Fluid and electrolyte balance during dietary restriction

by

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CERTIFICATE OF ORIGINALITY

This is to certify that I am responsible for the work submitted in this thesis, that the original work is my own except as specified in acknowledgments or in footnotes, and that neither the thesis nor the original work contained therein has been submitted to this or any other institution for a degree.

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Abstract

It is known that during fluid restriction, obligatory water losses continue and hypohydration develops and that restricted energy intake leads to a concomitant restriction of all other dietary components, as well as hypohydration, but the specific effects of periods of fluid and/ or energy restriction on fluid balance, electrolyte balance and exercise performance have not been systematically described in the scientific literature.

There were two main aims of this thesis. Firstly, to describe the effects of periods of severe fluid and/ or energy restriction on fluid and electrolyte balance; secondly, to determine the effect of electrolyte supplementation during and after energy restriction on fluid and electrolyte balance as well as energy exercise performance.

The severe restriction of fluid and/ or energy intake over a 24 h period all resulted in body mass loss (BML) and hypohydration, but whilst serum osmolality increases during fluid restriction (hypertonic hypohydration), serum osmolality does not change during energy restriction (isotonic hypohydration), despite similar reductions in plasma volume (Chapter 3). These differences in the tonicity of the hypohydration developed are most likely explainable by differences in electrolyte balance, with fluid restriction resulting in no change in electrolyte balance over 24 h (Chapter 3) and energy restriction (with or without fluid restriction) producing significant reductions in electrolyte balance by 24 h (Chapter 3; Chapter 4; Chapter 5; Chapter 6; Chapter 7).

Twenty four hour combined fluid and energy restriction results in large negative balances of both sodium and potassium, and whilst the addition of sodium chloride to a rehydration solution ingested after fluid and energy restriction increases drink retention, the addition of potassium chloride to a rehydration solution does not (Chapter 4).

Supplementation of sodium chloride and potassium chloride during periods of severe energy restriction reduces the BML observed during energy restriction and maintains plasma volume at pre-energy restriction levels (Chapter 5; Chapter 6; Chapter 7).

These responses to electrolyte supplementation during energy restriction appear to be related to better maintenance of serum osmolality and electrolyte concentrations and a consequential reduction in urine output (Chapter 5; Chapter 6; Chapter 7). Additionally, 48 h energy restriction resulted in a reduction in exercise capacity in a hot environment and an increase in heart rate and core temperature during exercise, compared to a control trial providing adequate energy intake. Whilst electrolyte supplementation during the same 48 h period of energy restriction prevented these increases in heart rate and core temperature and exercise capacity was not different from the control trial Chapter 8).

In conclusion, 24-48 h energy restriction results in large losses of sodium, potassium and chloride in urine and a large reduction in body mass and plasma volume and supplementation of these electrolytes during energy restriction reduces urine output, attenuates the reduction in body mass and maintains plasma volume and exercise capacity.

Key words: **fluid restriction, energy restriction, dietary manipulation, electrolyte balance, hypohydration, exercise capacity.**

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List of abstracts

Some of the results from this thesis have presented in the following abstracts:

James LJ and Shirreffs SM (2008) Effect of electrolyte consumption on fluid and electrolyte balance during 24 h energy restriction. *Proceedings of the Nutrition Society*. **67**, E333.

James LJ and Shirreffs SM (2009) Effect of NaCl and KCl supplementation on fluid and electrolyte balance during 24 hours energy restriction. *British Journal of Sports Medicine*, **43**, e2.

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List of Abbreviations

1RM	one repetition maximum
°C	degrees Celsius
ANOVA	analysis of variance
ATP	adenosine triphosphate
BML	body mass loss
CV	coefficient of variation
DR	dietary restriction
ER	energy restriction
F+ER	fluid and energy restriction
FFA	free fatty acid
FR	fluid restriction
g	gram
h	hour
K_2EDTA	potassium ethylenediamine tetra acetic acid
KCl	potassium chloride
kg	kilogram
l	litre
m	metre
mg	milligram
ml	millilitre
mmol	millimole
mosmol	milliosmole
min	minute
NaCl	sodium chloride
PCr	phosphocreatine
pg	picogram
pmol	picomole
RER	respiratory exchange ratio
RPE	rating of perceived exertion
RPM	revolutions per minute
s	second
SD	standard deviation

TC	thermal comfort
T_c	core temperature
T_{sk}	weighted mean skin temperature
$\dot{V}O_2$ max	maximum rate of oxygen uptake
$\dot{V}O_2$ peak	peak rate of oxygen uptake
y	years

Chapter 1

General Introduction

Human body water

Water is the most abundant molecule in the human body and in most healthy individuals makes up 45-75% of body mass, averaging ~60% (IOM, 2005). Differences in body composition account for most of the variation in relative body water and most of the variation can be explained by differences in the amount of adipose tissue present (Sawka *et al*., 2005). Whilst most lean tissues of the body have a water content of 70-80%, adipose tissue has a water content of ~10% (See Table 1.1) (Sawka, 1990), thus an individual with a higher percentage adipose tissue will have a lower relative total body water in comparison to an individual with a lower percentage adipose tissue. Similarly, when comparing men and women of the same body mass, women generally have lower relative body water, due to a higher relative adipose tissue content.

Table 1.1 Water content (%) of various tissues of the human body.

Tissue	% Water
Skin	72
Organs	76
Skeleton	22
Blood	83
Adipose	10
Muscle	76

Total body water is distributed between two main fluid compartments, the intracellular fluid and the extracellular fluid, with the extracellular fluid further divided into the interstitial and intravascular fluids. Approximately 2/3 total body water is contained in the intracellular fluid, with the remaining 1/3 contained in the extracellular fluid. Of this extracellular fluid, 3/4 contained in the interstitial fluid and 1/4 contained in the intravascular fluid (Sawka, 1990). For an average 70 kg male with a total body water content of 60% body mass (42 l), this would mean 28 l and 14

l water would be held in the intracellular and extracellular fluid compartments respectively, with 10.5 l in the interstitial fluid and 3.5 l in the intravascular fluid.

Regulation of body water

Despite the abundance of water within the human body, total body water is tightly regulated and even relatively small deviations either above or below normal resting levels can have a profound effect on both health and exercise performance (Maughan, 2003). Daily water balance is achieved by balancing water losses and water gains and in normal healthy populations total body water is regulated within ±0.22% body mass in temperate environmental conditions and ±0.48% body mass during hot environmental conditions and exercise (Greenleaf, 1992).

The main avenues of water gain are through water ingested through foods and fluids and via the production of metabolic water during substrate oxidation, whilst the main avenues of water loss are through respiration, perspiration, renal and faecal water losses. Metabolic water production and respiratory water loss are not regulated to maintain water homeostasis and being of similar magnitude (~12-13 $ml \cdot kcal^{-1}$) generally balance each other out (Sawka *et al*., 2007). In normal healthy individuals, faecal water losses are low (~100-200 $ml \cdot d^{-1}$) and thus daily water balance is principally determined by balancing water consumption with renal and perspiratory water losses. For sedentary individuals living in temperate environments, daily water losses through perspiration are relatively low (~0.5 $l \cdot d^{-1}$), but with exercise and or heat exposure water lost through perspiration can be much greater (>10 $l \cdot d^{-1}$) (Maughan, 2003). That said, the human body does not regulate water lost through perspiration to any great extent (i.e. sweat rate is not greatly decreased in a hypohydrated state) and thus fluid ingestion and renal water losses are the main factors that are regulated to determine daily water balance.

Euhydration or a state of body water balance can be represented by a sinusoidal wave, as water is continuously lost from the body, but only intermittently replaced, with hypohydration and hyperhydration representing new steady states below or above euhydration respectively (Greenleaf, 1992). Dehydration refers to the process of losing body water from hyperhydration to euhydration and then to hypohydration,

whilst rehydration refers to the process of gaining water from hypohydration to euhydration (Greenleaf, 1992).

Following a deviation in body water from euhydration, the thirst mechanism and the renal system act in concert to induce changes in water balance and in healthy free-living individuals water balance is generally well maintained (Sawka *et al*., 2005). The multiple factors effecting thirst have been described by Adolph *et al*. (1954) and include changes in serum osmolaltity and plasma volume. Hypohydration generally results in an increase in serum osmolality and/ or a decrease in plasma volume, although some methods used to induce hypohydration (diuretic administration/ energy restriction) don't lead to changes in serum osmolality. These changes stimulate the thirst sensation and fluid intake, theoretically leading to the correction of the water deficit (Adolph *et al*., 1954). In practice, this theory does not always hold true and it has been shown that in the initial few hours after a period of negative fluid balance an insufficient volume of fluid is ingested to re-establish euhydration (Rolls *et al*., 1980; Shirreffs *et al*., 2004b), but over a longer period (8-24 h) adequate fluid appears to be ingested to correct the water deficit (IOM, 2005).

The renal system's contribution to water balance is principally mediated through the hormone arginine vasopressin, which is secreted from the posterior pituitary gland in response to either hyperosmolality or hypotension, although it appears hyperosmolality is the more potent stimulus (Baylis, 1987). Arginine vasopressin (also known as antidiuretic hormone (ADH)) increases the permeability of the principal cells of the collecting duct of the kidney via the insertion of aquaporin-2 into the cells apical membranes. This results in the reabsorption of water from urine into the blood stream and the production of smaller volume of more concentrated urine, which in most healthy individuals can be concentrated up to ~1400 mosmol·kg^{-1}, although normal urine osmolality is in the range 442-1052 mosmol·kg^{-1} (Armstrong et al, 1994) Under most conditions, hypohydration results in an increase in serum osmolality and a decrease in blood volume, which both increase circulating concentrations of arginine vasopressin. It has been shown that serum omsolality is linearly related to arginine vasopressin concentration (Baylis, 1987) and that a 1 mosmol·kg^{-1} increase in plasma osmolality results in a 0.41 pmol·l^{-1} increase in arginine vasopressin concentration and that a 1 pmol·l^{-1} increase in plasma arginine

vasopressin concentration results in a 235 mosmol·kg^{-1} increase in urine osmolality, at least until urine concentration is maximal (Baylis, 1987). Thus a change in serum osmolality of 1 mosmol·kg^{-1} will induce a change in urine osmolality of ~100 mosmol·kg^{-1}. In contrast, larger changes in blood pressure are required to increase arginine vasopressin secretion, with a ~5% reduction in blood pressure producing an increase in arginine vasopressin concentration of ~1 pmol·l^{-1}. Aldosterone is a mineralocorticoid secreted from the zona glomerulosa of the adrenal cortex and is another hormone that plays a role in fluid balance. Aldosterone is secreted in response to a decrease in serum sodium concentration or an increase in serum potassium concentration, as well as due to a decrease in blood volume or blood pressure as part of the renin angiotensin system. A decrease in blood volume leads to the secretion of the hornmone renin from the kidneys, which results in the conversion of angiotensin to angiotensin I, which is then converted to angiotensin II by the angiotensin converting enzyme. Angiotensin II results in an increase in aldosterone secretion. Increased sympathetic nervous system activity can also increase aldosterone (Gordon *et al.*, 1967). An increase in the circulating concentration of aldosterone results an increased reabsorption of sodium in the distal convoluted tubule and collecting ducts of the kidneys, which consequently leads to reabsorption of water by osmosis (Martini, 2006).

Assessment of hydration status

Given the negative effects of hypohydration on health, as well as exercise and cognitive performance, the measurement of hydration status, as well as the identification of biomarkers to detect changes in hydration status has received much attention in the scientific literature and the numerous techniques available along with their appropriateness in a variety of settings have been described in a number of reviews (Kavouras, 2002; Oppliger and Bartok, 2002; Shirreffs, 2003; Manz and Wentz, 2003; Armstrong, 2005; Armstrong, 2007; Maughan and Shirreffs, 2008).

Hydration biomarkers should be sensitive enough to determine a change in total body water of ~3% (equivalent to a ~2% change in body mass) (Sawka *et al*., 2007), as this is the level of hypohydration that consistently degrades exercise and cognitive performance. The available hydration biomarkers can be divided into three main

categories: biomarkers that measure body water or surrogate markers of body water; haematological biomarkers and urinary biomarkers. Total body water can be determined by isotopic dilution with deuterium or tritium, or estimated using bioelectrical impedance spectroscopy. Body mass can also be used as a surrogate marker for total body water and changes in body mass, at least acutely in response to exercise or heat exposure over minutes or hours mainly reflect changes in body water stores (Maughan *et al*., 2007). Haematological markers include plasma osmolality and % change in plasma volume, but both might be subject to short-term variation due to acute postural, exercise or dietary effects (Maughan and Shirreffs, 2008). However, under well controlled laboratory conditions Popowski *et al*. (2001) demonstrated that plasma osmolality increased linearly by 5 mosmol·kg^{-1} for every 2% reduction in body mass, at least up to 5% body mass loss (BML). Urinary markers include urine osmolality, specific gravity, colour and 24 h urine volume. Urine colour is determined according to the 8 colour scale of Armstrong (2000). These urinary markers have been shown to not correlate well with hydration status after exercise when large volumes of fluid are consumed in a short period of time (Kovacs *et al*., 1999). Armstrong *et al*. (1994) demonstrated that urinary markers might be more sensitive to smaller changes in hydration status in the long-term and therefore might be of more use for assessing hydration status day-to-day, especially in an applied or occupational setting.

Recently, euhydration reference values for numerous hydration biomarkers were published (Armstrong *et al*., 2010) and some of these are summarised below in Table 1.2.

Table 1.2 Examples of euhydrated reference values for selected hydration biomarkers. Adapted from Armstrong *et al*. (2010).

Hydration biomarker	Euhydrated reference value
Morning serum osmolality	289-291 mosmol·kg^{-1}
24 h urine volume	1226-1525 ml
Morning urine specific gravity of the first sample of the day	1.024-1.026
Morning urine osmolality of the first sample of the day	818-924 mosmol·kg^{-1}
Morning urine colour (Armstrong et al, 2000) of the first sample of the day	5

Hypohydration

Deviations from euhydration can have a profound effect on human health, and water is without doubt the most essential of all dietary nutrients and the only nutrient for which abstinence from can prove fatal within days (Popkin *et al*., 2010). It is therefore clear that the vast majority of individuals maintain water balance on a daily basis, but whether this water balance is maintained around a suboptimally hydrated level is not well known. A large number of individuals including athletes and particularly weight categorised athletes (Shirreffs and Maughan, 1998), active individuals (Armstrong *et al*., 2010), recreational gym users (Peacock *et al*., 2011) and elderly populations (Strookey *et al*., 2005) present with either urinary or haematological markers that suggest hypohydration is present, but the extent of chronic hypohydration among the population is both difficult to define and determine.

Hypohydration can develop rapidly in as little as a few hours or gradually over days and weeks. Physical activity and/ or exposure to heat lead to sweat induced losses of body water, if fluids are not replaced at a similar rate to which they are being lost. During exercise, most individuals fail to fully replace water lost in sweat and thus hypohydration might develop both during and immediately after exercise (see Sawka *et al*., 2007 for review). The administration of diuretic drugs can similarly induce hypohydration rapidly (Caldwell *et al*., 1984; Armstrong *et al*., 1985; Viitasalo *et al*., 1987), whilst diarrhoeal disease can lead to significant loss of water from the gastrointestinal tract.

Hypohydration might also develop due to an inadequate water intake over a number of hours or days. Shirreffs *et al*. (2004b) demonstrated that the complete restriction of fluid intake for a period of 37 h resulted in a reduction in body mass of 2.7%, with approximately 1% of this BML occurring in the first 13 h of fluid restriction in temperate conditions. It appears that with complete fluid restriction a loss of ~2% of hydrated body mass occurs in 24 h (Phillips *et al*., 1993; Shirreffs *et al*., 2004b). This rate of BML appears to remain constant however long the fluid restriction period lasts, as Bosco *et al*. (1974) reported a 5.7% reduction in body mass following 72 h fluid restriction. In another study, Bosco *et al*. (1968) observed that restricting fluid intake to 900 $ml \cdot d^{-1}$ for 5 days, resulted in a 3.1% reduction in body mass. Smaller restrictions in water intake are not likely to result in hypohydration, unless water is restricted to a level that is below the volume of the obligatory daily loss of water in urine, faeces and perspiration.

Hypohydration and health

There is increasing evidence that inadequate water intake or poor hydration is linked to a number of morbidities including urinary stone formation and reoccurrence (Siener and Hesse, 2003) and constipation (Arnaud, 2003). Some evidence exists to implicate lower levels of water intake in the development of some forms of cancer, including bladder cancer (Michaed *et al*., 1999) and colon cancer (Shannon *et al*., 1996), but the evidence remains somewhat inconsistent (Alteiri *et al*., 2003)..

Hypohydration and exercise performance

Generally, it is advised that athletes and physically active individuals commence exercise euhydrated, but this might not always be the case (Sawka *et al*., 2007). Individuals might start exercise hypohydrated in situations where exercise bouts are undertaken in close proximity, where complete rehydration between bouts might not be possible and they are not intentionally trying to reduce body mass. Furthermore, it is well known that athletes competing in weight category sports intentionally dehydrate themselves prior to competition (discussed in detail later) and therefore might commence exercise hypohydrated.

There are two types of hypohydration that might be encountered during exercise: pre-existing hypohydration that is present at the onset of exercise and hypohydration that develops during exercise due to water intake being lower than water losses during exercise. When exercise is undertaken in temperate, warm or hot conditions hypohydration greater than 2% has been shown to reduce endurance exercise performance and capacity (Cheuvront *et al*., 2005) and increase physiological strain (increased core temperature, heart rate and rating of perceived exertion) is apparent (Sawka and Coyle, 1999). It appears that a larger reduction in body mass is required for a reduction in endurance performance in a cold environment (Cheuvront *et al*., 2005). Sawka *et al*. (2007) suggest that the physiological factors that contribute to the decrement in performance brought about by hypohydration include increased core body temperature, increased cardiovascular strain, increased glycogenolysis and altered metabolism and that these factors act in combination to augment a reduction in exercise performance.

Although there are fewer studies examining the effects of hypohydration on non-endurance based activities, Judelson *et al*. (2007) in review of the available literature concluded that hypohydration by 3-4% body mass consistently degrades strength, power and high-intensity endurance performance by approximately 2%, 4% and 10%, respectively.

The effect of hypohydration on cognitive performance is less well understood, but it appears that even very low levels of hypohydration can have an effect on some

aspects of cognitive function and wellbeing (Gopinathan *et al*., 1988; Ganio *et al*., 2011; Shirreffs *et al*., 2004b). Gopinathan *et al*. (1988) used exercise in the heat to dehydrate subjects by 1, 2, 3 and 4% body mass, demonstrating a reduction in performance of a number of cognitive tasks with dehydration >2%. More recently, Ganio *et al*. (2011) demonstrated that hypohydration greater than 1% body mass induced by exercise or diuretic administration, both in the absence of hyperthermia impaired certain aspects of cognitive function and also increased anxiety and fatigue. Shirreffs *et al*. (2004b) similarly reported that following just 13 h fluid restriction, subjective ratings of concentration and alertness were lower compared to a similar euhydration trial.

Similarly, the effect of hypohydration on skill related exercise performance is not well understood. McGregor *et al*. (1999) investigated the effects of hypohydration on soccer related skills performance. Subjects completed 90 min of the Loughborough Intermittent Shuttle Test (LIST) in moderate environmental conditions. The LIST involves shuttle running at a variety of intensities in an attempt to match the demands of soccer match play (Nicholas *et al*., 2000). Subjects completed two trials, during which fluid was ingested or restricted, with the restriction of fluid during the LIST resulting in ~2.4% reduction in body mass and a reduction in soccer related skills performance. Devlin *et al*. (2001) investigated the effects of hypohydration (~2.8%) induced by 1 h intermittent exercise in the heat on cricket bowling related performance among medium-fast bowlers. The cricketers bowled 6 overs of 6 balls (a total of 36 balls) at a target on the ground. Compared to a euhydrated condition, hypohydration resulted in no change in bowling velocity, but a reduction in bowling accuracy (line and length). More recently, Baker *et al*. (2007) examined the effect of hypohydration on basketball related skills performance. Seventeen basketball players completed 6 experimental trials, during which they walked for 3 h on a treadmill in a hot environment, with or without fluid replacement to induce hypohydration of 0% (either water or carbohydrate-electrolyte sports drink ingested), 1%, 2%, 3% or 4%. Subjects then completing 80 min of simulated basketball match play and basketball related skills performance was impaired in a linear fashion with increasing hypohydration, but was only significantly impaired with hypohydration ≥2% body mass. Similarly, Macleod and Sunderland (2010) demonstrated that field hockey skills performance was impaired when subjects started exercise in a hypohydrated state

compared to when they started in a euhydrated state, with the different conditions induced by exercise in the heat with or without fluid replacement on the previous day.

Rehydration after exercise

As most individuals drink less fluid during exercise than they lose in sweat and thus finish exercise in a mildly hypohydrated state (Sawka *et al*., 2007; Peacock *et al*., 2011), rehydration after exercise is necessary. If no further exercise is undertaken, it is likely rehydration can be achieved through normal dietary habits, but in situations where further exercise is performed in close proximity to the first session, specific rehydration is required if exercise performance is not to be impaired in the second bout (Judelson *et al*., 2007; Schoffstall *et al*., 2001).

The main factors affecting efficacy of rehydration after exercise have been identified as the volume and composition of the rehydration drink (Shirreffs *et al*., 2004a). The volume of fluid ingested must be greater than the volume of fluid lost through sweating to account for ongoing fluid losses in the post-exercise period. It has been shown that if the entire volume is ingested over a short time period (1 h), a volume equivalent to 150-200% of the sweat lost is required (Shirreffs *et al*., 1996). Although the rate at which a drink is ingested has been shown to affect drink retention and more of a drink is retained if the volume is ingested over a longer period (Archer and Shirreffs, 2001; Jones *et al*., 2010).

Sodium addition to ingested rehydration drinks has been shown to increase drink retention (Nose *et al*., 1988a; Maughan and Lieper, 1995; Shirreffs and Maughan, 1998; Merson *et al*., 2008). Furthermore, it has been shown that for complete recovery of fluid balance, enough sodium to replace that lost in sweat is required (Shirreffs and Maughan, 1998). Ingesting large volumes of plain water after exercise leads to a rapid expansion of plasma volume and an associated decrease in plasma tonicity (Nose *et al*., 1988a; Maughan and Lieper, 1995; Shirreffs and Maughan, 1998). This suppresses arginine vasopressin secretion and results in a large diuresis, which is attenuated when sodium is added to the rehydration drink (Nose *et al*., 1988a). Furthermore, sodium increases the drive to drink, meaning in situations where fluid is provided *ad libitum* more fluid might be ingested. The addition of potassium

to rehydration drinks has been suggested to increase rehydration by increasing fluid retention in the intracellular space (Yawata, 1990; Shirreffs *et al*., 2004a). The evidence from human experiments is equivocal (Maughan *et al*., 1994; Shirreffs *et al*., 2007), and whilst ingestion of potassium containing drinks results in a slower recovery of plasma volume, suggesting movement of water into the intracellular space, the effect on whole body net fluid balance appears to be inconsistent (Maughan *et al*., 1994; Shirreffs *et al*., 2007).

The addition of macronutrients and thus energy to a rehydration solution has been shown to increase the retention of a rehydration drink in comparison to macronutrient free or less energy dense solutions (Seifert *et al*., 2006; Evans *et al*., 2009; Osterberg *et al*., 2009). Ingesting a more energy dense rehydration solution has been shown to result in a greater plasma/ serum osmolality compared to a less energy dense solution (Seifert *et al*., 2006; Watson *et al*., 2008; Evans *et al*., 2009; Osterberg *et al*., 2009). This greater serum osmolality and increased drink retention of a more energy dense rehydration solution is likely due to the overall rate of fluid absorption from these solutions. Increasing the energy density of a rehydration solution will decrease the rate at which it empties from the stomach (Vist and Maughan, 1994; Vist and Maughan, 1995; Calbet and MacClean, 1997; Evans *et al*., 2011) and thus delay the overall rate of fluid uptake into the circulation (Evans *et al*., 2011). This delay in the rate of fluid uptake into the circulation attenuates the decline in serum osmolality that occurs when a large volume of a rehydration solution is ingested over a short space of time (Seifert *et al*., 2006; Watson *et al*., 2008; Evans *et al*., 2009; Osterberg *et al*., 2009).

Whole foods have also been shown to increase rehydration compared to ingesting fluids alone (Maughan *et al*., 1996; Ray *et al*., 1998). Maughan *et al*. (1996) compared ingestion of a carbohydrate-electrolyte sports drink to water accompanied by a whole meal, with both treatments providing the same total volume of water. The meal plus water resulted in a reduction in total urine production of ~300 ml over the 6 h post-exercise and the authors suggest that the reason for this increase in drink retention was related to the greater amounts of sodium (20 mmol) and potassium (14 mmol) ingested during the meal plus water trial, but it cannot be discounted that the

greater total energy, protein, carbohydrate or fat content of the meal plus water trial affected rehydration.

Dietary restriction

Dietary restriction refers to the restriction of one or more component of an individual's habitual diet, but in this thesis will be concerned about the severe restriction of either fluid and/ or total energy intake. The physiological effects of a period of dietary restriction will mainly be determined by the severity and length of the dietary restriction imposed. Whilst complete energy restriction can be tolerated for periods of days or weeks (Cahill, 1970; Runcie and Thompson, 1970; Runcie, 1971), complete restriction of fluid for even a few days may result in death (Maughan, 2003).

Dietary restriction and metabolism at rest and during exercise

Whilst the effects of fluid restriction on metabolism are unknown, the effects of energy restriction and especially total energy restriction are well documented and discussed in two review articles (Aragon-Vargas, 1993; Maughan *et al*., 2010). Whilst short term severe energy restriction or complete energy restriction are unlikely to alter basal metabolic rate (Consolazio *et al*., 1967; Maughan *et al*., 2010), longer periods (>9 days) of complete energy restriction (Consolazio *et al*., 1967), as well as severe (~50% energy requirements) prolonged (6 months) energy restriction (Taylor and Keys, 1950) have been shown to reduce basal metabolic rate. The post-absorptive state is the first phase of complete energy restriction and commences once all nutrients consumed at the last meal are absorbed from the small intestine, and during early stage of complete energy restriction (~24 h) adaptations occur to adjust to the absence of nutrient supply (Maughan *et al*., 2010). In the early stages of complete energy restriction, blood glucose concentration is relatively well maintained by hepatic glycogenolysis, which occurs at a rate of approximately 4 $g \cdot h^{-1}$ at this stage of complete energy restriction (Nilsson and Hultman, 1973). Although after 24 h complete energy restriction, liver glycogen content is greatly reduced (to ~20% of fed levels) and by 24-36 h of complete energy restriction, a reduction in blood glucose concentration is observed (Dohm *et al*., 1986; Zinker *et al*., 1991), which is accompanied by a reduction in plasma insulin concentration (Bjorkman and Eriksson,

1983; Dohm *et al*., 1986; Zinker *et al*., 1990). Although metabolic rate does not change during the initial few days of complete energy restriction (Consolazio *et al*., 1967), substrate utilisation is altered in an attempt to spare the body's limited carbohydrate stores. Carbohydrate oxidation decreases, whilst lipid oxidation increases to meet the body's metabolic demand, resulting in a lower Respiratory exchange ratio (RER) following complete energy restriction (Knapik *et al*., 1988). This reduction in RER may in part be due to the release of triglycerides from adipose tissue that occurs during complete energy restriction (Cahill *et al*., 1966), leading to an increased plasma concentration of free fatty acids (FFA) (Bjorkman and Eriksson, 1983; Dohm *et al*., 1986; Loy *et al*., 1986; Nieman *et al*., 1987; Knapik *et al*., 1988, Gleeson *et al*., 1988; Maughan and Gleeson, 1988; Zinker *et al*., 1990) and glycerol (Knapik *et al*., 1987, Gleeson *et al*., 1988; Maughan and Gleeson, 1988; Zinker *et al*., 1990). This increased in plasma FFA concentration likely reduces carbohydrate oxidation via a reduction in pyruvate dehydrogenase complex activity.

Glucose is needed for tissues of the body that have an obligate requirement (central nervous system and red blood cells), and in the early stages of complete energy restriction the body's requirement for glucose is ~105 $g \cdot d^{-1}$, falling to ~75 $g \cdot d^{-1}$ after a few days (Maughan *et al*., 2010). This glucose requirement is met by gluconeogenesis, as well as an increased availability of ketone bodies (Dohm *et al*., 1986; Loy *et al*., 1986; Knapik *et al*., 1987, Gleeson *et al*., 1988; Maughan and Gleeson, 1988; Zinker *et al*., 1990), which can be used by cardiac muscle, the brain and other tissues as a fuel source (Maughan *et al*., 2010). Glugoneogenic substrates include lactate, the carbon skeletons of certain amino acids and glycerol. During complete energy restriction, lactate used for gluconeogensis is primarily released by red blood cells, which can only respire anaerobically, whilst glycerol is readily available as a substrate due to the increased lipolysis from adipose tissue.

The metabolic response to exercise in a complete energy restricted state is characterised by an increased heart rate (Dohm *et al*., 1986; Knapik *et al*., 1987; Nieman *et al*., 1987) and RPE (Nieman *et al*., 1987) compared to a fed state at the same work load. This increased heart rate, likely reflects the reduced blood volume that accompanies complete energy restriction, as this would reduce stroke volume, meaning heart rate must increase to maintain cardiac output. Blood glucose

concentration seems to be maintained during exercise (Dohm *et al*., 1986; Loy *et al*., 1986; Knapik *et al*., 1988; Maughan and Gleeson, 1988; Zinker *et al*., 1990), which is likely to be partially explained by a reduction in glucose oxidation during exercise (Dohm *et al*., 1986; Nieman *et al*., 1987; Knapik *et al*., 1988; Maughan and Gleeson, 1988). Whilst fat oxidation is increased during complete energy restriction (Dohm *et al*., 1986; Nieman *et al*., 1987; Knapik *et al*., 1988; Maughan and Gleeson, 1988) compared to a fed state, plasma FFA levels remain elevated (Dohm *et al*., 1986; Loy *et al*., 1986; Knapik *et al*., 1988; Maughan and Gleeson, 1988; Zinker *et al*., 1990).

Dietary restriction and body mass/ body water

As discussed above there are a number of avenues of water loss and gain from and to the body, with fluid intake being the main avenue of water gain and obligatory losses of water (~25 $ml \cdot h^{-1}$ urine plus respiratory, cutaneous and faecal water) persisting even when fluid intake is completely restricted (Shirreffs *et al*., 2004). This continued loss of water, despite no intake results in a significant reduction in body mass, as well as plasma volume in most studies (Bosco *et al*., 1968; Bosco *et al*., 1974; Rolls *et al*., 1980; Zappe *et al*., 1993; Mack *et al*., 1994; Shirreffs *et al*., 2004b), but not all (Oliver *et al*., 2007). The only study to examine the effects of a more moderate restriction of fluid intake (Bosco *et al*., 1968) observed a 3.1% BML over 5 days, with water intake of 900 ml per day, resulting in a mean loss of body mass of 0.62 kg per day. Although it is difficult to determine the volume of fluid required to prevent a reduction in body mass, it appears that complete fluid restriction results in a loss of body mass of ~2% of euhydrated body mass per day in a sedentary individual (see above), suggesting that a fluid intake of ~2% body mass (1400 ml for a 70 kg individual) might lead to a state of fluid balance at rest.

The interaction between severe energy restriction and body water has been recognised since ancient times. Hippocrates wrote in his aphorisms “fasting should be prescribed for those persons who have humid flesh for fasting dries bodies”. A number of investigations have documented the effects of both short-term (<4 days) (Bosco *et al*., 1974; Oliver *et al*., 2007; Oliver *et al*., 2008) and long-term (>4 days) severe energy restriction or total complete energy restriction (Taylor *et al*., 1954; Brozek *et al*., 1957; Consolazio *et al*., 1967; Consolazio *et al*., 1968a; Consolazio *et al*., 1968b) on

changes in body mass and body water. Additionally, the majority of these studies (Taylor *et al*., 1954; Brozek *et al*., 1957; Consolazio *et al*., 1967; Consolazio *et al*., 1968a; Consolazio *et al*., 1968b) have allowed water intake *ad libitum*, with only a few documenting the effects of complete or very severe food and fluid abstinence (Bosco *et al*., 1974; Oliver *et al*., 2007; Oliver *et al*., 2008) over periods of more than 24 h.

Taylor *et al*. (1954) were among the first to document body mass and body water changes in response to a period of complete energy restriction. Subjects underwent 2, 4.5 day periods of complete energy restriction, whilst performing 3.25 $h \cdot d^{-1}$ of treadmill walking (3.5 mph, 10% gradient). Subjects lost a total of 6 kg (8.5% initial body mass) and 5.5 kg (7.8% initial body mass), with 36-38% of the total weight loss occurring during the first 24 h and ~65% during the first 48 h, with the rate of weight loss slowing for the remainder of the trial. This large reduction in body mass was accompanied by an ~18% reduction in plasma volume compared to before the period of complete energy restriction. Similarly, Consolazio *et al*. (1967) reported a 7.3 kg (9.5% of initial body mass) reduction in body mass, following 10 days of complete energy restriction, with *ad libitum* water intake. As observed by Taylor *et al*. (1954), daily BML was far greater at the beginning of the period of complete energy restriction compared to at the end (1.44 kg on day 1 compared to 0.35 kg on day 10) (Consolazio *et al*., 1967). In line with this, Consolazio *et al*. (1967) reported a negative water balance of 1212 ml on day 1 of the period of complete energy restriction, which gradually decreased to a negative water balance of 358 ml on day 10.

The effect of severe long-term energy restriction on body mass and body water was also examined by these two research groups in follow up studies (Brozek *et al*., 1957; Consolazio *et al*., 1968a). Brozek *et al*. (1957) reported the results of two separate experiments, during which energy intake was restricted to 580 $kcal \cdot d^{-1}$ (~2424 $kJ \cdot d^{-1}$) (Experiment 53) for a period of 12 days or 1010 $kcal \cdot d^{-1}$ (~4222 $kJ \cdot d^{-1}$) for 24 days (Experiment 54). During Experiment 53, the subjects lost a mean of 5.9 kg over the 12 days, with a large loss of body mass (1.4 kg, or 24% of the total BML) occurring over the first 24 h and after this point, daily body mass reduced in a linear manner, averaging 0.41 $kg \cdot d^{-1}$ (Brozek *et al*., 1957). Similarly, during experiment 54, a large

loss of body mass was observed over the initial few days of the 24 d period of energy restriction, with a mean BML of 1.0 kg, 0.7 kg and 0.7 kg on days 1, 2 and 3 of the energy restriction respectively, with 31% of the total BML occurring over the first 3 days of energy restriction. After day 4, daily BML was linear for the remainder of the period of energy restriction. Consolazio *et al*. (1968a) reported that a 10 day energy restriction period, with energy restricted to 420 kcal·d^{-1} (~1756 kJ·d^{-1}) resulted in a 5.62 kg reduction in body mass, with a large amount of this BML occurring over the initial 2 days of the energy restriction and a linear loss of body mass from day 3 onwards. In line with this, Consolazio *et al*. (1968a) also reported values for water balance over the 10 day energy restriction and observed that water balance was -720 g·d^{-1} over the first 2 days of energy restriction, but was markedly reduced for the remainder of the study (-76 to +90 g·d^{-1}). Additionally, a 19.1% reduction in plasma volume was observed over the 10 day energy restriction period. This suggests that the large reduction in body mass over the initial few days of severe energy restriction (Brozek *et al*., 1957; Consolazio *et al*., 1968a) or total complete energy restriction (Taylor *et al*., 1954; Consolazio *et al*., 1967) might be accounted for by a loss of body water at the onset of energy restriction.

In contrast, less information is available about the effects of combined severe or compete energy and fluid restriction on body mass and body water. As discussed previously, the complete restriction of fluid intake will likely result in death in a relatively short space of time and consequently the only studies to have documented the effects of complete (Bosco *et al*., 1974) and severe (Oliver *et al*., 2007; Oliver *et al*., 2008) combined fluid and energy restriction have done so over time periods ≤72 h. Bosco *et al*. (1974) used a mixed groups design and assigned subjects to either complete fluid restriction, starvation (complete fluid and energy restriction) or an energy maintaining control diet for 3 days. Complete fluid restriction resulted in a body mass reduction of 4.4 kg (5.7% of initial body mass), whilst starvation resulted in a body mass reduction of 4.5 kg (5.8% of initial body mass). Additionally, reductions in plasma volume were similar for both fluid restriction (-13.4%) and starvation (-15.8%). More recently, Oliver *et al*. (2007) were the first to separately examine the effects of fluid, energy or combined fluid and energy restriction in a cross over design. Mean (SD) BML over the 48 h trial period was 3.2 (0.5) %, 3.4 (0.3) % and 3.6 (0.3) % following fluid, energy and combined fluid and energy

restrictionm, respectively. There was no significant difference in the degree of BML between the energy and combined fluid and energy restriction trials, indicating that the same degree of body water loss occurred whether water was ingested or not. This was backed up by the data for change in plasma volume over the 48 h trials, and plasma volume decreased to a similar extent after energy restriction (-5.2 (3.2) %) and combined fluid and energy restriction (-5.1 (4.5) %).

Whilst is seems likely that the large reduction in body mass over the initial few days of energy restriction is related to changes in body water stores, another possible explanation might be due to changes in glycogen stores, although this seems far less plausible. The amount of glycogen that is utilised and thus the requirement for an exogenous carbohydrate source will primarily be determined by the amount and intensity of exercise that is being undertaken during the period of energy restriction. In the absence of exercise, 24 h complete energy restriction has been shown to have no effect on muscle glycogen stores (Maughan and Williams, 1981), but to result in a significant reduction (to ~20% of resting levels) in liver glycogen concentration (Nilsson and Hultman, 1973). It has been shown that ingesting some carbohydrate (100 $g \cdot d^{-1}$) during energy restriction, at least in the absence of exercise is antiketogenic, suggesting that liver glycogen levels are being maintained on a daily basis (Bloom, 1967).

In studies that have included some exercise during the period of energy restriction (Taylor *et al*., 1954; Brozek *et al*., 1957), it is very likely that muscle glycogen is utilised during exercise to some degree. At least during complete energy restriction, as seen in the experiment of Taylor *et al*. (1954), muscle glycogen utilised during exercise is unlikely to be replaced due to the lack of any exogenous carbohydrate source. The two experiments described by Brozek *et al*. (1957), provided some energy (580 $kcal \cdot d^{-1}$ and 1010 $kcal \cdot d^{-1}$) solely from carbohydrate, which equates to approximately 145 $g \cdot d^{-1}$ and 253 $g \cdot d^{-1}$ respectively. Whether this amount of carbohydrate was sufficient to replace muscle glycogen utilised during exercise is unknown, as neither muscle glycogen or plasma ketone levels were measured in this study. In contrast, the studies of Consolazio and colleagues (Consolazio *et al*., 1967; Consolazio *et al*., 1968a) did not include exercise during energy restriction, but did investigate the effects of complete energy restriction (Consolazio *et al*., 1967) and

also severe energy restriction to just 400 kcal·d^{-1} (100 g carbohydrate). Although Consolazio *et al*. (1967; 1968a) also did not measure muscle glycogen or plasma ketone levels, the amount of carbohydrate ingested in the study performed by Consolazio *et al*. (1968a) is the amount that has been shown previously to be antiketogenic (Bloom, 1967). This suggests that changes in body mass during the study of Consolazio *et al*. (1968a) are not likely to be explained by changes in glycogen content. Although these 5 experiments (Taylor *et al*., 1954; Brozek *et al*., 1957; Consolazio *et al*., 1967; Consolazio *et al*., 1968) have all imposed different levels of energy restriction (diets providing 0-1010 kcal·d^{-1}), as well as different lengths of energy restriction (4.5-24 days) and the inclusion or exclusion of exercise, the striking similarity between these experiments is the pattern of weight loss reported.

Dietary restriction and electrolyte balance

At present there is no data available for the effects of a period of fluid restriction on electrolyte balance, but micronutrient intake has been shown to be linearly related to total energy intake (van Erp-Baart *et al*., 1989). Thus, provided that energy intake remains unchanged, there is no reason to suspect electrolyte balance will be altered during a period of fluid restriction. This is supported by the data of Shirreffs *et al*. (2004b). Comparison of the mean values for electrolytes excreted in the urine, reveals that during fluid restriction there was a 29, 18 and 10% reduction in sodium, potassium and chloride excretion, respectively compared to the euhydration trial and this was accompanied by a 29% reduction in energy intake. Although electrolyte consumption was not reported, these calculated reductions in electrolyte restriction that are similar to, or less than the reduction in energy intake, suggest that electrolyte balance was not affected by the 37 h period of fluid restriction.

In studies investigating the long term effects of energy restriction on the excretion of electrolytes in the urine it has been observed that during the first 2 weeks of complete energy restriction, a sodium deficit of around 350 mmol develops, with little further excretion of sodium after this point. A potassium deficit of around 300 mmol also develops, but potassium appears to be less well conserved than sodium and its excretion continues throughout prolonged complete energy restriction (Lin *et al*.,

1997). As the major electrolyte in the extracellular space a loss of sodium from the body might be expected to result in a loss of water from the extracellular fluid. In line with this, blood volume decreases during the first 2 weeks of complete energy restriction and is accompanied by the continued excretion of sodium over this period (Rapoport *et al*., 1965). After this point, no further decrease in blood volume is observed and virtually no sodium is excreted in the urine (Rapoport *et al*., 1965). Conversely, as the major electrolyte in the intracellular space a loss of potassium might be expected to be accompanied by a loss of water from the intracellular fluid. The initial large loss of potassium at the onset of a period of energy restriction might be expected to be accompanied by a loss of intracellular water and may account for some of the large loss of body water during this time (Runchie, 1971, Rapoport *et al*., 1965). The continued excretion of potassium throughout a period of complete energy restriction might be explained by the breakdown of lean tissue, releasing potassium (Runchie, 1971).

Dietary restriction and exercise performance

The effects of hypohydration on exercise performance have been described above, so the specific effects of fluid restriction on exercise performance will be discussed here. Only three studies (Bosco *et al*., 1968; Bosco *et al*., 1974; Oliver *et al*., 2007) have examined the effect of fluid restriction on exercise performance. Bosco *et al*. (1968) observed a reduction in isometric strength during elbow flexion, but not during knee extension, leg extension, back extension or hand grip strength after 5 days with fluid restricted to 900 $ml \cdot d^{-1}$. Bosco *et al*. (1974) observed a significant reduction in maximal isometric strength during left elbow flexion, but not during right elbow flexion, right / left shoulder extension or right/ left knee extension after 3 days complete fluid restriction. Furthermore a reduction in the number of sit ups completed in a 2 min period was reported. The only study to examine the effects of fluid restriction on endurance performance (Oliver *et al*., 2007) reported a non-significant reduction in the distance covered in a 30 min treadmill test. The authors of this study (Oliver *et al*., 2007) reported the individual subject data for the 4 experimental trials. Reanalysis of the results indicates that 9 of the 13 subjects covered less distance after 48 h fluid restriction compared to a 48 h euhydration trial. The mean (SD) reduction was 2.8 (4.2) % and when compared with a paired t-test, this effect was significant (*P*

< 0.05), indicating the failure to find a significant reduction in performance during the study was caused by the reduced statistical power due to the multiple comparisons made.

The effects of energy restriction on endurance performance are well documented. Dohm *et al*. (1983) reported a significantly longer time to exhaustion for treadmill running in rats following 24 h complete energy restriction compared to an *ad libitum* fed control group. This performance enhancement in the complete energy restricted rats was attributed to increased FFA utilisation and sparing of muscle glycogen during exercise, as despite a lower muscle glycogen content at the beginning of exercise in the complete energy restricted rats, muscle glycogen was significantly greater at the end of exercise in the complete energy restricted compared to the fed rats. This ergogenic effect of complete energy restriction on endurance exercise performance has not been replicated in humans in a number of subsequent investigations.

