The Fluid Balance of Special Populations

By
Katherine Elizabeth Black

A Doctoral Thesis

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

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ACKNOWLEDGEMENTS

Firstly I would like to thank Peter Harrison and the Peter Harrison Foundation for their support through the Peter Harrison Centre for Disability Sport.

For everyone who participated in my studies and the coaches of the teams for allowing me to work with their players, I would like to express my thanks. In particular, I would like to thank Charlie Bethel at the Great Britain Wheelchair Basketball Asssociation, Great Britain Wheelchair Rugby, Glynn Tromans at U.K. Athletics and Shane Essau and Thomas Zochowoski at the Canadian Paralympic Committee for being so enthusiastic, co-operative and helpful about my studies. I would also like to thank Maurice Slapak, the British Transplant Games and the Edinburgh Organising Committee, for making me feel welcome and for their help with subject recruitment.

Thank you to Professor Ronald Maughan and Dr Susan Shirreffs for making my time as a PhD student interesting and informative. I really appreciate all the unique opportunities that I have received whilst studying for my PhD. I have never met anyone with such vast and in depth knowledge as Professor Ronald Maughan it is truly amazing, his opinions about my work I truly hold with high regard. Dr Susan Shirreffs has consistently answered all my questions throughout my MSc and the three years as a PhD student despite being continuously busy. Her dedication and enthusiasm is inspiring. She has also been cheerful and enthusiastic about my research, and about triathlons. She has made my time as a PhD student in the lab enjoyable.

Dr John Leiper for showing me how to analyse for deuterium oxide, using the IR- machine which I wont miss. Dr Philip Watson, for his help around the lab and with analysis.

I would like to thank my fellow PhD students Dr Gethin Evans, Lewis James, Ruth Hobson, Normah Jusoh, Javad Fallah, George Amphasis and Emma Clapp for their help and company whilst in the lab and office. Especially, I would like to thank Thomas Love who has been a great friend and help over the last four years and Marie Dannhaeuser thank you is not enough.

Thanks to my family who have always been there and helped me throughout my life, I know I am very lucky. Finally Kathleen Elizabeth Pearce without whose influence I would not have had the determination to persist with my studies. The knowledge and wisdom that she gave to me has

been invaluable and without her I would not be in a position to finish my PhD. I am eternally grateful to her.

Abstract

In humans, sweating is one of the most variable routes of water loss. This thesis investigates sweat volumes and sweat composition during single training sessions. Chapter 4 investigates the variability of sweat losses and sweat composition between four training sessions. 45 able-bodied individuals (20 males and 25 females) volunteered for the study and ranged in age from 18 -65 years. Data was collected during the participants normal training sessions, consisting of various recreational pursuits. Sweat losses were determined by changes in body mass over the training session, and corrected for any fluid intake and urine losses during training. A sample of participants' sweat was obtained via adhesive gauze patches and analysed for sodium, potassium and chloride concentrations. Sweat samples were obtained from the shoulder, chest, forearm and thigh. The 95 % confidence intervals determined from this study demonstrated the reliability of a single training session to estimate an individual's future hydration to within 2 % of initial body mass.

There is little published information examining the sweat volume and sweat composition of disabled athletes. Many disabled athletes follow hydration guidelines set for able-bodied athletes, which may be inappropriate. Chapter 5 studies the sweat volumes and sweat electrolyte losses of disabled athletes. The athletes involved participated in wheelchair rugby, wheelchair basketball and sprint athletics. The participants had both neurological and musculoskeletal disabilities. Sweat losses and sweat electrolyte compositions were measured in a manner similar to those used with able-bodied athletes, except only one training session was used. Sweat rates were mean±SD 0.60±0.27 $l.h^{-1}$, a value lower than values previously reported in able-bodied athletes. Sweat composition was similar to previous values published for able-bodied athletes. Sweat sodium concentrations ranged from 30-75 $mmol.l^{-1}$.

Total body water and water turnover rates can be determined by the use of an isotopic tracer-deuterium oxide. Chapters 6 and 7 investigate the water turnover rates and of individuals with a spinal cord injury. Chapter 6 is concerned with wheelchair rugby players during competition in the heat whereas, participants in Chapter 7 were undertaking normal activities in a cooler environment. Results showed total body water relative to body mass was lower than previously found able-bodied participants, reflecting a higher relative fat mass amongst those with a spinal cord injury. Water turnover rates tended to be similar to able-bodied participants. Non- renal losses were lower, but 24 hour urine losses were higher than able-bodied participants. This indicates a lower sweat loss amongst those with a spinal cord injury. The higher urine volumes

indicating a higher fluid intake amongst those with a spinal cord injury than able-bodied individuals.

Another population who may have a different water balance from the general healthy able-bodied population, are individuals who have received a kidney transplant. Prior to kidney transplantation patients are placed on a strict diet which limits water and sodium intake. It is unclear whether this strict pre-operation diet continues after transplantation. Chapter 8 studies water turnover rates of kidney transplant recipients during the British Transplant Games. All participants were participating in events at the Transplant Games. Results showed water turnover rates and 24 hour urine volumes were at the lower end of the normal range for the kidney transplant recipients when compared to ranges seen previously in the general population. Despite this, urine osmolality values indicated the participants were euhydrated throughout the study. This suggests that following a kidney transplant ,the ingestion of sodium and water remains low.

Therefore this thesis shows that there are groups for whom water and sodium intakes and requirements differ from those set out for the general, healthy, able-bodied, population.

Key Words: Spinal Cord Injury, Kidney Transplantation, Fluid Balance, Sweat Loss, Water Turnover, Deuterium Oxide

List of Abbreviations Used Throughout This Thesis

°C	Degrees centigrade
$\dot{V}O_2$	Volume of oxygen
$\dot{V}O_2$ peak	Peak Oxygen Uptake
ANOVA	Analysis of Variance
ANP	Atrial Natriuretic Peptide
BM	Body Mass
C	Cervical
$C_6H_{12}O_6$	Glucose
CO_2	Carbon dioxide
CV	Coefficient of variation
d	Day
D_2O	Deuterium oxide
ECF	Extracellular Fluid
g	Gram
h	Hour
H_2	Hydrogen
H_2O	Water
HR	Heart Rate
ICF	Intracellular Fluid
IPC	International Paralympic Committee
IWAS	International Wheelchair and Amputee Sports
IWBF	International Wheelchair Basketball Federation
K^+	Potassium
kcal	Kilocalorie
kg	Kilogram

L	Lumbar
l	Litre
max	Maximum
min	minimum
ml	Millilitre
mmol	Millimole
$mmol.l^{-1}$	Millimole per litre
$mosmol.kg^{-1}$	Milliosmole per kilogram
Na^+	Sodium
Na^+-K^+-ATPase	Sodium- Potassium Adenosine Triphosphatase (Sodium-Potassium pump)
NaCl	Sodium Chloride
NR	Non-renal
O_2	Oxygen
p.p.m.	Parts per million
s	Second
SCI	Spinal Cord Injury
SD	Standard Deviation
T	Thoracic
TBW	Total Body Water
WTR	Water Turnover Rate
y	Year

Chapter 1: General Introduction

1.1. Reasons And Aims For This Thesis

Fluid balance is important to athletes since both hypohydration and hyperhydration have been implicated with decreased performance and impaired health. Studies concerning the sweat losses and water turnover rates of able-bodied athletes are numerous, but there is a limited amount of information regarding the fluid balance of athletes with a disability and for individuals with a kidney transplantation. The British Government suggests that the U.K. population should consume 1.2 litres of water per day and that diets should include no more than 6 g of salt (NaCl) per day (Food standards Agency, 2008). These guidelines are based on information taken from studies using the general, healthy, able-bodied, population. Campaigns to reduce salt (NaCl) intake have received much publicity in the media ('Sid the Slug') (Food Standards Agency, 2006). It is possible that there are groups in society for whom these guidelines and the associated media campaigns are not applicable either because the targets are already being met, or due to differing nutritional requirements.

Individuals with a disability and those who have received a kidney transplantation may be groups for whom nutritional requirements are different to the general, healthy, able-bodied, population. Therefore, this thesis aims to study these populations and assesses whether their fluid and salt (NaCl) balance is different to values previously published concerning healthy, able-bodied, participants.

The two most variable routes of fluid loss between individuals are via sweating and urination. Both routes of sweat loss also result in the loss of sodium and other electrolytes. Therefore, this thesis is concerned with these two routes of loss. Firstly, sweat losses during exercise are studied to determine the potential fluid and electrolyte losses via this route. However, for this study to be valid, the reliability of a single sweat testing session needed to be confirmed. Secondly, to determine the degree to which non-renal losses in the form of sweat loss affect overall fluid losses, water turnover rates were studied using deuterium oxide. This allowed for the calculation of fluid balance, establishing both water intake and losses. By using 24 hour urine volume, water losses could be used to divide daily water losses into renal and non-renal losses. Knowing the sweat losses of those with a disability helped with the understanding of non-renal losses. Analysis of 24 hour urine samples allowed the calculation of 24 hour sodium losses, which is an indicator of sodium intake.

Chapter 4 of this thesis validates the use of a single testing session to determine sweat losses, particularly sweat volume and sweat composition, of subsequent training sessions. It was important to ascertain whether results from a single session could be generalised to future training sessions. If sweat losses were similar between training sessions, then the use of a single testing session could be used to determine the sweat losses of athletes with a disability. This was especially important for the purposes of this thesis, due to the time constraints of the athletes with a disability and their training schedules.

Chapter 5 of this thesis is concerned with the sweat losses of athletes with a disability. Existing research in this area is limited, with most of the research concentrating on the sweat losses in controlled laboratory exercise sessions. Unfortunately, these situations do not reflect athletes' normal training sessions. Since inappropriate fluid intake in relation to sweat losses has been shown to be detrimental to performance (ACSM, 2008), combined with the knowledge that sweat losses are one of the most variable sources of water loss from the body, information regarding sweat losses during a typical training session is important to an athlete's performance. This study used both wheelchair sports and whole body sport since not all athletes with a disability compete in a wheelchair.

Fluid balance is concerned with both water losses and the intake of water into the body, either in the form of water or food. It is possible to determine the rate of water turnover via the use of deuterium. The total body water and water turnover rates of individuals with a spinal cord injury were studied in Chapters 6 and 7, whereas Chapter 7 studies total body water and water turnover rates of individuals who have received a kidney transplantation.. These two groups were studied since it is believed that their fluid balance may be different in comparison to the general, healthy, able-bodied population due to the nature of their injury/ illness, and the potential effects that they has on the bodies' ability to loss body water. Indeed, it has been shown that individuals with a spinal cord injury are unable to sweat below the level of the lesion (Theisen and Vanlandewijck, 2002). For individuals who have received a kidney transplantation, the implications are equally important given that the kidney is responsible for filtering blood and removing excess salt, water and waste products from the body. When renal failure occurs, individuals are placed on a strict diet limiting water and salt intake. Following transplantation, this diet may continue and water turnover rates may be affected.

This thesis intends to contribute to the understanding of fluid balance amongst individuals with a spinal cord injury and those who have received a kidney transplant.

Aims

1) To determine the reliability of a single testing session for the sweat volume losses, sweat electrolyte losses and fluid intake patterns of future training sessions of a similar nature.

2) To provide a description of the sweat losses (volume and composition) and drinking patterns of athletes with a disability during training.

3) To provide descriptive data on the fluid balance of populations whose fluid intakes and loss of fluid may differ from the healthy, able-bodied, population.

4) To provide descriptive data on the sodium losses of populations whose sodium intakes may differ from the healthy, able-bodied, population.

Chapter 2: Literature Review

2.1. Disability Sport

Sport for individuals with a disability is becoming increasingly popular and many elite athletes with a disability are now able to access funding and support from national sporting organisations. However, the specific nutritional needs of athletes with a disability are still unknown with many nutritional recommendations based on data taken from able-bodied athletes. This tactic may not provide the athletes with a disability with the most accurate information.

Sir Ludwig Guttmann first conceived the idea of sport for individuals with a disability in 1948 when he organised sports competition at Stoke Mandeville Hospital for individuals with a spinal cord injury. The first Olympic style games did not take place until 1960 in Rome and it was a further 16 years before other disability groups were added. The Games 1988 Games held in Seoul marked the first time that the Paralympics were held in the same city as the Olympics. In 2001, a joint agreement signed between the International Paralympic Committee (IPC) and the International Paralympic Committee mandated that all future host cities must agree to host both the Olympic and Paralympic Games. Indeed, London 2012 promoted the Paralympic Games as a major factor in their bid to host the Games.

There are five separate disability groups competing at the Paralympic Games: amputee, cerebral palsy, visual impairment, spinal cord injury and those who do not fit into any of the previously mentioned categories- Les Autres. In Athens 2004, 3806 athletes from 136 countries competed at the Paralympics.

Classification of Paralympic Athletes

To allow athletes with different disabilities to compete against each other on a fair basis they are grouped together depending on their degree of function due to their disability. This process is called classification (International Paralympic Committee, 2006). Classes are determined by various assessments of technical and physical capacities which vary across sports (International Paralympic Committee, 2008).

2.1.1 Spinal Cord Injury

Although there are five disability groups represented at the Paralympic Games, athletes with a spinal cord injury, likely experience the greatest disruption in their ability to

thermoregulate. This disruption causes alterations in their fluid balance and body water content. These alterations are summarises in Table 2.3.1. and Table 2.3.2.

The spinal cord consists of the spinal tracts, which are areas of white matter surrounded by central areas of gray matter. The gray matter is organised into segments comprising both sensory and motor neurons (Maynard et al., 1997).

The spinal cord nerve has 31 segments, which are divided into 5 regions, starting from the base of the skull. These are:

1. The cervical region which has 8 segments. The nerves exits the spinal column above C1 and below C1-C7.

2. The thoracic region has 12 segments. Thoracic nerves exit the spinal column below T1-T12. The sympathetic outflow to the cardiovascular centre is located within this region.

3. The lumbar region contains 5 segments. The lumbar nerves exit the spinal column below L1-L5.

4. The sacral region has 5 segments, with the sacral nerves exiting the spinal column below S1-S5.

5. The coccygeal is the base of the spine, which only has 1 segment. The coccygeal nerves exit the spinal column at the coccyx.

In a medical setting, spinal cord injuries are classified according to the American Spinal Injury Association classification system (ASIA) (Maynard et al., 1997). Injury to the spinal cord can result in the complete or partial loss of somatic, sensory and autonomic functions below the level of the lesion.

Following injury, the sacral parasympathetic outflow from the nervous system is generally affected. This outflow innervates the bladder, colon, rectum and reproductive organs (Tortora and Grabowski, 2000). Depending on the level and completeness of the lesion, there may be some disruption of the sympathetic outflow from the thoracolumbar which include the thoracic and lumbar regions of the spinal cord (Tortora and Grabowski, 2000). The sympathetic nervous system is responsible for sympathetic tone, and its activity is regulated by impulses from the hypothalamus, brain stem and parts of central nervous system and is

mediated by peripheral feedback. Since vagal parasympathetic stimulation is absent, alterations in heart rate response to exercise have been observed, with maximal heart rate reduced to approximately 130 beats.min^{-1} in individuals with tetraplegia (Valent et al. 2007). Autonomic reflexes below the lesion can occur following a spinal cord injury but, they are no longer well controlled and may be inappropriate (Theisen and Vanlandewijck, 2002). As innervation of the bladder is disrupted there may be some effect on water turnover rates. As explained later in this thesis, individuals with a spinal cord injury are at greater risk of urinary tract infection and this may alter fluid intake.

An individual with a spinal cord injury is classed as either being a tetraplegic (also known as quadriplegic) or a paraplegic, depending on the level of the lesion. The level of the lesion is classified according to neurological and sensory levels. When defining a spinal cord injury the neurological level refers to the most caudal segment of the spinal cord, with normal sensory and motor function on both sides of the body since function usually differs between sides following a spinal cord injury (Maynard et al., 1997). Following injury to the spinal cord the sensory level is the most caudal segment of the spinal cord with normal sensory function on both sides of the body (Maynard et al., 1997).

Paraplegics- "Paraplegia is the impairment or loss of motor and or sensory function in the thoracic, lumbar or sacral segments of the spinal cord secondary to damage of neural elements within the spinal canal" (Maynard et al., 1997). Individuals with paraplegia tend to have full function of their upper limbs, but have some impairment in the lower limbs.

Tetraplegics- "Tetraplegia is the impairment or loss of motor and or sensory function in the cervical segments of the spinal cord due to damage to neural elements within the spinal canal" (Maynard et al., 1997). Injury resulting in tetraplegia has more involved complications than an injury occurring lower down the spinal column. For example, individuals with a spinal cord injury above the thoracic 6th vertebra have impaired sympathetic activity to the heart and stomach region. There is also an inability to activate the sympathetic efferent component of the baroreceptor reflex. Therefore there is an inability to increase blood pressure resulting in an increased greater risk of postural hypotension due to blood pooling in the lower limbs (Mathias and Frankel, 1988).

Therefore injuries above the 6th thoracic vertebrae risk autonomic dysreflexia which is an uncoordinated spinally mediated reflex response. If this happens then hypertension occurs

and heart rate falls (Schmid et al., 2001). If this happens during exercise, there is a release of catecholamines, an increase of heart rate, increased O_2 consumption, elevated blood pressure all combining to potentially result in improved athletic performance (Schmid et al., 2001). Even though an episode of autonomic dysreflexia has the potential to result in brain damage or death, some athletes purposely induce autonomic dysreflexia to enhance performance. Schmid and colleagues (2001) have demonstrated that autonomic dysreflexia can aid performance and in simulated wheelchair marathon racing with elite racers, with performance increasing by approximately. Athletes induce these effects of autonomic dysreflexia by causing an overdistention of the bladder, by sitting on sharp objects or tightly strapping their legs. This practice is generally referred to as 'boosting' and is banned by the International Paralympic Committee (IPC). All tetraplegics are subject to random blood pressure testing at any time during IPC sanctioned competitions. If they return a systolic pressure above 180 mmHg, they are immediately banned from competing (International Paralympic Committee, 2006a).

Incomplete/complete Injuries – Tetraplegic and paraplegic injuries are further categorised as being complete or incomplete. In some cases, there can be a partial preservation of sensory and/ or motor function below the neurological level of injury. If this sensation or motor control continues to the lowest sacral segment, then the injury is considered to be incomplete (Maynard et al., 1997). However, if no sensation or motor control is present at the lowest sacral segment, then the injury is defined as being complete (Maynard et al., 1997).

Secondary diseases associated with a spinal cord injury

There is a higher risk of secondary diseases such as urinary tract infections, pressure sores, hyponatraemia and obesity amongst the spinal cord injured population (Theisen and Vanlandewijck, 2002; Buchholz and Bugaresti, 2005). Indeed, coronary heart disease is the major cause of morbidity and mortality amongst the spinal cord injured population (Theisen and Vanlandewijck, 2002; Buchholz and Bugaresti, 2005). Both urinary tract infections and hyponatraemia have been linked to fluid intake (Manz and Wentz, 2005; Soni et al., 1994). When combined with the knowledge that obese individuals have lower total body water relative to body mass than lean individuals (Cardús et al., 1984), it is argued that these secondary diseases may affect water turnover rates, levels of hydration and total body water values for the spinal cord injured population.

In a review of the literature, Buchholz and Bugaresti (2005) found that percent body fat is 8-18 % greater in individuals with a spinal cord injury when compared to age, height and/or weight matched controls. Body fat percentage was determined using by dual energy X-ray absorptiometry (DXA), isotope dilution in a cross sectional study. A decrease in lean mass accompanies this increase in body fat. Other studies have measured body mass index as a classification of overweight/ normal weight in groups with spinal cord injury. Values for body mass index amongst those with a spinal cord injury have been reported as 20 to 27 this is inconsistent with the values reported for percent body fat (Buchholz and Bugaresti, 2005). The discrepancy between the values for percent body mass and body mass index probably reflects the difficulties in measuring height in those with a spinal cord injury and the unique decrease in lean mass. Therefore the use of body mass index to determine obesity is inappropriate amongst those with a spinal cord injury. There is often an underestimation of body fat in men with a spinal cord injury by as much as 9.4 % when using body mass index as a measure (Jones et al., 2003). There is also an alteration in the electrolytes – sodium and potassium – within total body water, accompanied by an alteration in their distribution within the extracellular and intracellular fluid (Claus-Walker and Halstead, 1981). Following spinal cord transaction the intracellular potassium concentrations in gastrocnemius muscle cells have been studied in rats and dogs, with results showing that paralysed cells had a lower potassium concentration and higher sodium concentrations compared to the muscle cells prior to transaction (Claus-Walker and Halstead, 1981). Exchangeable sodium is increased following a spinal cord injury and there is also an increase in the extracellular space but the increase in the extracellular space is still within the normal physiological range (Claus-Walker and Halstead, 1981).

