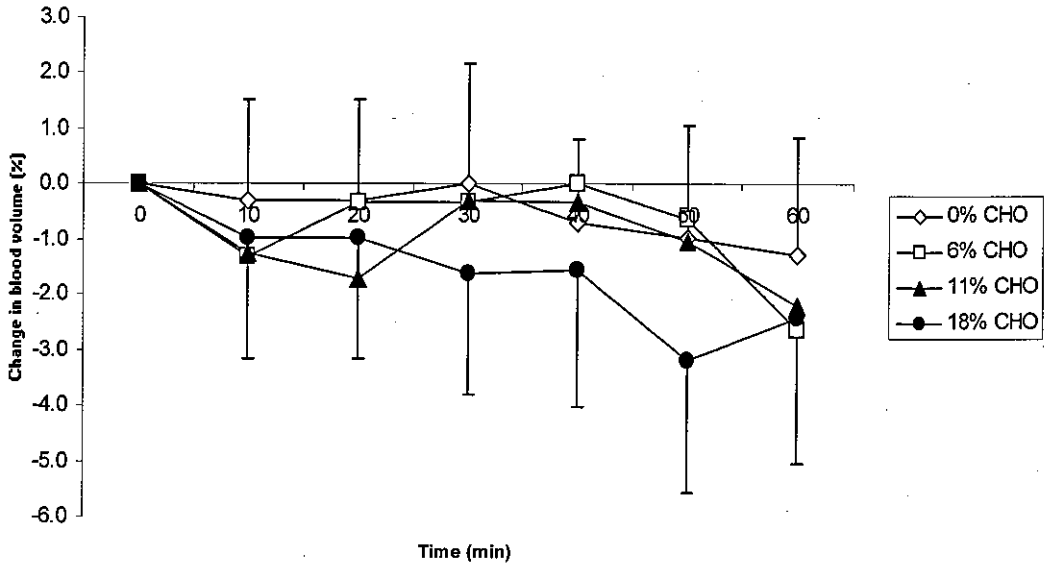


Figure 3.2 Estimated percentage changes in plasma volume following ingestion of 0, 6, 11 and 18% carbohydrate solutions. Points are mean ± SD. Data is shown in Appendix C.

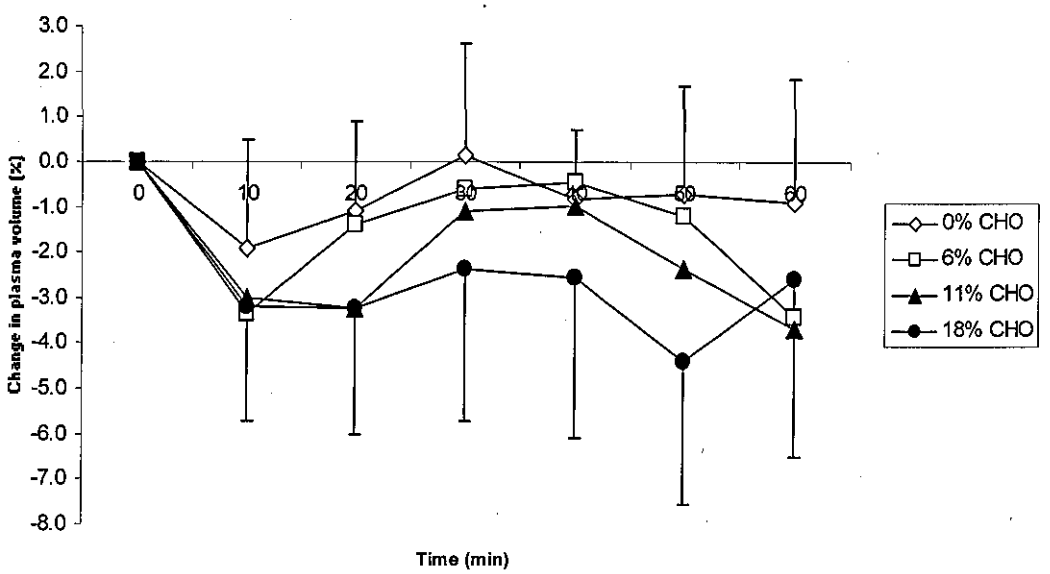
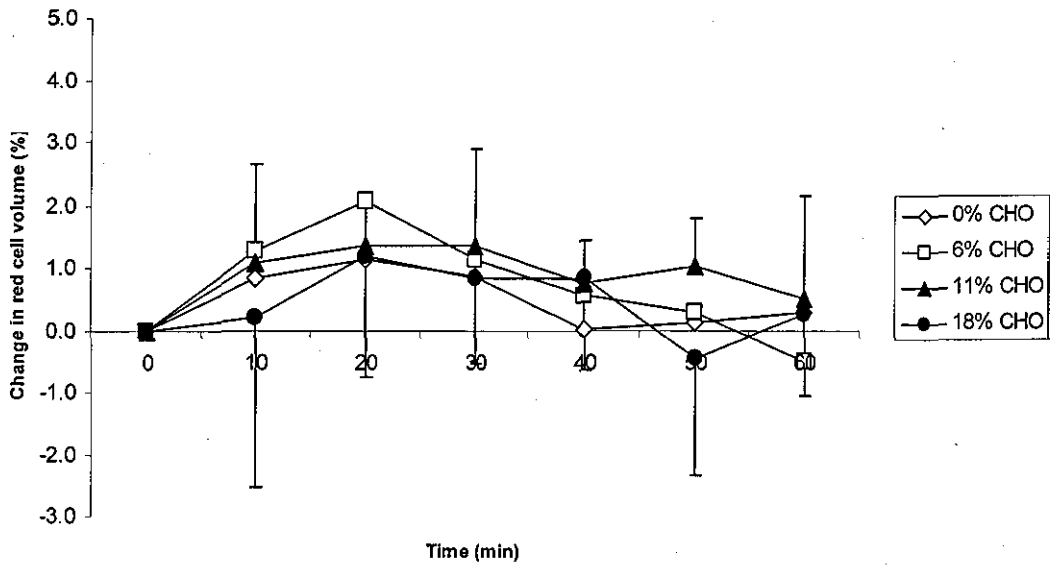


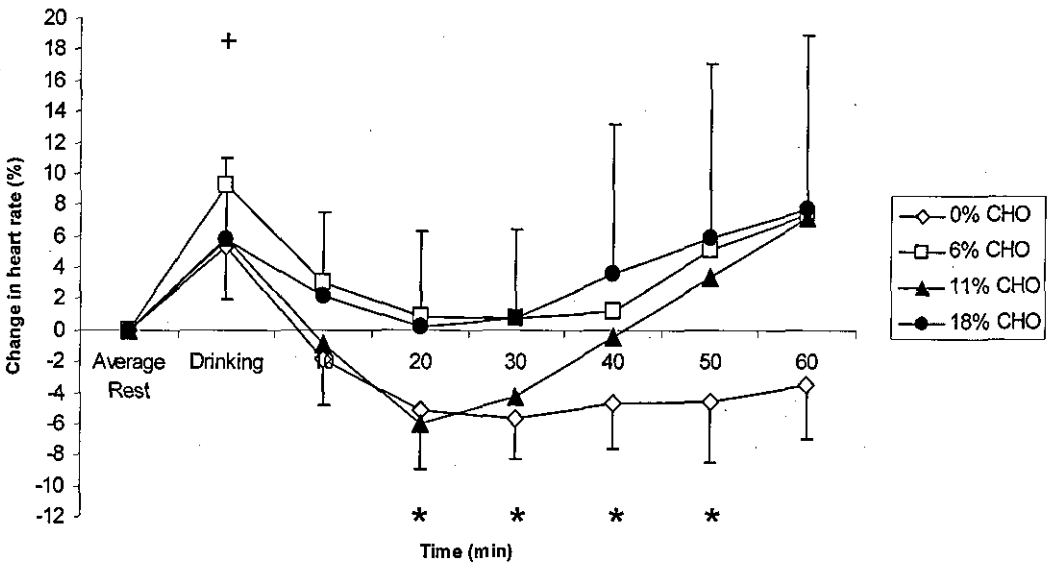
Figure 3.3 Estimated percentage changes in red cell volume following ingestion of 0, 6, 11 and 18% carbohydrate solutions. Points are mean ± SD. Data is shown in Appendix C.



Heart rate

Heart rate over the 30 minutes prior to drinking was averaged and taken as baseline. Two factor repeated ANOVA on heart rate data showed no main effect of trial ($P = 0.536$), a main effect of time ($P < 0.001$) and an interaction ($P = 0.002$). Heart rate was elevated above baseline values during the five minute drinking period on the 0 ($P = 0.002$), and 6 % ($P = 0.003$) carbohydrate trials and heart rate was significantly lowered from baseline values at 20, ($P = 0.003$), 30 ($P = 0.001$), 40 ($P = 0.007$) and 50 ($P = 0.008$) minutes following ingestion of the 0% carbohydrate solution. No significant deviations ($P > 0.05$) were observed during the other trials (Figure 3.4).

Figure 3.4 Percentage change in heart rate from baseline value following ingestion of 0, 6, 11 and 18% carbohydrate solutions. Points are mean ± SD. Data is shown in Appendix C. + denotes 0 and 6 % carbohydrate time points significantly different ($P < 0.05$) from baseline values. * denotes 0% carbohydrate time point significantly different ($P < 0.05$) from baseline value.



Blood glucose

Two factor repeated measures ANOVA on blood glucose data showed a main effect of trial ($P = 0.003$), a main effect of time ($P < 0.001$) and an interaction ($P < 0.001$). Blood glucose concentration was lower ($P < 0.05$) on the 0% carbohydrate trial at all time points after ingestion than on the 6, 11 and 18% carbohydrate trials with the exception of 60 minutes after ingestion when blood glucose concentration was only greater on the 18% carbohydrate trial compared to the 0% carbohydrate trial. Blood glucose concentration was elevated from baseline levels ($P < 0.05$) 10, 20, 30 and 40 min after ingestion of the 6% and 11% carbohydrate solutions and was significantly elevated ($P < 0.05$) at all time points after ingestion of the 18% carbohydrate solutions (Table 3.4).

Table 3.4 Blood glucose concentration (mmol L^{-1}) following ingestion of 0, 6, 11 and 18% carbohydrate solutions. Values are mean (SD). * indicated time point significantly different ($P < 0.05$) from pre-ingestion value.

Time (min)	0% CHO	6% CHO	11% CHO	18% CHO
0	5.6 (0.5)	5.7 (0.7)	6.0 (0.6)	5.7 (0.6)
10	5.4 (0.6)	6.6 (1.0)*	6.9 (1.1)*	6.6 (1.1)*
20	5.5 (0.6)	8.5 (1.1)*	8.4 (1.0)*	8.2 (1.2)*
30	5.7 (0.6)	8.8 (1.5)*	8.7 (1.3)*	9.0 (1.0)*
40	5.7 (0.6)	7.9 (1.4)*	7.7 (1.4)*	8.2 (1.0)*
50	5.8 (0.6)	6.9 (1.2)	6.9 (1.3)	8.0 (0.9)*
60	5.9 (0.6)	6.4 (0.9)	6.5 (1.3)	7.3 (1.0)*

Urine volume and osmolality

Two factor repeated measures ANOVA on urine volume data showed a main effect of trial ($P = 0.041$), a main effect of time ($P < 0.001$) but no interaction ($P = 0.469$) (Table 3.5).

Table 3.5 Urine volume (ml) before and after ingestion of 0, 6, 11 and 18% carbohydrate solutions. Values are mean (SD).

Time	0% CHO	6% CHO	11% CHO	18% CHO
Pre-	199 (184)	268 (199)	199 (162)	207 (139)
Post-	588 (183)	567 (152)	432 (192)	480 (245)

Two factor repeated measures ANOVA on urine osmolality data showed no main effect of trial ($P = 0.370$), a main effect of time ($P = 0.008$) but no interaction ($P = 0.609$) (Table 3.6).

Table 3.6 Urine osmolality (mosm kg^{-1}) before and after ingestion of 0, 6, 11 and 18% carbohydrate solutions. Values are mean (SD).

Time	0% CHO	6% CHO	11% CHO	18% CHO
Pre-	388 (235)	343 (177)	449 (230)	392 (241)
Post-	181 (30)	190 (45)	220 (74)	244 (110)

Discussion

The results of this study suggest that ingestion of strongly hypertonic drinks had a tendency to result in a decrease in plasma volume, as calculated from measured haemoglobin concentrations and haematocrit, most likely due to an acute net secretion of water into the small intestine.

Investigations that have involved perfusion of solutions directly into the intestine have shown that dilute hypotonic solutions result in a faster rate of water absorption relative to isotonic and hypertonic solutions. Shi *et al.* (1994) examined the effect of solution osmolality on intestinal fluid absorption in 6 resting male subjects. The results of this study showed that, when perfused into the duedenojejenum, a hypotonic solution with an osmolality of 186 ± 9 mosm kg^{-1} resulted in a 17% greater fluid absorption compared to a hypertonic solution with an osmolality of 403 ± 3 mosm kg^{-1}, and that this greater fluid absorption resulted in a faster increase in plasma volume. The results of the present study suggest that ingestion of the 6% carbohydrate drink had no effect on blood or plasma volume. This was observed in another study which suggested that plasma volume was significantly lower 20 minutes after ingestion of a 6% glucose solution compared to a 2% glucose solution (Merson *et al.* 2002).

Leiper and Maughan (1986) reported that perfusion of a hypertonic solution with an osmolality of 488 ± 53 mosm kg^{-1} in the jejunum resulted in net water secretion into the small intestine. The results of the present study suggest that oral ingestion of an 18% carbohydrate drink with an osmolality of 687 ± 16 mosm kg^{-1} may result in a reduction in fluid within the extracellular space. It is likely that any reduction in

extracellular fluid volume would be due to a transient movement of water into the intestinal lumen (Leiper and Maughan, 1986).

Intestinal absorption is highly variable between human subjects. Previous studies have reported large ranges in change in plasma volume following perfusion of different solutions (Gisolfi *et al.* 1992; Shi *et al.* 1994). The results of the present study are in agreement with this. Ingestion of the 18% carbohydrate drink resulted in a non significant average decrease in blood volume of 1.7 ± 2.2% 10 minutes after ingestion and 1.4 ± 2.6% 60 minutes after ingestion. However, the range at these time points was +0.6% to -6.4% and +0.6% to -3.8% respectively. At the same time points, plasma volume was reduced on average by -3.2 ± 2.5% and -2.6 ± 3.9% with ranges of +0.3% to -7.2% and +0.6% to -6.4% respectively. It is clear that a large variation between subjects exists. It is possible that this large variation is due to differences in the rate of gastric emptying that exist between individuals (Vist and Maughan, 1994; Vist and Maughan, 1995; Rehrer, 2001).

Another possible reason for such large variation between subjects is drink temperature. This variable was not measured during this study but the drink was taken out of the refrigerator approximately 10 minutes before it was given to the subject and consequently will have been quite cool. Imms and Lighten (1989) observed a significant decrease in skin temperature and a significant reduction in heart rate at rest following ingestion of one litre of water at 7°C compared to one litre of water at 37°C. This suggests that ingestion of cool drinks results in peripheral vasoconstriction and this may have interfered with heamodynamics causing more variation between subjects in the present study.

Studies (Gislofi *et al.* 1992; Shi *et al.* 1995) have observed that the type of carbohydrate present in a solution can affect the extent of water and solute uptake in the intestine. As the drinks investigated in this study were commercially available, total carbohydrate content will have consisted of different types of carbohydrate and may have affected the extent of water and solute uptake and/or secretion.

The extent of water uptake may be increased in the presence of sodium (Leiper, 1998; Schedl and Clifton, 1963). As there were differences in sodium concentration between

the drinks, this may provide another possible explanation for the lack of significant deviations from baseline values being observed in this study.

Decreases in the water content of the blood that result in decreases in plasma volume and increases in serum osmolality lead to an increased viscosity of the blood. In turn, this results in an increased total peripheral resistance, decreased venous return, a decreased end diastolic volume and decreased stroke volume. In order to maintain the necessary cardiac output, heart rate must increase. Although no significant increases in heart rate were observed following the 18% carbohydrate trial, the heart rate data obtained during this study supports the conclusions as heart rate was significantly lowered 20, 30, 40 and 50 min after ingestion of the 0% carbohydrate drink. This suggests that water absorption is greater following ingestion of low osmolality solutions. Heart rate was elevated by an average of 6 ± 11% (3 ± 10 bpm) and 8 ± 11% (4 ± 8 bpm) at 50 min and 60 min respectively following ingestion of the 18% carbohydrate drink. However, these changes were not significant. Maximum increase in heart rate at these time points was 29% (16 bpm) and 32% (18 bpm) respectively. These results again suggest that inter individual variation is high and may be the reason why the results are not significantly different from each other.

In summary, the results of this study suggest that ingestion of strongly hypertonic solutions may result in a decrease in estimated blood and plasma volume most likely due to a temporary net secretion of water from the extracellular fluid into the intestinal lumen.

Chapter 4

The acute effects of ingesting glucose solutions on estimated changes in blood and plasma volume

Introduction

The results of the study reported in chapter 3 suggested that ingestion of commercially available drinks with a high carbohydrate content and osmolality may cause a reduction in extracellular fluid volume that is most likely due to an acute net secretion of water into the small intestine. As a trend towards a decrease in plasma volume following ingestion of the highest carbohydrate and osmolality solution was observed, further investigation into this issue seems appropriate but with a greater level of control employed.

Gisolfi *et al.* (1992) and Shi *et al.* (1995) both observed that the type of carbohydrate in a solution influenced the extent of water and solute uptake following perfusion into the duodenojejunum. The drinks examined in chapter 3 were commercially available and differed in the type of carbohydrate(s) present in the solution. This may have had an effect on fluid absorption and/or secretion in the intestine.

The sodium concentration of the drinks consumed in chapter 3 were different and may have affected the extent of water uptake in the intestine (Leiper, 1998; Schedl and Clifton, 1963).

Imms and Lighten (1989) observed a significant decrease in skin temperature and a significant reduction in heart rate at rest following ingestion of one litre of water at 7°C compared to one litre of water at 37°C. This suggests that ingestion of cool drinks results in peripheral vasoconstriction and could potentially interfere with peripheral heamodynamics. Controlling the temperature of an ingested solution, therefore, seems necessary.

The aim of this study was to examine the effect that ingestion of different glucose solutions has on estimated changes in blood and plasma volume when electrolyte content of all drinks was the same and when solutions were warmed to a temperature of 37 °C.

Materials and Methods

Twelve healthy male subjects with a mean age of 25 ± 5 years, height of 176 ± 5 cm and body mass of 75.4 ± 12.9 kg volunteered to participate in this investigation. Subjects reported to the laboratory having consumed no food for at least six hours and after having drunk approximately 500 ml of water two hours prior to their arrival at the laboratory. Experimental trials were separated by a period of at least seven days, took place at the same time of the morning and subjects followed similar physical activity and nutritional patterns for the 24 hours prior to the start of each trial.

Each subject participated in four experimental trials that involved ingestion of 600ml of fluid containing distilled water, to which glucose was added to give concentrations of 0, 2, 5 or 10%. The order of experimental trials was randomly assigned using a Latin square design. No electrolytes were added to the drinks. Measured osmolalities of 0 ± 0 mosm kg^{-1}, 111 ± 1 mosm kg^{-1}, 266 ± 7 mosm kg^{-1} and 565 ± 5 mosm kg^{-1} were recorded for the 0, 2, 5 and 10% glucose solutions respectively.

Upon arrival at the laboratory, subjects provided a urine sample before body mass was measured wearing minimal clothing on a beam balance (Marsdens type 150, Marsdens Weighing Machines, London, UK) to the nearest 10 g. A heart rate monitor (Polar Team System, Polar, USA) was positioned before the subject was seated in a room maintained at 22.4 ± 0.5 °C for a period of ten minutes. A cannula, that remained in place for the duration of the trial and was kept patent between sample collection by flushing with heparinised isotonic saline, was inserted into a superficial forearm vein. Three resting 5 ml blood samples were obtained at intervals of ten minutes. The subject was then given 5 minutes to consume 600 ml of the test solution, all drinks were warmed to a temperature of 37 °C to avoid the peripheral vasoconstriction that can occur after the ingestion of cool drinks (Imms and Lighten, 1989). Blood samples (5ml) were collected at ten minute intervals for 60 minutes following ingestion of the test drink before a final urine sample was collected. Subjects remained seated for the duration of the trial to avoid the previously reported postural changes in plasma volume (Hagen *et al*, 1978; Shirreffs *et al.* 1994).

Sample analysis

Blood samples were analysed for haemoglobin concentration, haematocrit, blood glucose concentration, serum osmolality, serum sodium concentration and serum potassium concentration. Haemoglobin concentrations and haematocrit were used to calculate changes in blood, red cell and plasma volumes as described by Dill and Costill (1974).

Pre- and post- trial urine volume was measured and a sample retained for analysis of osmolality.

All analysis was performed as described in the general methods section of this thesis.

Statistical analysis

All data sets were tested for normal distribution using the Kolmogorov-Smirnov test. Parametric data are presented as mean ± standard deviation. Non parametric data are presented as median (range) values.

Two-factor repeated measures ANOVA were used to evaluate differences between trials. One-factor ANOVA followed by Tukey or Dunnetts pairwise comparisons were used as parametric post tests, depending on whether a clear control time point was present.

The Kruskal-Wallace test and Mann-Whitney pairwise comparisons were used as non-parametric post tests.

Statistical analysis was performed using SPSS 12.0 for windows.

Results

Pre-trial data is shown in table 4.1. Baseline measures were within the expected normal range for subjects following a period of fasting (Geigy Scientific Tables, 1962). The pre-trial serum osmolality, serum sodium concentration and urine

osmolality data collected in this study suggested that subjects were adequately hydrated at the onset of each trial (Armstrong *et al.* 1994; Shirreffs and Maughan, 1998b).

Table 4.1 Pre-trial body mass, Hb concentration, haematocrit, blood glucose concentration, serum osmolality, serum sodium concentration, serum potassium concentration and urine osmolality on all trials (Mean (SD)).

Measure	0% glucose	2% glucose	5% glucose	10% glucose	P-value
Body mass (kg)	75.4 (13.1)	75.5 (13.0)	75.3 (12.9)	75.2 (12.7)	1.000
Hb concentration ($g\ L^{-1}$)	152 (9)	149 (9)	152 (5)	149 (9)	0.841
Haematocrit (%)	43 (3)	42 (3)	43 (2)	42 (2)	0.476
Blood glucose concentration ($mmol\ L^{-1}$)	5.9 (0.3)	5.9 (0.4)	6.0 (0.3)	5.9 (0.4)	0.799
Serum osmolality ($mosm\ kg^{-1}$)	290 (4)	290 (4)	290 (4)	290 (5)	0.946
Serum sodium concentration ($mmol\ L^{-1}$)	139 (2)	139 (3)	139 (2)	139 (3)	0.927
Serum potassium concentration ($mmol\ L^{-1}$)	4.7 (0.3)	4.7 (0.4)	4.5 (0.4)	4.6 (0.3)	0.484
Urine osmolality ($mosm\ kg^{-1}$)	480 (307)	473 (327)	426 (246)	465 (267)	0.968

No difference in haemoglobin concentration ($P = 0.984$), haematocrit ($P = 0.947$) or blood glucose concentration ($P = 0.323$) were observed between the three resting blood samples (Table 4.2). As a result, the third sample was used as a baseline value, as this was the sample obtained immediately before drinking, and the other two resting samples were not included in statistical analysis and are not shown in figures.

Table 4.2 Haemoglobin concentration (Hb), haematocrit (Hct) and blood glucose concentration of three resting blood samples. Values are mean (SD).

Resting sample	Hb ($g\ L^{-1}$)	Hct (%)	Blood glucose ($mmol\ L^{-1}$)
1	151 (9)	42 (3)	5.8 (0.3)
2	151 (8)	42 (2)	5.9 (0.3)
3	150 (8)	42 (2)	5.9 (0.3)
P-value	0.984	0.947	0.323

Estimated changes in blood, red cell and plasma volumes

Two factor repeated measures ANOVA on blood volume data showed no main effect of trial ($P = 0.209$), a main effect of time ($P < 0.001$) and an interaction ($P = 0.041$). Blood volume was elevated from baseline levels by 1.8 (-0.7 to 6.7) % ($P = 0.003$) 20 minutes and by 1.4 (-2.0 to 5.3) % ($P = 0.005$) 30 minutes after ingestion of the 2% glucose solution and decreased by 1.1 (-3.2 to 1.4) % ($P = 0.001$) 60 minutes after ingestion of the 2% glucose solution (Figure 4.1).

Two factor repeated measures ANOVA on plasma volume data showed a main effect of trial ($P = 0.014$), a main effect of time ($P < 0.001$) and an interaction ($P = 0.006$). Plasma volume was lower ($P < 0.05$) on the 10% glucose trial than on the 2% glucose trial 10, 20 and 30 minutes after ingestion. Plasma volume was significantly increased from baseline values by 2.7 ± 2.4 % ($P = 0.006$) 20 minutes after ingestion of the 2% glucose solution. Plasma volume was decreased from baseline values by 2.9 ± 2.0 % ($P = 0.017$) and by 2.7 ± 2.0 % ($P = 0.036$) 10 and 60 minutes after ingestion of the 10% glucose solution respectively (Figure 4.2).

Two factor repeated measures ANOVA on red cell volume data showed no main effect of trial ($P = 0.269$), a main effect of time ($P = 0.006$) but no interaction ($P = 0.403$) (Figure 4.3).

Figure 4.1 Estimated percentage changes in blood volume following ingestion of 0, 2, 5 and 10% glucose solutions. Points are median values as data was not normally distributed. Data is shown in Appendix D. * denotes 2% glucose time point significantly different ($P < 0.05$) from baseline value.

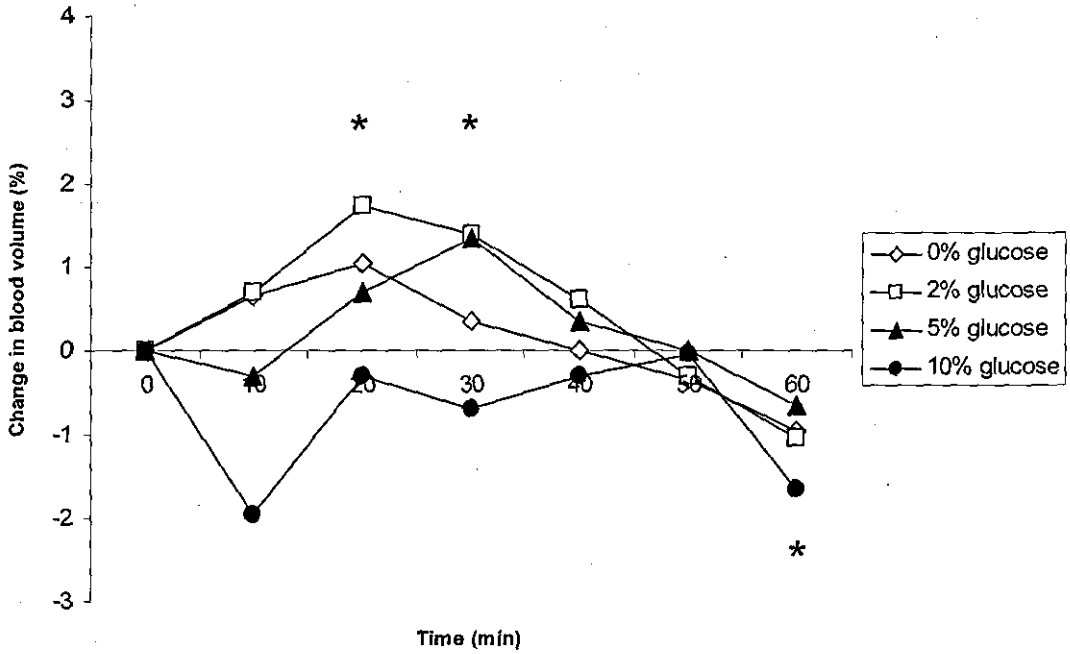


Figure 4.2 Estimated percentage changes in plasma volume following ingestion of 0, 2, 5 and 10% glucose solutions. Points are mean ± SD. Data is shown in Appendix D. + indicates 2% glucose time point significantly different ($P < 0.05$) from baseline value. * denotes 10% glucose time point significantly different ($P < 0.05$) from baseline value.

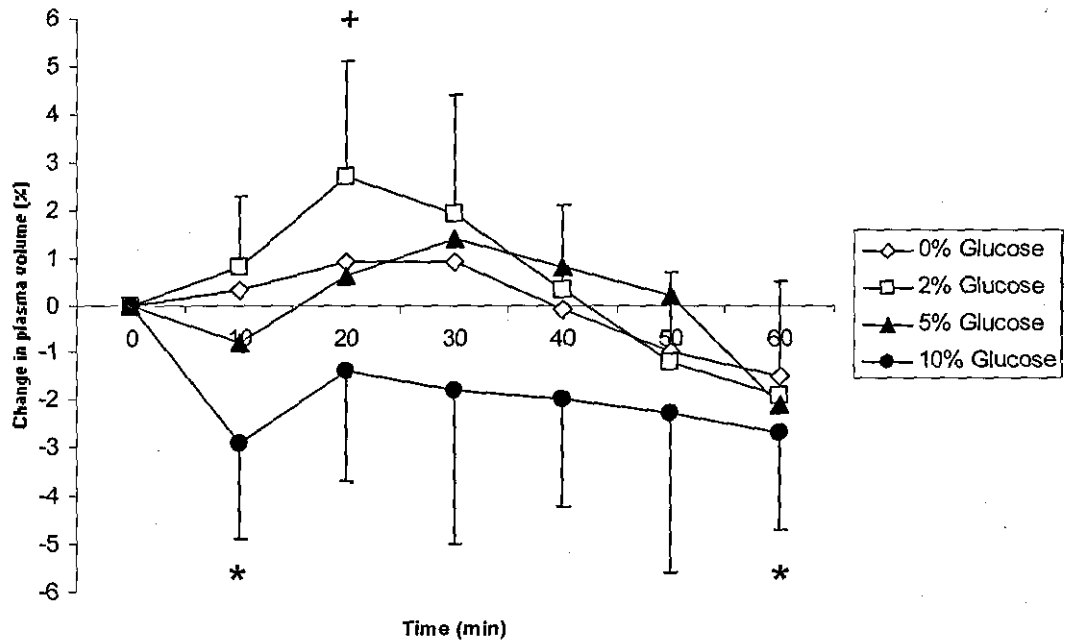
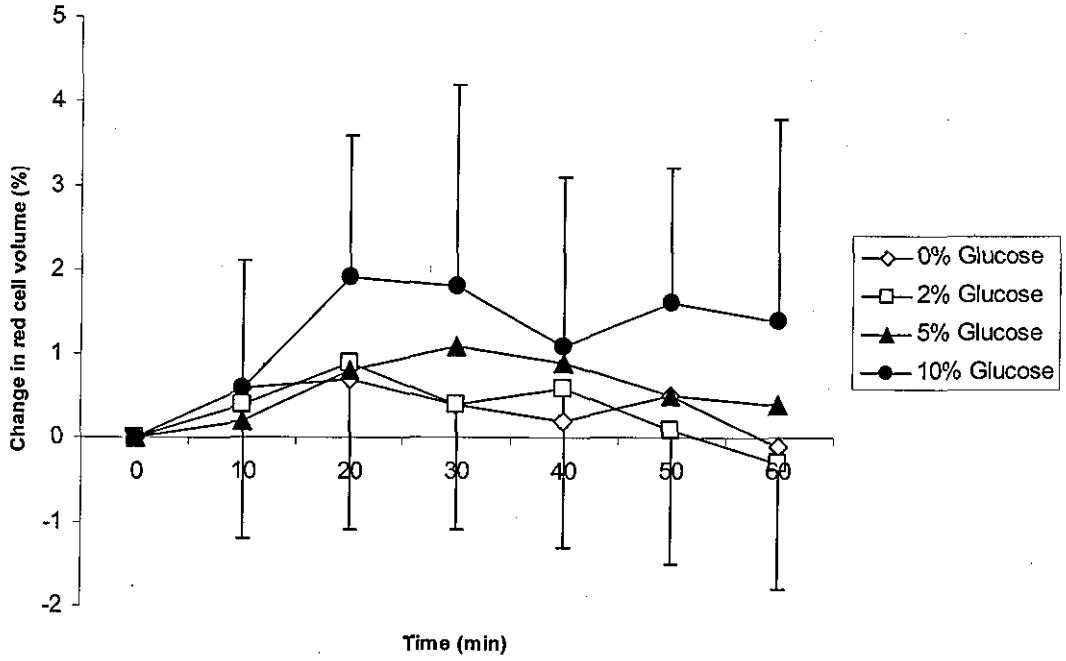


Figure 4.3 Estimated percentage changes in red cell volume after ingestion of 0, 2, 5 and 10% glucose solutions. Values are mean ± SD. Data is shown in Appendix D.

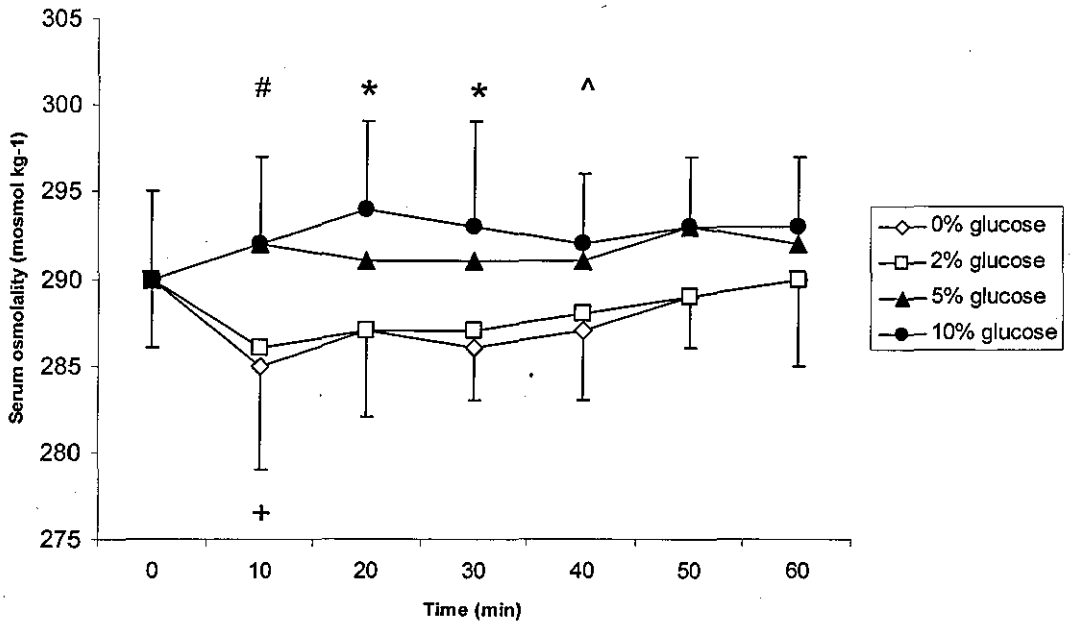


Serum osmolality

Two factor repeated measures ANOVA on serum osmolality data showed a main effect of trial ($P = 0.020$), time ($P < 0.001$) and interaction ($P = 0.008$). Serum osmolality was greater ($P < 0.05$) on the 5 and 10% glucose trials compared to the 0% glucose trial 10 minutes after drink ingestion and significantly greater ($P < 0.05$) on the 10% glucose trial compared to the 0 and 2% glucose trials 20 and 30 minutes after drink ingestion. 40 minutes after ingestion of the test solution, serum osmolality was greater ($P < 0.05$) on the 10% glucose trial compared to the 0% glucose trial (Figure 4.4).