Loy *et al*. (1986) investigated time to exhaustion at 79% (n = 4) and 86% (n = 4) $\dot{V}O_2$ max in 2 separate subject groups and observed that time to exhaustion was significantly reduced following 24 h complete energy restriction in both groups compared to when subjects consumed their normal diet. Muscle glycogen content was similar at rest and exhaustion and despite an increased plasma FFA concentration, RER values did not support an increased utilisation of fat during exercise. Nieman *et al*. (1987) investigated time to exhaustion for treadmill running at 70% $\dot{V}O_2$ max, which was performed either 3 h after a pre-exercise meal or following 27 h complete energy restriction and reported similar results to Loy *et al*. (1986), observing a reduction in time to exhaustion of 44.7 (5.8) % in the complete energy restricted trial compared to the fed trial. Additionally, despite an elevated level of blood free fatty acids and a lower RER, muscle glycogen degraded at a similar rate during the complete energy restricted and fed trials. Zinker *et al*. (1990) exercised subjects to exhaustion at 50% $\dot{V}O_2$ max on a cycle ergometer following either 36 h or a 12 h (overnight fasted) complete energy restriction. The 36 h complete energy restriction resulted in an increased blood FFA concentration and an increased utilisation of lipids (indicated by a lower RER) during exercise in comparison to the postabsorptive trial and resulted in a significantly decreased time to exhaustion of 88.9 (18.3) min (36 h

complete energy restriction) compared to 144.4 (22.6) min (12 h complete energy restriction). Maughan and Gleeson (1988) investigated the effects of 36 h complete energy restriction on time to exhaustion during cycling at 70% $\dot{V}O_2$ max and compared this to an overnight fast (postabsorptive), as well as following 36 h complete energy restriction and re-feeding with either glucose or glycerol (1 $g \cdot kg^{-1}$ body mass) 45 min prior to exercise. Time to exhaustion was lower in the complete energy restricted trial (77.7 (6.8) min) compared to the postabsorptive trial (119.5 (5.8) min) and was not significantly improved by re-feeding with glucose (92.4 (11.8) min) or glycerol (80.8 (3.6) min). Oliver *et al*. (2007) examined the effects of 48 h energy restriction (10% estimated requirement) and combined fluid (10% estimated requirement) and energy (10% estimated requirement) restriction on distance covered during 30 min treadmill running. Both trials resulted in a reduction in exercise performance when compared to the control trial. From these studies it is clear that unlike in rats (Dohm *et al*., 1983), there appears to be no ergogenic effect of complete energy restriction or severe energy restriction on endurance performance in humans, with all studies on humans demonstrating either a detrimental effect (Oliver *et al*., 2007, Loy *et al*., 1986, Nieman *et al*., 1987, Zinker *et al*., 1990, Maughan and Gleesen, 1988) or no effect on performance (Dohm *et al*., 1986, Knapik *et al*., 1987).

Gleeson *et al*. (1988) investigated the effect of 24 h complete energy restriction compared to a normal diet, on time to exhaustion during cycling exercise at a workload of 100% $\dot{V}O_2$ max. This workload was selected, as fatigue during exercise would not be expected to be as a result of muscle glycogen depletion. 24 h complete energy restriction resulted in a significant reduction in exercise time to fatigue compared to the normal diet condition. Plasma bicarbonate and blood base excess concentrations were decreased following 24 h complete energy restriction compared to the normal diet, although plasma pH was not significantly different. Additionally, plasma free fatty acid, blood glycerol and blood β-hydroxybutyrate concentrations were significantly elevated following 24 h complete energy restriction compared to the normal diet. Alterations in the composition of the diet have been shown to influence the acid-base status of the blood (Greenhaff *et al*., 1987, Greenhaff *et al*., 1988). Consuming a diet low in carbohydrate and high in protein and fat induces a metabolic acidosis (Greenhaff *et al*., 1987, Greenhaff *et al*., 1988) and results in a

reduction in high intensity exercise performance (100% $\dot{V}O_2$ max) (Greenhaff *et al*., 1987). It is likely that fatigue following 24 h complete energy restriction in the investigation of Gleeson *et al*. (1988) was as a result in of alterations in acid-base status, causing a metabolic acidosis prior to exercise. The effects of energy restriction on acid-base status and the subsequent effects on high intensity exercise performance warrants further investigation. Of particular interest is the reversal of the apparent metabolic acidosis that accompanies 24 h complete energy restriction via the consumption of a buffering agent such as $NaHCO_3$ and the effect that this reversal has on performance of high intensity exercise.

It is clear that energy restriction negatively impacts on endurance exercise performance in humans; however, the cause of this early onset of fatigue is not fully understood, but might be related to changes in glycogen stores, substrate availability during exercise or changes in acid-base status. One area that has yet to be investigated is the effect of the reduction in body water that accompanies severe or complete energy restriction on endurance exercise performance.

Whilst the effects of energy restriction on endurance performance are well documented, the effects of energy restriction on strength and power performance are less well understood. Knapik *et al*. (1987) observed no effect of a 3.5 day period of complete energy restriction, isometric strength or anaerobic capacity (Thorstenssen test), but isokinetic strength measured at velocities of 0.52 $rad \cdot s^{-1}$ and 3.14 $rad \cdot s^{-1}$ were significantly reduced 10.80 (2.98) % and 8.58 (2.95) % respectively, compared to following a 14 h overnight fast. Bosco *et al*. (1974) observed a reduction in some measures of maximal isometric strength (left elbow flexion, right knee extension), but not others (right/ left shoulder extension, right elbow flexion, left knee flexion), although there was a mean decrease in all of these measures of isometric strength after a 3 day period of complete energy restriction. Additionally, the number of sit-ups completed in a 2 min period was significantly decreased, indicating that muscular endurance might be impaired by energy restriction. The results of these 2 studies are somewhat difficult to interpret due to the apparent lack of a familiarisation trial for both studies. It would appear from the results of these 2 studies that there is some effect of short-term complete energy restriction on muscular strength and endurance;

however, it is clear that further investigation is required to fully evaluate the effects of severe energy restriction on muscular strength and power.

Dietary restriction and cognitive performance

Although no specific data are available, it is likely that restricting fluid intake would have a similar effect on cognitive function as observed with hypohydration brought about by exercise and/ or heat exposure. Whilst the effects of severe energy restriction on endurance (Oliver *et al*., 2007, Loy *et al*., 1986, Nieman *et al*., 1987, Zinker *et al*., 1990, Maughan and Gleesen, 1988, Dohm *et al*., 1986, Knapik *et al*., 1987) and strength (Knapik *et al*., 1987, Bosco *et al*., 1974) performance have been examined the effects on cognitive function and performance have not been investigated. Energy restriction is common among athletes competing in weight category sports such as boxing and wrestling where cognitive function and in particular reaction times are of vital importance for competitive success. Similarly, optimal cognitive function is of particular importance in an occupational military setting where personnel on sustained operations regularly experience periods of energy restriction (Nindl *et al*., 2002). The effects of sustained operations (sleep and food deprivation combined with sustained physical activity) on some aspects of cognitive functioning have been investigated, with investigators either reporting a decrease (Liebermann *et al*., 2006) or no effect (Nindl *et al*., 2002) on cognitive function. More recently, Lieberman *et al*. (2008) were the first to examine the effects of energy restriction on cognitive function. Subjects underwent three, 48 h experimental trials and the investigators attempted to blind the subjects as to whether they were receiving an energy restriction or adequate diet by providing low energy gels in the energy restricted trial. Energy restriction did not result in any impairment of cognitive function (Lieberman *et al*., 2008).

Electrolyte supplementation during energy restriction

As discussed above, the restriction of energy intake also results in the restriction of electrolytes and other micronutrients, unless supplements are provided during the period of energy restriction. Despite this restriction of electrolyte intake, their excretion in urine (and presumably faeces) continues throughout the period of energy restriction (Rapoport *et al*., 1965; Weinsier, 1970; Runcie, 1971; Lin *et al*., 1997).

Consolazio *et al*. (1968a; 1968b) reported the results of a 10 day period of energy restriction (1756 $kJ \cdot d^{-1}$), during which subjects were allocated to one of two experimental groups. Subjects either received a mineral supplement (3.93 $g \cdot d^{-1}$ sodium (171 mmol) and 2.41 $g \cdot d^{-1}$ potassium (62 mmol), as well as 798 $mg \cdot d^{-1}$ calcium, 269 $mg \cdot d^{-1}$ magnesium, 1.05 $g \cdot d^{-1}$ phosphorus and 5.4 $mg \cdot d^{-1}$ iron) during a 10 day period or received no supplement. Mineral supplementation resulted in a reduced BML over the 10 day period compared to when minerals were not supplemented. Additionally, sodium and potassium balance (Consolazio *et al*., 1968b), as well as plasma volume were better maintained (Consolazio *et al*., 1968a) in the group that received the mineral supplement compared to the group that did not receive any supplements.

Rapid weight loss in weight category sports

Weight category sports are sports whose competitors are separated into categories dependent on their body mass at the time of competition, although most weight category sports hold their official weigh-in some time before the actual competition. The purpose of separating competitors into weight categories based on their body mass is to reduce the risk of injury by eliminating any large differences in size and strength, as well as to allow competitors of various sizes to compete on a similar level (Horswill, 1992; Walberg-Rankin, 2000). Some examples of typical time intervals between weigh-in and competition are given in Table 1.3.

Table 1.3 Examples of typical times between weigh-in and competition in some common weight category sports.

Sport	Time between weigh-in and competition
Amateur wrestling	13-15 h
Amateur boxing	>3 h (generally 3-6 h)
Professional boxing	24-30 h
Light-weight rowing	2 h
Horse racing	30 min before each race
Judo	>2 h
Taekwondo	1-2 h
Professional Mixed martial arts	24-30 h
Olympic weightlifting	2 h

The designation of specific weight limits that athletes must adhere to for competition has meant that many athletes have resorted to rapid weight loss strategies in an attempt to manipulate their body mass in the days and hours leading up to the official weigh-in and to use the time between weigh-in and competition to recover, at least some of this lost body mass (Horswill, 1992; Walberg-Rankin, 2000; Smith, 2006). These athletes believe that these patterns of rapid weight loss and regain will offer a performance advantage over their competitor (Walberg-Rankin, 2000), but the two studies (Horswill *et al*., 1994; Wroble and Moxley, 1998) in the published literature

that have attempted to investigate whether there is any benefit of rapid weight loss in amateur wrestlers have provided conflicting results. Horswill *et al*. (1994) observed that neither acute weight gain between weigh-in and competition or weight difference between opponents was a significant predictor of success in the first round of a wrestling tournament in collegiate wrestlers. In contrast, Wroble and Moxley (1998) observed that the weight difference between first round opponents was significantly associated with success in the first round match in high school age wrestlers. Wroble and Moxley (1998) suggested that the discrepancy in findings between these two studies might be accounted for by an increased skill level in the more experienced collegiate wrestlers in the study of Horswill *et al*. (1994), which may have negated the effects of any weight discrepancy. It remains to be seen whether differences in weight loss and regain have any significant impact on performance in any of the other weight category sports and further research is required in this area.

Although rapid weight loss among athletes has been studied by the scientific community for many years, interest in the practices of weight categorised athletes increased following the untimely deaths of 3 collegiate wrestlers, who were undergoing rapid weight loss to make weight for a wrestling match in the months of November and December 1997 (CDC, 1998).

Methods of rapid weight loss in weight category sports

The use of rapid weight loss has been widely reported in the scientific literature, with studies dating back to the 1960's (Tipton and Tcheng, 1970; Steen and Brownwell, 1990; Maffulli, 1992; Oopik *et al*., 1996; Morris and Payne, 1996; Kingingham and Gorenflo, 2001; Moore *et al*., 2002; Oppliger *et al*., 2003; Smith, 2006). The results of some of the early studies on amateur wrestling have been reviewed previously (Horswill, 1992) and a wide range of techniques that induce rapid weight loss have been reported among wrestlers and other weight category sports athletes. These techniques include food restriction of complete energy restriction, partial or complete fluid restriction, exercise induced sweating, the wearing of rubberised suits during training to induce sweating, passive thermally induced sweating, spitting, vomiting, as well as the use of laxatives, diuretics and diet pills. The reported techniques used to induce rapid weight loss are similar among the athletes from different sports that have

been reported in the scientific literature, including amateur wrestlers (Kingingham and Gorenflo, 2001; Oppliger *et al*., 2003), amateur boxers (Smith, 2006), jockeys (Moore *et al*., 2002) and lightweight rowers (Morris and Payne, 1996).

Rapid weight loss and exercise performance

For the majority of weight categorised sports (except rowing and weightlifting) the ecological validity of the performance tests used is difficult to ascertain, due to the interactive nature of competition (Horswill, 1992) and thus most studies investigating the effects of rapid weight loss on performance tend to focus on one or more physiological performance variable.

Amateur wrestling (particularly high school and collegiate wrestling in the USA) has received the most attention in the scientific literature in relation to rapid weight loss and Horswill (1992) reviewed the early literature in this area. Whilst these studies are often difficult to interpret due to the multitude of methods used to induce rapid weight loss, Horswill (1992) concluded that whilst measures such as anaerobic power, strength and $\dot{V}O_2$ peak remain unchanged with rapid weight loss, sustained or repeated maximal efforts of more than 30 s seem to be impaired by rapid weight loss (Horswill *et al*., 1992; Webster *et al*., 1990). Tarnopolsky *et al*. (1996) instructed 6 competitive collegiate wrestlers to lose 5% of their body mass by self-selected means over a 3 day period. Although weight loss techniques were self-selected and all athletes chosen methods were slightly different, examination of the methods used demonstrated that all athletes used fluid and energy restriction alongside exercise, with some athletes also using sauna exposure or exercise in the heat or wearing heavy clothing to induce dehydration. The rapid weight loss period induced a 5.0 (0.1) % change in body mass and a 54% decrease in muscle glycogen. This indicates rapid weight loss using the techniques listed significantly reduces muscle glycogen stores.

Fogelholm *et al*. (1993) examined the effect of different weight loss strategies on performance in 10 experienced weight categorised athletes (7 wrestlers and 3 judoka). The athletes first lost 5% of their body mass over a 3 week period (Gradual reduction), via ~1000 $kcal \cdot d^{-1}$ deficit in energy intake, brought about mainly via a

reduction in high fat foods. Then two months later, the athletes lost 6% of their body mass over 59 h (rapid reduction), before they undertook a 5 h *ad libitum* recovery period, during which they could eat and drink what they wanted. The weight loss techniques used during rapid reduction were severe fluid and energy restriction, as well as exercise in a plastic suit to induce sweating. Performance during a 30 m sprint and a 1 min Wingate test was unaffected by either weight loss regimen, whilst vertical jump height was increased following gradual reduction and unchanged following rapid reduction. These results suggest, in experienced athletes at least, that either gradual or rapid means of reducing body mass in the run up to a competition does not impair performance, although the failure to randomise the trials and the lack of a 5 h *ad libitum* recovery period in the gradual reduction group make the data somewhat less convincing. Horswill *et al*. (1990) studied 12 highly trained collegiate wrestlers and in randomised order provided isoenergetic energy restriction liquid diets that where either high (HC) or low (LC) in carbohydrate for 4 days. The athletes trained daily and used a sauna to induce weight loss, with these practices standardised between trials. Following the 4 day period weight loss was the same in both trials (6.2 (0.3) % during HC and 6.3 (0.3) % during LC), but total work done in a 5 min arm cranking exercise protocol was reduced after LC, but not after HC. It is likely that these results can be explained by a lower level of muscle glycogen after the LC diet, but as the majority of weight category sports allow time for some recovery to occur after weigh-in (Table 1.3) the validity of the results in relation to weight category sports is questionable.

Slater *et al*. (2005) examined the effects of a 4% weight loss over 24 h by self-selected methods in 17 national level lightweight rowers on 2000 m rowing ergometer performance, with rapid weight loss resulting in a 2 s (0.7%) reduction ($P = 0.003$) in ergometer performance. Smith *et al*. (2000; 2001) investigated the effects of rapid weight loss on boxing performance using a purpose built boxing ergometer capable of simulating boxing performance. Smith *et al*. (2000) examined the effect of a 3-4% reduction in body mass induced by intermittent low intensity exercise in a hot environment compared to a euhydration trial on punching force during 3 x 3 min rounds, separated by 1 min breaks. Whilst mean punching force was lower after rapid weight loss, there was no significant difference observed between the trials, which appeared to be mainly due to one subject who increased their performance by 17.8%

after rapid weight loss. In a subsequent study, Smith *et al*. (2001) examined the impact of rapid weight loss on simulated championship amateur boxing performance. During major championships, amateur boxers must weigh in on the morning of each competitive match and Smith *et al*. (2001) attempted to mirror this. In randomised order, subjects completed 5 day trials of normal or restricted diet. During the restriction trial, energy intake was restricted to 1000 $kcal \cdot d^{-1}$ and water intake was restricted to 1000 $ml \cdot d^{-1}$. Simulated boxing bouts of 3 x 3 min rounds were completed using the boxing dynamometer described previously (Smith *et al*., 2000) on the mornings of day 3 and 5. The restriction trial resulted in a 3% reduction in body mass and although mean punching force was reduced compared to the normal trial, there was not a significant difference between the trials.

Whilst the above studies have documented some of the effects of rapid weight loss on exercise performance, the inclusion of a recovery period is common place in most weight category sports, therefore an important consideration from an exercise performance point of view is not if rapid weight loss impairs physical performance, but if rapid weight loss and subsequent recovery impair performance. This notion becomes even more complex if one considers the alternative to rapid weight loss, as the pertinent question is whether rapid weight loss and subsequent recovery gives the athlete a performance advantage compared to competing at their natural body mass? In sports such as amateur wrestling, boxing, judo, taekwondo and many other sports, which involve competing one-on-one against another competitor, this question is very difficult to answer.

A number of studies have examined the effects of rapid weight loss and subsequent recovery on exercise performance (Klinzing and Karpowicz, 1986; Burge *et al*., 1993; Walberg Rankin *et al*., 1996; Schoffstall *et al*., 2001; Slater *et al*., 2006). Klinzing and Karpowicz (1986) examined the effects of a 5% rapid weight loss regimen over 50 h (fluid and energy restriction combined with exercise in the heat) and subsequent recovery on the time to complete a wrestling specific performance task. Subjects completed a control (no weight loss) and three 5% weight loss trials, during which performance was assessed either immediately, 1 h or 5 h after weigh in. Subjects consumed food and fluids *ad libitum* during the 1 h and 5 h recovery periods and weight regain was 22% and 44% of the 5% weight loss respectively. Compared to the

no weight loss trial, performance in the wrestling specific task was impaired after rapid weight loss, as well as after 1 h recovery, but had returned to baseline values after 5 h recovery. Similarly, Walberg Rankin *et al*. (1996) studied 12 collegiate wrestlers who consumed a 3 day energy restricted formula diet (18 kcal·kg^{-1} body mass·d^{-1}) and were separated into 2 recovery groups, receiving isoenergetic high (75%) or moderate (47%) carbohydrate recovery diets (21 kcal·kg^{-1} body mass). Although there were no significant differences between groups ($P \geq 0.01$), there was a tendency for an enhanced recovery of exercise performance following ingestion of the high carbohydrate recovery diet compared to the moderate carbohydrate recovery diet.

Burge *et al*. (1993) and Slater *et al*. (2005) examined rowing performance in response to rapid weight loss and recovery. Burge *et al*. (1993) examined the effect of a 5% (-3.78 kg) reduction in body mass over 24 h, followed by the ingestion of 1.5 l of plain water compared to a normal weight trial. Plasma volume was decreased by ~12% following rapid weight loss and after recovery remained ~6% decreased. Additionally, after rapid weight loss and subsequent recovery muscle glycogen was ~33% lower compared to before the normal weight trial. 2000 m ergometer performance time was significantly slower after rapid weight loss and recovery compared to the normal weight trial, a finding that is most likely explained by the lower resting muscle glycogen levels. In contrast, Slater *et al*. (2005) observed that after a 4% body mass reduction over 24 h and a subsequent 2 h recovery period during which carbohydrate, sodium and water where consumed, rowing performance was not significantly impaired compared to no rapid weight loss. As water intake was similar to that administered in the study of Wallberg Rankin *et al*. (1996), it is likely that the recovery of exercise performance is due to the inclusion of carbohydrate in the recovery diet and the resynthesis of at least some of the muscle glycogen that would be expected to be utilised during rapid weight loss (Wallberg Rankin *et al*., 1996). In line with this, Schoffstall *et al*. (2001) reported that 1 repetition maximum bench press (1RM) was decreased following a body mass reduction of 1.7% decreased 1RM from 118 (7.6) kg to 114 (7.2) kg, but that after 2 h rehydration period during which subjects ingested water ad libitum, 1RM had recovery to euhydrated levels (117 (7.8) kg).

The effect of creatine supplementation during rapid weight loss and recovery from rapid weight loss has also been investigated (Oopik *et al*., 1998; Oopik *et al*., 2002). Creatine supplementation has been shown to result in increased intramuscular stores of creatine and phosphocreatine (Hultman *et al*., 1996) and to enhance high intensity exercise performance (Birch *et al*., 1994; Greenhaff, 1995), particularly during repeated high intensity activity (Bemben and Lamont, 2005). Given the high intensity, intermittent nature of many weight category sports, creatine supplementation might have the potential to augment weight category sports performance. One commonly reported side effect with creatine supplementation is an increase in body mass (Cassey and Greenhaff, 2000), likely caused by an increase in body water content due to the osmotically active nature of creatine and phosphocreatine. As such creatine supplementation during rapid weight loss might be considered undesirable and might hinder the athlete in their goal to reduce body mass. Oopik *et al*. (1998) studied 10 experienced karate competitors over two, 5 day rapid weight loss trials, during which they were asked to reduce their body mass by ~5% of starting body mass through self-selected methods. During 1 trial, subjects received 4 x 5 g portions of creatine, whilst in the other they received identical amounts of a glucose placebo. Body mass reduction over the 5 days was lower during the creatine trial (-2.2 (1.7) kg) than during the placebo trial (-3.3 (1.7) kg). Studies examining similar creatine supplementation protocols, but without the inclusion of body mass reduction, have reported increases in body mass of ~1 kg over the 5 days supplementation (Bemben and Lanmont, 2005), suggesting that the 1.1 kg lower body mass reduction during the creatine trial might be explained by the commonly reported increase in body mass that accompanies creatine supplementation. Similar results have also been observed with athletes consuming an energy restricted diet with or without creatine supplementation (Rockwell *et al*., 2001). Oopik *et al*. (1998) also examined isokinetic performance of the knee extensor muscles before and after both trials and observed no significant difference between the groups. Oopik *et al*. (2002) examined the effect creatine supplementation during recovery from rapid weight loss. On 2 separate occasions trained wrestlers reduced body mass by 4.5-5.3% body mass and recovered over 17 h with *ad libitum* food and fluid intake plus either 4 x 80 g glucose or 4 x 80 g glucose + 7.5 g creatine monohydrate. Performance was assessed by a 5 min isokinetic knee extension protocol involving intermittent submaximal and maximal work before and after rapid weight loss and after 17 h recovery. Whilst recovery of body mass was not

different between trials, combined creatine and glucose supplementation resulted in superior regain of maximal exercise performance compared with glucose alone. These results might be explained by two potential mechanisms. Firstly, creatine supplementation might have increased intramuscular phosphocreatine stores, thus increasing the available substrate for the resynthesis of ATP through the ATP-PCr pathway during high intensity exercise. Secondly, creatine supplementation combined with carbohydrate ingestion during recovery from glycogen depleting exercise has been shown to enhance muscle glycogen resynthesis compared to carbohydrate alone (Robinson *et al*., 1999). If glycogen resynthesis was increased during the 17 h recovery period in the study of Oopik *et al*. (2002), then this might also account for the performance enhancement observed with creatine supplementation.

Aims

- To document the effects of short periods of fluid and/ or energy restriction on fluid and electrolyte balance.
- To determine the effect of with sodium chloride and potassium chloride addition to rehydration drinks consumed after 24 h combined fluid and energy restriction.
- To determine the effect of supplementing with sodium chloride and potassium chloride during severe energy restriction on fluid and electrolyte balance.
- To determine the effect of electrolyte supplementation during energy restriction on exercise capacity.

Hypotheses

- Fluid and/ or energy restriction would result in hypohydration, but that energy restriction would also cause a negative electrolyte balance due to the restriction of electrolyte intake.
- Addition of sodium chloride and potassium chloride to rehydration drinks consumed after fluid and energy restriction would both enhance fluid balance compared to a placebo drink.

- Supplementation with sodium chloride and potassium chloride during energy restriction would prevent a negative electrolyte balance developing and maintain plasma volume.
- Supplementation with sodium chloride and potassium chloride during energy restriction would enhance exercise capacity compared to energy restriction alone.

Chapter 2

General Methods

Ethical approval

Subjects participating in all experiments described in this thesis were healthy male and female adults, aged 18-37 years.

Prior to the start of all experiments, ethical approval was granted by the Loughborough University Ethical Advisory Committee (specific ethical committee approval reference numbers are given in Chapters 3-8). Both written and verbal explanations of the nature of all experimental procedures involved in each experiment were given to subjects and they were informed of their right to withdraw at any time. After any questions they had related to the experiment had been answered, each subject gave their full written and verbal consent to participate. Additionally, for experiments described in Chapters 5-8, subjects gave their written consent prior to commencing each subsequent experimental trial.

Standardisation prior to and during experimental trials

All experiments reported in this thesis commenced in the morning following an overnight fast, after a 24-48 h period of dietary and physical activity control. Subjects were required to record their dietary and physical activity patterns for a period of 24-48 h prior to the first experimental trial of each study and then were asked to follow these same diet and physical activity patterns for the 24-48 h before the start of each subsequent experimental trial. This control period was 24 h for the studies reported in Chapter 3 and 4, and was 48 h for the studies reported in Chapters 5, 6, 7 and 8. In an attempt to ensure subjects began each trial in an euhydrated state, subjects were instructed to ingest 500 ml plain water, 2 h before commencing each experimental trial during the studies reported in Chapters 3, 4, 5 and 6. Similarly, during the studies reported in Chapters 7 and 8, the evening before commencing each experimental trial, subjects were instructed to ingest at least 500 ml plain water with their evening meal and 500 ml plain water before going to bed. Subjects refrained from any strenuous physical activity and consumption of alcohol for the 24 h prior to, as well as during each experimental trial.

Body mass measurement

During all the experiments described in this thesis, body mass was measured nude to the nearest 10 g (CFW150 digital scale; Adam, Milton Keynes, UK). BML (BML) (kg) was calculated as the change in nude body mass from baseline nude body mass for each trial. Percentage BML was also calculated relative to baseline nude body mass for each trial.

Urine collection and analysis

All experiments described in this thesis involved the collection of all urine produced over each trial (24-48 h). When urine samples were provided in the laboratory, subjects were provided with a plastic container and asked to completely empty their bladder and collect the entire volume of the urination. During the studies reported in Chapters 5 and 7, if subjects needed to urinate before the sample time point, this urine was retained, before being mixed thoroughly with urine produced at the samples specific time point. All studies reported in this thesis involved collection of all urine produced between laboratory visits. During the study reported in Chapter 3, subjects were provided with plastic containers and measuring cylinders and were required to collect the entire volume in the plastic container, measure and record the volume, and retain a 5 ml sample of each urination in a small plastic tube. During the studies reported in Chapters 5, 6, 7 and 8, subjects were provided with large urine collection containers and were required to collect all urine produced over the trial period in these containers.

Once the volume of each urine sample had been measured using a measuring cylinder (unless otherwise stated in the Chapter), at least one 5 ml sample was retained for analysis. All urine samples were analysed for osmolality by freezing point depression (Gonotec Osmomat 030 Cryoscopic Osmometer; Gonotec, Berlin, Germany), sodium and potassium concentrations by flame photometry (Corning Clinical Flame Photometry 410C; Corning Ltd., Halstead, Essex, UK), chloride concentration by coulometric titration (Jenway Chloride Meter; Jenway Ltd., Dunmow, Essex, UK) and creatinine concentration using the Jaffe reaction (Owen *et al*., 1954). All analysis was performed in duplicate, with mean values taken. Samples retained for analysis of

sodium, potassium and chloride were stored at 4°C, whilst samples retained for analysis of creatinine were stored at -20°C.

During the experiments reported in Chapters 3, 5, 6, 7 and 8 of this thesis sodium, potassium and chloride balance was calculated from losses in urine and gains through food consumption and drink ingestion.

Blood sampling and analysis

Blood samples were taken during the studies described in Chapters 3, 6, 7 and 8. The methods sections of each experimental Chapter gives specific details of the nature, timing and volume of blood samples taken. All blood samples, whether venepuncture or cannulation were taken with a 21-gauge butterfly needle and drawn into a plastic syringe, before being dispensed as described in the methods section of each experimental Chapter. Prior to each blood sample, subjects assumed an upright seated position for at least 15 min to avoid any postural changes in blood and plasma volume (Shirreffs and Maughan, 1994).

Blood glucose concentration, haemoglobin concentration and haematocrit

Whole blood was first dispensed into tubes containing K_2EDTA (1.75 mg·ml^{-1}). 100 µl of whole blood was mixed with 1000 µl of 0.3 N ice cold perchloric acid in duplicate, for deproteinisation. After centrifugation, the supernatant was used for the determination of blood glucose concentration by the glucose oxidase peroxidase amino antipyrine phenol method (Randox Laboratories Ltd., Crumlin, UK). Whole blood was also used for the determination of haematocrit by microcentrifugation (Hawksley micro-haematocrit centrifuge; Hawksley and Sons Ltd., Lancing, UK) and haemoglobin concentration by the cyanmethaemoglobin method. Haematocrit was performed in triplicate, whilst blood glucose and haemoglobin concentrations were determined in duplicate. In Chapters 3, 6, 7 and 8, haematocrit and haemoglobin concentrations were used to estimate changes in blood, plasma and red cell volume relative to the 0 h blood sample (Dill and Costill, 1974).

Serum electrolyte concentrations and osmolality

Whole blood was dispensed into plain tubes and allowed to clot at room temperature, before being centrifuged at 4°C and 1500 g for 15 min (ALC multispeed refrigerated centrifuge; Thompson Scientific, Aberdeen, UK). The serum was then separated and stored at 4°C before analysis.

Serum sodium, potassium and chloride concentrations, as well as osmolality were determined by the same methods described for the urine analysis. All measurements were made in duplicate.

Plasma aldosterone concentration

Whole blood was collected into pre-chilled tubes containing K_2EDTA (1.75 $mg.ml^{-1}$), before being centrifuged at 0°C and 1500 g for 15 min (ALC multispeed refrigerated centrifuge; Thompson Scientific, Aberdeen, UK). Plasma was then separated and stored at -80°C until analysis. Commercially available enzyme immunoassay (EIA) kits were used to measure plasma aldosterone concentration (Assay designs Aldosterone EIA kit, Cambridge Biosciences, Cambridge, UK). All measurements, except standards were made singularly.

Blood pH

Whole blood was collected into a pre-chilled tube containing lithium heparin and mixed thoroughly, before being analysed immediately with an automated blood gas analyser (ABL5 blood gas analyser, Radiometer Ltd, Crawley, UK). All measurements were made in duplicate.

Sweat collection and analysis

Sweat was collected during exercise sessions of the study described in Chapter 8 of this thesis. A small (2.5 cm x 4 cm) absorbent gauze patch (Tegaderm +Pad; 3M Health care, Loughborough, UK) was placed on the left of the subjects upper back

before exercise commenced. After exercise, the sweat patch was removed and transferred to a syringe, before sweat was aspirated into an epenndorf tube.

Sweat sodium, potassium and chloride concentrations were determined by the same methods described for the urine analysis. All measurements were made in duplicate.

Environmental temperatures

During all experimental trials reported in this thesis, daily environmental temperature recorded at the Met Office weather station situated at Sutton Bonnington, Loughborough, Leicestershire was noted for each experimental trial undertaken.

Statistical analysis

All data were first tested for normality of distribution using a Shapiro-Wilk test. After this, all data containing two independent variables were analysed using a two-way repeated measures analysis of variance (ANOVA) and if the assumption of sphericity was violated, the degrees of freedom where corrected using the Greenhouse-Geisser estimate. Where two-way ANOVA indicated significant main effects, *post-hoc* Bonferroni-adjusted t-tests for normally distributed data or post-hoc Bonferroni-adjusted Wilcoxon signed rank tests for non-normally distributed data were used to identify the location of differences. Normally distributed data containing one independent variable was analysed using a t-test or one-way repeated measures ANOVA with *post-hoc* Bonferroni-adjusted t-tests. Non-normally distributed data containing one independent variable was analysed using Wilcoxon signed rank test or Friedmans ANOVA with *post-hoc* Bonferroni-adjusted Wilcoxon signed rank tests. Pearson's product moment correlation coefficient (*r*) was calculated where appropriate. Normally distributed data are presented as mean (SD), whilst non-normally distributed data are presented as median (range). Data were accepted as being significantly different when $P \leq 0.05$.

Coefficient of variation for analytical procedures

Table 2.1 displays the coefficient of variation (CV) for analytical procedures described in this thesis. 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
Blood glucose concentration ($mmol \cdot l^{-1}$)	30	4.67	0.56	1.2
Haemoglobin concentration ($g \cdot l^{-1}$)	30	158	17	0.7
Haematocrit (%)	30	43	5	0.7
Serum osmolality ($mosmol \cdot kg^{-1}$)	30	285	4	0.3
Serum sodium concentration ($mmol \cdot l^{-1}$)	30	143	2	1.0
Serum potassium concentration ($mmol \cdot l^{-1}$)	30	4.3	0.3	2.3
Serum chloride concentration ($mmol \cdot l^{-1}$)	30	104	3	1.4
Plasma aldosterone concentration ($pg \cdot ml^{-1}$)	21	49	6	19.9
Urine osmolality ($mosmol \cdot kg^{-1}$)	30	493	302	2.8
Urine sodium concentration ($mmol \cdot l^{-1}$)	30	84	46	1.9
Urine potassium concentration ($mmol \cdot l^{-1}$)	30	57	35	2.4
Urine chloride concentration ($mmol \cdot l^{-1}$)	30	88	76	2.1
Urine creatinine concentration ($mmol \cdot l^{-1}$)	30	11.9	6.7	1.3

Chapter 3

Fluid and electrolyte balance during 24 h fluid and/or energy restriction

Abstract

This study examined fluid and electrolyte balance responses to 24 h fluid restriction (FR), energy restriction (ER) and fluid and energy restriction (F+ER) compared to a control trial (C). Twelve subjects (six male, six female) received 9.92 (1.41) MJ, 2770 (792) ml water (C), 9.93 (1.65) MJ, 277 (32) ml water (FR), 2.47 (0.40) MJ, 2770 (792) ml water (ER) and 2.47 (0.40) MJ, 277 (32) ml water (F+ER). Trials were completed in randomized counterbalanced order and subjects visited the laboratory at 0 h, 12 h and 24 h for blood and urine sample collection. Over the 24 h, body mass loss was 0.33 (0.33) % (C), 1.88 (0.52) % (FR), 1.97 (0.47) % (ER) and 2.44 (0.53) % (F+ER). Plasma volume was reduced ($P < 0.01$) at 24 h during FR, ER and F+ER, whilst serum osmolality was increased ($P < 0.05$) at 24 h for FR and F+ER and was greater at 24 h for FR compared to all other trials ($P < 0.05$). Over the 24 h, negative balances of sodium, potassium and chloride developed during ER and F+ER ($P < 0.01$), but not during C and FR. These results demonstrate that 24 h fluid and/ or energy restriction significantly reduces body mass and plasma volume, but has a disparate effect on serum osmolality, resulting in hypertonic hypohydration during FR and isotonic hypohydration during ER. These findings might be explained by the difference in electrolyte balance between the trials.

Introduction

The severe restriction of fluid and/ or energy intake is relatively common in a number of active populations, including athletes competing in weight category sports (Smith, 2006; Oppliger *et al*., 2003; Slater *et al*., 2005; Moore *et al*., 2002) and military personnel (Nindl *et al*., 2002).

Recently, Smith (2006) reported that in the 24 h immediately preceding the official weigh-in 78 (95%) and 82 (100%) out of 82 English international amateur boxers were restricting their fluid and energy intake respectively and that during this 24 h period the mean weight loss was 2.2 (0.3) % body mass. Additionally, military personnel undergo periods of limited fluid and energy supply, combined with a number of other operational stresses (e.g. increased energy expenditure, increased sweat losses and sleep deprivation (Nindl *et al*., 2002)).

Periods of fluid restriction have been used in a laboratory setting to induce hypohydration (Phillips *et al*., 1984; Pederson *et al*., 2001; Shirreffs *et al*., 2004b; Higgens *et al*., 2007) and the time course effects of such periods of fluid restriction on hydration status, as well as subjective responses have been examined (Shirreffs *et al*., 2004b). The effects of short term energy restriction or combined fluid and energy restriction on hydration status are less well known. Oliver and colleagues (2007, 2008) were the first to examine the singular and combined effects of short term (48 h) periods of severe fluid and energy restriction, primarily on exercise performance (Oliver *et al*., 2007), as well as some aspects of hydration status (Oliver *et al*., 2008). Oliver *et al*. (2007) observed large reductions in body mass with fluid and/ or energy restriction, as well as a reduction in plasma volume with energy restriction and combined fluid and energy restriction, but no change following fluid restriction. Other investigators have reported reduced plasma volume with fluid (Shirreffs *et al*., 2004b; Bosco *et al*., 1974; Zappe *et al*., 1993), energy (Consolazio *et al*., 1968a) and combined fluid and energy (Bosco *et al*. 1974) restriction. Whilst fluid restriction is associated with plasma hyperosmolality (Shirreffs *et al*., 2004b; Oliver *et al*., 2008; Zappe *et al*., 1993), combined fluid and energy restriction results in no change in plasma osmolality (Oliver *et al*., 2008), suggesting that fluid loss induced by restricted fluid intake results in hypertonic hypohydration, whilst fluid loss induced by

restricted fluid and energy intake results in isotonic hypohydration. It is likely that these differences are related to changes in electrolyte balance, but the separate and combined effects of fluid and/ or energy restriction on electrolyte balance are not known.

The present study therefore aimed to investigate the effects of a 24 h period of fluid, energy or combined fluid and energy restriction on fluid and electrolyte balance in humans and to compare this to the response of a control trial.

It was hypothesised that all fluid and/ or energy restriction would significantly reduce body mass and plasma volume, and that electrolyte excretion would continue in the urine in all trials, resulting in large negative balances during the trials involving energy restriction.

Methods

Twelve healthy volunteers (six men, six women) participated in this study, which was approved by the Loughborough University Ethical Advisory Committee (Reference No. R06-P142). Subjects' baseline physical characteristics were: age 25 (5) y, height 1.70 (0.08) m, body mass 66.69 (6.72) kg. Each subject completed an initial familiarisation trial followed by 4 experimental trials, which were completed in randomised counterbalanced order.

All trials (familiarisation and experimental) were undertaken on the same day of the week and separated by at least 7 days, with subjects continuing their daily activities whilst visiting the laboratory on 3 separate occasions (0 h, 12 h and 24 h). Trials were undertaken during the months of February, March, April and May in Loughborough, UK, with 24 h mean daily temperatures of 8.7 (2.9) °C. For all trials, subjects reported to the laboratory in the morning (0 h) following at least a 10 h overnight fast, with the exception of 500 ml tap water consumed two hours before arrival. The 12 h and 24 h visits were after at least a 4 h fast, although water intake was allowed up until 2 h before arrival.

During the initial familiarisation trial, subjects were fully familiarised with all procedures (urine sampling, blood sampling, body mass measurement). Kitchen scales accurate to the nearest gram were provided and subjects weighed all food and drink consumed over the 24 h period.

Each subject's 24 h weighed food records were analysed using a computerised dietary analysis package (CompEat Pro, version 5.8.0; Nutrition Systems, Banbury, UK) and energy, macronutrient, water, sodium, potassium and chloride intakes were estimated (Table 3.1). From this analysis the conditions for the 4 experimental trials were determined as follows: control trial (C), replication of the familiarisation trial diet; fluid restriction trial (FR), energy intake matched to C, water intake restricted (although a small amount of water was provided in foods); energy restriction trial (ER), ~25% energy of C, water intake matched to C; fluid and energy restriction trial (F+ER), ~25% energy of C, water intake matched to FR.

For each experimental trial subjects arrived at the laboratory for the 0 h visit and voided their bladder. Subject's nude body mass was then measured, before they assumed a seated position. After 15 min seated rest, a 3.5 ml blood sample was taken without stasis from an antecubital vein. Subjects were unaware of which trial they would complete each week before arriving for the 0 h visit, with the exception of the last visit, which subjects were able to identify by a process of elimination. For FR, ER and F+ER, all foods were provided to the subjects at the 0 h visit in the form of dry foods (e.g. pizza, crisps, cereal bars), along with the correct amount of tap water (where necessary, i.e. trials ER and F+ER). For trial C, subjects were provided with a copy of their familiarisation diet and a set of kitchen scales to allow them to precisely replicate the familiarisation diet, as well as a diary to record the exact weight of foods and drinks consumed. Subjects were also provided with urine collection equipment (A jug and measuring cylinder) and were instructed to measure and collect all urine produced whilst outside the laboratory for the duration of each trial. Subjects then left the laboratory, returning 12 h and 24 h later, when the same measurements were made as at 0 h.

Table 3.1 Dietary energy, macronutrient, water and electrolyte contents of experimental trials. Values are mean (SD).

	C	FR	ER	F+ER
Energy (MJ)	9.92 (1.41)	9.93 (1.65)	2.47 (0.40)	2.47 (0.40)
Protein (g)	97 (26)	71 (13)	20 (5)	20 (5)
Carbohydrate (g)	335 (61)	339 (63)	86 (17)	86 (17)
Fat (g)	72 (27)	82 (20)	19 (7)	19 (7)
Water (ml)	2770 (792)	277 (32)	2770 (792)	277 (32)
Sodium (mmol)	144 (47)	110 (18)	31 (11)	31 (11)
Potassium (mmol)	90 (26)	45 (10)	12 (4)	12 (4)
Chloride (mmol)	145 (49)	151 (22)	44 (13)	44 (13)

Analytical methods

1 ml of each blood sample was mixed with anticoagulant (K_2 EDTA, 1.75 $mg.ml^{-1}$) and was used for the determination of haematocrit, haemoglobin concentration and blood glucose concentration. The remaining 2.5 ml blood was allowed to clot and the serum was separated by centrifugation.

Serum samples were analysed for osmolality, as well as sodium, potassium and chloride concentrations.

Urine samples were analysed for osmolality, as well as sodium, potassium and chloride and creatinine concentrations.

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

Statistical analysis

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

Results

With the exception of haematocrit ($P < 0.001$), haemoglobin concentration ($P < 0.001$) and nude body mass ($P < 0.001$), after correction of electrolyte excretion for body mass, there were no differences between males and females ($P > 0.192$) for any of the other measured variables. Therefore data for both males and females are combined. 24 h urinary creatinine excretion was not different between trials ($P = 0.550$) and over all trials was 0.18 (0.04) mmol·kg^{-1} body mass·24 h^{-1}, which is indicative of a complete 24 h urine collection (Bingham and Cummings, 1985).

Pre-trial measurements

Pre-trial body mass ($P = 0.997$), urine osmolality ($P = 0.437$), serum osmolality ($P = 0.604$) and serum sodium concentration ($P = 0.642$) were not different between trials, indicating subjects started each trial in a similar state of hydration.

Body mass changes

BML (kg) over the four trials is displayed in Table 3.2. Over the 24 h trial period these reductions in body mass equate to losses of 0.33 (0.33) % (C), 1.88 (0.52) % (FR), 1.97 (0.48) % (ER) and 2.44 (0.53) % (F+ER) of the subjects initial body mass. BML was significant at 12 h and 24 h for FR, ER and F+ER ($P < 0.001$) and at 24 h for C ($P < 0.05$). At both 12 h and 24 h BML was greater during all three restriction trials compared to C ($P < 0.001$). Additionally, BML was greater during F+ER compared to ER at 12 h ($P < 0.05$) and 24 h ($P < 0.05$), as well as compared to FR at 24 h ($P < 0.05$.

Table 3.2 BML (BML) (kg) calculated as the change in body mass from 0 h. * Significantly different from 0 h. # Significantly different from trial C. † Significantly different from trial F+ER. Values are mean (SD).