Following a spinal cord injury properties of the venous vascular system alter in paralysed muscles. Hopman et al. (1994) investigated the differences between venous vascular system of male paraplegics and male able-bodied controls. They found that venous capacity in the left calf was significantly lower by approximately 50 % amongst paraplegics than able-bodied males as measured by a strain gauge occlusion plethysmography (Hopman et al., 1994). Hopman and colleagues (1994) thus posit that vascular flow resistance was higher amongst the group with paraplegia than amongst the able-bodied group (Hopman et al., 1994). Circulating blood volume is further affected by blood pooling in the lower limbs following a spinal cord injury, Raymond et al. (1997) examined the differences between upper body arm cranking and electrically stimulated lower body cycling and deduced from differences in $\dot{V}O_2$

and heart rate that their was a decrease in venous return during upper body exercise amongst paraplegics (T4-T12). Further the pooling of blood in the lower limbs was due to the lack of a skeletal muscle pump (Raymond et al., 1997). The study by Hopman et al. (1994) also demonstrated greater vascular resistance and atrophy in the lower limbs of individuals with a spinal cord injury when compared to able-bodied individuals.

2.2 The Importance Of Hydration

Water plays a vital role in the proper operation of many bodily functions by contributing to the maintenance of blood volume and acting as a medium for biochemical reactions. These actions contribute to the proper functioning of all the body's organs and facilitate osmolar equilibrium within and between cells. These roles allow for the optimal performance of the cardiovascular system and contribute to the maintenance of the internal homeostasis (Sawka and Montain, 2000; Sawka and Pandolf, 1990; Kay and Marino, 2000).

2.2.1 Distribution Of Body Water

A healthy human body is composed mainly of water; muscle mass is approximately 75 % water whereas adipose tissue is only 10 % water (Sawka, 1992). For average healthy male adult water represents about 60 % of the total body mass. Females generally have a greater percent of their body mass which is fat mass wiith water contributing 55 % of their body composition (Sawka, 1992). Therefore, a healthy able-bodied male weighing 75 kg would contain 45 litres of water, and a healthy 60 kg female would contain 33 litres of water (Sawka, 1992). Individuals with a spinal cord injury tend to have lower total body water relative to body mass than those of able-bodied participants (Cardús et al., 1984). This lower total body water was approximately 10 -15 % for paraplegics and tetraplegics when compared to age matched controls and has been attributed to both a decrease in muscle mass and a loss of muscle cells following spinal cord injury (Cardús et al., 1984). Castro et al. (1999) studied the muscle atrophy of the calf following spinal cord injury from 6 weeks after injury until 24 weeks after injury in 15 men. Between weeks 6 to 24 there was a 20-56 % decline in the cross sectional area of the type 1, 11a. and 11ax +11x (Castro et al., 1999). The greatest decrease came in weeks 6-11 (~22 %) compared to the smaller decrease seen in weeks 11-24 (~10 %) (Castro et al., 1999). This decrease tends to stabilise after 12-17 months (Olive et al., 2003). At 6 weeks after injury fibre size is approximately 60 % smaller than seen for able-bodied men. 24 weeks after injury fibre size is also reduced to approximately a third of able-bodied controls matched for age and body mass (Castro et al., 1999). A year after injury, femoral

artery cross sectional diameter is approximately 40 % lower than in able-bodied controls and a difference is still present when differences in muscle mass are accounted for (Olive et al., 2003).

Body water is distributed between the intracellular and extracellular fluid spaces. Extracellular space contains the interstitial fluid and the plasma. The intracellular space of an average 75 kg healthy male contains 30 litres of water, while the extracellular space contains 15 litres (Sawka, 1992). A third of total body water is thus, contained within the extracellular fluid space, and two thirds within the intracellular fluid space. Cardús et al. (1984) predicted intracellular volumes for tetraplegics (body mass 72.1±5.7 kg) and paraplegics (70.1±5.6 kg) by regression equations and then measured by tritium oxide and radiosulfate. They reported that there was a relative decrease in the volume of the intracellular space with values being approximately 10 litres lower than predicted values for tetraplegics and 5 litres lower for paraplegics. Further, an expansion of the extracellular space was observed, with corresponding values approximately 5 litres greater than predicted for tetraplegics and paraplegics (Cardús et al., 1984). Able-bodied participants (body mass 80.7±12.1 kg) in the same in the study by Cardús et al. (1984) had extracellular values which were 2 litres greater than predicted and intracellular values which were 2 litres lower than predicted. Thus extracellular fluid accounted for 42 % and 45 % of total body water for the paraplegics and tetraplegics respectfully. Further, intracellular volumes were 58 % and 55 % for paraplegics and tetraplegics (Cardús et al., 1984). It has been theorised that individuals with a spinal cord injury have an altered membrane function and muscle cells which may allow for an abnormal amount of electrolytes to accumulate in the extracellular space (mainly sodium), which may in turn account for the larger extracellular space (Cardús et al., 1985). To maintain homeostasis the accumulation of sodium results in an increase in the volume of water within the extracellular space.

Body water has an ability to redistribute itself within, and between, the extracellular and intracellular fluid compartments (Mallie et al., 2002). This ability to redistribute water between the fluid compartments is important when fluid losses resulting in water deficits occur, since redistribution minimises the effects of dehydration to be minimised (Mallie et al., 2002). It also means that both the intracellular and extracellular spaces can be affected by any degree of water loss (Sawka, 1992). When water losses are low, the greatest loss comes from the extracellular fluid. However, as water deficit increases, the proportion of water that is lost from the

intracellular fluid is increased (Costill et al., 1976). Costill et al. (1976) dehydrated 8 healthy able-bodied male participants by ~2, 4 and 6 % of their body mass (or 3, 6 and 9 % of total body water) (initial body mass 57.4-80.6 kg) via cycling exercise in the heat (39.5 °C, 25 % relative humidity). The results showed that plasma provided 10 % of the water loss for each stage of dehydration. At 2 % dehydration 60 % of the water loss came from the interstitial fluid and 30 % from the cellular water (Costill et al., 1976). However, at 4 % of body mass dehydration approximately 10 % was from plasma volume, 38 % from interstitial fluid and 52 % from intracellular fluid (Costill et al., 1976). At 6% of body mass loss 11 % of the water loss was from the plasma volume, 39 % from the interstitial waters and 50 % of the water loss came from the intracellular fluid (Costill et al., 1976). The body's ability to redistribute fluid is due to both the movement of sodium, the major electrolyte of the extracellular fluid and potassium, the major electrolyte of the intracellular compartment. Therefore, sodium and potassium concentrations determine both the extracellular and intracellular volumes respectively (Mallie et al., 2002).

2.2.2 Body Water Balance

Euhydration occurs when the body is in fluid balance. Under normal conditions, body water is controlled around this state (Greenleaf, 1992). It has been suggested that when resting in temperate environments, body water is controlled so that it is maintained within 0.22 % of euhydration (Greenleaf, 1992). In the average male (75 kg) with total body water of 45 litres, this would mean that body water is maintained within ±0.10 litres. For this equilibrium to be maintained, body water losses must equal body water gains. However, this is not always the case. Sometimes water is lost from the body at a greater rate than it is replaced. The process of losing body water is known as dehydration and results in hypohydration. When this loss from normal body water is replaced to restore euhydration, it is called rehydration. The addition of water to the body above euhydration levels resulting in greater than normal body water leads to hyperhydration.

The body is able to monitor and control body water by monitoring osmolality and blood volume. An increase in osmolality and decrease in blood volume results in a secretion of vasopressin which acts to conserve water via reabsorption in the kidneys. A decrease in osmolality results in an increased secretion of Atrial Natriuretic Peptide resulting in an increased excretion of water via urination. This is described in further detail in Section 2.3.3.

2.3 Body Water Losses

The body loses water through a variety of different routes. Insensible water loss includes the loss of water through the skin as well as respiratory water losses. Insensible water losses in a healthy able-bodied man are around 0.9 $l.d^{-1}$ (Silverthorn, 2001). This volume differs depending upon the temperature and humidity of the inspired air. Hall and Klemm (1963) measured the insensible water losses of clothed subjects at temperatures between 22-32 °C. They found that insensible water losses were only slightly increased (from 21 $g.min^{-1}$ to 26.5 $g.min^{-1}$) between temperatures of 22-26 °C. There was a more marked increase between temperatures of 26.7-32.2 °C from 26.5 $g.min^{-1}$ to 64 $g.min^{-1}$ .Due to the low ambient vapour pressure at altitude it is assumed the insensible water losses are increased (Westerp, 2001). Further to this large volumes of water are lost into the gastrointestinal tract each day, but faecal water losses have been reported to be approximately 0.1 $l.d^{-1}$ (Silverthorn, 2001). Therefore since faecal losses are low in a healthy human most of the water lost into the gastrointestinal tract must be reabsorbed further down the gastrointestinal tract. All of these routes of water loss are passive. However, the two major routes of water loss from the body are active processes. These routes are urinary losses and losses that occur through sweating, both of which are controlled by complex physiological systems.

2.3.1 Body Water Loss Via Urine

When at rest in a temperate environment, urine is the major source of water loss. Under these conditions, the control of urine loss is responsible for the maintenance of euhydration. The regulation of urine output is controlled by the kidney. In a healthy human, the kidney is able to produce urine ranging in osmolality from less than 50 $mosmol.kg^{-1}$ at its most dilute, to 1200 $mosmol.kg^{-1}$ at its most concentrated (Silverthorn, 2001).

2.3.2. Kidney Regulation Of Urine Output

A healthy human has two kidneys which are located in the superior lumbar region. The kidneys extend from around the level of the twelfth thoracic vertebra out to the third lumbar vertebra, with the rib cage offering some protection. Individuals who have received a kidney transplant only have one functioning kidney and it is usually located in the abdomen above the iliac crest (Danovitch, 2004). On average, a healthy human kidney weighs approximately 150 grams, is 12 cm long, 6 cm wide and 3 cm thick (Silverthorn, 2001). The lateral surface is convex, whilst the medial surface is concave. The kidney can be split into two layers: an inner

medulla and an outer cortex (Johnson and Bryne, 2003). A typical glomerular filtration rate for the kidneys is approximately 125 $ml.min^{-1}$; therefore based on this glomerular filtration rate the total volume of blood that can be filtered by the kidneys is around 7.5 $l.h^{-1}$, or 180 $l.d^{-1}$ (Schrier et al., 1999).

When considering the implications of kidney functioning for individuals with a spinal cord injury, findings by Ragnarsson et al. (1981) suggest that tetraplegics appear to have a significantly lower glomerular filtration rate (Ragnarsson et al., 1981). This is likely due to a decrease in blood volume and a reduction in renal blood flow. A decrease in glomerular filtrate rate may result in an altered urine excretion rate, and can cause differences in urine solute load which is illustrated in Table 2.3.1. Although the reabsorption of water in the kidney is also controlled by hormonal factors, there is no published literature to date on the effect of this reduced filtration rate on reabsorption.

A proper functioning kidney removes excess water and waste from the body via urination. Maximal urinary flow is approximately 1-2 $l.h^{-1}$ (Schrier et al., 1999). The functions of the kidney are regulated by the endocrine system, in particular vasopressin (antidiuretic hormone) and aldosterone alterations.

Nephron

Within the kidney, the nephrons are responsible for filtering the blood. This is the site where the volume of urine output is controlled.

Within the two kidneys, there are approximately two million functioning nephrons arranged in a parallel orientation. Approximately 80 % are located in the renal cortex, called cortical nephrons. The other 20 % cross into the medulla, and are called juxtamedullary nephrons (Silverthorn, 2001). Each nephron is an S-shaped, hollow tube with a membrane wall composed of highly specialised epithelial cells. The lumens of the nephron are about 20 μm in diameter and approximately 2 cm long (Seeley et al., 2003).

Water reabsorption from the kidney is a complex process since it is coupled with the reabsorption of solutes. Depending on the segment of nephron, this reabsorption is either active or passive. Active transport is primarily facilitated by sodium- and potassium-activated adenosine triphosphatase (Na^+-K^+- ATPase) (Knepper and Burg, 1983). Passive reabsorption occurs down an osmotic gradient, which is dependent on the state of water and

electrolyte homeostasis within the body. In the initial stages of the nephron sodium, transport is coupled with the transport of other secondary solutes, but in the final segments of the nephron the transport of sodium to the maintain homeostasis occurs (Knepper and Burg, 1983). Thus, the nephrons are important in the maintenance of homeostasis with respect to both water and electrolyte balance.

Renal Corpuscle and the Glomerulus

The flow through the glomerulus is driven by blood pressure which also provides the force for the ultrafiltration of fluid across the glomerular barrier, into Bowman's capsule and the renal tubules (Seeley et al., 2003). The capillary walls of the renal corpuscle are highly permeable, allowing the filtration of plasma to occur (Seeley et al., 2003). The filtrate flows from the renal corpuscle into the renal tubules where it is modified and the resultant urine which is produced that flows via the collecting ducts into the bladder. During this process, approximately 120 $ml.min^{-1}$ of filtrate can be filtered (Zambraski, 1990). Since daily urine volumes are not this high, much of the plasma must be reabsorbed in the renal tubules. This process of reabsorption is termed countercurrent multiplication (Morgan and Berliner, 1968).

Proximal Tubule

The microvilli characteristics of the proximal tubule section cause it to have an inner surface area of 25 m^2 (Silverthorn, 2001). As the filtrate flows through the proximal convoluted tubules sodium, potassium, glucose and amino acids are actively transported out of the tubule by transporters on the membrane walls. This reabsorption allows for the reabsorption of water via osmosis (Imai and Kokko, 1976). Approximately 70% of the filtered water, sodium, chloride and potassium are reabsorbed in this region (Imai and Kokko, 1976). Reabsorption is increased by an increase in sympathetic stimulation and is inhibited by the actions of angiotensin II (Seeley et al., 2003).

Loop of Henle

From the proximal tubules, the filtered fluid flows into the loop of Henle. The loop of Henle is the regulating unit of the nephron, and is composed of a hairpin shaped section dipping down from the cortex into the medulla and then back into the cortex (Silverthorn, 2001). It is composed of two limbs, a descending limb and an ascending limb. In isolated rabbit renal tubules, the descending limb is highly permeable to water but has low permeability to sodium

(Knepper and Burg, 1983). Therefore, the osmolality of the filtrate increases as it moves along the descending limb of the loop of Henle. The ascending limb is impermeable to water, but contains transporters for sodium, chloride, potassium and other electrolytes. Osmolality decreases as the filtrate moves along the ascending limb of the loop of Henle, osmolality decreases.

While the actual anatomy of the kidney does not alter in individuals who have received a kidney transplant, the preceeding anatomy discussion has been provided to give an overview of urine production since this thesis uses urine samples as a medium to determine electrolyte losses and total body water.

2.3.3. Hormonal Control Of Urine

Vasopressin

Vasopressin is also known as anti-diuretic hormone and has been implicated in the regulation of urine volume. Osmoreceptors situated in the cerebrospinal fluid monitor the osmolality. An increase in the osmolality stimulates vasopressin transcription from the paraventricular nucleus and the supraoptic nucleus of the hypothalamus. Vasopressin is released from the posterior lobe of the pituitary, and circulates in the bloodstream, mainly affecting the tubules of the kidney. The effect of dehydration on levels of plasma vasopressin was studied by Geelen et al. (1984). They placed healthy able-bodied participants on a 24 hour fluid restriction which induced mild dehydration. Following this restriction there was an increase in plasma vasopressin concentration which was associated with an increased plasma osmolality. Vasopressin release can also be stimulated via a fall in blood volume detected by the peripheral baroreceptors which act through afferent pathways on the vasopressin releasing cells in the hypothalamus. A drop in blood volume of 6 % showed increased plasma vasopressin concentrations (Antunes-Rodrigues et al., 2004). The circulating vasopressin binds to V2 receptors on the basolateral membranes of the collecting ducts. This binding causes the aquaporin-2 channels to relocate to the apical membrane thus increasing the permeability of the collecting ducts to water (Knepper, 1997; Wall et al., 1992). If vasopressin concentrations are low, the collecting ducts are relatively impermeable to water, resulting in the production of large volumes of dilute urine (Silverthorn, 2001). After maximal exercise there is an increase in plasma vasopressin concentrations (Maresh et al. 1985).

The Renin-Angiotensin System

The renin-angiotensin system responds to changes in circulating blood volume and body sodium content. Due to the close relationship between sodium reabsorption and water reabsorption by osmosis in the kidneys the renin-angiotensin system affects both sodium homeostasis and fluid balance (Lote, 1994). Renin is stored in the juxtaglomerular apparatus of the kidney (Lote, 1994). A fall in blood volume as detected by baroreceptors in the carotid bodies cause renin to be released from the afferent arterioles of the kidneys. This release is stimulated by increasing sympathetic nerve activity to these vessels (Lote, 1994). Blood pressure changes in the afferent arterioles of the kidney can also cause the release of renin, an action likely due to a decreased stretch of the arteriole walls (Lote, 1994). A decrease in the amount of sodium chloride (NaCl) delivered to the macula densa cells located in the distal convoluted tubule, stimulates the release of renin (Lote, 1994).

Renin is an enzyme whose actions take place on angiotensin. Angiotensin is a large protein which is produced in the liver. The action of renin on angiotensin causes the production of another protein called angiotensin I. Angiotensin I passes through the lungs where angiotensin converting enzyme convert it into angiotensin II. Angiotensin II then acts to retain water and sodium within the body. These effects are produced both directly and indirectly. It acts directly on the proximal tubule to increase sodium reabsorption. This also results in an increased release of aldosterone from the adrenal cortex (Antunes-Rodrigues et al., 2004). Its actions are further promoted by its stimulation of the release of vasopressin, further leading to an increase in the retention of body water (Antunes-Rodrigues et al., 2004). In a similar manner to plasma vasopressin there is an increase in the concentration of plasma renin activity at the end of maximal exercise season (Maresh et al., 1985).

Aldosterone

Aldosterone is secreted from the adrenal cortex is responsible for increasing sodium reabsorption from the lumen of the renal tubules (Silverthorn, 2001). Elevated levels of aldosterone increase the permeability of the renal tubules to sodium via the activity of the Na^+-K^+-ATPase pumps located at the basolateral membrane (Silverthorn, 2001).

Atrial Natriuretic Peptide (ANP)

Unlike the renin angiotensin and vasopressin, ANP acts to reduce an excess of fluid within the body. It is a hormone that is released from the cells of the atria of the heart. It is released in response to a stretching of the atrial walls, which occurs due to an increase in blood volume (Antunes-Rodrigues et al., 2004; Inoue et al., 2001). When ANP is released, it acts to increase the sodium and fluid output from the body, achieving this in two ways. Firstly, ANP increases glomerular filtration rate and kidney blood flow, however, Brenner et al. (1990) caution that this effect alone may not be great enough to have a significant effect on renal excretion rates Secondly, it has also been further suggested that ANP may act to reduce the action of vasopressin in the collecting ducts (Inoue et al., 2001).

2.3.4 Urine Output In Spinal Cord Injured (SCI)

Alterations in the pattern of urinary flow have been observed in those with a spinal cord injury. There is a similar urinary output during the night (between 22:00 and 08:00 h) as during the day (08:00- 22:00 h), whereas healthy able-bodied participants tend to demonstrate a diurnal rhythm for urine volume with volumes decreasing during the night (Kilinic et al., 1999). In able-bodied participants, there is a significantly higher urine osmolality at night and an increase in antidiuretic hormone. Conversely, results for those participants with a spinal cord injury reported no difference between urine osmolalities from samples obtained during the night and during the day (Killinic et al., 1999). A possible explanation for this nocturnal polyuria is an alteration in the blood volume between lying and sitting, posture changes result in a more pronounced change in blood volume for those with a spinal cord injury than healthy able-bodied participants (Wall et al., 1993). During the day blood pools in the lower limbs of individuals with a spinal cord injury due to their lack of a muscle pump and impaired sympathetic neural pathways. Upon lying down the blood redistributes and venous return increases. The resulting increase in blood volume is detected by the aortic baroreceptors. . Given that an increase in blood volume is usually due to an excessive intake of water, the body reacts to excrete the water load. Therefore the increased central blood volume upon lying prevents the normal decrease in vasopressin which is seen amongst able-bodied participants. This suggests that the diurnal variation for urine output is not present in individuals with a spinal cord injury.

The regulation of urine volume is not the only function which is affected following a spinal cord injury. Wall et al. (1993) found that following a water load, differences in urine

osmolality can also be observed. In a group of tetraplegics (C4-C7) placed in an erect position, urine osmolality did not decrease to levels seen in able-bodied participants following a water load. This inability to dilute urine to levels typically reported in able-bodied participants occurred despite a complete suppression of vasopressin (Wall et al., 1993). It was concluded that filtrate delivery to the diluting segments of the renal tubule is reduced, resulting in a reduction in the volume of fluid presented to the collecting ducts for reabsorption (Wall et al., 1993).

Table 2.3.1 The Effect of a Spinal Cord Injury On Body Water Compartments, Electrolytes and Hormones Controlling Water and Sodium Loss.