Serum osmolality was decreased from baseline levels 10 minutes ($P = 0.029$) after ingestion of the 0% glucose solution (Figure 4.4) but there was no significant deviations ($P > 0.05$) from baseline values during the 2, 5 or 10% glucose trials.

Figure 4.4 Changes in serum osmolality (mosm kg^{-1}) following ingestion of 0, 2, 5 and 10% glucose solutions. Points are mean ± SD. Data is shown in Appendix D. + denotes 0% glucose time point significantly different ($P < 0.05$) from baseline value. # denotes 0% glucose time point significantly different ($P < 0.05$) from 5 and 10% glucose time point. * denotes 10% glucose time point significantly different ($P < 0.05$) from 0 and 2% glucose time point. ^ denotes 0% glucose time point significantly different ($P < 0.05$) from 10% glucose time point.

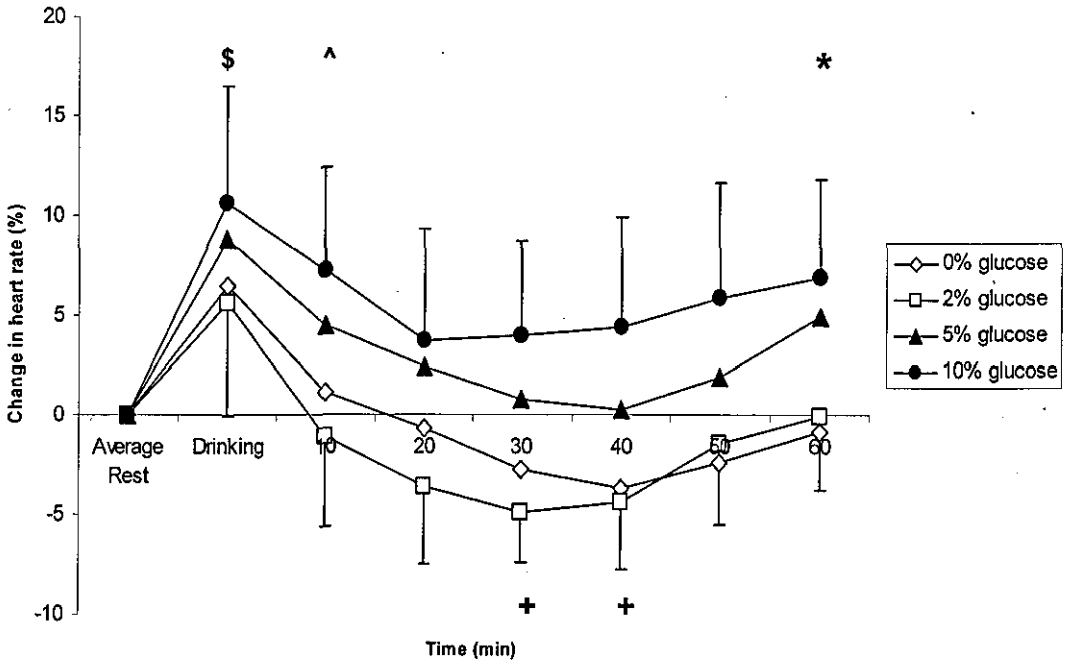


Heart rate

Heart rate over the 30 minutes prior to drinking was averaged and was taken as baseline. Two factor repeated measures ANOVA on heart rate data showed a main effect of trial ($P < 0.001$), time ($P < 0.001$) and interaction ($P = 0.014$). Heart rate was greater ($P < 0.05$) on the 10% glucose trial from 10 minutes after ingestion until the end of the experimental period than on the 0 and 2% glucose trials. Heart rate was elevated above baseline values during the five minute drinking period on the 0 ($P = 0.025$), 5 ($P = 0.009$) and 10% ($P = 0.002$) glucose trials, but not during the 2% glucose trial ($P = 0.163$), and remained elevated during the following 10 minutes after drinking the 5 ($P = 0.013$) and 10% ($P = 0.014$) glucose solutions. Sixty minutes after ingesting the 10% glucose solution, heart rate was elevated by 6.9 ± 4.9% ($P = 0.014$). Heart rate was reduced by 4.9 ± 2.5% ($P = 0.001$) and 4.4 ± 3.4% ($P = 0.027$) 30 and 40 minutes after drinking the 2% glucose solution respectively (Figure 4.5).

A significant moderate negative correlation was observed between percentage change in plasma volume and percentage change in heart rate (Pearson correlation = -0.512, P < 0.001).

Figure 4.5 Percentage change in heart rate from baseline value following ingestion of 0, 2, 5 and 10% glucose solutions. Points are mean ± SD. Data is shown in Appendix D. * denotes 10% glucose time point significantly different (P < 0.05) from baseline value. + denotes 2% glucose time point significantly different (P < 0.05) from baseline value. ^ denote 5 and 10% glucose time points significantly different (P < 0.05) from baseline value. $ denotes 0, 5 and 10% glucose time points significantly different (P < 0.05) from baseline value.



Blood glucose

Two factor repeated measures ANOVA on blood glucose data showed a main effect of trial (P < 0.001), time (P < 0.001) and interaction (P < 0.001). Blood glucose concentration was greater on the 10% glucose trial than on the 0 and 2% glucose trials from 10 minutes after ingestion until the end of the experimental period. Blood glucose concentration was elevated from baseline levels (P < 0.05) 10, 20, 30 and 40 min after ingestion of the 2% glucose solution as well as 10, 20, 30, 40 and 50 min

following ingestion of the 5% glucose solution and elevated from baseline levels ($P < 0.05$) at all time points following ingestion of the 10% glucose solution (Table 4.3).

Table 4.3 Blood glucose concentration (mmol L^{-1}) following ingestion of 0, 2, 5 and 10% glucose solutions. Values are mean (SD). * indicates time point significantly different ($P < 0.05$) from baseline value.

Time after ingestion (min)	0% glucose	2% glucose	5% glucose	10% glucose
0	5.9 (0.3)	5.9 (0.4)	6.0 (0.3)	5.9 (0.4)
10	5.8 (0.4)	7.1 (0.6)*	7.7 (0.9)*	7.9 (0.8)*
20	5.8 (0.4)	7.8 (0.6)*	9.2 (1.3)*	9.6 (1.1)*
30	5.9 (0.5)	7.5 (0.7)*	9.6 (1.4)*	9.8 (1.2)*
40	6.0 (0.6)	6.8 (0.7)*	9.0 (1.6)*	9.2 (1.8)*
50	6.1 (0.6)	6.2 (0.8)	8.0 (1.4)*	8.7 (1.6)*
60	6.0 (0.6)	6.0 (0.7)	7.4 (1.4)	8.0 (1.4)*

Serum electrolytes

Two factor repeated measures ANOVA on serum sodium data showed no main effect of trial ($P = 0.760$) a main effect of time ($P < 0.001$) but no interaction ($P = 0.316$) (Table 4.4).

Two factor repeated measures ANOVA on serum potassium data showed no main effect of trial ($P = 0.207$), a main effect of time ($P < 0.001$) but no interaction ($P = 0.324$) (Table 4.4).

Table 4.4 Serum sodium and potassium concentrations (mmol L^{-1}) following ingestion of 0, 2, 5 and 10% glucose solutions. Values are mean (SD).

Time after ingestion (min)	0% glucose	2% glucose	5% glucose	10% glucose
Serum sodium				
0	139 (2)	139 (3)	139 (2)	139 (3)
10	136 (1)	136 (3)	138 (3)	138 (5)
20	136 (1)	136 (4)	137 (2)	137 (4)
30	137 (2)	135 (4)	137 (3)	137 (5)
40	137 (2)	136 (4)	137 (2)	137 (5)
50	138 (3)	137 (4)	138 (3)	137 (5)
60	138 (2)	137 (3)	137 (2)	137 (4)
Serum potassium				
0	4.7 (0.3)	4.7 (0.4)	4.5 (0.4)	4.6 (0.3)
10	4.7 (0.4)	4.7 (0.5)	4.5 (0.4)	4.5 (0.4)
20	4.6 (0.4)	4.5 (0.4)	4.4 (0.4)	4.4 (0.4)
30	4.5 (0.3)	4.5 (0.4)	4.3 (0.5)	4.3 (0.4)
40	4.5 (0.3)	4.5 (0.5)	4.3 (0.5)	4.3 (0.4)
50	4.5 (0.4)	4.5 (0.4)	4.3 (0.4)	4.3 (0.4)
60	4.5 (0.3)	4.5 (0.4)	4.3 (0.5)	4.3 (0.4)

Urine volume and osmolality

Two factor repeated measures ANOVA on urine volume data showed no main effect of trial ($P = 0.216$), a main effect of time ($P = 0.001$) but no interaction ($P = 0.330$) (Table 4.5).

Two factor repeated measures ANOVA on urine osmolality data showed no main effect of trial (P = 0.680), a main effect of time (P = 0.002) but no interaction (P = 0.895) (Table 4.6).

Table 4.5 Urine volume (ml) before and after ingestion of 0, 2, 5 and 10% glucose solutions. Values are mean (SD).

Time	0% glucose	2% glucose	5% glucose	10% glucose
Pre-	206 (171)	265 (163)	247 (170)	181 (132)
Post-	620 (198)	493 (196)	568 (284)	482 (198)

Table 4.6 Urine osmolality (mosm kg^{-1}) before and after ingestion of 0, 2, 5 and 10% glucose solutions. Values are mean (SD).

Time	0% glucose	2% glucose	5% glucose	10% glucose
Pre-	480 (307)	473 (327)	426 (246)	465 (267)
Post-	175 (36)	191 (48)	162 (58)	189 (59)

Discussion

The main finding of this study is that ingestion of a glucose solution with a high energy density and osmolality resulted in a reduction in plasma volume, estimated from measured haemoglobin concentrations and haematocrit, that is most likely due to the net secretion of water into the small intestine.

Assuming that extracellular fluid accounts for one third of total body water and plasma volume constitutes one quarter of the extracellular fluid (Sawka, 1990), a 75 kg males total body water, extracellular fluid volume and plasma volume will be approximately 45, 15 and 3.75 litres respectively. The average reduction in plasma volume over the 60 minute period investigated in this study following ingestion of the highest osmolality solution amounted to 2.2%. This is equivalent to a reduction in plasma volume of approximately 83 ml in a 75 kg male. If total extracellular fluid is

reduced to a similar level as plasma volume this equates to a decrease in extracellular fluid volume of approximately 330 ml. It would require approximately 700 ml of pure water to dilute the 10% glucose solution to isotonicity. However, as water will be being absorbed following ingestion, the actual volume of pure water needed to dilute the 10% glucose solution to isotonicity in the small intestine will be less than 700 ml. It can, therefore, be concluded that the majority of water secreted into the small intestine following ingestion of hypertonic solutions is derived from the extracellular fluid.

A significant moderate negative correlation was observed between percentage change in plasma volume and percentage change in heart rate (Pearson correlation = -0.512, $P < 0.001$) and heart rate was significantly elevated on the 10% glucose trial when plasma volume was significantly lowered. This supports the conclusion that ingestion of hypertonic solutions reduces the volume of extracellular fluid.

A previous study has indicated that a dilute hypotonic solution (186 ± 1 mosm kg^{-1}) perfused directly into the intestine resulted in a more favourable rate of water absorption as fluid absorption was 17% faster when compared to a hypertonic solution (403 ± 3 mosm kg^{-1}) (Shi *et al.* 1994). In the present study, a 2% glucose solution with an osmolality of 111 ± 1 mosm kg^{-1} resulted in significant increases in blood volume 20 minutes and 30 minutes after ingestion. These results suggest that hypotonic solutions provide a greater rate of fluid absorption when compared to hypertonic solutions when drinks are ingested rather than perfused directly into the intestine.

Leiper and Maughan (1986) reported that perfusion of a hypertonic solution with an osmolality of 488 ± 53 mosm kg^{-1} in the jejunum resulted in net water secretion into the small intestine. Ingestion of a hypertonic solution in the present study resulted in significant decreases in plasma volume suggesting that ingestion of strongly hypertonic solutions results in a temporary net movement of water from the extracellular fluid into the intestinal lumen. This conclusion is supported by the serum osmolality data which was significantly greater on the 10% glucose trial than on the 0 and 2% glucose trials at a number of time points.

The results of this study are more convincing than those reported in chapter 3. This is likely to be due to a greater level of control being employed in this study. Drink temperature and ambient temperature were controlled in this study to a greater degree than in the study reported in chapter 3. In addition, the drinks used in the previous study were commercially available and differed not only in terms of osmolality but electrolyte composition and the type of carbohydrate present in the drink.

The results of this study suggest that ingestion of a hypotonic solution would be the most beneficial during exercise as it provided the most favourable rate of fluid uptake of the four solutions investigated. It has been shown that dehydration amounting to approximately 4% body mass leads to a reduction in blood flow to the exercising muscles of 8-14% as well as a reduction in forearm blood flow of approximately 40% (Gonzalez-Alonso *et al.* 1998). This results in a decreased ability to provide oxygen to exercising muscles as well as impairment of thermoregulation. Hypotonic solutions, and the fast rate of fluid absorption that their ingestion provides, are, therefore, likely to be of benefit during exercise. It would also seem that ingestion of an energy dense, high osmolality solution would not be ideal during exercise as the reduction in plasma volume observed increases heart rate and could potentially inhibit the ability of the cardiovascular system to transport heat to the periphery.

It has been suggested that hypertonic solutions are ineffective rehydration beverages (Leiper, 1998) due to the reduced rate of water uptake that occurs as a result of the secretion of water into the intestinal lumen. Current advice suggests that in order to properly rehydrate after exercise, an individual should drink a volume greater than that of body mass lost during exercise (Shirreffs *et al.* 1996) and that the drink consumed should be moderately hypotonic as this produces the greatest rate of fluid absorption (Leiper, 1998). However, as the results of this study suggest that hypotonic solutions result in a rapid acute increase in blood volume it is possible that, when large volumes of a hypotonic solution are ingested, this may result in an acute decrease in plasma arginine vasopressin and aldosterone concentrations (Kenefick *et al.* 2000; Melin *et al.* 1987). In terms of efficiency of rehydration, this may result in increased urine production and water loss. As ingestion of strongly hypertonic carbohydrate solutions results in a decrease in plasma volume, when large volumes are ingested, plasma arginine vasopressin and aldosterone concentrations may be

maintained or increased and have a potential positive effect on the efficiency of rehydration by aiding water retention in the kidneys.

In summary, the results of this study suggest that ingestion of strongly hypertonic glucose solutions results in a decrease in estimated plasma volume most likely due to a temporary net secretion of water from the blood into the intestinal lumen.

Chapter 5

The effectiveness of different glucose solutions on post-exercise rehydration

Introduction

Dehydration, as a result of water loss, is partly responsible for the onset of fatigue during prolonged exercise in the heat (Armstrong *et al.* 1985; Astrand and Saltin, 1964; Gonzalez-Alonso *et al.* 1997; Saltin, 1964). It is, therefore, important to replace water and electrolytes lost during exercise as effectively as possibly, especially if a further bout of exercise is to be performed.

In order to properly rehydrate following exercise a drink volume greater than body mass lost during exercise is needed (Shirreffs *et al.* 1996). Plain water is not considered to be a good rehydration solution as its ingestion results in a rapid acute reduction in plasma osmolality and serum sodium concentration causing a cessation of drinking and a diuresis (Nose *et al.* 1988a; Nose *et al.* 1988b).

Perfusion of hypertonic solutions has been shown to result in a net secretion of water and electrolytes into the intestinal lumen (Leiper and Maughan, 1986). For this reason, it has been suggested that hypertonic solutions may be ineffective rehydration beverages (Leiper, 1998). This may be the case during exercise however, the reduced rate of fluid uptake that results from the ingestion of hypertonic solutions may render them effective rehydration beverages in the post-exercise period.

Gonzalez-Alonso *et al.* (1992) showed that the presence of 6% carbohydrate and 20 mmol L^{-1} sodium in a rehydration solution was more effective than a caffeinated diet cola and water following a two hour rehydration period after exercise that induced a reduction in body mass of approximately 2.5%. Costill & Sparks (1973) reported that a hypertonic glucose-electrolyte solution (10.6g glucose per 100ml and 22 mEq L^{-1} sodium) was more effective in restoring whole body water levels than de-mineralized water, although neither solution restored body water to pre-dehydration levels. Lambert *et al.* (1992) observed that a 10% carbohydrate solution was as effective in restoring whole body water levels as a non-carbohydrate solution after exercise induced dehydration. Both of these studies employed a drink volume that amounted to that of body mass lost and this was ingested at a slow rate over a number of hours so subjects were dehydrated for the duration of the trials and can potentially explain why no differences between the trials was observed.

The results of the study reported in chapter 4 of this thesis showed that ingestion of a hypertonic glucose solution resulted in a reduction in estimated plasma volume. Following a period of exercise, the ingestion of large volumes of a hypertonic solution may result in a more gradual return to pre-dehydration levels of plasma volume and serum osmolality relative to a hypotonic solution. This could increase or maintain circulating levels of arginine vasopressin and aldosterone and may result in hypertonic solutions being effective rehydration beverages.

The aim of this study was to examine the effectiveness of a hypertonic glucose-electrolyte solutions on restoring and maintaining fluid balance following a period of exercise-induced dehydration.

Materials and Methods

Six healthy male subjects with a mean age of 26 ± 5 years, height of 173 ± 6 cm, body mass of 72.12 ± 5.87 kg and a $\dot{V}O_{2peak}$ of 3.6 ± 0.7 L min^{-1} volunteered to participate in this investigation. Subjects reported to the laboratory following a fasting period of at least 6 hours and after having consumed approximately 500 ml of water 2 hours prior to their arrival. Experimental trials were separated by a period of at least seven days, began at the same time in the morning and subjects followed similar physical activity and nutritional patterns for 24 hours prior to the start of each trial.

Two preliminary trials were performed prior to beginning the experimental trials. In the first of these, peak oxygen uptake ($\dot{V}O_{2peak}$) was determined as described in the general methods section of this thesis. During the second preliminary trial, subjects completed the dehydration and rehydration procedures that they would undertake in the experimental trials but were then free to leave the laboratory.

Each subject participated in three experimental trials and a solution with a different glucose concentration and osmolality was ingested during each trial. The order of experimental trials was randomly assigned using a Latin square design. Solutions contained distilled water, one in seven parts lemon squash and 0, 2 or 10% glucose. Electrolyte content of the solutions was 32 ± 1 mmol L^{-1} Na^+, 0.4 ± 0.1 mmol L^{-1} K^+

and 27 ± 2 mmol L^{-1} Cl^- giving osmolalities of 79 ± 4, 193 ± 5 and 667 ± 12 mosm kg^{-1} for the 0, 2 and 10% glucose solutions respectively.

Following arrival at the laboratory, subjects sat upright in a comfortable environment for a period of 15 minutes before a blood sample was obtained from an antecubital vein via venopuncture. A urine sample was collected before a subjective feelings questionnaire (Appendix B) was completed.

Subjects were weighed nude to the nearest 10 g on a digital scale (Adam Equipment, United Kingdom) before undertaking the dehydration protocol, which consisted of intermittent cycle exercise at an intensity of 58 ± 2 % $\dot{V}O_{2peak}$ at an ambient temperature of 35.3 ± 0.2 °C and a relative humidity of 59 ± 2 %. Subjects exercised for a period of 10 minutes before resting for 5 minutes, during which time nude body mass was measured, after drying as thoroughly as possible, to ascertain the extent of dehydration induced. When subjects approached a level of 2% dehydration, exercise was halted and the subjects were given 10 minutes to shower before a final nude body mass measurement was made. Subjects exercised on average for a period of 45 ± 8 minutes and lost 1.35 ± 0.15 kg during the dehydration phase. This is equivalent to 1.9 ± 0.1 % body mass. 15 minutes after completing the exercise, subjects sat in a room maintained at 23.8 ± 0.6 °C for a further 15 minutes during which time a cannula was inserted into a superficial forearm vein. This remained in position for the duration of the trial and was kept patent between sample collection by flushing with heparinised isotonic saline. Following this 15 minute period of upright rest, i.e. 30 minutes after cessation of exercise, blood and urine samples were obtained and a subjective feelings questionnaire completed.

The test drink was given in a volume that amounted to 150% of body mass loss and was given in four equal aliquots over a period of 60 minutes. One subject was unable to drink the required volume during his first trial and drank 130% of body mass loss during all trials. The responses of this subject were not different from those of the other five subjects, so he was not excluded from the results. Drinks were warmed to a temperature of 37 ± 0°C to avoid the peripheral vasoconstriction that may result from ingesting cool solutions (Imms and Lighten, 1989). Blood and urine samples were

collected 0, 1, 2, 3, 4 and 6 hours after the rehydration period. Subjective feelings questionnaires were also collected at these time points. Subjects remained seated in an upright position for 15 minutes prior to collection of blood samples so that previously reported changes in blood and plasma volume were avoided (Hagen *et al.* 1978; Shirreffs *et al.* 1994).

Sample analysis

Blood samples were analysed for haemoglobin concentration, haematocrit and blood glucose concentration. Haemoglobin concentrations and haematocrit were used to estimate changes in blood, red cell and plasma volumes as described by Dill and Costill (1974).

Serum samples were analysed for osmolality, sodium concentration, potassium concentration and chloride concentration.

Urine samples were analysed for osmolality, sodium concentration, potassium concentration and chloride concentration.

All analysis was performed as described in the general methods section of this thesis.

Statistical analysis

All data were found to be normally distributed using the Kolmogorov-Smirnov test and are, therefore, presented as mean ± standard deviation. Data were analysed using two-factor repeated measures ANOVA. One-factor ANOVA followed by Tukey or Dunnetts pairwise comparisons were used as parametric post tests, depending on whether a clear control time point was present.

Statistical analysis was performed using SPSS 12.0 for windows.

Results

Subjects pre-exercise body mass was the same on all trials (Table 5.1). Body mass lost during the dehydration phase was consistent at 1.35 ± 0.15 kg (P = 0.994) which is equivalent to a reduction in body mass of 1.9 ± 0.1 % (P = 1.000). Drink volume was the same on all trials (P = 0.911) and amounted to 1962 ± 247 ml.

Pre-trial data is shown in table 5.1. Baseline measures were within the expected normal range for subjects following a period of fasting with the exception of serum potassium concentrations which were higher than expected, possibly due to the method of sample collection (Geigy Scientific Tables, 1962). Pre-trial serum osmolality, serum sodium concentration and urine osmolality data collected in this study suggested that subjects were adequately hydrated at the onset of each trial (Armstrong *et al.* 1994; Shirreffs and Maughan, 1998b).

Table 5.1 Pre-trial body mass, Hb concentration, haematocrit, blood glucose concentration, serum osmolality, serum sodium concentration, serum potassium concentration, serum chloride concentration and urine osmolality on all trials (Mean (SD)).

Measure	0% glucose	2% glucose	10% glucose	P-value
Body mass (kg)	72.37 (6.01)	72.01 (5.97)	71.91 (5.64)	0.992
Hb concentration (g L^{-1})	150 (8)	147 (12)	146 (11)	0.776
Haematocrit (%)	45 (2)	44 (2)	45 (2)	0.843
Blood glucose concentration (mmol L^{-1})	5.0 (0.6)	5.1 (0.5)	5.2 (0.5)	0.835
Serum osmolality (mosm kg^{-1})	277 (5)	279 (3)	278 (4)	0.537
Serum sodium concentration (mmol L^{-1})	142 (3)	140 (2)	140 (2)	0.771
Serum chloride concentration (mmol L^{-1})	102 (1)	101 (4)	101 (3)	0.805
Serum potassium concentration (mmol L^{-1})	5.9 (0.7)	5.8 (0.7)	6.2 (0.8)	0.736
Urine osmolality (mosm kg^{-1})	504 (169)	528 (294)	521 (244)	0.984

The sodium concentration (P = 0.069), potassium concentration (P = 0.249) and chloride concentration (P = 0.605) of the ingested solutions was the same during all trials (Table 5.2).

Table 5.2 Drink characteristics (Mean (SD)).

Glucose content (%)	Na^+ concentration (mmol L^{-1})	Cl^- concentration (mmol L^{-1})	K^+ concentration (mmol L^{-1})	Osmolality (mosm kg^{-1})
0	32 (1)	27 (2)	0.5 (0.1)	79 (4)
2	32 (1)	26 (3)	0.4 (0.1)	193 (5)
10	31 (0)	27 (2)	0.4 (0.1)	667 (12)

Urine output and net fluid balance

Two factor repeated measures ANOVA on urine volume data showed a main effect of trial (P = 0.008), time (P < 0.001) and interaction (P = 0.039). On all trials, the greatest urine output occurred in the hour following the rehydration period (Figure 5.1). Urine volume at this time point was 585 ± 67, 528 ± 101 and 347 ± 121 for the 0, 2 and 10% glucose trials respectively and was lower on the 10% glucose trial compared to the 0% (P = 0.002) and 2% (P = 0.017) glucose trials. 6 hours after the end of the rehydration period, total urine output was not different (P = 0.228) between the trials (Table 5.3).

Figure 5.1 Urine output (ml) over time. Points are mean ± SD. Data is shown in Appendix E. * denotes 0, 2 and 10% time points significantly different ($P < 0.05$) from post-exercise time point.

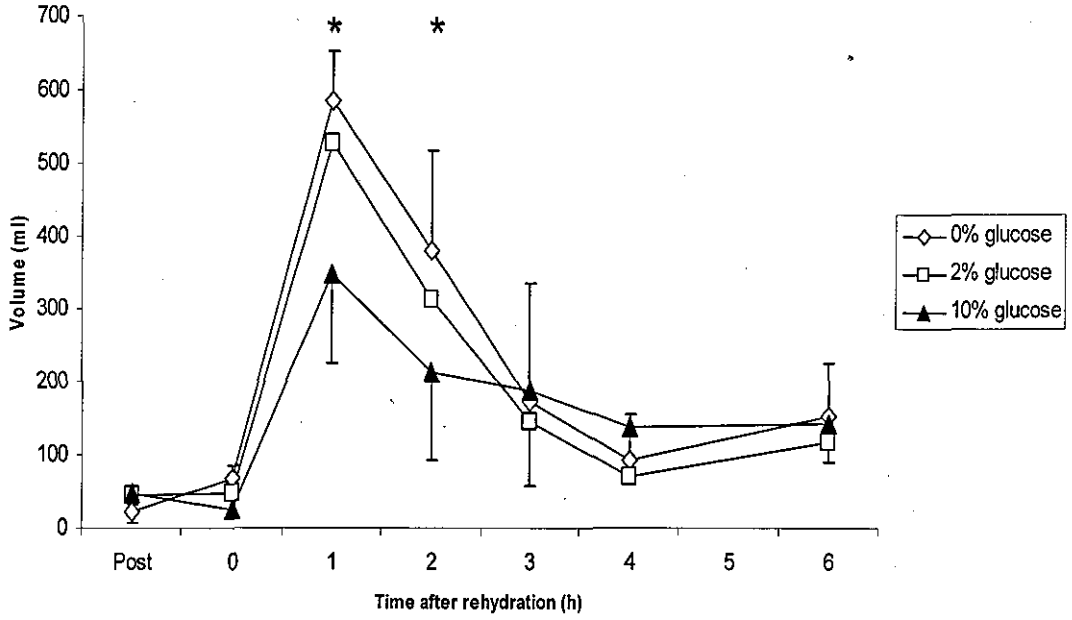


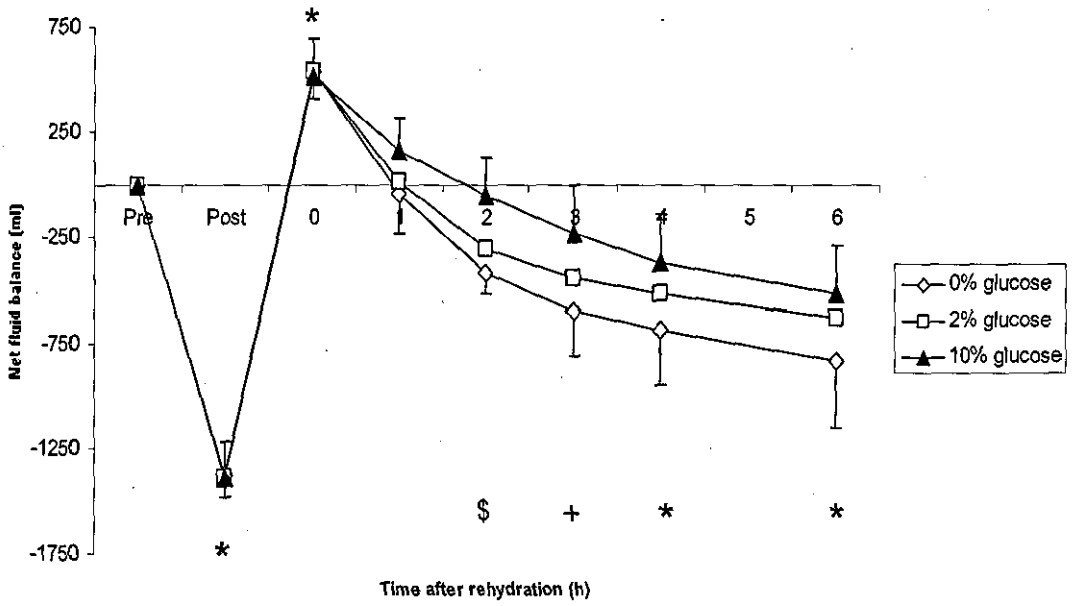
Table 5.3 Cumulative volume (ml) of urine produced following the rehydration period. Values are mean (SD).

Time after rehydration (h)	0% glucose	2% glucose	10% glucose
0	90 (15)	91 (49)	72 (48)
1	674 (75)	619 (93)	419 (102)
2	1053 (92)	932 (268)	631 (161)
3	1225 (240)	1076 (415)	818 (180)
4	1318 (297)	1146 (446)	955 (230)
6	1470 (328)	1263 (459)	1098 (252)

Net fluid balance (Figure 5.2) was calculated using data for sweat loss, drink volume ingested and urine production. Two factor repeated measures ANOVA showed a main effect of trial ($P = 0.010$), time ($P < 0.001$) and interaction ($P = 0.004$). Subjects were in negative fluid balance ($P < 0.05$) immediately after the dehydration procedure and in positive fluid balance ($P < 0.05$) immediately after the rehydration period during all

trials. During the 0% glucose trial, subjects returned to a state of negative fluid balance 2 hours after rehydration and remained in this state for the duration of the trial ($P < 0.05$). During the 2% glucose trial, subjects returned to negative fluid balance 3 hours after rehydration ($P < 0.05$). Subjects remained in positive fluid balance for one hour longer on the 10% glucose trial than on the 2% glucose trial.

Figure 5.2 Net fluid balance (ml) following ingestion of 0, 2 and 10% glucose solutions. Points are mean ± SD. Data is shown in Appendix E. * denotes 0, 2 and 10% glucose time points significantly different from pre-exercise. $ denotes 0% glucose time point significantly different from pre-exercise. + denotes 0 and 2% glucose time points significantly different from pre-exercise.



Fraction of ingested fluid retained

The percentage of ingested drink that was retained was calculated using drink volume and urine output data and is shown in Table 5.4. Two factor repeated measures ANOVA showed a main effect of trial ($P = 0.003$), time ($P < 0.001$) and interaction ($P = 0.003$). 6 hours after the end of the rehydration period, the fraction of ingested fluid retained during the 0% glucose trial was less ($P = 0.040$) than during the 10% glucose trial.

Table 5.4 Fraction of ingested fluid (%) retained after ingestion of 0, 2 and 10% glucose solutions. Values are mean (SD). * indicates 0% glucose trial significantly lower (P = 0.040) than 10% glucose trial.

Time after rehydration (h)	0% glucose	2% glucose	10% glucose
0	97 (1)	98 (1)	99 (1)
1	67 (7)	71 (6)	81 (6)
2	48 (3)	56 (8)	70 (6)
3	39 (8)	49 (12)	60 (7)
4	35 (10)	46 (13)	53 (8)
6	27 (13)*	40 (14)	46 (9)

Urinary electrolyte excretion

Two factor repeated measures ANOVA on total urinary sodium excretion showed no main effect of trial ($P = 0.251$), a main effect of time ($P < 0.001$) but no interaction ($P = 0.079$) (Table 5.5).

Two factor repeated measures ANOVA on total urinary potassium excretion showed no main effect of trial ($P = 0.146$), a main effect of time ($P < 0.001$) but no interaction ($P = 0.066$) (Table 5.5).

Two factor repeated measures ANOVA on total urinary chloride excretion showed no main effect of trial ($P = 0.137$), a main effect of time ($P < 0.001$) but no interaction ($P = 0.064$) (Table 5.5).

Table 5.5 Cumulative urinary excretion (mmol) of sodium, potassium and chloride following ingestion of 0, 2 and 10% glucose solutions. Values are mean (SD).

Time after rehydration (h)	0% glucose	2% glucose	10% glucose
Sodium			
0	8.3 (0.9)	9.0 (5.1)	6.9 (7.2)
1	18.7 (3.4)	17.7 (8.8)	13.2 (6.8)
2	26.9 (6.9)	26.0 (9.8)	19.0 (7.9)
3	34.9 (10.8)	33.7 (10.6)	24.9 (9.4)
4	44.6 (14.4)	41.8 (11.2)	31.9 (9.5)
6	64.2 (20.4)	58.1 (14.1)	45.2 (12.0)
Potassium			
0	9.1 (2.4)	8.3 (3.4)	7.1 (5.1)
1	23.0 (7.1)	17.1 (6.9)	13.4 (5.3)
2	31.3 (9.2)	25.1 (8.6)	18.4 (6.6)
3	37.8 (11.8)	31.0 (9.9)	25.5 (8.3)
4	43.7 (12.4)	36.3 (10.7)	31.9 (8.1)
6	54.5 (17.6)	44.9 (12.6)	42.6 (10.0)
Chloride			
0	6.3 (1.5)	7.3 (5.3)	5.9 (7.5)
1	19.7 (5.5)	16.2 (9.4)	11.3 (7.8)
2	26.9 (7.4)	22.4 (10.4)	16.6 (8.5)
3	32.9 (9.8)	29.5 (10.5)	22.3 (11.0)
4	39.9 (11.9)	36.1 (11.3)	27.7 (11.1)
6	52.6 (12.7)	47.5 (12.8)	37.1 (12.5)

Estimated changes in blood, red cell and plasma volumes

Two factor repeated measures ANOVA on blood volume data showed no main effect of trial ($P = 0.490$), a main effect of time ($P < 0.001$) and interaction ($P = 0.033$).

Blood volume was significantly increased from baseline values at the end of the rehydration period on the 2% glucose trial ($P = 0.017$) but no significant deviations ($P > 0.05$) from baseline values were detected on the other trials (Figure 5.3).