	0 h	12 h	24 h
C	0 (0)	0.13 (0.25)	0.23 (0.21) *
FR	0 (0)	0.82 (0.37) *#	1.26 (0.39) *#†
ER	0 (0)	0.85 (0.38) *#	1.32 (0.36) *#†
F+ER	0 (0)	1.16 (0.34) *#	1.64 (0.41) *#

Gross BML was defined as the observed BML over 24 h plus the weight of the dietary intake (food and water) and was greater for ER (4.22 (0.82) kg) compared to all trials ($P < 0.05$), as well as for C (3.40 (0.87) kg) compared to FR (2.03 (0.39) kg) and F+ER (2.01 (0.41) kg) ($P < 0.001$). Total urine losses accounted for a greater proportion of the gross BML during C (61 (19) %) and ER (67 (20) %), compared to FR (37 (8) %) and F+ER (38 (12) %) ($P < 0.001$). The remainder of the gross BML (1.29 (0.65) kg (C), 1.30 (0.39) kg (FR), 1.36 (0.72) kg (ER) and 1.24 (0.38) kg (F+ER)) can be accounted for by respiratory and cutaneous fluid losses, as well as faecal fluid and solid losses and was not different between trials ($P = 0.825$).

Urine volume, osmolality and electrolyte excretion

The total 24 h urine volume (Table 3.3) was greater for C and ER compared to FR and F+ER ($P < 0.001$), as well as for ER compared to C ($P < 0.05$). Additionally, larger volumes of urine were excreted during both C and ER compared to both FR and F+ER between 0-12 h ($P < 0.05$) and 12-24 h ($P < 0.05$). A greater volume of urine was excreted between 0-12 h than between 12-24 h for all trials ($P < 0.05$).

Table 3.3 Urine volume (ml) produced during the experimental trials for the entire 24 h, as well as between 0-12 h and 12-24 h. * Significantly different from 0-12 h. # Significantly different from trial C. ‡ Significantly different from ER. Values are mean (SD).

	Total	0-12 h	12-24 h
C	2118 (955)	1341 (741)	777 (540) *
FR	731 (145)	479 (141) #‡	252 (61) *#‡
ER	2866 (1118)	1878 (716)	979 (628) *
F+ER	766 (283)	554 (262) #‡	218 (74) *#‡

Urine osmolality (Figure 3.1) at 0 h was not different between trials (P = 0.312), and over all trials was 201 (78-877) mosmol·kg^{-1}. At 12 h, urine osmolality was increased compared to 0 h during C (584 (141-887) mosmol·kg^{-1}) (P < 0.01), FR (907 (598-1242) mosmol·kg^{-1}) (P < 0.001) and F+ER (852 (337-1068) mosmol·kg^{-1}) (P < 0.001), remaining increased at 24 h during FR (995 (706-1359) mosmol·kg^{-1}) and F+ER (997 (753-1095) mosmol·kg^{-1}). Furthermore, at 12 h urine osmolality was greater during FR compared to C and ER and during F+ER compared to ER (P < 0.05), whilst at 24 h urine osmolality was greater during either FR or F+ER compared to either C or ER (P < 0.01).

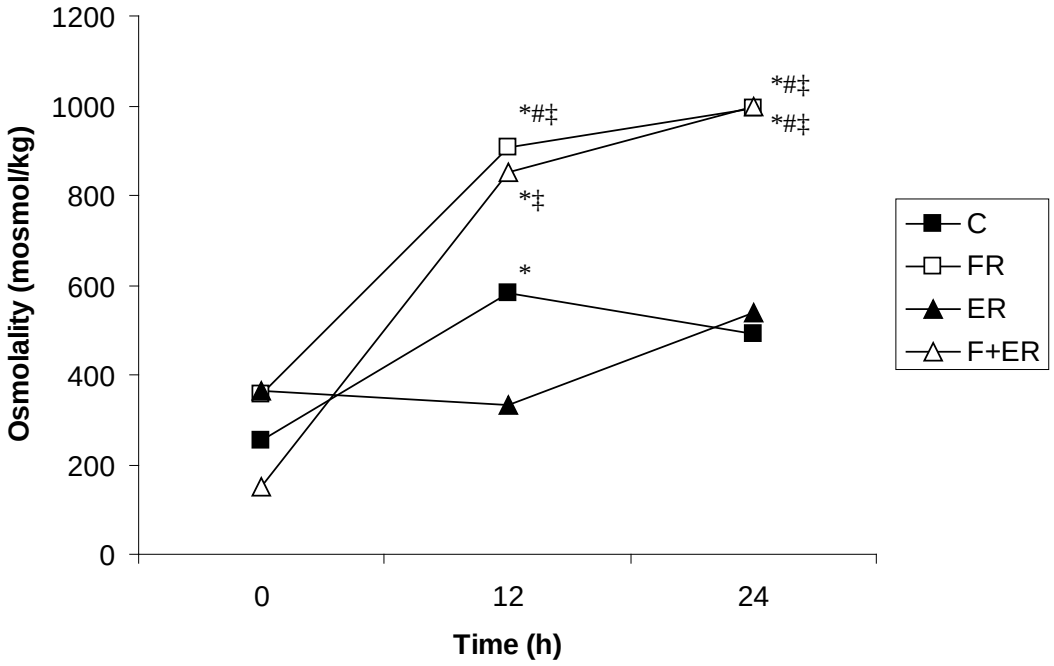

Figure 3.1 Urine osmolality (mosmol·kg^{-1}) of urine samples taken at 0, 12 and 24 h. * Significantly different from 0 h. # Significantly different from trial C. ‡ Significantly different from ER. Points are median values.

Urinary sodium excretion (Table 3.4a) was not different between trials over the whole 24 h period (P = 0.096) or between 0-12 h (P = 0.667), but a greater amount of sodium was excreted in the urine between 12-24 h for C compared to F+ER (P < 0.05) and urinary sodium excretion was greater between 0-12 h than between 12-24 h for all trials. The total amount of potassium excreted in the urine over the 24 h (Table 3.4b) was greater for C then FR (P < 0.01) or F+ER (P < 0.05), and tended to be greater than ER (P = 0.051). Additionally, urinary potassium excretion was greater for C compared to ER (P < 0.05) between 0-12 h and compared to FR (P < 0.05) and F+ER (P < 0.05) between 12-24 h. Urinary chloride excretion (Table 3.4c) showed a similar pattern to urinary sodium excretion and was not different between trials over the whole 24 h (P = 0.146) or between 0-12 h (P = 0.562), but was greater for ER compared to F+ER between 12-24 h (P < 0.05).

Table 3.4 Total, as well as 0-12 h and 12-24 h Sodium (mmol) (a), potassium (mmol) (b) and chloride (mmol) (c) excreted in the urine. * Significantly different from 0-12 h. † Significantly different from trial F+ER Values are median (range).

	Total	0-12 h	12-24 h
a) Sodium			
C	140 (67-186)	77 (34-107)	61 (3-91) *
FR	128 (80-188)	75 (38-110)	42 (26-83) *†
ER	135 (37-192)	76 (29-165)	32 (8-76) *
F+ER	78 (20-163)	46 (9-152)	20 (10-51) *
b) Potassium			
C	78 (15-182)	56 (10-90)	23 (1-43) *
FR	37 (28-70) #	29 (16-49) #	13 (4-27) *
ER	49 (28-89) #	30 (22-65) #	19 (7-26) *
F+ER	45 (23-104) #	34 (12-89)	12 (5-43) *
c) Chloride			
C	158 (86-198)	90 (38-131)	45 (8-70) *
FR	127 (68-172)	77 (39-130)	42 (18-68) *†
ER	157 (58-214)	87 (40-171)	35 (14-94) *
F+ER	76 (24-246)	49 (17-220)	25 (8-54) *

Electrolyte balance

Sodium balance (Table 3.5a) at 24 h over the study period was more positive for both C ($P < 0.05$) and FR ($P < 0.05$) compared to ER and F+ER. Potassium balance (Table 3.5b) showed a similar relationship and was more positive for C ($P < 0.001$) and FR ($P < 0.001$) compared with ER and F+ER, whilst chloride balance (Table 3.5c) was more positive for C than ER ($P < 0.001$) and more positive for FR than ER and F+ER

($P < 0.001$). Additionally, compared to 0 h sodium, potassium and chloride balances were all net negative at 24 h during ER ($P < 0.001$) and F+ER ($P < 0.01$)

Table 3.5 Sodium (mmol) (a), potassium (mmol) (b) and chloride (mmol) (c) balance over the 24 h trial period calculated from dietary intake and urinary excretion. * Significantly different from 0 h. # Significantly different from trial C. ¶ Significantly different from FR. Values are mean (SD).

	0 h	24 h
a) Sodium		
C	0 (0)	15 (47)
FR	0 (0)	-6 (40)
ER	0 (0)	-86 (44) *#¶
F+ER	0 (0)	-59 (35) *#¶
b) Potassium		
C	0 (0)	8 (31)
FR	0 (0)	-1 (18)
ER	0 (0)	-44 (19) *#¶
F+ER	0 (0)	-41 (23) *#¶
c) Chloride		
C	0 (0)	7 (57)
FR	0 (0)	35 (30) *
ER	0 (0)	-91 (44) *#¶
F+ER	0 (0)	-54 (50) *#¶

Estimated change in blood, plasma and red cell volume

Compared to 0 h, blood volume (Figure 3.2) was reduced at 12 h during F+ER ($P = 0.01$) and at 24 h during FR ($P < 0.001$), ER ($P < 0.001$) and F+ER ($P < 0.001$). Additionally, compared to trial C, blood volume was reduced at both 12 h ($P < 0.05$) and 24 h ($P < 0.01$) during F+ER, as well as at 24 h during FR ($P < 0.05$) and ER (P

= 0.01). Plasma volume (Figure 3.3) showed a similar response to blood volume and compared to 0 h was reduced at 12 h ($P < 0.01$) and 24 h ($P < 0.01$) during F+ER, as well as at 24 h during FR ($P < 0.01$) and ER ($P < 0.01$). Additionally, compared to trial C plasma volume was reduced at both 12 h ($P < 0.01$) and 24 h ($P < 0.01$) during F+ER, as well as at 24 h during FR ($P < 0.01$) and ER ($P = 0.01$). There was no significant change in red cell volume (Figure 3.4) either over time ($P = 0.335$) or between trials ($P = 0.723$).

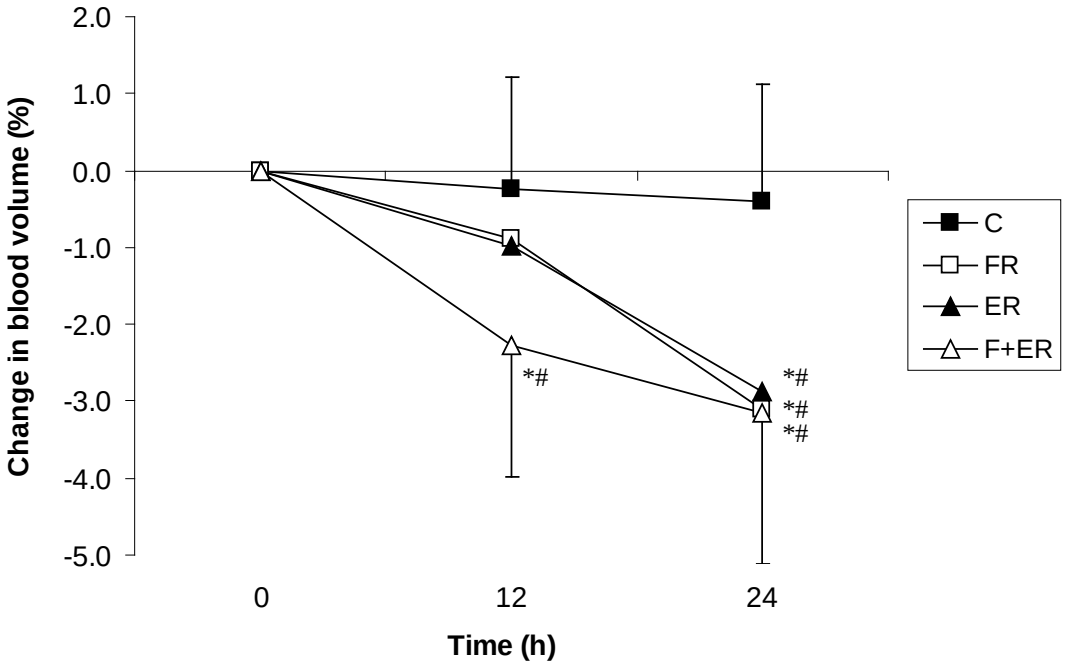

Figure 3.2 Estimated change in blood volume from 0 h (%).* Significantly different from 0 h. # Significantly different from trial C. Points are mean values. Error bars are SD.

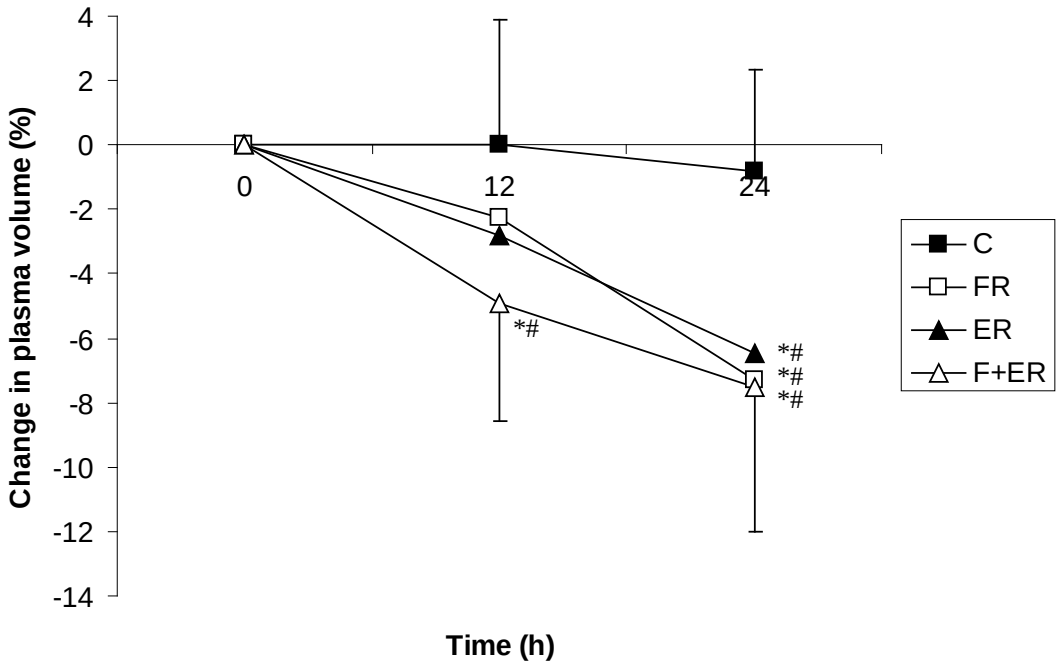

Figure 3.3 Estimated change in plasma volume from 0 h (%).* Significantly different from 0 h.[#] Significantly different from trial C. Points are mean values. Error bars are SD.

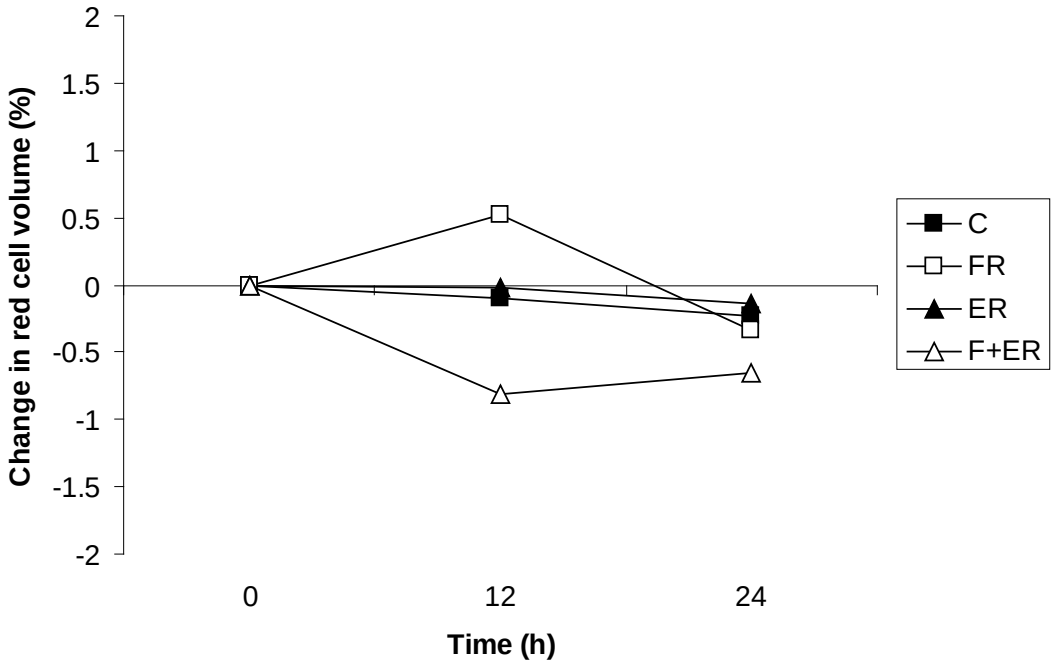

Figure 3.4 Estimated change in red cell volume from 0 h (%). Points are median values.

Serum osmolality, sodium, potassium and chloride concentrations

Serum osmolality (Table 3.6a) increased linearly by 5 mosmol·kg^{-1} from 0 h to 12 h and from 12 h to 24 h during FR and was elevated from 0 h values at both 12 h (P < 0.001) and 24 h (P < 0.001) during FR, as well as at 12 h during C (P < 0.05) and 24 h during F+ER (P < 0.01). Furthermore, serum osmolality was greater at 12 h during FR compared to ER (P < 0.05) and at 24 h for FR compared to all other trials (P < 0.05). Serum chloride concentration (Table 3.6b) demonstrated a similar pattern to serum osmolality and increased by 3 mmol·l^{-1} for each 12 h time period during FR and compared to 0 h was greater at both 12 h (P < 0.001) and 24 h (P < 0.001) during FR and at 24 h during ER (P < 0.05). Additionally, serum chloride concentration was greater at 24 h during FR compared to all other trials (P < 0.05). Compared to 0 h, serum sodium concentration (Table 3.7a) was increased at 12 h (P < 0.01) and 24 h (P < 0.001) during FR and at 24 h during F+ER (P < 0.05) and at 24 h was greater during FR than during C. No time (P = 0.056), trial (P = 0.598) or interaction (P = 0.491) effects for serum potassium concentration (Table 3.7b).

Table 3.6 Serum osmolality (mosmol·kg^{-1}) (a) and serum chloride concentration (mmol·l^{-1}) (b) at 0 h, 12 h and 24 h. * Significantly different from 0 h. ¶ Significantly different from FR. Values are mean (SD).

	0 h	12 h	24 h
a) Osmolality			
C	284 (3)	287 (4) *	286 (3) ¶
FR	285 (3)	290 (4)*	295 (4) *
ER	286 (3)	285 (3)¶	285 (3) ¶
F+ER	286 (4)	287 (2)	289 (5) *¶
b) Chloride			
C	104 (1)	104 (2)	105 (2) ¶
FR	103 (2)	106 (2)*	109 (1) *
ER	104 (2)	104 (3)	106 (2) *¶
F+ER	104 (2)	104 (2)	105 (2) ¶

Table 3.7 Serum sodium (a) and potassium (b) concentration (mmol·l^{-1}) at 0 h, 12 h and 24 h* Significantly different from 0 h. # Significantly different from trial C. Values are median (range).

	0 h	12 h	24 h
a) Sodium			
C	142 (139-146)	143 (140-146)	144 (140-146)
FR	142 (140-144)	145 (141-149) *	147 (143-150) *#
ER	141 (140-146)	142 (139-148)	143 (140-148)
F+ER	143 (140-146)	144 (139-147)	146 (140-149) *
b) Potassium			
C	4.3 (4.0-5.2)	4.3 (3.8-5.1)	4.4 (3.9-5.4)
FR	4.0 (3.5-5.0)	4.3 (3.8-5.0)	4.4 (4.1-5.2)
ER	4.3 (3.9-4.4)	4.2 (3.7-5.4)	4.3 (4.0-5.3)
F+ER	4.1 (3.9-4.9)	4.1 (3.8-4.6)	4.3 (3.9-4.8)

Blood glucose concentration

Blood glucose concentration (Figure 3.5) was not different between trials, but compared to 0 h showed a significant reduction at 12 h and 24 h during ER and F+ER ($P < 0.05$).

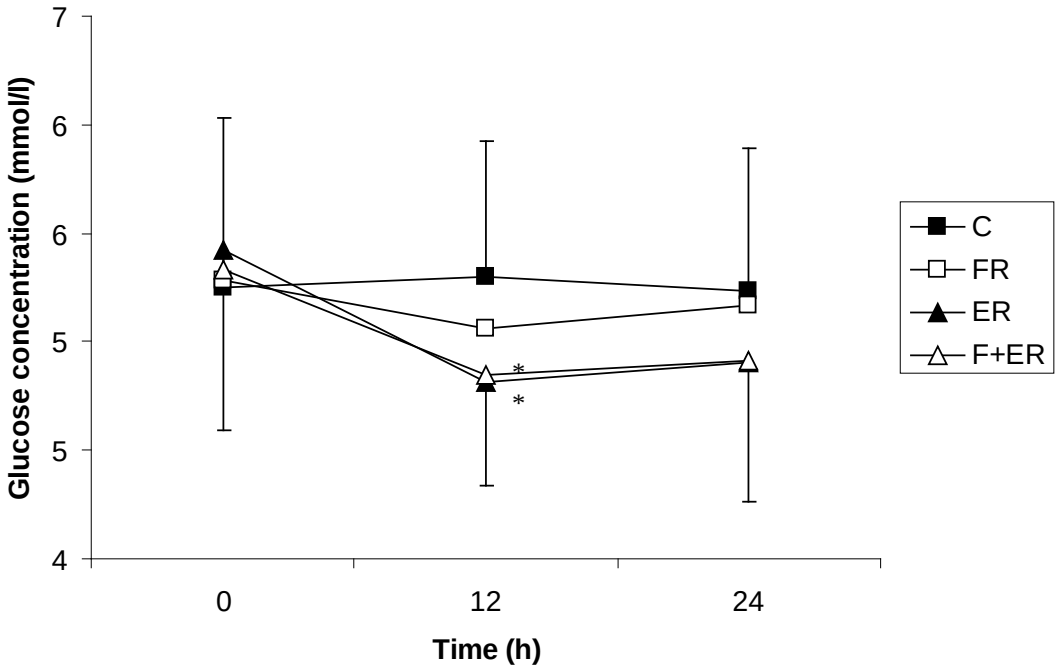

Figure 3.5 Blood glucose concentration ($mmol \cdot l^{-1}$). * Significantly different from 0 h. Points are mean values. Error bars are SD.

Markers of hydration status

Table 3.7 shows correlations between BML and the measured markers of hydration status for all urine and blood samples provided during laboratory visits for each trial. Change in plasma volume showed a good correlation with BML for all three restriction trials. All other markers of hydration status were well correlated with BML for FR, whilst urine osmolality was well correlated with BML for F+ER.

Table 3.14 Pearson's product moment correlation coefficients (R) between (BML) (kg) and estimated change in plasma volume (%), serum osmolality (mosmol·kg^{-1}), serum sodium concentration (mmol·l^{-1}) and urine osmolality (mosmol·kg^{-1}). * Significant correlation ($P < 0.05$).

	FR	ER	F+ER
		(BML (kg))	
Δ Plasma volume (%)	0.712*	0.748*	0.608*
Serum osmolality (mosmol·kg^{-1})	-0.704*	-0.152	-0.312
Serum sodium concentration (mmol·l^{-1})	-0.773*	-0.407*	-0.513 *
Urine osmolality (mosmol·kg^{-1})	-0.743*	-0.239	-0.785 *

Discussion

The results of this study demonstrate that a 24 h period FR, ER or F+ER significantly reduce body mass and plasma volume and that during ER and F+ER this is accompanied by the development of a negative balance of the electrolytes sodium, potassium and chloride caused by the continued loss of these electrolytes in urine despite only a small dietary intake. These varied losses of electrolytes during the

different dietary restrictions results in differences in the type of hypohydration that develops, with FR inducing hypertonic hypohydration, ER inducing isotonic hypohydration and F+ER inducing a mildly hypertonic hyperhydration.

24 h FR resulted in a BML of 1.88 (0.52) %, which is similar to reductions of 1.7-1.9% reported previously (Shirreffs *et al*., 2004b; Phillips *et al*., 1984; Phillips *et al*., 1993; Zappe *et al*., 1993). Similarly, the 1.97 (0.48) % BML after 24 h ER is similar to the 1.8% BML reported during the first 24 h of 12 days energy restriction (2424 $kJ \cdot d^{-1}$) (Brozek *et al*., 1957) and the 2.44 (0.53) % BML after 24 h F+ER agrees with the 2.4-2.9% BML observed after 24 h complete energy restriction (Ship and Fischer, 1999). BML at 24 h (0.32 (0.33) %) was significant for C, a finding reported previously (Oliver *et al*., 2007). A possible explanation in this group of subjects is that at 0 h subjects arrived 2 hours after consuming 500 ml plain water. Whereas at 24 h, only 2 out of the 12 subjects chose to consume water in the morning prior to visiting the laboratory, meaning 10 out of the 12 subjects arrived at 24 h after a complete overnight fast.

As observed with 48 h fluid and/ or energy restriction (Oliver *et al*., 2007), BML over 24 h was greater for F+ER than FR and ER. Whilst BML with F+ER is increased compared to either being restricted alone, the combined effect is not simply the additive effect of the two singular restriction treatments. Whilst FR-induced BML is due entirely to a loss of body water (hypohydration) (Shirreffs *et al*., 2004b; Higgins *et al*., 2007; Bosco *et al*., 1974), ER-induced BML is likely attributable to a number of mechanisms, namely: hypohydration (Oliver *et al*., 2007; Consolazio *et al*., 1968a); glycogen depletion (Nilsson and Hultman, 1973); reductions in body fat and muscle stores (Runcie and Hilditch, 1974); continued faecal losses, which due to the large reduction in food weight would only be partially replaced. Comparison of the FR and F+ER trials gives an indication of the mass loss during energy restriction attributable to mechanisms other than hypohydration. Urine, as well as mass losses via other avenues (cutaneous and respiratory fluid loss, and faecal fluid and solid loss) were not different between FR and F+ER, suggesting the 0.38 (0.30) kg difference in BML between these trials might be attributable to the 0.41 (0.07) kg reduction in the weight of the dietary intake during F+ER, and the 249 (43) g, 63 (12) g and 50 (7) g

reduction in carbohydrate, fat and protein intake, presumably leading to the utilisation and loss of endogenous energy stores (glycogen and fat).

Electrolyte (sodium, potassium and chloride) balance was calculated from estimated dietary intake and urinary excretion. Sodium, potassium and chloride losses in sweat and faeces over the 24 h period were not measured and therefore not included in the calculation of electrolyte balance. Consistent with other investigations comparing urinary electrolyte excretion and dietary intake (Pietinen, 1982), and reflecting the exclusion of electrolyte losses in sweat and faeces, 24 h electrolyte balances were all calculated to be slightly net positive during C, although not significantly different from 0 h. At 24 h during FR, both sodium and potassium balance was not different from 0 h, but chloride balance was significantly positive (Table 3.5). Although not measured in this study, this might be explained by an increased excretion of other anions in the urine to maintain electrolyte neutrality within the body. Indeed, if chloride was the only anion excreted together with sodium and potassium one would expect chloride balance to reflect the combined balance of sodium and potassium, which it does not for any of the trials. As reported previously during long term ER (Rapoport *et al*., 1965; Consolazio *et al*., 1968b; Runcie and Thompson, 1970; Runcie, 1971), 24 h ER or F+ER resulted in a significant negative balance of sodium, potassium and chloride. It appears that electrolyte balance during 24 h FR remains relatively neutral, indicating that whilst individuals might be hypohydrated, they do not appear to be electrolyte depleted, which is in contrast to exercise or heat induced hypohydration, where a significant negative balance of sodium and chloride develops (Shirreffs and Maughan, 1998; Shirreffs *et al*., 2007).

As the major electrolyte in the extracellular space, a loss of sodium from the body might be expected to result in a loss of water from the extracellular space. In line with this, plasma volume decreases during the first 2 weeks of a complete fast and is accompanied by a large excretion of sodium in the urine (Rapoport *et al*., 1965). After this point, no further decrease in plasma volume is observed and urinary sodium excretion is dramatically reduced to 1-15 $mmol \cdot d^{-1}$ (Rapoport *et al*., 1965; Weinsier, 1971). It seems likely that the reduction in plasma volume during short term ER observed in the present study and previously (Oliver *et al*., 2007; Bosco *et al*., 1974; Consolazio *et al*., 1968a) is caused by the continued excretion of sodium, combined

with virtually no intake. It is therefore logical that as the major electrolyte in the intracellular space, a loss of potassium during ER might represent a reduction in intracellular fluid volume. The urinary excretion of potassium remains high over the first 2 weeks of complete energy restriction (Rapoport *et al*., 1965; Runcie and Thompson, 1970; Runcie, 1971), after which, excretion is greatly reduced to ~10-15 $mmol \cdot d^{-1}$ for the remainder period of complete energy restriction (Weinsier, 1971). The continued excretion of potassium during prolonged complete energy restriction can be accounted for by losses of lean tissue, releasing potassium from the intracellular space (Weinsier, 1970). The larger loss of potassium during the early stages of ER, as observed in the present study is unlikely to be due to a loss of lean tissue. The ER imposed on the subjects caused the restriction of carbohydrate to 86 (17) g and whilst it is unlikely that muscle glycogen content was decreased, as subjects refrained from physical activity during trials (Maughan and Williams, 1981), it is likely liver glycogen was reduced (Nilsson and Hultman, 1973). Glycogen binds potassium in a ratio of 0.45 mmol potassium to 1 g glycogen (Patrick, 1977). A large reduction in liver glycogen might be expected to release a significant amount of potassium for excretion. Exercise induced dehydration results in losses of water from the intra and extracellular compartments (Costill *et al*., 1976). It seems likely that water will also be lost from the intracellular compartment during energy restriction and that this water might be expected to be accompanied by losses of potassium so as to maintain the osmotic equilibrium within the body.

Consolazio *et al*. (1968b) reported that compared to no supplementation, supplementation with 3.93 $g \cdot d^{-1}$ sodium (171 mmol) and 2.41 $g \cdot d^{-1}$ potassium (62 mmol) resulted in a positive sodium balance and a less negative potassium balance over a 10 day period, with energy intake restricted to 420 $kcal \cdot d^{-1}$ (1760 $kcal \cdot d^{-1}$) Additionally, this electrolyte supplementation resulted in a less negative water balance and a reduction in BML over the 10 day period (Consolazio *et al*., 1968a). The large reduction in body mass observed presently during ER might therefore be at least partially attributable to the concomitant restriction of electrolytes (in particular sodium and potassium) that occurs when food intake is dramatically reduced. It has been shown that during rehydration after a period of exercise-induced dehydration that the consumption of plain water, without the addition of sodium (Nose *et al*., 1988a; Maughan and Leiper, 1995; Shirreffs *et al*., 1998) or accompanying sodium-

containing foods (Maughan *et al*., 1996; Ray *et al*., 1998) results in a large dieresis. The consumption of large volumes of plain water combined with the restricted electrolyte intake during the ER trial, likely augmented the observed increase in urine production, leading to the reduction in plasma volume and body mass observed.

The negative electrolyte balance observed in the present study after 24 h ER or F+ER is relevant to individuals competing in weight categorised sports who regularly use ER (with or without FR) to rapidly reduce body mass in the days leading up to the competition (Smith, 2006; Oppliger *et al*., 2003; Slater *et al*., 2005; Moore *et al*., 2002). Recovery from a period of exercise and/ or heat induced dehydration has been well documented. Sweating makes the largest contribution to BML during exercise and/ or heat induced dehydration, with sweat containing greater concentrations of sodium (20-80 $mmol \cdot l^{-1}$) than potassium (4-8 $mmol \cdot l^{-1}$) (Shirreffs and Maughan, 1997). Consequently, a large loss of sweat, without electrolyte replacement results in a large negative balance of sodium, but only a small negative balance of potassium (Shirreffs and Maughan, 1998; Shirreffs *et al*., 2007). The addition of sodium to rehydration beverages has been shown to improve the retention of the ingested solution when consumed after exercise induced dehydration (Nose *et al*., 1988a; Maughan and Leiper, 1995; Shirreffs and Maughan, 1998; Merson *et al*., 2008). Whilst the addition of potassium to rehydration beverages appears to improve intracellular rehydration (Yawata, 1990; Maughan *et al*., 1994), its effect on whole body net fluid balance is equivocal (Maughan *et al*., 1994; Shirreffs *et al*., 2007). During 24 h ER or F+ER large negative balances of sodium and potassium accumulate and thus replacement of both these electrolytes might be required for the full and rapid recovery of fluid balance after weigh-in in athletes that have severely restricted energy intake prior to the weigh-in.

The assessment of hydration status and deviation from a euhydrated state has recently received much interest among the scientific community (see Shirreffs, 2003 for review). Periods of fluid and/ or energy restriction reduce body water and result in hypohydration (Oliver *et al*., 2007; Bosco *et al*., 1974; Consolazio *et al*., 1968a), but the assessment of the degree of hypohydration is difficult given the various possible causes of BML during energy restriction. Whilst some markers of hydration status have been assessed during FR (with or without ER) (Oliver *et al*., 2007; Oliver *et al*.,

2008), markers to assess hydration status during energy restriction have received little attention. From the results of the present study it is clear that the assessment of hydration status is problematic during periods of energy (with or without fluid) restriction. With the exception of urine osmolality during F+ER all urinary and haematological markers of hydration status showed little change from baseline values. This observation appears to extend to 48 h periods, with 48 h fluid and energy restriction resulting in an increase in urine osmolality (Oliver *et al*., 2007), but no change in plasma osmolality (Oliver *et al*., 2008) and 48 h energy restriction resulting in no change in urine osmolality (Oliver *et al*., 2007), when compared to euhydrated baseline values. However, the change in body mass and estimated change in plasma volume appear to respond well to changes in body water during 24-48 h periods energy or fluid and energy restriction.

In conclusion, 24 h fluid and/ or energy restriction results in a significant reduction in body mass and plasma volume, suggesting that all these restriction treatments resulted in a significant hypohydrated state by 24 h. The type of hypohydration incurred varied depending on the restriction imposed, with 24 h FR resulting in hypertonic hypohydration and 24 h ER resulting in isotonic hypohydration. Over 24 h ER and F+ER, a large negative balance of the electrolytes sodium, potassium and chloride developed, which was not apparent with FR. Replacement of these electrolytes lost might be vitally important for the rapid recovery of fluid balance and exercise performance after a period of restricted energy intake (with or without fluid restriction) and is of particular importance to weight categorised athletes.

Chapter 4

Restoration of fluid balance after 24 h severe fluid and energy restriction: effect of electrolyte addition to ingested fluids

Abstract

This study examined the effect of sodium chloride and potassium chloride addition to rehydration drinks ingested after 24 h fluid and energy restriction. Twelve subjects (six male, six female) completed three 30 h trials, consisting of 24 h fluid and energy restriction, during which they received food providing 21 $kJ \cdot kg^{-1}$ body mass and a volume of water equal to 5 $ml \cdot kg^{-1}$ body mass. Body mass loss (BML) during the 24 h fluid and energy restriction period was determined and subjects then ingested a volume of drink equivalent to 125% of BML in 6 equal aliquots over a 2 h rehydration period. The drinks ingested were a high sodium drink (Na), a high potassium drink (K) or a placebo drink (P) and subjects remained in the lab and collected all urine produced for the next 4 h. Ingestion of the Na drink resulted in a more positive sodium balance after drinking compared to P or K ($P < 0.001$), whilst ingestion of the K drink resulted in a more positive potassium balance after drinking compared to P or Na ($P < 0.001$). The cumulative volume of urine produced was lower for during trial Na than during trial P from 2 h after drinking onwards ($P < 0.05$) and at 4 h total urine volume was 1627 (540) ml (P), 1391 (388) ml (K) and 1150 (438) ml (Na). This meant that 33 (14-69) %, 22 (0-57) % and 6 (-10-64) % of drinks Na, K and P had been retained, respectively, with a greater drink retention for drink Na than drink P ($P < 0.05$). These results demonstrate that after 24 h fluid and energy restriction, that the ingestion of a sodium containing drink results in an increased sodium balance that augments greater drink retention compared to an electrolyte free drink. In contrast, whilst a potassium containing drink results in an increase in potassium balance, there was no increase in drink retention.

Introduction

The voluntary restriction of fluid and energy intake in the days leading up to the official weigh-in is common place among athletes competing in weight category sports (Tipton and Tcheng, 1970, Steen and Brownell, 1990, Moore *et al*., 2002, Oppliger *et al*., 2003, Slater *et al*., 2005, Smith, 2006). Although it is worth noting that all of these studies report a high prevalence of other rapid weight loss techniques, including increased exercise (particularly in a hot environment or wearing a rubber sweat suit), sauna use, as well as the use of laxatives and diuretics.

Whilst the restoration of fluid balance after periods of exercise and/ or heat induced hypohydration has been extensively examined (for review see Shirreffs *et al*., 2004a), the recovery of fluid balance after a period of combined fluid and energy restriction has not been investigated. Following exercise and/ or heat induced dehydration it has been shown that the volume of fluid ingested must be in excess of the volume lost, to allow for continued fluid losses in the post-exercise period (Shirreffs *et al*., 1996), and that the replacement of the sodium lost in sweat is necessary for the restoration of fluid balance (Nose *et al*., 1988a; Maughan and Leiper, 1995; Shirreffs and Maughan, 1998).

The addition of potassium to rehydration drinks ingested after exercise-induced dehydration might aid rehydration by increasing water retention in the intracellular space (Yawata, 1990; Maughan *et al*., 1994), but whilst Maughan *et al*. (1994) observed increased drink retention with a potassium containing drink (25 $mmol \cdot l^{-1}$) compared to an electrolyte free drink, consumed in volumes equivalent to the BML, Shirreffs *et al*. (2007) observed no difference in drink retention between a high potassium drink and water when the volume consumed was equal to 1.5 times the BML. The difference in the volume of drink consumed may account for the difference in findings between these two studies.

In contrast, little is known about the recovery of fluid balance after a period of fluid and energy restriction. Whilst it has been shown that fluid intake after weigh in is critical to subsequent exercise performance (Slater *et al*., 2007), the factors influencing the restoration of fluid balance after rapid weight loss have not been

systematically examined. It has been shown that periods of combined fluid and energy restriction result in a large reduction in body mass and plasma volume (Bosco *et al*., 1974; Oliver *et al*., 2007; Chapter 3), but an attenuated increase in plasma osmolality compared to fluid restriction (Oliver *et al*., 2008; Chapter 3). The isotonic hypohydration observed following combined fluid and energy restriction (Oliver *et al*., 2008; Chapter 3) might be explained by the continued loss of the electrolytes sodium, potassium and chloride in urine, despite little or no intake. Whilst exercise-induced dehydration is primarily a result of sweat loss, which contains large amounts of sodium and chloride (Shirreffs and Maughan, 1997), combined fluid and energy restriction results in losses of sodium, potassium and chloride (Chapter 3). Given the relative importance of sodium and potassium in the extracellular and intracellular water compartments of the human body, replacement of both sodium and potassium lost during severe fluid and energy restriction might be important for a rapid recovery of fluid balance.

Therefore, the purpose of this investigation was to investigate whether the addition of sodium chloride or potassium chloride to a rehydration drink consumed after 24 h fluid and energy restriction enhanced drink retention or fluid balance compared to the consumption of a placebo drink.

It was hypothesised that all addition of both sodium chloride and potassium chloride to the rehydration solutions would enhance fluid balance after drinking compared to the placebo drink.

Methods

This investigation was approved by the Loughborough University Ethical Advisory Committee (Reference No. R07-95) and 12 healthy subjects (6 male, 6 female) volunteered to participate. The subjects physical characteristics were age 24 (4) y, body mass 70.62 (15.08) kg and height 1.72 (0.13) m. Each subject completed a familiarisation trial followed by three experimental trials, with each experimental trial consisting of a 24 h period of dietary restriction (5 ml water·kg^{-1} body mass, 21 kJ·kg^{-1} body mass) (DR), followed by a 2 h rehydration period and a 4 h monitoring period. During the rehydration period subjects received one of three drinks (Table 4.1), either

a high sodium drink (Na), a high potassium drink (K) or a placebo drink (P). All drinks contained a small amount of lemon squash to help mask the contents and subjects were blinded to the trial order. Trials were separated by a minimum of 6 days and were administered in randomised counterbalanced order. Trials were undertaken during the months of January, February, March and April in Loughborough, UK, with 24 h mean daily temperatures of 7.2 (3.6) °C.

The familiarisation trial was completed after at least a 3 hour fast and was used to familiarise subjects with the rehydration and urine collection protocols involved. Subjects arrived at the laboratory and provided a urine sample (0 h), after which their nude body mass was measured. They then drank a volume of lemon flavoured squash equivalent to 3% of their body mass in 6 equal aliquots every 20 minutes. 3% was selected as it was estimated that this would be the approximate amount of fluid they would be required to drink during the experimental trials. Additional urine samples were obtained at 1 h and 2 h (i.e. halfway through and at the end of drinking), with a final urine sample collected 1 hour later (3 h).

Table 4.1 Rehydration drink osmolality (mosmol·kg^{-1}) and electrolyte content (mmol·l^{-1}).

	Drink P	Drink Na	Drink K
Osmolality (mosmol·kg^{-1})	28 (3)	125 (5)	83 (2)
Sodium (mmol·l^{-1})	5 (1)	57 (3)	5 (1)
Potassium (mmol·l^{-1})	1 (1)	1 (1)	32 (1)
Chloride (mmol·l^{-1})	1 (1)	53 (3)	31 (1)

For the three experimental trials subjects arrived at the laboratory in the morning (-26 h) after a 12 h overnight fast, with the exception of 500 ml plain water consumed approximately 2 h before arrival. Upon arrival at the laboratory, subjects voided their

bladders, collecting the entire volume. Subject's nude body mass was then measured, before were then provided with their food (American hard gums; Tesco, UK) (21 $kJ \cdot kg^{-1}$ body mass) and tap water (5 $ml \cdot kg^{-1}$ body mass) for the day before being free to leave the laboratory. Subjects were instructed to consume only the food and water provided, which had to be consumed before going to bed that day. The amount of food and volume of water was based on subject's body mass measured during the familiarisation trial, with 1523 (322) kJ, 7 (1) g protein, 84 (18) g carbohydrate, 0 (0) g fat and 366 (79) ml water provided for each 24 h period of DR. Additionally, subjects were provided with 24 h urine collection equipment and were instructed to collect all urine produced over the 24 h period and were asked to refrain from any physical activity. Subjects then returned to the laboratory the following morning (-2 h), exactly 24 h after the initial visit. Upon arrival, subjects again provided a urine sample and their nude body mass was measured. The change in body mass over the 24 h DR period was then used to calculate the volume of drink ingested during the rehydration period. The total volume of drink ingested (l) was calculated as 125% of the BML (kg) was consumed in 6 equal aliquots every 20 minutes, so that the rehydration period lasted a total of 2 h. Subjects then remained in the laboratory and sat quietly for a further 4 hour (monitoring period). At hourly intervals throughout the rehydration and monitoring periods (-1 h, 0 h, 1 h, 2 h, 3 h and 4 h), urine samples were provided. Additional questions related to the sensory characteristics of the drinks were included at the end of the rehydration period (2 h) (Appendix B). Urine samples were taken as described in the general methods section of this thesis.

The urine produced over the 24 h DR was weighed to the nearest g and volume was determined from the urine sample's specific gravity, which was determined from its osmolality using the equation in Appendix A. A sample of each urine sample (~5 ml) produced whilst in the laboratory was also retained. A sample (~5 ml) of each drink used was also retained for analysis. Urine and drink samples were analysed for sodium, potassium and chloride concentration, as well as osmolality.

Statistical analysis

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

Results

With the exception of nude body mass ($P < 0.001$), and after correction of electrolyte excretion for body mass, there were no differences between males and females ($P > 0.300$) for any of the other measured variables during DR and given that the menstrual cycle has been shown to not effect rehydration (Maughan *et al*., 1994) data for both males and females are combined. 24 h urinary creatinine excretion was not different between trials ($P = 0.960$) and over all trials was 0.15 (0.04) mmol·kg^{-1} body mass·24 h^{-1}, which is indicative of a complete 24 h urine collection (Bingham and Cummings, 1985).

Pre-trial measurements

Pre-trial body mass (p = 0.421) and urine osmolality (p = 0.500) were not different between trials, indicating subjects begin each trial in a similar state of hydration.