	Healthy Able-bodied	Spinal Cord Injured
Total Body Water	45-70 % of body mass Sawka, (1992)	Paraplegics 63 % Tetraplegics 59 % Cardús et al. (1984)
Intercellular Volume	67 % of total body water Sawka, (1992)	Paraplegics 58 % of total body water Tetraplegics 55 % of total body water Cardús et al. (1984)
Extracellular Volume	33 % of total body water Sawka, (1992) 238 ml.kg^{-1} Claus- Walker and Halstead, (1981)	Paraplegics 42 % of total body water Tetraplegics 45 % of total body water Cardús et al. (1984) Tetraplegics 3 months to 5 years after injury 258 ml.kg^{-1}* Claus- Walker and Halstead, (1981)
Interstitial Volume	75 % of extracellular fluid Åstrand, (1986)	
Plasma Volume	25 % of extracellular fluid Åstrand, (1986) 40 ml.kg^{-1} (Body mass = 80±12.1 kg) Claus- Walker and Halstead, (1981)	Tetraplegics 3 months to 5 years after injury 42 ml.kg^{-1} (Body Mass= 59.3 ±9.7 kg) Claus- Walker and Halstead, (1981)
Blood Volume	67 ml.kg^{-1} Claus- Walker and Halstead, (1981)	Tetraplegics 3 months to 5 years after injury 69 ml.kg^{-1} Claus- Walker and Halstead, (1981)

% Body Fat	Ideally 15 % of body mass Lohman, (1992)	24 % Sedentary George et al. (1988) 22 % Athletes Bulbulian et al. (1987)
Exchangeable Sodium	38.0±5.2 mmol.kg^{-1} Cardús et al. (1985)	Paraplegics= 48.8±6.2 mmol.kg^{-1} Tetraplegics = 47.5±8.4 mmol.kg^{-1} Cardús et al. (1985)
Exchangeable Potassium	47.1±7.3 mmol.kg^{-1} Cardús et al. (1985)	Paraplegics= 40.2±9.2 mmol.kg^{-1} Tetraplegics = 31.8±9.4 mmol.kg^{-1} Cardús et al. (1985)
Intracellular Potassium concentration	127 mmol.l^{-1} Cardús et al. (1985)	Paraplegics 106 mmol.l^{-1} Tetraplegics 102 mmol.l^{-1} Cardús et al. (1985)
Plasma Osmolality	Supine 290± 1.1 mosmol.kg^{-1} Wall et al. (1993)	Tetraplegics (C_4-C_7) Supine 284 ± 2.0 mosmol.kg^{-1} Wall et al. (1993)*
Urine Osmolality	Supine 843± 37 mosmol.kg^{-1} Wall et al. (1993)	Tetraplegics (C_4-C_7) 556± 50 mosmol.kg^{-1} Wall et al. (1993)
Creatinine Clearance following water load	Supine 125±8 ml.min^{-1} Erect 128±7 ml.min^{-1} Wall et al. (1993)	Tetraplegics (C_4-C_7) Supine 127±8 ml.min^{-1} Erect 113±8 ml.min^{-1} Wall et al. (1993)

Plasma Renin Activity		Tetraplegics
	Resting supine 1.04 ng.ml.h^{-1}	Resting supine 3.45 ng.ml.h^{-1}
	% change with sitting 63 %	% change with Sitting 91 %
	Claus- Walker et al. (1977)	Claus- Walker et al. (1977)
Plasma Renin Activity following a water load		Tetraplegics (C_4-C_7)
	Supine 0.40±0.10 ng.ml^{-1}.h^{-1}	Supine 1.11±0.30 ng.ml^{-1}.h^{-1}
	Erect 0.52±0.10 ng.ml^{-1}.h^{-1}	Erect 1.75±0.40 ng.ml^{-1}.h^{-1}
	Wall et al. (1993)	Wall et al. (1993)*
Plasma Aldosterone		Tetraplegics
	Resting Supine 54 pg.ml^{-1}	Resting Supine 94 pg.ml^{-1}
	% change with sitting 228 %	% change with sitting 83%
	Claus- Walker et al. (1977)	Claus- Walker et al. (1977)
Plasma Aldosterone following water load		Tetraplegics (C_4-C_7)
	Resting Supine 5.0 ± 0.9 ng.dl^{-1}	Resting Supine 9.8 ± 3.7 ng.dl^{-1}
	Erect 6.7± 1.7 ng.dl^{-1}	Erect 15.1± 2.5 ng.dl^{-1}
	Wall et al. (1993)	Wall et al. (1993)
Pattern of urinary Aldosterone excretion	Peak between 08:00 h and 14:00 h	Tetraplegics no peak
	Claus- Walker and Halstead, (1982)	Claus- Walker and Halstead (1982)
Urine Volume Pattern (Night (22:00-08:00 h Vs Day 08:00 – 22:00 h)	At night (22:00-08:00 h)	At night (22:00-08:00 h)
	⇧ Vasopressin	⇔Vasopressin
	⇩ Urine Volume	⇔ Urine Volume
	⇧ Urine osmolality	⇔ Urine Osmolality
		SCI groups- Above and Below T6
	Kilinic et al. (1999)	Kilinic et al. (1999)
Low sodium intake on plasma renin activity		Tetraplegics
	Sitting maximal values 3.30 ng.ml.h^{-1}	Sitting maximal values 4.79 ng.ml.h^{-1}
	Claus- Walker and Halstead, (1982)	Claus- Walker and Halstead, (1982)

** Significantly different from healthy able-bodied values*

Table 2.3.1. shows that there is a difference in the body water distribution and the relative total body water values for healthy able-bodied individuals and those with a spinal cord injury. The table also shows that the hormones responsible for regulating fluid balance may be affected by a spinal cord injury and this may result in an altered pattern of fluid intake and urine production.

Table 2.3.2. The Effects of a Spinal Cord Lesion Results from Animal Studies

Variable	Species	Normal Muscle	Paralysed Muscle
Potassium concentration in the muscle cell	Rat	175 mmol.l^{-1} Claus- Walker and Halstead, (1981)	Sciatic nerve transaction, 4 weeks of paralysis 159 mmol.l^{-1} Claus- Walker and Halstead, (1981)*
Sodium concentration in the muscle cell	Rat	15 mmol.l^{-1} Claus- Walker and Halstead, (1981)	Sciatic nerve transaction, 4 weeks of paralysis 23 mmol.l^{-1} Claus- Walker and Halstead, (1981)*
Cell membrane potential	Rat	88 mV Claus- Walker and Halstead, (1981)	Sciatic nerve transaction, 4 weeks of paralysis 62 mV Claus- Walker and Halstead, (1981)*
Blood flow of the Gastrocnemius	Dog	11.5 ml.min^{-1}.100g^{-1} Claus- Walker and Halstead, (1981)	Paraplegia 4 weeks after transaction 28.3 ml.min^{-1}.100g^{-1} Claus- Walker and Halstead, (1981)

**Significantly different from normal muscle values*

Table 2.3.2. shows that in animal species an injury to the spinal cord results in altered electrolyte composition within the extracellular and intracellular spaces. The table also demonstrates the alteration in blood flow following a spinal cord transaction. All of the experiments were performed on animals as they can be studied before and after a spinal cord

injury, which is not possible in human subjects. There may be some physiological differences which mean that these results may be different in humans.

2.4. THERMOREGULATION

Metabolic heat is produced in response to physical activity and unless this increase in core temperature is attenuated then performance will decrease (Sawka et al., 1989). Core temperature increases represent the storage of metabolic heat which is the by-product of the energy produced to perform skeletal muscle contractions (Sawka et al., 1989). Metabolic heat is lost from the body via dry heat exchange and sweat evaporation from the skin surface. The proportion that each route plays in thermoregulation is dependent on environmental conditions. In cool environments there is a large skin to ambient temperature gradient facilitating dry heat loss. Conversely, at higher temperatures sweat evaporation is the main route of heat loss (Sawka et al., 1989). There is a strong relationship between metabolic rate and core temperature for an individual (Sawka et al., 1989). There is also a large inter subject variability. It is possible to remove the inter subject variability by using relative exercise intensities rather than absolute workloads in study designs (Saltin and Hermansen, 1966).

During maximal upper body exercise, $\dot{V}O_2$ values are approximately 70 % of the $\dot{V}O_2$ produced during maximal lower body exercise (Sawka et al., 1984). If core temperature rises are determined by relative exercise intensity then core temperature would expect to be increased by a greater degree for a given metabolic rate than lower body exercise (Sawka et al., 1984). Conversely, if the surface area to mass ratio of the arm is greater than that of the lower limb and this will aid thermoregulatory responses (Sawka et al., 1984).

2.4.1. THERMOREGULATION AND SPINAL CORD INJURY

Upon commencing exercise, core temperature increases rapidly and then plateaus as heat loss equals heat production (Sawka et al., 1989). In those with a spinal cord injury, damage to the nervous system affects both sweating and the ability to regulate the peripheral vasculature via vasomotor and sudomotor responses (Sawka et al. 1989). Therefore there may be a decreased ability to thermoregulate amongst those with a spinal cord injury. The degree of impairment to the thermoregulatory system is dependent on the level of the lowest intact part of the sympathetic chain with thermoregulatory responses being proportional to the lesion level (Normell, 1974; Webborn et al., 2005). Gerner et al. (1992) exposed tetraplegics and paraplegics to environmental heat for 15 minutes. The results showed increased core

temperatures (rectal and oral) and reduced sweat rates amongst the participants with tetraplegia compared to those with paraplegia.

The skin and core temperatures of individuals with a spinal cord injury are influenced to a greater extent by the environmental conditions than they are for individuals without a spinal cord injury. Whilst resting in warm conditions (30 °C, 50 % relative humidity) for 30 minutes, those with a spinal cord injury had a greater increase in core (aural) temperature than their able-bodied counterparts (Petrofsky, 1992). The mean temperatures were 37.0°C for all participants at the start of the trial and remained at 37.0 °C for the able-bodied controls, but increased to 37.5°C for paraplegics and 37.6°C for tetraplegics. When exposed to 35 °C and 40 °C heat there was a significantly higher aural temperature amongst those with a spinal cord injury. Furthermore, this elevation in core temperature was related to the level of the lesion (Petrofsky, 1992). Similar findings had been found by Guttmann et al. (1958) who exposed individuals with a complete lesion causing tetraplegia to cold temperatures individuals with a complete lesion causing tetraplegia and found that they were unable to shiver when exposed to 18-20 °C. Further, participants in the study were unable to sweat below the lesion when exposed to 35-37 °C heat. Amongst those with an injury above T6, a rise in core temperature is necessary for sweating to occur, since a rise in local skin temperature is insufficient to cause a sweat response. This requirement is in contrast to the responses of able-bodied participants (Tam et al., 1978).

The ability to control core temperature is dependent on a balance between metabolic heat production, environmental temperature, the evaporation of sweat and the loss of heat through vasodilatation. The lower metabolic heat production seen amongst those with a spinal cord injury means that during 90 minutes of exercise at 80 % of heart rate peak those with a spinal cord injury experience no greater thermal strain than able-bodied participants (Price and Campbell, 1997). However, 80 % of peak heart rate could represent various percents of $\dot{V}O_2$ peak. This implies that despite an inability to sweat, metabolic heat production amongst those with a spinal cord injury in cool environments is not great enough to produce a thermal strain greater than reported in able-bodied athletes. Therefore, large core temperature increases are only likely to be seen in individuals with a spinal cord injury when they compete or train in hot environments since it is only in such situations, where heat gain and storage from the environment are likely to be greater than for able-bodied individuals. This is because the heat gains from the environment are the main contributors to heat strain amongst the tetraplegic population (Webborn et al., 2005).

The ability of an individual with a spinal cord lesion to vasodilate is also impaired below the level of the lesion thus attenuating this route of heat loss (Theisen et al., 2001). The core temperature at which the body reacts to increase skin blood flow is increased when there is a drop in blood volume (Theisen and Vandlandewijk, 2002). Some authors have suggested that blood pooling occurs in the lower limbs of those with a spinal cord injury (Kooner et al., 1988; Raymond et al., 1997) due to the lack of a muscle pump and an increased vascular resistance (Hopman et al., 1984). If there is blood pooling in the lower limbs and this results in a drop in blood volume.

Studies looking at the skin temperatures of those with a spinal cord injury have shown increases from measurements taken on the trunk, forearm and calf during 90 minutes of exercise at 50 % $\dot{V}O_2$ peak (Fitzgerald et al., 1990). In the same study, amongst the able-bodied participants there was a decrease at these sites. A similar finding was presented by Price and Campbell (1997) for skin temperatures on the calf during 90 minutes of arm cranking exercise at 80 % peak heart rate. Calf skin temperature increased by 1.4 (2.8) °C for those participants with paraplegia (T3/T4- L1) but decreased by 0.8 (2.0) °C for the able-bodied participants. The decrease in skin temperature amongst the able-bodied participants indicates an efficient thermoregulatory response and evaporative heat loss through sweating. The evaporation of sweat will cool the skin and this will also aid the heat gradient between the skin and the environment which is required for dry heat exchange. The increase in skin temperature seen amongst the participants with a spinal cord injury may indicate a transfer of heat from the core to the periphery by the blood as well as an inefficient dissipation of this heat due to an impaired sweat response (Fitzgerald et al., 1990). This transfer of heat from the core to the periphery may be of greater importance to those with a spinal cord injury than for able-bodied individuals. Sawka et al. (1989) suggested that individuals with a spinal cord injury need to rely more on dry heat exchange in an attempt to thermoregulate than do able-bodied participants. For dry heat exchange to occur a temperature gradient between the core and the skin needs to be present and also a temperature gradient between the skin and the environment needs to be present. Therefore greater core temperatures seen amongst those with a spinal cord injury may actually aid this dry heat exchange (Theisen and Vandlandewijk, 2002). The theory of increased dry heat exchanged has also been promoted as a reason for the lower stoke volumes and higher heart rates seen amongst those with a spinal cord injury during exercise. The mechanism behind this theory is that a larger volume of blood is diverted

to the skin. There is therefore a decrease in venous return, meaning that stroke volume is unable to increase to values seen amongst able-bodied participants (Fitzgerald et al., 1990).

At rest, differences in skin temperature exist with Price and Campbell, (1997) reporting higher skin temperatures on the upper body of paraplegics when compared to their lower body skin temperatures. This is in contrast to the results of able-bodied participants for whom similar temperatures on the upper and lower body were seen (Price and Campbell, 1997). This difference in resting skin temperature may represent the decreased metabolic heat produced by the lower limbs following a spinal cord injury (Price and Campbell, 1997).

2.5. Body Water Losses Via Sweating

Sweating is one of the most variable routes of body water loss, making up the greatest single contribution to total body water losses during exercise (Cheuvront et al., 2002). Energy required to perform physical movement also generates heat. The more intense the exercise, the greater the metabolic energy required and the larger the amount of heat that needs to be removed from the muscles (Armstrong, 2000). Cycling is the most efficient form of exercise and is only about 20 % efficient meaning that of the energy produced only 20 % is transformed into mechanical work. The remaining 80 % of energy is converted to heat energy which needs to be dissipated from the body (Hopman and Binkhorst, 1997). The evaporation of sweat from the skin's surface is important for the dissipation of this heat, with the evaporation of sweat at a rate of 1 $l.h^{-1}$ resulting in a heat loss of 580 $kcal.h^{-1}$ (Rehrer and Burke, 1997). If this evaporation did not occur, then core temperature would increase and performance would decrease. If this core temperature increase were to continue, an athlete's health would ultimately be compromised. Unfortunately, as well as dissipating heat from the body, sweat secretion also results in the loss of vital fluids that can reduce blood volume. A loss of fluid from the body which results in hypohydration produces a decreased stoke volume and so to maintain cardiac output heart rate needs to increase (Montain and Coyle, 1992b). Electrolytes including sodium, chloride and potassium are also lost in sweat, which can have a negative effect on performance. Therefore, it is necessary to replace both body water and sodium following exercise (Noakes, 1993).

2.5.1. Determining Sweat Losses

It is commonly assumed that changes in body mass over a short period of time are due to a loss in body water, and it is generally assumed that 1 litre of sweat equates to 1 kg of body mass (Shirreffs et al., 2006). However this is not the case as the specific gravity of sweat is

greater than 1.00 and therefore 1 litre of sweat will have a mass greater than 1.00 kg, depending on the sweat composition it is likely that 1 litre of sweat will have a mass of 1.001 - 1.008 kg (Lenter, 1981). However, it is argued that this error is less than the precision of the measurement of body mass in humans and can therefore can be ignored (Maughan et al., 2007). Thus the assumption that 1 litre of sweat equals 1 kg can be used to determine the sweat losses of athletes during their normal training or in competition. Previous studies concerning the sweat losses of athletes during their normal activities have employ the measurement of nude body mass before and after training, followed by correcting for any fluid consumed or urine produced during the testing/training session in order to calculate sweat losses (Shirreffs et al., 2005; Maughan et al., 2005).

However, this common field technique does not differentiate between eccrine and non-eccrine fluid loss. Measurement of nude body mass eradicates the problem of sweat being trapped in clothing as sweat losses can become trapped in the athletes clothing (running vest/ tee-shirt, shorts, socks and shoes). Values of this trapped sweat have been reported to be 0.27±0.22 kg (range 0.05-0.66 kg) when sweat losses were 2.91±0.73 litres (range 1.49-3.88 litres) in an environment of warm (30°C). In cool conditions (14°C) trapped sweat was 0.22±0.14 kg (0.03-0.46 kg) when sweat losses were 1.91±0.58 litres (range (1.31-2.94 litres). These results were obtained from 8 female marathon runners (age 37±4 years) undertaking a 30 km treadmill run at 42 km race pace (Cheuvront et al., 2002).

"Sweat loss = body mass loss + ingested fluid – substrate oxidation + metabolic water – respiratory water loss"

Maughan et al. (2007)

Due to the equipment required to determine respiratory losses it is not possible to undertake such testing in the field. The following equation can be used to estimate respiratory water losses.

$$M_e = 0.019\ \dot{V}O_2\ (44\text{-}Pa)$$

Ingelstedt, (1956)

M_e = Rate of evaporative water loss ($g.min^{-1}$)

$\dot{V}O_2$ = Oxygen uptake ($l.min^{-1}$)

Pa = Ambient water vapor pressure (mm Hg)

This equation shows, it is possible to estimate respiratory water losses if the $\dot{V}O_2$ of the exercise is known but there are flaws in the calculation of respiratory water losses as the relationship between O_2 uptake and respiratory water loss is not linear above 70 % $\dot{V}O_2$max (Mitchell et al., 1972). The increase in ventilation-$\dot{V}O_2$ ratio at work rates higher than this results in respiratory water rates greater than estimated by the calculation (Mitchell et al., 1972).

While the deviation is small up to workloads of 80 % $\dot{V}O_2$max and has been shown to be within 0.25 g.min^{-1} when compared to direct measurements of humidity in the expired air, Mitchell et al. (1972).

However, the equation relies on the absolute $\dot{V}O_2$ of the exercise being performed to calculate the rate of evaporative respiratory water losses. Furthermore, when absolute $\dot{V}O_2$ values are low respiratory water losses can be overestimated by 1-2 ml.min^{-1} (Mitchell et al., 1972). This is because the relationship between respiratory water loss as a function of oxygen consumed is not linear (Mitchell et al. 1972). $\dot{V}O_2$ peak has been found to be 2.65 l.min^{-1} in international wheelchair basketball players (Goosey-Tolfrey, 2005). This is lower than the $\dot{V}O_2$ max reported in the study by Mitchell et al. (1972) and as workloads which elicited $\dot{V}O_2$ values of upto 3 l.min^{-1} resulted in a lower respiratory water loss than was estimated by the equation (Mitchell et al. 1972) it is likely that any estimates for athletes with a disability will be an overestimation. Especially as, upper body exercise elicits lower absolute $\dot{V}O_2$ values (e.g. absolute $\dot{V}O_2$peak is lower therefore % $\dot{V}O_2$ peak is also lower in absolute terms) (Hooker et al., 1993 Pimentel et al., 1984). Additional difficulties with using this equation in an applied setting emerge when considering the practicalities of measuring $\dot{V}O_2$. It has been reported that the respiratory rate in response to a heat stress at rest for individuals with a spinal cord injury is not the same as found in able-bodied participants (Wilsmore et al., 2006). This increase in breathing frequency may decrease the amount of water lost per breath (McFadden, 1988) but, the greater number of total breaths taken in total may result in an increase in respiratory water losses. A theoretical calculation of respiratory water loss is shown in Chapter 5. Despite this error when calculating sweat losses, an athlete needs to know all of their water losses and therefore this error is of minimal significance when determining an athletes fluid needs.

If a value of 1-2 g.min^{-1} is used as an average respiratory weight loss then it would equate to 60-120 g.hour^{-1}. Mean sweat losses calculated from changes in body mass of professional male footballers have been reported to be 1.3 l.h^{-1} (Shirreffs et al., 2006) thus suggesting that respiratory water losses would account 3-6 % of the decrease in body mass. For groups whose sweat losses are likely to be lower such as those with a spinal cord injury then this avenue of body mass change will be of greater significance. Indeed in some cases, this amount of body mass change could equate to all of the water loss implying that the calculations of electrolyte losses are incorrect.