Two factor repeated measures ANOVA on plasma volume data showed no main effect of trial ($P = 0.177$), a main effect of time ($P < 0.001$) and interaction ($P = 0.026$). Plasma volume was significantly increased from baseline values at the end of the rehydration period on the 2% glucose trial ($P < 0.001$) but no significant deviations ($P > 0.05$) from baseline values were detected on the other trials (Figure 5.4).

Two factor repeated measures ANOVA on red cell volume data showed no main effect of trial ($P = 0.524$), time ($P = 0.066$) or interaction ($P = 0.552$) (Figure 5.5).

Figure 5.3 Estimated percentage changes in blood volume following ingestion of 0, 2 and 10% glucose solutions. Points are mean ± SD. Data is shown in Appendix E. * denotes 2% glucose time point significantly different ($P < 0.05$) from pre-exercise.

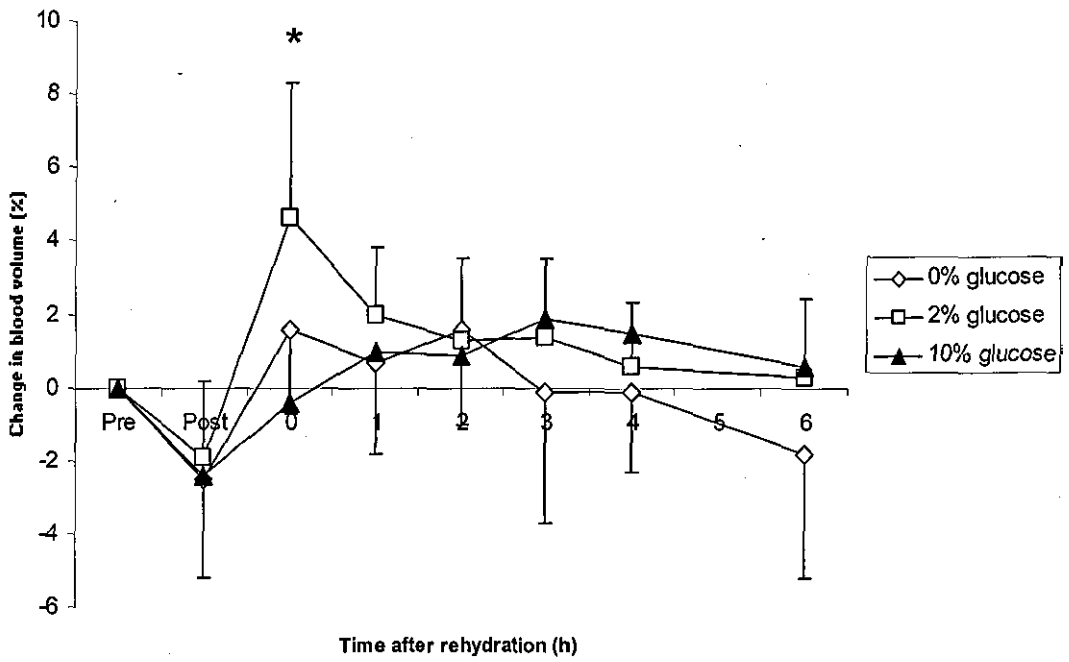


Figure 5.4 Estimated percentage changes in plasma volume following ingestion of 0, 2 and 10% glucose solutions. Points are mean ± SD. Data is shown in Appendix E. * denotes 2% glucose time point significantly different ($P < 0.05$) from pre-exercise.

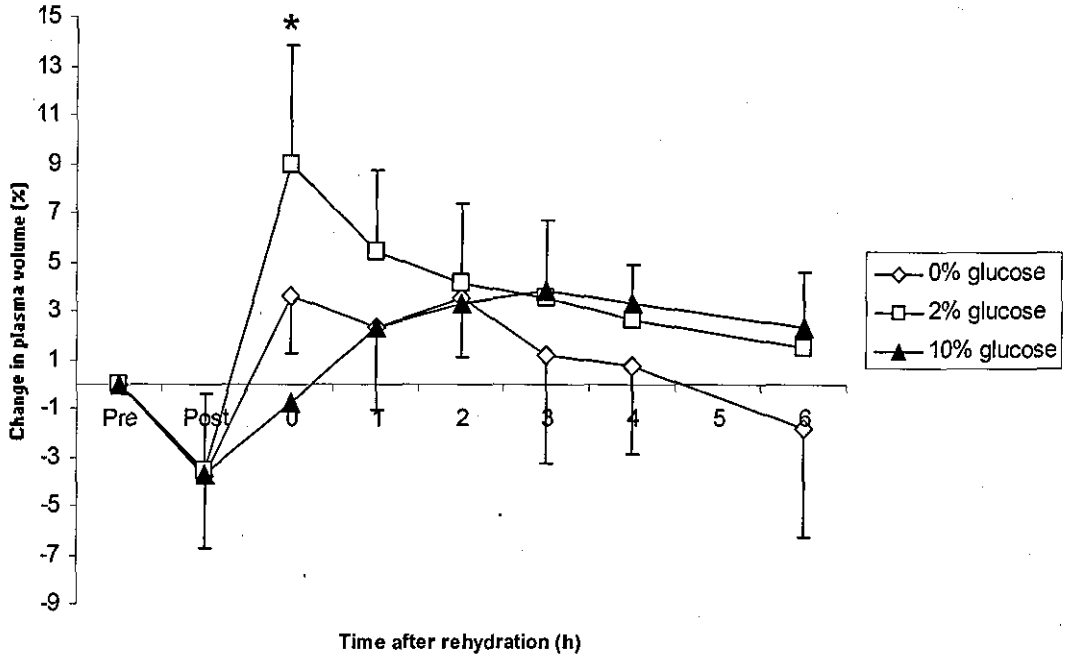
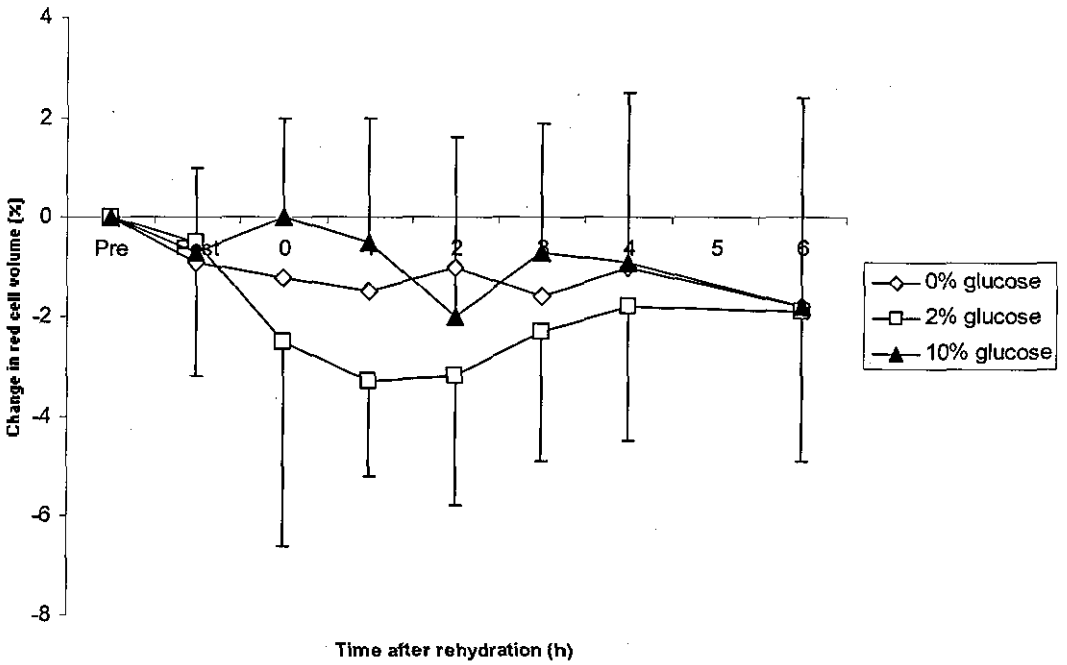


Figure 5.5 Estimated percentage changes in red cell volume following ingestion of 0, 2 and 10% glucose solutions. Points are mean ± SD. Data is shown in Appendix E.



Blood glucose concentration

Two factor repeated measures ANOVA on blood glucose data showed a main effect of trial ($P = 0.017$), time ($P < 0.001$) and interaction ($P = 0.001$). Blood glucose concentration was significantly elevated from baseline levels immediately after ($P < 0.001$) ingesting the 2% glucose solution as well as immediately after ($P < 0.001$) and 1 hour ($P = 0.001$) after ingesting the 10% glucose solution (Table 5.6).

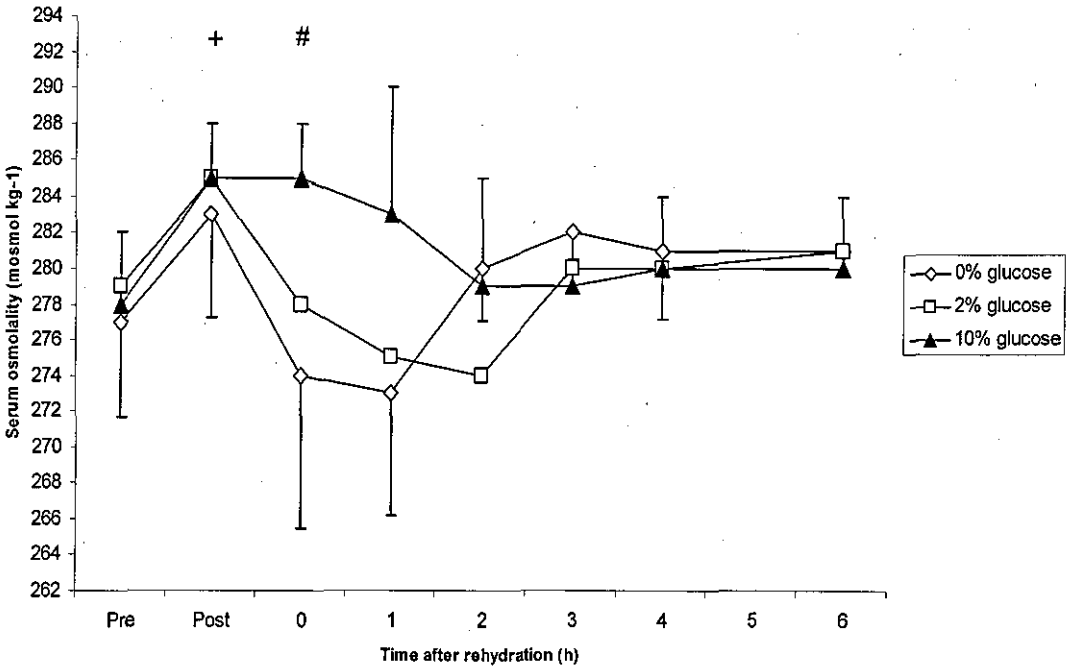
Table 5.6 Blood glucose concentration (mmol L^{-1}) following ingestion of 0, 2 and 10% glucose solutions. Values are mean (SD). * indicates time point significantly greater ($P < 0.05$) than pre-exercise value.

Time after rehydration (h)	0% glucose	2% glucose	10% glucose
Pre	5.0 (0.6)	5.1 (0.5)	5.2 (0.5)
Post	5.6 (0.7)	5.6 (0.5)	5.5 (0.4)
0	5.0 (0.6)	7.9 (0.6)*	9.6 (1.1)*
1	5.1 (0.6)	5.2 (0.8)	7.7 (1.0)*
2	5.2 (0.5)	4.9 (0.6)	6.3 (1.1)
3	5.5 (0.3)	5.3 (0.4)	5.9 (1.6)
4	5.4 (0.5)	5.6 (0.9)	5.2 (1.0)
6	5.9 (0.7)	6.0 (0.4)	6.1 (0.6)

Serum osmolality

Two factor repeated measures ANOVA on serum osmolality data showed no main effect of trial ($P = 0.509$), a main effect of time ($P = 0.001$) and an interaction ($P = 0.008$). Serum osmolality was significantly increased from baseline levels on the 2% glucose trial ($P = 0.042$) and tended to be increased on the 10% glucose trial ($P = 0.052$). Immediately after the rehydration period, serum osmolality was increased from baseline levels on the 10% glucose trial ($P = 0.045$) (Figure 5.6).

Figure 5.6 Serum osmolality (mosm kg^{-1}) following ingestion of 0, 2 and 10% glucose solutions. Points are mean ± SD. Data is shown in Appendix E. + denotes 2% glucose time point significantly different ($P < 0.05$) from pre-exercise value. # denotes 10% glucose time point significantly different ($P < 0.05$) from pre-exercise value.



Serum electrolytes

Two factor repeated measures ANOVA on serum sodium concentration data showed no main effect of trial ($P = 0.809$), a main effect of time ($P = 0.019$) but no interaction ($P = 0.329$) (Table 5.7).

Two factor repeated measures ANOVA on serum potassium concentration data showed no main effect of trial ($P = 0.755$), a main effect of time ($P < 0.001$) but no interaction ($P = 0.261$) (Table 5.7).

Two factor repeated measures ANOVA on serum chloride concentration data showed no main effect of trial ($P = 0.425$), no main effect of time ($P = 0.066$) or interaction ($P = 0.343$) (Table 5.7).

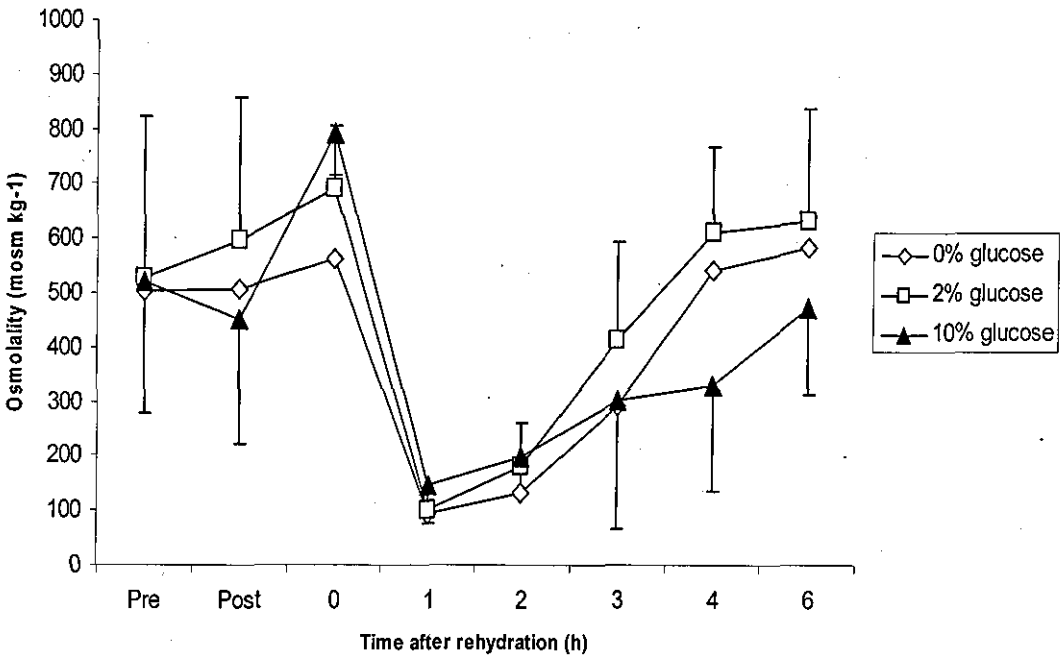
Table 5.7 Serum electrolyte concentrations (mmol L^{-1}) following ingestion of 0, 2 and 10% glucose solutions. Values are mean (SD).

Time after Rehydration (h)	0% glucose	2% glucose	10% glucose
Sodium			
Pre	141 (2)	140(2)	140 (2)
Post	142 (3)	143 (3)	142 (2)
0	139 (5)	139 (3)	140 (3)
1	141 (5)	140 (3)	142 (2)
2	141 (4)	140 (3)	141 (2)
3	141 (4)	141 (3)	141 (3)
4	140 (4)	141 (3)	141 (3)
6	140 (5)	140 (3)	142 (3)
Potassium			
Pre	5.9 (0.7)	5.8 (0.7)	6.2 (0.8)
Post	5.5 (0.7)	5.2 (0.6)	5.7 (0.6)
0	5.3 (0.7)	4.9 (0.6)	5.2 (0.4)
1	5.0 (0.4)	4.6 (0.3)	4.8 (0.5)
2	4.6 (0.4)	4.7 (0.3)	4.6 (0.4)
3	4.6 (0.1)	4.5 (0.3)	4.5 (0.4)
4	4.5 (0.3)	4.5 (0.3)	4.3 (0.3)
6	4.3 (0.1)	4.5 (0.3)	4.2 (0.3)
Chloride			
Pre	102 (1)	101 (4)	101 (3)
Post	103 (1)	102 (3)	104 (3)
0	101 (1)	101 (3)	103 (2)
1	102 (1)	102 (2)	103 (2)
2	103 (2)	102 (2)	104 (2)
3	103 (2)	101 (3)	102 (3)
4	102 (2)	101 (2)	102 (2)
6	101 (2)	102 (2)	101 (2)

Urine osmolality

Pre-exercise urine osmolality was 504 ± 169, 528 ± 294 and 521 ± 244 mosm kg^{-1} for the 0, 2 and 10% glucose trials respectively. Two factor repeated measures ANOVA showed no main effect of trial (P = 0.095), a main effect of time (P = 0.001) but no interaction (P = 0.157) (Figure 5.7).

Figure 5.7 Urine osmolality (mosm kg^{-1}) following ingestion of 0, 2 and 10% glucose solutions. Points are mean ± SD. Data is shown in Appendix E.

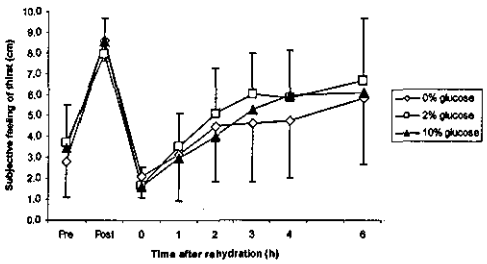


Subjective feelings questionnaires

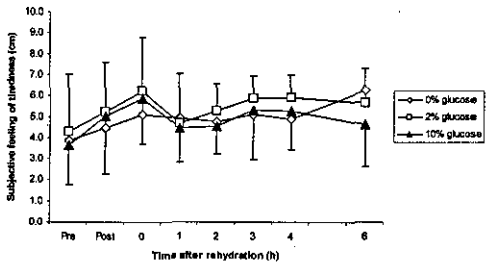
Subjects reported no significant differences at any time between the 0, 2 or 10% glucose trials in measures of thirst (P = 0.507), hunger (P = 0.197), tiredness (P = 0.733), alertness (P = 0.534), concentration (P = 0.317) or head soreness (P = 0.387). However, subjects did report a greater feeling of stomach fullness (P < 0.05) on the 10% glucose trial compared to the 0 and 2% glucose trials 0, 1 and 2 hours after the end of the rehydration period (Figure 5.8 (b)) and a greater feeling of being bloated (P < 0.05) on the 10% glucose trial compared to the 0 and 2% glucose trials 0 and 1 hours after the end of the rehydration period (Figure 5.8 (c)).

Figure 5.8 Subjective feelings (cm) of (a) thirst (b) stomach fullness (c) bloatedness (d) hunger (e) tiredness (f) alertness (g) concentration (h) head soreness following ingestion of 0, 2 and 10% glucose solutions. Points are mean ± SD. Data is shown in Appendix E. * indicates 10% glucose time point significantly greater than 0 and 2% glucose time points.

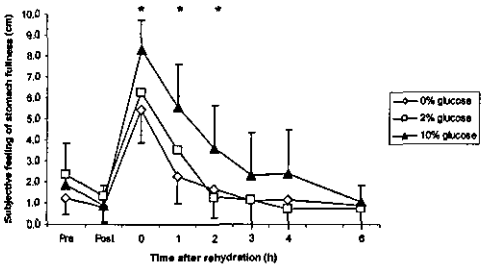
(a)



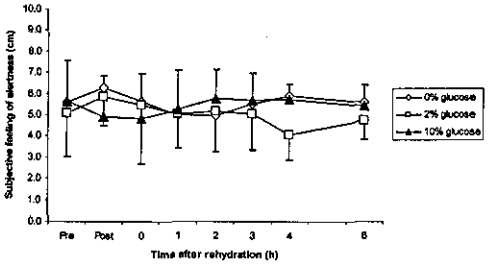
(e)



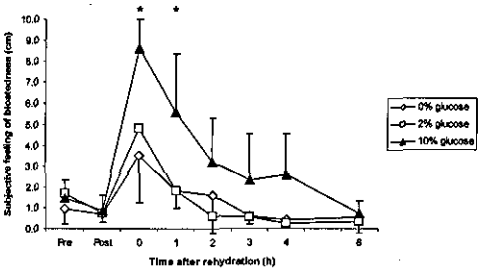
(b)



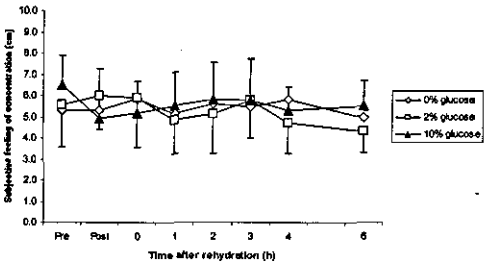
(f)



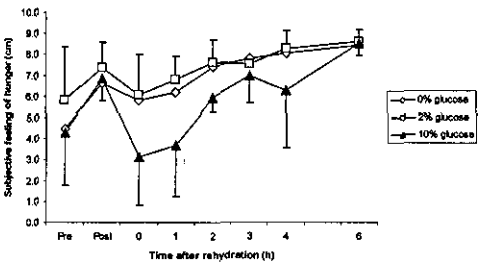
(c)



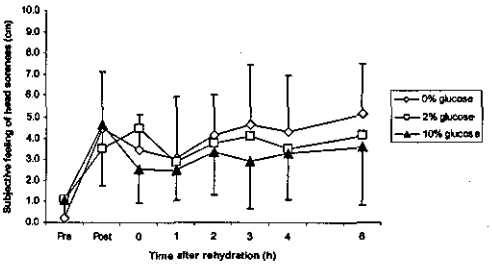
(g)



(d)



(h)



Discussion

The results of this study suggest that a hypertonic glucose-electrolyte solution with an osmolality of 667 ± 12 mosm kg^{-1} may be more effective in promoting and maintaining post-exercise rehydration than are more dilute solutions with the same electrolyte content. Following exercise-induced dehydration of 1.9 ± 0.1% body mass, subjects retained significantly more of the volume ingested during the 10% glucose trial than on the 0% glucose trial, as well as remaining euhydrated for one hour longer during the 10% glucose trial than during the 2% glucose trial.

Previous studies have demonstrated that perfusion of hypotonic carbohydrate-electrolyte solutions results in rapid acute increases in plasma volume (Gisolfi *et al.* 1992; Shi *et al.* 1994) which is largely due to active co-transport of carbohydrates and sodium leading to enhanced water uptake in the proximal small intestine (Schedl and Clifton, 1963). The present study supports these observations, as plasma volume increased relative to the pre-exercise value by 9.0 ± 4.9% immediately after the rehydration phase following ingestion of the 2% glucose solution. As expected, the same was not true following ingestion of the 10% solution which seems to offer a more gradual return to pre-dehydration levels of plasma volume, as can be seen from the estimated change in plasma volume data. Hypertonic solutions result in a net secretion of water into the small intestine (Leiper and Maughan, 1986). As the results obtained during the studies described in chapters 3 and 4 of this thesis show, ingestion of energy dense hypertonic solutions results in a reduction in extracellular fluid volume due to the osmotic gradient that is established following ingestion of these solutions. For this reason, it has been suggested that hypotonic solutions are the most effective rehydration solutions and that hypertonic solutions are ineffective in restoring and maintaining body fluid balance (Leiper, 1998). This would seem to be the case during exercise as plasma volume expansion is beneficial for cardiovascular function and thermoregulation but the results of this study show that the delay in fluid uptake following ingestion of hypertonic solutions is beneficial when considering post-exercise rehydration.

The present study suggests that ingestion of energy dense hypertonic solutions are effective for the maintenance of hydration status following exercise-induced

dehydration as subjects remained euhydrated for a longer period of time following ingestion of the hypertonic 10% glucose solution compared to the hypotonic 2% glucose solution. Urine output, although not significantly different, was greater during the 2% glucose trial compared to the 10% glucose trial in 5 of the 6 subjects that participated in this study which may be due to changes in circulating concentrations of arginine vasopressin and aldosterone.

Arginine vasopressin release is influenced by plasma osmolality and, to a lesser extent, hypovolemia. Linear regression analysis has suggested that circulating concentrations of arginine vasopressin fluctuate by 0.41 pmol L^{-1} per unit change in plasma osmolality whereas a decrease in blood volume of 8-10% in necessary to stimulate arginine vasopressin release (Baylis, 1987). It would seem that any differences in circulating arginine vasopressin concentration in the present study are likely to be due to differences in serum osmolality observed between trials rather than changes in estimated blood and plasma volume.

It is now accepted that in order to return to a state of euhydration it is necessary to allow for ongoing obligatory water losses by ingesting a volume greater than that of the body mass loss as described by Shirreffs *et al.* (1996). It is also generally accepted that, provided that an appropriate amount of fluid is ingested, sodium intake has a major role in restoring fluid homeostasis (Maughan and Leiper, 1995; Nose *et al.* 1988b; Shirreffs and Maughan, 1998a). The results of the present study suggest that the carbohydrate content and osmolality of ingested drinks do have a major role in restoring fluid balance following exercise induced dehydration. As subjects were in a state of negative fluid balance before the end of all trials it would appear that the amount of sodium ingested is still the main consideration when formulating rehydration solutions.

Although the results of the present study suggest that hypertonic glucose-electrolyte solutions are effective post-exercise rehydration solutions, it is important to consider these results in an applied sense. Ingestion of the 10% glucose solution resulted in subjects feeling more bloated following the rehydration period compared to the 0% glucose solutions. In practice, where *ad libitum* fluid intake is allowed, this may result

in a cessation of fluid ingestion before positive fluid balance has been attained, thereby hindering the rehydration process.

In conclusion, the results of this study demonstrate that a hypertonic 10% glucose-electrolyte solution with an osmolality of 667 ± 12 mmol kg^{-1} and sodium concentration of 32 ± 1 mmol L^{-1} was more effective in maintaining a state of euhydration following exercise-induced dehydration of 1.9 ± 0.1% body mass than a 2% glucose solution and a 0% glucose solution containing the same amount of sodium with osmolalities of 193 ± 5 mosm kg^{-1} and 79 ± 4 mosm kg^{-1} respectively. It is concluded that the carbohydrate content and osmolality are important factors when considering post-exercise rehydration solutions.

Chapter 6

The effectiveness of different glucose solutions on post-exercise rehydration after *ad libitum* fluid ingestion

Introduction

The results reported in chapter 5 of this thesis showed that a hypertonic glucose-electrolyte solution was more effective in restoring and maintaining whole body fluid balance following a period of exercise-induced dehydration than a hypotonic glucose-electrolyte solution and an electrolyte solution containing no glucose. However, subjects did report a greater feeling of bloatedness following ingestion of the 10% glucose solution when compared to the 0% glucose solution. In practice, this may lead to a cessation of fluid intake of a high carbohydrate solution that could reduce it's effectiveness as a rehydration solution.

Little evidence is available on *ad libitum* fluid ingestion during recovery from exercise that results in a significant level of dehydration. Wemple *et al.* (1997) subjected volunteers to a three hour *ad libitum* rehydration period, following 90 minutes of treadmill exercise in the heat that resulted in a reduction in body mass of 3%, using an artificially sweetened water or a flavoured 6% sucrose drink containing either 25 or 50 mmol L^{-1} sodium chloride. Total fluid intake amounted to 123 ± 16, 130 ± 19 and 105 ± 15% body mass lost for the sweetened water, low sodium and high sodium trials respectively. Total fluid intake was significantly greater during the low sodium trial compared to the sweetened water trial and the low sodium trial was more effective in restoring whole body water levels than both the sweetened water and high sodium trials. Maughan & Leiper (1993) observed that subjects ingested significantly greater volumes of a sports drink and an orange juice/lemonade drink than a 90 mM glucose solution and a high sodium and potassium solution in a two hour rehydration period following exercise induced dehydration. The taste of the sports drink and orange juice/lemonade drink was perceived to be better than the other two solutions. These studies demonstrate the importance of taste preference and electrolyte content of solutions in situations of *ad libitum* fluid restoration.

The rate of fluid intake in a rehydration period also appears to be an important factor when considering the effectiveness of a rehydration solution. Kovacs *et al.* (2002) observed that urine output in the first two hours following a rehydration period with a high rate of fluid ingestion of a volume amounting to body mass lost during exercise was greater when compared to a low rate of fluid ingestion. Archer & Shirreffs (2001)

observed that ingesting a volume of a sports drink equivalent to 150% body mass lost during exercise over a 30 minute period resulted in a significantly greater cumulative urine output and, consequently, subjects were in a more negative state of dehydration at the end of a four hour recovery period than when the same volume was ingested over a 90 minute period. In situations of *ad libitum* fluid ingestion, the rate at which a solution is consumed may have a large effect on the effectiveness of the solution in maintaining body fluid balance.

The aim of this study was to investigate the effectiveness of a hypertonic glucose-electrolyte solution on restoring and maintaining whole body fluid balance following a rehydration period during which time *ad libitum* fluid ingestion is allowed. The solutions used in this investigation were the same as those used in the study reported in chapter 5 of this thesis.

Materials and Methods

Six male and three female subjects with a mean age of 23 ± 2 years, height of 172 ± 8 cm, body mass of 76.84 ± 8.34 kg and a $\dot{V}O_{2peak}$ of 3.7 ± 0.8 L min^{-1} volunteered to participate in this investigation. Subjects reported to the laboratory following a fasting period of at least 6 hours and after having consumed approximately 500 ml of water two hours prior to their arrival. Subjects were unaware of the aims of this study. Experimental trials were separated by a period of at least seven days, began at the same time in the morning and subjects followed similar physical activity and nutritional patterns for 24 hours prior to the start of each trial.

Two preliminary trials were performed prior to beginning the experimental trials. In the first of these, peak oxygen uptake ($\dot{V}O_{2peak}$) was determined as described in the general methods section of this thesis. During the second preliminary trial, subjects completed the dehydration and rehydration procedures that they would undertake in the experimental trials but were then free to leave the laboratory.

Each subject participated in three experimental trials and a solution with a different glucose concentration and osmolality was ingested during each trial. The order of

experimental trials was randomly assigned using a Latin square design. Solutions contained distilled water, one in seven parts lemon squash and 0, 2 or 10% glucose. Electrolyte content of the solutions was 31 ± 1 mmol L^{-1} Na^+, 0.6 ± 0.1 mmol L^{-1} K^+ and 27 ± 1 mmol L^{-1} Cl^- giving osmolalities of 74 ± 1, 188 ± 3 and 654 ± 4 mosm kg^{-1} for the 0, 2 and 10% glucose solutions respectively. Aspartame was added to the 0% (0.1538 g L^{-1}) and 2% (0.123 g L^{-1}) glucose trials so that the sweetness of all drinks was comparable, the intention being that the taste of the drink would not have a large effect on the volume of fluid ingested.

Following arrival at the laboratory, subjects sat upright in a comfortable environment for a period of 15 minutes before a blood sample was obtained from an antecubital vein via venopuncture. A urine sample was collected before a subjective feelings questionnaire (Appendix B) was completed.

Subjects were weighed nude to the nearest 10 g on a digital scale (Adam Equipment, United Kingdom) before undertaking the dehydration protocol, which consisted of intermittent cycle exercise at an intensity of 58 ± 3 % at an ambient temperature of 35.3 ± 0.3 °C and a relative humidity of 66 ± 2 %. An absorbent sweat patch (3M, Loughborough, United Kingdom) was placed on the subjects back. When subjects reached a level of approximately 1% dehydration, this patch was removed and another positioned just below the previous patch. This was then removed at the end of exercise. Sweat patches were put into a syringe and sweat was collected into a sealed epindorph tube and retained for analysis of Na^+, K^+ and Cl^- concentration. Subjects exercised for a period of 10 minutes before resting for 5 minutes, during which time nude body mass was measured, after drying as thoroughly as possible, to ascertain the extent of dehydration induced. When subjects approached a level of 2% dehydration, exercise was halted and the subjects were given 10 minutes to shower before a final nude body mass measurement was made. Subjects exercised on average for a period of 50 ± 16 minutes and lost 1.53 ± 0.18 kg during the dehydration phase. This is equivalent to 1.99 ± 0.07 % body mass. 15 minutes after the cessation of exercise, subjects sat in a room maintained at 26.1 ± 0.9 °C for a further 15 minutes during which time a cannula was inserted into a superficial forearm vein. This remained in position for the duration of the trial and was kept patent between sample collection by flushing with heparinised isotonic saline. Following this period of upright rest, i.e. 30

minutes after cessation of exercise, blood and urine samples were obtained and a subjective feelings questionnaire completed.

A 120 minute rehydration period then began during which time the test solution was drunk *ad libitum.* Subjects were given drinks bottles at intervals of 15 minutes during which time they were unaware of the volume that they were ingesting. Drinks bottles were weighed before and after each 15 minute period to determine the volume of fluid ingested. Drinks were warmed to a temperature of 37 ± 0 °C to avoid the peripheral vasoconstriction that may result from ingesting cool solutions (Imms and Lighten, 1989). Blood and urine samples were collected midway through the rehydration period, immediately after the rehydration period and 0, 1, 2, 3, and 5 hours after drinking. Subjective feelings questionnaires were also completed at the these time points. Subjects remained seated in an upright position for 15 minutes prior to the collection of a blood sample so that previously reported postural changes in blood and plasma volume were avoided (Hagen *et al.* 1978; Shirreffs *et al.* 1994).

Sample analysis

Blood samples were analysed for haemoglobin concentration, haematocrit and blood glucose concentration. Haemoglobin concentrations and haematocrit were used to estimate changes in blood, red cell and plasma volumes as described by Dill and Costill (1974).

Serum samples were analysed for osmolality, sodium concentration, potassium concentration and chloride concentration.

Urine samples were analysed for osmolality, sodium concentration, potassium concentration and chloride concentration.

Sweat samples were analysed for osmolality, sodium concentration, potassium concentration and chloride concentration. Coefficient of variations for sweat electrolyte concentrations are shown in table 6.1.

Table 6.1 Mean, SD and Coefficient of variation (%) of duplicates obtained for sweat electrolyte concentrations

Assay	n	Mean	SD	CV
Sweat sodium concentration (mmol L^{-1})	30	61.0	19.0	2.6
Sweat chloride concentration (mmol L^{-1})	30	53.6	24.0	4.4
Sweat potassium concentration (mmol L^{-1})	30	5.2	0.9	3.6

All analysis was performed as described in the general methods section of this thesis.

Statistical analysis

Data that were found to be normally distributed using the Kolmogorov-Smirnov test are presented as mean ± standard deviation. This data was analysed using two-factor repeated measures ANOVA. One-factor ANOVA followed by Tukey or Dunnetts pairwise comparisons were used as parametric post tests, depending on whether a clear control time point was present.