DR period

BML (kg), urine volume, as well as sodium, potassium and chloride excretion over the 24 h DR (Table 4.2) were not different between trials ($P > 0.373$). Over all trials the BML was 1.49 (0.34) kg, which equates to a percentage BML of 2.13 (0.48) % in this group of subjects. Over all trials the total urine volume, sodium, potassium and chloride excretion over the 24 h DR were 821 (226) ml, 69 (27) mmol, 49 (24) mmol and 84 (32) mmol respectively. As BML was not different between trials, neither was the volume of drink ingested during the rehydration period and totalled 1863 (356) ml (P), 1810 (329) ml (K) and 1865 (368) ml (Na).

Table 4.2 Body mass loss (BML) (kg), urine volume (ml), as well as urinary sodium (mmol), potassium (mmol) and chloride (mmol) excretion over the 24 h DR period. Values are mean (SD)

	P	Na	K	P value
BML (kg)	1.48 (0.29)	1.45 (0.26)	1.49 (0.45)	0.861
Urine volume (ml)	848 (219)	823 (241)	794 (234)	0.743
Urine sodium (mmol)	67 (25)	74 (33)	62 (21)	0.465
Urine potassium (mmol)	53 (26)	49 (25)	43 (20)	0.360
Urine chloride (mmol)	90 (22)	82 (40)	76 (28)	0.364

Urine osmolality, cumulative urine output and drink retention

Urine osmolality (Figure 4.1) was increased at -2 h compared to -26 h values on all trials ($P < 0.001$) and at this time was 879 (555-1053) mosmol·kg^{-1} over all trials. Additionally, compared to -26 h, urine osmolality was decreased during P at 0 h and 1 h ($P < 0.001$), and tended to be decreased at 2 h ($P = 0.07$). During trial K, urine osmolality was decreased at 0 h and 1 h ($P < 0.05$) compared to -26 h, and during trial Na was decreased 0 h ($P < 0.01$) and increased at 4 h ($P < 0.05$), with a tendency to also be increased at 3 h ($P = 0.063$). Compared to trial P, urine osmolality was greater during trial K at 0 h, 1 h, 2 h and 3 h ($P < 0.05$) and during trial Na at 1 h, 2 h, 3 h and 4 h ($P < 0.05$), with no differences between trials Na and K.

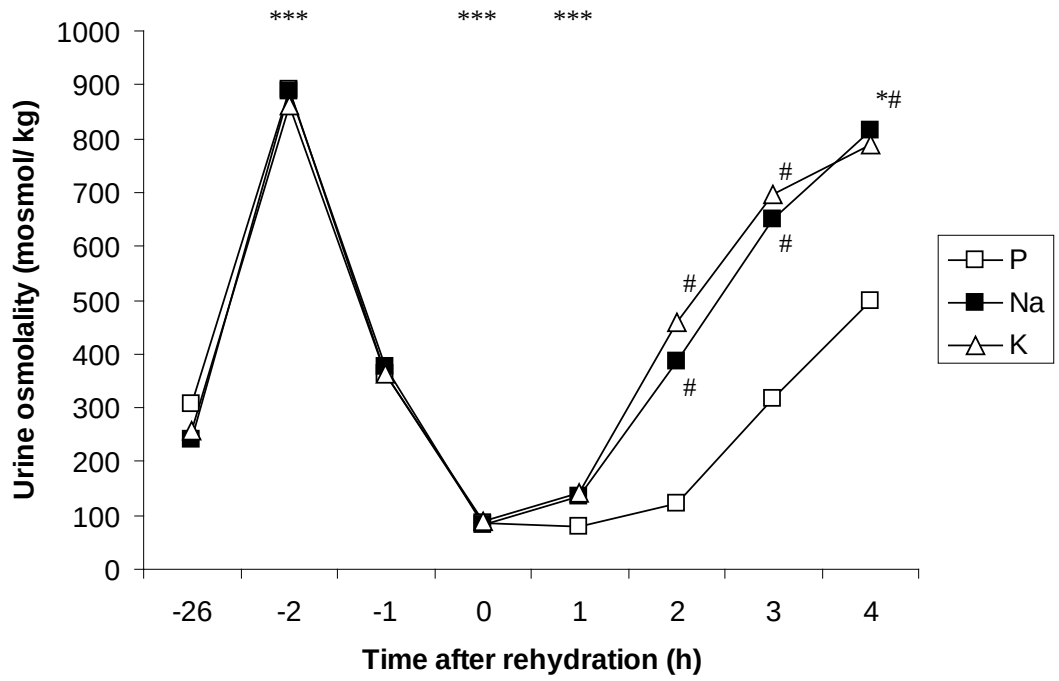

Figure 4.1. Urine osmolality (mosmol·kg^{-1}). *** All trials significantly different from 0 h. * Significantly different from 0 h. # Significantly different from trial P. Points are median values.

Total cumulative urine output over the rehydration and monitoring periods (Figure 4.2) was 1627 (540) ml (P), 1391 (388) ml (K) and 1150 (438) ml (Na) and from 2 h was greater for trial P than trial Na ($P < 0.05$). In line with this, the fraction of the ingested drink retained (Figure 4.3) showed the opposite trend and at the end of the monitoring period was 33 (14-69) % (Na), 22 (0-57) % (K) and 6 (-10-64) % (P). Compared to trial P, the fraction of the ingested drink retained was greater from 2 h onwards during trial Na ($P < 0.05$). Cumulative urine output and the fraction of the ingested drink retained were not different between trial Na and trial K ($P > 0.082$) or between trial K and trial P ($P > 0.539$).

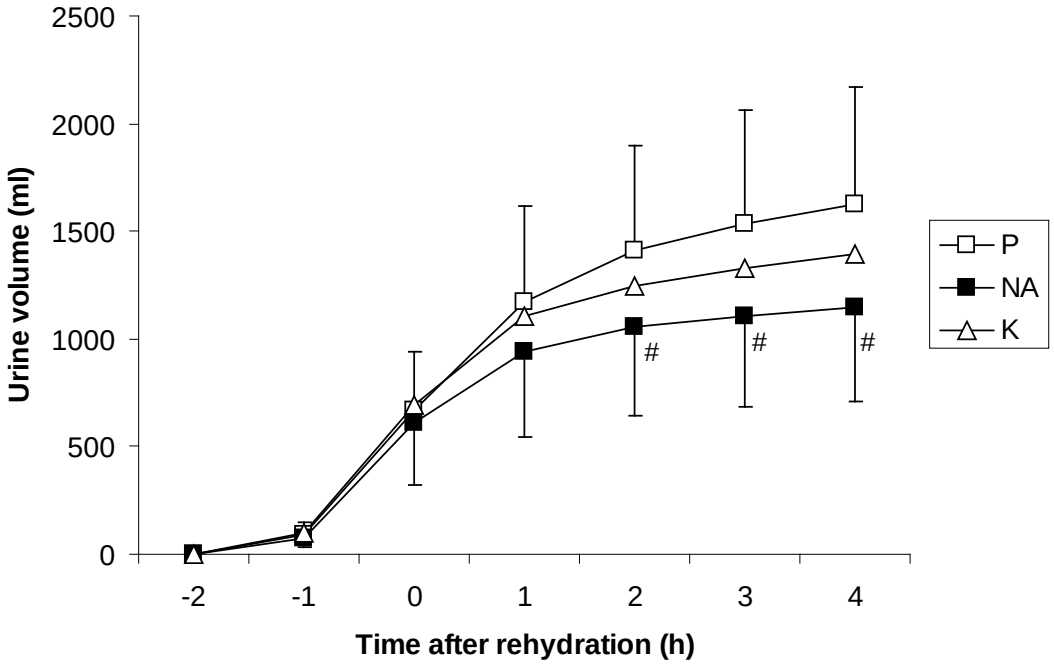

Figure 4.2. Cumulative urine output (ml). # Significantly different from trial P. Points are mean values. Error bars are SD.

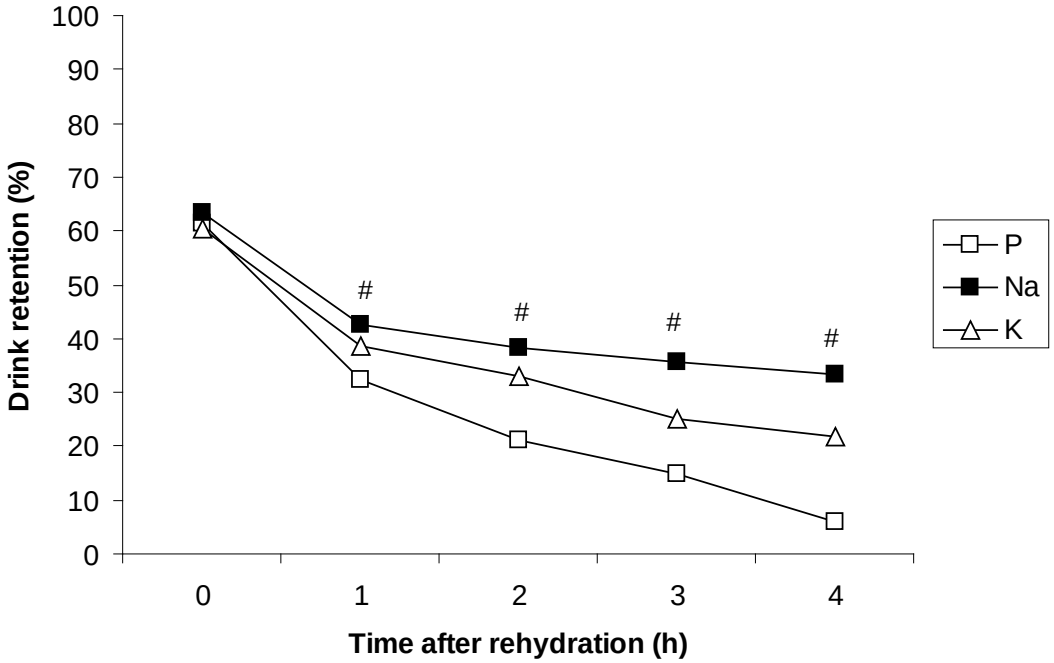

Figure 4.3. Fraction of the ingested drink retained (%).# Significantly different from trial P. Points are median values.

Electrolyte excretion and balance

Whilst sodium ingestion during the rehydration period was greater during trial Na (102 (17) mmol), compared to trials P (10 (2) mmol) and K (10 (3) mmol) (P < 0.001), cumulative sodium excretion (Table 4.3a) over the rehydration and monitoring periods was not different between trials (p = 0.085). In contrast, potassium consumption during rehydration was greatest during trial K (59 (13) mmol) (P < 0.001), with virtually no intake during trial P (1 (1) mmol) and trial Na (1 (1) mmol), with cumulative potassium excretion (Table 4.3b) also greatest during trial K (P < 0.001). This meant that during trial Na, 62 (19) % of the ingested sodium was retained, whilst during trial K 31 (19) % of the ingested potassium was retained. Total cumulative chloride excretion (Table 4.3c) was not different between trials (P > 0.243), but was greater during trial K than trial P at 0 h and 2 h (P < 0.05), with a tendency to be greater at 1 h (P = 0.060).

Table 4.3 Cumulative urinary sodium (mmol) (a), potassium (mmol) (b) and chloride (mmol) (c) excretion (mmol) over the rehydration and monitoring periods. # Significantly different from trial P. † Significantly different from trial Na. Values are median (range).

a) Sodium

Time (h)	P	Na	K
-2	0 (0-0)	0 (0-0)	0 (0-0)
-1	3 (0-10)	2 (0-6)	4 (1-6)
0	7 (1-22)	9 (1-21)	16 (5-24)
1	11 (2-34)	17 (2-34)	24 (8-36)
2	15 (4-41)	24 (6-47)	29 (8-47)
3	21 (5-54)	28 (8-64)	31 (9-55)
4	30 (7-72)	33 (10-78)	35 (11-62)

b) Potassium

-2	0 (0-0)	0 (0-0)	0 (0-0)
-1	2 (1-6)	2 (1-10)	3 (1-11)
0	6 (2-12)	5 (1-18)	12 (6-30) #†
1	11 (4-19)	9 (1-24)	23 (9-52) #†
2	13 (4-28)	13 (2-29)	31 (11-66) #†
3	14 (5-35)	16 (2-35)	37 (14-74) #†
4	16 (5-48)	19 (3-39)	40 (16-79) #†

c) Chloride

-2	0 (0-0)	0 (0-0)	0 (0-0)
-1	4 (1-12)	3 (0-10)	4 (2-12)
0	10 (1-24)	14 (2-28)	22 (8-32) #
1	16 (1-41)	24 (5-56)	40 (17-50)
2	20 (8-50)	31 (10-65)	48 (20-67) #
3	25 (12-62)	37 (14-75)	54 (24-77)
4	33 (13-90)	44 (17-88)	60 (28-85)

Sodium, potassium and chloride balances were calculated from losses in urine over the whole trial period and gains from food consumed during DR and drinks ingested during rehydration. Sodium balance (Figure 4.4) was net negative at all time points after DR during trials P and K. During trial Na, 102 (17) mmol of sodium was ingested during the rehydration period, which was in excess of the 74 (34) mmol deficit observed following DR. This meant that by the end of the rehydration period (0 h) sodium balance was net positive and from -1 h until the end of trial Na was not different from -26 h. Sodium balance was also more positive for trial Na than trials P and K from -1 h onwards. Potassium balance (Figure 4.5) at the end of DR was negative during all trials ($P < 0.001$), remaining so during trials P and Na ($P < 0.001$). During trial K, the ingestion of 59 (13) mmol potassium during the rehydration period was greater than the 44 (20) mmol deficit observed at -2 h and at the end of the rehydration period (0 h) subjects were in net positive potassium balance (1 (20) mmol), but by 3 h had become significantly negative ($P < 0.05$). Potassium balance was also more positive for trial K than trials P and Na from -1 h onwards. Chloride balance (Figure 4.6) was net negative at all time points after DR during all trials. Although, whilst during trials P and K this negative chloride balance was significant at all time points ($P < 0.001$), it was only significant during trial Na at -2 h and -1 h. Chloride balance was greater at -1 h during the Na and K trials compared to the P trial ($P < 0.001$) and all trials were different from each other from 0 h onwards ($P < 0.05$).

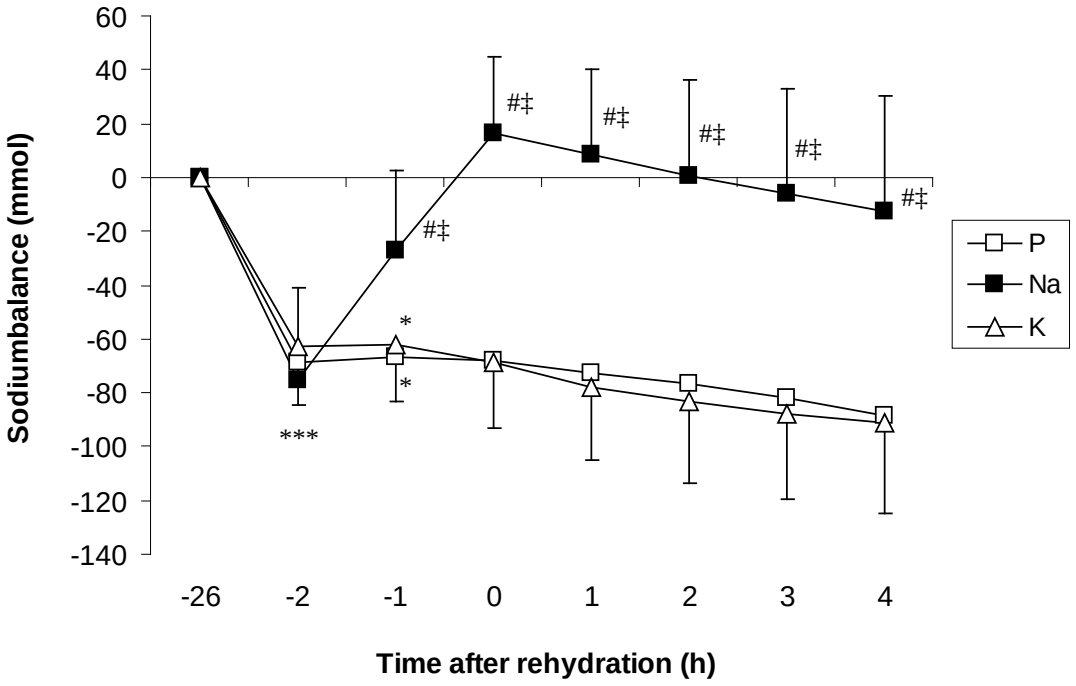

Figure 4.4. Sodium balance (mmol). *** All trials significantly different from 0 h. * Significantly different from 0 h. # Significantly different from trial P. ‡ Significantly different from trial K. Points are mean values. Error bars are SD.

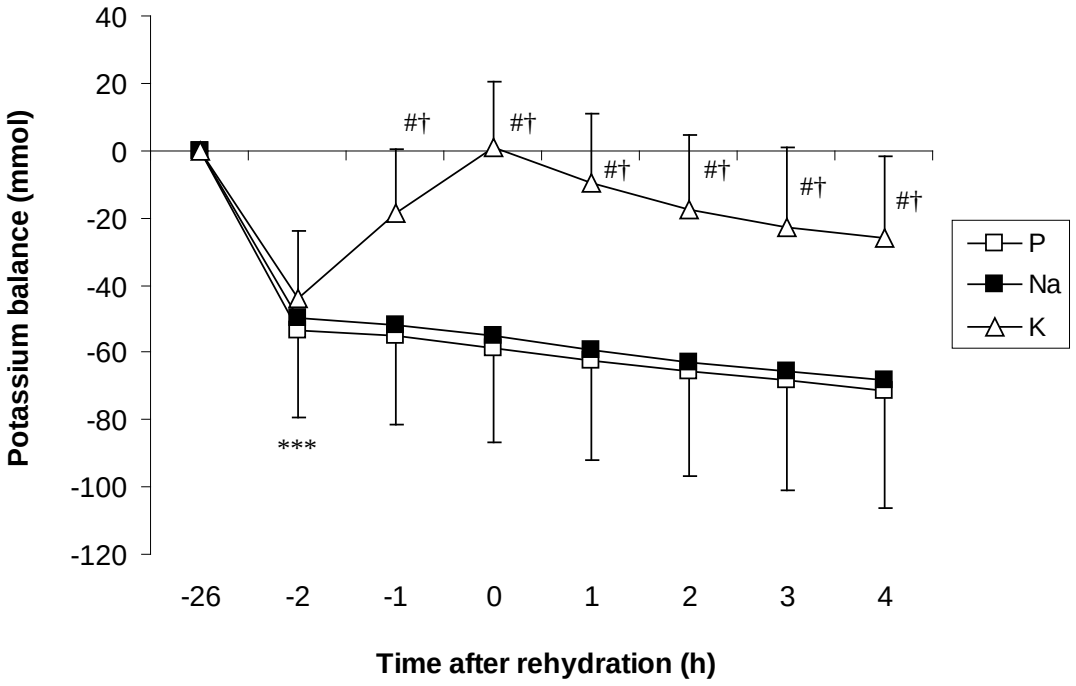

Figure 4.5. Potassium balance (mmol). *** All trials significantly different from 0 h. * Significantly different from 0 h. # Significantly different from trial P. † Significantly different from trial Na. Points are mean values. Error bars are SD.

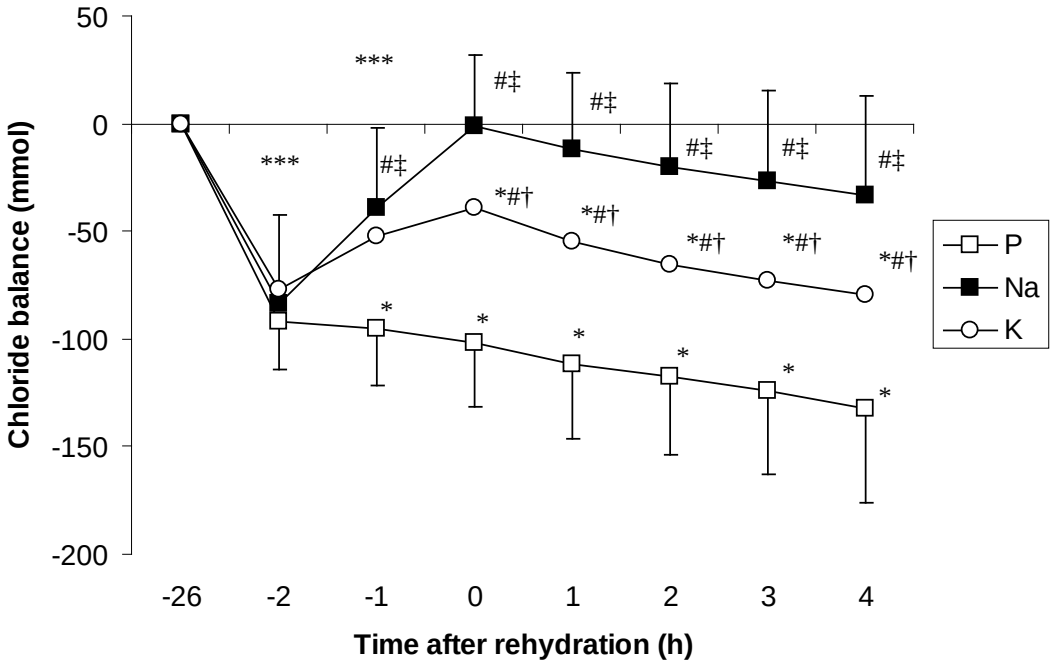

Figure 4.6. Chloride balance (mmol). *** All trials significantly different from 0 h. * Significantly different from 0 h. # Significantly different from trial P. † Significantly different from trial Na. ‡ Significantly different from trial K. Points are mean values. Error bars are SD.

Discussion

To our knowledge this is the first study to investigate the effectiveness of different composition rehydration drinks ingested after a period of fluid and energy restriction, although post-exercise rehydration has been extensively examined (for review see Shirreffs *et al.*, 2004a). The results of this study suggest that following a 24 h period of severe fluid and energy restriction, ingestion of a sodium-containing drink (57 (3) $mmol \cdot l^{-1}$ Na, 1 (1) $mmol \cdot l^{-1}$ K) resulted in a reduced urine output and consequently a greater retention of the ingested drink compared to a placebo drink (5 (1) $mmol \cdot l^{-1}$ Na, 1 (1) $mmol \cdot l^{-1}$ K), whilst a potassium-containing drink (5 (1) $mmol \cdot l^{-1}$ Na, 32 (1) $mmol \cdot l^{-1}$ K) was not different from either the sodium-containing drink or placebo drink in terms of cumulative urine output or drink retention.

Over all trials the 24 h period of DR resulted in losses of the electrolytes sodium, potassium and chloride of 68 (27) mmol, 48 (23) mmol and 83 (31) mmol respectively. The continued loss of these electrolytes in the urine during DR despite

very little dietary intake (1 (0) mmol sodium, (0 (0) mmol potassium and 0 (0) mmol chloride) accounts for the negative balance and has been reported previously (Consolazio *et al*., 1968a; Runchie 1971). Oliver *et al*. (2007) recently reported that following a 48 h period of severe fluid and energy restriction, body mass was reduced 3.6 (0.3) % and plasma volume was estimated to have decreased by 5.1 (4.5) %. Oliver *et al*. (2008) demonstrated that the same 48 h period of fluid and energy restriction resulted no change in plasma osmolality compared to pre-restriction, findings that are supported by the results of the study reported in Chapter 3 of this thesis. It is therefore likely in the present study that at the end of the 24 h DR, subjects were hypohydrated and hypovolaemic, but isosmotic compared to before DR. This is a situation that is not encountered following exercise-induced dehydration, which results in hypovolemic, hyperosmotic hypohydration (Maughan and Leiper, 1995; Shirreffs and Maughan, 1998). It is logical that the sodium lost during the DR period is lost from the extracellular space and that the continued loss of sodium (and chloride) during a period of severe fluid and energy restriction accounts for the lack of an increase in serum osmolality reported previously (Oliver *et al*., 2008).

During trial Na, 102 (17) mmol sodium was consumed during rehydration compared to the 10 (2) mmol and 10 (3) mmol consumed during trials P and K. Despite this large difference in sodium ingestion, cumulative sodium excretion was not different between trials (Table 5.3a) and therefore sodium balance was greater during trial Na and was net neutral from -1 h onwards. As observed following exercise-induced dehydration (Maughan and Leiper, 1995; Shirreffs and Maughan, 1998; Merson *et al*., 2008), the addition of sodium to a rehydration drink, ingested after 24 h fluid and energy restriction resulted a reduction in urine output and consequently a greater drink retention compared to a low sodium drink. The inclusion of sodium in a rehydration beverage reduces the haemodilution that occurs with the ingestion of a large volume of sodium free fluid (Nose *et al*., 1988a). This haemodilution results in a rapid reduction in plasma sodium concentration and osmolality (Nose *et al*., 1988a), leading to reductions in plasma renin activity and plasma aldosterone concentration, resulting in a large diuresis (Nose *et al*., 1988b). Despite the low sodium content of drink K (5 (1) mmol) there was no significant difference in terms of drink retention or urine output in comparison to drink Na.

During trial K, 59 (13) mmol potassium was consumed during rehydration, compared to the 1 (1) mmol and 1(1) mmol consumed during trials P and Na. This difference in potassium intake resulted in an increased excretion of potassium in urine during trial K compared to both trial P and Na (Table 5.3b), although potassium balance was significantly greater during trial K compared to the other two trials, only becoming net negative 3 h after rehydration. Although mean cumulative urine output was lower during trial K than trial P, and greater than during trial Na throughout the trials, it was not significantly different at any time during trial K compared to the other two trials. Similarly, median drink retention was greater during trial K than trial P, and lower than during trial Na throughout the trials, there were no significant difference between in drink retention during trial K compared to the other two trials.

Exercise induced hypohydration only results in a small loss of potassium in sweat (4-8 $mmol \cdot l^{-1}$ (Shirreffs and Maughan, 1997)), such that a 70 kg individual dehydrated by 2% of their body mass via sweat production would lose approximately 6-11 mmol of potassium via sweat production. In contrast, the 24 h period of fluid and energy restriction employed in the present study resulted in a much larger negative balance of potassium (-49 (24) mmol). By the end of trial K, potassium balance was 45 (33) mmol and 42 (23) mmol less negative compared to trials P and Na respectively. The extra potassium retained in trial K was likely retained in the intracellular space and it is logical to speculate that water would be drawn into the intracellular space to maintain the osmotic equilibrium, although this increased retention of potassium resulted in no significant change in urine output or drink retention compared to the placebo drink.

The observed negative balance of potassium following 24 h fluid and energy restriction in the present study might be explained by two potential mechanisms. Firstly, the utilisation and loss of liver glycogen stores during energy restriction would result in the liberation of associated potassium, with approximately 0.45 mmol of potassium liberated for every gram of glycogen lost (Patrick, 1977). Or, secondly, as the major cation in the intracellular space, any loss of water stores from the intracellular fluid might result in a concomitant loss of potassium. The addition of potassium to rehydration drinks has previously been suggested to increase fluid retention by aiding rehydration of the intracellular space (Shirreffs *et al.*, 2004a).

Yawata (1990) dehydrated rats by approximately 9% of body mass via heat exposure (36°C) and observed that compared to animals that ingested a NaCl drink, those that ingested a KCl drink showed a tendency for increased restoration of the intracellular fluid. In men hypohydrated by approximately 2% of body mass by exercise in the heat, Maughan *et al*. (1994) observed better restoration of fluid balance following ingestion of a 25 $mmol \cdot l^{-1}$ KCl drink in comparison to an electrolyte free 90 $mmol \cdot l^{-1}$ glucose solution, but no difference between the KCl drink and a 60 $mmol \cdot l^{-1}$ NaCl drink or a drink containing all three ingredients (90 $mmol \cdot l^{-1}$ glucose, 60 $mmol \cdot l^{-1}$ NaCl, 25 $mmol \cdot l^{-1}$ KCl). All drinks were consumed in a volume equivalent to the BML and whilst there were no differences between electrolyte containing drinks in terms of net fluid balance, the restoration of plasma volume was slowest during the KCl trial, indicating that the intracellular space might have been preferentially restored following ingestion of the KCl drink. More recently, Shirreffs *et al*. (2007) reported that following dehydration by approximately 2% body mass, a potassium containing drink (Apfelschorle 30 (1) $mmol \cdot l^{-1}$) showed a slower recovery of plasma volume, but did not result in any benefit to whole body fluid balance compared to mineral water. Drinks were ingested in a volume equal to 150% of the BML (Shirreffs *et al*., 2007) compared to the 100% used previously by Maughan *et al*. (1994).

In the present investigation, drink Na was best retained, but its retention (37 (14-69)%) was much lower than that reported for a drink of similar sodium concentration ingested in a volume equal to 150% of the BML after exercise-induced dehydration by 1.9% body mass (Maughan and Leiper, 1995). 69 (4) (mean (SEM)) of a 52 $mmol \cdot l^{-1}$ NaCl drink was retained after a 0.5 h rehydration period and 5.5 h monitoring period (Maughan and Leiper, 1995). Although the present investigation employed a 2 h rehydration period in comparison to the 0.5 h used by Maughan and Leiper (1995), this longer rehydration period would be expected to increase drink retention (Archer and Shirreffs, 2001; Jones *et al*., 2010). This difference in drink retention after a period of fluid and energy restriction or exercise-induced dehydration might be explained by the difference in serum osmolality at the onset of rehydration. As urine production is dramatically reduced to basal levels during fluid and energy restriction (Chapter 3), it seems likely that circulating concentrations of arginine vasopressin would have been increased compared to normal resting levels. The isosmotic state observed after fluid and energy restriction (Oliver *et al*., 2008; Chapter

3), compared to the hyperosmotic state observed after exercise-induced dehydration (Maughan and Leiper, 1995) might result in a more rapid reduction in arginine vasopressin, as well as other hormones associated with fluid balance (Nose *et al*., 1988b) at the onset of rehydration, resulting in a larger diuresis after fluid and energy restriction than after exercise-induced dehydration and consequently a reduction in the amount of drink retained.

Whilst the composition of a rehydration beverage is important, the most important factor in the effectiveness of a rehydration solution is the volume consumed (Shirreffs and Maughan, 1998). The volume of fluid consumed must be in excess of the volume of sweat lost during dehydration so as to attenuate obligatory urine losses after rehydration (Shirreffs and Maughan, 1998; Shirreffs *et al*., 1996; Mitchell *et al*., 1994). For effective rehydration after exercise-induced dehydration a volume of fluid equivalent approximately 150% of BML should be ingested (Shirreffs *et al*., 1996; Mitchell *et al*., 1994). In the present study the volume of drink consumed in litres was 125% of the 24 h DR BML (in kg). A volume smaller than the 150% previously suggested as optimal was selected as during the 24 h DR not all the BML would be as a result of water loss.

In a laboratory setting, ensuring an adequate volume of fluid is ingested for effective rehydration is not an issue, as drink volume can be set, but in a practical setting, the palatability of the ingested drink is of vital importance as individuals consume larger amounts of fluids they find more palatable (Wemple *et al*., 1997). Whilst drink Na was perceived to be saltier than drink P, there were no perceived differences in sweetness, bitterness or pleasantness. Although subjects only rated the drinks as 38 (25) (drink P), 33 (26) (drink Na) and 38 (19) (drink K) out of 100 for pleasantness, suggesting subjects might not have ingested sufficient amounts of any of the drinks in a practical setting where fluid is ingested *ad libitum*.

In conclusion, a 24 h period of fluid and energy restriction results in a significant reduction in body mass that is accompanied by large losses of sodium, potassium and chloride in the urine. As has been reported following exercise induced dehydration, the addition of sodium chloride to a rehydration drink ingested after 24 h fluid and energy restriction results in an increase in the retention of the ingested drink, via a

reduction in urine output compared to a placebo drink. The addition of potassium chloride resulted in no significant benefit in terms of the recovery of fluid balance.

Chapter 5

Fluid and electrolyte balance during 24 h energy restriction: effect of NaCl and KCl supplementation

Abstract

This study examined the fluid and electrolyte balance effects of sodium chloride and potassium chloride supplementation during 24 h energy restriction. Ten subjects (five male, five female) completed two 24 h trials, during which energy intake was restricted to ~1650 $kJ \cdot d^{-1}$ and they received 2800 $ml \cdot d^{-1}$ water. Subjects visited the laboratory at 0 h, 12 and 24 h for blood samples and collected all urine produced during the trials. Subjects ingested capsules at 0 h, 6 h and 12 h containing either placebo (~3 g glucose) (trial P) or 3.05 g NaCl and 2.29 g KCl (trial E). Total urine volume excreted over the study period was greater during P (3230 (216)) than E (2441 (280)) ($P < 0.001$) and consequently the degree of body mass loss was attenuated in E (-0.71 (0.43) kg) compared to P (1.54 (0.45) kg) ($P < 0.001$), although both trials resulted in a reduction in body mass ($P < 0.001$). Sodium, potassium and chloride balance were negative during P ($P < 0.001$), but not during E, whilst serum sodium, potassium and chloride concentrations all decreased at 12 h compared to 0 h during P ($P < 0.05$), but remained unchanged during E. Compared to 0 h, plasma volume at 24 h was decreased during P ($P < 0.001$), but maintained during E. These results suggest that 24 h energy restriction results in a significant reduction in body mass and that this can be attenuated by the consumption of 9.15 g NaCl and 6.87 g KCl. It appears that the supplementation of NaCl and KCl during energy restriction prevents the decline in serum electrolytes that occurs and presumably attenuates any decrease in arginine vasopressin concentrations leading to the reduction in urine output observed,

Introduction

Long term energy restriction leads to a reduction in body mass caused by a reduction in body fat and body muscle tissue (Owen *et al*., 1998), but over the initial 24-72 h of severe energy restriction, a disproportionately large reduction in body mass is observed (Taylor *et al*., 1954; Brozek *et al*., 1957; Consolazio *et al*., 1967; Consolazio *et al*., 1968a).

The large reduction in body mass observed with severe energy restriction might be accounted for by a combination of four main avenues of weight loss: 1) Loss of body fat and muscle tissue (Owen *et al*., 1998; Kryzywicki *et al*., 1972). 2) Depletion of glycogen stores (Hultman, 1967; Maughan and Williams, 1981). 3) A reduction in intestinal contents due to the dramatically reduced food intake during severe energy restriction. 4) A reduction in body water stores, indicated by a reduction in plasma volume (Oliver *et al*., 2007; Consolazio *et al*., 1968a). Whilst the depletion of glycogen stores and the reduction in intestinal contents are relatively unavoidable during severe or complete ER, the reduction in body water stores could possibly be prevented by supplementing with electrolytes during the energy restriction period (Consolazio *et al*., 1968a).

During severe (Consolazio *et al*., 1968b) and complete (Runchie, 1971) energy restriction, the continued excretion of sodium and potassium in the urine, despite little or no intake results in a net negative balance of these electrolytes (Consolazio *et al*., 1968a; Runchie, 1971) and a reduction in blood and plasma volume (Consolazio *et al*., 1968a; Oliver *et al*., 2007; Chapter 3) and presumably total body water. It has previously been shown that the consumption of mineral supplements (3.93 $g \cdot d^{-1}$ sodium (171 mmol) and 2.41 $g \cdot d^{-1}$ potassium (62 mmol), as well as 798 $mg \cdot d^{-1}$ calcium, 269 $mg \cdot d^{-1}$ magnesium, 1.05 $g \cdot d^{-1}$ phosphorus and 5.4 $mg \cdot d^{-1}$ iron) during 10 days energy restriction (1756 $kcal \cdot d^{-1}$) attenuates the BML and plasma volume reduction during the period of energy restriction (Consolazio *et al*., 1968a), indicating that this reduction in the observed BML might be due to extra water retained in the blood as a result of the electrolytes consumed. Consolazio *et al*. (1968a; 1968b) gave water *ad libitum* during the energy restriction period and thus the consumption of sodium in the mineral supplements might have influenced thirst and fluid intake

(Adolph *et al*., 1954) and consequently body water content. The specific acute effects of electrolyte supplementation during the initial 24 h of energy restriction, when the degree of BML is greatest (Taylor *et al*., 1954; Brozek *et al*., 1957; Consolazio *et al*., 1967; Consolazio *et al*., 1968a) and body water stores might be significantly depleted (Oliver *et al*., 2007; Bosco *et al*., 1974, Consolazio *et al*., 1968) on whole body fluid and electrolyte balance is not fully understood. In certain occupational settings (e.g. military recruits under operational stress) energy restriction might be unavoidable and the supplementation of electrolytes (particularly sodium and potassium) during a energy restriction might reverse the reduction in body water stores observed and thus reduce the degree of BML and potentially attenuate the decrement in performance that has been reported.

The aim of the present investigation was therefore to determine the effects of sodium chloride and potassium chloride supplementation during a 24 h period of severe energy restriction.

It was hypothesised that the supplementation of sodium chloride and potassium chloride would maintain electrolyte balance and plasma volume and attenuate the observed body mass loss.

Methods

Ten healthy subjects (five male, five female) (age 23 (3) y, body mass 66.33 (9.01) kg, height 1.70 (0.09) m) volunteered to participate in this study, which was approved by the Loughborough University Ethical Advisory Committee (Reference number R07-P130).

Subjects completed an initial familiarisation trial, during which they were fully familiarised with all experimental procedures involved. Subjects then completed two 24 h energy restriction trials in randomised counterbalanced order, during which energy intake was restricted to ~1650 $kJ \cdot d^{-1}$. Energy was provided almost entirely as carbohydrate and this level of energy restriction was chosen as it provided the amount of carbohydrate (~100 g carbohydrate) that has been shown to be adequate to prevent the onset of ketosis with sedentary activity (Bloom, 1967). The two experimental

trials were carried out on the same day of the week and were separated by exactly 7 days. During each 24 h trial, subjects visited the laboratory on four separate occasions 0h, 6 h, 12 h and 24 h. Trials were undertaken during the months of January and February in Loughborough, UK, with 24 h mean daily temperatures of 6.3 (2.6) °C.

Subjects arrived in the morning (0 h) after at least a 10 h overnight fast, with the exception of 500 ml plain water consumed 2 h before arriving at the laboratory. Upon arrival at the laboratory, subjects voided their bladder, collecting the entire volume of this void. They were then weighed nude, before assuming a seated position. After 15 min in a seated position, a 5 ml blood sample was drawn without stasis from an antecubital vein. Subjects then consumed one third of their energy intake for the day, as well as 300 ml plain tap water and capsules containing 3.05 g NaCl and 2.29 g KCl (E) or 3 g glucose (P). Trials were isoenergetic so that during trial P, less food was given (without the subjects knowledge) so as to balance the 3 g glucose consumed in the capsules. After consuming the food and water, subjects were provided with 700 ml plain tap water to be consumed between 0-5 h and were free to leave the laboratory. Subjects returned to the laboratory 6 h after the morning visit and again consumed one third of their energy intake for the day, as well as 300 ml plain tap water and either electrolyte or placebo capsules as appropriate. They were then provided with a further 700 ml water to consume between 6-11 h and left the laboratory, before returning exactly 12 h after the 0 h visit (12 h) and undergoing exactly the same procedures as at 0 h, with the exception that before leaving the laboratory they were provided with 500 ml plain tap water to consume before going to bed. Subjects then returned to the laboratory the following morning exactly 24 h after the 0 h visit (24 h) and again underwent the same procedures as the 0 h visit. During visits to the laboratory at 0 h, 12 h and 24 h the entire volume of the urination produced was measured and a small sample (~5 ml) retained for analysis. Whilst outside the laboratory subjects collected all urine produced in a large 24 h urine collection container. This urine collection container was brought to the laboratory for each visit, the volume measured, a sample retained and a fresh container provided. The dietary intake for each trial is given in Table 5.1.

Table 5.1 Dietary intake over each 24 h trial. Values are mean (SD)

	P	E
Energy (kJ)	1648 (33)	1665 (24)
Protein (g)	8 (1)	9 (1)
Carbohydrate (g)	89 (1)	89 (1)
Fat (g)	0 (0)	0 (0)
Water (ml)	2800 (0)	2800 (0)
Sodium (mmol)	1 (0)	157 (0)
Potassium (mmol)	0 (0)	93 (0)
Chloride (mmol)	0 (0)	250 (0)

Analytical methods

1 ml of each blood sample was mixed with anticoagulant (K_2 EDTA, 1.75 $mg \cdot ml^{-1}$) and was used for the determination of haematocrit, haemoglobin concentration and blood glucose concentration. The remaining 2.5 ml blood was allowed to clot and the serum was separated by centrifugation.

Serum samples were analysed for osmolality, as well as sodium, potassium and chloride concentrations.

Urine samples were analysed for osmolality, as well as sodium, potassium, chloride and creatinine concentrations.

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

Statistical analysis

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

Results

With the exception of haematocrit ($P < 0.001$), haemoglobin concentration ($P < 0.001$) and nude body mass ($P < 0.001$), after correction of electrolyte excretion and body mass, there were no differences between males and females ($P > 0.116$) for any of the other measured variables. Therefore data for both males and females are combined. 24 h urinary creatinine excretion was not different between trials ($P = 0.579$) and over all trials was 0.21 (0.08) mmol·kg^{-1} body mass·24 h^{-1}, which is indicative of a complete 24 h urine collection (Bingham and Cummings, 1985).

Pre-trial measurements

Pre-trial body mass ($P = 0.842$), urine osmolality ($P = 0.545$), serum osmolality ($P = 0.372$) and serum sodium concentration ($P = 0.472$) were not different between trials, indicating subjects started each trial in a similar state of hydration.

BML and urine output

Trial P resulted in approximately linear BML over the 24 h trial period of 0.73 (0.37) kg (1.07 (0.54) %) and 1.54 (0.45) kg (2.31 (0.55) %) at 12 h ($P < 0.001$) and 24 h ($P < 0.001$) respectively. During trial E, body mass remained unchanged at 12 h (-0.02 (0.37) kg (0.00 (0.58) %)), but decreased by a similar amount to P between 12 h and 24 h and by 24 h subjects had lost 0.71 (0.43) kg (1.04 (0.58) %) ($P < 0.01$). BML was greater during P than E at both 12 h ($P < 0.01$) and 24 h ($P < 0.001$).

Total urine volume (Table 5.2) was greater during trial P than during trial E ($P < 0.001$). This difference between trials was largely due to a greater urine volume between 0-12 h during trial P compared to trial E ($P < 0.001$), with similar urine volumes produced between 12-24 h during both trials ($P = 0.462$). During both trial P

($P < 0.01$) and trial E ($P < 0.001$) a greater volume of urine was produced between 0-12 h than between 12-24 h.

Table 5.2 Urine volume (ml) excreted over the whole 24 h period, as well between 0-12 h and between 12-24 h. * Significantly different from 0-12 h. # Significantly different from trial P. Values are mean (SD).

	P	E
Total	3230 (216)	2441 (280) #
0-12 h	2185 (220)	1457 (303) #
12-24 h	1046 (191) *	984 (178) *

Gross BML over the 24 h trial period (calculated from the observed BML, food, capsule and water intake) was greater during trial P (4.45 (0.49) kg) than during trial E (3.63 (0.43) kg) ($P < 0.001$). This difference was entirely explained by the difference in urine output between the trials, as weight loss caused by mechanisms other than urine production was 1.22 (0.40) kg and 1.19 (0.28) kg during trials P and E respectively ($P = 0.777$).

Urine osmolality, electrolyte excretion and electrolyte balance

Urine osmolality (Figure 5.1) was greater at 24 h during trial E than trial P ($P < 0.05$) and additionally, during trial E was greater at 24 h compared to 0 h ($P < 0.05$).