It is also possible to estimate the loss of body mass which is due to differences in between CO_2 losses and O_2 uptake by using the following equation

$$m_r = 0.53 \dot{V}O_2$$

Nadel et al., (1971).

Further, the equations used to calculate the difference between CO_2 losses and O_2 uptake is based on the moles of both substances being lost and gained by the body during exercise. This respiratory quotient is dependent on the workload of the exercise and thus the $\dot{V}O_2$. In an applied setting it is not possible to measure the respiratory quotient of the athletes. Therefore, the equation assumes that the respiratory quotient of the exercise is 1.00 and this will not be the case during athletic events and games due to different energy sources.

Due to the production of water by the oxidation of carbohydrate and fat during exercise respiratory water losses tend to be ignored since they are balanced by the increased metabolic water production (Sawka et al., 2005; Shirreffs et al., 2006).

The metabolic water production during exercise depends on the substrate and upon the exercise intensity. Metabolic water production was 1.1, 1.7 and 2.4 g.min^{-1} at 37, 56 and 74 % of maximum workloads which corresponded to respiratory water losses of 0.7, 1.1, 1.4 g.min^{-1} (Pivarnik et al., 1984). One gram of carbohydrate oxidation results in a net mass loss of 0.40 g and produces 0.60 g of water, whereas the oxidation of 1 gram of fat increases body mass by 0.13 g and produces 1.17 g of water (Maughan et al., 2007). During exercise at 30 % of $\dot{V}O_2$ max carbohydrate contributes 45 % to the substrate metabolism and fat contributes to the remaining 55 %. This corresponds to an 82 % contribution from carbohydrate and an 18 % contribution from fat at 80 % $\dot{V}O_2$ max this is assuming that protein does not contribute to

the oxidative metabolism (Maughan et al., 2007). These proportions are also dependent on the aerobic fitness and diet prior to exercise (Maughan et al., 2007)A calculation of the mass loss associated with substrate metabolism for disabled athletes is shown in Chapter 5.

2.5.2. Sweat Composition

Determining the electrolyte composition of sweat during training and matches is important in order to provide estimates in changes in extracellular fluid, as well as for nutritional replacement (Patterson et al., 2000). The loss of electrolytes from the body whilst exercising can also impact on performance and result in injury. Heat-induced muscle cramps have been linked to sodium losses, but it is argued that the supporting evidence is not strong (Maughan et al., 2005). Stofan et al. (2005) found that national collegiate American football players who were prone to cramping lost almost twice as much sodium in their sweat during training than team-mates who had not previously suffered from cramping. Further anecdotal evidence of a link between sodium loss and heat-induced cramping is illustrated by the use of a sodium supplementation as a method used to alleviate cramp (Stofan et al., 2005). If there is a link between sweat sodium losses and cramp exists, then knowledge of sodium losses would be of importance to an athlete. It is known that sweat sodium concentrations can vary widely amongst individuals undertaking the same exercise (Maughan et al., 2005; Shirreffs et al., 2005) and that sweat sodium concentrations are altered in an individual by acclimatisation and following changes in training status (Allan and Wilson, 1971; Nadel et al., 1974).

The major electrolyte of sweat is sodium. Other electrolytes present in smaller quantities within sweat are chloride and potassium (Sawka and Montain, 2000). Generally mean sweat sodium concentrations equal 35 $mmol.l^{-1}$ although this may range from 10-80 $mmol.l^{-1}$ (Sawka and Montain, 2000). These variations can be attributed to the effects of diet, sweating rate, hydration status and acclimatization (Sawka and Montain, 2000). Sweat potassium concentrations average 5 $mmol.l^{-1}$, ranging from 3-15 $mmol.l^{-1}$ (Sawka and Montain, 2000).

Shirreffs and Maughan, (1997) describe a whole-body wash down technique for determining sweat electrolyte composition during exercise. Although the whole-body wash down technique can provide accurate and reproducible results with respect to sweat sodium concentration, it is only really of practical use in a laboratory setting. Patterson et al. (2000) compared this wash down technique with a regional sweat collection method using adhesive gauze patches during 90 minutes of cycling. From this study, the following weighted equation based on the collection of a sweat sample from four sites was proposed:

'Mean whole- body concentration = 28.2% chest + 28.2% Scapula + 11.3% forearm + 32.3% thigh'

Patterson et al. (2000).

The use of this equation amongst individuals with a disability may not be practical, especially for amputees and those with a spinal cord lesion, since they may not sweat over all the sites in the equation. Since values for the thigh would be zero if there was no sweat produced this would result in an underestimation in the calculation of whole body sweat electrolyte concentrations. Strong correlations were evident between the regional mean and whole body sweat sodium concentrations and sweat chloride concentrations (Patterson et al., 2000). The gradient coefficient for the foot was 0.93 (Patterson et al., 2000). It is suggested that the sweat sodium and sweat chloride concentrations of the foot most closely represent the absolute levels of the whole body. The thigh region was the next best single representation of both whole body sweat sodium concentration and chloride concentration (Patterson et al., 2000). It could therefore be considered that a single site, such as the forearm, thigh or calf could be used to estimate whole body concentrations due to a high correlation coefficient (Patterson et al., 2000). Verde et al. (1982) also found that the recovery of the mineral ions by use of a regional collection method was satisfactory and reproducible. Additionally, they also showed that the sweating response is symmetrical indicating that patches only need to be placed on one side of the body (Verde et al., 1982). Research specific to individuals with a disability conducted by Unnithan and Wilk, (2005) amongst eight boys with spastic cerebral palsy has shown a difference in the pattern of sweat loss between dominant and non-dominant forearms. While a higher sweat gland density was observed, there was a lower drop area on the non-dominant forearm than for the dominant forearm. Since sweat gland population density is related to body surface area, the higher density of sweat glands on the non-dominant arm is probably due to a smaller surface area on this arm compared to the dominant arm (Unnithan and Bogdan, 2005). Drop area is assumed to be a function of the rate of sweat production from the sweat gland (Unnithan and Bogdan, 2005). Given that sweat sodium and sweat chloride concentration are affected by sweat rate, it can be argued that there may be some differences in sweat electrolyte composition between dominant and non-dominant sides of the body in those with cerebral palsy. However, despite an increase in sweat sodium and sweat chloride concentrations for an individual when whole body sweat rates are increased, differences in sweat rates over regional sites of the body did not result in different sweat sodium or sweat chloride concentrations between the sites of sweat collection

(Verde et al., 1982). It is therefore unlikely that differences in the sweat rate of the glands between the dominant and non-dominant side of the body will have a significant impact on the calculation of sweat electrolyte losses.

Despite a good correlation between the regional collection of sweat and whole body sweat collection, the regional method seems to overestimate sweat sodium losses by 30-40 % depending on the site of regional sweat collection (Patterson et al., 2000; Shirreffs and Maughan, 1997). Theories concerning elevated sweat sodium and chloride values with regional site collection involve the suppression of sweat evaporation at regional collection sites. A suppression of sweat evaporation, may lead to elevated local skin temperature, which in turn may affect the sweat rate (Nadel et al., 1971; Palacios et al., 2003). This likely accounts for the greater sodium and chloride concentrations seen from regional site collections compared to whole-body collections (Patterson et al., 2001) since within a single person sodium and chloride concentrations are increased with greater sweat rates within a given skin region (Sato and Dobson, 1970; Alan and Wilson, 1997). It is therefore uncertain whether or not regional sites can predict whole-body sweat constituent concentrations at higher sweat rates induced by alterations in exercise intensity and environmental conditions, (Patterson et al., 2000). However, whole-body sweat rates amongst athletes with a disability especially those with a spinal cord injury are likely to be low. Sweat rates during a wheelchair basketball match were 0.9 $l.h^{-1}$ (Burnham et al., 1999), therefore the use of regional collection sites for the sweat is a valid method.

2.5.3. Sweat Glands

The human body has 3-4 $*10^6$ sweat glands (Saga, 2002). There are two generally recognised types of sweat glands: appocrine and eccrine. The eccrine sweat glands are important for the control of body temperature. The sweat gland consists of the secretory and ductal portion. The secretory coil is 60-80μm in diameter and 2-5mm long (Saga, 2002). The ductal portion is composed of a coiled duct, intradermal straight duct and an intraepidermal duct called the acrosyringium. The coiled duct and the intradermal duct are known as the dermal duct and are composed of two layers of cells: the basal or outer layer of cells, and the luminal or inner cells (Saga, 2002). The secretory section of the eccrine sweat gland is composed of three cell types: the dark secretory cells which line the lumen, clear secretory cells which are believed to be responsible for the secretion of water and electrolytes, and myoepithelial cells which surround the secretory cells (Saga, 2002). The eccrine sweat gland has two major functions;

firstly it secretes a plasma-like precursor fluid by the secretory coil in response to acetylcholine. Secondly, the duct acts to reabsorb sodium in excess of water to produce a hypotonic solution which is secreted onto the skin as sweat (Saga, 2002). When sweat losses are high, the reabsorption of sodium is important for the maintenance of homeostasis.

2.5.4. Nervous Control Of Sweat Glands

The preoptic area and anterior hypothalamus in the brain contain many thermosensitive neurons that detect changes in temperature and initiate the responses to secrete sweat (Boulant, 1981). While core temperature is a major determinant of sweat secretion, skin temperature via afferent feedback also plays a relatively minor role in the activation of sudomotor activity whilst at rest (Nadel et al., 1971). During exercise, it appears that the role of skin temperature has a greater role to play in sweat rates than when at rest (Tam et al., 1978). The stimulation to secrete isotonic sweat in the eccrine sweat gland is induced by cholinergic agonists, alpha-adrenergic agonists and beta-adrenergic agonists, with the sensitivity to each agonist in a human sweat gland being in the of ratio 5:1:1. In contrast to ordinary sympathetic innervation, acetylcholine and norepinephrine are the main neurotransmitters secreted from the sympathetic nerves surrounding eccrine sweat glands. Large numbers of cholinergic terminals and a few adrenergic terminals innervate the human sweat gland (Uno, 1977). At the sweat gland Na^+-K^+-ATPase in the basolateral cell membranes may be involved in the production of primary sweat. In vitro studies have demonstrated that cholinergic and alpha-adrenergic stimulation induce the movement of calcium from the extracellular fluid into the secretory cells, triggering a series of intracellular events for sweat secretion to occur (Saga, 2002).

The efferent sudomotor pathway for the stimulation of sweat production begins in the cerebral cortex. From there, it descends through the hypothalamus to the medulla where it crosses to the lateral horn of the spinal cord to sympathetic ganglia and then to the sweat glands as postganglionic C fibres (Saga, 2002). For individuals with a spinal cord injury, this pathway is disrupted at the point of injury and therefore the stimulation for sweat production does not reach the sweat glands below the lesion.

Secretion from the sweat gland is not continuous at ordinary room temperatures, or at high temperatures of 35 °C. It has been shown that varying numbers of glands may be activated in

what appear to be phased discharge over true secretary nerve fibres (Randall, 1946b). Active sweating of individual glands is usually of very short duration (15 sec or less), but may be longer under suitable circumstances of stimulation (Randall, 1946b).

2.5.5. Electrolyte Movement In The Sweat Gland

Through active transport the sweat gland has the ability to reabsorb some of the sodium from the primary sweat. As such, sweat secreted onto the skin surface is hypotonic. As sweat rate increases, the body's ability to reabsorb sodium does not subsequently increase (Sawka and Montain, 2000), thus increasing sweat sodium and sweat chloride concentrations. Persons who have acclimatized to heat do however appear to have an improved ability to reabsorb sodium. This adaptation leads to a lower sweat sodium concentration with a greater than 50% reduction in sweat sodium concentration at any specific sweating rate (Sawka and Montain, 2000). Kirby and Convertino, (1986) found that following heat acclimation there was a 59 % decrease in sweat sodium concentration despite a 12 % increase in sweat rate. It would appear that physical training also affects the secretary activity of the human sweat gland in that physical training increases secretary activity of the sweat gland (Buono and Sjoholm, 1988). The increased ability of sweat glands to secrete sweat following physical training may not only be the result of an increased peripheral sensitivity to a central thermoregulatory drive, but may also be intrinsic to the sweat glands themselves (Buono and Sjoholm, 1998). These findings suggest that the sweat glands respond to chronic exposure to high sweat losses, resulting in a greater ability to reabsorb electrolytes culminating in more dilute sweat being secreted onto the skin surface.

2.5.6. Spinal Cord Injury And Sweating

For able-bodied persons, sweat evaporation is limited predominantly by ambient water vapour pressure. In hot and humid environments, the ability to dissipate heat is thus impaired, limiting exercise capacity in such conditions (Dennis and Noakes, 1999). For athletes with a spinal cord injury, the level of the lesion is an important factor in determining the ability to secrete sweat and so dissipate heat as the relative area of skin available for sweat production is proportional to the level of the spinal cord injury (Theisen and Vanlandewijck, 2002).

Observational and anecdotal evidence of an inability to sweat below the lesion amongst war veterans in a hospital setting was reported by Kuhn (1950) who discussed the absence of a sweat response on the insensate skin. Guttmann (1958) observed a different sweat response in those individuals with a spinal cord injury when compared to the response seen in healthy able-bodied individuals. When exposed to heat (35-37°C) individuals with a mid cervical region injury displayed no sweat response (Guttmann, 1958). It was later shown that there may be some sweating below the level of the lesion even if the lesion is complete (Silver et al. 1991). This would suggest that there are some thermoregulatory sudomotor responses of the isolated cord to intensive heat stimulation. This phenomenon was also seen by Freund et al. (1984) who studied individuals with spinal cord lesions between the 1st thoracic and the 11th thoracic vertebrae by passively heating the insensate skin by having participants wear a suit with circulating water at 47 °C. Results showed that sweat production was low on the upper body, and minimal on the lower body. The low sweat rate above the lesion level or upper body represents an inability of the peripheral heat sensors below the lesion to relay the information about the increase in temperature to the central nervous system for an afferent response of sweating to occur. This is further highlighted by the large sweating response seen over the upper body when the whole body was heated (Freund et al., 1984). The heating of the whole body is likely more reflective of daily living in that an increase in heat across the whole body results in a large volume of sweat being produced over the sensate skin. It is generally accepted that the whole bodied sweat loss of individuals with a spinal cord injury is lower than that for able-bodied individuals (Petrofsky, 1992). A further complication in those with a lesion above the 6th thoracic vertebrae involves a loss of supraspinal control of the sympathetic nervous system. This results in a decreased catecholamine release, ultimately reducing catecholamine induced sweat rates in regions above the level of injury (Hagobian et al., 2004).

While exercising in 10°C at 40 % of peak power is not a thermoregulatory strain for able-bodied individuals, those with paraplegia below T1 level will experience a loss of body heat (Hopman and Binkhorst, 1998). At rest, in cool conditions (22.4±1.1°C and 46.2±8.6 % relative humidity) no differences were seen between paraplegic (T4/5-T11) and able-bodied participants with respect to aural temperature (Price and Goosey-Tolfrey, 2008). Similarly, there were no differences in the skin temperatures of the forehead, upper arm, back, chest or abdomen between paraplegic and able-bodied groups. Differences were observed when comparing thigh temperatures, with paraplegics having lower thigh temperatures compared

to able-bodied participants (28.9±2.5 °C for paraplegics and 31.1±0.7°C for able-bodied). A similar difference was seen in the skin temperature at the calf (26.4±1.3 °C for paraplegics and 31.7±0.9 °C for able-bodied) (Price and Goosey-Tolfrey, 2008). Overall, heat exposure resulted in a greater increase in skin temperatures in the lower limbs for paraplegic participants when compared to able-bodied participants but there was a smaller increase in aural temperature during the 90 minutes of heat exposure in the paraplegics compared to able-bodied participants (Price and Goosey-Tolfrey, 2008). However, the validity of aural temperature has been questioned and was found to differ significantly from rectal temperature (Casa et al., 2007). Although it is noted that for those with a spinal cord injury rectal temperature may not be a valid measurement (Gass and Camp, 1989).

For able-bodied individuals, whole-body sweat rates increase as environmental temperature increases. This rate of increase is not as steep, or pronounced, for those with paraplegia (Price, 2006). For tetraplegics exposed to heat (40 °C), sweat rates were double the sweat rates observed for able-bodied controls at rest in 30 °C conditions (Petrofsky, 1992). Since nearly two thirds of the sweat glands were unable to secrete sweat due to the lack of innervation, it was theorised that local sweat rates amongst those with tetraplegia were almost six times greater than observed amongst able-bodied controls (Petrofsky, 1992). However, the value of such an increase in regional sweat rates is somewhat attenuated by the observation that most of the sweat produced ran off the skin surface thereby not promoting a loss of body temperature (Petrofsky, 1992).

Fitzgerald and colleagues (1990) found that in a group of low level (T9/T12-L3/L4) paraplegic women, thermoregulatory strain was greater than in able-bodied female controls during 90 minutes of wheelchair ergometry at 50-55 % $\dot{V}O_2$ peak at 24-25 °C and 38-52 % relative humidity. This was evidenced by higher oral and skin temperatures amongst the women with paraplegia compared to able-bodied controls (Fitzgerald et al., 1990).

Differences between tetraplegics and paraplegics have been demonstrated there is a more rapid increase in core temperature amongst low level paraplegics (below T7) compared to high level paraplegics (T1-T6) and tetraplegics (C5/6-C7/8). This probably relates to differences in the recruitable muscle mass (Price and Campbell, 2003). As exercise continues there is greater heat storage amongst tetraplegics undertaking 60 minutes of wheelchair ergometry at 60 % $\dot{V}O_2$ peak compared to low level paraplegics. (Price and Campbell, 2003).

This greater increase in heat storage by the tetraplegics was observed during from 30-60 minutes of exercise in conditions of 31.5±1.7°C, 42.9±8.0 % relative humidity (Price and Campbell, 2003).

As well as the disruption of motor output from the spinal cord the afferent information is also impaired. Freund et al. (1984) demonstrated this by passively heating the insensate skin of five participants with a spinal cord injury at T1-T11. This heating resulted in only a mild sweat response over the area above the lesion, with very limited sweating below the lesion. Further, participants' forearm blood flow was also only slightly elevated (Freund et al. 1984). The response seen amongst the spinal cord injured participants was in contrast to that seen in the able-bodied participants for whom the heating resulted in a large thermoregulatory response.

Sweat losses during physical activity are usually determined by changes in body mass while accounting for any fluid consumption or urinary loss. The change in body mass during exercise is used as an indication of the percentage of dehydration that has occurred over the training session. Therefore, a reduced whole body sweat rate may mean that during exercise those with a spinal cord injury may be less likely to suffer from excessive dehydration. This reduced risk may be even greater than would first appear if the findings of Price and Campbell (1999) are examined. They found that during 60 minutes of arm cranking at 60 % $\dot{V}O_2$ peak in cool conditions (21.5±1.7 °C 47±7.8 % relative humidity) body mass change and fluid consumption were similar between paraplegics and able-bodied participants (0.38±0.39 kg and 598± 433 ml; 0.38±0.31 kg and 403± 368 ml respectively). However, there was a greater decrease in plasma volume amongst the able-bodied participants compared to the paraplegic participants (-9.8±5.8 % and 4.36±4.9 % respectively) (Price and Campbell, 1999). Since there are factors which may affect body mass during exercise which will not affect fluid balance (see Chapter 5) plasma volume may be a better indication of fluid balance. Therefore, the findings of Price and Campbell (1999) may indicate that there is a smaller disruption of hydration amongst those with a spinal cord injury than able-bodied participants. It is cautioned, however, that this hypothesis requires further investigation.

2.6. Blood Redistribution And Spinal Cord Injury

Individuals with a spinal cord injury have an impaired ability to redistribute blood flow below the level of the lesion and an impaired ability to produce sweat below the lesion level. As a result, lower limbs become a potent site for heat storage (Price and Campbell, 1999), which

may affect their ability to lose heat since the gradient of temperature between the skin and the environment is reduced. The ability of the lower limbs to vasodilate during exercise depends to a certain extent on the level of the lesion. The pathway for lower limb vasodilatation is at the thoracic 10th vertebra. Amongst paraplegic men, there is an attenuation of skin blood flow in response to hyperthermia when compared to able-bodied men (Freund et al., 1984). This route of dissipating excess heat is thus impaired since the ability to vasodilate at the skin surface of the lower limb is impaired for those with a spinal cord injury above T10 (Freund et al., 1984). The impaired thermoregulatory responses amongst those with a spinal cord injury, both decreased sweat response and an inability to initiate an appropriate vascular response, is due to deficits of the vasomotor sudomotor functions over the sympathetically uncontrolled areas and a decrease in the thermoregulatory effecter drive for a given core temperature. This is likely due to a lack of afferent input into the hypothalamic control centres (Sawka et al., 1989).