Urine osmolality data were not normally distributed and are, therefore, presented as median (range). Data was analysed using two-factor repeated measures ANOVA. Kruskal-Wallace test followed by Mann-Whitney pairwise comparisons were used as post hoc tests to locate differences.

Statistical analysis was performed using SPSS 12.0 for windows.

In figures and tables, the 120 minute rehydration period is represented by the points "-1" and "0".

Results

Body mass lost during the dehydration phase was consistent at 1.53 ± 0.18 kg (P = 0.932) which is equivalent to a reduction in body mass of 1.99 ± 0.07 % (P = 0.696).

Pre-trial data is shown in table 6.2. Baseline measures were within the expected normal range for subjects following a period of fasting with the exception of serum potassium concentrations which were higher than expected, possibly due to the method of sample collection (Geigy Scientific Tables, 1962). Pre-trial serum osmolality, serum sodium concentration and urine osmolality data collected in this study suggested that subjects were adequately hydrated at the onset of each trial (Armstrong *et al.* 1994; Shirreffs and Maughan, 1998b).

Table 6.2 Pre-trial body mass, Hb concentration, haematocrit, blood glucose concentration, serum osmolality, serum sodium concentration, serum potassium concentration, serum chloride concentration and urine osmolality on all trials (Mean (SD)).

Measure	0% glucose	2% glucose	10% glucose	P-value
Body mass (kg)	76.64 (8.44)	77.01 (8.47)	76.88 (8.12)	0.995
Hb concentration (g L^{-1})	144 (15)	142 (13)	145 (16)	0.889
Haematocrit (%)	43 (4)	43 (4)	43 (4)	0.996
Blood glucose concentration (mmol L^{-1})	5.3 (0.3)	5.0 (0.2)	5.1 (0.3)	0.143
Serum osmolality (mosm kg^{-1})	278 (4)	280 (2)	277 (4)	0.243
Serum sodium concentration (mmol L^{-1})	139 (1)	139 (1)	139 (2)	0.950
Serum chloride concentration (mmol L^{-1})	104 (2)	104 (2)	104 (2)	0.783
Serum potassium concentration (mmol L^{-1})	6.4 (0.9)	6.5 (0.7)	5.9 (0.8)	0.197
Urine osmolality (mosm kg^{-1})	362 (242)	413 (297)	389 (226)	0.914

The sodium concentration ($P = 0.591$), potassium concentration ($P = 0.489$) and chloride concentration ($P = 0.607$) of the ingested solutions was the same during all trials (Table 6.3).

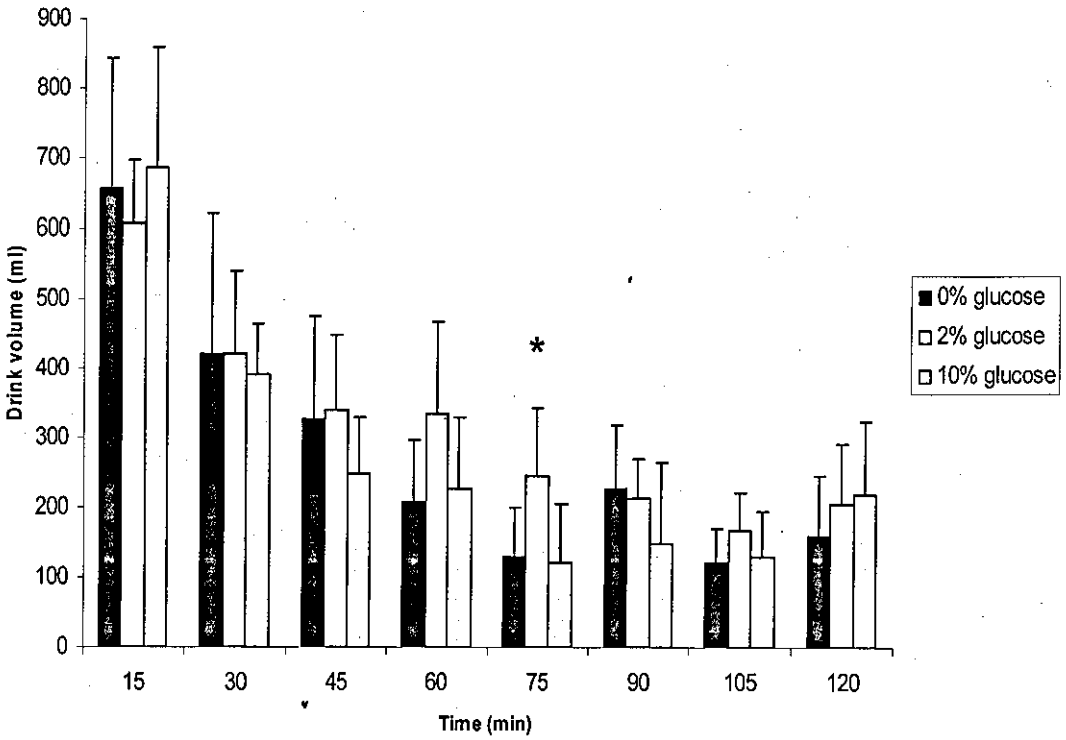
Table 6.3 Drink characteristics (Mean (SD)).

Glucose content (%)	Na^+ concentration (mmol L^{-1})	Cl^- concentration (mmol L^{-1})	K^+ concentration (mmol L^{-1})	Osmolality (mosm kg^{-1})
0	31 (1)	27 (1)	0.6 (0.1)	74 (1)
2	31 (1)	26 (1)	0.6 (0.1)	188 (3)
10	31 (1)	27 (1)	0.6 (0.1)	654 (4)

Fluid intake

The volume of test solution consumed during each 15 minute period is presented in Figure 6.1. Total fluid intake over the 120 minute rehydration period was not different between trials (P = 0.170) and amounted to 2254 ± 516 ml, 2539 ± 436 ml and 2173 ± 252 ml on the 0, 2 and 10% glucose trials respectively. This was equivalent to 150 ± 36 %, 165 ± 26 % and 143 ± 21 % body mass lost during exercise for each trial. Total fluid intake after 60 minutes of the rehydration period was not different between trials (P = 0.701) and amounted to 1614 ± 472 ml, 1706 ± 356 ml and 1554 ± 298 ml on the 0, 2 and 10% glucose trials respectively. No differences were reported in the perception of sweetness (0%: 6.0 ± 2.3 cm; 2%: 7.1 ± 2.1 cm; 10%: 6.8 ± 2.8 cm (P = 0.595)), bitterness (0%: 4.3 ± 2.5 cm; 2%: 3.8 ± 2.5 cm; 10%: 4.1 ± 3.2 cm (P = 0.905)) or pleasantness (0%: 4.7 ± 2.2 cm; 2%: 5.5 ± 2.8 cm; 10%: 3.3 ± 2.5 cm (P = 0.192)) of the drinks.

Figure 6.1 Drink volume (ml) during each 15 minute period of the rehydration phase. Points are mean ± SD. Data is shown in Appendix F. * denotes 2 % glucose time point significantly different (P < 0.05) from 10% glucose time point.



Urine output and net fluid balance

Two factor repeated measures ANOVA on urine volume data showed no main effect of trial (P = 0.052), a main effect of time (P < 0.001) and an interaction (P < 0.001). Urine volume was significantly greater (P < 0.05) than post-exercise values immediately after and one hour after the rehydration period on the 0% and 2% glucose trials but urine volume was not significantly elevated (P < 0.05) from post-exercise values until one, two and three hours after ingesting the 10% glucose drink (Figure 6.2). No difference (P > 0.05) in total urine output was observed between any of the trials but, although not significantly different, total urine volume at the end of the experimental period tended to be greater on the 2% glucose trial than on the 10% glucose trial (P = 0.100) (Table 6.4).

Figure 6.2 Urine output (ml) over time. Points are mean ± SD. Data is shown in Appendix F. * denotes 0 and 2 % glucose time points significantly different ($P < 0.05$) from post-exercise time point. # denotes 0, 2 and 10% glucose time points significantly different ($P < 0.05$) from post-exercise time point. + denotes 10% glucose time point significantly different ($P < 0.05$) from post-exercise time point.

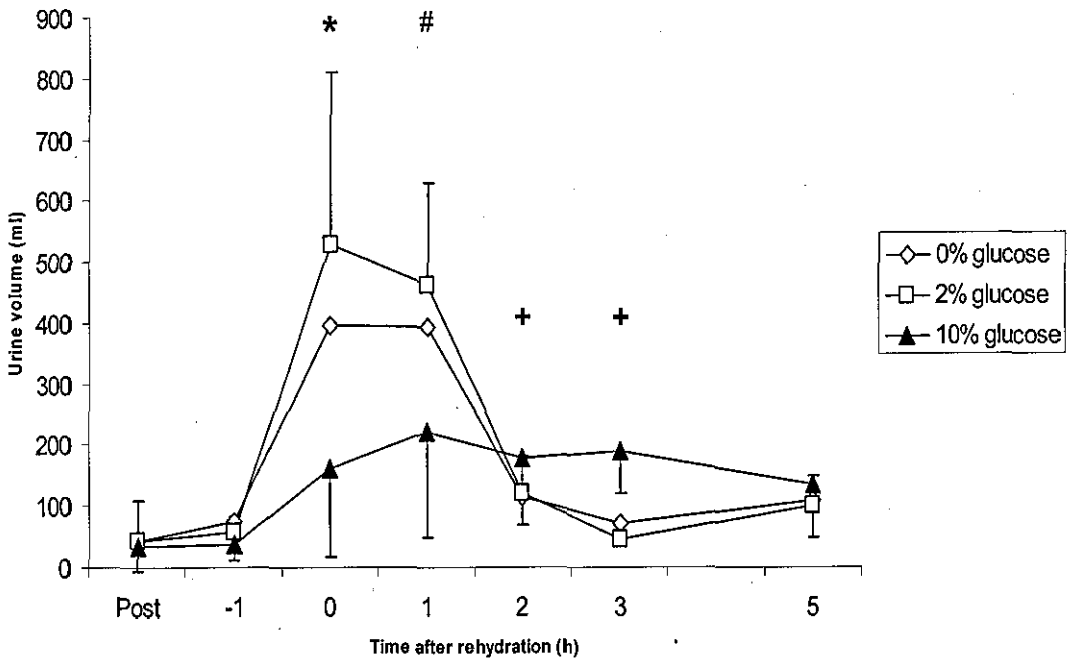
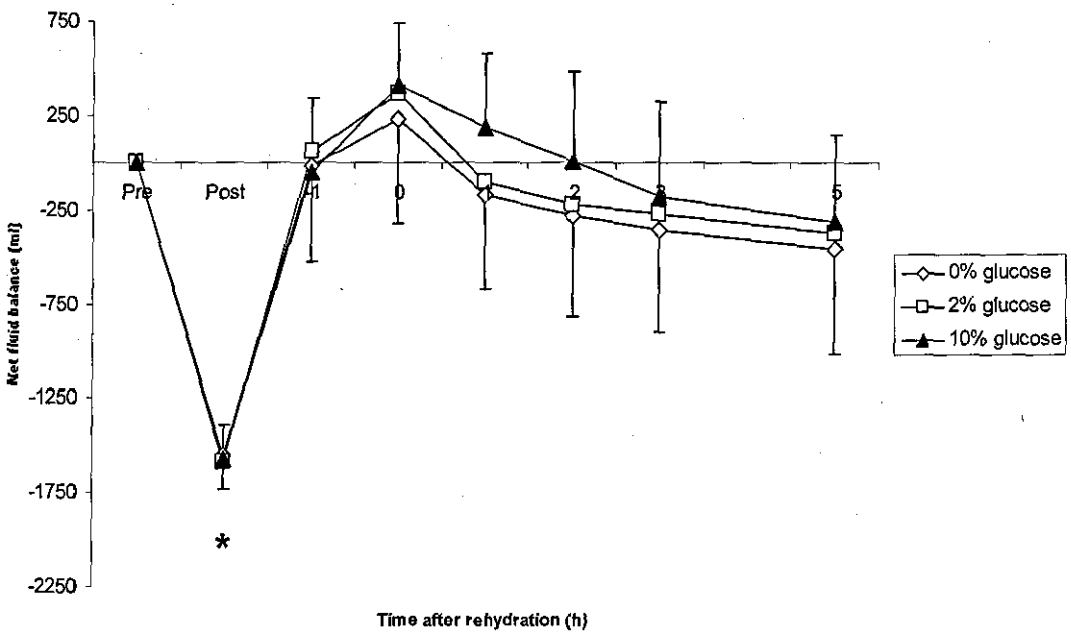


Table 6.4 Cumulative volume (ml) of urine produced following the rehydration period. Values are mean (SD).

Time after rehydration (h)	0% glucose	2% glucose	10% glucose
-1	118 (84)	100 (78)	72 (54)
0	514 (273)	630 (325)	233 (170)
1	907 (400)	1094 (427)	453 (289)
2	1023 (455)	1215 (443)	631 (337)
3	1094 (454)	1261 (451)	822 (371)
5	1202 (473)	1363 (471)	957 (347)

Net fluid balance was calculated using data for sweat loss, drink volume ingested and urine production. Two factor repeated measures ANOVA showed no main effect of trial ($P = 0.095$), a main effect of time ($P < 0.001$) and an interaction ($P = 0.013$). Subjects were in a state of negative fluid balance ($P < 0.05$) immediately after the dehydration period during all trials but this was the only significant deviation from pre-exercise values (Figure 6.3).

Figure 6.3 Net fluid balance (ml) following ingestion of 0, 2 and 10% glucose solutions. Points are mean ± SD. Data is shown in Appendix F. * denotes 0, 2 and 10% glucose time points significantly different from pre-exercise.



Fraction of ingested fluid retained

Two factor repeated measures ANOVA showed a main effect of trial ($P = 0.008$), a main effect of time ($P < 0.001$) and an interaction ($P = 0.016$). No difference ($P = 0.307$) in the fraction of ingested fluid retained was observed between the trials 5 hours after the rehydration period (Table 6.5).

Table 6.5 Fraction of ingested fluid (%) retained after ingestion of 0, 2 and 10% glucose solutions. Values are mean (SD).

Time after rehydration (h)	0% glucose	2% glucose	10% glucose
-1	96 (3)	98 (1)	98 (1)
0	78 (11)	77 (10)	91 (6)
1	59 (17)	59 (12)	81 (12)
2	54 (21)	54 (12)	72 (15)
3	50 (20)	53 (12)	63 (16)
5	45 (21)	49 (13)	57 (15)

Urine electrolyte loss and electrolyte balance

Two factor repeated measures ANOVA on total urinary sodium excretion showed a main effect of trial ($P = 0.006$), a main effect of time ($P < 0.001$) and an interaction ($P = 0.016$). No difference ($P = 0.105$) in total urinary sodium excretion was observed between the trials at the end of the experimental period (Table 6.6).

Two factor repeated measures ANOVA on total urinary chloride excretion showed a main effect of trial ($P < 0.001$), a main effect of time ($P < 0.001$) and an interaction ($P < 0.0001$). Total urinary chloride excretion was greater ($P = 0.003$) on the 0% glucose trial compared to the 10% glucose trial at the end of the experimental period (Table 6.6).

Two factor repeated measures ANOVA on total urinary potassium excretion showed a main effect of trial ($P = 0.022$), a main effect of time ($P < 0.001$) and an interaction ($P = 0.018$). No difference ($P = 0.522$) in total urinary potassium excretion was observed between the trials at the end of the experimental period (Table 6.6).

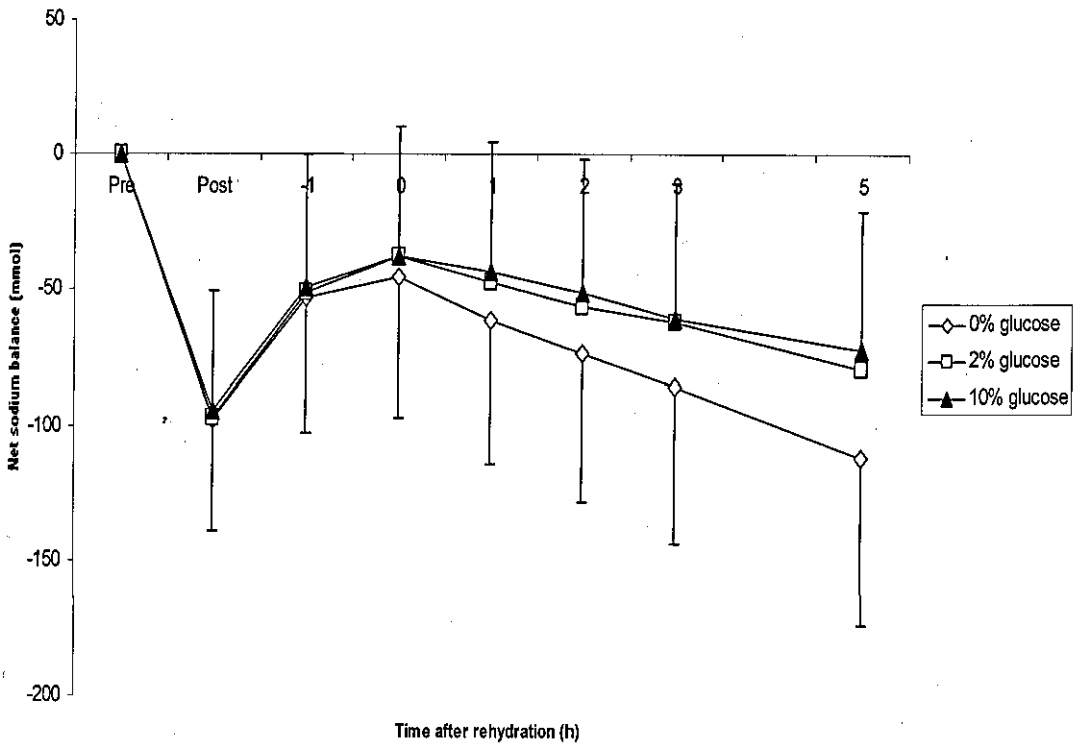
Table 6.6 Cumulative urinary excretion (mmol) of sodium, potassium and chloride following ingestion of 0, 2 and 10% glucose solutions. Values are mean (SD). * indicates time point significantly greater than 10% glucose time point. + indicates time point significantly greater than 2% glucose time point.

Time after rehydration (h)	0% glucose	2% glucose	10% glucose
Sodium			
-1	12.1 (6.7)	9.8 (5.0)	6.2 (3.7)
0	26.8 (13.4)	21.8 (9.1)	13.6 (5.7)
1	36.8 (15.5)	31.8 (12.4)	19.8 (8.4)
2	44.2 (18.9)	40.4 (14.8)	27.1 (9.5)
3	52.8 (21.7)	46.4 (15.8)	36.8 (12.2)
5	70.7 (27.4)	63.5 (20.8)	48.5 (14.4)
Chloride			
-1	9.3 (5.8)	7.0 (4.6)	4.1 (3.1)
0	20.9 (11.0)	15.6 (7.2)	9.4 (5.3)
1	30.5 (12.8)	23.7 (8.9)	14.5 (7.1)
2	38.5 (15.4)	31.1 (11.2)	19.5 (7.9)
3	46.5 (17.4)	36.1 (13.4)	25.3 (9.5)
5	60.4 (20.4)*	48.1 (15.4)	32.7 (10.8)
Potassium			
-1	7.9 (3.5)	6.9 (2.8)	5.3 (2.8)
0	20.2 (10.3)	15.0 (7.2)	11.1 (4.3)
1	30.6 (13.8)	23.3 (11.2)	16.3 (7.1)
2	36.6 (15.3)	30.0 (13.4)	22.6 (9.5)
3	41.6 (16.6)	33.7 (12.7)	31.4 (12.2)
5	50.7 (18.3)	42.1 (17.8)	43.1 (15.3)

Electrolyte balance was calculated using sweat electrolyte loss, drink volume, drink electrolyte and urinary electrolyte excretion data.

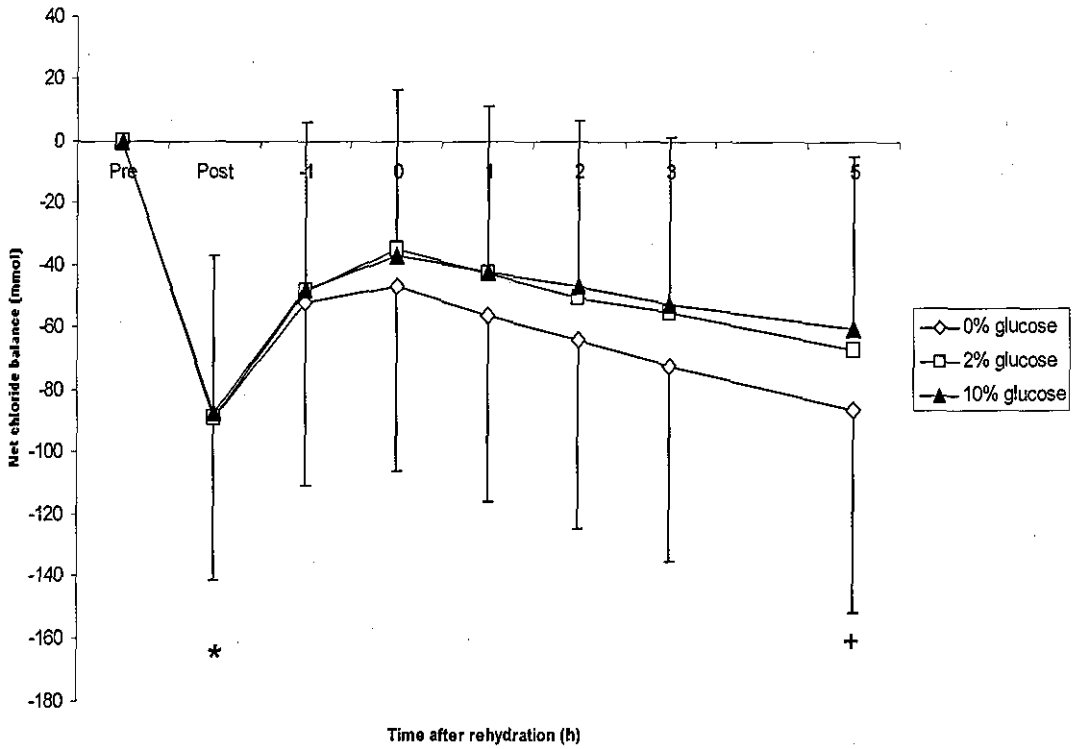
Two factor repeated measures ANOVA on sodium balance data showed no main effect of trial ($P = 0.281$), a main effect of time ($P < 0.001$) but no interaction ($P = 0.176$) (Figure 6.4).

Figure 6.4 Sodium balance (mmol) following ingestion of 0, 2 and 10% glucose solutions. Points are mean ± SD. Data is shown in Appendix F.



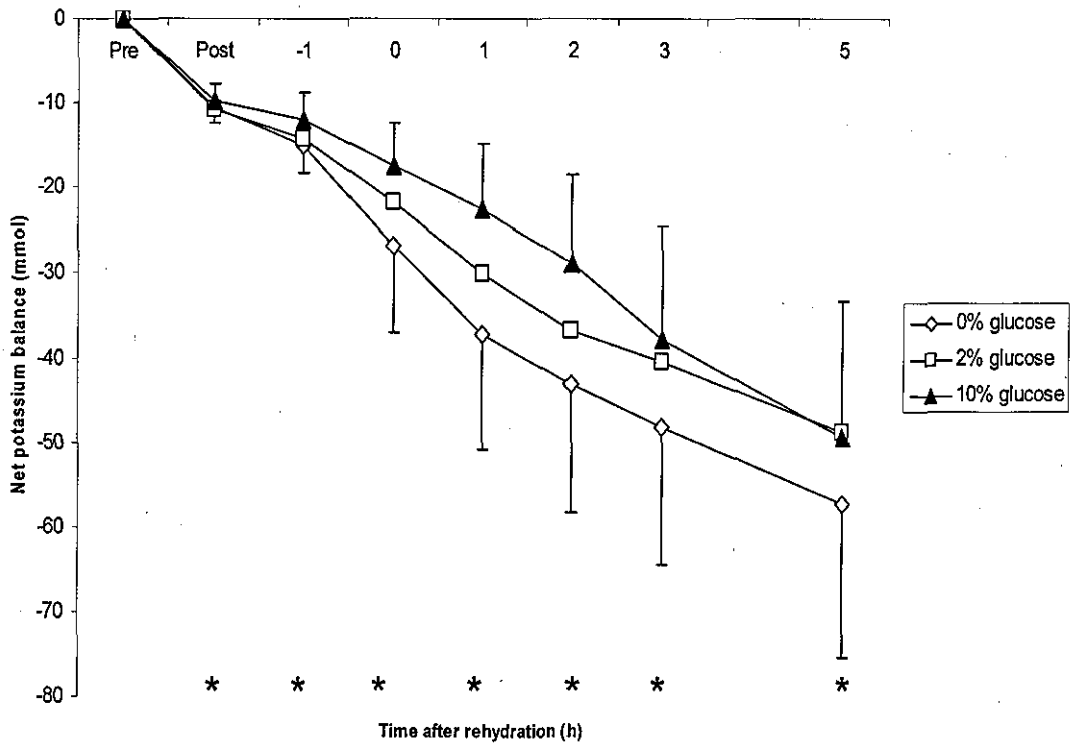
Two factor repeated measures ANOVA on chloride balance data showed no main effect of trial ($P = 0.225$), a main effect of time ($P = 0.002$) and an interaction ($P = 0.029$). Subjects were in a state of negative chloride balance immediately after the dehydration phase on all trials ($P < 0.05$) and returned to negative chloride balance ($P < 0.05$) 5 hours after the rehydration period on the 0 and 2% glucose trials (Figure 6.5).

Figure 6.5 Chloride balance (mmol) following ingestion of 0, 2 and 10% glucose solutions. Points are mean ± SD. Data is shown in Appendix F. * indicates 0, 2 and 10% glucose time points significantly different ($P < 0.05$) from pre-exercise time point. + indicates 0 and 2% glucose time points significantly different ($P < 0.05$) from pre-exercise time point.



Two factor repeated measures ANOVA on potassium balance data showed no main effect of trial ($P = 0.062$), a main effect of time ($P < 0.001$) and an interaction ($P = 0.015$). Subjects were in a state of negative potassium balance ($P < 0.05$) at each time point on all trials (Figure 6.6)

Figure 6.6 Potassium balance (mmol) following ingestion of 0, 2 and 10% glucose solutions. Points are mean ± SD. Data is shown in Appendix F. * indicates 0, 2 and 10% glucose time points significantly different ($P < 0.05$) from pre-exercise value.



Calculated changes in blood, red cell and plasma volumes

Two factor repeated measures ANOVA on blood volume data showed no main effect of trial ($P = 0.541$), time ($P = 0.063$) or interaction ($P = 0.185$) (Figure 6.7).

Two factor repeated measures ANOVA on plasma volume data showed no main effect of trial ($P = 0.373$), a main effect of time ($P = 0.030$) and an interaction ($P = 0.001$). Plasma volume was significantly elevated ($P < 0.05$) above baseline values midway through the rehydration period, at the end of the rehydration period and 1 and 2 hours after rehydration on the 2% glucose trial. On the 0% glucose trial, plasma volume was significantly elevated ($P < 0.05$) from baseline values 1 and 2 hours after rehydration. No significant deviations ($P > 0.05$) from baseline values were observed on the 10% glucose trial (Figure 6.8).

Figure 6.7 Estimated percentage changes in blood volume following ingestion of 0, 2 and 10% glucose solutions. Points are mean ± SD. Data is shown in Appendix F.

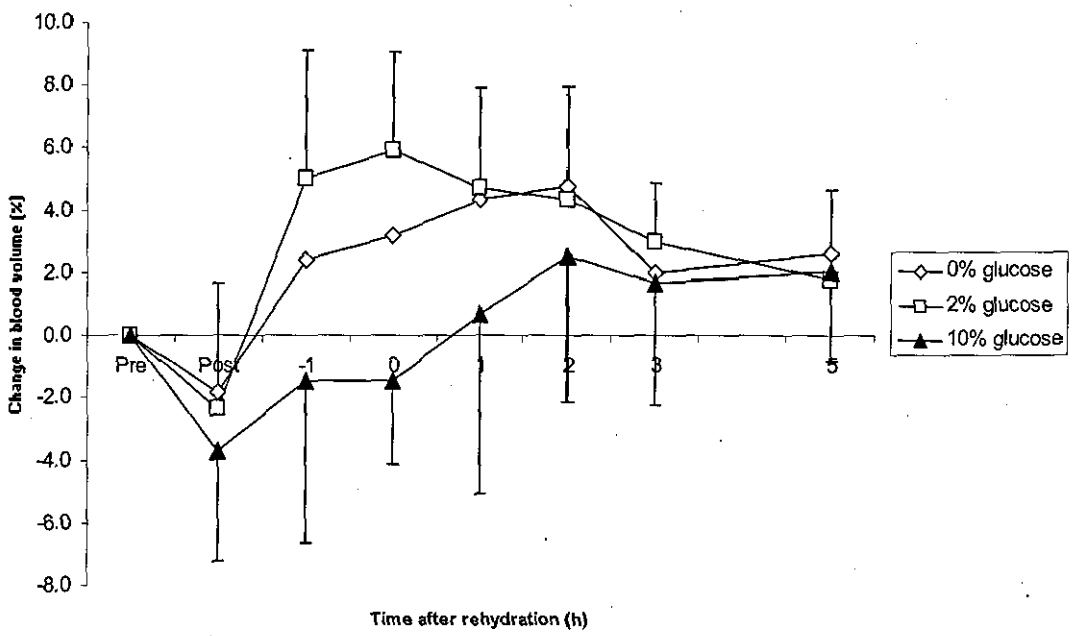
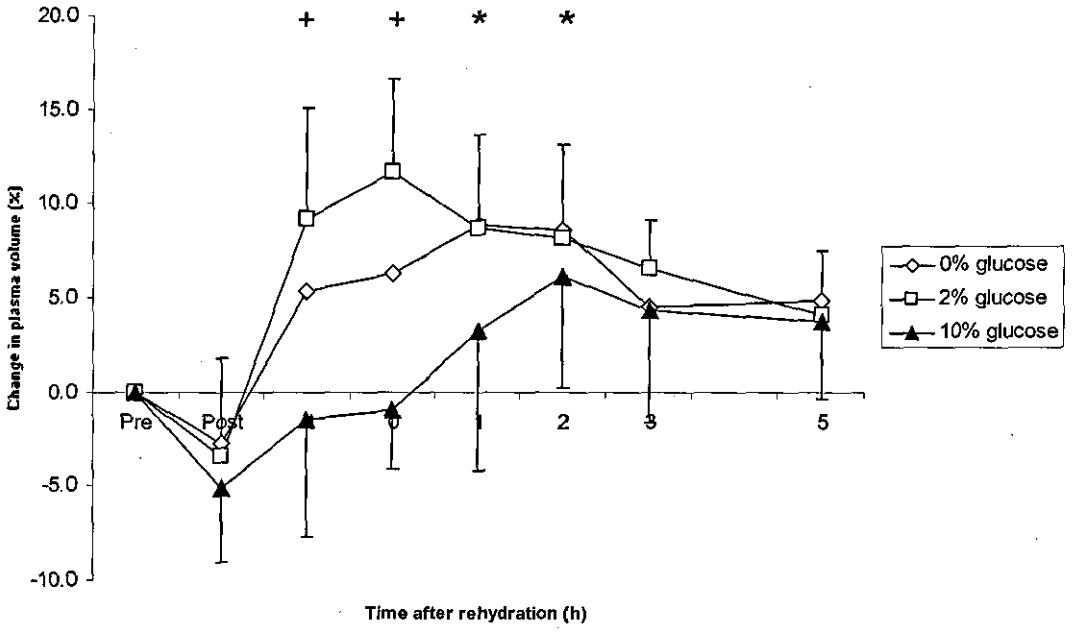
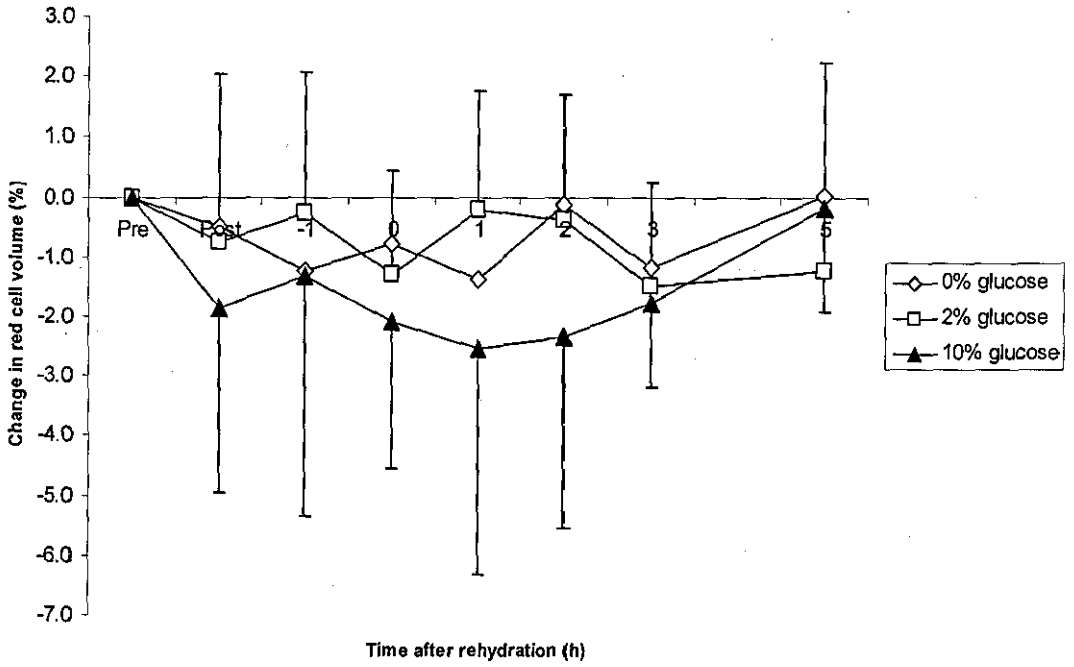


Figure 6.8 Estimated percentage changes in plasma volume following ingestion of 0, 2 and 10% glucose solutions. Points are mean ± SD. Data is shown in Appendix F. + indicates 2% glucose time point significantly different ($P < 0.05$) from pre-exercise value. * indicates 0 and 2% glucose time points significantly different ($P < 0.05$) from pre-exercise value.



Two factor repeated measures ANOVA on red cell volume data showed no main effect of trial (P = 0.710), time (P = 0.607) or interaction (P = 0.363) (Figure 6.9).

Figure 6.9 Estimated percentage changes in red cell volume following ingestion of 0, 2 and 10% glucose solutions. Points are mean ± SD. Data is shown in Appendix F.



Blood glucose concentration

Two factor repeated measures ANOVA on blood glucose data showed a main effect of trial (P = 0.003), time (P < 0.001) and interaction (P < 0.001). Blood glucose concentration was significantly elevated (P < 0.05) from baseline levels mid-way through, at the end and one and two hours after the rehydration period on the 10% glucose trial. Blood glucose concentration was significantly elevated (P < 0.05) from baseline levels mid-way through the rehydration period and at the end of the rehydration period on the 2% glucose trial but no differences (P > 0.05) were observed on the 0% glucose trial (Table 6.7).