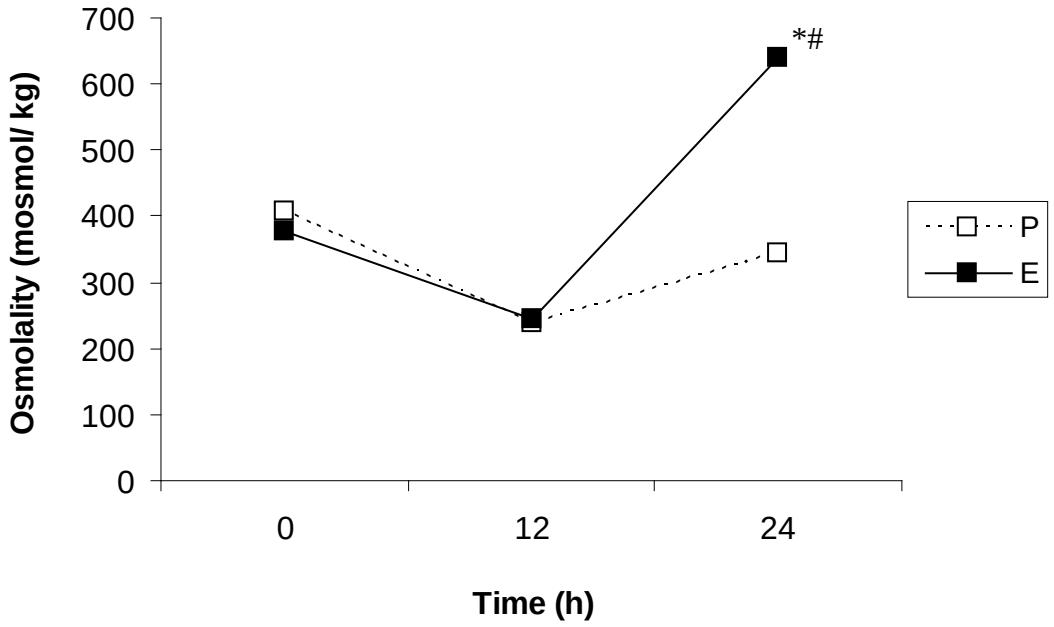

Figure 5.1 Urine osmolality (mosmol·kg^{-1}) of urine samples produced at 0 h, 12 h and 24 h. * Significantly different from 0 h. # Significantly different from trial P. Points are median values.

The total urinary excretion of sodium (Table 5.3a) ($P < 0.01$), potassium (Figure 5.3b) ($P < 0.01$) and chloride (Figure 5.3c) ($P < 0.01$) was greater during trial E than trial P. Between 0-12 h, the excretion of potassium ($P < 0.01$) and chloride ($P < 0.01$) was greater during trial E than trial P, whilst there was a tendency for greater sodium excretion ($P = 0.056$). Between 12-24 h, the excretion of sodium ($P < 0.001$) and chloride ($P < 0.05$) was greater during trial E than trial P, whilst there was a tendency for greater potassium excretion ($P = 0.051$). During trial P, sodium ($P < 0.05$), potassium ($P = 0.05$) and chloride ($P < 0.05$) excretion was greater between 0-12 h than between 12-24 h. During trial E only potassium excretion was greater between 0-12 h than between 12-24 h ($P < 0.01$).

Table 5.3 Sodium (mmol) (a), potassium (mmol) (b) and chloride (mmol) (c) excreted in the urine over the whole 24 h period, as well between 0-12 h and between 12-24 h. * Significantly different from 0 h. # Significantly different from trial P. Values are mean (SD).

	P	E
a) Sodium		
Total	86 (33-132)	163 (70-200) #
0-12 h	53 (12-91)	74 (23-119)
12-24 h	23 (4-64) *	73 (39-106) #
b) Potassium		
Total	65 (34-87)	100 (58-141) #
0-12 h	42 (23-81)	72 (49-89) #
12-24 h	10 (6-45) *	26 (10-52) *#
c) Chloride		
Total	107 (32)	245 (82) #
0-12 h	70 (26)	145 (57) #
12-24 h	37 (23) *	100 (51) #

Sodium (Table 5.4a), potassium (Table 5.4b) and chloride (Table 5.4c) balances were calculated from losses in urine and gains from food intake and electrolyte supplements. The balance of all electrolytes were significantly negative at both 12 h ($P < 0.001$) and 24 h ($P < 0.001$) during trial P and were not different from 0 h during trial E, although sodium balance at 12 h tended towards being positive ($P = 0.078$). Additionally, sodium, potassium and chloride balances were lower at both 12 h ($P < 0.01$) and 24 h ($P < 0.01$) during trial P than during trial E.

Table 5.4 Sodium (mmol) (a), potassium (mmol) (b) and chloride (mmol) (c) balance over the 24 h trial period calculated from dietary intake and urinary excretion. * Significantly different from 0 h. # Significantly different from trial P. Values are mean (SD).

	P	E
a) Sodium		
0 h	0 (0)	0 (0)
12 h	-50 (25) *	29 (38) *#
24 h	-76 (33) *	10(49) #
b) Potassium		
0 h	0 (0)	0 (0)
12 h	-46(20) *	-8 (14) #
24 h	-62 (20) *	-7 (21) #
c) Chloride		
0 h	0 (0)	0 (0)
12 h	-69 (26) *	22 (57)#
24 h	-106 (32) *	5 (82) #

Blood, plasma and red cell volume changes

Compared to 0 h, blood volume (Figure 5.2) was reduced at both 12 h ($P < 0.05$) and 24 h ($P < 0.001$) during trial P, but did not change from 0 h values during trial E ($P > 0.234$). Similarly, plasma volume (Figure 5.3) was reduced at both 12 h ($P < 0.01$) and 24 h ($P < 0.001$) compared to 0 h during trial P, but during trial E was increased at 12 h ($P < 0.05$) and was not different to 0 h at 24 h. Both blood volume and plasma volume were greater at 12 h ($P < 0.01$) and 24 h ($P < 0.001$) during trial E compared to trial P. There were no main effects for time ($P = 0.191$), trial ($P = 0.209$) or interaction ($P = 0.450$) for red cell volume (Figure 5.4).

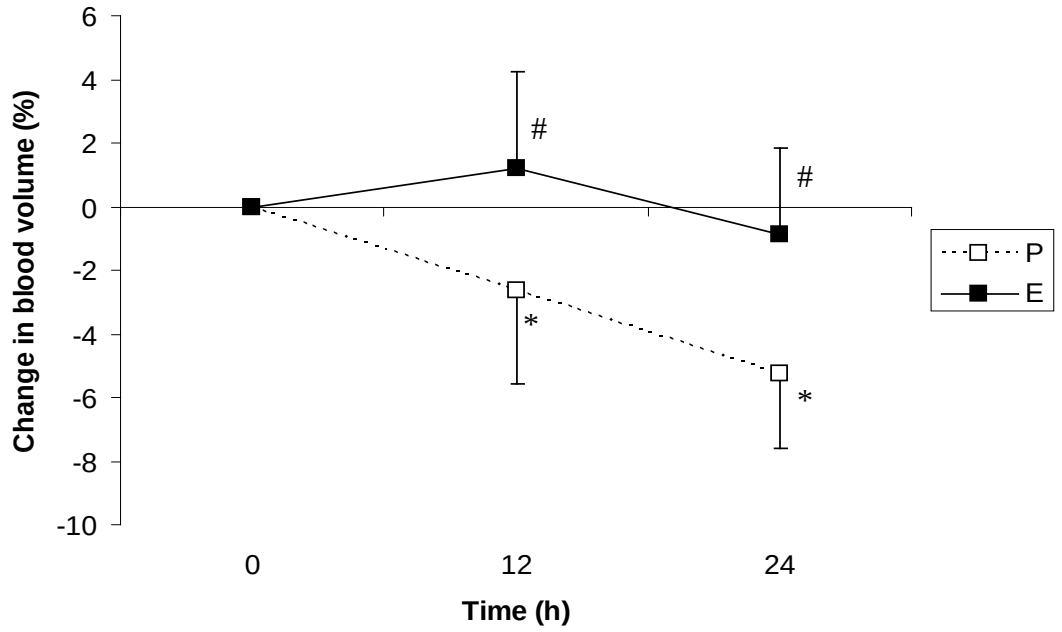

Figure 5.2 Estimated change in blood volume from 0 h (%). * Significantly different from 0 h. # Significantly different from trial P. Points are mean values. Error bars are SD.

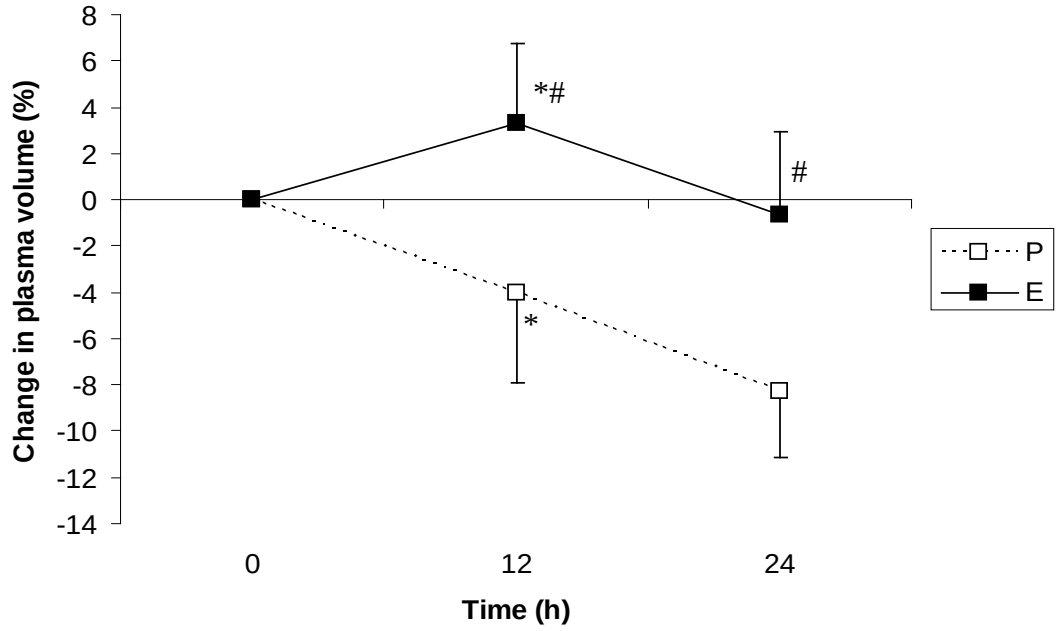

Figure 5.3 Estimated change in plasma volume from 0 h (%).* Significantly different from 0 h. # Significantly different from trial P. Points are mean values. Error bars are SD.

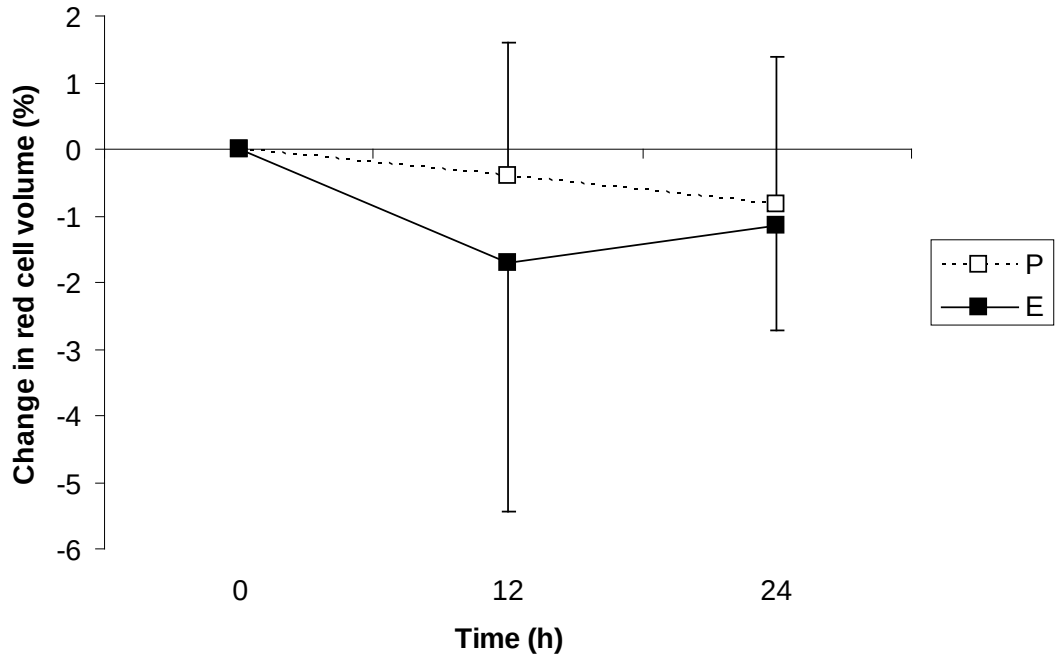

Figure 5.4 Estimated change in red cell volume from 0 h (%). Points are mean values. Error bars are SD.

Serum osmolality and electrolyte concentration

Serum osmolality (Table 5.5a), as well as serum sodium (Table 5.5b), potassium (Table 5.5c) or chloride (Table 4.4d) concentrations were all decreased at 12 h compared to 0 h, all returning to baseline values by 24 h. Serum sodium concentration at 12 h was significantly lower during P than E, but there were no other between trial differences for any of the serum measures

Table 5.5 Serum osmolality ($mosmol \cdot kg^{-1}$) (a), sodium ($mmol \cdot l^{-1}$) (b), potassium ($mmol \cdot l^{-1}$) (c) and chloride ($mmol \cdot l^{-1}$) (d) concentration at 0 h, 12 h and 24 h. * Significantly different from 0 h. # Significantly different from trial P. Values are mean (SD).

	P	E
a) Osmolality		
0 h	285 (3)	284 (3)
12 h	283 (4)	285 (3)#
24 h	286 (4)	286 (3)
b) Sodium		
0 h	144 (3)	143 (2)
12 h	141 (2)*	144 (3) #
24 h	144 (1)	143 (2)
c) Potassium		
0 h	4.5 (0.3)	4.3 (0.3)
12 h	4.2 (0.2) *	4.3 (0.2)
24 h	4.6 (0.2)	4.4 (0.2)
d) Chloride		
0 h	105 (2)	104 (3)
12 h	102 (3)*	105 (4)
24 h	104 (3)	106 (3)

Blood glucose concentration

Blood glucose (Figure 5.5) was not different between trials (P = 0.922), but compared to 0 h was decreased ($P < 0.001$) at 12 h during trial P.

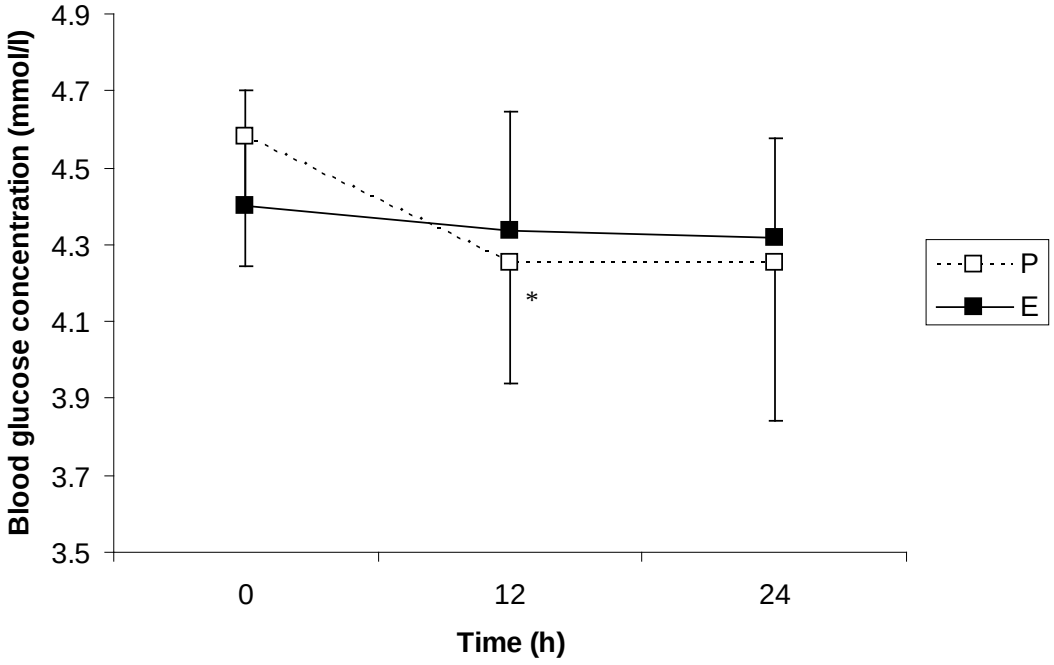

Figure 5.5 Blood glucose concentration (mmol·l^{-1}). * Significantly different from 0 h. Points are mean values. Error bars are SD.

Discussion

The main findings of this study were that supplementation of sodium chloride and potassium chloride during 24 h energy restriction attenuated body mass loss by reducing total cumulative urine output. Additionally, sodium, potassium and chloride balance were maintained with electrolyte supplementation and consequently, plasma volume was also maintained at pre-trial levels.

During the trial P, where energy intake was restricted to ~1600 kJ·d^{-1} and 2800 ml·d^{-1} water was provided, subjects lost 1.54 (0.45) kg (2.31 (0.55) % of their initial body

mass) over the 24 h trial period. This degree of body mass reduction is similar to the 1.8% BML reported by Brozek *et al*. (1957) during the first 24 h of a 12 day period of ER (2424 $kJ \cdot d^{-1}$). The consumption of 157 mmol NaCl and 93 mmol KCl during the same 24 h period of ER resulted in a 0.83 (0.35) kg reduction in the amount of body mass lost. In line with the present findings, Consolazio *et al*. (1968a) reported that 10 days severe energy restriction (420 $kcal \cdot d^{-1}$) resulted in an 8.0% (5.62 kg) reduction in body mass, whilst supplementation with 3.93 $g \cdot d^{-1}$ sodium (171 mmol) and 2.41 $g \cdot d^{-1}$ potassium (62 mmol), as well as 798 $mg \cdot d^{-1}$ calcium, 269 $mg \cdot d^{-1}$ magnesium, 1.05 $g \cdot d^{-1}$ phosphorus and 5.4 $mg \cdot d^{-1}$ iron during the same 10-day energy restriction resulted in a 5.9% (4.1 kg) reduction in body mass.

Total urine production over the 24 h trial period was 789 (310) ml greater during trial P than during trial E, whilst weight loss caused by mechanisms other than urine production was not different between trials. Given that 1 ml of urine weighs approximately 1 g, these results indicate that the difference in urine output between the two trials accounts for the 0.83 (0.35) kg difference in BML observed.

The total urinary excretion of the electrolytes sodium, potassium and chloride over the 24 h trial period was greater during trial E than during trial P (Table 4.2), but the consumption of NaCl and KCl during trial E meant that the balance of all of these electrolytes was not different from 0 h at the end of the 24 h trial period. Whilst subjects were in net sodium, potassium and chloride balance at the end of trial E, the continued excretion of these electrolytes in the urine during trial P, combined with little to no intake resulted in a significant net negative balance of all three electrolytes at the end of the 24 h trial period. This is a finding consistent with previous studies (Chapter 3, Consolazio *et al*., 1968b). Although at 24 h, subjects were in net balance of the measured electrolytes during trial E, this balance was calculated using dietary intake and urinary excretion only. It is likely that losses of these electrolytes from the body via routes other than urinary excretion would continue during the 24 h trial period. Small amounts of sodium (~2 $mmol \cdot d^{-1}$) and potassium (~10 $mmol \cdot d^{-1}$) (Holbrook *et al*., 1984), as well as presumably chloride are excreted in faeces and it is likely that these faecal losses persist during energy restriction, at least during the initial few days. Due to the abstinence of exercise and the cool environmental conditions during trials, it is likely that sweat losses were minimal (~500 $ml \cdot d^{-1}$), but

some electrolyte losses will have occurred through perspiration. During exercise and/ or heat exposure sweat sodium, potassium and chloride concentrations of 20-80 $mmol \cdot l^{-1}$, 4-8 $mmol \cdot l^{-1}$ and 20-60 $mmol \cdot l^{-1}$ respectively have been reported (Maughan and Shirreffs, 1998) and it has been shown that sweat sodium concentration is linearly related to sweat rate (Buono *et al*., 2008). Although little is known about the composition of sweat secreted in sedentary individuals or the effects of acute changes in dietary electrolyte consumption on sweat electrolyte composition, it is likely that the very slow daily rates of sweat production under sedentary conditions allow more time for reabsorption of electrolytes in the sweat gland. In line with this, Heer *et al*. (2000) reported that sweat losses of sodium in sedentary individuals during diets containing 50, 200 and 550 mmol $sodium \cdot d^{-1}$ were 2.88 (0.35) $mmol \cdot d^{-1}$, 3.38 (0.28) $mmol \cdot d^{-1}$ and 4.92 (0.28) $mmol \cdot d^{-1}$ respectively. In the present investigation the difference in sodium consumption between trials P and E (156 mmol), was similar to the difference in sodium consumption between the 50 and 200 $mmol \cdot d^{-1}$ trials in the study of Heer *et al*. (2000), suggesting that sodium losses through perspiration was likely to be similar between trials.

Despite a marked retention of sodium, potassium and chloride during trial E, serum concentrations of these electrolytes remained unchanged, indicating that water was retained along with these electrolytes so that no rise in their serum concentrations was observed. This is supported by the estimated changes in plasma volume during the trials, which show that plasma volume remained unchanged (-0.7 (3.7) %) from 0 h at 24 h during trial E, but was decreased 8.3 (2.8) % during trial P. If one assumes that all sodium lost came from the extracellular space and that serum sodium concentration, which remained constant (143 (3) $mmol \cdot l^{-1}$), is representative of total extracellular sodium concentrations then it is possible to calculate that the 88 (56) mmol difference in sodium balance between the trials might be expected to retain an extra 616 (392) ml water in the extracellular space during trial E. Additionally, if one assumes that all potassium lost came from the intracellular space, as serum potassium concentration was not different at 0 h or 24 h either between trials or over time and that the intracellular potassium concentration was 150 $mmol \cdot l^{-1}$ (Maughan and Shirreffs, 1998) then the 56 (26) mmol difference in potassium balance between the trials might be expected to retain an extra 373 (175) ml water in the intracellular space during trial E. Given the assumptions made in these calculations the estimated total

extra water retained with the sodium and potassium (986 (378) ml) is fairly similar to the both the difference in BML (0.83 (0.35) kg) and urine output (789 (310) ml) between the two trials.

It has been clearly demonstrated that the addition of sodium to a rehydration drink ingested after exercise-induced dehydration results in an increased drink retention compared to a sodium free drink (Maughan and Leiper, 1995; Shirreffs and Maughan, 1998; Merson *et al*., 2008) and that correction of the negative sodium balance incurred through sweat losses is necessary for correction of fluid balance after exercise (Shirreffs and Maughan, 1998). Ingestion of large volumes of sodium free drinks after exercise-induced dehydration results in a rapid reduction in plasma osmolality and sodium concentration (Nose *et al*., 1888a), a decrease in plasma renin activity and plasma aldosterone concentration (Nose *et al*., 1988b), and consequently a large diuresis. Addition of sodium to the drink prevents this cascade and the associated large diuresis (Nose *et al*., 1988a; Nose *et al*., 1988b). The addition of potassium to rehydration drinks consumed after exercise-induced dehydration has been hypothesised to improve fluid retention via increased intracellular rehydration (Shirreffs *et al*., 2004), but recent studies show conflicting results (Maughan *et al*., 1994; Shirreffs *et al*., 2007).

As with post-exercise rehydration, the consumption of water and the restriction of electrolytes during 24 h energy restriction in trial P resulted in a reduction in serum sodium concentration and serum osmolality at 12 h, would be expected to have reduced circulating levels of arginine-vasopressin (Baylis, 1987), which would account for the increased urine production in this trial, particularly between 0- 12 h, when the majority (2000 out of 2800 ml) of the water was ingested.

In conclusion, 24 h energy restriction, with energy intake restricted to ~1650 $kJ \cdot d^{-1}$ results in a large reduction in body mass and the continued excretion of the electrolytes sodium, potassium and chloride in urine. The consumption of 9.05 g NaCl (156 (0) mmol) and 6.79 g KCl (93 (0) mmol) during the same 24 h period of ER maintained serum osmolality and electrolyte concentrations at 12 h, and whole body net sodium, potassium and chloride balance over the 24 h trial, resulting in a reduced

total urine output and a reduction in the degree of BML compared to the placebo trial, as well as the maintenance of baseline blood and plasma volume at 24 h.

Chapter 6

Fluid balance response to NaCl and KCl supplementation during 24 h severe energy restriction

Abstract

This study examined the fluid and electrolyte balance effects of sodium chloride and potassium chloride supplementation during 24 h energy restriction. Eight male subjects completed two 24 h trials, during which energy intake was restricted to ~150 $kJ \cdot d^{-1}$ and they received 2995 (217) $ml \cdot d^{-1}$ water. Subjects arrived at the laboratory after an overnight fast (0 h) and remained there for 12 h, before going home to sleep and returning the following morning after an overnight fast, exactly 24 h after their first visit. Subjects ingested capsules at 0 h, 4 h, 8 h and 12 h containing either 0.02 $g \cdot kg^{-1}$ body mass glucose (P) 0.55 $mmol \cdot kg^{-1}$ body mass NaCl (E) and half of the water ingested during trial E contained 20 $mmol \cdot l^{-1}$ KCl. Blood and urine samples were taken at 0 h, 2 h, 4 h, 6 h, 8 h, 10 h, 12 h and 24 h and all urine produced was collected. Total urine volume excreted over the study period was greater during trial P (3519 (401)) than during trial E (2746 (594)) ($P < 0.001$) and consequently the degree of body mass loss was attenuated in E (-0.92 (0.44) kg) compared to P (1.72 (0.59) kg) ($P < 0.001$), although both trials resulted in a reduction in body mass ($P < 0.001$). Serum electrolyte concentrations and osmolality, as well electrolyte balance, and blood and plasma volumes were better maintained during trial E than during trial P. These results demonstrate that the supplementation of NaCl and KCl during 24 h severe energy restriction attenuates the decline in serum osmolality and electrolyte concentrations that occurs due to the acute ingestion of water, thus reducing the amount of urine produced and leading to better maintenance of body mass and plasma volume.

Introduction

Some of the fluid balance effects of electrolyte supplementation during severe energy restriction have been described previously (Consolazio *et al*., 1968a; Consolazio *et al*., 1968b; Chapter 5). Conzolazio *et al*. (1968a; 1968b) reported the effects of supplementing with 3.93 $g \cdot d^{-1}$ sodium (171 mmol) and 2.41 $g \cdot d^{-1}$ potassium (62 mmol), as well as 798 $mg \cdot d^{-1}$ calcium, 269 $mg \cdot d^{-1}$ magnesium, 1.05 $g \cdot d^{-1}$ phosphorus and 5.4 $mg \cdot d^{-1}$ iron on body mass and body water (Consolazio *et al*., 1968a) and electrolyte balance (Consolazio *et al*., 1968b) during 10 days energy restriction, with energy restricted to 420 $kcal \cdot d^{-1}$ (1760 $kJ \cdot d^{-1}$) in a placebo controlled study design with 4 subjects in each group. These studies suggested that some of the large loss of body water observed during severe energy restriction might be prevented by providing an electrolyte supplement. The study reported in Chapter 5 of this thesis demonstrated that supplementing with sodium chloride (157 mmol) and potassium chloride (93 mmol) during 24 h severe energy restriction (~1650 $kJ \cdot d^{-1}$), reduced urine output and BML.

The difference in response between electrolyte supplementation and placebo occurred between 0-12 h and therefore the purpose of the present investigation was to monitor subjects over the initial 12 h of 24 h severe energy restriction and determine the acute response to water and electrolyte ingestion during energy restriction.

It was hypothesised that the supplementation of sodium chloride and potassium chloride would maintain serum electrolyte concentrations and serum osmolality leading to a reduction in urine volume.

Methods

Eight healthy males (age 25 (6) y, body mass 74.81 (5.43) kg, height 1.77 (0.08) m) volunteered to take part in this study, which was approved by the Loughborough University Ethical Advisory Committee (Reference number R08-P119).

Subjects completed an initial familiarisation trial, during which they were fully familiarised with all experimental procedures involved in the study. Subjects then

completed two 24 h energy restriction trials in a randomised counterbalanced order, during which energy intake was restricted to 153 $kJ \cdot d^{-1}$. This was to represent complete energy restriction, with the only energy supplied that of the glucose contained in capsules (or added to drinks to match capsule glucose intake). The two experimental trials were carried out on the same day of the week and were separated by exactly 7 or 14 days. Trials were undertaken during the months of February, March and April in Loughborough, UK, with 24 h mean daily temperatures of 7.7 (2.7) °C.

Subjects arrived in the morning after at least a 12 h overnight fast and having consumed 500 ml plain water with their evening meal the day before the trial and 500 ml 10 h before arriving at the laboratory. Upon arrival, subjects voided their bladder, collecting the entire volume of this void. They were then weighed nude, before assuming a seated position. After approximately 10 min in a seated position, a 21-gauge butterfly needle was inserted into a superficial forearm vein for repeated blood sampling and after 15 min seated rest, a 7 ml blood sample was drawn (0 h). Subjects then ingested 5 $ml \cdot kg^{-1}$ body mass of either a 20 $mmol \cdot l^{-1}$ KCl, 3 $g \cdot l^{-1}$ glucose solution (Trial E) or a $1g \cdot l^{-1}$ glucose solution (Trial P), as well as capsules containing either 0.55 $mmol \cdot kg^{-1}$ body mass NaCl (Trial E) or 0.02 $g \cdot kg^{-1}$ body mass glucose (Trial P). They then rested in the laboratory (24.1 (1.5) °C, 29.5 (3.8) % relative humidity) for the next 12 h. Subjects were provided with the same drinks and capsules at 4 h, 8 h and 12 h, as well as being provided with 5 $ml \cdot kg^{-1}$ body mass of the same composition drink to consume between 0-4 h, 4-8 h and 8-12 h, as well as at 14 h. Total dietary intake for the two trials is provided in Table 6.1.

Further blood samples were drawn at 2 h, 4 h, 6 h, 8 h, 10 h and 12 h, with no drink consumed in the 15 min prior to each blood sample. Urine was collected in 2 h blocks whilst subjects were in the laboratory, with subjects providing urine samples immediately after each blood sample. This urine was pooled with any urine produced over the preceding 2 h. After the 12 h urine sample, subjects were weighed and allowed to leave the laboratory.

Subjects returned to the laboratory at 24 h and provided a urine sample, after which their body mass was measured, they completed a questionnaire and after 15 min seated rest, a blood sample was drawn by venepuncture of an antecubital vein. All

urine produced between 12 h and 24 h was collected by the subjects and brought with them to the laboratory at 24 h.

Table 6.1 Dietary intake over each 24 h trial. Values are mean (SD)

	P	E
Energy (kJ)	153 (4)	153 (11)
Protein (g)	0 (0)	0 (0)
Carbohydrate (g)	9 (1)	9 (0)
Fat (g)	0 (0)	0 (0)
Water (ml)	2995 (217)	2995 (217)
Sodium (mmol)	0 (0)	165 (11)
Potassium (mmol)	0 (0)	60 (4)
Chloride (mmol)	0 (0)	225 (15)

Analytical methods

1 ml of each blood sample was mixed with anticoagulant (K_2 EDTA, 1.75 $mg \cdot ml^{-1}$) and was used for the determination of haematocrit, as well as haemoglobin and glucose concentrations.

1 ml of each blood sample was mixed with ice cold lithium heparin and used for the determination of blood pH.

2.5 ml of the remaining blood was mixed with pre-chilled anticoagulant (K_2 EDTA, 1.75 $mg \cdot ml^{-1}$) and plasma was separated by centrifugation, before being frozen at -80°C. The remaining 2.5 ml blood was allowed to clot and the serum was separated by centrifugation. Plasma samples were analysed for aldosterone concentration, whilst serum samples were analysed for osmolality, as well as sodium, potassium and chloride concentrations.

Urine samples were analysed for osmolality, as well as sodium, potassium chloride and creatinine concentrations.

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

Statistical analysis

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

Results

24 h urinary creatinine excretion was not different between trials (P = 0.366) and over all trials was 0.24 (0.08) mmol·kg^{-1} body mass·24 h^{-1}, which is indicative of a complete 24 h urine collection (Bingham and Cummings, 1985).

Pre-trial measurements

Pre-trial body mass (P = 0.202), urine osmolality (P = 0.472), serum osmolality (P = 0.342) and serum sodium concentration (P = 0.565) were not different between trials, indicating subjects started each trial in a similar state of hydration.

BML and urine output

Baseline (0 h) body mass was similar (P = 0.202) between trials and for trials P and E was 74.97 (5.43) kg and 74.54 (5.26) kg respectively. BML (kg) (Table 6.2) became significant at 12 h during trial P and at 24 h during trial E. BML was greater at both 12 h (P < 0.05) 24 h (P < 0.01) during trial P than during trial E. Over the 24 h trial period the BML equated to losses of 1.24 (0.60) % and 2.30 (0.79) % of subjects initial body mass during trial E and P, respectively.

Table 6.2 Body mass loss (BML) (kg), calculated as the change in body mass from 0 h. * Significantly different from 0 h. # Significantly different from trial P. Values are mean (SD).

	0 h	12 h	24 h
P	0 (0)	0.86 (0.66)	1.72 (0.59) *
E	0 (0)	0.29 (0.39)	0.92 (0.44) *#

Cumulative urine output (Figure 6.1) was greater at 24 h during trial P compared to trial E ($P < 0.01$) and tended to be greater at 10 h ($P = 0.070$) and 12 h ($P = 0.058$). Over the 24 h, total urine volume amounted to 3519 (401) ml and 2746 (594) during trials P and E respectively.

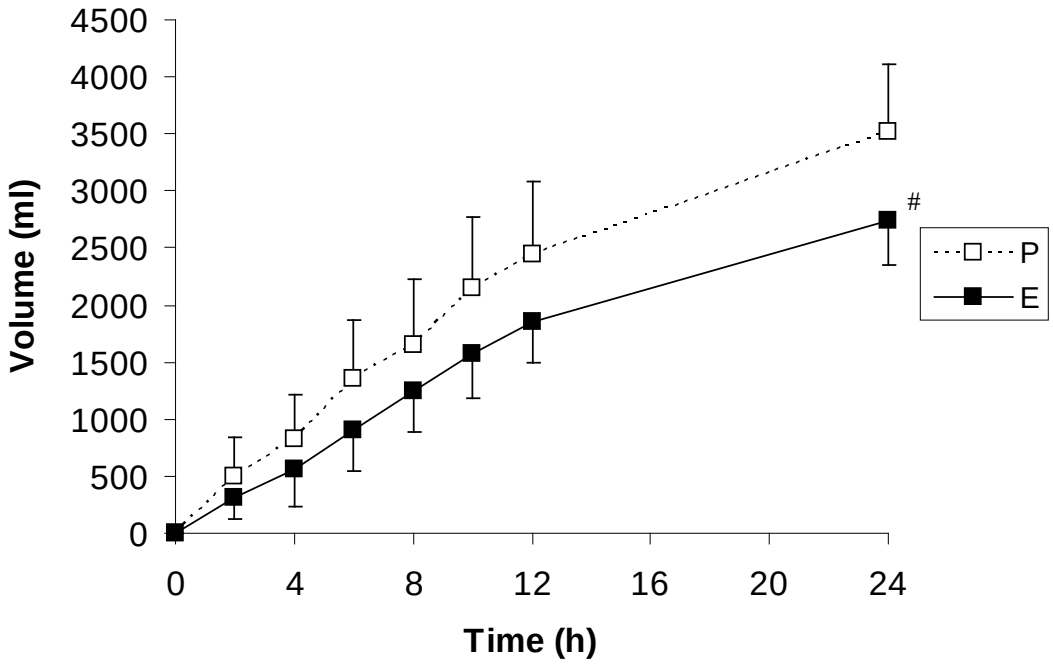

Figure 6.1 Cumulative urine output (ml). # Significantly different from trial P. Points are mean values. Error bars are SD.

The difference in BML between the trials at 12 h (0.57 (0.59 kg) and 24 h (0.80 (0.48) kg) was mostly attributable to the difference in urine output between the trials at 12 h

(592 (459 ml) and 24 h (773 (371) ml). Consistent with this, there were significant relationships between the difference in BML between the trials and the difference in urine output between the trials at both 12 h (r = 0.921, P < 0.01) and 24 h (r = 0.806, P < 0.05).

Urine osmolality, electrolyte excretion and electrolyte balance

Compared to 0 h, urine osmolality (Figure 6.2) was reduced between 2-12 h during trial P (P < 0.01), but only at 6 h and 8 h during trial E (P < 0.05) and at 6 h was lower during trial P than during trial E (P < 0.01).

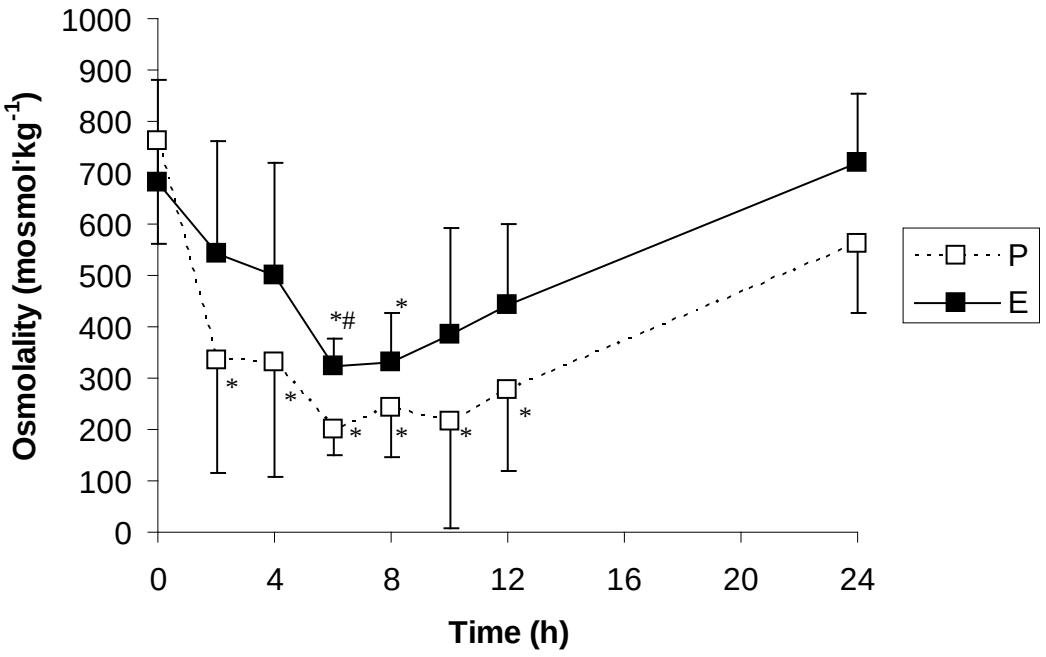

Figure 6.2 Urine osmolality (mosmol·kg^{-1}). * Significantly different from 0 h. # Significantly different from trial P. Points are mean values. Error bars are SD.

Urine sodium (Table 6.3a) and chloride (Table 6.3b) excretion followed similar patterns and were both greater during trial E from 8 h onwards (P < 0.05). Urine potassium excretion (Table 6.4) was not different between trials. Sodium balance (Figure 6.3) was greater during trial E than during trial P from 2 h onwards (P <

0.05). Compared to 0 h, sodium balance was negative from 2 h onwards during trial P ($P < 0.05$), but only at 24 h during trial E ($P < 0.05$). Chloride balance (Figure 6.4) followed a similar pattern to sodium balance and was greater during trial E than during trial P from 2 h onwards ($P < 0.05$) and was negative compared to 0 h from 2 h onwards during trial P ($P < 0.05$). Similarly, potassium balance (Figure 6.5) was greater during trial E than during trial P from 6 h onwards ($P < 0.05$) and was negative compared to 0 h, from 2 h onwards during trial P ($P < 0.05$). Potassium ($P > 0.112$) and chloride ($P > 0.082$) balance did not change significantly from baseline at any time point during trial E.

Table 6.3 Cumulative urine sodium (mmol) (a) and chloride (mmol) (b) excretion. # Significantly different from trial P. Values are mean (SD).

	0 h	2 h	4 h	6 h	8 h	10 h	12 h	24 h
a) Sodium								
Trial E	0	25	41	63	86 #	111 #	138 #	213 #
	(0)	(12)	(17)	(22)	(26)	(30)	(30)	(36)
Trial P	0	17	30	43	53	67	81	119
	(0)	(9)	(15)	(16)	(18)	(21)	(23)	(36)
b) Chloride								
Trial E	0	30	52	78	104 #	131 #	162 #	266 #
	(0)	(12)	(18)	(22)	(26)	(29)	(30)	(43)
Trial P	0	27	44	59	69	82	94	140
	(0)	(16)	(20)	(19)	(21)	(23)	(26)	(47)

Table 6.4 Cumulative urine potassium (mmol) excretion. Values are median (range).

	0 h	2 h	4 h	6 h	8 h	10 h	12 h	24 h
Trial E	0 (0-0)	16 (4-27)	27 (8-44)	39 (14-55)	52 (20-67)	64 (25-78)	73 (29-97)	101 (43-125)
Trial P	0 (0-0)	16 (6-45)	24 (10-60)	32 (16-76)	36 (18-88)	40 (23-94)	43 (26-98)	57 (38-112)

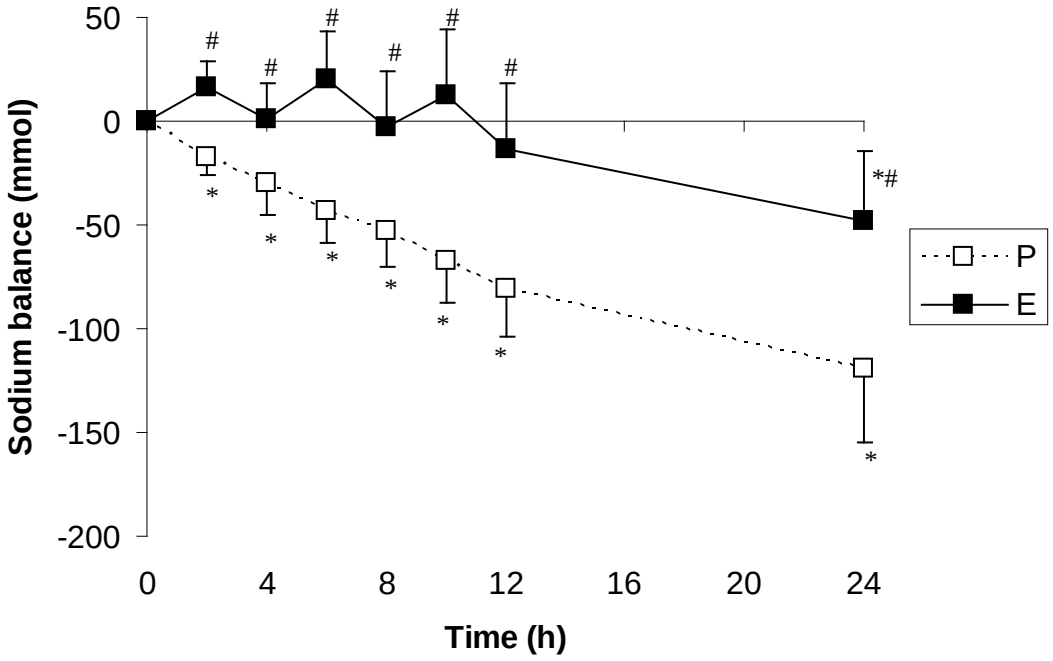

Figure 6.3 Sodium balance (mmol). * Significantly different from 0 h. # Significantly different from trial P. Points are mean values. Error bars are SD.

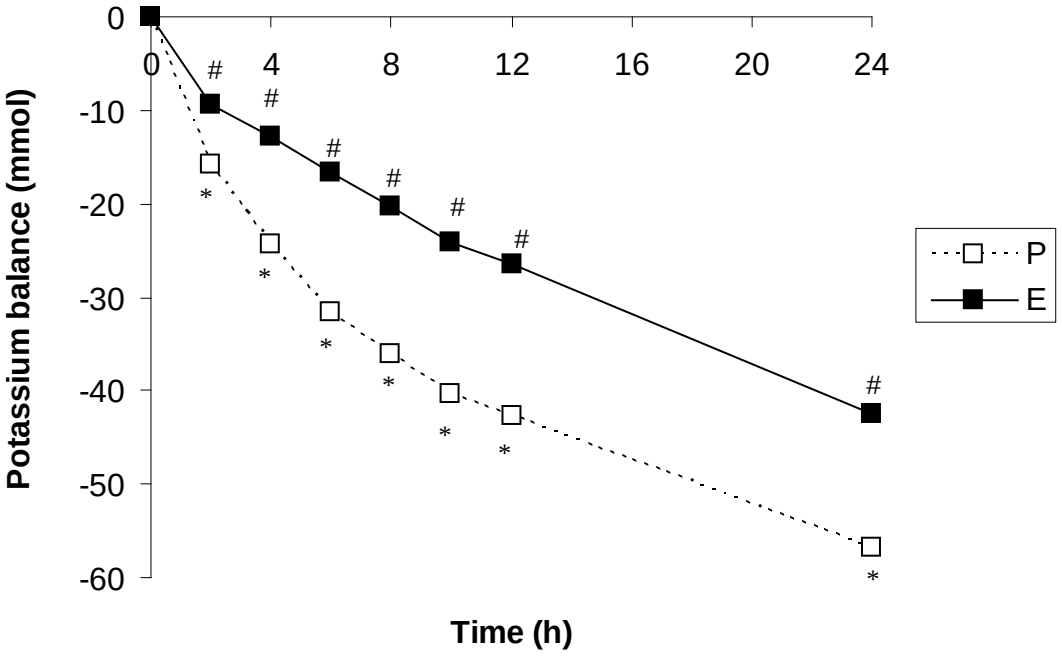

Figure 6.4 Potassium balance (mmol). * Significantly different from 0 h. # Significantly different from trial P. Points are median values.