2.7. Body Water Gains

Although the mechanisms for thirst and fluid consumption of individuals are not directly studied in this thesis, the water turnover rates and non-renal losses are calculated and therefore some understanding of body water gains is required.

Many factors such as hormonal and habitual habits determine fluid intake. Water intake is dependent on the volume and composition of foods and drinks consumed as well as the by-products of metabolic processes. The volume of fluid consumed by an individual is dependant on the taste of the fluid, its temperature, carbonation, electrolyte concentration and the feeling it produces in the mouth (Maughan and Murray, 2000). Once fluid has entered the body, for it to be of any use, it needs to be absorbed from the intestine. Before absorption can take place, it has to pass through the stomach. The rate of availability of any ingested fluid is thus dependent on the rate of emptying from the stomach and on the rate of absorption from the intestine.

2.7.1. Thirst

The body has mechanisms in place to prevent the occurrence of hypohydration and to encourage water intake. Thirst is 'the desire to ingest water' (Nadel, 1992). Thirst is normally the main driver for fluid intake; however, fluid consumption is also influenced by habit and social issues rather than by a physiological drive to drink (Maughan and Shirreffs, 2004). Under normal resting conditions, hypohydration is prevented by increased sensations of

thirst which are strong enough to produce a drive to drink (Greenleaf, 1992). Thirst is regulated by an increase in the osmotic pressure of the extracellular space. Sensations of thirst occur when there is an increase in plasma osmolality and decreased blood volume is detected by osmoreceptors and stretch receptors in the atria (Nadel, 1992). It is possible that due to blood pooling in the lower limbs of individuals with a spinal cord injury, a constantly low blood volume may exist, which may have an effect on feelings of thirst.

During exercise, able-bodied athletes can experience involuntary dehydration which occurs despite an increase in extracellular osmotic pressure, increased blood osmolality and higher angiotensin II (Reid, 1984). Even with unlimited access to fluid, humans do not replace all of the body water that is lost during prolonged exercise (Noakes, 1993; Armstrong et al., 1997: Kay and Marino, 2000). It has also been proposed that the rennin-angiotensin II aldosterone system plays a role in the thirst driven drive to drink (Greenleaf, 1983). Blood volume, blood pressure and hormonal responses may be altered following a spinal cord injury.

Although previous studies have shown that tetraplegics have a greater fluid intake than able-bodied participants during exercise (Price and Campbell, 2003; Goosey-Tolfrey et al. ,2008), many factors such as habit can influence fluid intake. Goosey-Tolfrey et al. (2008) found similar decreases in plasma volume during wheelchair propulsion with and without the effect of head and neck cooling. Despite these similar decreases in plasma volume, fluid intake decreased more when participants were cooled than when they were not. This would suggest that despite the same degree of dehydration, as shown by the change in plasma volume, the desire to drink (thirst) was decreased by approximately 42 % in the cool trial. This would indicate that thirst is not the only mechanism responsible for the fluid intakes volume of those with tetraplegia. It would appear that fluid intake may be related to a psychological attempt to attenuate rises in core temperature and to aid feelings of thermal comfort. Conversely, there may be a delay in the emptying of the fluid from the stomach (Gondim et al., 1999). For further discussion on gastric emptying rates, see Section 2.7.2. To date there has not been any published research which is specific to those with a spinal cord injury regarding their sensations of thirst (Sawka and Montain, 2000). Research in this area is merited, especially when considering the differences in vasopressin and blood volume between those with a spinal cord injury and able-bodied participants have been reported (Wall et al. 1993; Kilinic et al. 1999). It is suggested that these factors could influence feelings of thirst amongst those with a spinal cord injury.

2 .7.2. Gastric Emptying And Intestinal Absorption Of Ingested Fluids

Gastric emptying is the rate at which fluids passes from the stomach to the small intestine and is considered a limiting factor in fluid replacement. It can be influenced by numerous factors including volume, carbohydrate concentration, osmolality and temperature, solute acidity, exercise activity and heat stress (Shi and Gisolfi, 1998). A greater volume of fluid in the stomach results in a faster rate of gastric emptying. As the volume in the stomach decreases (as a result of the stomach empting) so does the absolute rate of gastric emptying. Increased distention and pressure created by increased volume of the stomach are mechanisms which determine the volume- dependent gastric emptying rate. These mechanisms stimulate receptors in the gastric musculature to increase the rate of gastric emptying (Shi and Gisolfi, 1998).

Intestinal fluid absorption is governed primarily by luminal osmolality and solute transport. Solute transport is influenced by the concentration of carbohydrate, type of carbohydrate and number of transportable carbohydrates in the drink (Shi and Gisolfi, 1998).

Solute transport in carbohydrate electrolyte drinks has been attributed to the movement of carbohydrate, sodium and potassium across the wall of the small intestine. Solutes can be transported against or down a concentration gradient with movement of the solutes affecting the osmotic gradient and thus also water absorption (Shi and Gisolfi, 1998). Therefore, water absorption is highly correlated with carbohydrate absorption via the sodium-glucose co-transporters (Shi and Gisolfi, 1998).

The effects of spinal cord injury on gastric emptying is inconclusive, with differences in age, gender, time since injury and type of injury all affecting the results (Datz et al., 1987; Segal et al., 1995). However, any impairment of the rate of gastric emptying may affect the volumes of fluid that are consumed by those with a spinal cord lesion. If there is a slower emptying of fluid then there will be a lag between drinking and becoming adequately hydrated. This may mean that those with a spinal cord injury may experience the thirst sensation for a longer period of time (Gondim et al., 1999).. An impaired rate of gastric emptying at rest amongst individuals with an injury in the cervical region has been shown (Segal et al., 1995; Chia-Hung et al., 1999). Since gastric emptying is controlled by parasympathetic and sympathetic nervous innervation, and that the vagus nerve increases motility, the disruption of these pathways following injury could have an affect the rate of gastric emptying (Chia-Hung et al.,

1999). Innervation of the stomach arises from T5-T12 therefore injuries above this level are likely to have a prolonged gastric emptying time (Chia-Hung et al., 1999).

2.8. Fluid Balance

2.8.1. Hypohydration

On one side of the water imbalance equation is hypohydration. In the early part of the last century, many people involved with the production of optimal athletic performance believed that fluid restriction during endurance events was the best strategy to aid performance (Barr, 1999). The military first recognized the importance of fluid ingestion on physical performance, when it was identified that many major battles were won or lost based on the availability of water (Sawka and Pandolf 1990; Kay and Marino, 2000). Towards the end of the 1960s, the importance of hydration status and fluid consumption on human performance was recognised (Kay and Marino, 2000). Research provided evidence that dehydration as a result of fluid restriction had a detrimental effect on endurance type activities. Reductions in performance appeared to be proportional to the degree of dehydration (Barr, 1999). Indeed, a 1-2 % loss in body mass due to dehydration leads to an elevation in core temperature and cardiovascular strain during exercise (Hoffman et al., 1994,; Kay and Marino, 2000). A rise of approximately 0.4 °C in rectal temperature has been attributed to each 1 % loss in body mass above a 2 % body mass loss (Cheuvront and Hayes, 2001). However, it is unlikely that a 1-3% reduction in body weight will affect strength since losses of up to 5 % can be tolerated without a decrease in maximal strength (Armstrong, 2000). Acute dehydration equating to 3 % in the cold or 2-4 % in the heat, or chronic hypohydration which lasts more than 4 hours without rehydration reduces endurance performance regardless of hyperthermia or environmental temperature (Armstrong, 2000).

Although all sweat losses need to be replaced between exercise sessions, it is not necessary for them to be replaced during the training session (Shi and Gisolfi, 1998). Some degree of fluid deficit is tolerable without having any negative effects on physiological function or exercise capacity. In fact, some sporting events such as long jump and high jump in which body mass has to be moved against gravity and small amounts of fluid deficit may be beneficial to performance in these sports. In these situations, athletes' power is maintained, but the mass to be moved is reduced (Maughan and Shirreffs, 2004).

2.8.2. Hyperhydration

On the other side of the water imbalance equation is hyperhydration. If an individual consumes too much water, hyperhydration can occur. With the low sweat losses observed in individuals with a spinal cord injury, hyperhydration is more likely to occur than hypohydration.

Hyperhydration provides no thermoregulatory advantage over euhydration. It does not affect rectal temperature, oesophageal temperature, mean sweat rate or heart rate responses (Latzka et al., 1997). Indeed, some investigators caution against excessive fluid intakes during physical activity, especially those slower athletes in endurance events where excessive over consumption of fluids has previously resulted in severe health problems, and in extreme cases, death (Noakes 2003; Maughan and Shirreffs, 2004).

In fact, for some individuals, excessive intakes of water without the replacement of sodium can lead to hyponatraemia, characterised by a serum sodium concentration below 130 $mmol.l^{-1}$. This is especially concerning for the spinal cord injured population, for whom the incidence of hyponatraemia is higher than that seen in the general population.

2.8.3. Hyponatraemia And Spinal Cord Injury

Hyponatraemia is a condition in which the water-sodium balance is disturbed, resulting in a dilution of the sodium concentration. This occurs because of an increase in total body water, or due to a loss of sodium or a combination of these factors. In healthy individuals, normal serum sodium concentrations are in the range of 135-145 $mmol.l^{-1}$ (Weschler et al., 2005). However, in the hyponatraemic state serum sodium concentrations fall below 135 $mmol.l^{-1}$, when corrected for hyperglycemia (Peruzzi et al., 1994). It is noted that other texts classify hyponatraemia in individuals with serum sodium concentrations lower than 130 $mmol.l^{-1}$ (Montain, 2001; Soni et al., 1994). For the purposes of this thesis a value of 135 $mmol.l^{-1}$ will be used. The lowering of the extracellular sodium concentration results in an inflow of water to the cells altering cellular volume, which can lead to the damage and death of cells, particularly in the central nervous system (Peruzzi et al., 1994; Lin et al., 2005).

The symptoms associated with hyponatraemia are multiple and depend on the type of hyponatraemia, the speed of the fall in sodium concentration, and the severity of the fall. When the onset of hyponatraemia is rapid, the symptoms occur at higher sodium concentrations than when an individual is in a chronic state of hyponatraemia (Weschler et al., 2005). In general, the symptoms of hyponatraemia are not seen until serum sodium

concentrations have fallen below 125 mmol.l^{-1} (Adler et al., 2006). Typically, symptoms associated with hyponatraemia are mainly neurological in nature and include headaches, confusion, muscle weakness, vomiting, nausea and/or aphasia, fatigue, irritability and disorientation (Montain and Wenger, 2001; Adler and Verbalis, 2006; Lin et al., 2005).

In the general population of the United States, the prevalence of hyponatraemia is 1% (Lin et al., 2005), although this figure varies depending on the criteria used to diagnose hyponatraemia. In patients with a spinal cord injury, the incidence of hyponatraemia during the acute phase of injury (< 1 week) was much higher, with 29% suffering some degree of hyponatraemia (Peruzzi et al., 1994). For this group of hyponatraemic SCI patients, 1% suffered from severe hyponatraemia in which serum sodium concentration was between 120-124 mmol.l^{-1}, 20% suffered from moderate hyponatraemia, with serum sodium concentrations between 125-129 mmol.l^{-1}, and the remaining 79% suffered with mild hyponatraemia with serum sodium concentrations between 130-134 mmol.l^{-1}. Although hypoosmolar hyponatraemia is a common problem in the SCI population, its causes have not been fully explained (Silca and Culpepper, 1990).

While injury level was not associated with occurrence rate of hyponatraemia, Frankel's classification of SCI does seem to be related to the occurrence rate of hyponatraemia. During the acute phase of a spinal cord injury, a large amount of muscle wastage and bone demineralisation occurs, resulting in the excretion of sodium and a reduction of the body's sodium stores. The body must to adapt to these new levels, which may contribute to the development of hyponatraemia (Peruzzi et al., 1994). If the body does not react appropriately to these large sodium losses then this may be a mechanism for hyponatraemia.

Chronic Spinal Cord Injury

There also appears to also be a higher incidence of hyponatraemia in the chronic phase of a spinal cord injury, with a prevalence rate of approximately 5-10% (Soni, et al., 1994; Leehey et al., 1988; Sica et al., 1984; Sica et al., 1989).

Unlike the acute phase, where injury level did not appear to be related to the occurrence rate of hyponatraemia, there does appear to be some link in the chronic phase. Tetraplegics, and those with high level paraplegia, have a greater occurrence rate than low level paraplegic individuals.

Leehey et al. (1988) studied 5 hyponatraemic tetraplegics (plasma sodium 129±1.6 mmol.l^{-1}) compared to age and gender matched healthy controls, they found higher 24 hour urine volumes amongst the tetraplegics. The higher urine volumes were attributed to higher habitual fluid intakes. Further to this there is an inability by the tetraplegics to excrete a water load (20 ml.kg^{-1}). This was believed to be due to a reduction in the delivery of filtrate to the distal diluting segments of the nephrons (Leehey et al., 1988). Wall et al. (1993) found that a lower plasma osmolality stimulated the release of vasopressin amongst tetraplegics when compared to healthy able-bodied participants following a water load in the erect position. This would mean that water is retained in the body at lower levels of dehydration amongst tetraplegics in the erect position compared to healthy able-bodied individuals.

Fluid intake

From the literature on able-bodied athletes, it is known that drinking excess fluid can lead to hyponatraemia (Weschler et al., 2005). Following SCI, there is an emphasis placed on the need to consume fluid in order to reduce the risk of urinary tract infections and decrease the likelihood of developing kidney stones and prevent catheter blockages (Burr et al., 1993; Kahn 2003) Leehey et al. (1988) found high daily urine volumes (4.454±0.624 l) in 5 tetraplegic participants who suffered repeated hyponatraemia. They attributed the high urine volumes to their large fluid intakes. High fluid intakes of 6-10 litres in this population are further supported by Silca et al. (1989) who attributed most hyponatraemic incidents in 14 patients with a spinal cord injury to excessive fluid intake. For individuals with a spinal cord injury, it is possible that the body's ability to handle the excess fluid is impaired, due to decreases in glomerular filtration rates and the release of vasopressin at lower plasma osmolalities whilst in the erect position. It is suggested that these mechanisms could, in turn, lead to an increased risk of hyponatraemia. A further complication of an altered fluid intake amongst those with a high level spinal cord injury is autonomic dysreflexia in response to an abnormal water load (Claydon et al. 2006).

Arginine vasopressin dependent mechanisms (AVP)

In humans, the osmotic threshold for the release of AVP is 280-290 mosmol.kg^{-1} (Robertson, 1987). When serum osmolality is greater than this threshold, there is a 2-4 fold increase in AVP for every 1% increase in serum osmolality (Dunn et al., 1973).

As the region responsible for vagal tone, the thoracic 6th (T6) vertebra is the crucial level of injury for sympathetic flow to the heart and major cardiovascular systems. Individuals with lesions above this level are more likely to have blood pressures which are towards the low end of the normal range when in the seated or upright position, and are therefore at greater risk of hypotension as a result of tilting (Peruzzi et al., 1994; Soni et al., 1994). A complication of this resultant low blood pressure is its potential to act as a stimulus for AVP release via the activation of the carotid and aortic baroreceptors (Pham et al., 2006; Peruzzi et al., 1994). In this case, water will be conserved even if plasma osmolality is low. In those with a spinal cord injury, the interruption of the descending sympathetic pathways and the inability to contract the leg muscles results in an absence of a pumping action in the leg muscles causing blood to pool in the lower limbs. This decrease in central blood volume results in an altered haemodynamic balance which can act as a stimulus for AVP release, altering the normal pattern of urine output, which is frequently reduced in the sitting position (Soni et al., 1994). A decrease in blood pressure will also have a direct effect on the blood flowing through the kidney and thus reducing the rate of glomerular filtration.

2.9. Deuterium Oxide (Heavy Water)

First discovered by Harold Urey in 1931, deuterium oxide (D_2O) is a stable heavy isotope of hydrogen and is denoted by the symbols ^2H or D. Due to its larger molecular mass, it is commonly known as heavy water (H_2O has molecular weight of 18: D_20 heavy water - has a molecular of 20). In small quantities, it is a non toxic tracer and is distinguishable from H_2O by its additional neutron (Fjeld et al., 1988). Its physical and chemical properties are somewhat similar to those of water, H_2O but there are, however, slight alterations in the freezing and boiling points from H_2O (light water). The freezing and boiling points are higher for D_2O than those seen for H_2O (3.82°C and 101.4°C respectively) and it is 10.6% denser than normal water.

2.9.1. Total Body Water (TBW)

The interpretation of fluctuations in body weight and changes in the state of hydration requires knowledge of the total amount of water in a given organism (Schloerb et al., 1950). An understanding of the amount of water (total body water) is important for the interpretation of body mass changes over a period of days (Schloerb et al., 1950).

Deuterium oxide has the ability to diffuse into all of the fluid compartments of the body within a short time. Evidenced by equal concentrations in blood and urine samples taken simultaneously, it is not secreted, selectively stored or metabolised in the body (Davis et al., 1987). This means total body water can be determined by measuring the concentration of deuterium oxide in a sample of blood, urine or saliva (Davis et al., 1987; Schloerb et al., 1950). Deuterium oxide (D_20) has been used to measure total body water (TBW) since Heversey and Hofer (1934) first reported that an injected volume of deuterium oxide, into human participants, is able to distribute itself into a volume of water that closely matches total body water (Westerterp, 1999; Van Marken Lichtenbelt et al., 1994; Solomon et al., 1950). When using deuterium oxide to measure total body water, the equilibrium is actually being described. As cautioned by Schloerb and colleagues (1950), this volume may vary to a minor extent from the actual total body water.

TBW has previously been estimated to be between 51.9% and 75% of body mass in healthy adult participants (Schloerb et al., 1950). A summary of previous studies measuring total body water is shown in Table 2.9.1.

It is possible to determine the % body fat of an individual by using TBW measurements. Pace and Rathbun (1945) found that in guinea pigs, body water and electrolyte content bear a constant relationship to fat free tissue or lean body mass. When applied to humans, calculation of lean body mass and body fat from TBW is possible by means of the following formula:

$$\% \text{ fat} = 100 - \%H_2O / 0.732$$

(Schloerb et al., 1950).

The main source of error when using deuterium to measure TBW lies in the exchange of deuterium atoms with labile hydrogen atoms of organic molecules (Schoenheimer and Rittenberg, 1935; Smith et al., 1936). Not only does deuterium exchange with water to form DHO, the D atoms will also exchange with exchangeable H atoms of organic molecules, resulting in an overestimation of total body water. These additional exchanges are chiefly hydrogen in carboxyl, hydroxyl and amino groups where the H atom is not bound to carbon (Schloerb et al., 1950). Owing to these exchanges deuterium will be diluted into a volume greater than that of body water alone, resulting in an equilibrium dilution which is lower that which would be observed if D_2O exchanged only with water.

According to Westerterp (1999) the dilution space for deuterium is assumed to be 4% larger than total body water space. Due to this difference a correction factor of 1.04 is used to correct for this error when determining total body water by deuterium dilution. Deuterium can be administered orally or via injection. Oral consumption is easier for participants and provides an opportunity to observe the absorption of water from the alimentary tract. Its concentration can be measured precisely in small quantities of fluid by infrared spectrometry, gas chromatography, nuclear magnetic resonance and mass spectrometry.

The doses of deuterium oxide administered to human participants have no toxic manifestations. However, at doses greater than 10% of body mass some toxic effects are seen. In mice toxic effects are seen when serum deuterium concentrations reach 25%. Further, when mice were given 100% deuterium oxide with no other water source available it was lethal within a week. Rest assured, such high doses are not required for the measurement of total body water in humans, as a 100 g dose of deuterium oxide in a human subject produces a serum concentration of only 0.2%. No ill effects have been observed following D_2O administration to either healthy and sick patients (Schloerb et al., 1950). 5g of deuterium is sufficient to increase the isotopic excess to approximately 500% above background levels (Battistini et al., 1995) and 20g of deuterium represents approximately 0.04% of total body water (Davis et al., 1987)

Serum has long been considered the medium of choice for measuring isotope enrichment because of the rapid tracer equilibration in this medium (Schoeller et al., 1982). However, urine or saliva sampling is less burdensome for the subject and allows for participants to maintain their normal daily activities.