Table 6.7 Blood glucose concentration (mmol L^{-1}) following ingestion of 0, 2 and 10% glucose solutions. Values are mean (SD). * indicates time point significantly different ($P < 0.05$) from pre-exercise value

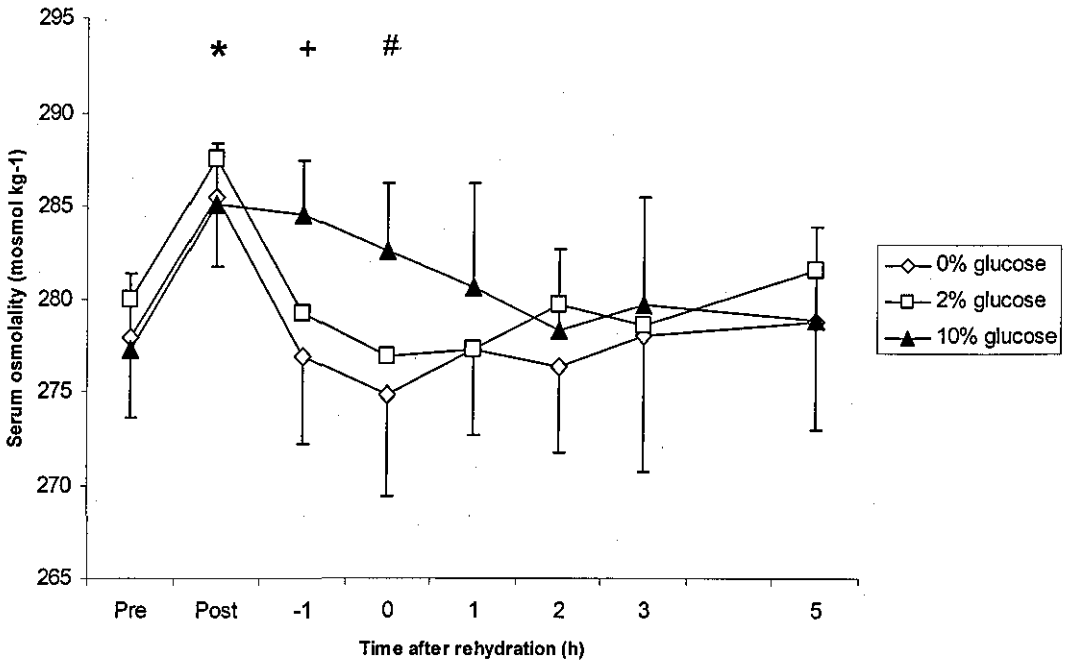
Time after rehydration (h)	0% glucose	2% glucose	10% glucose
Pre-	5.3 (0.3)	5.0 (0.2)	5.1 (0.3)
Post-	5.8 (0.6)	5.4 (0.5)	5.5 (0.5)
-1	5.1 (0.4)	6.7 (0.9)*	7.5 (0.9)*
0	5.1 (0.5)	5.9 (0.5)*	7.0 (0.9)*
1	5.1 (0.4)	4.8 (0.5)	6.1 (0.4)*
2	5.3 (0.5)	4.9 (0.3)	6.2 (0.6)*
3	5.6 (0.6)	5.5 (0.4)	5.3 (0.9)
5	5.7 (0.6)	5.7 (0.4)	5.8 (0.4)

Serum osmolality

Two factor repeated measures ANOVA on serum osmolality data showed no main effect of trial ($P = 0.288$), a main effect of time ($P < 0.001$) and an interaction ($P < 0.001$). Serum osmolality was significantly elevated ($P < 0.05$) post-exercise during all trials and remained elevated ($P = 0.014$) midway through the rehydration period during the 10% glucose trial but returned to baseline levels in the 0% and 2% glucose trials. Serum osmolality was reduced from pre-exercise values ($P = 0.045$) at the end of the rehydration period on the 2% glucose trial (Figure 6.10).

A more gradual return to pre-exercise serum osmolality values was observed during the 10% glucose trial compared to the 0 and 2% glucose trials. Serum osmolality was significantly reduced ($P < 0.05$) from post-exercise values at all time points on the 2% glucose trial and all time points except 5 hours after the end of the rehydration period on the 0% glucose trial. In contrast, serum osmolality was not significantly different ($P > 0.05$) from post-exercise values at any time point following ingestion of the 10% glucose solution.

Figure 6.10 Serum osmolality (mosm kg^{-1}) following ingestion of 0, 2 and 10% glucose solutions. Points are mean ± SD. Data is shown in Appendix F. * denotes 0, 2 and 10% glucose time points significantly different ($P < 0.05$) from pre-exercise value. # denotes 2% glucose time point significantly different ($P < 0.05$) from pre-exercise value. + denotes 10% glucose time point significantly different ($P < 0.05$) from pre-exercise value.



Serum electrolytes

Two factor repeated measures ANOVA on serum sodium concentration data showed no main effect of trial ($P = 0.332$), a main effect of time ($P = 0.016$) but no interaction ($P = 0.143$) (Table 6.8).

Two factor repeated measures ANOVA on serum potassium concentration data showed no main effect of trial ($P = 0.137$), a main effect of time ($P < 0.001$) but no interaction ($P = 0.222$) (Table 6.8).

Two factor repeated measures ANOVA on serum chloride concentration data showed no main effect of trial ($P = 0.514$), a main effect of time ($P = 0.004$) but no interaction ($P = 0.129$) (Table 6.8).

Table 6.8 Serum concentrations (mmol L^{-1}) of sodium, potassium and chloride following ingestion of 0, 2 and 10% glucose solutions. Values are mean (SD).

Time after rehydration (h)	0% glucose	2% glucose	10% glucose
Sodium			
Pre	139 (1)	139 (1)	139 (2)
Post	142 (3)	142 (2)	142 (2)
-1	138 (2)	139 (2)	141 (2)
0	138 (2)	139 (2)	142 (2)
1	139 (3)	139 (3)	141 (3)
2	139 (1)	141 (2)	140 (2)
3	142 (2)	141 (1)	141 (3)
5	143 (3)	141 (3)	142 (3)
Potassium			
Pre	6.4 (0.9)	6.5 (0.7)	5.9 (0.8)
Post	5.8 (0.8)	5.8 (0.5)	5.4 (0.8)
-1	5.6 (0.7)	5.3 (0.5)	4.9 (0.8)
0	5.1 (0.6)	5.0 (0.5)	4.6 (0.8)
1	5.0 (0.5)	4.9 (0.2)	4.6 (0.4)
2	4.6 (0.4)	4.8 (0.2)	4.3 (0.4)
3	4.4 (0.4)	4.5 (0.2)	4.2 (0.4)
5	4.3 (0.3)	4.5 (0.1)	4.2 (0.4)
Chloride			
Pre	104 (2)	104 (2)	104 (2)
Post	107 (2)	107 (3)	106 (3)
-1	104 (2)	104 (2)	107 (2)
0	105 (2)	104 (2)	107 (3)
1	106 (2)	105 (3)	105 (2)
2	105 (3)	105 (3)	105 (2)
3	105 (1)	104 (2)	105 (2)
5	104 (2)	105 (2)	104 (3)

Urine osmolality

Two factor repeated measures ANOVA on urine osmolality data showed no main effect of trial ($P = 0.596$), a main effect of time ($P < 0.001$) and an interaction ($P = 0.041$). Urine osmolality was significantly reduced from pre-exercise values one hour after ($P = 0.038$) drinking the 0% glucose solution and was significantly elevated after the five hour recovery period ($P = 0.012$). On the 2% glucose trial, urine osmolality was significantly reduced immediately after ($P = 0.009$) and one hour ($P = 0.004$) after the rehydration period. A significant increase ($P = 0.016$) in urine osmolality was observed midway through the rehydration period during the 10% glucose trial (Table 6.9).

Table 6.9 Urine osmolality (mosm kg^{-1}) following ingestion of 0, 2 and 10% glucose solutions. Points are median (range). * indicates time point significantly different ($P < 0.05$) from pre-exercise value.

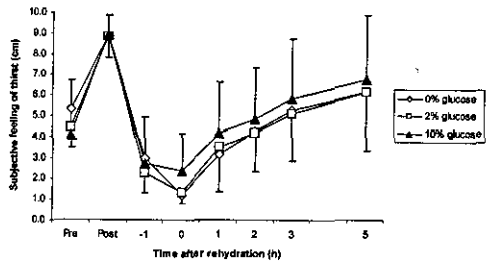
Time after rehydration (h)	0% glucose	2% glucose	10% glucose
Pre	360 (87 - 744)	345 (81 - 853)	367 (98 - 739)
Post	584 (140 - 700)	542 (155 - 670)	487 (120 - 637)
-1	630 (321 - 840)	709 (401 - 862)	683 (406 - 864)*
0	114 (77 - 569)	91 (65 - 501)*	324 (108 - 935)
1	112 (61 - 348)*	100 (64 - 157)*	258 (67 - 858)
2	431 (132 - 650)	420 (146 - 609)	209 (122 - 806)
3	624 (399 - 657)	617 (430 - 848)	213 (146 - 248)
5	724 (566 - 750)*	732 (551 - 807)	612 (161 - 867)

Subjective feelings

No significant differences between trials were observed in subjects perceived feelings of thirst ($P = 0.411$), stomach fullness ($P = 0.054$), bloatedness ($P = 0.144$), hunger ($P = 0.375$), tiredness ($P = 0.287$), alertness ($P = 0.134$), concentration ($P = 0.323$) or head soreness ($P = 0.081$) between trials (Figure 6.11).

Figure 6.11 Subjective feelings (cm) of (a) thirst (b) stomach fullness (c) bloatedness (d) hunger (e) tiredness (f) alertness (g) concentration (h) head soreness following ingestion of 0, 2 and 10% glucose solutions. Points are mean ± SD. Data is shown in Appendix F.

(a)



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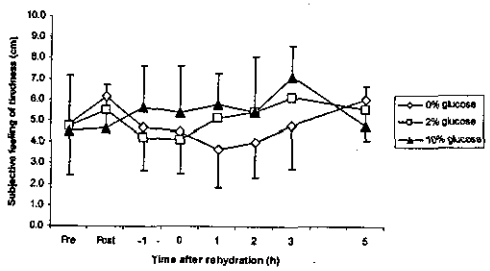

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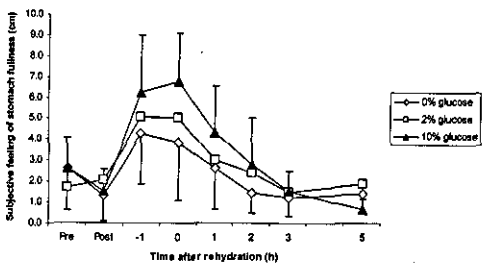
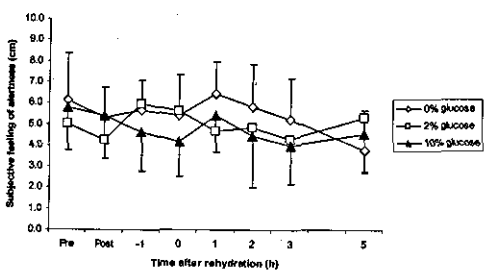


(f)



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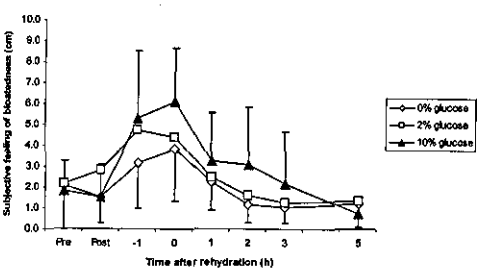

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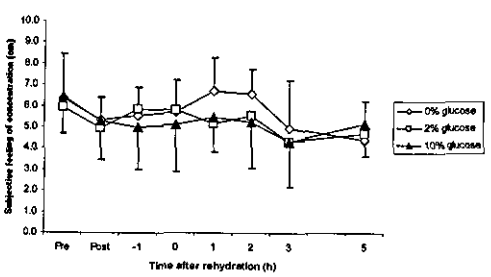
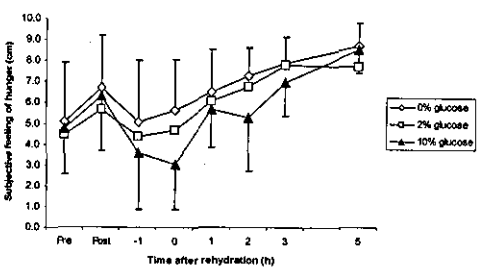
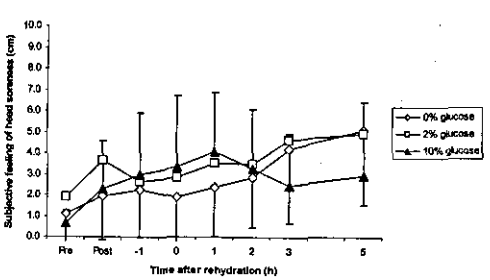


(d)



(h)



Discussion

The results of this study suggest that, in situations of voluntary fluid intake, a 10% glucose-electrolyte solution with an osmolality of 654 ± 4 mosm kg^{-1} and sodium concentration of 31 ± 1 mmol L^{-1} was as effective in restoring and maintaining whole body water levels as 0% and 2% glucose-electrolyte solutions with similar sodium concentrations and osmolalities of 74 ± 1 mosm kg^{-1} and 188 ± 3 mosm kg^{-1} respectively.

Previous studies have suggested that high carbohydrate solutions were more effective (Costill and Sparks, 1973) or as effective (Lambert *et al.* 1992) in restoring whole body net fluid balance as water however, these studies employed a drink volume equal to that lost during exercise and was consumed over a relatively long period. In addition, neither study compared a hypertonic solution to a hypotonic solution.

The results presented in chapter 5 of this thesis reported that a 10% glucose-sodium solution with an osmolality of 667 ± 12 mosm kg^{-1} was more effective in maintaining a state of euhydration following a moderate level of dehydration than a 0% glucose-sodium solution with an osmolality of 79 ± 4 mosm kg^{-1} and a 2% glucose-sodium solution with an osmolality of 193 ± 5 mosm kg^{-1} when a volume of 150 (130-150) % (median (range)) body mass lost during exercise was ingested over a period of one hour. The results of the present study suggest that hypertonic glucose-electrolyte solutions can be as effective as non-glucose solutions and low glucose-electrolyte solutions, in situations of voluntary fluid ingestion.

During this study, subjects consumed 2254 ± 516, 2539 ± 436 and 2173 ± 252 ml during the 2 hour rehydration period on the 0, 2 and 10% glucose trials respectively. Similar ingested volumes of 1796 ± 758, 1750 ± 559, 2492 ± 765 and 2488 ± 269 ml were reported by Maughan and Leiper (1993) when a glucose-electrolyte drink, water, a sports drink and an orange juice/lemonade drink was consumed respectively during a 2 hour *ad libitum* rehydration period. In contrast, Wemple *et al.* (1997) reported considerably lower volumes of 123 ± 16, 130 ± 19 and 105 ± 15% body mass lost during a 3 hour rehydration period when sweetened water, a low sodium solution and a high sodium solution were ingested respectively. The difference in the reported

volume of fluid intake during this study may be due to the dehydration and rehydration protocols used. Wemple *et al.* (1997) reduced body mass by 3.0 ± 0.2 % via continuous treadmill exercise in the heat before allowing a 3 hour rehydration period whereas Maughan and Leiper (1993) employed a dehydration and rehydration protocol similar to that reported in the present study.

Studies that have been performed investigating the effectiveness of solutions during situations of *ad libitum* fluid ingestion highlight the importance of taste preference and electrolyte content (Maughan and Leiper, 1993; Wemple *et al.* 1997). Although no statistical differences in total fluid intake were observed between the trials, 7 of the subjects consumed a larger volume of the 2% glucose solution than the 10% glucose solution. Aspartame was added to the 0 and 2% glucose solutions so that the taste of all of the drinks would be similar. The reported subjective feelings suggested that there was no difference in the sweetness or bitterness of the drinks and there was also no difference in the plesantness of the drinks.

The results of the study reported in chapter 5 showed that subjects reported a greater feeling of bloatedness and stomach fullness following ingestion of a 10% glucose-electrolyte solution. In the present study, no significant differences in subjective feelings of bloatedness or stomach fullness were observed although all subjects did report a greater feeling of these variables on the 10% glucose trials compared to the 0 and 2% glucose trials. This may be a reason for most subjects ingesting a lower volume on the 10% glucose trial compared to the 2% glucose. One of the aims of this study was to examine whether a greater feeling of bloatedness and/or stomach fullness following ingestion of the 10% glucose solution would reduce it's effectiveness as a rehydration solution. This does not seem to be the case as subjects ingested a large enough volume of the 10% glucose solution to restore and maintain fluid balance as effectively as the other solutions examined.

There was no differences observed between the trials in net fluid balance during the present study. Although not significantly different ($P = 0.100$), all subjects produced less urine on the 10% glucose trial than on the 2% glucose trial. Results reported in chapter 5 of this thesis suggested that ingestion of a high glucose solution resulted in a more gradual return to pre-dehydration levels of plasma volume and serum osmolality

which may have maintained circulating levels of arginine vasopressin and aldosterone. During the present study, serum osmolality remained elevated during the 10% glucose trial midway through the rehydration period when serum osmolality had returned to pre-exercise levels in the 0 and 2% glucose trials, despite their being no difference between the trials in the volume of fluid ingested during the first 60 minutes of rehydration. As serum osmolality is the main determinant of arginine vasopressin release (Baylis, 1987), it is likely that the tendency towards a reduced urine output on the 10% glucose trial compared to the 2% glucose trial was due to differences in circulating concentrations of arginine vasopressin.

The reason for this more gradual return to pre-dehydration levels of plasma volume and serum osmolality is still unclear. The reduced rate of gastric emptying that accompanies the ingestion of high carbohydrate drinks (Vist and Maughan, 1995) and the movement of water into the small intestine (Leiper and Maughan, 1986) from the extracellular fluid (chapters 3 and 4 of this thesis) may both contribute to the reduced rate of water uptake observed.

It is generally accepted that a volume of fluid greater than body mass lost during exercise is required to adequately rehydrate due to obligatory urine losses that persist during the recovery period (Shirreffs *et al.* 1996). The rate of fluid ingestion also appears to have a major role in the effectiveness of a rehydration solution. Archer & Shirreffs (2001) observed that drinking 150% body mass lost during exercise over a period of 30 minutes resulted in a significantly reduced state of net fluid balance compared to when the same volume of fluid was ingested over a period of 90 minutes. Kovacs *et al.* (2002) observed that a high rate of fluid intake resulted in an increased urine output over the subsequent two hours when compared to a low rate of fluid intake. In the study reported in chapter 5 of this thesis, despite the 10% glucose solution maintaining a state of euhydration for longer than the other two drinks studied, subjects were in a state of negative fluid balance during all trials four hours after the end of the rehydration period. The drinks were similar in the present study to those investigates in the previous chapter, yet subjects were in a state of euhydration five hours after the end of the rehydration period. This is likely to be due to the rate at which the drink was ingested.

In summary, the results of this study demonstrate that a 10% glucose-electrolyte solution with an osmolality of 654 ± 4 mosm kg^{-1} was as effective in restoring and maintaining whole body water levels as a 0% glucose-electrolyte solution with an osmolality of 74 ± 1 mosm kg^{-1} and a 2% glucose-electrolyte solution with an osmolality of 188 ± 3 mosm kg^{-1} when sodium content of the drinks were the same.

Chapter 7

The rate of gastric emptying and blood deuterium accumulation following repeated ingestion of glucose-electrolyte solutions

Introduction

The results reported in chapter 5 of this thesis showed that a glucose-electrolyte solution with an osmolality of 667 ± 12 mosm kg^{-1} was more effective than a glucose-electrolyte solution with an osmolality of 193 ± 5 mosm kg^{-1} at maintaining whole body fluid balance following exercise-induced dehydration when a fixed volume of fluid was consumed. In addition, the results reported in chapter 6 of this thesis showed that a glucose-electrolyte solution with an osmolality of 654 ± 4 mosm kg^{-1} was as effective as a glucose-electrolyte solution with an osmolality of 188 ± 3 mosm kg^{-1} when *ad libitum* fluid ingestion was allowed. The effectiveness of a hypertonic glucose-electrolyte solution in maintaining fluid balance following exercise appears to be due to a relatively low rate of fluid absorption that results in the avoidance of large rapid increases in plasma volume and falls in serum osmolality that will lead to a diuresis. However, the main reason for this low rate of fluid absorption is unclear. One potential explanation is the reduced rate of gastric emptying of high carbohydrate solutions and the slower increase in plasma volume and decrease in serum osmolality that will occur as a result. Another explanation is the reduction in extracellular fluid volume that occurs following ingestion of such solutions (chapters 3 and 4 of this thesis).

It has been well established that total stomach volume (Hunt and Spurrell, 1951; Hunt and MacDonald 1954; Murray, 1987; Noakes *et al.* 1991; Rehrer *et al.* 1989; Rehrer *et al.* 1990), carbohydrate content (Costill and Saltin, 1974; Murray *et al.* 1999; Simpson *et al.* 2001; Vist and Maughan, 1994) and, to a lesser extent, solution osmolality (Vist and Maughan, 1995) have an effect on the rate of gastric emptying of ingested solutions. However, few investigations have examined the gastric emptying characteristics of solutions following repeated fluid ingestion (Rehrer *et al.* 1990; Mitchell and Voss, 1990; Duchman *et al.* 1990).

The addition of deuterium oxide (D_2O) to orally ingested fluids and the subsequent accumulation rate of deuterium (^2H) in the blood has been proposed as a non-invasive measure of total fluid absorption of a test solution. Although it would seem that the measurement of ^2H accumulation in the blood does not give an accurate indication of water movement within the intestine (Gisolfi *et al.* 1990) it would seem that addition

of deuterium oxide to an ingested solution and subsequent ^{2}H accumulation in the blood is a good indicator of the total rate of fluid absorption of an ingested solution (Davies *et al.* 1987; Gisolfi *et al.* 1992a).

The aim of this study was to evaluate the gastric emptying characteristics of hypotonic and hypertonic glucose-electrolyte solutions following repeated fluid ingestion and to assess the fluid absorption patterns of these solutions. Solutions are similar to those used in chapters 5 and 6 of this thesis and a similar fluid ingestion pattern to that employed in chapter 5 is used.

Materials and Methods

3 male and 5 female subjects with a mean age of 26 ± 6 years, height of 170 ± 10 cm and body mass of 65.99 ± 8.96 kg volunteered to participate in this investigation. Experimental trials were separated by a period of at least seven days, began at the same time of day and subjects followed similar physical activity and nutritional patterns for 24 hours prior to the start of each trial.

A preliminary trial was performed in order to familiarise subjects with the experimental procedures. This trial involved positioning of a gastric tube before the stomach was emptied and washed. A recovery test, similar to that reported by Hassan and Hobsley (1970), was carried out to ensure that the gastric tube was appropriately positioned. Briefly, this involves instillation of 100ml of distilled water before mixing and aspiration of the gastric contents. If more than 80 ml was aspirated, the gastric tube was considered to be in the correct position at the base of the stomach. If less than 80ml was aspirated, the gastric tube was repositioned and the recovery test performed again. The final part of the familiarisation trial involved subjects ingesting approximately 500ml of water to ensure that they were able to drink with the gastric tube was in place. If this was not possible, drinks were instilled via the gastric tube during the experimental trials.

Two experimental trials were undertaken by each subject and a solution with a different carbohydrate content was ingested during each trial. The order of experimental trials was randomly assigned using a Latin square design. Solutions

contained distilled water, one in seven parts lemon squash and 2 or 10% glucose. Electrolyte content of the solutions was 32 ± 1 mmol L^{-1} Na^+, 0.6 ± 0.1 mmol L^{-1} K^+ and 27 ± 1 mmol L^{-1} Cl^- giving osmolalities of 189 ± 3 and 654 ± 3 mosm kg^{-1} for the 2 and 10% glucose solutions respectively.

13 hours before each trial, subjects entered the laboratory and provided a urine sample before nude body mass was obtained to the nearest 10g (Adam Equipment, United Kingdom). Subjects were instructed to ingest approximately 500 ml of water two hours before arrival at the laboratory in an attempt to ensure that they were adequately hydrated. During the first experimental trial, a cheese and tomato pizza was provided (711 ± 103 g) and subjects ate *ad libitum.* The same amount of pizza was consumed on the subsequent trial. Average food intake on both trials was 430 ± 183 g. 200 ml of water was provided on each trial before subjects left the laboratory and were instructed to refrain from eating or drinking anything further until arrival at the laboratory the following morning.

13 hours after the initial body mass measurement, subjects arrived at the laboratory for the experimental trial. A urine sample was obtained and nude body mass was measured in order to establish the extent of body mass lost during the period of fluid restriction. Following this, subjects sat in a comfortable environment maintained at 24.5 ± 1.3 °C and inserted the gastric tube. The stomach was emptied, washed and the recovery test performed. Subjects then sat in an upright position with one hand immersed in water heated and maintained to a temperature of >42°C for a period of 15 minutes, during which time a cannula was inserted into a vein in the back of the hand. This remained in position for the duration of the trial and was kept patent between sample collection by flushing with heparinised isotonic saline. A 7 ml blood sample was obtained before the stomach was emptied for a final time. Five minutes after the initial blood sample, a further resting sample was obtained. 495 ml of the test solution containing 10.0177 ± 0.0054 g deuterium oxide (D_2O) and 50 mg L^{-1} phenol red was then consumed over a period of 1 minute. Further boli of the test solution, containing no D_2O or phenol red, were consumed 15, 30 and 45 minutes after the initial bolus to replicate the drink volumes consumed during the study described in chapter 5 of this thesis. These boli were ingested over a period of 1, 5 and 5 minutes respectively. Total drink volume amounted to 1986 ± 274 ml which is equivalent to

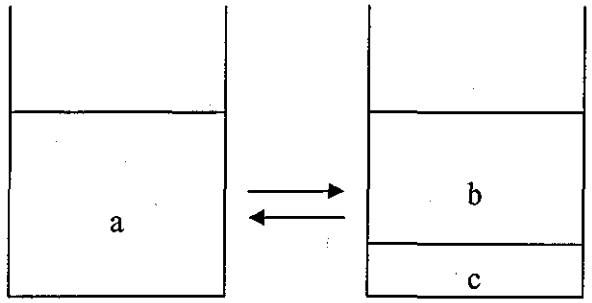
3.0 ± 0.0 % of body mass during the 2% glucose trial and 3.0 ± 0.0 % of body mass during the 10% glucose trial. Blood samples were obtained 2, 5, 10, 15, 20, 30, 45, 60, 75, 90, 105 and 120 minutes after ingestion of the first bolus of solution. The subjects hand was immersed in water that was warmed to 42°C for the duration of the experimental period to arterialise the venous blood (Forster *et al.* 1972). A urine sample was obtained at the end of the trial.

Measurement of residual stomach volume, total stomach volume, test meal volume and gastric secretions

Gastric volumes were measured at 15 minutes intervals throughout the duration of the trial using a modified double sampling technique of George (1968) by Beckers *et al.* (1988).

Following ingestion of the initial bolus of test solution the total volume of fluid in the stomach (volume "a" in figure 7.1) is equal to the sum of the residual stomach volume (volume "c" in figure 7.1) and the volume of test meal ingested (volume "b" in figure 7.1). To calculate residual stomach volume a sample of the first bolus of the test solution was retained for analysis of phenol red concentration. Immediately after ingestion of the first bolus of the test solution, the contents of the stomach were mixed by removing and immediately aspirating between 30 and 50 ml at least 10 times. A sample of the stomach contents was then obtained and analysed for phenol red concentration. Residual stomach volume can then be calculated as it is the volume of fluid required to dilute the concentration of phenol red in the test drink to the concentration of phenol red following mixing in the stomach. This was calculated as shown in Appendix H.

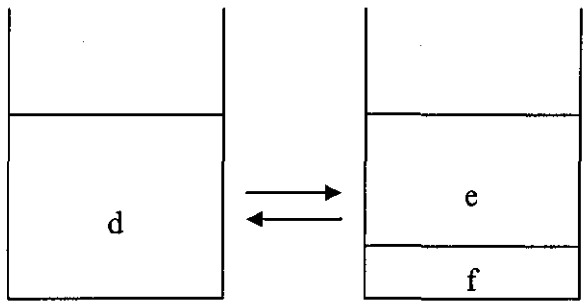
Figure 7.1 Calculation of residual stomach volume after ingestion of first bolus of test drink.



Twelve and a half minutes after ingestion of the first bolus of test solution, the contents of the stomach were mixed and a sample obtained for analysis of phenol red concentration. A known quantity and volume of phenol red was added to the stomach before mixing and a sample was obtained for analysis of phenol red concentration 15 minutes after ingestion of the initial bolus of the test solution. The total volume of fluid in the stomach at this time point (volume "d" in figure 7.2) can then be calculated using the equations described in Appendix H.

The total volume of fluid in the stomach at this time point consists of the remaining volume of the original test drink administered (volume "e" in figure 7.2) and the volume of gastric secretions present in the stomach at this time point (volume "f" in figure 7.2). The volume of gastric secretions produced can be calculated as described in Appendix H. Consequently, the volume of original test meal remaining in the stomach can be calculated by subtracting the volume of gastric secretions (volume "f" in figure 7.2) from the total volume of fluid in the stomach (volume "d" in figure 7.2.

Figure 7.2 Calculation of total stomach volume, test meal volume and gastric secretions.



This process was repeated for each sampling point.

5ml of 500 mg L^{-1} phenol red was added to calculate stomach and drink volumes at time points 15, 30 and 45 minutes. 2.5 ml of 2000 mg L^{-1} phenol red was added at time points 60 and 75 minutes and 5 ml of 2000 mg L^{-1} phenol red was added at time points 90, 105 and 120 minutes. The stomach was emptied at the end of each trial and the volume remaining in the stomach at the end of the trial was recorded.

Sample Analysis

Blood samples were analysed for haemoglobin concentration, haematocrit and blood glucose concentration using the procedures described in the general methods section of this thesis. Haemoglobin concentrations and haematocrit were used to estimate changes in blood, red cell and plasma volumes as described by Dill and Costill (1974).

Serum samples were analysed for osmolality and sodium concentration using the procedures described in the general methods section of this thesis.

Concentrations of ^2H in the blood and gastric aspirate samples were measured using infrared spectrophotmetry and was performed in duplicate. Water and ^2H were initially separated from the blood by vacuum sublimation as described by Lukaski and Johnson (1985). The maximum concentration of ^2H observed in the blood has been designated C_{max} and the time at which this concentration occurred is called T_{max}. If the ^2H concentration at the previous time point from C_{max} was within 10ppm, this was taken as C_{max} as it was assumed that a plateau in the blood ^2H response had occurred. The rate of accumulation of ^2H in the blood has been called "slope".

Gastric aspirate samples were analysed for phenol red concentration by spectrophotometry after dilution (1:20) with NaOH:$NaHCO_3$ (200:500 mmol L^{-1}) buffer and for osmolality by freezing point depression. The methods used for calculating total fluid volume in the stomach, test drink volume in the stomach and gastric secretions are shown in Appendix H.

Coefficient of variations for blood ^{2}H concentrations, gastric aspirate ^{2}H concentrations, gastric aspirate phenol red concentrations and gastric aspirate osmolality are shown in table 7.1.

Table 7.1 Mean, SD and Coefficient of variation (%) of duplicates obtained for blood ^{2}H concentrations, gastric aspirate ^{2}H concentrations, gastric aspirate phenol red concentrations and gastric aspirate osmolality

Assay	n	Mean	SD	CV
Blood ^{2}H concentration (ppm)	30	185	46	2.1
Gastric Aspirate Phenol Red concentration (mg L^{-1})	30	31.3	22.8	1.8
Gastric Aspirate Osmolality (mosm kg^{-1})	30	387	212	0.6
Gastric Aspirate ^{2}H concentration (ppm)	30	227	131	3.6

Urine samples were analysed for osmolality using the procedure described in the general methods section of this thesis.

A sample of the test solution was obtained before addition of deuterium oxide and analysed for osmolality and electrolyte concentrations as described in the general methods section of this thesis.

Statistical analysis

All data sets were tested for normal distribution using the Kolmogorov-Smirnov test. Parametric data are presented as mean ± standard deviation.

Two-factor repeated measures ANOVA were used to evaluate differences between trials. One-factor ANOVA followed by parametric post tests were used to evaluate differences in one trial over time. Tukey or Dunnetts pairwise comparisons were used depending upon whether a clear control time point was present. Paired t-tests were used to establish differences between trials at a given time point.

Results

Subjects pre-trial body mass was the same (P = 0.280) on both trials (2%: 66.11 ± 9.15 kg; 10%: 65.86 ± 8.77 kg). Body mass lost during the 13 hour fluid restriction period was consistent at 0.60 ± 0.29 kg (P = 0.244) which is equivalent to a reduction in body mass of 0.89 ± 0.38 % (P = 0.219).

Urine osmolality prior to fluid restriction (2%: 241 ± 193 mosm kg^{-1}; 10%: 350 ± 150 mosm kg^{-1}) was not different (P = 0.206) between trials and suggested that subjects were adequately hydrated prior to the fluid restriction period (Armstrong *et al.* 1994; Shirreffs and Maughan, 1998b).

Table 7.2 shows baseline measurements obtained after the 13 hour fluid restriction period. No difference in these parameters were observed between trials and are within the expected normal range for subjects following a period of fasting and fluid restriction with the exception of the serum osmolality data which was lower than expected (Geigy Scientific Tables, 1962). Urine osmolality shown in table 7.2 suggested that subjects were somewhat dehydrated following the period of fluid restriction (Armstrong *et al.* 1994; Shirreffs and Maughan, 1998b).

Table 7.2 Baseline measurements following 13 hours fluid restriction (Mean (SD)).

Measure	2% glucose	10% glucose	P-value
Hb concentration (g L^{-1})	136 (16)	137 (18)	0.850
Haematocrit (%)	39 (4)	39 (5)	0.492
Blood glucose concentration (mmol L^{-1})	5.9 (0.5)	6.0 (0)	0.545
Serum osmolality (mosm kg^{-1})	281 (8)	282 (7)	0.201
Serum sodium concentration (mmol L^{-1})	142 (3)	141 (1)	0.654
Urine osmolality (mosm kg^{-1})	879 (113)	895 (88)	0.638

The sodium concentration ($P = 0.111$), potassium concentration ($P = 0.598$) and chloride concentration ($P = 0.064$) of the ingested solutions was the same during both trials (Table 7.3).