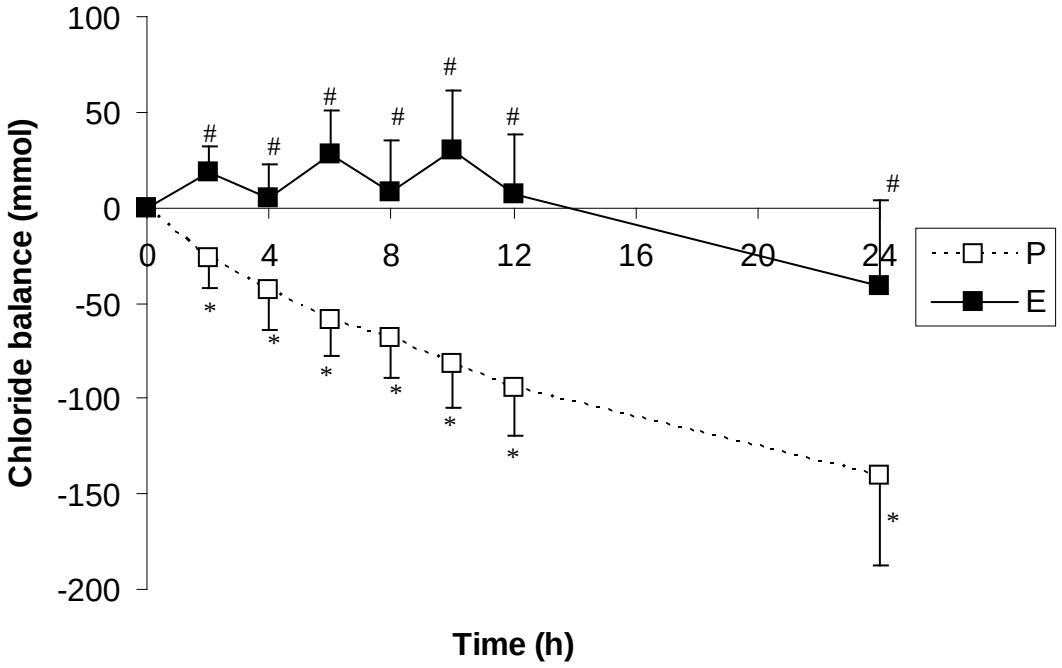

Figure 6.5 Chloride balance (mmol). * Significantly different from 0 h. # Significantly different from trial P. Points are mean values. Error bars are SD.

Blood, plasma and red cell volume changes

There were no main effects of trial for the estimated change in plasma volume (Figure 6.7) (P = 0.090) or red cell volume (Figure 6.8) (P = 0.130), but at 24 h, estimated blood volume was greater for trial E than for trial P (P = 0.029). Furthermore, compared to 0 h, estimated blood and plasma volume were decreased at 24 h (P < 0.01) during trial P, but were not different from 0 h during trial E (P > 0.191).

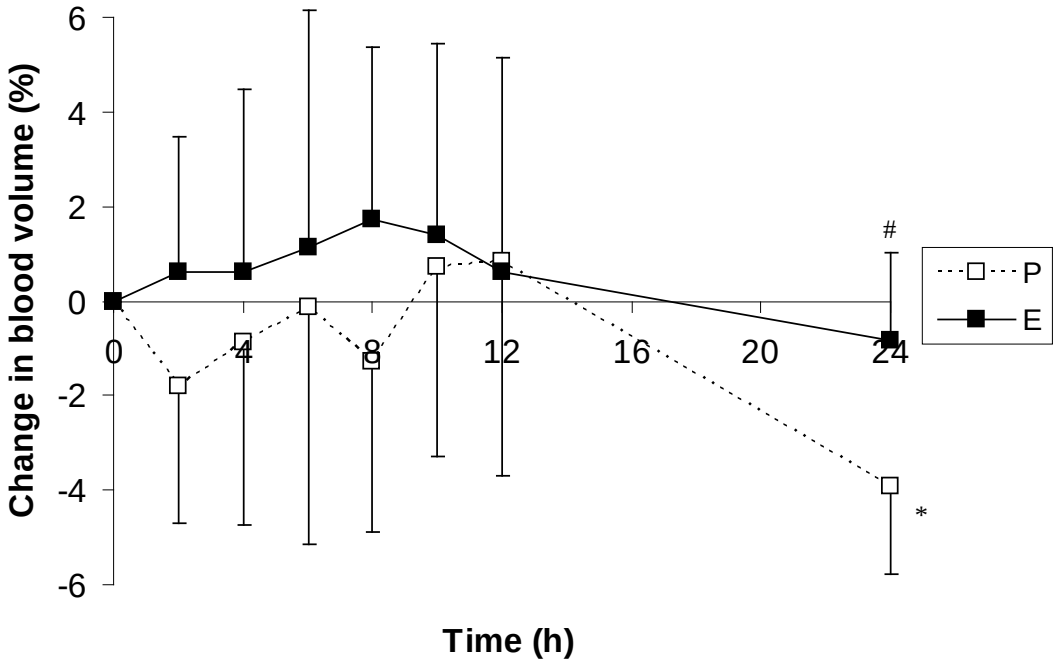

Figure 6.6 Estimated change in blood volume from 0 h (%).* Significantly different from 0 h. # Significantly different from trial P. Points are mean values. Error bars are SD.

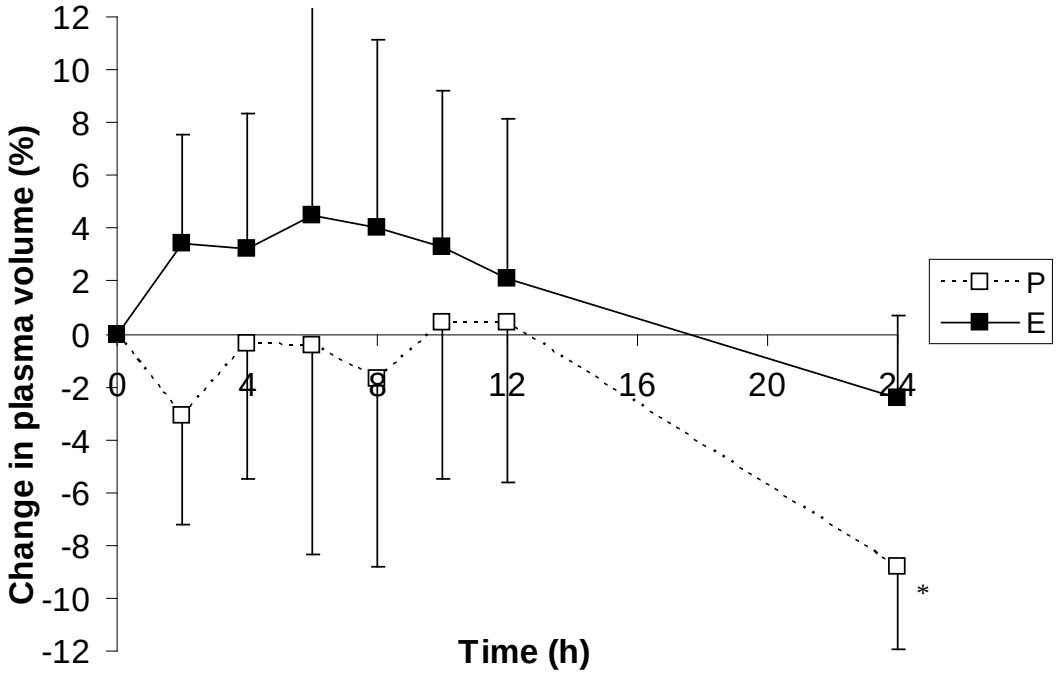

Figure 6.7 Estimated change in plasma volume from 0 h * Significantly different from 0 h. Points are mean values. Error bars are SD.

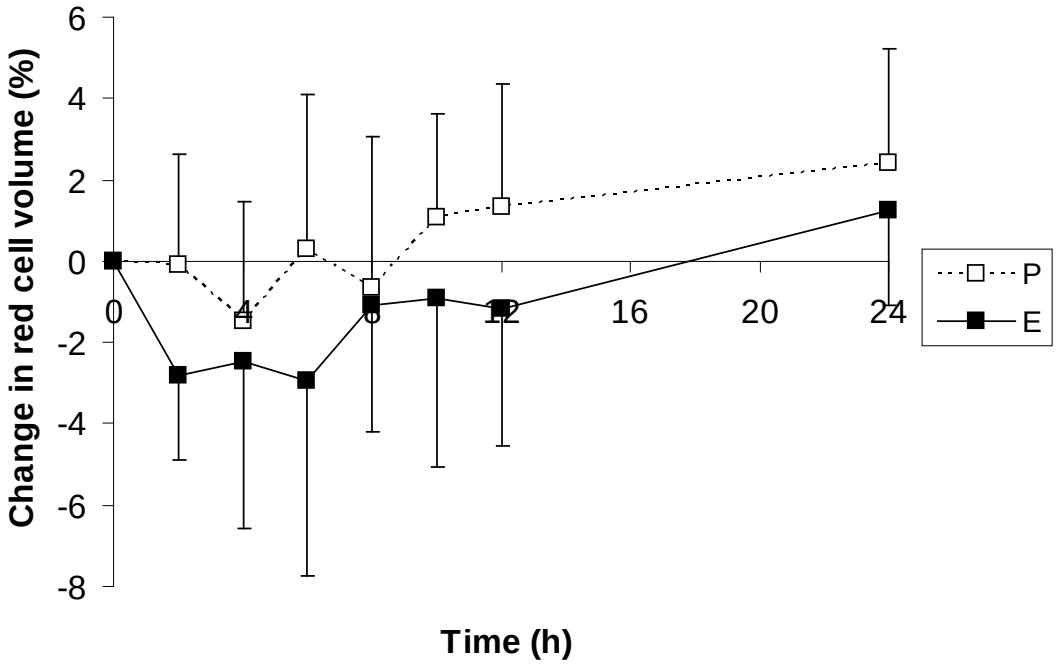

Figure 6.8 Estimated change in red cell volume from 0 h (%). Points are mean values. Error bars are SD.

Serum osmolality and electrolyte concentrations

Serum osmolality (Table 6.4a) at 6 h ($P < 0.01$) and 10 h ($P < 0.05$) and serum potassium concentration (Table 6.4b) at 4 h ($P < 0.05$) and 12 h ($P < 0.05$) where greater during trial E than during trial P. Serum sodium (Table 6.5a) and chloride (Table 6.5b) concentrations were not significantly different between trials, although there was a tendency for serum sodium concentration at 6 h ($P = 0.056$) and 12 h ($P = 0.056$) and serum chloride concentration at 6 h ($P = 0.056$) and 10 h ($P = 0.056$) to be greater during trial E compared to trial P. There were no differences in serum osmolality or serum electrolyte concentrations over time.

Table 6.5 Serum osmolality (mosmol·kg^{-1}) (a) and serum potassium concentration (mmol·l^{-1}) (b). # Significantly different from trial P. Values are mean (SD).

	0 h	2 h	4 h	6 h	8 h	10 h	12 h	24 h
a) Osmolality								
Trial E	285 (3)	287 (4)	287 (3)	288 # (2)	283 (4)	286 # (3)	288 (3)	286 (4)
Trial P	286 (4)	282 (5)	284 (5)	282 (4)	283 (5)	280 (4)	283 (5)	284 (2)
b) Potassium								
Trial E	4.3 (0.2)	4.6 (0.2)	4.7 # (0.3)	4.7 (0.4)	4.6 (0.3)	4.6 (0.4)	4.7 # (0.3)	4.6 (0.3)
Trial P	4.3 (0.2)	4.4 (0.3)	4.3 (0.1)	4.3 (0.2)	4.2 (0.2)	4.2 (0.1)	4.2 (0.1)	4.6 (0.3)

Table 6.5 Serum sodium ($mmol \cdot l^{-1}$) (a) and serum chloride concentration ($mmol \cdot l^{-1}$) (b). Values are median (range).

	0 h	2 h	4 h	6 h	8 h	10 h	12 h	24 h
a) Sodium								
Trial E	144 (137-145)	144 (139-147)	144 (142-147)	143 (142-146)	143 (140-145)	143 (140-145)	143 (142-147)	142 (140-144)
Trial P	143 (140-143)	141 (139-146)	142 (138-143)	139 (138-142)	140 (138-147)	141 (137-142)	140 (137-142)	142 (139-143)
b) Chloride								
Trial E	105 (103-108)	105 (103-109)	106 (103-107)	106 (102 108)	106 (100-107)	106 (104 109)	106 (102 108)	104 (102 108)
Trial P	104 (101-105)	102 (98-104)	102 (99-106)	102 (99-106)	102 (99-104)	102 (98-105)	103 (100-106)	103 (101-107)

Plasma aldosterone concentration

There was no difference between trials for plasma aldosterone concentration (Table 6.9) ($P = 0.371$), but concentrations did change significantly with time and compared to 0 h was reduced at 12 h ($P < 0.05$) and increased at 24 h during trial A ($P < 0.05$) and tended to be increased at 24 h during trial P ($P = 0.074$).

Table 6.6 Plasma aldosterone concentration ($pg \cdot ml^{-1}$). * Significantly different from 0 h. Values are median (range).

	0 h	2 h	4 h	6 h	8 h	10 h	12 h	24 h
Trial E	56 (12-108)	42 (23-82)	41 (9-57)	47 (28-79)	35 (10-72)	20 (9-116)	33 * (8-58)	92 * (24-171)
Trial P	50 (24-99)	61 (31-83)	50 (15-76)	44 (19-66)	41 (22-83)	21 (12-141)	41 (10-103)	97 (35-267)

Blood glucose concentration

Blood glucose concentration (Figure 6.9) was not different between trials (P = 0582) and did not change significantly over time (P = 0.147).

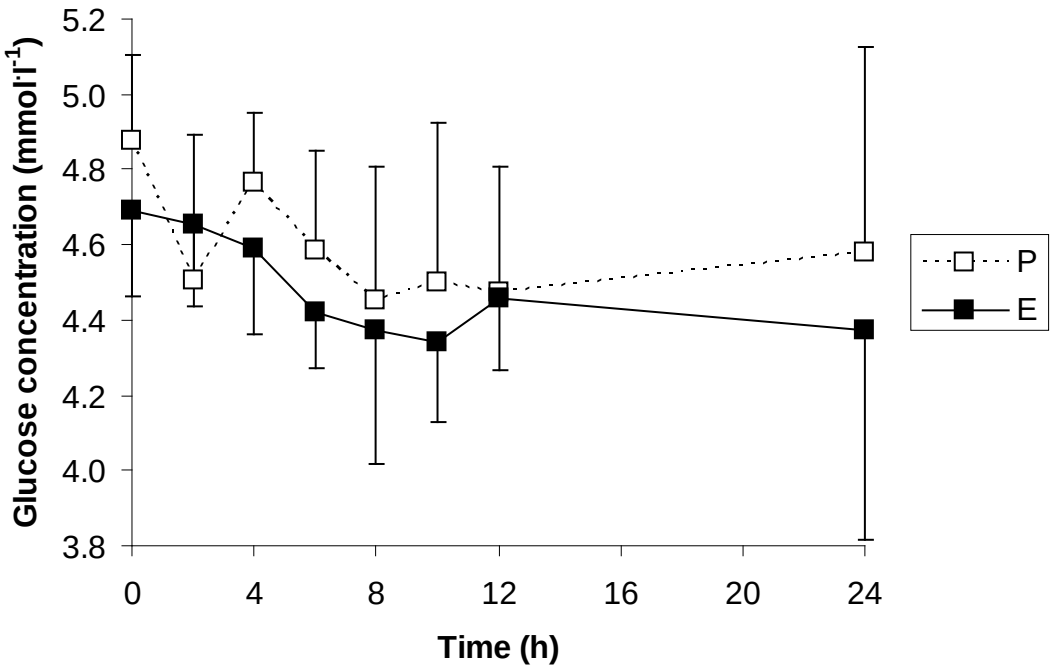

Figure 6.9 Blood glucose concentration (mmol·l^{-1}). Points are mean values. Error bars are SD.

Blood pH

Blood pH (Figure 6.10) was not different between trials (P = 0.106) and did not change significantly over time (P = 0.064).

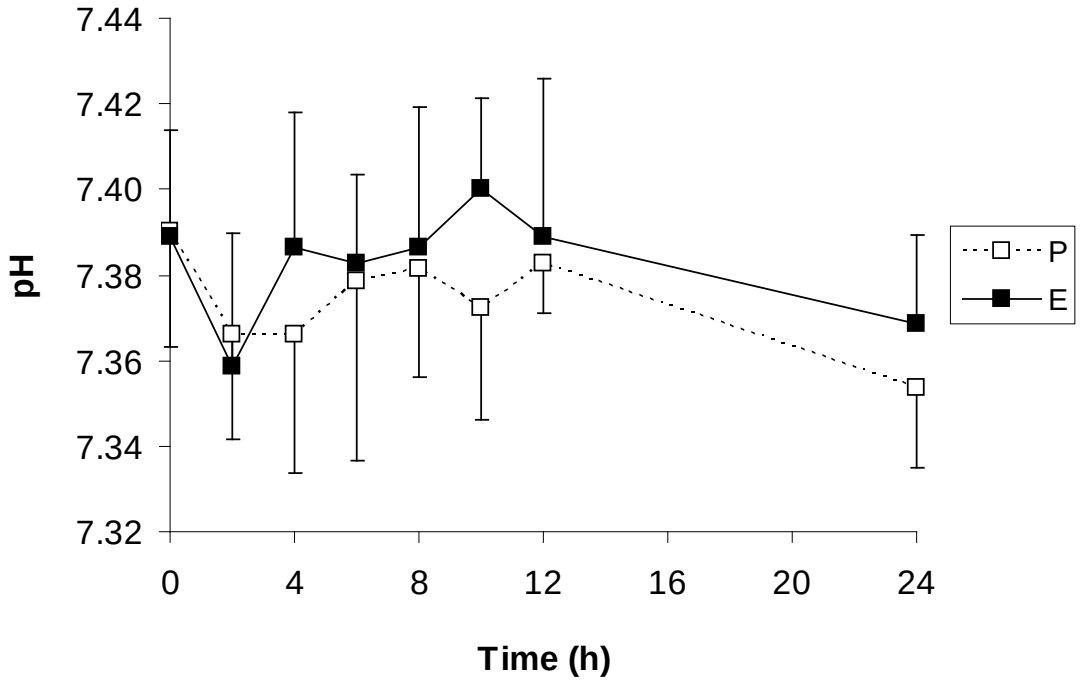

Figure 6.10 Blood pH. Points are mean values. Error bars are SD.

Discussion

The main findings of this study demonstrate that the supplementation of 165 (11) mmol sodium chloride and 60 (4) mmol potassium chloride during energy restriction attenuates the decline in serum osmolality and serum electrolyte concentrations, consequently reducing urine production and attenuating the reduction in body mass that occurs with energy restrction.

In this subject group, 24 h energy restriction, with energy restricted to 153 $kJ \cdot d^{-1}$ resulted in a BML of 1.72 (0.59) kg (2.30 (0.79) %). Supplementation of 165 (11) mmol NaCl and 60 (4) mmol KCl over the first 12 h of the 24 h period of energy restriction resulted in a 0.80 (0.44) kg reduction in the observed BML, but body mass

was still significantly reduced by 0.92 (0.44) kg over 24 h. In line with this difference in BML, total urine volume produced over the 24 h was 773 (371) ml greater during trial P compared to trial E. It appears that the difference in BML between the trials can be accounted for by the difference in urine output between the trials. These findings confirm those reported in Chapter 5 of this thesis and demonstrate that supplementation of sodium chloride and potassium chloride can attenuate the rapid and large reduction in body mass that has been reported during the initial few days of energy restriction (Taylor *et al*., 1954; Brozek *et al*., 1957; Consolazio *et al*., 1967; Consolazio *et al*., 1968a). During trial P, subjects excreted 119 (36) mmol sodium, 147 (47) mmol chloride and 57 (38-112) mmol potassium and this combined with the lack of ingestion of any of these electrolytes over the 24 h trial, meant subjects were in negative sodium (-119 (36) mmol), chloride (-140 (47) mmol) and potassium (-57 (38-112) mmol) balance at the end of the trial. This continued loss of large amounts of electrolytes in urine over the initial few days of energy restriction has been reported previously (Consolazio *et al*., 1968b; Runchie, 1971; Weisier, 1971; Chapter 3; Chapter 5). Chapter 3 of this thesis reported that during 24 h energy restriction (energy intake of 2.47 (0.40) $MJ \cdot d^{-1}$) 140 (67-186) mmol sodium, 158 (86-198) mmol chloride and 78 (15-182) mmol potassium were excreted in the subjects' urine. Chapter 5 of this thesis reported losses of 86 (33-132) mmol sodium, 107 (32) mmol chloride and 65 (34-87) mmol potassium in the urine during a 24 h trial with energy restricted to 1648 (33) $kJ \cdot d^{-1}$. Some, all be it small intake of these electrolytes occurred during the studies reported in Chapters 3 and 5 of this theses, whereas no intake occurred in the present study. Taken together, these results suggest that there is a fairly large interindividual variation in the excretion of these electrolytes during a 24 h period of severe energy restriction. Some of the factors that might influence urinary electrolyte losses during energy restriction might be: electrolyte intake during energy restriction, electrolyte intake in the days leading up to the period of energy restriction, environmental conditions during energy restriction and physical exercise during energy restriction. The supplementation of 165 (11) mmol sodium, 225 (15) mmol chloride and 60 (4) mmol potassium during trial E increased excretion of these electrolytes in the urine, but attenuated the negative balance incurred. At the end of the study, balance of sodium, potassium and chloride were less negative during trial E than during trial P by 70 (51) mmol, 99 (81) and 27 (29) mmol respectively, which agrees with results of previous studies (Consolazio *et al*., 1968b; Chapter 5). Making

the assumption that all this extra sodium was retained in the extracellular fluid and all the extra potassium was retained in the intracellular fluid and given there was no significant change in serum sodium concentration, and assuming there was no change in intracellular potassium concentration, it is possible to estimate the extra water that would be expected to be retained with these electrolytes. With a mean serum sodium concentration of 142 $mmol \cdot l^{-1}$, the extra 70 (51) mmol sodium retained during trial E would be expected to retain an additional 498 (357) ml water in the extracellular fluid compared to trial P. Similarly, assuming an intracellular potassium concentration of 150 $mmol \cdot l^{-1}$ (Maughan and Shirreffs, 1998), the extra 27 (29) mmol potassium retained during trial E would be expected to retain an additional 177 (196) ml water in the intracellular fluid compared to trial P. The total water that might be expected to be retained with the less negative sodium and potassium balance observed during trial E compared to trial P, given the assumptions discussed above would be 675 (402) ml. This estimation is very similar to the difference in urine output (773 (371) ml) between the trials.

The difference in BML between the trials (0.80 (0.44) kg) equates to 1.24 (0.60) % of subjects pre-study body mass, which is very similar to the 0.83 (0.35) kg (1.27 (0.58) %) difference in BML between the placebo and electrolyte supplementation trials in the study reported in Chapter 5 of this thesis. A number of recent studies have shown reductions in endurance exercise performance following short term (24-48 h) periods of severe or complete energy restriction (Loy *et al*., 1986; Nieman *et al*., 1987; Gleeson *et al*., 1988; Zinker *et al*., 1990; Oliver *et al*., 2007) and it is well documented that a reduction in body mass of greater than 2% might impair endurance exercise performance in temperate, warm or hot environments (Cheuvront *et al*., 2003). Whilst it is likely that the effects of energy restriction on exercise performance (Loy *et al*., 1986; Nieman *et al*., 1987; Gleeson *et al*., 1988; Zinker *et al*., 1990; Oliver *et al*., 2007) are at least partially due to the reduced energy (and thus macronutrient) intake, the hypohydration that accompanies energy (dietary) restriction might play an important role and warrants further investigation (Aragon-Vargas, 1993).

In conclusion, the restriction of energy intake to 153 $kJ \cdot d^{-1}$ results in a large loss of body mass, blood and plasma volume and the electrolytes sodium, potassium and

chloride. The supplementation of 165 (11) mmol NaCl and 60 (4) mmol KCl during the same 24 h period of energy restriction attenuates the reductions in body mass and electrolytes and maintained estimated blood and plasma volume at pre-energy restriction levels, as well as a better maintenance of serum osmolality and electrolytes, reducing urine production and BML.

Chapter 7

NaCl and KCl supplementation during 48 h severe energy restriction: effect on exercise capacity in the heat

Abstract

This study examined the effects of sodium chloride and potassium chloride supplementation during 48 h energy restriction on exercise capacity in the heat. Nine male subjects completed three 48 h trials, consisting of a control trial during which they received their estimated energy requirement (Trial C) and two energy restriction trials during which they received 33% of their estimated energy requirements (Trial P and E). During trial E, subjects received NaCl and KCl supplements in capsules and drinks to match electrolyte intakes to trial C, whilst during trial P, they received glucose placebo capsules. At 48 h, subjects performed a cycling exercise capacity test at 61 (4)% $\dot{V}O_2$ peak in a hot (35.2°C), humid (61.5% relative humidity) environment. Over the 48 h trial, body mass decreased on trial P (2.16 (0.36) kg) ($P < 0.001$) and trial E (1.43 (0.47) kg) ($P < 0.001$), but didn't change significantly from 0 h during trial C (0.39 (0.68) kg). Body mass loss at 48 h was greater for trial P than trial E and C ($P < 0.01$), as well as greater during trial E than trial C ($P < 0.01$). Plasma volume decreased during trial P ($P < 0.001$), but was maintained at pre-trial levels during trial C and trial E. Exercise capacity was greater during trial C (73.6 (13.5) min) and E (67.0 (17.2) min) than during trial P (56.5 (13.1) min) ($P < 0.01$), but was not different between trials C and E ($P = 0.237$). Heart rate during exercise, was lower during trial C and trial E than trial P at 10, 20 and 30 min ($P < 0.05$), whilst core temperature was lower during trial C and trial E than trial P at 20 min ($P < 0.05$). These results suggest that supplementation of NaCl and KCl during energy restriction prevents the reduction in exercise capacity that occurred with energy restriction alone. Supplementation maintained plasma volume at pre-trial levels and consequently prevented the increased heart rate and core temperature observed with energy restriction alone, suggesting that supplementation reduced thermal strain and thereby attenuated the decline in exercise capacity.

Introduction

The effects of short term periods of severe or complete energy restriction on exercise capacity have been well documented in the scientific literature. Energy restriction in rats has been shown to increase endurance exercise capacity, by increasing fat oxidation during exercise and thus sparing muscle glycogen (Dohm *et al*., 1983). Whilst an increase in fat oxidation has been demonstrated in humans after complete energy restriction, an improvement in endurance exercise capacity or performance has not been demonstrated (for review see Aragon-Vargos, 1993; Maughan *et al*., 2010). In fact, with the exception of two studies (Dohm *et al*., 1986; Knapik *et al*., 1988), which reported no difference in exercise capacity, studies that have investigated short term severe or complete energy restriction have shown a significant impairment of exercise capacity (Loy *et al*., 1986; Nieman *et al*., 1987; Gleeson *et al*., 1988; Maughan and Gleeson, 1988; Zinker *et al*., 1990) and performance (Oliver *et al*., 2007). These impairments of endurance exercise capacity or performance have been found whether the mode of exercise is treadmill running (Oliver *et al*., 2007; Nieman *et al*., 1987) or cycle ergometry (Loy *et al*., 1986; Gleeson *et al*., 1988; Zinker *et al*., 1990) and over a range of exercise intensities (50-100% $\dot{V}O_2$ max). All of these studies have investigated exercise capacity in a moderate environment (room temperature) and the effect of energy restriction on exercise capacity in a hot environment is unknown.

It has been shown that short term energy restriction also results in a negative balance of the electrolytes sodium and potassium, as well as a reduction in body water and plasma volume (Consolazio *et al*., 1968a; Consolazio *et al*., 1968b; Chapter 3). This negative sodium and potassium balance results from the continued excretion of these electrolytes in urine, despite little or no intake and can be off set or prevented by the supplementation of these electrolytes during energy restriction (Colsolazio *et al*., 1968b; Chapter 5; Chapter 6). The results presented in Chapter 5 and Chapter 6 of this thesis demonstrate that supplementing with sodium chloride and potassium chloride during severe energy restriction can attenuate the rapid reduction in body mass that is observed over the first 24 h of energy restriction and that blood and plasma volumes are maintained at pre-energy restriction levels (Chapter 5; Chapter 6).

The aim of this study was to investigate the effects of supplementing with sodium chloride and potassium chloride during 48 h severe energy restriction on exercise capacity at ~60% $\dot{V}O_2$ peak in a hot environment (~35°C, ~60% relative humidity) in comparison to the same 48 h period of energy restriction without supplementation, as well as to a control trial, where adequate energy was provided. An exercise intensity of ~60% $\dot{V}O_2$ peak was used because fatigue is likely to be caused by hypohydration and hyperthermia.

It was hypothesised that the supplementation of sodium chloride and potassium chloride would maintain attenuate the decline in exercise capacity caused by energy restriction.

Methods

Nine healthy males (age 25 (4) y, body mass 76.98 (4.45) kg, height 1.76 (0.08) m, $\dot{V}O_2$ peak 56.3 (6.7) $ml \cdot kg^{-1}$) volunteered to participate in this study, which was approved by the Loughborough University Ethical Advisory Committee (Reference number R08-P127).

Subjects initially completed a discontinuous incremental exercise test to volitional fatigue on an electronically braked cycle ergometer (Gould Corival 300, Groningen, Holland) to determine their peak oxygen uptake ($\dot{V}O_2$ peak). All subjects completed an initial 5 min stage at 100 W, followed by 4 min stages at progressively increasing intensities, with all subjects completing a total of 4-5 stages (17-21 min exercise). Heart rate and rating of perceived exertion (RPE) were taken 15 s before the end of each stage and were used to determine the work rate of the subsequent stage. During the final 2 min of the initial stage and final 1 min of each subsequent stage, expired air samples were collected into a Douglas bag. 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) were determined on each expired air sample.

During a second preliminary trial, subjects were familiarised with the exercise capacity test and the blood and urine sampling procedures, all of which are described in detail below.

Subjects then completed three experimental trials, which were completed in a randomised counterbalanced order and were conducted on the same days of the week, with at least 7 d separating trials. Trials were undertaken during the months of April, May, June, July and August in Loughborough, UK, with 24 h mean daily temperatures of 14.9 (3.6) °C.

For each experimental trial, subjects completed a 48 h period of dietary control and manipulation, before completing an exercise capacity test on a cycle ergometer. Subjects visited the laboratory in the morning on 3 consecutive days (0 h, 24 h and 48 h). On each of these visits, subjects arrived in 10 h overnight complete energy restricted state and voided their bladder and their nude body mass was then measured. Subjects then assumed a seated position and after 15 min seated rest, a 7 ml venous blood sample was drawn without stasis from an antecubital vein. At the 0 h and 24 h visits, subjects were provided their food and drink for the next 24 h, as well as instructions of when to consume each item and equipment to complete a 24 h urine collection (5 litre urine collection container and jug). At the 48 h visit, immediately after the nude body mass measurement and before assuming a seated position prior to blood sampling, subjects positioned a rectal thermistor (YSI 400 series, YSI Ltd., Farnborough, UK) 10 cm beyond the anal sphincter for measurement of core body temperature (T_c). Skin thermistors (YSI 400 series) were then attached at 4 sites (chest, triceps, thigh and calf) and were used to determine weighted mean skin temperature (T_{sk}) and a heart rate telemetry band (Polar Beat, Kempele, Finland) was positioned on the subject's chest. Resting core temperature, skin temperature and heart rate measurements, as well as perceived thermal comfort (TC) were taken after 15 min seated rest at room temperature (23.5 (1.3) °C, 44.6 (8.2) % relative humidity). The area of skin over the subject's right scapula was cleaned with distilled water and thoroughly dried using sterile gauze, before a small absrbent gauze patch (Tergaderm +Pad, 3M Healthcare, Loughborough, UK) was attached for the collection of sweat secreted during exercise. Subject's then entered a temperature (35.2 (0.2) °C) and humidity (61.5 (3.6) % relative humidity) controlled

environmental chamber (Weiss Gallenkamp, Loughborough, UK) and completed an exercise capacity test on an electronically braked cycle ergometer. During the exercise capacity test, subjects exercised at a predetermined fixed intensity equivalent to approximately 60% $\dot{V}O_2$ peak and continued until they could no longer maintain a pedal cadence of 60 RPM, despite verbal encouragement. 1 min expired gas samples were collected every 15 min during exercise. RPE and TC were taken every 10 min during exercise, whist core temperature, skin temperature and heart rate were measured every 10 min and at the point of fatigue. After exercise, the absorbent gauze patch was removed using forceps and the sweat was aspirated into an eppendorf tube using a 10 ml sterile syringe. After thoroughly towel drying, a final nude body mass measurement was taken. The same investigator supervised subjects during exercise for all experimental trials and verbal encouragement to continue exercise was provided to all subjects.

Dietary conditions

During the three experimental trials, three different dietary conditions of varying energy and electrolyte content were applied (Table 7.1). Subjects were provided with their food and drink for each 24 h period of dietary control during their morning visit to the laboratory (0 h and 24 h). They were provided with 4 meals and 8 drinks daily to consume at specified times. During each 24 h period, subjects consumed a meal at 0 h, 4 h, 8 h and 12 h, whilst they ingested a volume of drink equivalent to 5 $ml \cdot kg^{-1}$ body mass at 0 h, 0-4 h, 4 h, 4-8 h, 8 h, 8-12 h, 12 h and 14 h. The drinks provided were tap water, whilst drinks provided with meals contained a small amount of sugar-free black current squash. Subjects' energy requirements were determined by estimating subjects resting energy expenditure (Mifflin *et al*., 1990) and multiplying this by a physical activity level of 1.5 (light activity). The energy intake of the trials was prescribed so that subjects consumed an amount of energy equivalent to their estimated energy requirement during the control trial (C) or an energy intake equivalent to ~33% of their estimated energy requirement during the two energy restriction trials (P and E). Sodium and potassium intake was also manipulated so that subjects consumed 2.2 $mmol \cdot kg^{-1}$ body $mass \cdot d^{-1}$ sodium and 0.8 $mmol \cdot kg^{-1}$ body $mass \cdot d^{-1}$ potassium during trials C and E. This intake of electrolytes was provided in

foods consumed during each trial, with additional sodium in the form of sodium chloride in capsules consumed at 0 h, 4 h, 8 h and 12 h and additional potassium in the form of potassium chloride added to drinks and capsules consumed at 0 h, 4 h, 8 h and 12 h to provide the required electrolyte content. During trial P, the only electrolytes consumed were those provided by the foods during the trial. To determine the electrolyte content of each food item used in the study, a sample of each food was weighed and homogenised with a known amount of water, with a sample of each homogenate analysed for sodium and potassium content before the start of the study.

Table 7.1 Dietary intake between 0-24 h (a), 24-48 h (b) and the average 24 h intake over the study (c). Values are mean (SD)

	C	P	E
a) 0-24 h			
Energy (kJ)	11131 (894)	3703 (230)	3700 (229)
Protein (g)	69 (3)	21 (1)	21 (1)
Carbohydrate (g)	381(33)	130 (13)	130 (13)
Fat (g)	99 (9)	31 (1)	31 (1)
Water (ml)	3074 (181)	3085 (174)	3086 (174)
Sodium (mmol)	172 (11)	17 (1)	173 (11)
Potassium (mmol)	63 (7)	12 (1)	61 (8)
b) 24-48 h			
Energy (kJ)	11300 (888)	3683 (211)	3693 (214)
Protein (g)	69 (3)	21 (1)	21 (1)
Carbohydrate (g)	381 (33)	129 (14)	129 (13)
Fat (g)	99 (9)	31 (2)	31 (2)
Water (ml)	3074 (182)	3085 (174)	3085 (174)
Sodium (mmol)	172 (12)	17 (1)	172 (11)
Potassium (mmol)	62 (7)	12 (1)	61 (8)
c) Average			
Energy (kJ)	11301 (890)	3693 (219)	3696 (221)
Protein (g)	69 (3)	21 (1)	21 (1)
Carbohydrate (g)	381 (33)	129 (13)	130 (13)
Fat (g)	99 (9)	31 (2)	31 (2)
Water (ml)	3074 (182)	3085 (174)	3086 (174)
Sodium (mmol)	172 (11)	17 (1)	173 (12)
Potassium (mmol)	62 (7)	12 (1)	61 (8)

Analytical methods

1 ml of each blood sample was mixed with anticoagulant (K_2 EDTA, 1.75 mg·ml^{-1}) and was used for the determination of haematocrit, as well as haemoglobin and glucose concentrations.

1 ml of each blood sample was mixed with ice cold lithium heparin and used for the determination of blood pH.

2.5 ml of the remaining blood was mixed with pre-chilled anticoagulant (K_2 EDTA, 1.75 mg·ml^{-1}) and plasma was separated by centrifugation, before being frozen at -80°C. The remaining 2.5 ml blood was allowed to clot and the serum was separated by centrifugation. Plasma samples were analysed for aldosterone concentration, whilst serum samples were analysed for osmolality, as well as sodium, potassium and chloride concentrations.

Urine samples were analysed for osmolality, as well as sodium, potassium, chloride and creatinine concentrations, whilst sweat samples were analysed for sodium and potassium concentration.

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

Statistical analysis

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

Results

24 h urinary creatinine excretion was not different between trials for 0-24 h (P = 0.142) or 24-48 h (P = 0.070) and over all trials was 0.22 (0.03) mmol·kg^{-1} body mass·24 h^{-1}, and 0.24 (0.05) mmol·kg^{-1} body mass·24 h^{-1} between 24-48 h, which is indicative of a complete 24 h urine collection (Bingham and Cummings, 1985).

Pre-trial measurements

Pre-trial body mass (P = 0.855), urine osmolality (P = 0.472), serum osmolality (P = 0.342) and serum sodium concentrations (P = 0.565) were not different between trials, indicating subjects started each trial in a similar state of hydration.

BML and urine output

Baseline (0 h) body mass was 76.47 (3.76) kg (trial C), 76.67 (4.29) kg (trial P) and 76.53 (4.31) kg (trial E). BML (kg) (Table 7.2) was significant at 24 h and 48 h during P and E, but did not change significantly during C. BML was greater at 24 h (P < 0.01) and 48 h (P < 0.01) during both P and E compared to C, and was greater during P compared to E at 48 h (P < 0.01). These amounts of BML at 48 h equate to changes in body mass of -0.54 (0.91) % (C), -2.84 (0.56) % (P) and -1.89 (0.72) % (E).

Table 7.2 Body mass loss (BML) (kg), calculated as the change in body mass from 0 h. * Significantly different from 0 h. # Significantly different from trial C. † Significantly different from trial P. Values are mean (SD).

	0 h	24 h	48 h
C	0 (0)	0.26 (0.55)	0.39 (0.68)
P	0 (0)	1.24 (0.42) *#	2.16 (0.36) *#
E	0 (0)	0.86 (0.44) *#	1.43 (0.47) *#†

Cumulative urine output (Table 7.3) between 0-24 h ($P < 0.01$) and 24-48 h ($P < 0.05$) of the study was greater during P than during C. Additionally there was a tendency between 0-24 h for urine output to be greater during P than E ($P = 0.057$), as well as during E than C ($P = 0.057$). Total urine output over the 48 h study period was greater during P than during either C ($P < 0.01$) or E ($P < 0.05$), but was not different between C and E ($P = 0.178$). Additionally, urine output during E was greater between 0-24 h than between 24-48 h ($P < 0.05$).

Table 7.3 Total (0-48 h), as well as 0-24 h and 24-48 h urine output (ml). * Significantly different from 0-24 h. # Significantly different from trial C. † Significantly different from trial P. Values are mean (SD).

.

	0-24 h	24-48 h	0-48 h
C	2052 (657)	2162 (370)	4214 (975)
P	3103 (402) #	2724 (349) #	5828 (242) #
E	2488 (245)	2320 (298) *	4808 (471) #†

Urine osmolality, electrolyte excretion and electrolyte balance

There were no time ($P = 0.309$), trial ($P = 0.064$) or interaction effects ($P = 0.796$) for urine osmolality (Figure 7.7).

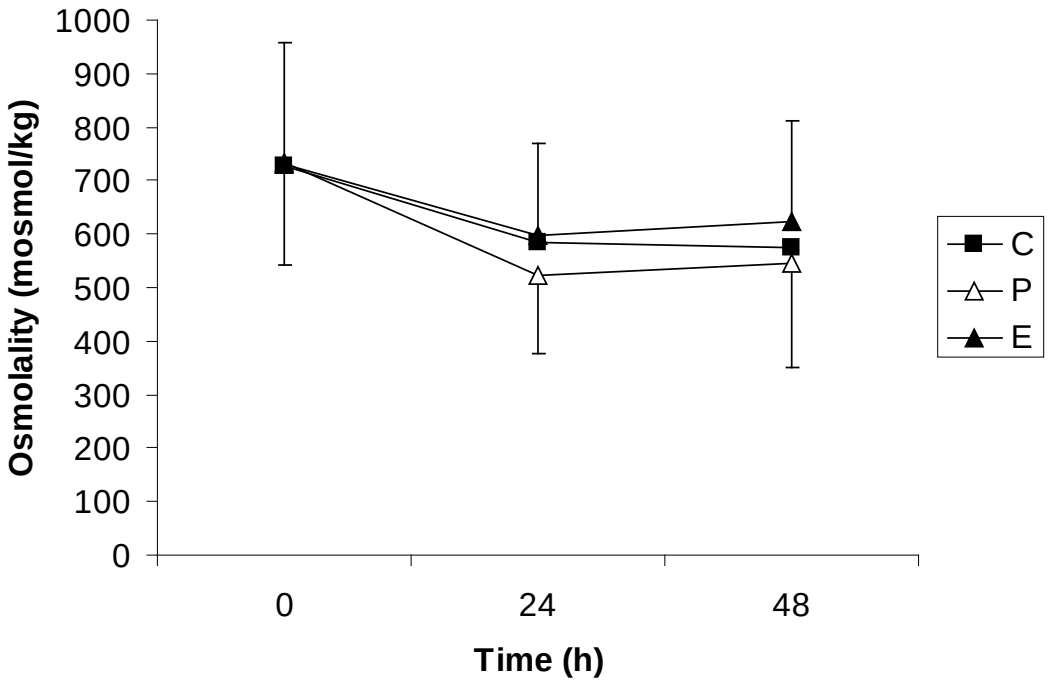

Figure 7.1 Urine osmolality (mosmol·kg^{-1}) at 0 h, 24 h and 48 h. Points are mean values. Error bars are SD.

Urine sodium excretion (Table 7.4a) was greater for trial C than for trial P between 24-48 h ($P < 0.01$) and was greater for trial E than for trial P for 0-24 h ($P < 0.05$), 24-48 h ($P < 0.05$) and over the entire study (0-48 h) ($P < 0.05$). Additionally, urine sodium excretion tended to be greater during 0-24 h for trial E than for trial C ($P = 0.061$), as well as tending to be greater during the entire study period for trial C than for trial P ($P = 0.068$). Urine sodium excretion during trial P was greater between 0-24 h than between 24-48 h ($P < 0.05$). Urine potassium excretion (Table 7.4b) was greater during trials C and E than during trial P for 0-24 h ($P < 0.05$) and over the entire study (0-48 h) ($P < 0.05$) and was greater during trial E than during trial P for 24-48 h ($P < 0.05$). Additionally, urine potassium excretion during trial C was greater between 0-24 h than between 24-48 h ($P < 0.05$). Urine chloride excretion (Table 7.4c) was greater during both trials C and E, than during trial P for 0-24 h, 24-48 h and for the whole study period (0-48 h) ($P < 0.05$). There were no differences in urine chloride excretion between trials C and E.