One study has shown that the measurement of isotope enrichment from urine samples was less reliable than in serum (Schoeller et al., 1980). This discrepancy is may be due to the longer time period for isotopic equilibration of the bladder contents relative to serum (Schoeller et al., 1980). This would help to explain why the ^2H enrichment values tended to be lower in urine than in saliva or serum. There were no significant differences in isotope enrichment between the three media - urine, serum and saliva - over a longer time frame of 2-6 hours (Jankowski et al., 2004).There does seem to be some debate in the literature regarding the optimal time for equilibration of deuterium oxide with all the body fluids; however it is to ensure that both the timing of the dose administration and sample collection time are correct (Van Marken Lichtenbelt et al., 1994). Additionally, as discussed by Janowski

et al. (2004) the method of sample collection will also affect the time required between deuterium intake and sample collection. Some authors suggest that it takes as little as 2-3 hours between deuterium ingestion and sampling time (Raman et al., 2004). Wong et al. (1989) found that the isotopic enrichment from urine samples collected at 3, 4, 5 and 6 hours after ingestion of deuterium oxide were within 3% of each other. Westerterp et al. (1995) found an underestimation of total body water with isotope dilution after 4 hours (a discrepancy which increases with the size of total body water) and believed that a 10 hour equilibration time was more accurate. A longer period of time between ingestion and sample collection is further supported by the findings of some studies in which random results have been seen from urine samples. in some individuals, when the period between ingestion of deuterium and sample collection is too short and in some individuals it can take over 6 hours for 100% equilibration to occur (Wong et al., 1989; Schoeller et al., 1980; Van Marken Lichtenbelt et al., 1994). Further to this Hevesy and Hofer (1934) considered the isotope concentration in urine after the first day of D_2O ingestion to be identical to body water concentration. Due to deuterium's exchange with liable hydrogen atoms of organic molecules, mainly hydrogen in carboxyl, hydroxyl, amino and any other groups where the H atom is not bound to carbon there is an overestimation of total body water (Schloerb et al. ,1950). Hence a correction factor (1.04) is included in the calculation for total body water (Westerterp, 1999).

Equilibrium time is further influenced by the feeding status of the subject (Van Marken Lichtenbelt et al., 1994). If the subject is in the fasted state between the intake of deuterium and the collection of the first sample then the calculated total body water was significantly lower (at 6 hours post-dose) than when participants were in the fed state and this effect was even greater if participants were allowed to eat between the time of dose administration and sample collection Van Marken Lichtenbelt et al. (1994). This is probably due to a slower equilibration time, given that in the fed state, there may be greater intestinal transport and absorption through the intestinal wall which speeds the equilibration process (Van Marken Lichtenbelt et al., 1994). To avoid any contamination or dilution of the sample when measuring total body water Van Marken Lichtenbelt et al. (1994) suggest that the second urine sample after ingestion should be used to avoid dilution of the deuterium with the contents of the bladder.

Previous studies

Table 2.9.1 summarises previous studies examining total body water. When considering results relevant to this thesis, these studies show that in elite athletes, total body water of elite athletes was found to be 63.2±1.9 % of body mass and was 60.2±1.9 % of body mass for non elite athletes (Battistini et al., 1995).

2.9.2. Water Turnover Rate (WTR)

Body water turnover refers to the replacement of body water in a day or other given period, and also shows body water homeostasis (Shimamoto and Komiya, 2000). In order to measure Water Turnover Rate (WTR), a bolus of deuterium oxide is consumed, similar to the method used to measure total body water.

This then equilibrates into all the body's fluid compartments and is diluted by the body water. The labelled water is then excreted from the body though all routes of water loss e.g. sweat, respiratory loss and urine loss, and the deuterium is further diluted over time by water intake. By collecting samples over a period of time (usually 7 days although some studies have used different time frames) the time course of labelled water dilution provides a measure of water turnover (input and output) per unit of time (Fjeld et al., 1988). The calculation of water intake from deuterium oxide elimination is based on the assumption that:-

1) Deuterium equilibrates uniformly within a single compartment,

2) The size of this compartment is constant or changing in a uniform manner,

3) All water regardless of the route of exit carries deuterium in proportion to deuterium in that single compartment, and

4) Deuterium exits only as water.

The optimal observational interval for the measurement of water turnover rate is one to thee biological half lives of the isotope (Shimamoto and Komiya, 2000). This can be as short as 3 days for individuals who are undertaking a lot of endurance exercises, to a period up to 40 days for those individuals who are extremely sedentary participants and/or have a low water intake (Shimamoto and Komiya, 2000). Sweat loss and metabolic water production are affected by physical activity and therefore activity levels can influence the rate of water turnover (Van Marken Lichtenbelt et al., 1994).

While water content can fluctuate over the course of a day in a healthy adult, consistencies do occur across days, when measured at the same time of day (Adolph et al., 1947). Additionally, even if any fluctuations do occur then they will have only have a minimal effect on the estimation of water turnover using deuterium (Lifson and McClintock, 1966; Nagy, 1980). Since this method of analysing water turnover rate does not directly measure water intake, and given that preformed water intake constitutes only 80-85% of water turnover volume, it is necessary for values need to be corrected for metabolic water, inspired water and transcutaneous water which are estimated to constitute 16-18% of water influx volume to provide accurate results (Raman et al., 2004). A summary of previous studies concerning water turnover rates is shown in Table 2.9.1.

Table 2.9.1. Total Body Water, Water Turnover Rates and Non-Renal Losses Seen Amongst Healthy Participants, Special Populations and Athletes from Previous Research.

Study	Subject	Body mass	TBW	Relative TBW to Body mass	WTR	Relative WTR to body mass	Non-renal
		(kg)	(l)	(%)	($l.d^{-1}$)	($ml.kg.d^{-1}$)	($l.d^{-1}$)
Fush et al. (1996)	13 healthy participants (11 males, 2 females), age 30.2±5.4 years, at altitude	73.5±10.3	43.1±7.3	58.6±3.4		45±7	
Raman et al. (2004)	458 healthy participants (251 male, 207 female), age 40-79 y				Males 40-49 yrs 3.81±1.24 50-59 yrs 3.63±0.89 60-69 yrs 3.55±0.92 70-79 yrs 3.55±0.78 Females 40-49 yrs 3.26±0.78 50-59 yrs 3.03±0.77 60-69 yrs 2.87±0.66		Males 40-49 yrs 1.61±0.79 50-59 yrs 1.53±0.74 60-69 yrs 1.00±0.67 Females 40-49 yrs 1.11±0.68 50-59 yrs 0.65±0.43 60-69 yrs 0.49±0.54
Schoeller et al. (1982)	5 males, 4 females age 14.2±2.1 y,	116.8±15.3	45.5±10.2	38.9			
Spinal Cord injury and Controls							
Cardús et al. (1984)	12 Healthy Controls	80.7±12.1	49.3±7.3	61.0			
Cardús et al. (1984)	22 Paraplegics >3 months after injury	70.1±5.6	39.0±4.6	55.6			
Cardús et al. (1984)	23 Tetraplegics >3 months after injury	72.1±5.7	34.9±7.4	48.4			
Elderly							
Leiper et al. (2005)	Independent 9 males 13 females, aged 75 (69-88) y summer 15 (11-19) °C winter 4(0-7) °C	Summer 67.5 (43.0- 87.5) Winter 65.3 (44.0-86.5)	Summer 27.4 (19.4-41.8) Winter 28.6 (20.7-38.8)		Summer 2.2 (1.3-3.6) Winter 2.1 (1.4- 3.6)		Summer 0.39(0.3-1.4) Winter 0.49 (0.15-0.87)
Leiper et al. (2005)	Dependent 4 males, 11 females, age 83 (72-93) y summer 15 (11-19) °C winter 4(0-7) °C	Summer 62.1 (39.7-87.0) Winter 60.5 (38.8-87.9)	Summer 28.8 (16.8-32.9) Winter 27.0 (17.1-31.8)		Summer 1.5 (0.9-2.9) Winter 1.6 (1.0-2.8)		Summer 0.37 (0.15-0.87 Winter 0.47 (0.11-1.11)
Athletes and Controls							
Battistini et al., (1994)	Elite footballers,	82.8±8.0	52.3±5.0	63.2±1.9			
Battistini et al., (1994)	Non competitive participants	76.5±6.9	46.1±4.2	60.2±1.9			
Leiper et al., 2001	Cyclists		54.3 (43.2-62.3)	70.1 (65.5-73.9)	3.5(2.9-4.9)		1.4(1.1-3.0)
Leiper et al., 2001	controls		41.4(35.8-49.0)	63.5(52.7-71.0)	2.3 (2.1-3.5)		0.4(0.3-1.5)
Leiper and Maughan, (2004)	Swimmers 4 males, 4 females age 17±2 y	67.6±6.7	38.7±5.6	20.1±6.3	3.7±1.2	54+18 9.4±3.0 %	2.7±1.4
Leiper and Maughan, (2004)	Controls males, 4 females 2, age 17±2 y	60.1±7.7	37.5±8.0	17.5±1.4	1.7±1.4	28+21 4.5±3.0 %	0.9±1.3
Stofan et al. (2007)	American football varsity players training twice a day temperature 19.3-25.6 °C	118.3±20 (88.6-145.0)	76.8±9.1 (60.5-89.9)		10.3±2.2	88±18 $ml.kg.d^{-1}$ (13% TBW)	
Stofan et al. (2007)	Controls, injured American football varsity players temperature 19.3-25.6 °C	100.7±10.7 (86.4-113.6)	66.7±9.0 (60.3-80.9)		7.0±1.0	71±4 $ml.kg.d^{-1}$ (10 % TBW)	
Fusch et al. (1998)	11 males 4 females; age 27.7±2.8 y; 7 day trek across the Swiss Alps, 120.5 Km, altitude 1285-3317 m	71.0±10.4	45.3±7.3	63.6±4.1	5.7±1.8	78.7±17.5 $ml.kg.d^{-1}$	
Hill and Davies, (2001)	37 y male. Ultra marathon 14964 km, 63.1 kg	53.0			6.08		

2.10. Pre Exercise Hydration

Burke (1997) states it is important for an individual to be well hydrated before undertaking physical activity or exercise. This is supported by Barr's (1999) findings that dehydration before exercise also results in a decrease in endurance performance.

Conversely, Maresh et al. (2004) believe that pre-exercise dehydration is primarily responsible for differences in thirst and thus fluid intake meaning that dehydration before low intensity exercise in the heat increases thirst-driven drinking. However, this relationship has not been seen in field studies on football players in cold environments (5.1±0.7 °C, 81 ± 6 % relative humidity) (Maughan et al., 2005) or in warm environments (24-29 °C, 46-64 % relative humidity) (Maughan et al., 2004). According to Maresh and his colleagues (2004), this means that due to the increased fluid intake the negative influences of pre-exercise hypohydration on plasma volume and fluid regulatory hormonal measures are attenuated. At present, there is no definite scientific consensus for the assessment of hydration status (Cheuvront and Sawka 2005). Current methods of assessment include the dilution of an ingested isotope, plasma osmolality, concentrations in the plasma of the hormones that regulate body water e.g. vasopressin, urine colour, urine osmolality or urine specific gravity (Cheuvront and Sawka, 2005). The use of measures associated with blood sampling means that a trained phlebotomist and can result in infection, bruising or vein damage (Oppliger and Bartok, 2002). Therefore, although it provides an accurate measure of hydration status it also carries risks to the athletes/ subjects and also requires specialist investigators and equipment. The use of isotope dilution to measure body water is time consuming and therefore not practical in the field setting where speed and efficiency is of importance. Plasma markers of hydration are also impractical in the field where conditions may be less than hygienic and again, the time taken to obtain a blood sample would delay the testing process. The use of urine osmolality (<800 mosmol.kg^{-1}), specific gravity (1.013-1.029) or urine colour (paleish yellow) is a simple, quick and efficient method of assessing hydration status. It has also been shown that urine markers are reliable for the assessment of hydration status and thresholds for appropriate hydration levels have been defined (Armstrong et al., 1994; Bartok et al., 2004; Shirreffs and Maughan, 1998). Other measures for monitoring hydration include the daily measurement of nude body mass as daily changes in body mass reflect alterations in fluid volumes, providing calorific intake matches expenditure (Oppliger and Bartok, 2002). Measuring daily alterations in body mass is a simple and inexpensive method for measuring daily changes in body mass over a period of time (Oppliger and Bartok, 2002).

2.11. Field Based Testing Versus Laboratory Based Testing

Research can be defined into two categories depending on whether it answers 'basic' or 'applied' questions (Atkinson and Nevill, 2001). Basic research is often designed to prove or disprove a theory of underlying mechanisms for a particular phenomenon. This type of research allows investigators the flexibility to control of other confounding variables (Atkinson and Nevill, 2001). Conversely, applied research is concerned with 'real world' settings and whether a particular variable, without controlling for other factors, effects performance (Atkinson and Nevill, 2001). Both types of research are equally valid in research, but it is merely the type of validity which differs. Basic research is considered to have internal validity whereas applied research has external validity (Thomas and Nelson, 1996). Therefore, the research question and aim of the study need to be considered before deciding on the type of research to be undertaken.

Given that this thesis is concerned with providing descriptive data concerning the 'normal' activities of the participants either during exercise or daily living, a more applied type of research is to be undertaken. Therefore the number of controls placed on the participants is limited and they are encouraged to continue their training/ days as normal. It is hoped that by doing this a true description of their drinking behaviour will be obtained. Therefore the studies in this thesis will have external validity but will lack internal validity.

It would have been possible to prescribe exercise workloads for participants and to control the conditions to assess sweat losses during exercise, but this does not occur during normal training sessions. Athletes train and compete at different intensities throughout training and during matches. By using normal training sessions, it is possible to determine the amount and composition of the sweat participants lose and to assess the volumes of fluid that they consume. These values, obtained from normal activities, will provide the greatest value to athletes since the data is reflective of activities and habits that they do on a regular basis, enabling them to adjust their behaviours depending on the findings. However, it is not possible to confirm with absolute certainty why these factors differ between athletes as it could be due to exercise intensity, disability type or pre-training diet.

2.12 Conclusion Of Literature Review

As discussed, the kidney is responsible for the maintenance of homeostasis within the body with respect to fluid and electrolyte balance. When considering 'special populations', a logical question therefore follows: what happens to the habits of those individuals who have had kidney failure and subsequently receive a kidney transplantation? Does the awareness of the body's ability to maintain homeostasis and the restrictive diets they are placed on during kidney failure result in an altered pattern of fluid and sodium intake after transplantation?

The literature review also shows that individuals with a spinal cord injury have impaired abilities to thermoregulate and thus exhibit differences in the regional and whole body sweat response. How does this effect sweat losses during a normal training session? If whole body sweat rates are reduced, but sweat rates on the sensate skin are increased, does this result in an increased sweat sodium and sweat chloride concentration? As sweat sodium and sweat chloride concentrations are related to sweat rate (Allan and Willson, 1971). Further, individuals with spinal cord injuries are not the only impairment group to participate in disability sport. Research on children with cerebral palsy has shown that they too have an altered sweat response, which may also mean a difference in sweat composition. Differences in the sweat rates of disabled athletes may be due to the upper body exercise involved with wheelchair sports since upper body and lower body exercise can result in altered thermoregulatory responses (Sawka et al. 1984). Therefore, the inclusion of a whole body type sport will help to determine if differences exist between wheelchair and whole body exercise.

The literature shows large fluid intakes amongst those with tetraplegia compared to able-bodied groups (Price and Campbell, 2003). Does this only occur during exercise? To further explore this, the 24 hour fluid balance of those with tetraplegia will be examined. Alterations in vasopressin and the pattern of urine excretion have also been shown amongst hospitalised patients with spinal cord injuries. Does this remain when individuals with a spinal cord injury are away from hospital setting and have greater control over their fluid intakes? This will also be examined in participants with a spinal cord injury during free living.

Chapter 3: General Methods

3.1. Ethical Approval

All of the studies presented within this thesis were granted ethical approval by the Ethical Advisory Committee at Loughborough University. Approval was obtained before the commencement of any subject recruitment or testing. All potential volunteers were first issued with an information sheet describing the experimental protocol in detail; this sheet also outlined any risks involved. Volunteers were free to discuss the testing with their families and doctors before commencing the studies, volunteers were also encouraged to discuss the testing with their coaches. All volunteers were free to ask any question regarding the study and questions were answered before the trials commenced. Prior to the start of all the studies participants were asked to provide written consent on an informed consent form and to complete a health screening questionnaire. All participants were free to withdraw from the study at any time without providing any reason for their withdrawal. All participants were informed of this right to withdraw prior to signing the informed consent form. Since a description of normal free living behaviour was required, trials were not based in the laboratory, and participants were provided with contact details for the investigator.

3.2. Participants

Details of the subject recruitment methods and specific participant characteristics are described in detail in each Chapter. At the time of all the studies, the participants self-reported both in writing and verbally that they were healthy and free from any urinary tract infections. Female participants in Chapter 6 were not studied during the menses period of menstruation. Participants were excluded if they were under 16 years of age or over 65 years of age. Research in 1966 by Dill and colleagues showed no impairment of thermoregulation with age. Participants were also excluded if they were unable to provide written informed consent due to mental illness, pregnant women were also excluded. For Chapters 5 and 6 participants were only included if they had a spinal cord injury and all the participants in Chapter 7 had received a kidney transplant.

3.3. Experimental Design

All studies were field based in which no pre-study standardisation took place. The studies in this thesis were designed to provide descriptive accounts of individuals and athletes during their normal activities. Therefore, no interference in their normal patterns was desired and no standardisation was put in place. The data in Chapters 4 and 5 were collected over the

participants' normal training session (for Chapter 4 the data was collected over 4 training sessions). No restraints or interference with the training session was undertaken. Heart rate and the duration of the training sessions were recorded so this data could be factored into the analysis.

Chapter 4 was concerned with the variability in pre-training hydration, sweat volume losses and sweat composition and no dietary restrictions or controls were put on the participants prior to each exercise session. It is possible that by controlling for dietary factors before each session, potential variations in one or more of the variables may have been masked, underestimating pre-training hydration and the true variability of sweat composition. Exercise intensity and duration, as well as environmental conditions and clothing worn, can all affect sweat losses and fluid intake patterns. Therefore for an accurate assessment of the degree of difference in sweat loss and fluid intake between training sessions to be ascertained, these factors were not controlled for.

The aim of Chapter 5 was to describe the training practises of athletes with a disability. The parameters measured included pre-training hydration levels, sweat losses, fluid intakes and sweat composition. Since the practises of a normal training session were of importance, exercise intensity and duration were not controlled and participants were asked to train as they normally would. As with methods pertaining to Chapter 4, no dietary controls were put in place since this may have affected the fluid intakes of the participants, pre-training hydration or influenced sweat composition.

The data in Chapters 6 and 8 was collected over the course of a multi day event away from the participants' normal residence. Participants were free to consume their normal foods and undertake any physical activity during the course of the study. The physical activities of these participants was observed by the investigator and also reported by the participants to the investigator on each morning of the study. The data in Chapter 7 was collected by participants whilst living at home again no restrictions were placed on the participants regarding their diet or physical activity patterns. However, participants were asked to refrain from any physical activity during the period of the study and all participants confirmed verbally to the researcher that they had adhered to this restriction.

The studies concerning water turnover rates placed no restrictions on diet since the normal fluid losses of the participants were required to describe the normal activities of the

participants. Any restrictions placed on the participants would have affected these results. Recording dietary intake sometimes results in a change in dietary patterns and so was not undertaken. Physical activity would affect water turnover rates as it is likely to increase non-renal losses. As such any restrictions placed on the participants' normal daily living activities would have resulted in an alteration of the normal water turnover rates of the participants.

3.4. Collection, Handling And Analysis Of Urine Samples

Urine collection in Chapters 4 and 5 was facilitated by asking the participants to empty their bladders/catheter bags into a plastic jug and to then pour a sample of approximately 20 ml into a labelled universal tube. This was done in private and with the remainder of the urine being discarded.

Urine collection in Chapter 6 involved participants emptying their catheter bags into a large (2 .5 litre) plastic 24 hour urine container (Western Laboratory Services, U.K.) with a screw top. Participants emptied their catheter bags in the same manner as if they were using the toilet so catheter bags were emptied whenever they were required to do so. This container was weighed every morning by the investigator and the 24 hour urine volume determined by subtracting the weight of the empty container from the weight each morning. A sample of approximately 20 ml was retained from each container in a screw top sterile labelled universal tube. If a subject urinated more than 2.5 litres in a 24 hour period, then they were provided with an additional 24 hour urine container. Containers were washed with deionised water and dried between each use. To ensure that containers were not contaminated by improper cleaning a test was performed on 15 'clean' containers. An amount of 400 ml of deionised water were added to 15 'clean' 24 hour urine containers and then analysed for sodium, potassium and chloride concentration in a similar manner to urine analysis. The resulting concentrations were 0±1 $mmol.l^{-1}$ for sodium, 1±1 $mmol.l^{-1}$ for potassium and 0±0 $mmol.l^{-1}$ for chloride. When a sodium chloride standard at the top end of the range for urinary sodium (150 $mmol.l^{-1}$) was added to 15 clean containers and then analysed the sodium concentration was 149±1 $mmol.l^{-1}$ and the chloride concentration was 151±1 $mmol.l^{-1}$. When a potassium chloride standard of 150 $mmol.l^{-1}$ was added to 15 clean containers and analysed the potassium concentration was 150±0 $mmol.l^{-1}$. These results show that urine samples were not contaminated by the 24 hour urine containers in which they were stored.