Table 7.3 *Drink characteristics (Mean (SD)).*

Glucose content (%)	Na^+ concentration (mmol L^{-1})	Cl^- concentration (mmol L^{-1})	K^+ concentration (mmol L^{-1})	Osmolality (mosm kg^{-1})
2	32 (1)	27 (1)	0.6 (0.1)	189 (3)
10	31 (1)	26 (1)	0.6 (0.1)	654 (3)

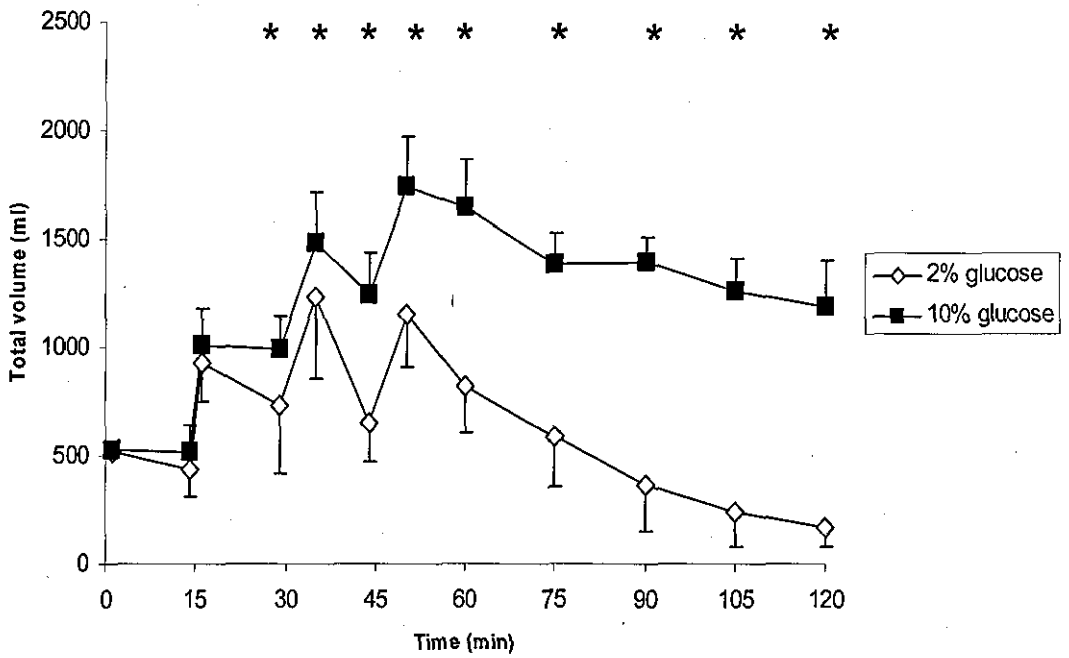
Residual gastric volume

Gastric residual volumes calculated from changes in phenol red concentration following ingestion of the first bolus of the test solution were not different between trials ($P = 0.684$) and amounted to 26 ± 17 and 30 ± 25 ml for the 2% and 10% glucose trials respectively.

Total stomach volume

Two factor repeated measures ANOVA on total stomach volume data showed a main effect of trial ($P < 0.001$), time ($P < 0.001$) and interaction ($P < 0.001$). The total volume of fluid in the stomach was greater ($P < 0.05$) on the 10% glucose trial compared to the 2% glucose trial from 30 minutes after ingestion of the initial 495 ml bolus until the end of the experimental period (Figure 7.3).

Figure 7.3 Total gastric volume (ml) at each time point following ingestion of 2 and 10% glucose solutions. Points are mean ± SD. Data is shown in Appendix G. * denotes gastric volume significantly different ($P < 0.05$) between trials.

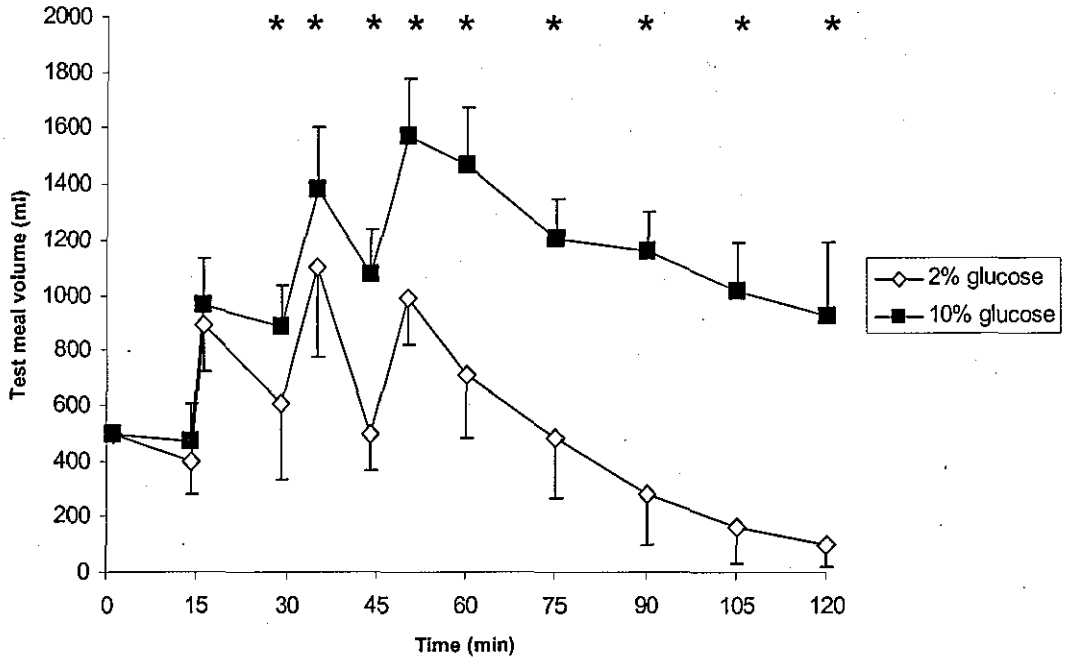


The total volume of fluid aspirated from the stomach at the end of each trial was 108 ± 100 and 1110 ± 187 ml for the 2 and 10% glucose solutions respectively. These values were not different (2%: $P = 0.098$; 10%: $P = 0.390$) from the final values calculated from the change in phenol red concentrations which were 167 ± 88 and 1189 ± 215 ml for the 2 and 10% glucose trials respectively.

Test meal volume in stomach

Two factor repeated measures ANOVA on test meal volume data showed a main effect of trial ($P < 0.001$), time ($P < 0.001$) and interaction ($P < 0.001$). The test meal volume remaining in the stomach was greater ($P < 0.05$) during the 10% glucose trial compared to the 2% glucose trial from 30 minutes after ingestion of the initial 495 ml bolus until the end of the experimental period (Figure 7.4).

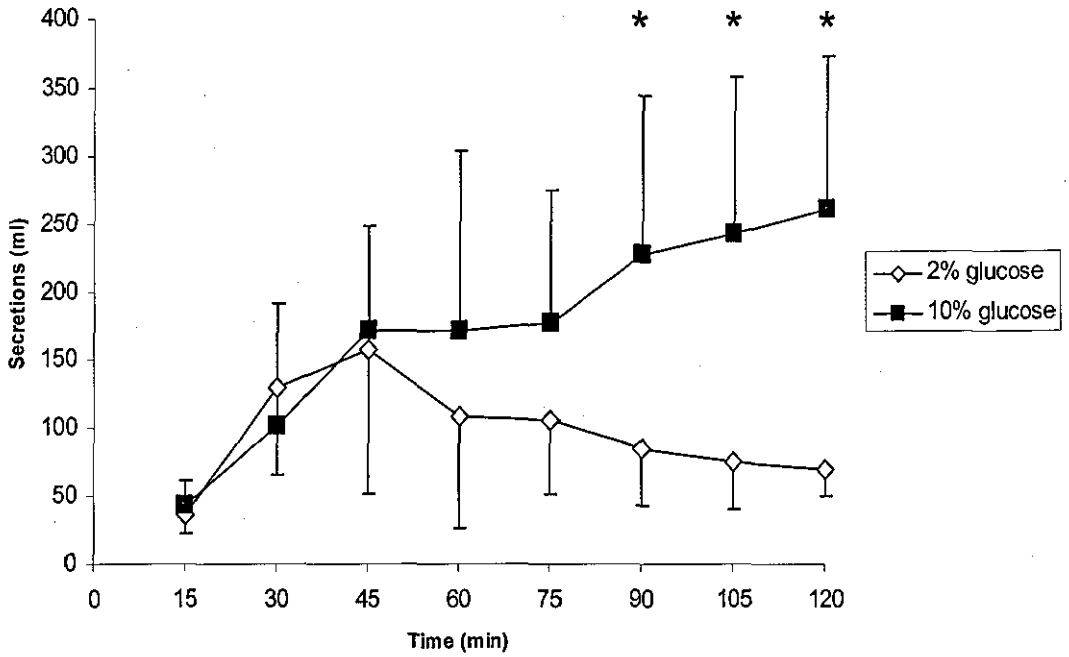
Figure 7.4 Test meal volume remaining in the stomach (ml) at each time point following ingestion of 2 and 10% glucose solutions. Points are mean ± SD. Data is shown in Appendix G. * denotes test meal volume significantly different ($P < 0.05$) between trials.



Gastric secretions

Two factor repeated measures ANOVA on gastric secretion data showed a main effect of trial ($P = 0.014$), time ($P = 0.001$) and an interaction ($P < 0.001$). The volume of gastric secretions was greater ($P < 0.05$) during the 10% glucose trial compared to the 2% glucose trial 90, 105 and 120 minutes after ingestion of the initial bolus of 495 ml (Figure 7.5). The total volume of gastric secretions at the end of the experimental period was not different ($P = 0.085$) between the trials and amounted to 751 ± 356 and 1395 ± 685 ml on the 2 and 10% glucose trials respectively.

Figure 7.5 Gastric secretions (ml) at each time point following ingestion of 2 and 10% glucose solutions. Points are mean ± SD. Data is shown in Appendix G. * denotes gastric secretions significantly different ($P < 0.05$) between trials.



Gastric aspirate osmolality

Two factor repeated measures ANOVA on gastric aspirate osmolality data showed a main effect of trial ($P < 0.001$), time ($P < 0.001$) and interaction ($P = 0.009$). The osmolality of the gastric contents was greater throughout the 10% glucose trial compared to the 2% glucose trial ($P < 0.001$). During the 10% glucose trial, gastric osmolality was lowered ($P < 0.05$) 1, 14, 15, 30, 90, 104, 105, 119 and 120 minutes after ingestion of the first bolus of test solution. During the 2% glucose trial, gastric aspirate osmolality was increased ($P < 0.05$) 29, 30, 44, 45, 59, 60, 74 and 75 minutes after ingestion of the first bolus of test solution (Table 7.4).

Table 7.4 Gastric aspirate osmolality (mosm kg^{-1}) at each time point following ingestion of 2 and 10% glucose solutions. Values are mean (SD). # indicates 2% glucose time point significantly different ($P < 0.05$) from drink osmolality. ^ indicates 10% glucose time point significantly different ($P < 0.05$) from drink osmolality.

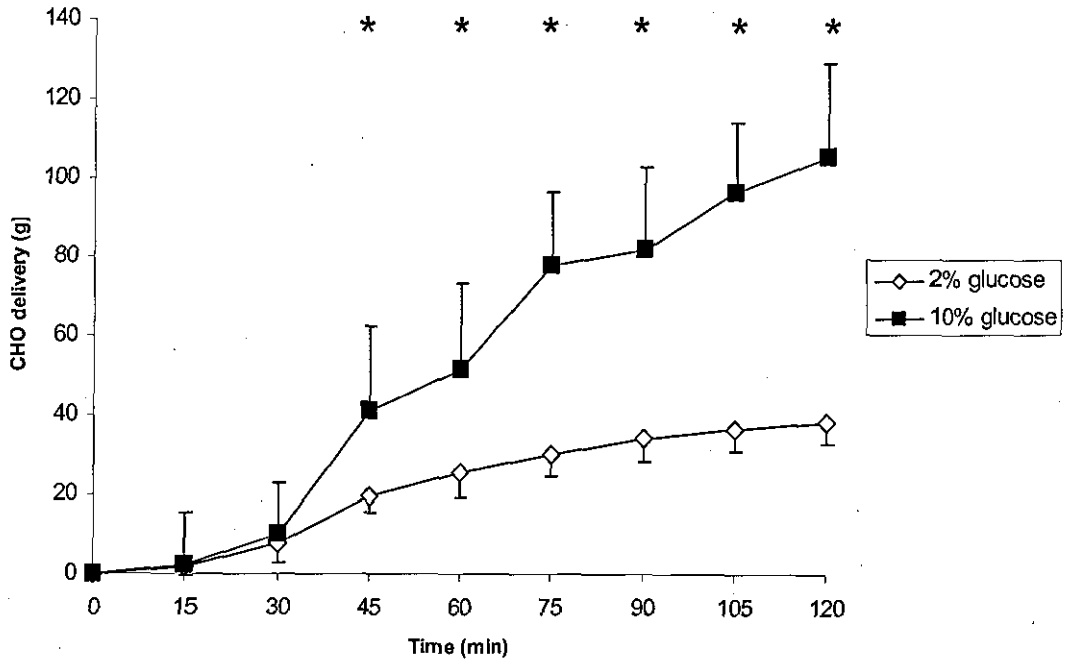
Time (min)	2% glucose	10% glucose
Drink	153 (2)	613 (7)
1	151 (2)	577 (6)^
14	159 (3)	565 (13)^
15	158 (4)	555 (14)^
29	180 (4)#	587 (11)
30	176 (4)#	579 (12)^
44	184 (4)#	592 (13)
45	183 (4)#	588 (12)
59	186 (4)#	594 (13)
60	185 (4)#	590 (14)
74	188 (6)#	585 (15)
75	187 (6)#	582 (17)
89	186 (15)	576 (15)
90	184 (13)	573 (16)^
104	182 (20)	560 (20)^
105	175 (23)	562 (17)^
119	186 (22)	560 (16)^
120	177 (23)	553 (15)^

Carbohydrate delivery

The amount of carbohydrate emptied from the stomach was calculated as the difference between the amount of carbohydrate in the stomach between two sample points. Two factor repeated measures ANOVA on carbohydrate delivery data showed a main effect of trial ($P < 0.001$), time ($P < 0.001$) and an interaction ($P < 0.001$). The total amount of carbohydrate emptied from the stomach was greater than baseline values ($P < 0.05$) from 45 minutes after ingestion of the first bolus of test solution

until the end of the experimental period on both trials and was greater ($P < 0.05$) on the 10% glucose trial than on the 2% glucose trial from this time point onwards (Figure 7.6).

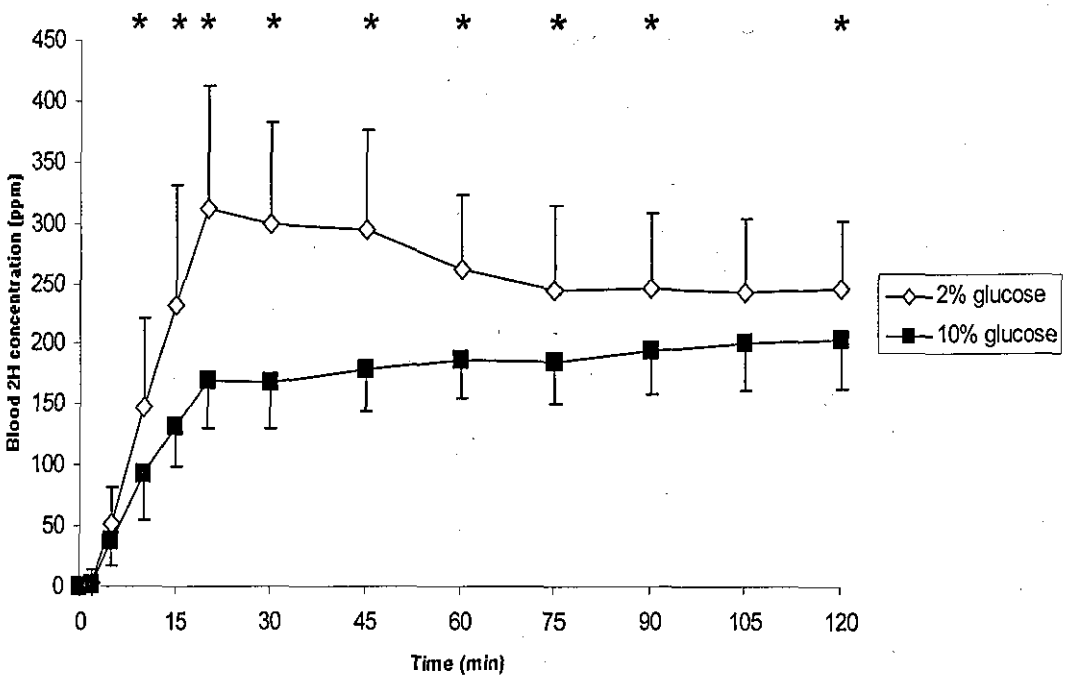
Figure 7.6 The amount of CHO emptied from the stomach (g) at each time point following ingestion of 2 and 10% glucose solutions. Points are mean ± SD. Data is shown in Appendix G. * *indicates 2% glucose time point significantly different (P < 0.05)* from 10% glucose time point.



Blood ^2H concentration

Two factor repeated measures ANOVA on blood ^2H data showed a main effect of trial ($P = 0.011$), time ($P < 0.001$) and an interaction ($P = 0.002$). Blood ^2H accumulation was significantly increased ($P < 0.05$) from baseline values on both trials from 5 minutes after ingestion of the initial bolus of test solution until the end of the experimental period. Blood ^2H concentration was significantly higher ($P < 0.05$) from 10 minutes after ingestion of the initial 495 ml bolus of the test solution on the 2% glucose trial compared to the 10% glucose trial with the exception of 105 minutes after ingestion of the initial 495 ml bolus which showed a tendency ($P = 0.057$) towards a greater blood ^2H concentration on the 2% glucose trial compared to the 10% glucose trial (Figure 7.7).

Figure 7.7 Blood ^{2}H concentration (ppm) at each time point following ingestion of 2 and 10% glucose solutions. Points are mean ± SD. Data is shown in Appendix G. * indicates 2% glucose time point significantly different ($P < 0.05$) from 10% glucose time point.



The maximum concentration of ^{2}H accumulated in the blood (Table 7.5) was significantly higher during the 2% glucose trial compared to the 10% glucose trial ($P = 0.005$) and the time at which the maximum blood ^{2}H concentration occurred (Table 7.5) was different between the trials ($P = 0.011$). As a result the rate of blood ^{2}H accumulation (Table 7.5) was faster on the 2% glucose trial compared to the 10% glucose trial ($P = 0.005$).

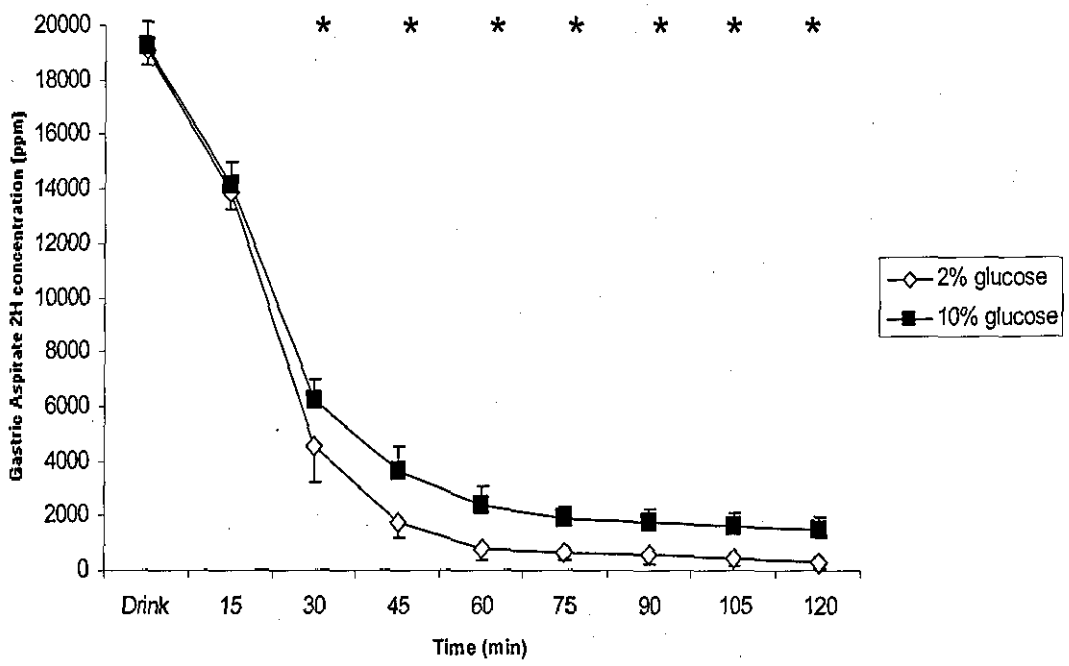
Table 7.5 Cmax, Tmax and Slope of ^{2}H accumulation in the blood following ingestion of 2 and 10% glucose solution. Values are mean (SD). * indicates 10% glucose value significantly different ($P < 0.05$) from 2% glucose value.

	2% glucose	10% glucose
Cmax (ppm)	324 (88)	212 (37)*
Tmax (min)	29 (13)	74 (33)*
Slope (ppm min^{-1})	13.7 (7.3)	3.7 (2.6)*

Gastric aspirate ^{2}H concentration

The concentration of ^{2}H present in the initial 495 ml bolus of test solution amounted to 19168 ± 686 ppm and was not different between the 2 and 10% glucose trials (P = 0.623). Two factor repeated measures ANOVA on gastric aspirate ^{2}H data showed a m main effect of trial (P = 0.001), time (P < 0.001) and an interaction (P = 0.018). 15 minutes after ingestion of the initial bolus of test solution, the concentration of ^{2}H present in the gastric aspirate was not different between the 2 and 10% glucose trials (P = 0.182). From 30 minutes after ingestion of the initial bolus of test solution until the end of the experimental period, the concentration of ^{2}H present in the gastric contents was greater on the 10% glucose trial compared to the 2% glucose trial (P < 0.05) with the ^{2}H concentration being 279 ± 205 and 1475 ± 455 ppm at the end of the experimental period on the 2 and 10% glucose trials respectively (Figure 7.8). The amount of $^{2}H_2O$ remaining in the stomach at the end of the experimental period was 0.0437 ± 0.0537 and 1.7237 ± 0.5526 g on the 2 and 10% glucose trials respectively.

Figure 7.8 Gastric aspirate ^{2}H concentration (ppm) at each time point following ingestion of 2 and 10% glucose solutions. Points are mean ± SD. Data is shown in Appendix G. * indicates 2% glucose time point significantly different (P < 0.05) from 10% glucose time point.



Estimated changes in blood, red cell and plasma volumes

Two factor repeated measures ANOVA on blood volume data showed a main effect of trial ($P = 0.004$), no main effect of time ($P = 0.351$) and an interaction ($P = 0.004$). Blood volume was significantly greater ($P < 0.05$) during the 2% glucose trial compared to the 10% glucose trial 30, 45, 60, 75, 90 and 105 minutes following ingestion of the first bolus of test solution (Figure 7.9).

Two factor repeated measures ANOVA on plasma volume data showed a main effect of trial ($P < 0.001$), no main effect of time ($P = 0.485$) and an interaction ($P = 0.001$). Plasma volume was significantly greater ($P < 0.05$) during the 2% glucose trial compared to the 10% glucose trial 20, 30, 45, 60, 75, 90, 105 and 120 minutes following ingestion of the first bolus of test solution (Figure 7.10).

Two factor repeated measures ANOVA on red cell volume data showed no main effect of trial ($P = 0.406$), time ($P = 0.103$) or interaction ($P = 0.589$) (Figure 7.11).

Figure 7.9 Estimated percentage change in blood volume at each time point following ingestion of 2 and 10% glucose solutions. Points are mean ± SD. Data is shown in Appendix G. * denotes blood volume significantly different ($P < 0.05$) between trials.

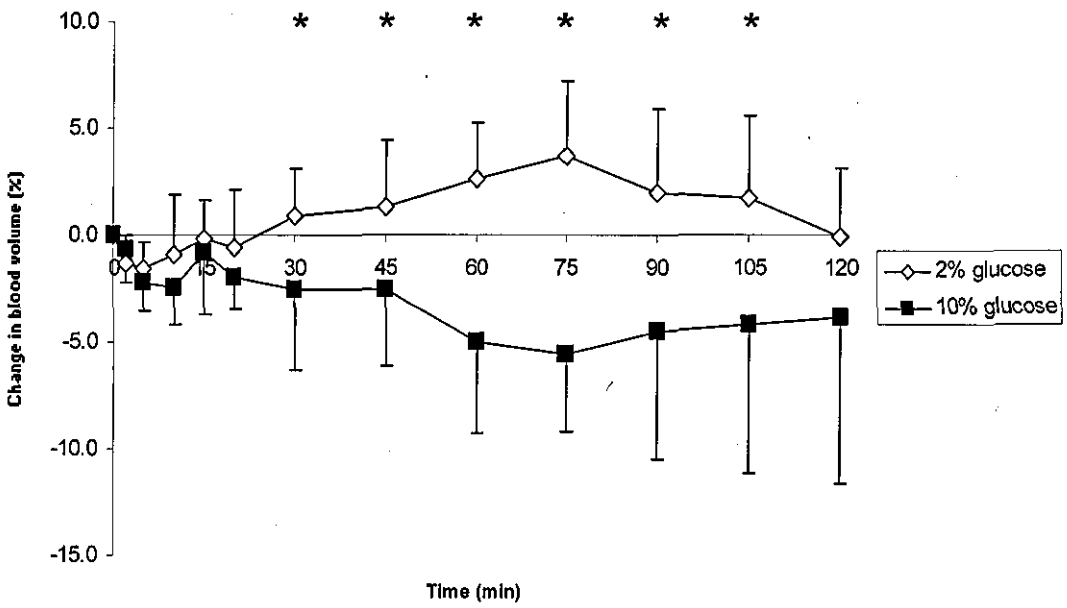


Figure 7.10 Estimated percentage change in plasma volume at each time point following ingestion of 2 and 10% glucose solutions. Points are mean ± SD. Data is shown in Appendix G. * denotes plasma volume significantly different ($P < 0.05$) between trials.

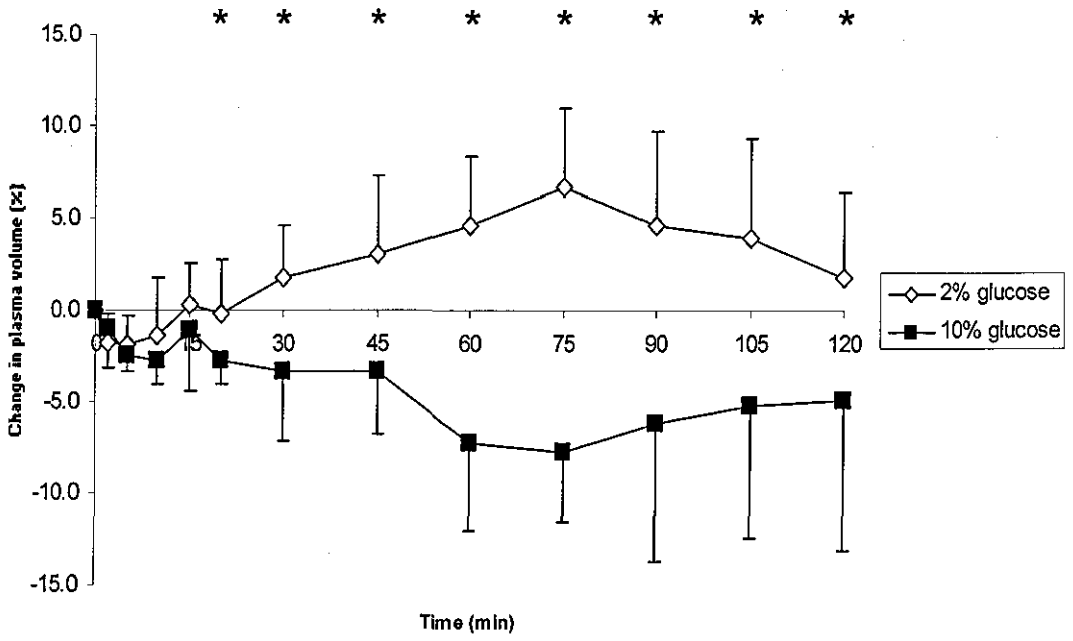
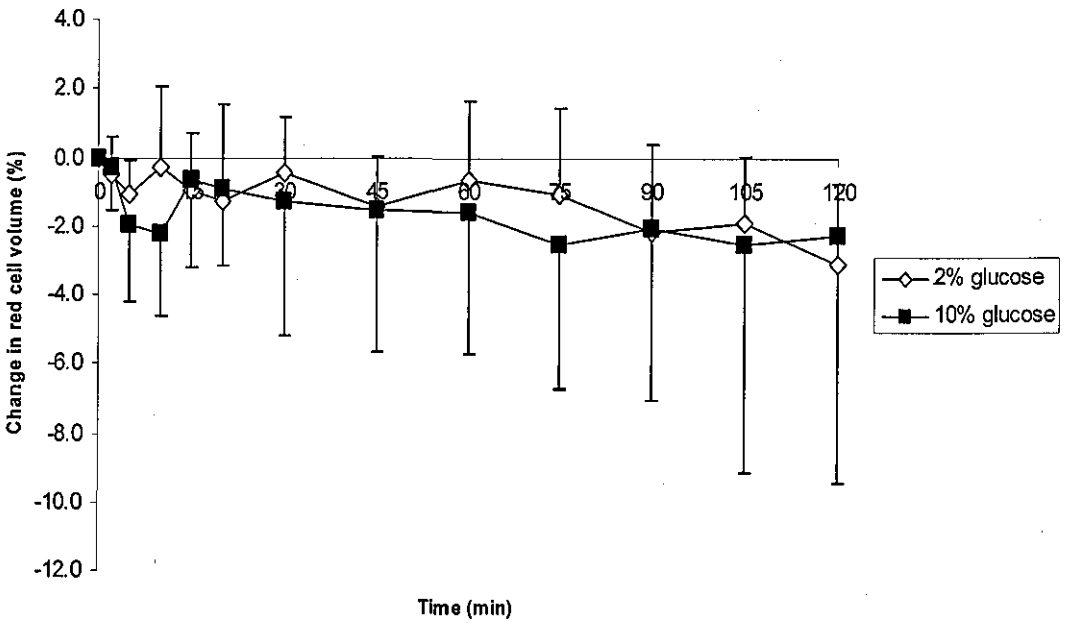


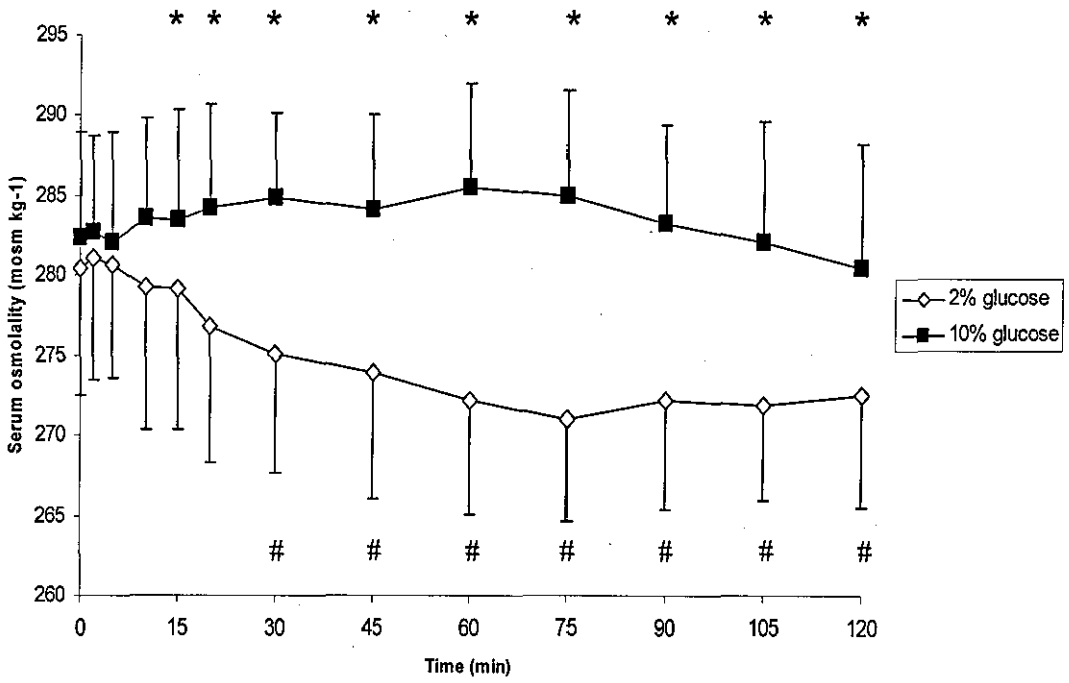
Figure 7.11 Estimated percentage change in red cell volume at each time point following ingestion of 2 and 10% glucose solutions. Points are mean ± SD. Data is shown in Appendix G.



Serum osmolality

Two factor repeated measures ANOVA on serum osmolality data showed a main effect of trial ($P = 0.003$), time ($P < 0.001$) and an interaction ($P < 0.001$). Serum osmolality was significantly lower ($P < 0.05$) during the 2% glucose trial compared to the 10% glucose trial from 15 minutes after ingestion of the initial 495 ml bolus of test solution until the end of the experimental period. Reductions ($P < 0.05$) in serum osmolality from baseline values were observed from 30 minutes after ingestion of the initial bolus of test solution until the end of the experimental period on the 2% glucose trial. No deviations from baseline values were observed at any time point on the 10% glucose trial (Figure 7.12).

Figure 7.12 Serum osmolality (mosm kg^{-1}) at each time point following ingestion of 2 and 10% glucose solutions. Points are mean ± SD. Data is shown in Appendix G. * denotes plasma volume significantly different ($P < 0.05$) between trials. # denotes 2% glucose time point significantly different ($P < 0.05$) from baseline value.



Serum sodium concentration

Two factor repeated measures ANOVA on serum sodium concentration data showed no main effect of trial ($P = 0.911$), a main effect of time ($P = 0.025$) and an interaction

(P = 0.043). Serum sodium concentration was significantly lowered (P = 0.034) from baseline values 90 minutes after ingestion of the initial bolus of test solution on the 2% glucose trial (Table 7.6).

Table 7.6 Serum sodium concentration (mmol L^{-1}) at each time point following ingestion of 2 and 10% glucose solutions. Values are mean (SD). + indicates 2% glucose time point significantly different (P < 0.05) from time point "0".

Time (min)	2% glucose	10% glucose
0	142 (3)	141 (1)
2	141 (3)	141 (2)
5	141 (4)	140 (2)
10	141 (4)	140 (2)
15	139 (4)	139 (2)
20	140 (3)	140 (2)
30	139 (3)	140 (2)
45	137 (3)	140 (2)
60	138 (3)	140 (2)
75	138 (2)	141 (2)
90	137 (2)+	141 (2)
105	138 (2)	140 (2)
120	139 (2)	140 (2)

Blood glucose concentration

Two factor repeated measures ANOVA on blood glucose data showed a main effect of trial (0.020), time (P = 0.001) and an interaction (P = 0.025). Blood glucose concentration was significantly elevated (P < 0.05) from baseline values 20, 30, 45, 60 and 75 minutes after ingestion of the initial bolus of test solution on the 2% glucose trial and elevated from baseline values from 15 minutes after ingestion of the initial bolus of test solution until the end of the experimental period on the 10% glucose trial (Table 7.7).

Table 7.7 Blood glucose concentration (mmol L^{-1}) at each time point following ingestion of 2 and 10% glucose solutions. Values are mean (SD). * indicates time point significantly different ($P < 0.05$) from time point "0".