Table 7.4 Total (0-48 h), as well as 0-24 and 24-48 h urine sodium (mmol) (a), potassium (mmol) (b) and chloride (mmol) (c) excretion. * Significantly different from 0-24 h. # Significantly different from trial C. † Significantly different from trial P. Values are mean (SD).

	0-24 h	24-48 h	0-48 h
a) Sodium			
C	156 (49)	166 (24)	323 (51)
P	112 (44)	73 (47)* #	185 (82) #
E	197 (33)	169 (34) †	366 (50) †
b) Potassium			
C	96 (26)	80 (16) *	176 (38)
P	57 (17)#	52 (21)	109 (35) #
E	98 (28) †	84 (22) †	181 (45) †
c) Chloride			
C	174 (43)	196 (17)	370 (45)
P	84 (34)#	65 (32) #	149 (60) #
E	170 (58) †	209 (76) †	379 (107) †

Sodium balance compared to 0 h (Figure 7.8), estimated from sodium lost in urine and sodium gained from food and drink was not different at either 24 h or 48 h during trial C ($P > 0.796$) or trial E ($P > 0.187$), but was negative at both 24 h ($P < 0.001$) and 48 h ($P < 0.001$) during trial P. Sodium balance was greater at both 24 h and 48 h during trial C ($P < 0.05$) and trial E ($P < 0.01$) than during trial P. Relative to 0 h, potassium balance (Figure 7.9) was negative at both 24 h ($P < 0.05$) and 48 h ($P < 0.05$) during all trials, but was not different between trials ($P = 0.315$).

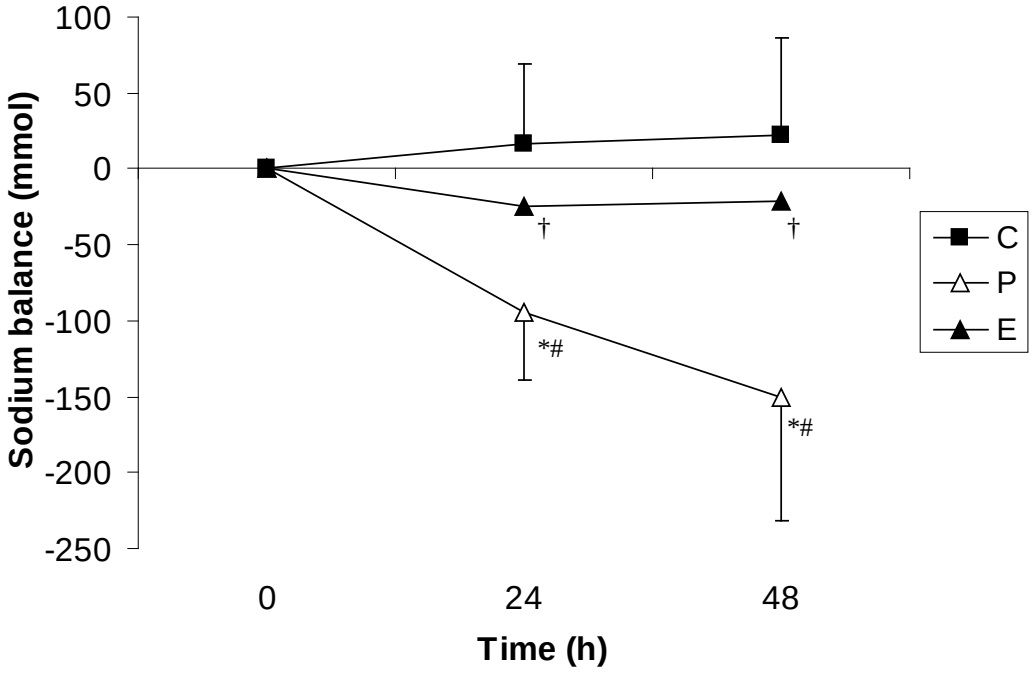

Figure 7.2 Sodium balance (mmol) calculated from urinary excretion and dietary intake. * Significantly different from 0 h. # Significantly different from trial C. † Significantly different from trial P. Points are mean values. Error bars are SD.

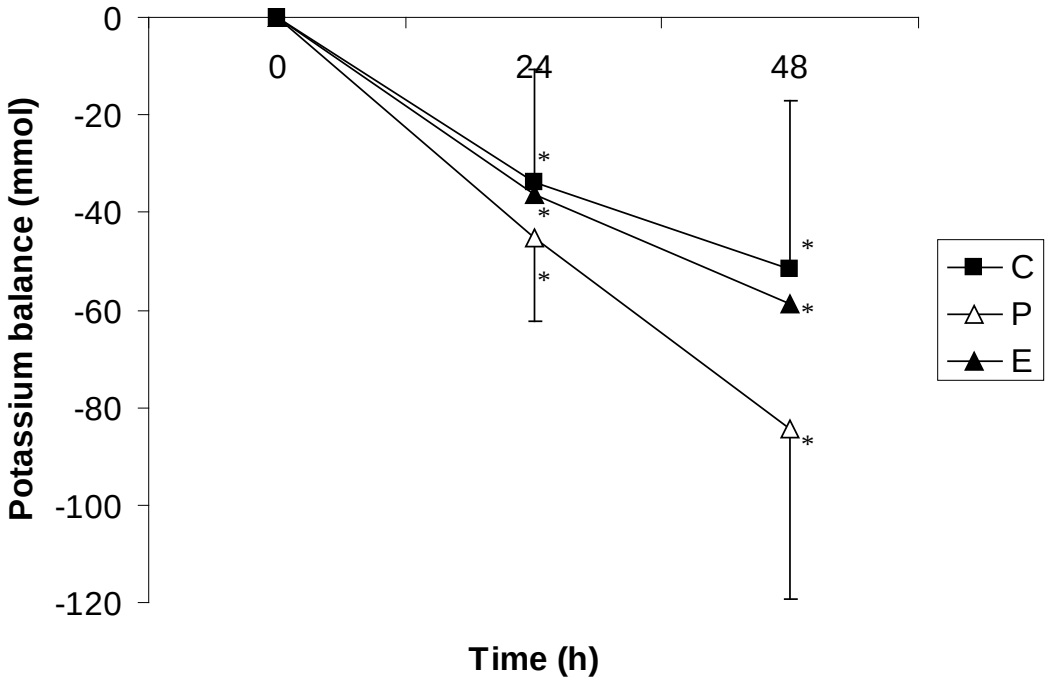

Figure 7.3 Potassium balance (mmol) calculated from urinary excretion and dietary intake. * Significantly different from 0 h. Points are mean values. Error bars are SD.

Blood, plasma and red cell volume changes

There were main effects for time ($P < 0.001$), trial ($P < 0.001$) and time by trial interaction ($P < 0.01$) for estimated changes in blood volume (Figure 7.10). Blood volume did not change ($P < 0.195$) throughout the study for trial C or E, but compared to 0 h, there was a decrease in estimated blood volume at 24 h ($P < 0.01$) and 48 h ($P < 0.001$) during trial P. Additionally, estimated blood volume was greater during trial C and E compared to trial P at 24 h ($P < 0.01$) and 48 h ($P < 0.05$). Estimated change in plasma volume (Figure 7.11) showed a similar response as blood volume with main effects for time ($P < 0.001$), trial ($P < 0.001$) and time by trial interaction ($P < 0.01$). Estimated plasma volume did not change ($P > 0.219$) throughout the study for trial C or E, but compared to 0 h, there was a decrease in estimated plasma volume at 24 h ($P < 0.01$) and 48 h ($P < 0.001$) during trial P. Additionally, estimated plasma volume was greater during trial C and E compared to trial P at 24 h ($P < 0.01$) and 48 h ($P < 0.05$). There were no main effects for time ($P = 0.891$), trial ($P = 0.617$) or time by trial interaction ($P = 0.870$) for estimated change in red cell volume (Figure 7.12).

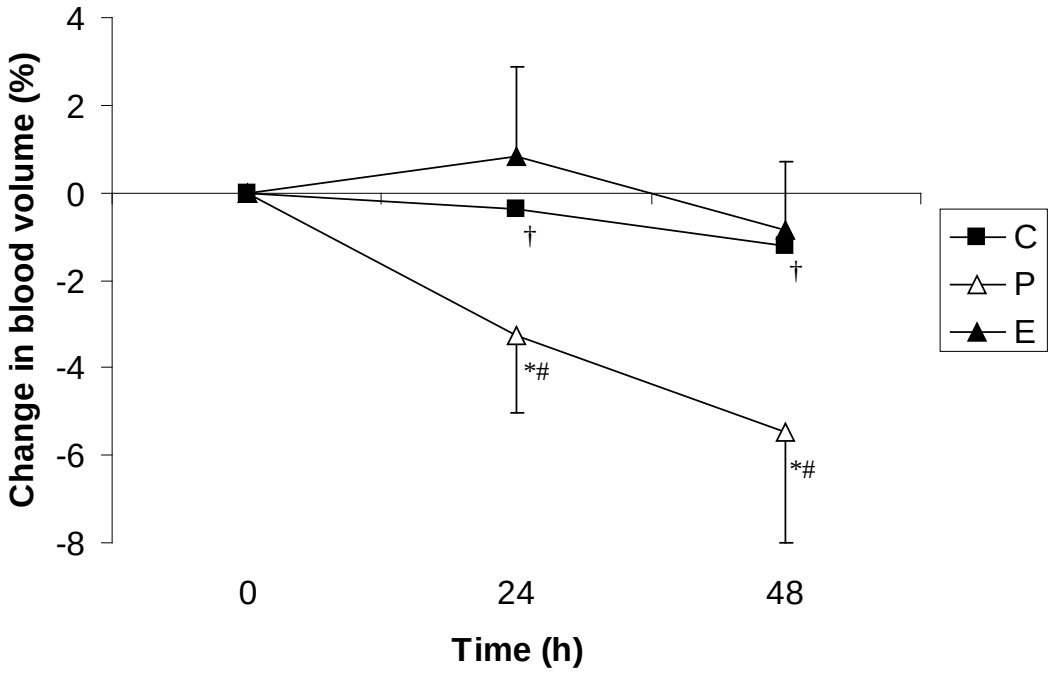

Figure 7.4 Estimated change (%) in blood volume from 0 h. * Significantly different from 0 h. # Significantly different from trial C. † Significantly different from trial P. Points are mean values. Error bars are SD.

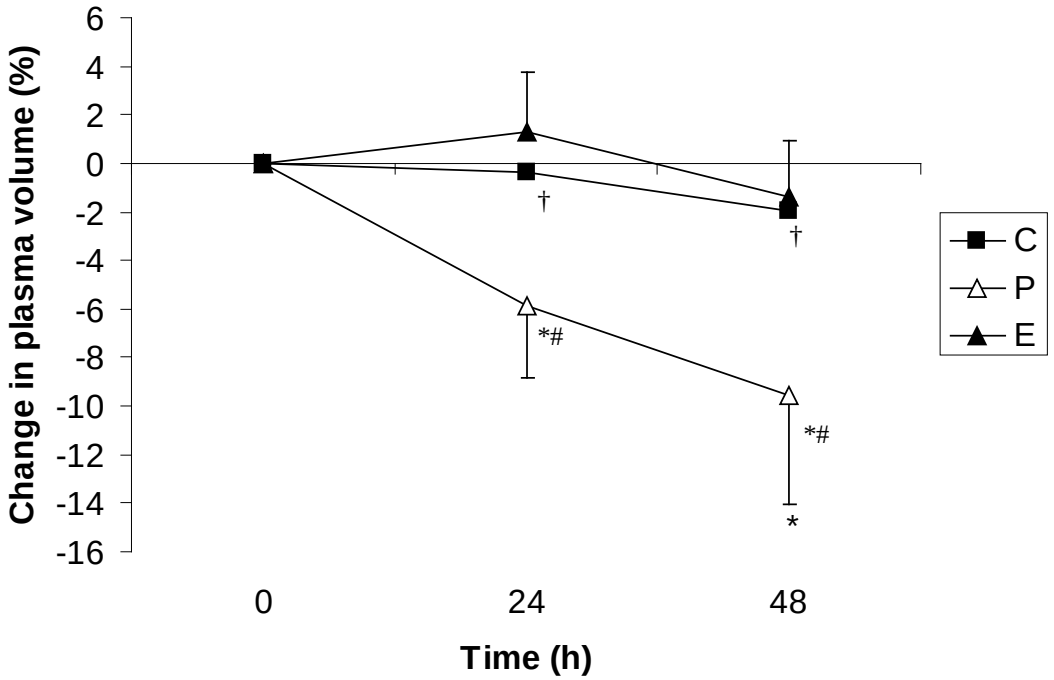

Figure 7.5 Estimated change (%) in plasma volume from 0 h. * Significantly different from 0 h. # Significantly different from trial C. † Significantly different from trial P. Points are mean values. Error bars are SD.

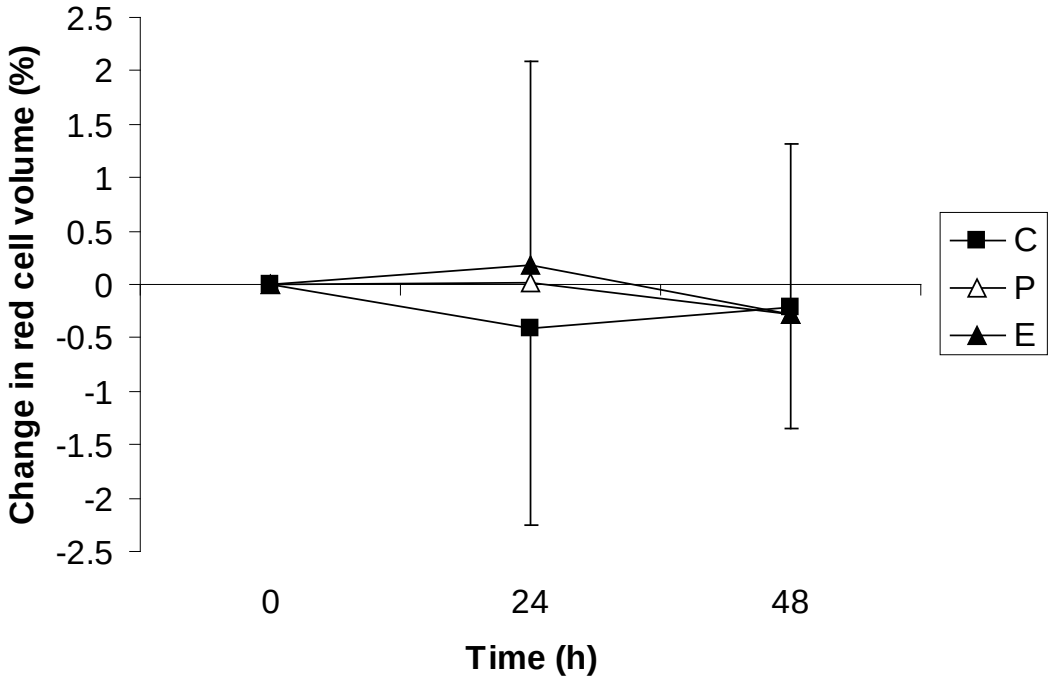

Figure 7.6 Estimated change (%) in red cell volume from 0 h. Points are mean values. Error bars are SD.

Serum osmolality and electrolyte concentrations

There were no main effects for time ($P > 0.226$), trial ($P > 0.137$) or time by trial interaction ($P > 0.092$) for serum osmolality (Table 7.5a), serum sodium concentration (Table 7.5b), serum potassium concentration (Table 7.5c) or serum chloride concentration (Table 7.5d).

Table 7.5 Serum osmolality (mosmol·kg^{-1}) (a), sodium concentration (mmol·l^{-1}) (b), potassium concentration (mmol·l^{-1}) (c) and chloride concentration (mmol·l^{-1}) (d) at 0 h, 24 h and 48 h. Values are mean (SD).

	0 h	24 h	48 h
a) Osmolality			
C	287 (5)	286 (4)	286 (6)
P	286 (4)	284 (4)	284 (4)
E	286 (3)	284 (4)	285 (2)
b) Sodium			
C	142 (3)	143 (1)	142 (2)
P	143 (2)	142 (2)	143 (3)
E	142 (2)	143 (2)	143 (1)
c) Potassium			
C	4.4 (0.3)	4.6 (0.3)	4.6 (0.3)
P	4.5 (0.2)	4.5 (0.4)	4.5 (0.3)
E	4.6 (0.4)	4.8 (0.6)	4.6 (0.3)
d) Chloride			
C	104 (2)	105 (2)	103 (3)
P	104 (3)	102 (2)	102 (2)
E	106 (3)	103 (2)	103 (3)

Plasma aldosterone concentration

There was no main effect of time (P = 0.781) for plasma aldosterone concentration (Table 7.6), but there was a main effect for trial (P < 0.05). Whilst there was no difference between the trials at 0 h (P > 0.492), plasma aldosterone concentration tended to be greater at 48 h during trial P than during trial C (P = 0.060)

Table 7.6 Plasma aldosterone concentration ($pg \cdot ml^{-1}$) at 0 h, 24 h and 48 h. Values are median (range).

	0 h	24 h	48 h
C	65 (12-111)	58 (18-132)	42 (17-108)
P	46 (42-102)	56 (32-138)	73 (45-163)
E	40 (25-113)	40 (24-61)	33 (9-134)

Blood glucose concentration

There were no main effects for time (P = 0.124), trial (P = 0.445) or time by trial interaction (P = 0.339) for blood glucose concentration (Figure 7.7).

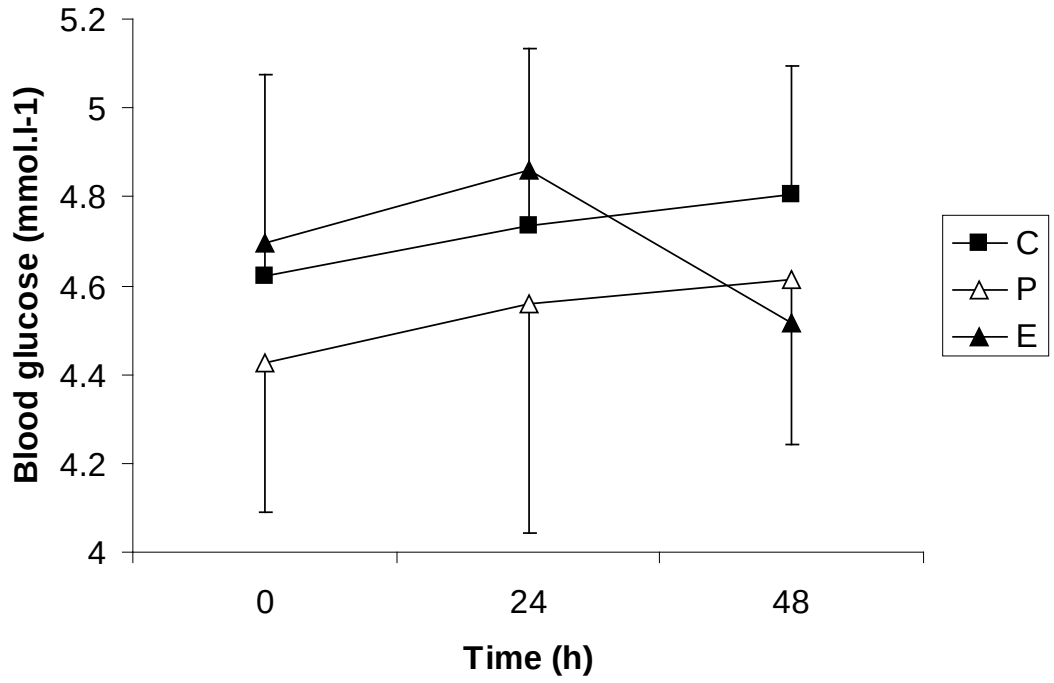

Figure 7.7 Blood glucose concentration (mmol·l^{-1}). Points are mean values. Error bars are SD.

Blood pH

There were no main effects for time (P = 0.535), trial (P = 0.913) or time by trial interaction (P = 0.361) for blood pH (Figure 7.8).

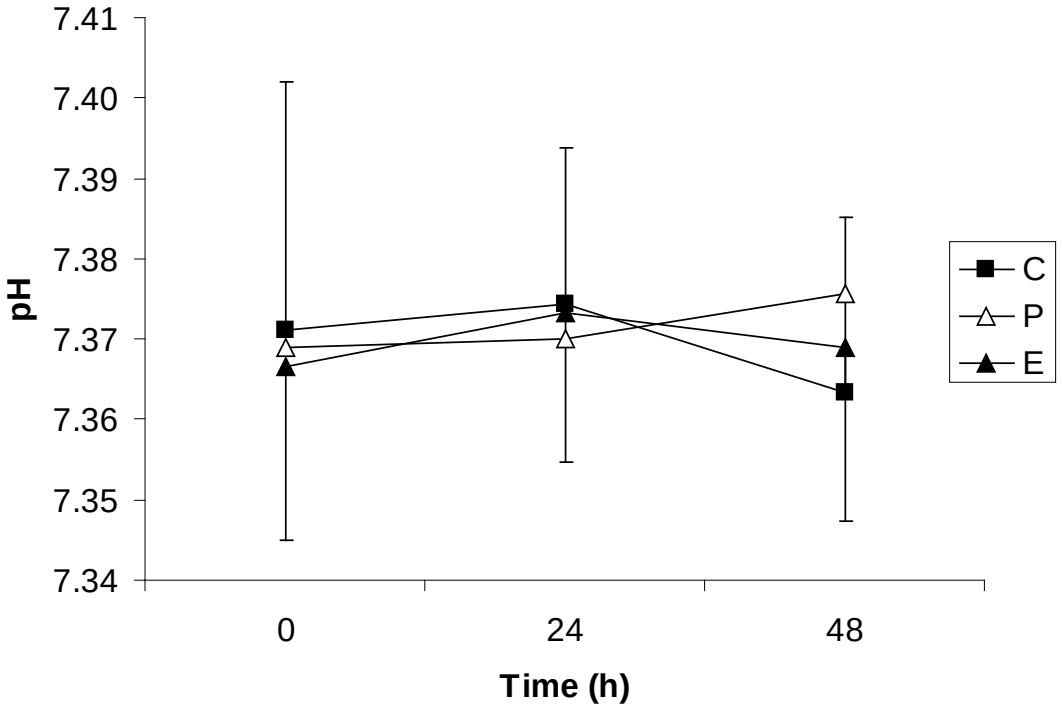

Figure 7.8 Blood pH. Points are mean values. Error bars are SD.

Exercise capacity test, thermoregulation, RPE and heart rate

The environmental temperature and humidity during the exercise capacity test was not different between trials (P = 0.164) and over all trials were 35.2 (0.2) °C and 61.5 (3.6) % relative humidity. Mean exercise intensity over all trials was 61 (4) % of $\dot{V}O_2$ peak and was not different between trials (P = 0.471) and there was no main effects of time (P = 0.541), trial (P = 0.334) or time by trial interaction (P = 0.584) for RER. Exercise capacity (Figure 7.9) was greater during trials C (73.6 (13.5) min) and E (67.0 (17.2) min) compared to trial P (56.5 (13.1) min) (P < 0.01), but was not different between trials C and E (P = 0.237).

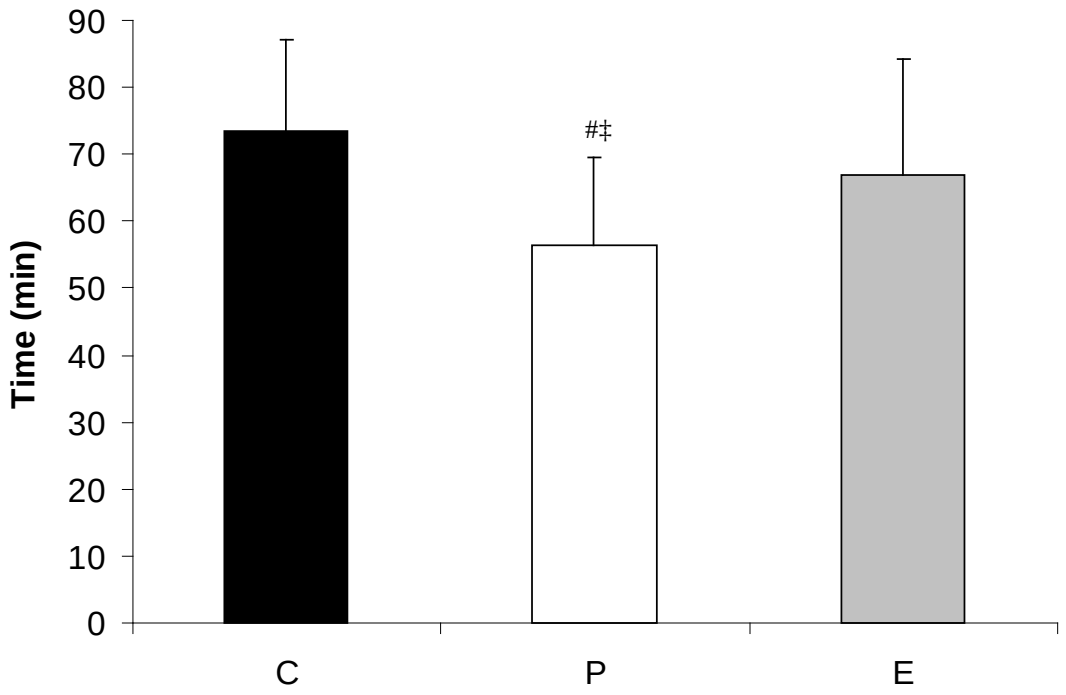

Figure 7.9 Exercise capacity (s). * Significantly different from 0 h. # Significantly different from trial C. ‡ Significantly different from trial E. Bars are mean values. Error bars are SD.

There were significant main effects of time for both T_c ($P < 0.001$) (Figure 7.10) and T_{sk} ($P < 0.001$) (Figure 7.11) during exercise. Compared to trial P, T_c was significantly lower at 20 min ($P < 0.05$) during trial C and at 20 min ($P < 0.05$) and 30 min ($P < 0.01$) during trial E, but there were no differences between the trials at exhaustion. T_{sk} was not different between trials ($P = 0.485$).

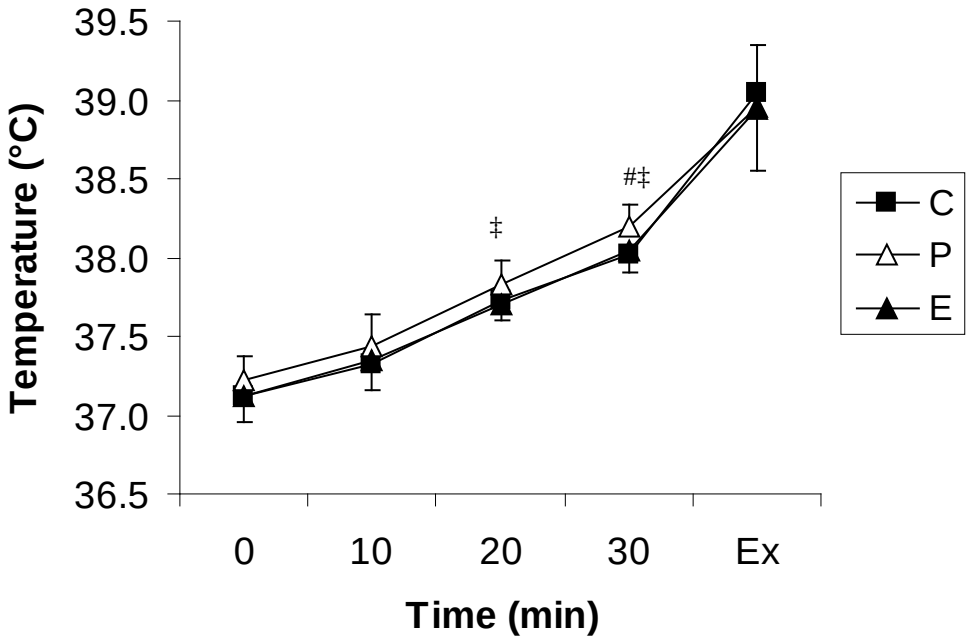

Figure 7.10 Core temperature (°C) (T_c) during exercise. * Significantly different from 0 h. # Significantly different from trial C. ‡ Significantly different from trial E. Points are mean values. Error bars are SD.

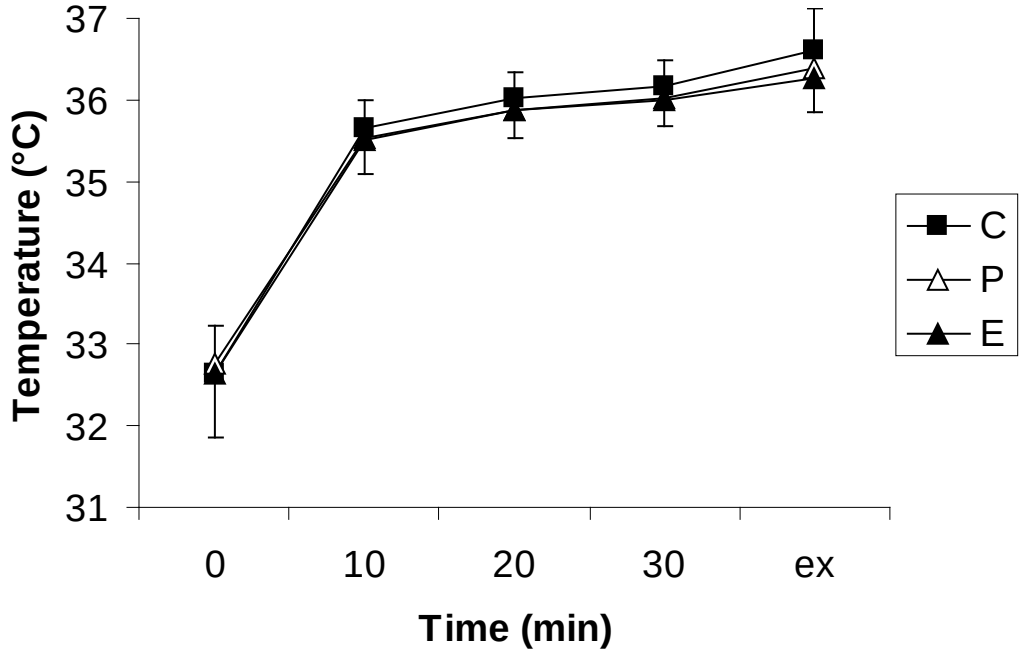

Figure 7.11 Weighted mean skin temperature (°C) (T_{sk}) during the exercise capacity test. Points are mean values. Error bars are SD.

Compared to trial C, RPE (Figure 7.12) was greater for Trials P ($P < 0.05$) and E ($P < 0.05$) at 10 min and 20 min and remained greater during trial P at 30 min ($P < 0.05$). There was no difference in perceived thermal comfort (Figure 7.13) between the trials ($P = 0.173$). Resting heart rate (Figure 7.14) was lower for trial E than trial P ($P < 0.05$) and during exercise was lower at 10 min, 20 min and 30 min for both trial C and trial E compared to trial P ($P < 0.05$).

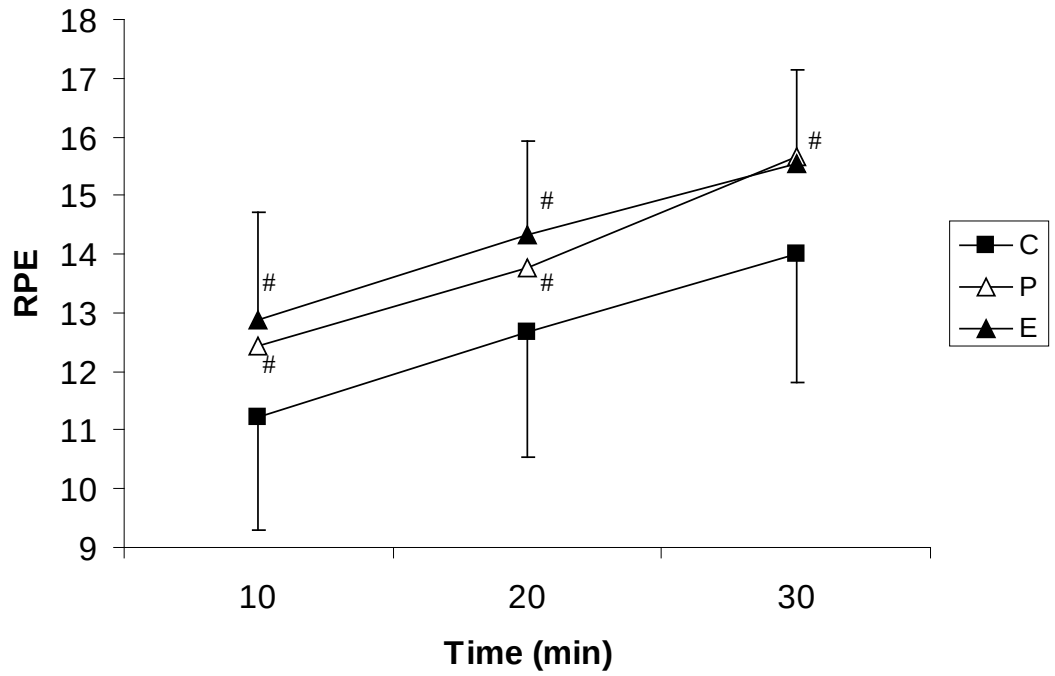

Figure 7.12 Rating of perceived exertion (RPE) during the exercise capacity test. # Significantly different from trial C. Points are mean values. Error bars are SD.

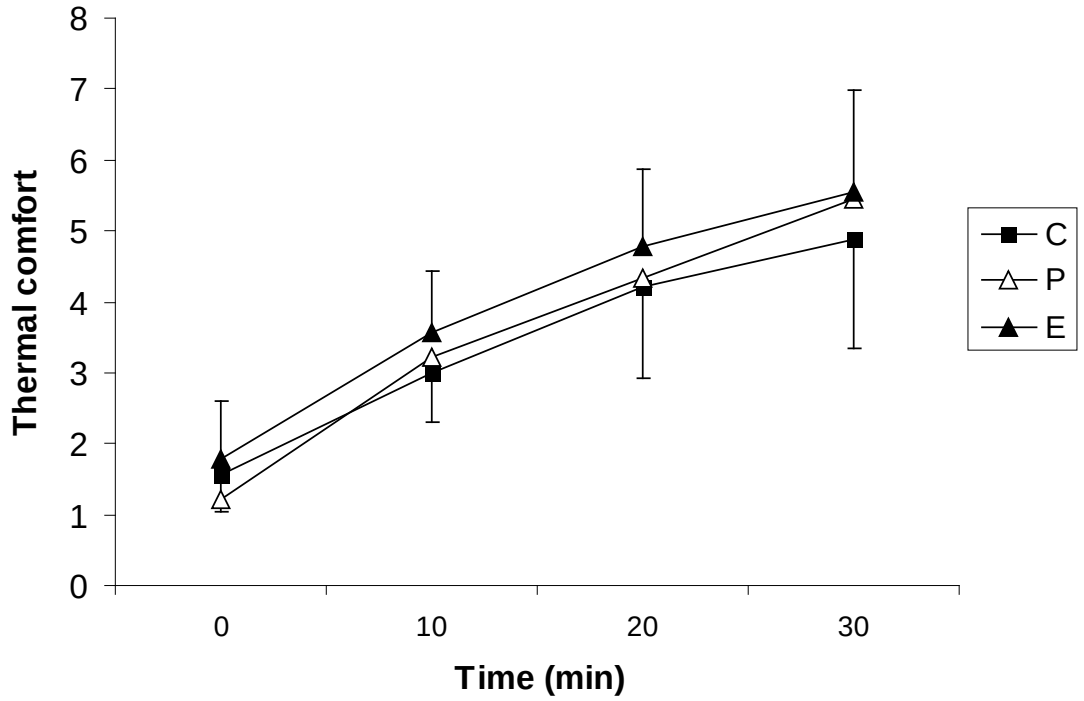

Figure 7.13 Perceived thermal comfort during the exercise capacity test. Points are mean values. Error bars are SD.

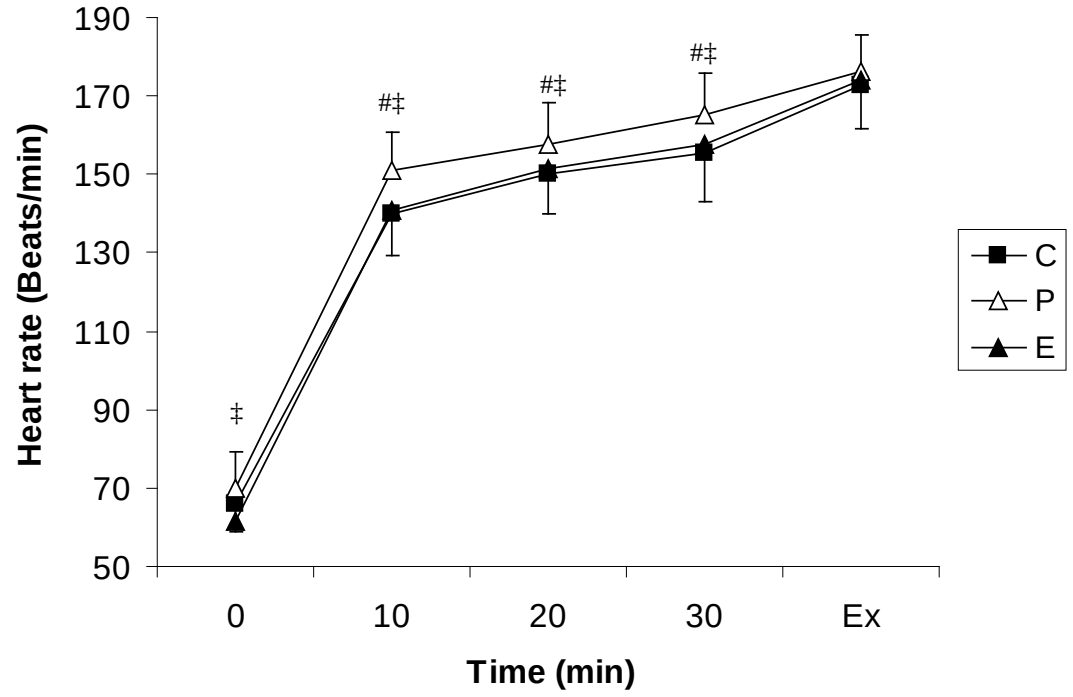

Figure 7.14 Heart rate (beats·min^{-1}) before and during the exercise capacity test. # Significantly different from trial C. ‡ Significantly different from trial E. Points are mean values. Error bars are SD.

Sweat response

Total sweat loss during exercise (Table 7.7) (determined from the change in body mass during exercise) was greater during trials C and E compared to trial P ($P < 0.01$), but was not different between trials C and E ($P = 0.103$). The difference in exercise time meant there was no difference in sweat rate between trials ($P = 0.944$). Similarly, there was no difference in sweat sodium ($P = 0.384$) or potassium ($P = 0.160$) concentration between the trials (Table 7.8).

Table 7.6 Total sweat loss (l) and sweat rate ($l \cdot h^{-1}$) during exercise. # Significantly different from trial C. ‡ Significantly different from trial E. Values are mean (SD).

	C	P	E
Total sweat loss (l)	1.67 (0.44)	1.28 (037) #‡	1.51 (0.45)
Sweat rate ($l \cdot h^{-1}$)	1.36 (0.20)	1.36 (0.21)	1.35 (0.17)

Table 7.7 Sodium and potassium concentration ($mmol \cdot l^{-1}$) of sweat secreted during exercise. Values are mean (SD).

	Sodium ($mmol \cdot l^{-1}$)	Potassium ($mmol \cdot l^{-1}$)
C	64 (9)	5.6 (0.9)
P	59 (17)	5.6 (0.9)
E	65 (14)	5.2 (0.8)

Discussion

The main findings of this study were that in comparison to a control trial, during which the subjects estimated energy requirement, was provided, the restriction of energy intake to 33 (2) % of estimated energy requirements for 48 h reduced exercise

capacity, but that supplementation with sodium chloride and potassium chloride during the same period of energy restriction prevented this decline in exercise capacity. The decline in exercise capacity with severe energy restriction is well documented, but this is the first study to demonstrate that the reduction in body water that occurs with energy restriction might play a role in the reduction in exercise capacity, and that supplementation of electrolytes that prevents the decrease in plasma volume and attenuates BML can also prevent this decline in exercise capacity, at least during exercise in the heat.

These results demonstrate that a 48 h period of energy restriction, which provided approximately 33 (2) % of subject's estimated energy requirements (3693 (219) $kJ \cdot d^{-1}$) resulted in a significant reduction in body mass of 2.16 (0.36) kg (2.84 (0.56) % of subjects initial body mass). This degree of BML is similar to that observed during 48 h energy restriction with energy restricted to 290 (20) $kcal \cdot d^{-1}$ (~1200 kJ d^{-1}) (Oliver *et al*., 2007). When subjects were supplemented with an additional 156 (11) $mmol \cdot d^{-1}$ sodium chloride and 49 (8) $mmol \cdot d^{-1}$ potassium chloride, this BML was reduced to 1.43 (0.47) (1.89 (0.72) % of the subjects initial body mass). In line with this, the volume of urine produced over the 48 h energy restriction trial was reduced when subjects were supplemented with sodium chloride and potassium chloride (trial E). Whilst the supplementation of sodium chloride and potassium chloride during trial E increased urinary excretion of these electrolytes compared to during trial P, sodium balance was greater at both 24 h and 48 h during trial E, which agrees with the results of previous studies (Chapter 5; Chapter 6; Consolazio *et al*., 1968b). The findings for a reduction in the excretion of sodium in the urine are supported by the data for plasma aldosterone concentration, which tended (P = 0.060) to be greater at 48 h during trial P than during trial C. Although there were no significant changes over time for plasma aldosterone concentration, the median values decreased over the trial during C and E, whilst increasing over trial P. The greater plasma aldosterone concentration at 48 h during P likely explains the difference in sodium excretion in the urine, via an increase in renal sodium reabsorption. As reported after 24 h energy restriction in Chapters 5 and 6 of this thesis, there was no change in serum osmolality or sodium, potassium and chloride concentrations during either energy restriction (P) or energy restriction with the supplementation of electrolytes (E), but at 48 h there was a 9.6 (4.5) % reduction in estimated plasma volume during trial P, which was

maintained at pre-trial levels during trial E (-1.4 (2.3) %). It appears that changes in serum osmolality and electrolytes occur as transient responses acutely after nutrient ingestion and that these effects are no longer present in an overnight complete energy restricted state as observed in the present study (Chapter 5; Chapter 6).

It has been shown that during a period of severe energy restriction, both body mass and plasma volume are reduced to a similar degree whether water intake is restricted or not (Oliver *et al*., 2007, Chapter 3) and that excretion of the electrolytes sodium, potassium and chloride is similar whether water is restricted or not, resulting in similar negative balances of these electrolytes (Chapter 3). Supplementation of sodium, potassium and chloride during periods of severe energy restriction has been shown to reduce urine output and prevent the reduction in blood and plasma volume associated with energy restriction (Chapter 5; Chapter 6; Consolazio *et al*., 1968a). It seems reasonable that the supplementation of sodium during trial E resulted in a more positive sodium balance, which in turn resulted in water being retained in the extracellular fluid, preventing the decline in plasma volume observed during trial P (Chapter 5; Chapter 6; Consolazio *et al*., 1968b).

It is well documented that a period of severe energy restriction reduces exercise capacity in humans, at a variety of different exercise intensities (Loy *et al*., 1986; Nieman *et al*., 1987; Gleeson *et al*., 1988; Zinker *et al*., 1990). Similarly, Oliver *et al*. (2007) demonstrated that a 48 h period of either energy restriction or combined energy and fluid restriction reduce exercise performance to a similar extent compared to a control trial (~10% and ~15% respectively).