The urine collection in Chapters 7 and 8 was done by asking the participants to urinate/ empty their catheter bag into a plastic jug. Participants were then asked to pour the urine into a measuring cylinder (of various sizes) and recorded the volume of urine. For individuals using catheter bags, participants emptied their bags directly into the measuring cylinder. Participants were also asked to record the time of urination on a specific recording sheet. This recording sheet was formatted to have specific columns for time, volume and sample number. Participants were also asked to pour a sample of approximately 20 ml into a screw top sterile labelled universal tube. Catheter bags/ bladders were emptied prior to the commencement of the study.

All of the urine samples were then analysed for osmolality. Osmolality was measured in duplicate via freezing point depression using an Osmomat 030 (Genotec, YSI, Farnborough, UK). The urine samples collected in Chapters 4, 5 and 6 were also analysed for sodium, potassium and chloride concentrations. The sodium and potassium concentrations of the urine samples were also measured in duplicate and were analysed by flame photometry using a Corning Flame photometer 410C (Corning, Halstead, Essex, UK). A Corning M805 Dilutor (Corning, Halstead, Essex, UK) was used to dilute the urine to the correction concentration for the flame photometer. Urinary chloride concentrations were determined in duplicate by coulometric titration using a Jenway PCLM 3 Chloride Meter (Jenway Ltd, Gransmore, Essex, UK).

The urine collected in Chapters 6, 7 and 8 was also analysed for deuterium concentration. This was done in duplicate by infra red spectrometry (Miran-1a, The Foxboro Company, Connecticut, USA). Urine samples first underwent vacuum sublimation as described by Lukaski and Johnsonn (1985). This method requires urine samples to be frozen in liquid nitrogen before being placed in a J-shaped tube and sealed with a rubber bung; a needle attached to the vacuum was then inserted through the rubber bung removing all the air from the J-shaped tube. The needle was then removed from the J-shaped tube and the samples were left for the water to sublimate across the J-shaped tube, separating the water from the solute. The separated water from the samples was then injected into the infra red spectrometer (Miran-1a, The Foxboro Company, Connecticut, USA) and the deuterium concentration was recorded.

A calibration curve of known standards was determined. From the calibration curve and the analysis of the urine samples total body water was determined, (correcting for baseline measures of deuterium) using the equation of Schloerb et al. (1950) as shown below.

$$V_2 = \frac{C_1V_1 - C_UV_U}{C_2}$$

Where

V_2 = the volume of water into which the deuterium has diffused and equilibrated

C_1 = the concentration of the administered Deuterium

V_1 = the volume of Deuterium ingested

C_u = the concentration of Deuterium in the baseline urine sample

V_u= the volume of water excreted prior to equilibrium

C_2 = Urine deuterium concentration at equilibrium

To determine total body water the value of V_2 was multiplied by a correction factor of 0.96 to account for non- aqueous exchange of the labile hydrogen with other body constituents (Schloerb et al., 1950).

Daily water turnover rates were calculated using the rate of decline of deuterium in the subsequent urine samples as described by Lifson and McClintock (1966). The deuterium content of each morning urine sample (the sample which completed a 24 hour collection) was determined and corrected for baseline levels taken from the urine sample prior to ingestion. From these samples, a linear regression of deuterium decline is plotted from this the equation of the linear regression can be determined. This is then used in the equation of Lifson and McClintock (1966) to determine water turnover rate as shown below:

$$\text{Water Turnover Rate } (l.d^{-1}) = \text{Total Body Water } (l) \times k$$

Lifson and McClintock (1966)

K= the slope of the regression line of the natural logarithm for the decline of deuterium in the morning urine samples after correction for baseline levels.

Non-renal losses were calculated from the difference between water turnover rate and urinary losses.

$$\text{Non-renal loss } (l.d^{-1}) = \text{Water turnover rate } (l.d^{-1}) - \text{24 hour urine volume (litres)}$$

The urine samples collected in Chapter 7 were also analysed for creatinine. This analysis was done to confirm a full 24 hour urine volume. This was done by spectrophotometry (Shimadzu UV-Visible recording spectrophotometer UV-160, Kyoto, Japan) following the methods described by Vitro Scient (Hanover, Germany) which are based on the Jaffé reaction (1886), whereby the rate of dye formation, over 120 s, is directly proportional to the creatinine concentration.

Based on the equation

$$\text{Creatinine + Picric acid} \xrightarrow{\text{Alkaline Ph}} \text{Creatinine- picric acid complex}$$

3.5. Collection, Handling And Analysis Of Sweat Samples

Regional sweat samples were collected in Chapters 3 and 4 with the use of sterile adhesive absorbent patches (Tegaderm, 3M, Loughborough, UK). Patches were 40 mm by 27 mm and consisted of an absorbent patch in the middle with an adhesive plastic covering. The adhesive covering was attached to the absorbent patch and analysis was performed with the adhesive backing attached to the absorbent patch. By adding 1.5 ml of deionised water to 30 unused patches and placing 15 in temperatures of 21 °C and 15 in 30 °C for 2 hours with measures for mass taken after 1 hour and 2 hours the patches were found to be permeable to water at a rate of 0.0148±0.0080 $g.h^{-1}$ and 0.0200±0.0105 $g.h^{-1}$ for each temperature.

Deionised water was added to 15 unused patches, followed by treatment in a similar manner to the sweat patches after sweat collection, sealed in a universal tube and deionised water was added. When analysed for sodium, potassium and chloride the values were 0±0, 0.0±0.0 and 0±1 $mmol.l^{-1}$ respectively. When known standards at the lower (20 $mmol.l^{-1}$) and upper end (80 $mmol.l^{-1}$) of the range for sweat sodium were added to 15 unused patches and analysed the values obtained for sodium concentration were 20±0 $mmol.l^{-1}$ and 81±1 $mmol.l^{-1}$. When this was repeated using known standards at either end of the same range for sweat chloride concentrations were 20±1 $mmol.l^{-1}$ and 80±2 $mmol.l^{-1}$. For potassium, values obtained following the addition of a 5 $mmol.l^{-1}$ standard solution were 5.0±0.2 $mmol.l^{-1}$.

The self adhesive patches were positioned on the skin surface and since the position of the patches differed between the chapters full details of the locations of these patches are described in Chapters 4 and 5 respectively. In all cases the skin at the site where the patch was to be placed was first washed with gauze wipes and deionised water in order to remove any cosmetic products or residual electrolytes on the skin surface. The area was then dried with further gauze wipes. Sweat patches were then attached to the skin surface ensuring that an air tight seal with the skin was obtained all around the adhesive plastic backing. The patches remained in place throughout the training sessions. At the end of training patches were removed with tweezers which had previously been cleaned with deionised water. Patches were placed in sterile screw top labelled universal tubes of known weights, which were airtight. Samples were then stored for later analysis.

Prior to use a random sample of 15 patches from each pack of 50 patches were weighed individually with the packaging to determine the mass to the nearest 0.0001 g, on an electronic balance (Mettler AC100, Mettler Instruments, Zurich). The mass of the packaging was determined following the use of the sweat patches and this was subtracted from the mass of the patch plus packaging to determine the mass of the patch. Calculation of patch mass was done in this manner since removing the patch from the packaging prior to use could have lead to the contamination of the patch.

Mass of Patch (g) = [mass of patch+packaging (g)] – mass of packaging (g).

The mass of the universal tubes used for storing the sweat samples was measured to the nearest 0.0001 g, on an electronic balance (Mettler AC100, Mettler Instruments, Zurich). These were then reweighed for mass following sample collection. Assuming that 1 g = 1 ml, the mass of the storage tube before sample collection and the mass of the sweat patches were subtracted from the post collection tube mass to determine the volume of sweat on the patches.

Sweat mass (g)=[universal tube mass +patch after use (g)]–[universal tube mass pre use (g)] - [patch mass (g)]

While it is assumed that the mass of sweat in the tube would be equal to that of the volume of sweat in the patch (1 g = 1 ml), as described in Chapter 2: Literature Review, it is cautioned that this assumption is not entirely accurate since 1 ml of sweat is greater than 1 g (1.001-1.008 g). The sweat sample is then diluted by the addition of 0.75 - 1.5 ml of deionised water, using a manual pipette, to produce a volume great enough for analysis. Due to the small sample volume

as well as the concentrations of the electrolytes relative to the sensitivity of the machine only a small volume of additional water is required to provide an adequate volume for analysis. If excessive water is added to the sample then the samples become to dilute for analysis.

In order to determine the exact volume of deionised water added to the universal tubes, the universal tubes were measured as previously discussed. A dilution factor was then calculated by adding the volume of sweat to the volume of deionised water and dividing by the volume of sweat. The sweat and water in the tubes were then mixed thoroughly on an autovortex mixer (Stuart Scientific Co., UK). Immediately after mixing as much of the sample as possible was pipetted into eppendorf tubes which were refrigerated until further analysis.

Sodium and potassium concentrations were determined by flame photometry in the same manner as for urine sample, except sweat samples were diluted to the correct concentrations using a Hamilton Microlab 500 automatic pipette (Hamilton Bonaduz AG, Bonaduz, Switzerland) for the sweat samples obtained in Chapter 4 and a Hamilton Microlab 1000 series automatic pipette (Hamilton Bonaduz AG, Bonaduz, Switzerland) for sweat samples obtained in Chapter 5. The samples were analysed for chloride by coulometric titration in exactly the same manner as for urine samples.

The electrolyte concentrations of the sweat and water mix were then multiplied by the dilution factor to determine the sweat sodium, potassium and chloride concentrations.

3.6. Body Mass

For Chapter 3 and 4 body mass change was used to determine changes in fluid balance. After correction for fluid intake and any urine loss, it was assumed that the change in body mass was solely due to sweat loss. Body mass was measured to ±20 g on electronic scales (Adams Equipment Co., Milton Keynes, UK). Since participants taking part in the studies discussed in Chapter 5 had impairments which made the use of standard 'standing scales' impractical or impossible, the scale was adapted so that a plastic chair could be securely placed on the scales, enabling participants to sit on the scale, rather than stand on it. The scale was recalibrated after the chair had been positioned. The measurement of able-bodied participants prior to and after the chair had been added resulted in the same body mass being recorded. Prior to measuring of body mass participants were asked to towel themselves dry and participants were then weighed in some clothing. The types of clothing worn differed between the chapters details are provided in Chapter 4 and 5. Participants were permitted to wear clothing during body mass

measurements as logistical and privacy issues often precluded the measurement of nude body mass. Cheuvront et al. (2002) found that not correcting body mass for sweat trapped in the clothing provided an accurate assessment of sweat losses (within 0.2 %) can still be obtained, even if body mass is not corrected for sweat trapped in the clothing. As described in Chapter 2: Literature Review, respiratory water losses and substrate oxidation will have an effect on the calculated volume of sweat losses. However due to the logistical requirements of accurately measuring the volumes of these water losses/ gains the respiratory water loss and metabolic water production was not corrected for in these studies.

3.7 Statistical Analysis

Statistical analysis was performed using the SPSS version 7 software package. Details of the statistical tests for each Chapter are explained in the appropriate methods sections.

All data were first analysed for normative distribution using a Kolomogorov- Smirnov test. Normally distributed data is described using mean± standard deviation and data which was not normally distributed is described as median (range). Differences were deemed to be significantly different when $P<0.05$.

3.8 Coefficients Of Variation For The Methods Used In This Thesis

The intra-assay coefficient of each method used in this thesis is shown in Table 3.8.1. To calculate the coefficient of variation the mean and standard deviation of the difference between the duplicates of 15 samples was used.

The coefficient of variation of the flame photometer is 1.5 % for sodium and potassium at 140 mmol.l^{-1} of sodium and 5 mmol.l^{-1} of potassium. The coulometric titration is accurate to within ± 0.08 mmol.l^{-1}.

The mean coefficient of variation for urine osmolality was 1.96 % and the mean urine osmolality for this thesis was 508 mosmol.kg^{-1}. It can be assumed that the urine osmolality values presented in this thesis are within 10 mosmol.kg^{-1} of the correct value. As urine osmolality values can range from 100 mosmol.kg^{-1} to 1200 mosmol.kg^{-1} this coefficient of variation is low and therefore the results are reliable.

In this thesis the mean coefficient of variation with respect to sweat sodium concentration was 1.71 % and mean sweat sodium concentrations was 58 mmol.l^{-1}. This means that results were accurate to ± 1.0 mmol.l^{-1}. The range for these values was between 36 and 89 mmol.l^{-1} therefore

the coefficient of variation is low. With respect to urinary sodium concentrations the mean coefficient of variation was 1.78 %, the mean urinary sodium concentration was 92 $mmol.l^{-1}$, therefore the degree of variation is low as it is within 1.6 $mmol.l^{-1}$.

With respect to sweat potassium the mean concentration was 3.5 $mmol.l^{-1}$ with a coefficient of variation of 2.48 %. Therefore values were within 0.09 $mmol.l^{-1}$ a small value. The results presented in this thesis for sweat potassium concentration are reliable. Measurement of urinary potassium concentration resulted in a mean coefficient of variation of 1.74 % as the mean urinary potassium concentration was 54 $mmol.l^{-1}$. This would mean that urinary potassium concentrations were within 0.9 $mmol.l^{-1}$. Considering the range of urine potassium concentrations is 8-138 $mmol.l^{-1}$, this value is small and therefore the results for urinary potassium concentration are also accurate.

Mean sweat chloride concentration coefficients was 2.5 % and mean sweat chloride concentrations were 27 $mmol.l^{-1}$. These values mean that sweat chloride concentrations were measured to within 0.7 $mmol.l^{-1}$ as the range of sweat chloride concentrations was 18-41 $mmol.l^{-1}$ this degree of variance is small. For urinary chloride concentration the mean value was 66 $mmol.l^{-1}$ with a coefficient of variation of 2.1 $mmol.l^{-1}$. This means that the variability between duplicates was 1.4 $mmol.l^{-1}$. As the coulometric titration machine has an accuracy of ± 3 $mg.l^{-1}$. This coefficient of variation is low.

Measurement of deuterium concentration via infra-red spectrometry resulted in a mean coefficient of variation of 2.7 % however the values seen for urine deuterium concentration resulted in a mean value of 377 p.p.m. Therefore the variability was 10 p.p.m. considering the values of urine deuterium concentration this variation is small.

The determination of urine creatinine resulted in a coefficient of variation of 1.60 %. The mean value for urine creatinine is 0.037 $mmol.kg^{-1}d^{-1}$ with a range of 0.007-0.063 $mmol.kg^{-1}d^{-1}$. The coefficient of variation for this assay shows that the variability between duplicate is 0.001 $mmol.kg^{-1}day^{-1}$ which is small.

Table 3.8.1: Mean, Standard Deviations, Range of Samples Used to Determine Coefficient Of Variation for Assays Employed in Chapters 4, 5, 6, 7 and 8.

Assay	Method	Mean	Standard Deviation	Range	Coefficient Of Variation
	Chapter 4				
Urine Osmolality (mosmol.kg^{-1})	Freezing Point Depression	551	301	84-1141	1.43
Sweat/ water Sodium concentration (mmol.l^{-1})	Flame photometry	15	7	6-29	1.88
Calculated Sweat Sodium (mmol.l^{-1})	Flame photometry	54	19	18-90	2.45
Sweat/ Water Potassium Concentration (mmol.l^{-1})	Flame photometry	1.8	0.6	0.7-3.0	1.60
Calculated Sweat Potassium (mmol.l^{-1})	Flame Photometry	5.7	1.3	3.2-9.0	2.47
Sweat/ water chloride concentration (mmol.l^{-1})	Coulometric titration	23	8	10-41	2.00
Calculated Sweat Chloride concentration (mmol.l^{-1})	Coulometric titration	50	16	22-84	3.16
	Chapter 5				
Urine Osmolality (mosmol.kg^{-1})	Freezing point depression	618	282	181-925	2.02
Sweat/ water sodium concentration (mmol.l^{-1})	Flame photometry	12	4	5-18	1.44
Sweat sodium concentration (mmol.l^{-1})	Flame photometry	58	18	35-89	1.70
Sweat/ water potassium concentration (mmol.l^{-1})	Flame photometry	0.9	0.5	0.2-1.8	2.50
Calculated sweat potassium concentration (mmol.l^{-1})	Flame photometry	4.5	2.7	1.0-10.0	2.50
Sweat/ water chloride concentration (mmol.l^{-1})	Coulometric titration	31	8	18-42	2.90
Calculated sweat chloride concentration (mmol.l^{-1})	Coulometric titration	58	15	31-80	2.70

Chapter 6					
Urine Deuterium concentration (p.p.m.)	Infra red spectrometry	385	248	28-753	2.99
Calculated urinary deuterium concentration (p.p.m)	Infra red spectrometry	290	55	216-338	1.45
Urinary sodium concentration (mmol.l^{-1})	Flame photometry	112	22	39-162	1.89
Urinary Potassium Concentration (mmol.l^{-1})	Flame Photometry	41	17	8-89	1.52
Urinary chloride concentration (mmol.l^{-1})	Coulometric titration	68	34	10-160	2.52
Urinary Osmolality (mosmol.kg^{-1})	Freezing point depression	478	217	145-1324	1.92
Chapter 7					
Urine Deuterium concentration (p.p.m.)	Infra red spectrometry	226	105	7-414	3.52
Calculated deuterium concentration (p.p.m.)	Infra red spectrometry	231	77	70-345	3.41
Urinary sodium concentration (mmol.l^{-1})	Flame photometry	66	31	14-150	2.05
Urinary Potassium Concentration (mmol.l^{-1})	Flame Photometry	50	23	13-138	2.30
Urinary chloride concentration (mmol.l^{-1})	Coulometric titration	51	42	8-148	2.17
Urinary Osmolality (mosmol.kg^{-1})	Freezing point depression	361	197	117-934	2.77
Chapter 8					
Urine Deuterium concentration (p.p.m.)	Infra red spectrometry	519	189	300-973	1.67
Calculated Urine deuterium concentration (p.p.m.)	Infra red spectrometry	376	231	109-896	1.41
Urinary sodium concentration (mmol.l^{-1})	Flame photometry	99	18	30-184	1.41
Urinary Potassium Concentration (mmol.l^{-1})	Flame Photometry	71	23	15-73	1.18
Urinary chloride concentration (mmol.l^{-1})	Coulometric titration	79	26	34-120	1.70
Urinary Osmolality (mosmol.kg^{-1})	Freezing point depression	535	91	144-798	1.67
Creatinine Concentration	Spectrophotometry	0.037	0.022	0.007-0.063	1.60

Chapter 4: Variability of Sweat Quantity and Sweat Electrolyte Concentration during 4 Self Selected Training Sessions.

4.1. INTRODUCTION

In a press release, The American College of Sports Medicine (ACSM) (2005) stated that athletes should know their hourly sweat losses and drink appropriate amounts according to their sweat losses. Due to the variability in sweat rates and sweat electrolyte composition, individualised fluid consumption regimes are recommended (ACSM, 2007). The goal of any drinking regime during training should be to limit excessive dehydration, keeping losses to < 2% of body mass and to minimise any disturbances in the electrolyte balance (ACSM, 2007). Athletes must drink the correct volumes of fluid since either drinking too much or drinking too little can have a detrimental effect on performance. Consuming too much fluid results in an increase in body mass, which can be detrimental in sports where body mass has to be moved against gravity and could result in a decreased power output per kilogram of body mass (Coyle, 2004). Conversely, not ingesting enough fluids can result in dehydration, which has also been known to result in decreased performance. Ultimately, the magnitude of the detrimental effect on exercise is proportional to the degree of dehydration (Barr, 1999). However, the question remains: how much do sweat losses change from session to session? At present, very little published data exists regarding the normal variability of an individual's sweat losses, fluid intake volumes, or sweat electrolyte concentrations between training sessions. This knowledge is crucial given that large variations in sweat losses and or fluid intakes would require athletes to know the variability of their sweat losses between sessions, making planning appropriate drinking strategies extremely difficult. If sweat loss quantity and composition variability between training sessions is low, then one off testing could provide athletes with useful insights into their future fluid and salt (NaCl) needs during training, making it easier to successfully plan future drinking strategies.