Time (min)	2% glucose	10% glucose
0	5.9 (0.5)	6.0 (0.1)
2	5.9 (0.5)	6.0 (0.1)
5	6.0 (0.5)	6.3 (0.3)
10	6.5 (0.6)	7.1 (0.7)
15	7.0 (0.8)	7.9 (0.9)*
20	7.5 (0.8)*	8.7 (0.8)*
30	8.8 (0.8)*	9.9 (0.7)*
45	9.6 (0.7)*	10.3 (1.0)*
60	9.1 (1.1)*	10.1 (1.3)*
75	8.1 (1.4)*	9.6 (1.3)*
90	6.9 (1.5)	8.9 (1.0)*
105	6.2 (1.3)	8.5 (0.9)*
120	5.9 (1.0)	8.0 (0.7)*

Urine measurements

Two factor repeated measures ANOVA on urine volume data showed a main effect of trial ($P = 0.010$), time ($P = 0.002$) and an interaction ($P = 0.021$). No significant differences in urine volume were observed before ($P = 0.169$) or after ($P = 0.804$) the fluid restriction phase between trials. A greater volume of urine was passed at the end of the 2% glucose trial compared to the 10% glucose trial ($P = 0.024$) (Table 7.8).

Two factor repeated measures ANOVA on urine osmolality data showed a main effect of trial ($P = 0.033$), time ($P < 0.001$) and an interaction ($P = 0.043$). No significant differences in urine osmolality were observed before ($P = 0.231$) or after ($P = 0.760$) the fluid restriction phase between trials. Urine osmolality was significantly lower ($P = 0.021$) at the end of the 2% glucose trial compared to the 10% glucose trial (Table 7.9).

Table 7.8 Urine volume (ml) before and after fluid restriction and at the end of each trial. Values are mean (SD). * indicates 10% glucose value significantly different ($P < 0.05$) from 2% glucose value.

Time	2% glucose	10% glucose
Pre-fluid restriction	286 (226)	159 (99)
Post-fluid restriction	89 (125)	105 (114)
Post-fluid ingestion	741 (221)	413 (293)*

Table 7.9 Urine osmolality (mosm kg^{-1}) before and after fluid restriction and at the end of each trial. Values are mean (SD). * indicates 10% glucose value significantly different ($P < 0.05$) from 2% glucose value.

Time	2% glucose	10% glucose
Pre-fluid restriction	241 (193)	350 (150)
Post-fluid restriction	879 (113)	895 (88)
Post-fluid ingestion	156 (50)	357 (214)*

Discussion

The results of this study suggest that repeated ingestion of a 10% glucose-electrolyte solution with an osmolality of 654 ± 3 mosm kg^{-1} results in a reduced rate of fluid uptake compared to a 2% glucose-electrolyte solution with an osmolality of 189 ± 3 mosm kg^{-1}. Based on the gastric emptying characteristics and blood ^{2}H accumulation patterns of the solutions investigated, this is primarily due to a relatively low rate of gastric emptying following ingestion of the high osmolality solution resulting in the avoidance of large rapid increases in plasma volume and falls in serum osmolality that lead to an increase in urine formation.

Shirreffs *et al.* (2004) reported that a period of 13 hours fluid restriction, during which time food with a low water content was consumed *ad libitum*, resulted in a reduction in body mass of approximately 1%. The period of 13 hours fluid restriction in the present study resulted in a reduction in body mass of 0.89 ± 0.38%. In addition, urine

osmolality and serum sodium concentration data obtained following 13 hours of fluid restriction in the present study were similar to those reported by Shirreffs *et al.* (2004) and suggested that subjects were moderately dehydrated before ingestion of the test solutions. The serum osmolality data obtained following the 13 hour period of fluid restriction during this study was lower than expected due to large variation that existed between subjects.

The gastric emptying characteristics of both solutions during the one hour period after fluid ingestion followed expected patterns. Vist and Maughan (1995) reported that ingestion of a single bolus of two dilute glucose solutions (40 g L^{-1}) resulted in an exponential decrease in fluid volume remaining in the stomach over the following 60 minute period. In contrast, ingestion of two concentrated glucose solutions (188 g L^{-1}) resulted in a more linear decrease in the total fluid volume. The results of the present study are in agreement with this as an exponential decrease in total stomach volume and test meal volume was observed in the hour following repeated ingestion of the 2% glucose solution and a more linear reduction in these variables was observed following repeated ingestion of the 10% glucose solution.

In the hour following ingestion of the test solutions, the volume of the original test solution remaining in the stomach was reduced on average by 39 ± 5 and 11 ± 6 % every 15 minutes on the 2 and 10% glucose trials respectively.

The total volume of test meal ingested over the first hour of the experimental period amounted to 1986 ± 274 ml during each trial. At the end of the experimental period, it was calculated that 97 ± 77 and 928 ± 264 ml of the original test meal remained in the stomach on the 2 and 10% glucose trials respectively. This means that approximately 96 ± 4 and 54 ± 10 % of the test solution had emptied from the stomach on the 2 and 10% glucose trials respectively and was, therefore, available for fluid absorption in the small intestine.

Gastric aspirate osmolality at the end of the first hour examined was 187 ± 4 and 590 ± 14 mosm kg^{-1} on the 2 and 10% glucose trials respectively. This was reduced over the following hour to 177 ± 23 and 553 ± 15 mosm kg^{-1} for each trial. The osmolality of gastric secretions ranges from 160 – 440 mosm kg^{-1} at rest (Geigy Scientific

Tables) and, as such, a volume of between 278 and 763 ml would need to be secreted into the stomach on the 2% glucose trial to account for the change in gastric aspirate osmolality observed. Similarly, gastric secretions would have to amount to between 699 and 1922 ml to account for the decrease in gastric aspirate osmolality observed on the 10% glucose trial. The volume of gastric secretions produced during the second hour of this study amounted to 391 ± 91 and 907 ± 431 ml on the 2 and 10% glucose trials respectively and are, therefore, within the expected range. From these numbers, it can be calculated that the average osmolality of the gastric secretions produced over the second hour of this investigation was approximately 321 ± 138 and 340 ± 119 mosm kg^{-1} on the 2 and 10% glucose trials respectively.

The ^{2}H accumulation data obtained during this study also followed expected patterns. Ingestion of $^{2}H_2O$ labelled beverages generally results in a linear increase in blood ^{2}H concentration before reaching a plateau (Davis *et al.* 1990; Gisolfi *et al.* 1990; Lambert *et al.* 1999; Maughan *et al.* 2004). Addition of 10.0177 ± 0.0054 g of $^{2}H_2O$ to the first bolus of test solution in the present study resulted in a faster rate of increase in blood ^{2}H accumulation and a greater maximum ^{2}H concentration following ingestion of the 2% glucose solution compared to ingestion of the 10% glucose solution. ^{2}H accumulation was greater on the 2% glucose trial compared to the 10% glucose trial from 10 minutes after ingestion of the initial bolus until the end of the experimental period with the exception of one time point. This is likely to be due to the reduced rate of gastric emptying following ingestion of the 10% glucose solution compared to the 2% glucose solution as the amount of $^{2}H_2O$ emptied from the stomach was also less on the 10% glucose trial. The blood ^{2}H accumulation data also shows that, although the rate of fluid absorption was greater on the 2% glucose trial, a significant amount of fluid absorption did occur on the 10% glucose trial.

It has been shown that perfusion of high osmolality solutions into the small intestine results in a net secretion of water into the intestinal lumen (Leiper and Maughan, 1986) and the results presented in chapters 3 and 4 of this thesis suggest that this results in a reduction in extracellular fluid volume. As a result, this will lead to a reduced rate of net fluid uptake. In the present study, the rate of ^{2}H accumulation in the blood was significantly greater 15 minutes after ingestion of the initial bolus of test solution on the 2% glucose trial compared to the 10% glucose trial. The total

volume of fluid and test meal volume present in the stomach 15 minutes after ingestion of the initial bolus of test solution was not different between trials and the ^{2}H concentration of the gastric contents was also not different between the trials. The reason for the faster rate of fluid uptake during this period on the 2% glucose trial must therefore be due to the faster rate of intestinal absorption. Further evidence for this can be seen from the plasma volume and serum osmolality data which shows plasma volume to be greater and serum osmolality lower 20 and 15 minutes respectively after ingestion of the initial bolus of test solution on the 2% glucose trial compared to the 10% glucose trial.

Although the rate of intestinal absorption appears to have been the main factor in reducing the total rate of fluid uptake on the 10% glucose trial in the early stages of this investigation, the gastric aspirate ^{2}H concentration would seem to suggest that the reduced rate of gastric emptying on the 10% glucose trial is the main reason for the reduced rate of total fluid uptake after the initial 15-30 minute period.

Both plasma volume and blood volume were significantly higher at numerous time points on the 2% glucose trial compared to the 10% glucose trial and significant deviations from baseline values were observed on both trials. In addition, serum osmolality was significantly lower at numerous time points on the 2% glucose trial compared to the 10% glucose trial. Circulating arginine vasopressin concentrations are primarily influenced by changes in the osmolality of the extracellular fluid and, to a lesser extent, changes in blood and plasma volume (Baylis, 1987). It is likely that the increased urine volume and decreased urine osmolality observed on the 2% glucose trial compared to the 10% glucose trial in the present study is due to alterations in arginine vasopressin concentrations resulting from observed changes in serum osmolality and blood and plasma volumes.

The results reported in chapter 5 of this thesis showed that a hypertonic glucose-electrolyte solution was more effective in restoring and maintaining whole body fluid balance following a period of exercise-induced dehydration than a hypotonic glucose-electrolyte solution and an electrolyte solution containing no glucose when a fixed volume of 150% body mass lost was consumed over a one hour period. In addition, the results presented in chapter 6 of this thesis showed that a hypertonic glucose-

electrolyte solution was as effective in restoring and maintaining whole body fluid balance following a period of exercise-induced dehydration than a hypotonic glucose-electrolyte solution and an electrolyte solution containing no glucose when fluid was ingested *ad libitum* over a two hour period. The results of the present study suggest that the success of hypertonic glucose-electrolyte solutions in maintaining fluid balance is due to a combination of a reduced rate of gastric emptying and a reduced rate of intestinal absorption when compared to hypotonic glucose-electrolyte solutions. Of these, the reduced rate of gastric emptying following ingestion of a hypertonic glucose-electrolyte solution seems to have the greatest impact on the total rate of fluid absorption.

Chapter 8

General Discussion and Conclusions

Effect of solution osmolality on body water

Previous investigations have shown that perfusion of hypertonic solutions into the intestine results in secretion of water and electrolytes into the lumen due to the osmotic gradient that is established between the intestinal contents and the extracellular fluid (Leiper and Maughan, 1986). The effects of water secretion into the intestine on body water compartments has not been extensively researched. Merson *et al.* (2002) reported that ingestion of a hypertonic 12% glucose solution resulted in a lower estimated plasma volume compared to ingestion of a 2% glucose solution. This suggested that ingestion of high carbohydrate hypertonic solutions may result in reductions in plasma and extracellular fluid volume.

The studies reported in chapters 3 and 4 of this thesis were designed to investigate the effects of ingesting solutions with different carbohydrate concentrations and osmolalities on changes in blood and plasma volume and offer further insight into the physiological consequences of intestinal water secretion. From the data presented in these chapters, it was concluded that ingestion of low energy hypotonic solutions results in increases in plasma volume, whereas energy dense hypertonic solutions result in reductions in plasma volume which is most likely due to an acute net secretion of water into the intestinal lumen.

In situations where plasma volume expansion in desirable to maintain muscle blood flow and effective thermoregulation (Gonzalez-Alonso *et al.* 1998), i.e. during prolonged exercise, it would seem that low energy hypotonic solutions should be the drink of choice and energy dense hypertonic solutions should be avoided. Although not studied in this thesis, the reduction in plasma and extracellular fluid volume observed following ingestion of energy dense hypertonic solutions may have a negative effect on thermoregulation during exercise. Further investigation into this topic seems appropriate.

Restoration of fluid balance following exercise-induced dehydration

Previous investigations have highlighted the need to ingest a volume of fluid greater than that lost during exercise in order to effectively restore fluid balance after a period

of exercise-induced dehydration (Mitchell *et al.* 1994; Shirreffs *et al.* 1996). This is again demonstrated by the results reported in chapter 5 of this thesis. During this study a fixed volume of 150 (130-150) % of body mass lost during exercise was consumed over a period of one hour and, as a result, subjects were in a state of positive fluid balance on all trials following the rehydration period.

Rehydration strategies similar to the one used in the study reported in chapter 5 are required to understand mechanisms behind the efficacy of rehydration solutions however, they are not necessarily strategies that are used in applied situations. *Ad libitum* fluid ingestion studies have shown that fluid intake is highly variable between subjects (Maughan and Leiper, 1993; Wemple *et al.* 1997). It is likely that this high variability in fluid intake during the post-exercise period will be observed in common sporting situations and, as such, care should be taken when considering the results of rehydration studies.

The study reported in chapter 6 allowed *ad libitum* fluid ingestion over a two hour period following cessation of exercise in the heat and is, therefore, likely to be closer to rehydration strategies used by athletes in applied situations. Despite total fluid intake exceeding fluid losses incurred during exercise on all trials, at no point were subjects considered to be in positive fluid balance. In addition, the results of this study again highlight the large variation in fluid intake between subjects during the post-exercise period.

Due to the relatively large variability in total fluid intake observed between subjects (Maughan and Leiper, 1993; Wemple *et al.* 1997), the effect of an individuals taste preference on fluid intake (Maughan and Leiper, 1993) and previously reported effects of the rate of fluid intake on whole body fluid balance (Archer and Shirreffs, 2001; Kovacs *et al.* 2002), it seems important to consider the results of rehydration studies in an applied sense. While the results of some studies may suggest that a drink is an effective rehydration solution in a laboratory setting, this does not necessarily mean that it will be an effective rehydration solution in applied situations. For example, the addition of sodium to an ingested solution has been extensively researched (including Shirreffs *et al.* 1998; Mitchell *et al.* 2000) and these investigations have suggested that large quantities of sodium are necessary to

maintain fluid balance for a long duration. Other studies have shown that increasing the sodium concentration of a solution from 25 mmol L^{-1} to 50 mmol L^{-1} results in a reduction in voluntary fluid intake and that the 25 mmol L^{-1} sodium solution was more effective in restoring fluid balance as a result (Wemple *et al.* 1997).

The role of glucose in the maintenance of fluid balance following exercise-induced dehydration

The role of carbohydrate in post-exercise rehydration has been largely ignored despite the promising results of some studies (Gonzalez-Alonso *et al.* 1992; Nielsen *et al.* 1986; Costill and Sparks 1973; Lambert *et al.* 1992). However, the methodologies of these investigations differed substantially and, as a result, the role of carbohydrate in the maintenance of fluid balance following dehydration has not been systematically examined.

The results of the study reported in chapter 5 showed that fluid balance was maintained for a longer period of time following ingestion of a 10% glucose-electrolyte solution compared to a 2% and 0% glucose-electrolyte solution when a fixed volume of 150 (130-150) % body mass lost during exercise was consumed over a period of one hour. Similarly, the results of the study reported in chapter 6 showed that there was no difference in net fluid balance between a 0, 2 or 10% glucose-electrolyte solution when a two hour *ad libitum* fluid intake was allowed. The results of these studies suggest that there is a role for the addition of large amounts of glucose to a rehydration solution. The results of the study reported in chapter 7 suggest that this is due to a relatively low rate of gastric emptying and the avoidance of large increases in plasma volume and falls in serum osmolality as a result. As the rate of gastric emptying is reduced when energy density increases, the results of this study can be translated to other energy dense solutions.

The success of energy dense hypertonic solutions in maintaining fluid balance following exercise-induced dehydration is the result of a relatively slow rate of fluid uptake and the avoidance of large acute increases in plasma volume and falls in serum osmolality that will ultimately affect the extent of urine production due to their role in

maintaining circulating concentrations of arginine vasopressin and other fluid retention hormones (Baylis, 1987; Nose *et al.* 1988a; Nose *et al.* 1988b).

The rate of fluid uptake of an ingested solution is dependent on the combined rates of gastric emptying and intestinal absorption. The rate of gastric emptying of a solution is affected by its energy density (Costill and Saltin, 1974; Simpson *et al.* 2001; Vist and Maughan, 1994) and, to a lesser extent, its osmolality (Brouns *et al.* 1995; Simpson *et al.* 2002; Vist and Maughan, 1995). However, as shown in chapters 3 and 4 of this thesis, the ingestion of hypertonic solutions results in reductions in plasma volume and, therefore, a reduction in the rate of intestinal absorption compared to hypotonic solutions.

The results of the study described in chapter 7 suggested that the relatively low rate of fluid uptake of a hypertonic solution was primarily due to the relatively low rate of gastric emptying, although the rate of intestinal absorption did have a measurable affect on fluid absorption during the initial stages of the investigation. This suggests that the gastric emptying characteristics of a solution are of importance when formulating rehydration solutions and that a relatively low rate of gastric emptying is beneficial as this will reduce the rate of fluid absorption and help avoid a diuresis.

The rehydration studies described in this thesis used drinks that differed only in the concentration of glucose present in the solutions. As it seems that low rates of gastric emptying are beneficial for the maintenance of fluid balance following dehydration and the osmolality of a solution has less effect on the rate of gastric emptying than the energy density (Vist and Maughan, 1995), the drinks used during the rehydration studies in this thesis could be modified but still remain effective rehydration solutions.

The substitution of maltodextrins for glucose would reduce the osmolality of a solution but have little effect on the rate of gastric emptying (Vist and Maughan, 1995). Consequently, such solutions should be effective in maintaining fluid balance following dehydration as their ingestion should result in similar physiological responses of plasma volume and serum osmolality observed during the rehydration studies described in this thesis.

The addition of sodium to rehydration solutions has been extensively studied. Shirreffs and Maughan (1998) reported that subjects remained in a state of positive fluid balance 6 hours after ingesting a 100 mmol L^{-1} sodium solution in a volume greater than body mass lost during exercise. However, large amounts of potassium were excreted and this resulted in significant potassium deficit. It would seem that the addition of sodium is still the main consideration when formulating rehydration solutions however, the carbohydrate content of the solution should also be considered as this does not seem to have any additional detrimental effect on whole body electrolyte balance.

Conclusions

The experiments described within this thesis have extended previous knowledge concerning the effects of glucose ingestion on water movement and availability at rest and following exercise resulting in moderate reductions in total body water content. The main conclusions that can be drawn from this work are:

1) Ingestion of strongly hypertonic solutions results in a reduction in plasma and extracellular fluid volume which can most likely be explained by the secretion of water into the intestinal lumen as a result of the osmotic gradient that is established following ingestion of such solutions.

2) Ingestion of commercially available hypertonic carbohydrate solutions may not result in reductions in extracellular fluid volume due to the characteristics of an ingested solution. In particular, the type of carbohydrate and electrolyte content of a drink may have an effect on the extent of both fluid uptake and/or secretion.

3) If an aim of fluid ingestion is plasma volume expansion, as is likely to be the case during prolonged exercise, then hypotonic solutions should be the drink of choice as fluid is available at the fastest rate. Hypertonic solutions are likely to be ineffective rehydration solutions if plasma volume expansion is desirable.

4) Hypertonic glucose-electrolyte solutions are more effective than hypotonic glucose-electrolyte and electrolyte only solutions following a period of exercise-induced dehydration when a fixed volume of fluid is consumed. This is due to a relatively low rate of fluid uptake that results in relatively low rates of urine production.

5) Hypertonic glucose-electrolyte solutions are as effective at maintaining whole body fluid balance as hypotonic-electrolyte and electrolyte only solutions following a period of exercise-induced dehydration when fluid is consumed *ad libitum*.

6) It would seem that sodium content of an ingested solution is the main consideration when formulating rehydration solutions. However, the carbohydrate content of a rehydration solution is an important consideration due to the provision of fluid, substrate and the avoidance of negative effects on whole body electrolyte balance.

7) The reduced rate of total fluid uptake observed following repeated ingestion of a hypertonic glucose-electrolyte solution is primarily due to the rate of gastric emptying rather than the rate of intestinal absorption.

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Appendix A

Ref No: R04/P68

LOUGHBOROUGH UNIVERSITY

ETHICAL ADVISORY SUB-COMMITTEE

RESEARCH PROPOSAL
INVOLVING HUMAN PARTICIPANTS

Title: **Acute effects of ingestion of different drinks on blood volume**

Applicants: Professor R Maughan, Dr S Shirreffs, Dr P Watson

Department: School of Sport and Exercise Sciences

Date of clearance: 27 September 2004

Comments of the Sub-Committee:

The Sub-Committee agreed to issue clearance to proceed subject to the following conditions:

- That references in the Participant Information to the University's 'Ethics Committee' were changed to 'Ethical Advisory Committee.'
- That the investigators provided more information on the water/glucose test drink to be used in study 1. This would help the Sub-Committee to determine whether the University's insurers would require the study to be notified on the basis that the drink might be considered a 'food supplement' rather than a 'food or nutrient' under the insurer's scheme. The Sub-Committee felt it would probably be necessary, once more information was provided, to approach the insurers for a view on the status of the drink.
- That the investigators consider whether some of the language in the Participant Information sheet (references to 'high osmality' for example) was appropriate for the layman.

Ref No: G04/P1

LOUGHBOROUGH UNIVERSITY

ETHICAL ADVISORY SUB-COMMITTEE

RESEARCH PROPOSAL
INVOLVING HUMAN PARTICIPANTS

Title: Post exercise rehydration in man

Applicants: SM Shirreffs, R Maughan, J Foolchand, C Sharkey

Department: School of Sport and Exercise Sciences

Date of clearance: 16 April 2004

Comments of the Sub-Committee:

The Sub-Committee agreed to issue clearance to proceed, subject to the following condition:

- That the investigators changed the title to the gender neutral: 'Post exercise rehydration in humans.'

Ref No: R06/P18

LOUGHBOROUGH UNIVERSITY

ETHICAL ADVISORY COMMITTEE

RESEARCH PROPOSAL
INVOLVING HUMAN PARTICIPANTS

Title: Post-exercise rehydration in man: the role of carbohydrate content and solution osmolality

Applicant: Dr S Shirreffs, Professor R Maughan, G Evans

Department: SSES

Date of clearance: 24 February 2006

Comments of the Committee:

The Committee agreed to issue clearance to proceed, but noted that the Secretary would refer the proposal to UMAL in compliance with its Clinical Trial Questionnaire procedures.

Ref No: R06/P96

LOUGHBOROUGH UNIVERSITY

ETHICAL ADVISORY SUB-COMMITTEE

RESEARCH PROPOSAL
INVOLVING HUMAN PARTICIPANTS

Title:	Rate of gastric emptying and blood deuterium oxide accumulation following ingestion of 2 and 10% glucose-electrolyte solutions
Applicant:	Professor R Maughan, Dr S Shirreffs, G Evans, Dr J Leiper
Department:	SSES
Date of clearance:	12 October 2006

Comments of the Sub-Committee:

The Sub-Committee agreed to issue clearance to proceed subject to the following conditions:

- That the participant information sheet was amended to explain fully what was involved in positioning a gastric tube.
- The Sub-Committee expressed some concern about:

(i) the volume of liquid that participants would be asked to drink,
(ii) the total amount of glucose that participants would be ingesting, and
(iii) the frequency of blood samples to be taken.

The investigators were asked to confirm that the volumes of liquid and sugar referred to in (i) and (ii), and the frequency of samples in (iii) were standard and/or similar to those employed in other studies.

Appendix B

The following subjective feelings questionnaire was completed at the time points described in chapters 5 and 6 of this thesis. Subjects were asked to place a vertical line at the point which they believe corresponded to their feelings at that time.

Subjective feeling

How thirsty do you feel now?

not at all thirsty _____ very thirsty

How full does your stomach feel now?

not at all full _____ very full

How bloated do you feel now?

not at all bloated _____ very bloated

How hungry do you feel now?

not at all hungry _____ very hungry

How tired do you feel now?

not at all tired _____ very tired

How alert do you feel now?

not at all alert _____ very alert

How well can you concentrate just now?

not at all well _____ very well

How does your head feel now?

not at all sore _____ very sore

The following subjective feelings questionnaire was completed immediately after the two hour rehydration phase described in chapter 6 of this thesis. The procedure for completing the questionnaire was the same as described above.

Subjective feeling

How sweet did your drink taste?

not at all sweet _____ very sweet

How bitter did your drink taste?

not at all bitter _____ very bitter

How pleasant did your drink taste?

not at all pleasant _____ very pleasant

How refreshed do you feel now?

not at all refreshed _____ very refreshed

Appendix C

The data presented in this appendix is a tabulated version of the data used to construct figures shown in chapter 3.

Table C.1 Estimated percentage changes in blood volume following ingestion of 0, 6, 11 and 18% carbohydrate solutions. Values are Mean (SD).

Time (min)	0% CHO	6% CHO	11% CHO	18% CHO
0	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
10	-0.3 (1.8)	-1.3 (0.8)	-1.3 (0.9)	-1.0 (2.2)
20	-0.3 (1.8)	-0.3 (2.2)	-1.7 (1.9)	-1.0 (2.2)
30	0.0 (2.2)	-0.3 (2.0)	-0.3 (1.5)	-1.6 (2.2)
40	-0.7 (1.5)	0.0 (1.8)	-0.3 (1.6)	-1.6 (2.5)
50	-1.0 (2.0)	-0.7 (1.7)	-1.0 (1.9)	-3.2 (2.4)
60	-1.3 (2.1)	-2.6 (1.4)	-2.2 (2.0)	-2.4 (2.6)

Table C.2 Estimated percentage changes in plasma volume following ingestion of 0, 6, 11 and 18% carbohydrate solutions. Values are Mean (SD).

Time (min)	0% CHO	6% CHO	11% CHO	18% CHO
0	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
10	-1.9 (2.4)	-3.4 (1.8)	-3.0 (2.2)	-3.2 (2.5)
20	-1.1 (2.0)	-1.4 (3.4)	-3.2 (3.5)	-3.2 (2.8)
30	0.1 (2.5)	-0.6 (3.4)	-1.1 (3.0)	-2.4 (3.4)
40	-0.8 (1.5)	-0.5 (2.8)	-1.0 (2.6)	-2.5 (3.5)
50	-0.7 (2.4)	-1.2 (2.6)	-2.4 (3.6)	-4.4 (3.2)
60	-0.9 (2.8)	-3.4 (2.0)	-3.7 (3.5)	-2.6 (3.9)

Table C.3 Estimated percentage changes in red cell volume following ingestion of 0, 6, 11 and 18% carbohydrate solutions. Values are Mean (SD).

Time (min)	0% CHO	6% CHO	11% CHO	18% CHO
0	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
10	0.8 (2.1)	1.3 (1.2)	1.1 (1.6)	0.2 (2.7)
20	1.1 (1.9)	2.1 (1.7)	1.4 (0.8)	1.2 (1.9)
30	0.9 (2.3)	1.1 (1.4)	1.4 (1.5)	0.8 (1.4)
40	0.0 (2.4)	0.5 (1.2)	0.7 (0.7)	0.8 (1.5)
50	0.1 (2.3)	0.3 (1.2)	1.0 (0.8)	-0.4 (1.9)
60	0.3 (1.8)	-0.5 (1.2)	0.5 (1.6)	0.3 (1.3)

Table C.4 Percentage change in heart rate from baseline value following ingestion of 0, 6, 11 and 18% carbohydrate solutions. Values are Mean (SD). * indicates time point significantly different ($P < 0.05$) from average rest.

Time (min)	0% CHO	6% CHO	11% CHO	18% CHO
Average rest	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
Drinking	5.3 (3.4)*	9.2 (6.0)*	5.9 (7.7)	5.7 (5.3)
10	-1.9 (2.9)	3.0 (6.2)	-0.9 (4.1)	2.2 (5.3)
20	-5.1 (3.8)*	0.8 (6.1)	-6.0 (4.2)	0.2 (6.1)
30	-5.7 (2.6) *	0.7 (6.1)	-4.3 (3.6)	0.7 (5.7)
40	-4.7 (2.9)*	1.2 (4.5)	-0.5 (4.2)	3.6 (9.5)
50	-4.6 (3.9)*	5.1 (6.2)	3.4 (5.6)	5.9 (11.2)
60	-3.5 (3.5)	7.4 (6.3)	7.2 (6.5)	7.7 (11.2)

Appendix D

The data presented in this appendix is a tabulated version of the data used to construct figures shown in chapter 4.

Table D.1 Estimated percentage changes in blood volume following ingestion of 0, 2, 5 and 10% glucose solutions. Values are Median (Range). * indicates time point significantly different ($P < 0.05$) from pre-ingestion value.

Time (min)	0% glucose	2% glucose	5% glucose	10% glucose
0	0.0	0.0	0.0	0.0
	(0.0 – 0.0)	(0.0 – 0.0)	(0.0 – 0.0)	(0.0 – 0.0)
10	0.7	0.7	-0.3	1.5
	(-1.9 – 2.9)	(-2.0 – 2.6)	(-2.7 – 1.4)	(-3.7 – 0.7)
20	1.1	1.8*	0.7	1.8
	(-3.2 – 2.9)	(-0.7 – 6.7)	(-0.7 – 3.5)	(-2.8 – 3.7)
30	0.4	1.4*	1.4	2.6
	(-1.7 – 2.5)	(-2.0 – 5.3)	(-2.4 – 4.2)	(-3.7 – 5.3)
40	0.0	0.6	0.4	1.8
	(-1.3 – 1.9)	(-2.0 – 3.2)	(-3.0 – 5.7)	(-3.3 – 2.2)
50	-0.4	-0.3	0.0	2.5
	(-3.1 – 1.9)	(-3.8 – 0.7)	(-2.0 – 5.7)	(-6.5 – 1.9)
60	-1.0	-1.1*	-0.7	2.0
	(-3.7 – 1.3)	(-3.2 – 1.4)	(-4.6 – 2.0)	(-3.1 – 2.5)

Table D.2 Estimated percentage changes in plasma volume following ingestion of 0, 2, 5 and 10% glucose solutions. Values are Mean (SD). * indicates time point significantly different ($P < 0.05$) from pre-ingestion value.

Time (min)	0% glucose	2% glucose	5% glucose	10% glucose
0	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
10	0.3 (2.3)	0.8 (1.5)	-0.8 (1.9)	-2.9 (2.0)*
20	0.9 (2.8)	2.7 (2.4)*	0.6 (1.7)	-1.4 (2.3)
30	0.9 (2.1)	1.9 (2.5)	1.4 (2.4)	-1.8 (3.2)
40	-0.1 (1.5)	0.3 (1.8)	0.8 (3.3)	-2.0 (2.2)
50	-1.0 (1.9)	-1.2 (1.9)	0.2 (3.1)	-2.3 (3.3)
60	-1.5 (1.6)	-1.9 (2.4)	-2.1 (3.0)	-2.7 (2.0)*

Table D.3 Estimated percentage changes in red cell volume following ingestion of 0, 2, 5 and 10% glucose solutions. Values are Mean (SD).

Time (min)	0% glucose	2% glucose	5% glucose	10% glucose
0	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
10	0.6 (1.1)	0.4 (1.6)	0.2 (1.8)	0.6 (1.5)
20	0.7 (1.9)	0.9 (2.0)	0.8 (1.3)	1.9 (1.7)
30	0.4 (1.3)	0.4 (1.5)	1.1 (1.7)	1.8 (2.4)
40	0.2 (1.4)	0.6 (1.9)	0.9 (2.0)	1.1 (2.0)
50	0.5 (2.1)	0.1 (1.6)	0.5 (1.9)	1.6 (1.6)
60	-0.1 (2.4)	-0.3 (1.5)	0.4 (2.0)	1.4 (2.4)

Table D.4 Changes in serum osmolality (mosm kg^{-1}) following ingestion of 0, 2, 5 and 10% glucose solutions. Values are mean (SD). + denotes 0% glucose time point *significantly different* ($P < 0.05$) from baseline value. # denotes time point significantly different ($P < 0.05$) from 0% glucose time point. * denotes time point significantly different ($P < 0.05$) from 0 and 2% glucose time point. ^ denotes time point significantly different ($P < 0.05$) from 0% glucose time point.

Time (min)	0% glucose	2% glucose	5% glucose	10% glucose
0	290 (4)	290 (4)	290 (4)	290 (5)
10	285 (6)+	286 (5)	292 (5)#	292 (5)#
20	287 (5)	287 (4)	291 (4)	294 (5)*
30	286 (3)	287 (5)	291 (4)	293 (6)*
40	287 (4)	288 (4)	291 (5)	292 (4)^
50	289 (3)	289 (5)	293 (4)	293 (4)
60	290 (5)	290 (4)	292 (4)	293 (4)

Table D.5 Percentage change in heart rate from baseline value following ingestion of 0, 2, 5 and 10% glucose solutions. Values are Mean (SD). * indicates time point significantly different ($P < 0.05$) from average resting value.

Time (min)	0% glucose	2% glucose	5% glucose	10% glucose
Average rest	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
Drinking	6.5 (5.0)*	5.6 (5.7)	8.8 (5.9)*	10.6 (5.9)
10	1.1 (3.8)	-1.1 (4.5)	4.5 (3.1)*	7.2 (5.2)*
20	-0.7 (3.5)	-3.6 (3.9)	2.4 (2.8)	3.7 (5.6)
30	-2.8 (3.4)	-4.9 (2.5)*	0.8 (4.2)	4.0 (4.7)
40	-3.7 (3.6)	-4.4 (3.4)*	0.3 (4.8)	4.4 (5.5)
50	-2.4 (4.5)	-1.5 (4.0)	1.9 (4.4)	5.9 (5.7)
60	-0.9 (4.5)	-0.1 (3.7)	4.9 (4.9)	6.9 (4.9)*

Appendix E

The data presented in this appendix is a tabulated version of the data used to construct figures shown in chapter 5.

Table E.1 *Urine output* (ml) over time. Values are mean (SD). * indicates time point significantly different ($P < 0.05$) from post-exercise value.

Time after rehydration (h)	0% glucose	2% glucose	10% glucose
Post-exercise	23 (10)	44 (48)	47 (39)
0	67 (18)	47 (16)	25 (13)
1	585 (67)*	528 (101)*	347 (121)*
2	379 (139)*	313 (214)*	212 (119)*
3	172 (164)	144 (166)	187 (130)
4	93 (63)	70 (33)	137 (73)
6	152 (74)	118 (38)	143 (54)

Table E.2 Net fluid balance (ml) following ingestion of 0, 2 and 10% glucose solutions. Values are mean (SD). * indicates time point significantly different ($P < 0.05$) from pre-exercise value.

Time after rehydration (h)	0% glucose	2% glucose	10% glucose
Pre-exercise	0 (0)	0 (0)	0 (0)
Post-exercise	-1372 (110)*	-1394 (216)*	-1389 (170)*
0	541 (136)*	539 (106)*	512 (183)*
1	-43 (190)	11 (143)	165 (151)
2	-422 (94)*	-302 (198)	-47 (173)
3	-594 (214)*	-447 (331)*	-233 (228)
4	-687 (264)*	-516 (363)*	-370 (227)*
6	-839 (318)*	-634 (384)*	-514 (228)*

Table E.3 Estimated percentage changes in blood volume following ingestion of 0, 2 and 10% glucose solutions. Values are mean (SD). * indicates time point significantly different ($P < 0.05$) from pre-exercise value.