All these studies examined exercise in a temperate environment and the results of the present study suggest that when exercise is performed in a hot environment (~35ºC, ~60% relative humidity) energy restriction also reduces exercise capacity. Exercise capacity was reduced by ~24% during trial P compared to trial C. This reduction in exercise performance was accompanied by a greater heart rate and core body temperature during trial P, suggesting that the reduction in plasma volume following energy restriction increased thermoregulatory strain during exercise. In contrast, when electrolytes were supplemented during trial E, plasma volume was maintained at pre-energy restriction levels and heart rate at rest and during exercise, as well as core

body temperature were not different to the control trial. It remains to be seen if supplementation of electrolytes during energy restriction can maintain exercise performance in temperate conditions.

The reason for a reduction in exercise performance following short term periods of severe or complete energy restriction is not fully understood, but is likely to be multifactoral in nature. Severe energy restriction results in reductions in liver glycogen (Nilsson and Hultman, 1973) and alterations in substrate availability and utilisation at rest and during exercise (Dohm *et al*., 1986; Nieman *et al*., 1987; Knapik *et al*., 1988; Maughan and Gleeson, 1988), as well as a reduction in plasma volume (Consolazio *et al*., 1968a; Oliver *et al*., 2007; Chapter 3; Chapter 5; Chapter 6) and an increase in heart rate (Dohm *et al*., 1986; Knapik *et al*., 1987; Nieman *et al*., 1987) and RPE (Nieman *et al*., 1987) during exercise.

The majority of these studies have examined the effects of complete energy restriction, whilst in the present study, subjects consumed 3693 (219) $kJ \cdot d^{-1}$ and 129 (13) $g \cdot d^{-1}$ carbohydrate. During the 48 h energy restriction period in the present study, subjects performed no physical exercise and thus the 129 (13) $g \cdot d^{-1}$ carbohydrate consumed by the subjects was likely to have been sufficient to supply the body with its obligate requirement for carbohydrate (Maughan *et al*., 2010) and to maintain liver glycogen at resting levels and prevent the ketoacidosis associated with complete energy or carbohydrate restriction (Bloom, 1967; Maughan *et al*., 2010). Given no exercise was undertaken during the period of energy restriction, muscle glycogen levels were unlikely to be affected (Maughan and Williams, 1981), a notion that is further supported by the finding that there was no difference in RER during exercise, suggesting that unlike in previous studies (Dohm *et al*., 1986; Nieman *et al*., 1987; Knapik *et al*., 1988; Maughan and Gleeson, 1988), fuel utilisation was unaffected by the imposed energy restriction. As reported previously, the present study observed a reduction in plasma volume following energy restriction (Taylor *et al*., 1954; Brozek *et al*., 1957; Consolazio *et al*., 1967; Consolazio *et al*., 1968a; Oliver *et al*., 2007) and that this plasma volume reduction was prevented by sodium chloride and potassium chloride supplementation during energy restriction (Consolazio *et al*., 1968a; Chapter 5; Chapter 6). Energy restriction also resulted in a greater heart rate and T_c during exercise compared to an energy balanced diet and that supplementation of sodium

chloride and potassium chloride during energy restriction reversed these changes, although sweat rate, sweat electrolyte composition and T_{sk} weren't different between trials. It therefore seems that the most plausible explanation for the reduction in exercise performance observed with energy restriction in the present study might be related to an increased cardiovascular strain brought about by the observed reduction in plasma volume, which appeared to lead to an increased T_c during exercise (Coyle, 2004). It is also well known that the effects of reduced body water on exercise performance are more profound in hot environments, than in cool environments (Maughan and Shirreffs, 2004). The present study demonstrates that preventing the reduction in plasma volume that occurs during energy restriction via the supplementation of sodium chloride and potassium chloride, prevents the reduction in endurance exercise capacity that occurs after energy restriction, at least when exercise is performed in a hot environment, where hypohydration has a more deleterious effect on endurance performance (Maughan and Shirerffs, 2004). It remains to be seen whether supplementing with electrolytes during a period of energy restriction to maintain pre-energy restriction plasma volume can prevent the associated decline in endurance performance in a temperate environment (Loy *et al*., 1986; Nieman *et al*., 1987; Gleeson *et al*., 1988; Zinker *et al*., 1990; Oliver *et al*., 2007).

Sweat sodium and potassium concentrations were not different between any of the experimental trials, despite a large difference in both sodium (~155 $mmol \cdot d^{-1}$) and potassium (~50 $mmol \cdot d^{-1}$) intake. Heat acclimation has been shown to reduce sweat sodium concentration (Kirby and Convertino, 1986; Robinson *et al*., 1950; Armstrong *et al*., 1985; Allsopp *et al*., 1998; Eichner, 2008), but this appears to be related to the negative sodium balance that develops due to the repeated daily loss of sodium in sweat. It has also been shown that during heat acclimation there is an interaction between dietary sodium intake and sweat sodium concentration (Robinson *et al*., 1950; Armstrong *et al*., 1985; Allsopp *et al*., 1998) and if sufficient sodium is ingested during heat acclimation to prevent a negative sodium balance developing, sweat sodium concentration does not decrease (Armstrong *et al*., 1985). Although the present investigation is complicated by the introduction of energy restriction, comparison of the two energy restriction trials allows the effects of dietary electrolyte intake on sweat composition to be examined. Whilst the difference in sodium balance between trials P and E over the 48 h trial (~130 mmol) was significant, there was no

difference in sweat sodium concentration between the trials, suggesting either that the difference in sodium balance between the trials was not large enough to produce the previously described reduction in sweat sodium concentration or that dietary sodium restriction, without heat acclimation involving exercise in the heat, does not produce the same effects on sweat sodium concentration, at least not after only 48 h.

In conclusion the present study demonstrates that the supplementation of sodium chloride and potassium chloride during a 48 h period of energy restriction (33 (2) % of subjects estimated energy requirement) prevented the reduction in plasma volume and negative sodium balance that occurred without electrolyte supplementation. During energy restriction, electrolyte supplementation also resulted in a lower T_c during exercise in the heat and significantly increased performance compared to energy restriction alone.

Chapter 8

General Discussion

Effect of severe dietary restriction on water balance in the human body

It is clear that both water and energy restriction can have a profound effect on water balance within the human body. Whilst in healthy, free living individuals water balance is regulated on a day-to-day basis within relatively narrow limits (~0.5% body mass) (Greenleaf, 1992), the restriction of either water or energy intake can reduce body water and result in hypohydration.

Although the urinary system is capable of dramatically reducing urine production, even during complete water restriction obligatory losses of water from the body persist, such that a state of negative water balance in unavoidable (Bosco *et al*., 1974; Shirreffs *et al*., 2004b; Oliver *et al*., 2007; Chapter 3). Similarly, during combined water and energy restriction, obligatory water losses continue and a hypohydrated state develops (Bosco *et al*., 1974; Oliver *et al*., 2007; Chapter 3; Chapter 4). In contrast, energy restriction results in an increase in urine production compared to a diet containing adequate energy. This increase in urine results in a decrease in plasma volume which is similar to combined water and energy restriction (Chapter 3; Oliver *et al*., 2007).

During severe fluid restriction, serum hyperosmolality is observed (Chapter 3; Shirreffs *et al*., 2004b; Oliver *et al*., 2008) and thus fluid restriction induces a hypertonic hypohydration. In contrast, combined water and energy restriction does not result in the same degree of hyperosmolality and serum osmolality appears to remain relatively unchanged compared to an overnight complete energy restricted state (Chapter 3; Oliver *et al*., 2008). Combined water and energy restriction therefore appears to result in isotonic hypohydration. With energy restriction, a large reduction in body mass, plasma volume and body water is observed over the initial few days of the energy restriction, whether water intake is allowed *ad libitum* or is prescribed. (Consolazio *et al*., 1968a; Consolazio *et al*., 1968b; Oliver *et al*., 2007; Chapter 3; Chapter 5; Chapter 6; Chapter 7) After this initial large reduction, body water appears to be lost at a far slower rate (Brozek *et al*., 1957) or maintained (Consolazio *et al*., 1968a) for the remainder of the energy restriction period. As with combined water and energy restriction, energy restriction alone results in no change in serum osmolality (Chapter 3), suggesting isotonic hypohydration.

It is likely that these differences in the type of hypohydration that develop during water and/ or energy restriction are related to electrolyte balance during the period of dietary restriction (discussed in detail below).

Effect of severe dietary restriction on electrolyte balance in the human body

As discussed above, whilst the short-term restriction of water and/ or energy augment an acute reduction in body mass and the development of a hypohydrated state, the type of hypohydration that develops is different depending on the dietary restriction that is imposed. Similarly, the effect that the different conditions of dietary restriction have on electrolyte balance also varies.

In situations where fluid alone is restricted, electrolyte balance should be unaffected (Chapter 3), although Shirreffs *et al*. (2004b) reported that completely restricting fluid intake over 37 h also produced a 29% reduction in energy intake, which is likely to impact on electrolyte balance acutely. Shirreffs *et al*. (2004b) allowed the subjects complete control of their dietary intake during fluid restriction, but restricted their food choices to dried foods only, meaning the subjects may have reduced their food intake, as their food choices were limited to food they might not habitually consume in such large amounts, without drink accompaniment. It is difficult to determine therefore whether fluid intake and hydration status per se have an effect on energy intake.

During energy restriction, the reduced intake of food results in an accompanying reduced intake of all food components (except water), including the electrolytes sodium, potassium and chloride. The continued urinary excretion of these electrolytes means that a progressively increasing negative balance of these electrolytes develops as the energy restriction period continues (Rapoport *et al*., 1965; Consolazio *et al*., 1968a; Consolazio *et al*., 1968b; Runchie, 1971; Weinsier *et al*., 1965). It is difficult to measure electrolytes lost in sweat in free living individuals, but under conditions where physical activity is minimal, these losses appear to be low (Heer *et al*., 2000) and do not appear to be greatly affected by dietary sodium intake. The balance of electrolytes in the human body in situations where sweat losses are not a factor, can be measured by dietary intake and urinary excretion (Holbrook *et al*., 1984), although

it must be noted that some, albeit a very small amount of some electrolytes are excreted in faeces and measurement of faecal electrolyte excretion would increase the accuracy. From the studies that have investigated electrolyte balance during prolonged complete energy restriction, it seems that the majority of the negative electrolyte balance that accumulates during energy restriction occurs over the first 5-10 days (Runcie, 1971; Weinsier, 1971). Although the urinary excretion of these electrolytes is greatest over the initial few days of prolonged complete energy restriction, their excretion never completely ceases and there is an obligatory loss at least for sodium and potassium for the duration of the period of energy restriction.

Recovery from periods of severe water and energy restriction

Whilst there is much research that has investigated the recovery from bouts of exercise, there is relatively little information about recovery and rehydration after periods of water and/ or energy restriction. It is intuitive that for the human body to completely recover from any situation one must know what stores or compartments have been depleted or altered. The lack of research on the area of water and/ or energy restriction means that further work is required before we have a full understanding of what is required for complete recovery from such periods of dietary restriction.

After a period of water restriction (as long as energy intake is not also restricted), recovery will solely be focussed on recovering body water stores that have been lost during the restriction period (rehydration). If electrolyte intake is sufficient, electrolyte balance should be maintained during fluid restriction (Shirreffs *et al.*, 2004). Provided energy and carbohydrate intake are sufficient and no physical exercise has taken place, liver and muscle glycogen stores should be at normal resting levels, provided glycogen stores were not depleted before fluid restriction started. The type of hypohydration that is induced by a period of fluid restriction is similar to that induced by exercise without fluid replacement, in that during both situations, hypohydration induces a reduction in plasma volume and hyperosmolality. It is likely that given that the types of hypohydration are similar and that following fluid restriction only water has been lost from the body that rehydration after fluid restriction will be dictated by the same factors that influence rehydration after exercise. The main factors that influence rehydration after exercise are the volume of

drink ingested and its composition (Shirreffs *et al*., 2004a), although the rate at which the rehydration solution is ingested might also play an important role (Archer and Shirreffs, 2001; Jones *et al*., 2010). It has been shown that after a period of fluid restriction and given free access to fluid; subjects do not ingest sufficient fluid to replace that lost during fluid restriction (Shirreffs *et al*., 2004b). Therefore, if urgent recovery is needed, the volume of drink to be ingested during recovery should be prescribed based on the amount of fluid that has been lost and would need to be a volume equivalent to approximately 150% of the body mass reduction during fluid restriction (Shirreffs *et al*., 1996). The addition of sodium to rehydration solutions ingested after exercise-induced dehydration has been shown to increase the retention of the ingested solution (Maughan and Lieper, 1995; Shirreffs and Maughan, 1998; Merson *et al*., 2008) by attenuating the reduction in plasma sodium concentration and therefore plasma osmolality and renin activity (Nose *et al*., 1988a). Other compositional factors that have been shown to increase the amount of a rehydration drink that is retained are potassium (Maughan *et al*., 1994), carbohydrate (Evans *et al*., 2009; Osterberg *et al*., 2009) and protein (Seifert *et al*., 2006), although the mechanisms for these effects have not been fully elucidated. It is likely that the same volume and compositional factors will influence drink retention after a period of fluid restriction.

The factors that will affect recovery after energy restriction of combined energy and fluid restriction are not well understood. Short term (<4 days) complete energy restriction will result in a reduction in body stores of all dietary components. The restriction of carbohydrate will initially reduce liver glycogen stores (Maughan and Williams, 1981) and then slowly muscle glycogen stores as the period of energy restriction progresses, although no data exists for the isolated effects of energy restriction on muscle glycogen levels. Therefore after a period of complete or very severe energy restriction where carbohydrate is also completely or very severely restricted, some carbohydrate will be necessary to replace the lost glycogen stores (Walberg Rankin *et al*., 1996). As water stores have been shown to be decreased whether water is restricted or not during energy restriction (Oliver *et al*., 2007; Chapter 3), water is needed for rehydration after a period of energy restriction. Energy restriction results in a negative balance of the electrolytes sodium, potassium and chloride (Consolazio *et al*., 1968b; Ruchie, 1971; Weisnier, 1971) and it is likely that

these electrolytes would need to be replaced if recovery and particularly rehydration is to be complete after a period of energy restriction. Shirreffs and Maughan (1998) demonstrated that after exercise-induced dehydration, rehydration solutions should contain enough sodium to replace that lost in sweat, if whole body net fluid balance was to be restored. Similarly, the results of the study presented in Chapter 4 of this thesis demonstrate that after a 24 h period of severe fluid and energy restriction, the addition of sodium to a rehydration solution increased the retention of the ingested solution. In contrast, whilst severe fluid and energy restriction results in a significant negative balance of potassium, the inclusion of potassium in a rehydration drink did not significantly increase fluid retention compared to an electrolyte free solution (Chapter 4).

Effect of electrolyte supplementation during energy restriction on water and electrolyte balance in the human body

As discussed above, the restriction of energy intake results in a concomitant reduction in electrolyte intake, and results in a large negative balance of the electrolytes sodium and potassium over the initial few days of energy restriction, as well as a large reduction in body water stores whether fluid is also restricted. Consolazio *et al*. (1968b) reported the effects of electrolyte supplementation during energy restriction with a between subjects design and observed that in the group that received the electrolyte supplement, the large reduction in body water that is apparent during the early of stages of energy restriction was attenuated. The results presented in Chapters 5, 6 and 7 of this thesis agree with these findings and demonstrate that when electrolytes (sodium chloride and potassium chloride) are ingested during energy restriction, the reduction in plasma volume that occurs during severe energy restriction (Consolazio *et al*., 1968a; Oliver *et al*., 2007) can be prevented. Additionally, supplementation of sodium chloride and potassium chloride can attenuate the large reduction in body mass that occurs at the onset of severe energy restriction (Taylor *et al*., 1954; Brozek *et al*., 1957; Consolazio *et al*., 1967; Consolazio *et al*., 1968a; Consolazio *et al*., 1968b; Oliver *et al*., 2007).

No differences were observed either over time or between trials for serum osmolality or for serum sodium, potassium or chloride concentration, when subjects were in an

overnight complete energy restricted state (Chapter 5; Chapter 6; Chapter 7). In contrast, over the first 12 h of energy restriction, changes over time were observed for serum osmolality, as well as serum sodium, potassium and chloride concentration (Chapter 5), and between trials for serum sodium (Chapter 5) and serum osmolality (Chapter 6). From the results presented in Chapters 5 and 6 of this thesis, it appears that supplementation of sodium chloride and potassium chloride during severe energy restriction maintains serum osmolality, as well as serum electrolyte concentrations and that transient changes occur as an acute response to water and electrolyte ingestion.

It is well known that supplementation of electrolytes during rehydration from exercise induced dehydration increases water retention, via a reduction in urine production (Nose *et al*., 1988a; Maughan *et al*., 1994; Maughan and Lieper, 1995; Shirreffs and Maughan, 1998; Merson *et al*., 2008). These studies generally involve ingesting a large volume of fluid (1500-2500 ml) over a relatively short time period (30-60 min), in comparison to the ingestion of ~3000 ml over a ~14 h period during the studies reported in Chapters 5, 6 and 7 of this thesis, but the findings are similar. Retention of a rehydration solution ingested after exercise has been shown to be linearly related to its sodium content (Maughn and Lieper, 1995; Shirreffs and Maughan, 1998). As the major cation in the extracellular fluid, the addition of sodium to a rehydration solution prevents the decline in serum sodium concentration and consequently serum osmolality that occurs after the ingestion of a large volume of a sodium free solution (Nose *et al*., 1988a). Attenuating the decline in serum osmolality prevents a decrease in circulating levels of AVP and reduces renal water losses, as more water is reabsorbed (Nose *et al*., 1988a). It is likely the same effect is apparent during energy restriction, with the supplementation of sodium chloride maintaining serum sodium concentration and consequently serum osmolality (Chapter 5; Chapter 6), thus maintaining AVP secretion (Baylis, 1987) and reducing urine production during the period of energy restriction (Consolazio *et al*., 1968a).

Similarly, the addition of potassium to rehydration solutions has been shown to increase whole body fluid retention compared to an electrolyte free solution (Maughan *et al*., 1994), although another study showed no difference (Shirreffs *et al*., 2007). It has been suggested that as the major cation in the intracellular fluid, the

addition of potassium to rehydration solutions might aid fluid retention in the intracellular space (Yawata, 1990) and this is supported by the data of Maughan *et al*. (1994) and Shirreffs *et al*. (2007), both of whom observed a slower recovery of plasma volume over the early stages of rehydration with a potassium containing solution, but no difference in urine production, indicating that extra water might have been retained in the intracellular space.

It seems likely that during energy restriction the supplementation of sodium chloride and potassium chloride maintains tissue electrolyte concentrations, resulting in a greater electrolyte balance within the body and that this retention of electrolytes retains water and consequently reduces urine production. In situations where athletes are using severe energy restriction in an attempt to rapidly reduce body mass for a competition weight category, the ingestion of sodium chloride or potassium chloride is not advisable, as it will reduce the degree of BML that occurs.

Effect of energy restriction on exercise performance

Severe or complete energy restriction lasting >24 h appears to have a detrimental effect on endurance exercise performance and this impairment of exercise performance is apparent over a variety of exercise intensities (50-100% $\dot{V}O_2$ max) and whether the mode of exercise is running or cycling. In line with the results of Oliver *et al*. (2007), who restricted energy intake to ~10% of subject's estimated energy requirements for 48 h, the study reported in Chapter 7 of this thesis demonstrates that when energy intake is restricted to ~33% of energy requirements for 48 h, exercise performance is impaired. Additionally, exercise after a period of severe energy restriction in a hot environment appears to be degraded in a manner similar to when exercise is undertaken in a temperate environment, although we have not measured exercise in a temperate environment and therefore cannot make a direct comparison. The degradation in exercise performance after a period of short-term energy restriction is likely to be due to a number of factors, including glycogen availability, acid-base status, hypohydration and the subjective response to exercise. It is likely in situations where exercise is not performed during short-term energy restriction, that muscle glycogen availability

is unlikely to limit exercise performance (Maughan and Williams, 1981), although liver glycogen availability might be limited (Nilsson and Hultman, 1973), which might impair endurance exercise performance (Hargreaves *et al*., 2004). With complete energy restriction, the production of ketoacids results in ketoacidosis and although Gleeson *et al*. (1988) observed no significant decrease in blood pH following 24 h complete energy restriction, it is likely that as the energy restriction period continues pH/ blood bicarbonate would decrease and that this might impair high intensity exercise performance (Greenhaff *et al*., 1987; Greenhaff *et al*., 1988). Consumption of ~100 $g \cdot d^{-1}$ carbohydrate prevents the increased production of ketoacids and thus during energy restriction, where no exercise is performed and a small amount of carbohydrate is consumed acid-base balance is unlikely to impair exercise performance. The most consistent finding with periods of severe of complete energy restriction is a large reduction in body water or plasma volume, which might increase cardiovascular strain during exercise (Coyle, 2004) and thus exercise performance, particularly in the heat. The data presented in Chapter 7 of this thesis supports this hypothesis as preventing the decline in plasma volume via supplementation of Sodium chloride and potassium chloride during energy restriction prevented the decline in exercise capacity in the heat observed with energy restriction alone.

Chapter 9

Conclusions and practical applications

Conclusions

The studies reported in this thesis extend previous understanding regarding the effects of periods of severe dietary restriction on fluid and electrolyte balance in humans. The main conclusions from this work can be summarised as follows:

1. A 24 h period of severe fluid and/ or energy restriction results in a significant reduction in body mass and similar reductions in plasma volume, which is most likely explained by reductions in body water of similar magnitude (Chapter 3).

2. Whilst during fluid restriction urine losses of sodium, potassium and chloride are similar to gains through dietary intake, during energy restriction urine loss far exceeds any intake through the diet. This means that during fluid restriction, balance of these electrolytes is generally maintained, but during energy restriction a significant negative electrolyte balance develops within 12 h (Chapter 3; Chapter 4; Chapter 5; Chapter 6; Chapter 7).

3. Fluid restriction results in hypertonic hypohydration, whilst energy restriction results in isotonic hypohydration and these differences are likely caused by the difference in electrolyte balance between the different treatments of dietary restrictions (Chapter 3; Chapter 5; Chapter 6; Chapter 7).

4. The supplementation of sodium chloride and potassium chloride during a period of energy restriction leads to better maintenance of the balance of sodium, potassium and chloride, as well as maintenance of plasma volume. Additionally, the large reduction in body mass observed with energy restriction is attenuated by supplementation of sodium chloride and potassium chloride (Chapter 6; Chapter 7; Chapter 8).

5. The addition of sodium chloride to a drink ingested after a 24 h period of fluid and energy restriction increases drink retention, but despite a large negative balance of potassium after fluid and energy restriction, addition of potassium chloride to a drink does not increase fluid retention (Chapter 4).

6. As observed with exercise in a temperate environment, energy restriction results in a reduction in endurance exercise performance undertaken in a hot environment, and the supplementation of sodium chloride and potassium chloride during energy restriction prevents this decline in endurance exercise performance in a hot environment (Chapter 7).

Practical applications

1. The results of the study reported in chapter 3 of this thesis demonstrate that fluid and/ or energy restriction result in divergent losses of fluid and electrolytes from the body and this might be of practical importance to athletes competing in weight category sports who's specific recovery will depend on the nature of the methods they have used to "make weight".

2. The results of the study reported in chapter 4 of this thesis demonstrate that during recovery from a period of fluid an energy restriction as is typical for weight category athletes, the retention of a rehydration solution is enhanced if sodium chloride is added to the solution, but not if potassium chloride is added to the solution.

3. The results of the studies reported in chapters 5, 6 and 7 of this thesis demonstrate that during energy restriction as typically observed in certain military operational stresses, the supplementation of sodium chloride and potassium chloride can attenuate body mass loss, maintain plasma volume and prevent a decline in exercise capacity in the heat.

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Appendix A

This appendix presents the relationship between urine osmolality and specific gravity measured on urine samples collected at laboratory visits during all trials of the study presented in chapter 3 (n=144).

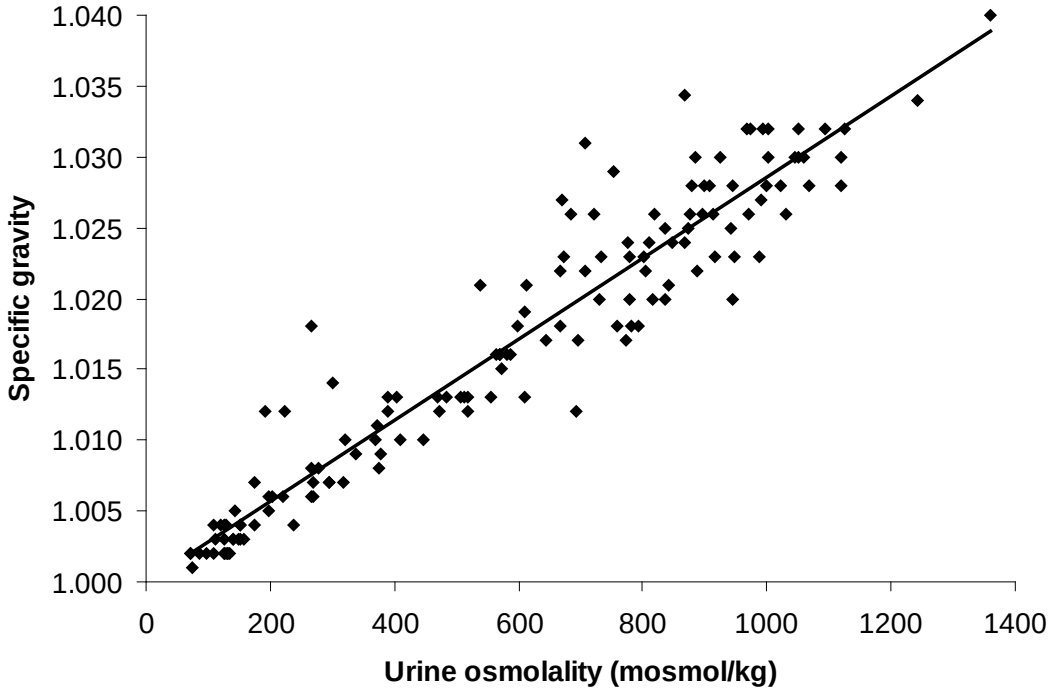

Figure A.1 Urine osmolality (mosmol·kg^{-1}) vs. urine specific gravity.

A significant correlation was observed between urine osmolality and urine specific gravity (r = 0.954, p < 0.001), resulting in a coefficient of determination (R^2) of 0.909. The equation of the linear regression model between urine osmolality and specific gravity is given below.

Equation A.1 **Equations for determining urine specific gravity (Usg) from urine osmolality (mosmol·kg^{-1}) (Uosm) (a) and for determining urine osmolality (mosmol·kg^{-1}) (Uosm) from urine specific gravity (Usg) (b) determined by linear regression analysis.**

a) $Usg = ((2.9 \times 10^{-5}) \times Uosm) + 1$

b) $Uosm = (Usg - 1) / (2.9 \times 10^{-5})$

Appendix B

Rating of perceived exertion

6	
7	Very very light
8	
9	Very light
10	
11	Fairly Light
12	
13	Fairly hard
14	
15	Hard
16	
17	Very hard
18	
19	Extremely hard
20	Maximum

Thermal comfort scale

-10	Cold impossible to bear
-9	
-8	Very cold, shivering hard
-7	
-6	Cold, light shivering
-5	
-4	Most areas of the body feel cold
-3	
-2	Some areas of the body feel cold
-1	
0	Neutral
1	
2	Some areas of the body feel warm
3	
4	Most areas of the body feel hot
5	
6	Very hot, uncomfortable
7	
8	Extremely hot, close to limit
9	
10	Heat impossible to bear

Appendix C

The data presented in this appendix is a tabulated version of the data used for Figures presented in chapter 3.

Table C.1 Urine osmolality (mosmol·kg^{-1}) of urine samples taken at 0, 12 and 24 h. Values are median (range).

	Total	0-12 h	12-24 h
C	253 (85-685)	584 (141-887)	490 (119-867)
FR	356 (72-877)	907 (598-1242)	995 (706-1359)
ER	219 (98-836)	335 (75-777)	540 (221-1119)
F+ER	150 (72-842)	852 (337-1068)	997 (753-1095)

Table C.2 Estimated change in blood volume from 0 h (%). Values are mean (SD).

	Total	0-12 h	12-24 h
C	0 (0)	-0.2 (1.5)	-0.4 (1.5)
FR	0 (0)	-0.9 (1.6)	-3.1 (1.6)
ER	0 (0)	-1.0(1.3)	-2.9 (1.3)
F+ER	0 (0)	-2.3 (1.7)	-3.1 (2.0)

Table C.3 Estimated change in plasma volume from 0 h (%). Values are mean (SD).

	Total	0-12 h	12-24 h
C	0 (0)	-0.0 (3.8)	-0.8 (3.1)
FR	0 (0)	-2.3 (3.6)	-7.3(3.2)
ER	0 (0)	-2.8 (3.2)	-6.4 (1.9)
F+ER	0 (0)	-4.9 (3.6)	-7.5 (4.5)

Table C.4 Estimated change in red cell volume from 0 h (%). Values are mean (SD).

	Total	0-12 h	12-24 h
C	0 (0)	-0.7 (1.4)	-0.2 (1.1)
FR	0 (0)	0.2 (1.5)	-0.4 (2.0)
ER	0 (0)	0.3 (2.1)	-0.9 (2.5)
F+ER	0 (0)	-0.9 (1.9)	-0.5 (1.9)

Table C.5 Blood glucose concentration ($mmol \cdot l^{-1}$) at 0 h, 12 h and 24 h. Values are mean (SD).

	Total	0-12 h	12-24 h
C	5.25 (0.78)	5.30 (0.63)	5.23 (0.65)
FR	5.28 (0.81)	5.14 (0.67)	5.17 (0.75)
ER	5.42 (0.83)	4.82 (0.48)	4.90 (0.46)
F+ER	5.33 (0.73)	4.85 (0.46)	4.92 (0.56)

Appendix D

The data presented in this appendix is a tabulated version of the data used for Figures presented in chapter 4.

Table D.1 Urine osmolality (mosmol·kg^{-1}). Values are median (range).

	P	Na	K
-26 h	306 (89-615)	242 (115-787)	257 (149-800)
-2 h	890 (623-1028)	889 (555-1048)	861 (661-1053)
-1 h	363 (235-890)	378 (226-955)	362 (165-772)
0 h	86 (43-163)	82 (54-275)	91 (53-212)
1 h	81 (53-121)	136 (67-464)	141 (58-226)
2 h	123 (69-502)	386 (98-877)	460 (142-797)
3 h	318 (78-727)	650 (249-973)	697 (140-923)
4 h	498 (121-850)	814 (415-1000)	788 (171-1009)

Table D.2 Cumulative urine output (ml). Values are mean (SD).

	P	Na	K
-2 h	0 (0)	0 (0)	0 (0)
-1 h	89 (57)	76 (40)	101 (55)
0 h	652 (285)	614 (292)	694 (254)
1 h	1152 (462)	946 (398)	1103 (352)
2 h	1394 (506)	1059 (411)	1248 (334)
3 h	1518 (546)	1113 (423)	1330 (356)
4 h	1611 (559)	1154 (436)	1391 (388)

Table D.3 Drink retention (%). Values are median (range).

	P	Na	K
0 h	62 (48-90)	63 (55-95)	60 (46-86)
1 h	32 (7-81)	43 (29-86)	39 (13-74)
2 h	21 (3-72)	38 (21-78)	33 (6-64)
3 h	15 (-5-68)	36 (18-72)	25 (2-60)
4 h	6 (-10-64)	33 (14-69)	22 (0-57)

Table D.4 Sodium balance (mmol). Values are mean (SD).

	P	Na	K
-26 h	0 (0)	0 (0)	0 (0)
-2 h	-66 (25)	-73 (33)	-61 (21)
-1 h	-65 (26)	-25 (29)	-60 (21)
0 h	-66 (28)	19 (28)	-67 (24)
1 h	-71 (30)	10 (31)	-76 (27)
2 h	-75 (31)	3 (35)	-81 (30)
3 h	-80 (33)	-4 (39)	-85 (32)
4 h	-86 (36)	-11 (42)	-89 (33)

Table D.5 Potassium balance (mmol). Values are mean (SD).

	P	Na	K
-26 h	0 (0)	0 (0)	0 (0)
-2 h	-53 (26)	-49 (25)	-43 (20)
-1 h	-54 (26)	-51 (27)	-18 (19)
0 h	-58 (28)	-55 (29)	2 (19)
1 h	-62 (29)	-59 (30)	-9 (20)
2 h	-65 (31)	-62 (31)	-17 (22)
3 h	-67 (32)	-65 (33)	-22 (23)
4 h	-70 (35)	-67 (34)	-25 (24)

Table D.6 Chloride balance (mmol). Values are mean (SD).

	P	Na	K
-26 h	0 (0)	0 (0)	0 (0)
-2 h	-90 (22)	-49 (25)	-43 (20)
-1 h	-94 (25)	-51 (27)	-18 (19)
0 h	-100 (29)	-55 (29)	2 (19)
1 h	-110 (35)	-59 (30)	-9 (20)
2 h	-116 (36)	-62 (31)	-17 (22)
3 h	-123 (38)	-65 (33)	-22 (23)
4 h	-131 (43)	-67 (34)	-25 (24)

Appendix E

The data presented in this appendix is a tabulated version of the data used for Figures presented in chapter 5.

Table E.1 Urine osmolality (mosmol·kg^{-1}) of urine samples taken at 0 h, 12 h and 24 h. Values are median (range).

	0 h	12 h	24 h
P	407 (139-704)	238 (127-336)	345 (114-775)
E	378 (103-648)	245 (202-388)	640 (312-790)

Table E.2 Estimated change in blood volume from 0 h (%). Values are mean (SD).

	0 h	12 h	24 h
P	0 (0)	-2.6 (3.0)	-5.2 (2.4)
E	0 (0)	1.2 (3.0)	-0.9 (2.7)

Table E.3 Estimated change in plasma volume from 0 h (%). Values are mean (SD).

	0 h	12 h	24 h
P	0 (0)	-4.0 (3.8)	-8.3 (2.8)
E	0 (0)	3.3 (3.5)	-0.9 (2.7)

Table E.4 Estimated change in red cell volume from 0 h (%). Values are mean (SD).

	0 h	12 h	24 h
P	0 (0)	-0.4 (2.0)	-0.8 (2.2)
E	0 (0)	1.7 (3.7)	-1.2 (1.6)

Table E.5 Blood glucose concentration ($mmol \cdot l^{-1}$) at 0 h, 12 h and 24 h. Values are mean (SD).

	0 h	12 h	24 h
P	4.58 (0.34)	4.25 (0.31)	4.25 (0.41)
E	4.40 (0.30)	4.34 (0.31)	4.32 (0.26)

Appendix F

The data presented in this appendix is a tabulated version of the data used for Figures presented in chapter 6.

Table F.1 Cumulative urine volume (ml). Values are mean (SD).

	P	E
0 h	0 (0)	0 (0)
2 h	505 (194)	319 (335)
4 h	818 (324)	557 (399)
6 h	1351 (351)	902 (516)
8 h	1647 (361)	1242 (584)
10 h	2152 (391)	1570 (623)
12 h	2437 (356)	1845 (649)
24 h	3519 (401)	2746 (594)

Table F.2 Urine osmolality (mosmol·kg^{-1}). Values are mean (SD).

	P	E
0 h	760 (217)	680 (201)
2 h	334 (182)	542 (218)
4 h	329 (112)	501 (220)
6 h	202 (47)	324 (53)
8 h	243 (57)	330 (95)
10 h	217 (76)	384 (208)
12 h	276 (136)	442 (157)
24 h	560 (280)	720 (133)

Table F.3 Sodium balance (mmol). Values are mean (SD).

	P	E
0 h	0 (0)	0 (0)
2 h	16 (13)	-17 (9)
4 h	1 (18)	-30 (15)
6 h	20 (23)	-43 (16)
8 h	-3 (27)	-53 (18)
10 h	13 (32)	-67 (21)
12 h	-14 (32)	-81 (23)
24 h	-48 (33)	-119 (36)

Table F.4 Potassium balance (mmol). Values are mean (SD).

	P	E
0 h	0	0
2 h	-9 (-19 to 3)	-16 (-45 to -6)
4 h	-13 (-28 to 7)	-24 (-60 to -10)
6 h	-17 (-32 to 9)	-32 (-76 to -16)
8 h	-20 (-30 to 11)	-36 (-88 to -18)
10 h	-24 (-43 to 14)	-40 (-94 to -23)
12 h	-26 (-54 to 18)	-43 (-98 to -26)
24 h	-43 (-69 to 19)	-57 (-112 to -38)

Table F.5 Chloride balance (mmol). Values are mean (SD).

	P	E
0 h	0 (0)	0 (0)
2 h	19 (13)	-27 (16)
4 h	5 (18)	-44 (20)
6 h	28 (23)	-59 (19)
8 h	8 (27)	-69 (21)
10 h	30 (32)	-82 (23)
12 h	7 (32)	-94 (26)
24 h	-41 (33)	-140 (47)

Table F.6 Estimated change in blood volume from 0 h (%). Values are mean (SD).

	P	E
0 h	0 (0)	0 (0)
2 h	-1.8 (2.9)	0.6 (2.8)
4 h	-0.9 (3.9)	0.6 (2.2)
6 h	-0.1 (5.0)	1.1 (2.2)
8 h	-1.3 (3.6)	1.7 (3.6)
10 h	0.7 (4.0)	1.4 (3.7)
12 h	0.8 (4.5)	0.6 (3.9)
24 h	-3.9 (1.9)	-0.8 (2.6)

Table F.7 Estimated change in red cell volume from 0 h (%). Values are mean (SD).

	P	E
0 h	0 (0)	0 (0)
2 h	-0.1 (2.7)	-2.8 (2.1)
4 h	-1.5 (2.9)	-2.5 (4.1)
6 h	0.3 (3.8)	-2.9 (4.8)
8 h	-0.6 (3.7)	-1.1 (3.1)
10 h	1.1 (2.5)	-0.9 (4.2)
12 h	1.3 (3.0)	-1.2 (3.4)
24 h	2.4 (2.8)	1.2 (2.3)

Table F.8 Estimated change in plasma volume from 0 h (%). Values are mean (SD).

	P	E
0 h	0 (0)	0 (0)
2 h	-3.4 (4.2)	3.4 (5.2)
4 h	-0.4 (5.1)	3.2 (5.4)
6 h	-0.5 (7.9)	4.5 (4.8)
8 h	-1.7 (7.1)	4.0 (7.4)
10 h	0.5 (5.9)	3.3 (6.5)
12 h	0.4 (6.1)	2.1 (7.5)
24 h	-8.8 (3.1)	-2.4 (4.8)

Table F.9 **B**lood glucose concentration (mmol·l^{-1}) at 0 h, 12 h and 24 h. Values are mean (SD).

	P	E
0 h	4.88 (0.22)	4.69 (0.23)
2 h	4.51 (0.39)	4.65 (0.21)
4 h	4.77 (0.19)	4.59 (0.23)
6 h	4.58 (0.27)	4.42 (0.15)
8 h	4.45 (0.36)	4.37 (0.35)
10 h	4.50 (0.42)	4.34 (0.21)
12 h	4.48 (0.34)	4.46 (0.19)
24 h	4.58 (0.55)	4.37 (0.56)

Table F.10 Blood pH. Values are mean (SD).

	P	E
0 h	7.39 (0.03)	7.39 (0.03)
2 h	7.36 (0.02)	7.37 (0.03)
4 h	7.39 (0.03)	7.37 (0.03)
6 h	7.38 (0.04)	7.38 (0.02)
8 h	7.38 (0.03)	7.38 (0.03)
10 h	7.37 (0.03)	7.37 (0.02)
12 h	7.38 (0.01)	7.38 (0.04)
24 h	7.35 (0.02)	7.35 (0.02)

Appendix G

The data presented in this appendix is a tabulated version of the data used for Figures presented in chapter 7.

Table G.1 Urine osmolality (mosmol·kg^{-1}) at 0 h, 24 h and 48 h. Values are mean (SD).

	C	P	E
0 h	726 (231)	733 (191)	732 (226)
24 h	586 (173)	522 (145)	545 (193)
48 h	574 (155)	598 (173)	623 (189)

Table G.2 Sodium balance (mmol) calculated from urinary excretion and dietary intake. Values are mean (SD).

	C	P	E
0 h	0 (0)	0 (0)	0 (0)
24 h	16 (54)	-94 (44)	-24 (39)
48 h	22 (64)	-150 (82)	-21 (61)

Table G.3 Potassium balance (mmol) calculated from urinary excretion and dietary intake. Values are mean (SD).

	C	P	E
0 h	0 (0)	0 (0)	0 (0)
24 h	-34 (23)	-45 (17)	-36 (32)
48 h	-52 (34)	-84 (35)	-59 (54)

Table G.4 Estimated change (%) in blood volume from 0 h. Values are mean (SD).

	C	P	E
0 h	0 (0)	0 (0)	0 (0)
24 h	-0.4 (2.0)	-3.3 (1.7)	0.8 (2.1)
48 h	-1.2 (2.0)	-5.5 (2.5)	-0.9 (1.6)

Table G.5 Estimated change (%) in red cell volume from 0 h. Values are mean (SD).

	C	P	E
0 h	0 (0)	0 (0)	0 (0)
24 h	-0.4 (1.8)	0.0 (1.3)	0.2 (1.9)
48 h	-0.2 (1.1)	-0.3 (1.1)	-0.3 (1.6)

Table G.6 Estimated change (%) in red cell volume from 0 h.. Values are mean (SD).

	C	P	E
0 h	0 (0)	0 (0)	0 (0)
24 h	-0.4 (3.1)	-5.9 (3.0)	1.3 (2.4)
48 h	-2.0 (3.3)	-9.6 (4.5)	-1.4 (2.3)

Table G.7 Blood glucose concentration ($mmol \cdot l^{-1}$) at 0 h, 24 h and 48 h. Values are mean (SD).

	C	P	E
0 h	4.62 (0.45)	4.43 (0.34)	4.70 (0.29)
24 h	4.73 (0.40)	4.56 (0.51)	4.86 (0.37)
48 h	4.80 (0.29)	4.62 (0.37)	4.52 (0.39)

Table G.8 Blood pH. Values are mean (SD).

	C	P	E
0 h	7.37 (0.03)	7.37 (0.02)	7.37 (0.02)
24 h	7.37 (0.02)	7.37 (0.03)	7.37 (0.02)
48 h	7.36 (0.02)	7.38 (0.02)	7.37 (0.02)

Table G.9 Exercise capacity (min). Values are mean (SD).

	C	P	E
Exercise capacity (min)	73.58 (13.46)	56.48 (13.06)	67.03 (17.24)

Table G.10 Core temperature (°C) before and during exercise. Values are mean (SD).

	C	P	E
0 min	37.1 (0.2)	37.2 (0.2)	37.1 (0.2)
10 min	37.3 (0.1)	37.4 (0.2)	37.3 (0.2)
20 min	37.7 (0.1)	37.8 (0.1)	37.7 (0.1)
30 min	38.0 (0.2)	38.2 (0.1)	38.0 (0.1)
Exhaustion	39.0 (0.4)	39.0 (0.4)	38.9 (0.4)

Table G.11 Weighted mean skin temperature (°C) during the exercise capacity test. Values are mean (SD).

	C	P	E
0 min	32.6 (0.6)	32.8 (0.8)	32.6 (0.8)
10 min	35.6 (0.4)	35.5 (0.5)	35.5 (0.4)
20 min	36.0 (0.3)	35.9 (0.5)	35.9 (0.3)
30 min	36.2 (0.3)	36.0 (0.5)	36.0 (0.3)
Exhaustion	36.6 (0.5)	36.6 (0.6)	36.6 (0.4)

Table G.12 Rating of Perceived Exertion during the exercise capacity test. Values are mean (SD).

	C	P	E
10 min	10 (2)	12 (1)	13 (2)
20 min	13 (2)	14 (2)	14 (2)
30 min	14 (2)	15 (2)	16 (2)

Table G.13 Perceived Thermal Comfort during the exercise capacity test. Values are mean (SD).

	C	P	E
0 min	2 (1)	1(1)	2 (1)
10 min	3 (1)	3 (1)	3 (1)
20 min	4 (1)	4 (1)	4 (1)
30 min	5 (2)	5 (2)	6 (2)

Table G.14 Heart rate (beats·min^{-1}) before and during the exercise capacity test. Values are mean (SD).

	C	P	E
0 min	66 (7)	70(8)	62 (1)
10 min	140 (11)	149 (10)	141 (1)
20 min	150 (10)	157 (10)	151 (1)
30 min	155 (12)	165 (11)	157 (2)
Exhaustion	174 (10)	176 (9)	174 (10)