Time after rehydration (h)	0% glucose	2% glucose	10% glucose
Pre-exercise	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
Post-exercise	-2.5 (2.7)	-1.9 (2.1)	-2.4 (2.3)
0	1.6 (2.3)	4.6 (3.7)*	-0.4 (1.9)
1	0.7 (2.5)	2.0 (1.8)	1.0 (2.0)
2	1.6 (2.6)	1.3 (2.2)	0.9 (2.2)
3	-0.1 (3.6)	1.4 (2.1)	1.9 (3.3)
4	-0.1 (2.2)	0.6 (1.7)	1.5 (2.2)
6	-1.8 (3.4)	0.3 (2.1)	0.6 (4.6)

Table E.4 Estimated percentage changes in plasma volume following ingestion of 0, 2 and 10% glucose solutions. Values are mean (SD). * indicates time point significantly different ($P < 0.05$) from pre-exercise value.

Time after rehydration (h)	0% glucose	2% glucose	10% glucose
Pre-exercise	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
Post-exercise	-3.8 (2.9)	-3.6 (3.2)	-3.7 (3.4)
0	3.6 (2.3)	9.0 (4.9)*	-0.8 (2.2)
1	2.3 (3.4)	5.4 (3.3)	2.3 (4.1)
2	3.5 (2.4)	4.1 (3.3)	3.3 (3.0)
3	1.2 (4.5)	3.5 (3.2)	3.8 (4.2)
4	0.7 (3.6)	2.6 (2.3)	3.3 (1.7)
6	-1.8 (4.5)	1.5 (3.1)	2.3 (5.0)

Table E.5 Estimated percentage changes in red cell volume following ingestion of 0, 2 and 10% glucose solutions. Values are mean (SD).

Time after rehydration (h)	0% glucose	2% glucose	10% glucose
Pre-exercise	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
Post-exercise	-0.9 (2.7)	-0.5 (2.7)	-0.7 (1.7)
0	-1.2 (4.0)	-2.5 (4.1)	0.0 (2.0)
1	-1.5 (2.7)	-3.3 (1.9)	-0.5 (2.5)
2	-1.0 (3.2)	-3.2 (2.6)	-2.0 (3.6)
3	-1.6 (3.0)	-2.3 (2.6)	-0.7 (2.6)
4	-1.0 (2.3)	-1.8 (2.7)	-0.9 (3.4)
6	-1.8 (2.3)	-1.9 (3.0)	-1.8 (4.2)

Table E.6 Serum osmolality (mosm kg^{-1}) following ingestion of 0, 2 and 10% glucose solutions. Values are mean (SD). * indicates time point significantly different ($P < 0.05$) from pre-exercise value. + indicates 10% glucose time point significantly greater ($P = 0.007$) than 0% glucose time point.

Time after rehydration (h)	0% glucose	2% glucose	10% glucose
Pre-exercise	277 (5)	279 (3)	278 (4)
Post-exercise	283 (6)	285 (3)*	285 (3)
0	274 (9)	278 (3)	285 (3)*
1	273 (7)	275 (6)	283 (7)
2	280 (3)	274 (4)	279 (6)
3	282 (2)	280 (3)	279 (3)
4	281 (4)	280 (4)	280 (4)
6	281 (3)	281 (3)	280 (4)

Table E.7 Urine osmolality (mosm kg^{-1}) following ingestion of 0, 2 and 10% glucose solutions. Values are mean (SD).

Time after rehydration (h)	0% glucose	2% glucose	10% glucose
Pre-exercise	504 (169)	528 (294)	521 (244)
Post-exercise	507 (122)	596 (262)	451 (228)
0	562 (118)	692 (114)	790 (74)
1	95 (14)	99 (31)	145 (67)
2	134 (65)	181 (79)	198 (64)
3	291 (132)	414 (179)	303 (238)
4	541 (176)	612 (154)	328 (194)
6	583 (178)	631 (204)	472 (161)

Table E.8 (a) Subjective feeling (cm) of thirst following ingestion of 0, 2 and 10% glucose solutions. Values are mean (SD).

Time after rehydration (h)	0% glucose	2% glucose	10% glucose
Pre-exercise	2.8 (1.7)	3.7 (1.8)	3.5 (2.2)
Post-exercise	8.6 (1.0)	8.0 (1.7)	8.6 (1.2)
0	2.1 (1.0)	1.7 (0.9)	1.6 (1.1)
1	3.2 (2.2)	3.5 (1.6)	3.0 (2.3)
2	4.5 (2.6)	5.1 (2.2)	4.0 (1.9)
3	4.7 (2.8)	6.0 (2.0)	5.3 (2.7)
4	4.8 (2.7)	5.9 (2.2)	6.0 (1.9)
6	5.9 (3.2)	6.7 (3.0)	6.1 (2.8)

Table E.8 (b) Subjective feeling (cm) of stomach fullness following ingestion of 0, 2 and 10% glucose solutions. Values are mean (SD). * indicates 10% glucose time point significantly greater ($P < 0.05$) than 0 and 2% glucose time points.

Time after rehydration (h)	0% glucose	2% glucose	10% glucose
Pre-exercise	1.2 (0.8)	2.3 (2.7)	1.9 (2.0)
Post-exercise	0.8 (0.7)	1.3 (1.8)	0.9 (1.0)
0	5.4 (1.5)	6.3 (2.1)	8.3 (1.4)*
1	2.3 (1.3)	3.5 (1.6)	5.6 (2.0)*
2	1.7 (1.3)	1.3 (1.5)	3.6 (2.0)*
3	1.1 (1.5)	1.2 (1.3)	2.3 (2.0)
4	1.1 (1.4)	0.7 (1.2)	2.4 (2.1)
6	0.9 (1.3)	0.7 (1.3)	1.0 (0.8)

Table E.8 (c) Subjective feeling (cm) of bloatedness following ingestion of 0, 2 and 10% glucose solutions. Values are mean (SD). * indicates 10% glucose time point significantly greater ($P < 0.05$) than 0 and 2% glucose time points.

Time after rehydration (h)	0% glucose	2% glucose	10% glucose
Pre-exercise	1.0 (0.8)	1.7 (1.3)	1.5 (0.9)
Post-exercise	0.7 (0.4)	0.7 (0.4)	0.9 (0.8)
0	3.5 (2.3)	4.8 (1.7)	8.6 (1.4)*
1	1.8 (0.8)	1.8 (0.6)	5.6 (2.8)*
2	1.6 (1.8)	0.6 (0.3)	3.2 (2.1)
3	0.6 (0.4)	0.6 (0.3)	2.3 (2.2)
4	0.4 (0.4)	0.3 (0.2)	2.6 (2.0)
6	0.6 (0.8)	0.3 (0.2)	0.8 (0.6)

Table E.8 (d) Subjective feeling (cm) of hunger following ingestion of 0, 2 and 10% glucose solutions. Values are mean (SD).

Time after rehydration (h)	0% glucose	2% glucose	10% glucose
Pre-exercise	4.5 (2.8)	5.8 (2.6)	4.3 (2.5)
Post-exercise	6.6 (2.1)	7.4 (1.2)	6.8 (1.0)
0	5.8 (1.7)	6.1 (2.0)	3.1 (2.3)
1	6.2 (1.5)	6.8 (1.1)	3.7 (2.5)
2	7.4 (1.2)	7.7 (1.1)	6.0 (0.7)
3	7.8 (1.2)	7.5 (0.3)	7.0 (1.3)
4	8.1 (1.1)	8.3 (0.9)	6.3 (2.7)
6	8.5 (1.0)	8.6 (0.6)	8.6 (0.6)

Table E.8 (e) Subjective feeling (cm) of tiredness following ingestion of 0, 2 and 10% glucose solutions. Values are mean (SD).

Time after rehydration (h)	0% glucose	2% glucose	10% glucose
Pre-exercise	3.9 (2.1)	4.3 (2.8)	3.7 (1.9)
Post-exercise	4.5 (2.7)	5.2 (2.4)	5.0 (2.8)
0	5.1 (2.1)	6.2 (2.6)	5.9 (2.2)
1	4.9 (1.9)	4.7 (2.3)	4.5 (1.6)
2	4.8 (2.3)	5.3 (1.3)	4.5 (1.3)
3	5.1 (2.2)	5.9 (1.1)	5.3 (2.4)
4	4.9 (1.6)	5.9 (1.1)	5.3 (1.8)
6	6.3 (1.3)	5.7 (1.6)	4.6 (2.0)

Table E.8 (f) Subjective feeling (cm) of alertness following ingestion of 0, 2 and 10% glucose solutions. Values are mean (SD).

Time after rehydration (h)	0% glucose	2% glucose	10% glucose
Pre-exercise	5.6 (1.6)	5.1 (2.0)	5.7 (1.9)
Post-exercise	6.3 (1.9)	5.9 (1.4)	4.9 (1.9)
0	5.6 (1.3)	5.5 (2.8)	4.8 (2.1)
1	5.0 (2.2)	5.1 (1.6)	5.3 (1.8)
2	5.0 (2.6)	5.2 (1.9)	5.8 (1.4)
3	5.5 (1.6)	5.0 (1.7)	5.7 (1.3)
4	5.9 (1.5)	4.1 (1.2)	5.7 (0.7)
6	5.6 (1.6)	4.7 (0.9)	5.4 (1.0)

Table E.8 (g) Subjective feeling (cm) of concentration following ingestion of 0, 2 and 10% glucose solutions. Values are mean (SD).

Time after rehydration (h)	0% glucose	2% glucose	10% glucose
Pre-exercise	5.3 (2.1)	5.6 (2.0)	6.5 (1.4)
Post-exercise	5.3 (2.3)	6.0 (1.6)	4.9 (2.4)
0	5.9 (1.6)	5.9 (2.3)	5.2 (1.5)
1	5.1 (2.3)	4.9 (1.6)	5.5 (1.6)
2	5.6 (2.4)	5.1 (1.9)	5.8 (1.7)
3	5.5 (1.6)	5.8 (1.8)	5.8 (2.0)
4	5.8 (2.1)	4.7 (1.4)	5.3 (1.1)
6	5.0 (2.1)	4.3 (1.0)	5.5 (1.2)

Table E.8 (h) Subjective feeling (cm) of head soreness following ingestion of 0, 2 and 10% glucose solutions. Values are mean (SD).

Time after rehydration (h)	0% glucose	2% glucose	10% glucose
Pre-exercise	0.2 (0.1)	1.1 (1.3)	1.1 (0.8)
Post-exercise	4.5 (2.6)	3.5 (2.9)	4.7 (2.9)
0	3.5 (1.7)	4.5 (2.7)	2.6 (1.6)
1	3.1 (2.9)	2.9 (2.1)	2.5 (1.4)
2	4.2 (1.9)	3.8 (2.4)	3.4 (2.1)
3	4.7 (2.8)	4.1 (2.6)	2.9 (2.3)
4	4.3 (2.6)	3.5 (3.5)	3.3 (2.2)
6	5.2 (2.4)	4.2 (3.3)	3.6 (2.8)

Appendix F

The data presented in this appendix is a tabulated version of the data used to construct figures shown in chapter 6.

Table F.1 Drink volume (ml) during each 15 minute period of the rehydration phase. Values are mean (SD). * indicates 2% glucose time point significantly different ($P < 0.05$) from 10% glucose time point.

Time	0% glucose	2% glucose	10% glucose
15	657 (186)	608 (88)	686 (174)
30	422 (200)	422 (119)	393 (72)
45	327 (149)	341 (108)	249 (80)
60	208 (89)	334 (133)	226 (103)
75	129 (70)	246 (96)*	122 (84)
90	228 (91)	212 (57)	150 (115)
105	122 (48)	168 (55)	130 (63)
120	160 (85)	206 (86)	218 (107)

Table F.2 Urine output (ml) over time. Values are mean (SD). * indicates time point significantly different ($P < 0.05$) from post-exercise value.

Time after rehydration (h)	0% glucose	2% glucose	10% glucose
Post-exercise	42 (45)	43 (67)	35 (40)
-1	76 (50)	57 (28)	37 (26)
0	396 (211)*	530 (280)*	160 (144)
1	393 (224)*	464 (165)*	220 (170)*
2	116 (77)	121 (59)	179 (111)*
3	71 (37)	47 (28)	190 (70)*
5	108 (41)	102 (48)	135 (85)

Table F.3 Net fluid balance (ml) following ingestion of 0, 2 and 10% glucose solutions. Values are mean (SD). * indicates time point significantly different ($P < 0.05$) from pre-exercise value.

Time after rehydration (h)	0% glucose	2% glucose	10% glucose
Pre-exercise	0 (0)	0 (0)	0 (0)
Post-exercise	-1557 (173)*	-1587 (198)*	-1568 (177)*
-1	-15 (507)	61 (326)	-51 (393)
0	229 (546)	364 (322)	409 (330)
1	-164 (509)	-100 (327)	189 (395)
2	-280 (530)	-221 (321)	10 (476)
3	-351 (545)	-267 (324)	-181 (502)
5	-459 (561)	-369 (345)	-316 (463)

Table F.4 Sodium balance (mmol) following ingestion of 0, 2 and 10% glucose solutions. Values are mean (SD).

Time after rehydration (h)	0% glucose	2% glucose	10% glucose
Pre-exercise	0 (0)	0 (0)	0 (0)
Post-exercise	-96.3 (41.6)	-97.3 (36.9)	-94.5 (43.8)
-1	-54.5 (49.4)	-51.2 (34.8)	-49.3 (49.4)
0	-49.0 (52.3)	-37.3 (34.4)	-37.3 (47.8)
1	-59.1 (53.6)	-47.4 (34.3)	-43.5 (48.1)
2	-66.5 (55.1)	-56.0 (34.3)	-50.8 (49.0)
3	-75.1 (57.8)	-61.9 (33.7)	-60.6 (50.1)
5	-93.0 (61.6)	-79.1 (35.5)	-72.2 (51.3)

Table F.5 Chloride balance (mmol) following ingestion of 0, 2 and 10% glucose solutions. Values are mean (SD). * indicates time point significantly different ($P < 0.05$) from pre-exercise value.

Time after rehydration (h)	0% glucose	2% glucose	10% glucose
Pre-exercise	0 (0)	0 (0)	0 (0)
Post-exercise	-89.2 (52.4)*	-88.7 (43.5)*	-87.2 (50.2)*
-1	-52.3 (58.6)	-48.3 (41.9)	-48.0 (54.2)
0	-46.7 (59.5)	-34.9 (41.8)	-36.7 (53.2)
1	-56.3 (59.8)	-42.9 (41.1)	-41.9 (53.3)
2	-64.3 (60.8)	-50.4 (40.0)	-46.9 (53.7)
3	-72.3 (62.8)	-55.4 (39.3)	-52.7 (54.1)
5	-86.3 (65.6)*	-67.4 (39.6)*	-60.1 (55.3)

Table F.6 Potassium balance (mmol) following ingestion of 0, 2 and 10% glucose solutions. Values are mean (SD). * indicates time point significantly different ($P < 0.05$) from pre-exercise value.

Time after rehydration (h)	0% glucose	2% glucose	10% glucose
Pre-exercise	0 (0)	0 (0)	0 (0)
Post-exercise	-10.5 (1.9)*	-10.7 (3.4)*	-9.9 (2.2)*
-1	-15.0 (3.3)*	-14.3 (3.3)*	-12.0 (3.2)*
0	-26.9 (10.1)*	-21.8 (7.0)*	-17.4 (5.1)*
1	-37.3 (13.6)*	-30.2 (10.9)*	-22.7 (7.8)*
2	-43.2 (15.1)*	-36.8 (13.2)*	-28.9 (10.3)*
3	-48.3 (16.3)*	-40.5 (12.6)*	-37.8 (13.1)*
5	-57.4 (18.1)*	-48.9 (17.6)*	-49.5 (16.2)*

Table F.7 Estimated percentage changes in blood volume following ingestion of 0, 2 and 10% glucose solutions. Values are mean (SD).

Time after rehydration (h)	0% glucose	2% glucose	10% glucose
Pre-exercise	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
Post-exercise	-1.8 (1.8)	-2.4 (4.0)	-3.7 (3.5)
-1	2.4 (2.5)	5.0 (4.1)	-1.4 (5.2)
0	3.2 (2.9)	5.9 (3.2)	-1.4 (2.7)
1	4.3 (4.2)	4.7 (3.2)	0.7 (5.8)
2	4.7 (4.0)	4.4 (3.6)	2.5 (4.6)
3	2.0 (3.4)	3.0 (1.9)	1.7 (3.9)
5	2.6 (4.3)	1.8 (2.9)	2.0 (2.9)

Table F.8 Estimated percentage changes in plasma volume following ingestion of 0, 2 and 10% glucose solutions. Values are mean (SD). * indicates time point significantly different ($P < 0.05$) from pre-exercise value.

Time after rehydration (h)	0% glucose	2% glucose	10% glucose
Pre-exercise	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
Post-exercise	-2.7 (2.6)	-3.5 (5.2)	-5.2 (3.9)
-1	5.3 (4.5)	9.2 (5.9)*	-1.5 (6.2)
0	6.3 (4.6)	11.7 (4.9)*	-1.0 (3.2)
1	8.8 (6.5)*	8.7 (4.9)*	3.2 (7.4)
2	8.6 (6.6)*	8.2 (5.0)*	6.1 (5.9)
3	4.5 (4.9)	6.6 (2.6)	4.3 (6.0)
5	4.8 (4.9)	4.1 (3.4)	3.7 (4.2)

Table F.9 Estimated percentage changes in red cell volume following ingestion of 0, 2 and 10% glucose solutions. Values are mean (SD).

Time after rehydration (h)	0% glucose	2% glucose	10% glucose
Pre-exercise	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
Post-exercise	-0.5 (1.0)	-0.8 (2.8)	-1.9 (3.1)
-1	-1.2 (1.5)	-0.3 (2.3)	-1.3 (4.0)
0	-0.8 (2.8)	-1.3 (1.7)	-2.1 (2.5)
1	-1.4 (2.3)	-0.2 (2.0)	-2.5 (3.8)
2	-0.1 (2.4)	-0.4 (2.1)	-2.4 (3.2)
3	-1.2 (2.0)	-1.5 (1.7)	-1.8 (1.4)
5	0.0 (4.0)	-1.2 (3.5)	-0.2 (1.7)

Table F.10 Serum osmolality (mosm kg^{-1}) following ingestion of 0, 2 and 10% glucose solutions. Values are mean (SD). * indicates time point significantly different ($P < 0.05$) from pre-exercise value.

Time after rehydration (h)	0% glucose	2% glucose	10% glucose
Pre-exercise	278 (4)	280 (2)	277 (4)
Post-exercise	285 (4)*	288 (3)*	285 (3)*
0	277 (5)	279 (3)	285 (3)*
1	275 (5)	277 (1)*	283 (4)
2	277 (5)	277 (2)	281 (6)
3	276 (5)	280 (3)	278 (4)
4	278 (7)	279 (3)	280 (6)
6	279 (6)	282 (3)	279 (5)

Table F.11 (a) Subjective feeling (cm) of thirst following ingestion of 0, 2 and 10% glucose solutions. Values are mean (SD).

Time after rehydration (h)	0% glucose	2% glucose	10% glucose
Pre-exercise	5.3 (1.8)	4.4 (2.3)	4.1 (2.6)
Post-exercise	8.8 (1.1)	8.8 (0.9)	8.9 (0.9)
-1	3.0 (1.7)	2.3 (1.7)	2.7 (2.2)
0	1.2 (0.4)	1.3 (1.1)	2.4 (1.8)
1	3.2 (1.8)	3.5 (2.2)	4.2 (2.4)
2	4.3 (1.9)	4.2 (2.4)	4.8 (2.5)
3	5.3 (2.4)	5.1 (2.7)	5.8 (2.9)
5	6.2 (2.8)	6.1 (3.6)	6.8 (3.1)

Table F.11 (b) Subjective feeling (cm) of stomach fullness following ingestion of 0, 2 and 10% glucose solutions. Values are mean (SD).

Time after rehydration (h)	0% glucose	2% glucose	10% glucose
Pre-exercise	2.6 (2.0)	1.7 (0.8)	2.6 (1.4)
Post-exercise	1.3 (1.2)	2.0 (2.5)	1.5 (1.0)
-1	4.3 (2.4)	5.1 (2.4)	6.2 (2.7)
0	3.8 (2.7)	5.0 (2.7)	6.8 (2.3)
1	2.6 (2.0)	3.0 (2.2)	4.3 (2.2)
2	1.5 (1.0)	2.4 (2.0)	2.8 (2.2)
3	1.2 (0.9)	1.5 (1.1)	1.5 (0.9)
5	1.4 (0.9)	1.9 (2.0)	0.6 (0.5)

Table F.11 (c) Subjective feeling (cm) of bloatedness following ingestion of 0, 2 and 10% glucose solutions. Values are mean (SD).

Time after rehydration (h)	0% glucose	2% glucose	10% glucose
Pre-exercise	2.1 (2.1)	2.2 (1.9)	1.8 (1.4)
Post-exercise	1.5 (1.2)	2.8 (3.2)	1.5 (1.6)
-1	3.2 (2.2)	4.7 (1.7)	5.3 (3.2)
0	3.8 (2.5)	4.4 (2.5)	6.1 (2.6)
1	2.3 (1.3)	2.5 (2.0)	3.3 (2.3)
2	1.2 (0.9)	1.6 (0.9)	3.1 (2.7)
3	1.0 (0.8)	1.3 (1.2)	2.2 (2.5)
5	1.2 (1.1)	1.4 (1.4)	0.8 (0.5)

Table F.11 (d) Subjective feeling (cm) of hunger following ingestion of 0, 2 and 10% glucose solutions. Values are mean (SD).

Time after rehydration (h)	0% glucose	2% glucose	10% glucose
Pre-exercise	5.1 (2.8)	4.5 (2.1)	4.8 (2.2)
Post-exercise	6.7 (2.5)	5.7 (2.9)	6.3 (2.6)
-1	5.1 (2.9)	4.4 (1.9)	3.6 (2.7)
0	5.6 (2.5)	4.7 (2.3)	3.0 (2.2)
1	6.5 (2.1)	6.1 (2.1)	5.7 (1.8)
2	7.3 (1.3)	6.7 (1.7)	5.3 (2.5)
3	7.9 (1.3)	7.8 (1.1)	7.0 (1.6)
5	8.7 (1.1)	7.7 (1.9)	8.5 (1.2)

Table F.11 (e) Subjective feeling (cm) of tiredness following ingestion of 0, 2 and 10% glucose solutions. Values are mean (SD).

Time after rehydration (h)	0% glucose	2% glucose	10% glucose
Pre-exercise	4.8 (2.4)	4.7 (2.5)	4.5 (2.6)
Post-exercise	6.2 (1.7)	5.5 (2.1)	4.6 (2.1)
-1	4.7 (2.0)	4.2 (1.7)	5.7 (1.9)
0	4.5 (2.0)	4.1 (1.5)	5.4 (2.2)
1	3.6 (1.8)	5.1 (2.5)	5.8 (1.5)
2	4.0 (1.7)	5.4 (1.9)	5.4 (2.7)
3	4.8 (2.0)	6.1 (1.6)	7.1 (1.5)
5	6.0 (1.9)	5.6 (2.4)	4.8 (1.9)

Table F.11 (f) Subjective feeling (cm) of alertness following ingestion of 0, 2 and 10% glucose solutions. Values are mean (SD).

Time after rehydration (h)	0% glucose	2% glucose	10% glucose
Pre-exercise	6.1 (2.2)	5.0 (2.3)	5.8 (2.0)
Post-exercise	5.3 (1.4)	4.3 (2.3)	5.4 (2.0)
-1	5.7 (1.4)	6.0 (1.8)	4.6 (1.8)
0	5.4 (1.9)	5.6 (1.6)	4.2 (1.6)
1	6.4 (1.6)	4.7 (2.5)	5.4 (1.7)
2	5.8 (2.0)	4.9 (1.9)	4.5 (2.5)
3	5.2 (2.0)	4.3 (2.0)	4.0 (1.8)
5	3.8 (1.9)	5.3 (1.7)	4.6 (1.8)

Table F.11 (g) Subjective feeling (cm) of concentration following ingestion of 0, 2 and 10% glucose solutions. Values are mean (SD).

Time after rehydration (h)	0% glucose	2% glucose	10% glucose
Pre-exercise	6.4 (2.1)	5.9 (2.0)	6.5 (1.8)
Post-exercise	5.3 (1.1)	5.0 (2.4)	5.3 (1.9)
-1	5.5 (1.3)	5.9 (2.2)	5.0 (2.0)
0	5.7 (1.5)	5.8 (1.6)	5.1 (2.2)
1	6.7 (1.6)	5.2 (2.4)	5.5 (1.6)
2	6.6 (1.1)	5.6 (1.7)	5.3 (2.2)
3	5.0 (2.3)	4.3 (2.0)	4.3 (2.2)
5	4.4 (1.9)	4.7 (1.9)	5.2 (1.5)

Table F.11 (h) Subjective feeling (cm) of head soreness following ingestion of 0, 2 and 10% glucose solutions. Values are mean (SD).

Time after rehydration (h)	0% glucose	2% glucose	10% glucose
Pre-exercise	1.1 (1.9)	1.9 (1.8)	0.7 (0.6)
Post-exercise	2.0 (2.1)	3.6 (2.5)	2.3 (2.3)
-1	2.3 (2.3)	2.7 (2.3)	3.0 (2.9)
0	1.9 (2.2)	2.8 (3.3)	3.4 (3.3)
1	2.4 (2.4)	3.5 (3.4)	4.1 (2.8)
2	2.9 (2.4)	3.5 (3.4)	3.3 (2.8)
3	4.2 (3.5)	4.6 (3.9)	2.5 (2.5)
5	5.1 (3.6)	4.9 (4.1)	2.9 (3.5)

Appendix G

The data presented in this appendix is a tabulated version of the data used to construct figures shown in chapter 7.

Table G.3 Total gastric volume (ml) at each time point following ingestion of 2 and 10% glucose solutions. Values are mean (SD). * indicates 2% glucose time point significantly different ($P < 0.05$) from 10% glucose time point.

Time (min)	2% glucose	10% glucose
1	521 (17)	525 (25)
14	435 (123)	515 (131)
16	928 (176)	1009 (172)
29	732 (308)	989 (154)*
35	1228 (371)	1485 (230)*
44	654 (183)	1245 (196)*
50	1151 (243)	1741 (229)*
60	818 (212)	1639 (230)*
75	590 (234)	1381 (148)*
90	366 (216)	1388 (122)*
105	238 (155)	1262 (152)*
120	167 (88)	1189 (215)*

Table G.4 Test meal volume remaining in the stomach (ml) at each time point following ingestion of 2 and 10% glucose solutions. Values are mean (SD). * indicates 2% glucose time point significantly different ($P < 0.05$) from 10% glucose time point.

Time (min)	2% glucose	10% glucose
1	495 (0)	495 (0)
14	399 (118)	471 (133)
16	893 (170)	965 (167)
29	603 (270)	887 (151)*
35	1099 (325)	1384 (220)*
44	496 (134)	1074 (164)*
50	993 (172)	1570 (205)*
60	708 (227)	1468 (205)*
75	485 (217)	1205 (145)*
90	281 (184)	1161 (144)*
105	162 (128)	1019 (173)*
120	97 (77)	928 (264)*

Table G.5 Gastric secretions (ml) at each time point following ingestion of 2 and 10% glucose solutions. Values are mean (SD). * indicates 2% glucose time point significantly different ($P < 0.05$) from 10% glucose time point.

Time (min)	2% glucose	10% glucose
15	36 (13)	44 (17)
30	129 (64)	101 (89)
45	158 (106)	171 (76)
60	109 (83)	171 (133)
75	106 (54)	176 (99)
90	85 (43)	227 (117)*
105	76 (35)	242 (116)*
120	70 (21)	261 (113)*

Table G.6 The amount of CHO emptied from the stomach (g) at each time point following ingestion of 2 and 10% glucose solutions. Values are mean (SD). * indicates 2% glucose time point significantly different ($P < 0.05$) from 10% glucose time point.

Time (min)	2% glucose	10% glucose
0	0 (0)	0 (0)
15	2 (2)	2 (13)
30	8 (5)	10 (12)
45	20 (4)	41 (21)*
60	25 (6)	51 (22)*
75	30 (6)	78 (19)*
90	34 (6)	82 (21)*
105	36 (5)	96 (18)*
120	38 (6)	105 (24)*

Table G.7 Blood ^{2}H concentration (ppm) at each time point following ingestion of 2 and 10% glucose solutions. Values are mean (SD). * indicates 2% glucose time point significantly different ($P < 0.05$) from 10% glucose time point.

Time (min)	2% glucose	10% glucose
0	0 (0)	0 (0)
2	3 (11)	2 (32)
5	51 (31)	38 (21)
10	147 (74)	92 (37)*
15	232 (99)	132 (33)*
20	312 (100)	170 (39)*
30	300 (82)	167 (37)*
45	295 (82)	179 (35)*
60	261 (61)	187 (31)*
75	245 (71)	185 (35)*
90	247 (63)	195 (37)*
105	244 (61)	201 (40)
120	247 (55)	204 (42)*

Table G.8 Gastric aspirate ^{2}H concentration (ppm) at each time point following ingestion of 2 and 10% glucose solutions. Values are mean (SD). * indicates 2% glucose time point significantly different ($P < 0.05$) from 10% glucose time point.

Time (min)	2% glucose	10% glucose
Drink	19087 (517)	19261 (876)
15	13891 (630)	14112 (890)
30	4525 (1322)	6176 (825)*
45	1761 (589)	3621 (932)*
60	738 (391)	2349 (729)*
75	636 (290)	1868 (462)*
90	556 (371)	1768 (488)*
105	398 (277)	1619 (448)*
120	279 (205)	1475 (455)*

Table G.9 Estimated percentage change in blood volume at each time point following ingestion of 2 and 10% glucose solutions. Values are mean (SD). * indicates 2% glucose time point significantly different ($P < 0.05$) from 10% glucose time point.

Time (min)	2% glucose	10% glucose
0	0.0 (0.0)	0.0 (0.0)
2	-1.3 (1.3)	-0.7 (1.6)
5	-1.6 (1.3)	-2.2 (1.3)
10	-0.9 (2.8)	-2.5 (1.7)
15	-0.2 (1.9)	-0.8 (2.8)
20	-0.6 (2.8)	-2.0 (1.5)
30	0.9 (2.2)	-2.5 (3.8)*
45	1.3 (3.1)	-2.6 (3.6)*
60	2.6 (2.7)	-5.0 (4.3)*
75	3.7 (3.5)	-5.6 (3.6)*
90	2.0 (3.9)	-4.5 (6.0)*
105	1.7 (3.9)	-4.2 (7.0)*
120	-0.1 (3.2)	-3.8 (7.8)

Table G.10 Estimated percentage change in plasma volume at each time point following ingestion of 2 and 10% glucose solutions. Values are mean (SD). * indicates 2% glucose time point significantly different ($P < 0.05$) from 10% glucose time point.

Time (min)	2% glucose	10% glucose
0	0.0 (0.0)	0.0 (0.0)
2	-1.8 (1.6)	-1.0 (2.1)
5	-1.9 (1.6)	-2.5 (0.8)
10	-1.4 (3.1)	-2.7 (1.3)
15	0.3 (2.2)	-1.1 (3.4)
20	-0.2 (2.9)	-2.7 (1.3)*
30	1.7 (2.9)	-3.3 (3.9)*
45	3.0 (4.3)	-3.3 (3.4)*
60	4.6 (3.8)	-7.3 (4.8)*
75	6.7 (4.3)	-7.7 (3.9)*
90	4.7 (5.1)	-6.2 (7.6)*
105	3.9 (5.4)	-5.2 (7.2)*
120	1.8 (4.6)	-4.9 (8.2)*

Table G.11 Estimated percentage change in red cell volume at each time point following ingestion of 2 and 10% glucose solutions. Values are mean (SD).

Time (min)	2% glucose	10% glucose
0	0.0 (0.0)	0.0 (0.0)
2	-0.5 (1.1)	-0.3 (1.3)
5	-1.1 (1.0)	-2.0 (2.2)
10	-0.3 (2.4)	-2.2 (2.4)
15	-1.0 (1.7)	-0.6 (2.6)
20	-1.3 (2.8)	-0.9 (2.3)
30	-0.5 (1.6)	-1.3 (4.0)
45	-1.4 (1.5)	-1.6 (4.1)
60	-0.7 (2.3)	-1.6 (4.1)
75	-1.1 (2.5)	-2.5 (4.2)
90	-2.2 (2.6)	-2.1 (4.9)
105	-1.9 (2.0)	-2.5 (6.6)
120	-3.1 (2.3)	-2.3 (7.2)

Table G.12 Serum osmolality (mosm kg^{-1}) at each time point following ingestion of 2 and 10% glucose solutions. Values are mean (SD). * indicates 2% glucose time point significantly different ($P < 0.05$) from 10% glucose time point. # indicates 2% glucose time point significantly different ($P < 0.05$) compared to time point "0".

Time (min)	2% glucose	10% glucose
0	281 (8)	282 (7)
2	281 (8)	283 (6)
5	281 (7)	282 (7)
10	279 (9)	284 (6)
15	279 (9)	284 (7)*
20	277 (8)	284 (6)*
30	275 (8)#	285 (5)*
45	274 (8)#	284 (6)*
60	272 (7)#	286 (7)*
75	271 (6)#	285 (7)*
90	272 (7)#	283 (6)*
105	272 (6)#	282 (7)*
120	273 (7)#	281 (8)*

Appendix H

The following method was used to calculate the total volume of fluid in the stomach, the volume of test solution present in the stomach and gastric secretions at a given time point during the study reported in chapter 7 of this thesis.

The total volume of fluid present in the stomach at a given time point was calculated using the method described by George (1968):

$$V_1 = V_2 * (C_2 - C_3)/ (C_3 - C_1) \qquad \text{[Equation 1]}$$

Where:

V_1 is the volume to be determined at each time point

V_2 is the volume of phenol red added to determine V_1

C_1 is the concentration of phenol red in V_1

C_2 is the concentration of phenol red added

C_3 is the concentration of phenol red after addition to V_1

The residual volume of the gastric contents was calculated from Equation 1 by taking:

V_2 as the volume of test solution given during the first bolus

C_1 as the concentration of phenol red before drinking the test solution e.g. zero

C_2 as the concentration of phenol red in the first bolus of the test solution

C_3 as the phenol red concentration of the gastric contents following ingestion of the test solution

The volume of the test solution present in the stomach at a given time point was calculated using the method described by Beckers *et al.* (1988):

$$V^t_n = V^t_{n-1} * (V^1_n * C^1_n)/(V^1_{n-1} * C^3_{n-1}) \qquad \text{[Equation 2]}$$

Where:

V^t_n is the volume of test solution present in the stomach at a given time point

V^t_{n-1} is the volume of test solution present in the stomach at the previous time point

V^1_n is the total volume of fluid in the stomach at a given time point

C^1_n is the concentration of phenol red at a given time point before further addition of dye

V^1_{n-1} is the total volume of fluid in the stomach at the previous time point

C^3_{n-1} is the concentration of phenol red at the previous time point after addition of dye

The volume of gastric secretions was calculated by subtracting test meal volume (Equation 2) from total volume of fluid in the stomach (Equation 1).