Many factors alter sweat volume and sweat electrolyte concentration, including the intensity and duration of the exercise being performed as well as the core body temperature of the athlete which is dependent on environmental conditions and the work being performed (Adams et al., 1975). It is questionable whether these factors result in significant changes in the volume and composition of sweat losses between training sessions? At present, nearly all of the field based papers (Table 4.1.1.) have been based on a single training session. Zetou et al. (2008) investigated the sweat losses of 46 elite (had played internationally) and non-elite male volleyball players occurring from 3 beach volleyball games at an official tournament. Unfortunately, all data was pooled and only group mean±SD values along with the range for the each of the three games was provided with no data regarding individual players given. Stofan et al. (2005) investigated the sweat losses of

national collegiate American footballers during a training camp with 2 a day training sessions, in an attempt to indentify predictors of cramp. No individual data was provided by Stofan et al. (2005), but they did show the mean±SD values for the morning and evening session. The results were split between those players who had a history of heat cramp and those players who had not previously suffered from cramp. For those who had previously suffered from cramp, the variability of their sweat losses was low 0.17 $l.h^{-1}$ (AM 1.49±0.6 $l.h^{-1}$ and PM 1.66±0.27 $l.h^{-1}$) between the 2 training sessions. For those who had not previously suffered from cramp, the difference in the volume of sweat produced between the morning session and evening session was larger 0.81 $l.h^{-1}$ (AM 0.99±0.41 $l.h^{-1}$ and PM 1.80±1.48 $l.h^{-1}$). Since the paper was concerned with potential causes of heat cramp no reasons for the differences between the sweat rate responses to the two sessions of the groups was postulated by the authors. Providing only mean and standard deviations masks the individual differences between the two sessions, hence the large standard deviations. The number of participants in this study was small, with only 5 participants in each group, and there was a wide range of sweat volumes during the evening training session being recorded amongst the group of non-crampers as evidenced by the high standard deviation, which masks any individual differences between the sessions. Individual data concerning sweat volume losses between exercise sessions has been demonstrated by Adams et al. (1975) who reported the values of a 38 year old male with a body mass of 70 kg during treadmill running in temperatures of 10, 22 and 35 °C, and a relative humidity of 28-30 %. Sweat loss was calculated by body mass changes accounting for fluid intake, blood withdrawn, CO_2-O_2 exchange and respiratory water loss (Adams et al. 1975). Each run was completed on 3 occasions which differed only in duration: once before heat acclimatisation and twice following heat acclimatisation. The volume of sweat lost per hour before acclimatisation in the cool conditions (10 °C) ranged from 0.69-0.72 $l.h^{-1}$, a difference of 0.03 $l.h^{-1}$ or ~ 4%. In moderate conditions, the range was 1.21-1.25 $l.h^{-1}$ (difference 0.04 $l.h^{-1}$; ~ 3 %), and in hot conditions the sweat loss range was 1.91-1.98 $l.h^{-1}$ (difference 0.06 $l.h^{-1}$; ~ 3%). Following acclimatisation, sweat losses in the moderate conditions was 1.33-1.35 $l.h^{-1}$ (difference 0.02 $l.h^{-1}$; ~1 %), and in the hot conditions it was 2.02-2.13 $l.h^{-1}$. This equates to a difference between the two runs under these conditions of 0.11 $l.h^{-1}$, or ~ 5 %. These data tend to suggest that when conditions are kept constant between the exercise sessions, then differences in sweat rates are low. However, the effect of environmental temperature could alter the volume of sweat rate by 1.29 $l.h^{-1}$ (i.e. between the hot and cold trials before acclimatisation) (Adams et al., 1975). Acclimatisation can also affect sweat rate produced during the same exercise session. For example, (Adams et al., 1975) found that the difference in sweat volume produced during the run in moderate conditions before

acclimatisation was ~0.14 $l.h^{-1}$ a value which was lower than after heat acclimatisation. Given that athletes do not train under the same conditions for each of their training sessions, the laboratory based study of Adams and colleagues (1975) may not reflect the consistency or variability of sweat rates amongst athletes during their normal daily training sessions. Further the study by Adams and colleagues (1975) had only one participant therefore findings can not be generalised. There is even less published data regarding differences in sweat electrolyte composition between training sessions. It is known that the sweat electrolyte composition is variable within an individual since a variety of factors influence concentrations of the electrolytes, including sweat rate and state of acclimatisation (Maughan and Nadel, 2001). Yet, the magnitude of the effect these variables will have on a participants sweat composition is unclear. Additionally since, to this as sweat rate has an effect on sweat electrolyte composition, all variables which can potentially effect the sweat rate of an athlete will also have an influence on their sweat electrolyte composition. Stofan et al. (2005) compared sodium losses of American football players who had a history of heat associated cramp to players who had not previously suffered from heat cramps by analysing their sweat losses during 2 training sessions. Both sessions were conducted on the same day, and each session lasted 2.5 hours. In their findings Stofan and colleagues (2005) reported that sodium concentrations in the players with cramp were 30±9 $mmol.l^{-1}$ during morning practise (sweat rates were 1.49±0.60 $l.h^{-1}$) and 28±7 $mmol.l^{-1}$ during evening practise (sweat rates were 1.66±0.27 $l.h^{-1}$). For players who did not have a history of heat associated cramp, sweat sodium concentrations were 12±5 $mmol.l^{-1}$ (sweat rates were 0.99+0.41 $l.h^{-1}$) during morning practise, and 16±7 $mmol.l^{-1}$ during evening practise (sweat rates were 1.80±1.48 $l.h^{-1}$). During the morning session the crampers thus lost 5.0±2.6 g of sodium, and in the evening session they lost 5.3±2.2 g of sodium, a difference of 0.3 g. Conversly, the non- crampers lost 1.5±0.5 g in the morning session and 3.1±2.0 g in the evening session (Stofan et al., 2005). The sodium losses for the crampers did not differ by a large amount between morning and evening training sessions as 0.3 g would equate to approximately 0.75 g of salt (NaCl). These differences reflect the differences in sweat rates and the larger sodium loss amongst the crampers is due to the greater sweat rates. If these athletes are advised to replace the additional sodium losses based on the first session i.e. they are advised to replace approximately 5.0 g (advise bases on individuals losses) after each training session then the difference of 0.3 g would have a minimal effect on performance. This is because their normal diet of collegiate athletes is likely to contain 2.9±1.3 g of sodium per day (Hinton et al., 2004) However, for the non-crampers, the sodium loss difference between the morning and evening session was 1.6 g which is approximately 4 g of salt (NaCl). It is not known if salt losses were replaced between the morning and afternoon session. The

amount of variation is greater than the reported intakes of American male collegiate athletes of 2.9±1.3 g (Hinton et al., 2004). This amount is potentially greater than their intakes (if intakes are at the lower end of the range indicated by Hinton et al. (2004)) and may require athletes to alter their diet to ensure they ingest enough salt (NaCl) to match these losses, this finding indicates that there are large differences in salt losses between training sessions and therefore salt intakes may need to be tailored to each training session. Although, it is cautioned not all athletes will consume the same amount of salt and some may already consume sufficient salt in their diet to replace sweat losses. However, since sweat sodium concentrations for this group were similar, it is argued that the large variation in sweat loss between the two training sessions accounted for the difference in the total sodium loss. Unfortunately this study did not provide any individual data and so individual variability cannot be determined.

The aim of the present study is to determine if there is any significant difference in the sweat loss and drinking behaviour between 4 freely chosen training sessions amongst healthy able-bodied recreationally active participants. If findings suggest that variability is minimal then the use of a single testing session will be considered a valid method to determine sweat losses and sweat composition of athletes. This information is vital as Chapter 5 requires the use of a single training session to determine sweat losses.

Table 4.1.1. Sweat rates for different groups of Athletes seen in the Previous Literature

Study	Participants	Exercise	Temperature (°C), Relative Humidity (%)	Sweat Rates
Godek et al. (2005)	National Collegiate Cross country runners, age 19-26 yrs, body mass 71.2±8.9 kg	Practice runs	26-34, 36-71	1.77±0.44 $l.h^{-1}$
Godek et al. (2005)	National Collegiate football players, age 19-26 yrs, body mass 116.6±16.3 kg	Pre season training	26-34, 36-71	2.4±0.53 $l.h^{-1}$
Maughan et al. (2005)	Dutch Premier league footballers, age 24±4 yrs, Body mass 78.1±6.8 kg	Football training	5.1±0.7, 81±6	1.13±0.30 (0.71-1.77 $l.h^{-1}$)
Greenhaff and Clough (1989)	19 males, age 27±2 (20-45) yrs, body mass 68.7±1.7 kg	Cycling 1 h at 192±8 (140-265) Watts equated to 70±1 (66-74) % $\dot{V}O_2$ max	22.5±0.0, 85±0	0.91±0.08 (0.43-1.67) $l.h^{-1}$
Shirreffs et al. (2005)	26 successful professional footballers, age 26±4 yrs, body mass 77±5 kg	Pre season training lasting 90 minutes	32±3, 20±5	1.346±0.24 (1.12-2.09) $l.h^{-1}$
Maughan et al. (2004)	24 male footballers at an English Premier league team	Pre season lasting 90 minutes	24-29, 46-64	1.36±0.28 $l.h^{-1}$
Stofan et al. (2005)	5 Division 1 National collegiate American footballers, age 20.2±1.6 yrs, body mass 113±20 kg, had suffered cramp at least 5 times during the previous season	2 a day training sessions morning (AM) and evening (PM). Training sessions lasted 2.5 h	23-26, 72-93	AM 1.49±0.6 $l.h^{-1}$ PM 1.66±0.27 $l.h^{-1}$
Stofan et al. (2005)	5 Division 1 National collegiate American footballers, age 19.6±0.6 yrs, body mass 110±20 kg, no history of cramp	2 a day training sessions morning (AM) and evening (PM). Training sessions lasted 2.5 h	23-26, 72-93	AM 0.99±0.44 $l.h^{-1}$ PM 1.80±1.48 $l.h^{-1}$

4.2. METHOD

Following the approval from the Ethical Advisory Committee at Loughborough University, 45 recreationally active individuals (20 males and 25 females) volunteered to participate in the study.

Full details of the participants' characteristics are shown in Table 4.2.1. Mean ± standard deviation (SD) body mass prior to the first training session was 71.89±16.60 kg and mean ± SD for age was 34±15 years. They were all healthy and physically active at the time of the study, either training in structured or non structured exercise sessions for health and general fitness or for competitive means. Training took place both outdoor and indoors.

Table 4.2.1. Mean±SD of the Age (y), Body Mass (kg) Before The First Session And Before The Fourth Session And Subject Height (cm)

	Male	Female	Overall
Age (y)	42±20	27±8	34±15
Number of participants in each age group			
18-27			16
28-37			4
38-47			4
48-57			6
58+			15
Body mass pre-training session 1 (kg)	83.1±14.1	64.0±13.6	72.0±16.6
Body mass pre session 4 (kg)	82.6±13.8	64.3±13.3	71.80±16.2
Height (cm)	180±9	163±9	168±12

Participants were tested over 4 of their normal training sessions. All data was collected in the U.K. over an 8 month period between the months of May and December. Mean ± SD environmental temperature was 14.3±10.9 °C and mean ± SD relative humidity was 58±22 %. No more than 6 weeks lapsed between an individual's first and last sessions, and no participant was tested twice during a single day. Training sessions lasted between 1-2 hours. Mean± SD heart rates during training were 139±17 beats.min^{-1}. 27 participants trained in the gym, 2 female participants undertook basketball training (indoors), 12 males aged 18-27 years performed circuit training (indoors), 1 male subject aged 18-27 years played football (outdoors), 3 (2 males aged 18-27 and 1 female aged 18-27 years) were long distance runners (outdoors) and 2 males (1 aged 18-27 years and 1 aged 28-37 years) were triathletes (outdoors).

On each occasion the following procedure was followed:
Before each of these training sessions:

1) Participants were first asked to provide a urine sample, which was later analysed to assess pre-training hydration status via osmolality (Osmomat 030, Germany).

2) Participants' drinks containers were weighed before the start of each session and participants were instructed to only drink from the containers that had been weighed.

3) Participants were weighed for body mass (Adams CE) prior to the start of each session. This was done in either nude or in minimal clothing (underwear) since some measures were taken in a public settings making it logistically difficult to obtain nude body mass.

4) The participant's skin was washed with deionised water and dried before gauze adhesive patches (3M Health Care, Loughborough, UK.) were attached to the skin surface. Further details are described in Chapter 3: General Methods of this thesis. The patches were positioned on the front of the mid thigh, forearm, shoulder and chest. In all cases, this was on the right hand side of the body.

5) A Polar Team heart rate monitor (Polar Electro Oy, Finland) was positioned around the chest and activated.

Participants were then free to train as they normally would, during the training session and there was no interference from the investigators. Any further urine produced during the training sessions was retained by the subject and weighed by the investigator. If additional drinks were required during the session then these were also weighed.

Upon completion of their training session.

1) The gauze patches were removed with sterile tweezers and stored in labelled sterile sealed containers of known weights for later analysis.
2) Drinks containers were reweighed.
3) The participants were asked to towel dry before body mass was taken again in the same clothing as worn prior to training.

The gauze patches were later analysed for sodium and potassium concentration by flame photometry (Corning, 410, New York, USA) and for chloride concentration by coulometric titration (Jenway PCLM 3, Essex, UK). The method for analysing sweat patches is described in Chapter 2: General Methods. Sweat losses were calculated from the change in body mass during the training

session and corrected for fluid intake and urinary losses which took place during the training session.

Statistical Analysis

All data were tested for normal distributions using a Kolomogorov- Smirnov test. All data were normally distributed and are presented as mean ± standard deviation (SD). A one way ANOVA with repeated measures was performed with a Bonferri post hoc test to determine any differences in the means between the 4 training sessions. Statistical significance was set at an alpha level of $p<0.05$. The first training session was selected to be the training session by which future training sessions would be compared since a single sweat testing session would be most similar to this session in that participants would be unaware of the testing procedure. To determine the reproducibility of the sweat testing protocol, 95% confidence intervals were calculated for each individual participant between session 1 and the other 3 training sessions for each individual subject in accordance with Bland and Altman (1986).

4.3. RESULTS

The primary finding of this study was the narrow 95% confidence intervals with respect to sweat volume, sweat sodium concentration, total sweat sodium chloride loss sweat chloride concentration and fluid intake between the 4 training sessions. Additionally, there were no significant differences for the group mean between the 4 training sessions with respect to sweat volume ($p=0.803$), body mass change ($p=0.699$), sweat sodium concentration ($p=0.629$), sweat potassium concentration ($p=0.783$), sweat chloride concentration ($p=0.181$) or fluid intake ($p=0.803$).

Sweat loss

Table 4.3.1. Absolute Mean, Standard Deviation, Median, Minimum and Maximum Sweat Loss (l) Values For Each of the Four Training Sessions.

Training Session	Mean	Standard Deviation	Median	Minimum	Maximum	95 % Confidence Interval	95 % Confidence Interval relative to body mass
	(l)	(l)	(l)	(l)	(l)	(l)	(%)
1	0.72	0.43	0.62	0.06	1.29		
2	0.68	0.49	0.52	0.10	1.55	0.59	0.58
3	0.65	0.42	0.53	0.10	1.47	0.46	0.60
4	0.68	0.45	0.51	0.14	1.55	0.50	0.45

The mean sweat loss for the four sessions was 0.68±0.43 litres, the group mean±SD, median (range) for each of the four training sessions are shown in Table 4.3.1. The lowest individual sweat loss was 0. 06 litres, and the greatest individual sweat loss from one session was 1.55 litres. The difference in sweat loss between session 1 and the other training sessions for each subject are shown in Figure 4.3.1. 95 % confidence intervals for the group between the first training session and sessions two, three and four are also shown in Table 3.3.1. the largest 95 % confidence interval is 0.59 litres. Differences in sweat loss relative to body mass between the first training session and sessions two, three and four are shown in Table 3.1.1. the largest 95 % confidence intervals between the first session and any of the other sessions was 0.60 %. The largest individual difference in sweat loss relative to body mass was 0.87 %. This was a 0.59 litre difference in sweat loss from session 1.

There was no significant correlation between sweat loss and age ($p=0.159$, $r^2= 0.046$). There was no significant difference between the males and female participants with respect to sweat loss ($p=0.296$)

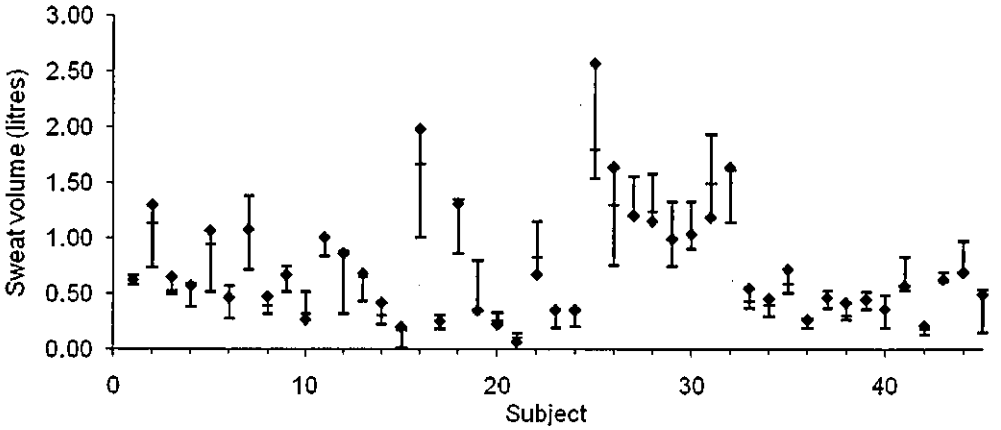


Figure 4.3.1. Difference in Sweat Losses (l) Between Session 1 and the Maximal and Minimal Values of the Other 3 Sessions.

The mean±SD of the individual differences between session 1 and the subsequent sessions was 0.23±0.15 litres. The greatest absolute individual difference for sweat loss between the first training session and any subsequent training session was 0.57 litres (sweat losses were 0.58 litres in session 1, which ranged to 1.15 litres in a subsequent training session). Expressed as a percent of body mass, this difference in sweat loss was 0.63 %. The greatest individual difference in sweat loss relative to body mass between the first session and a subsequent session was 0.87 %. The 95 % confidence intervals between session 1 and the other sessions are shown in Table 4.3.2. 95 % confidence intervals show that sweat loss from the first training session is within 0.59 litres of future sweat losses.

Calculating 95 % confidence intervals for each subject using training session 1 as the value to which training sessions 2, 3 and 4 were compared resulted in a mean ± SD 0.26 ±0.17 litres median (range) 0.19 (0.04- 0.69) litres. The largest 95 % confidence interval represents 0.78 % of body mass. 95 % confidence intervals for each subject are shown in Figure 3.3.2. These values relative to body mass showed a mean ± SD 0.36 ± 0.20 % and median (range) 0.25 (0.06-0.81) %.

There were significant correlations between session 1 and session 2 ($p<0.01$, $r^2=0.795$), session 1 and session 3 ($p<0.01$, $r^2=0.835$) and between session 1 and session 4 ($p<0.01$, $r^2=0.834$). Sweat loss during training sessions was significantly correlated to body mass change for session 1 ($p<0.001$, $r^2=0.521$), for session 2 ($p<0.001$, $r^2=0.316$), for session 3 ($p<0.001$, $r^2=0.412$) and for session 4 ($p<0.001$, $r^2=0.346$). These correlations are shown in Figure 3.3.3.

There was no significant differences with respect to gender for fluid intake (p=0.218) and there was no significant correlation between age and fluid intake (p=0.484 , R^2= 0.011).

Table 4.3.2. Absolute and Relative (to Body Mass) Mean, Standard Deviation and Median Differences Between the First Training Session and Sessions Two, Three and Four with Respect to Sweat Losses (l) During the Training Sessions.

Absolute			
	Mean	SD	Median
	(l)	(l)	(l)
Session 1-2	-0.03	0.21	-0.07
Session 1-3	-0.05	0.25	-0.04
Session 1-4	-0.04	0.22	-0.05
Relative			
	Mean	SD	Median
	(%)	(%)	(%)
Session 1-2	-0.04	0.30	0.1
Session 1-3	-0.08	0.31	0.05
Session 1-4	-0.05	0.23	0.06

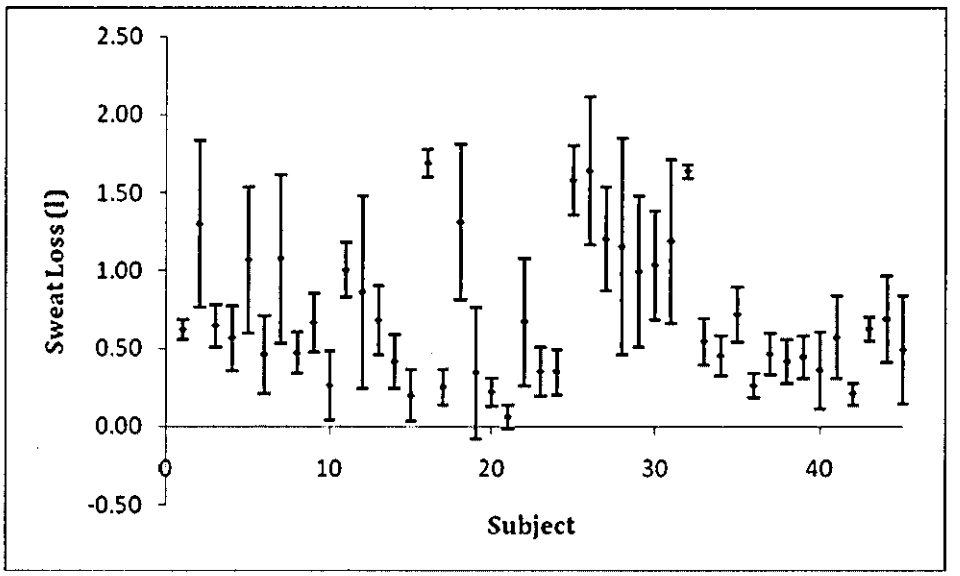
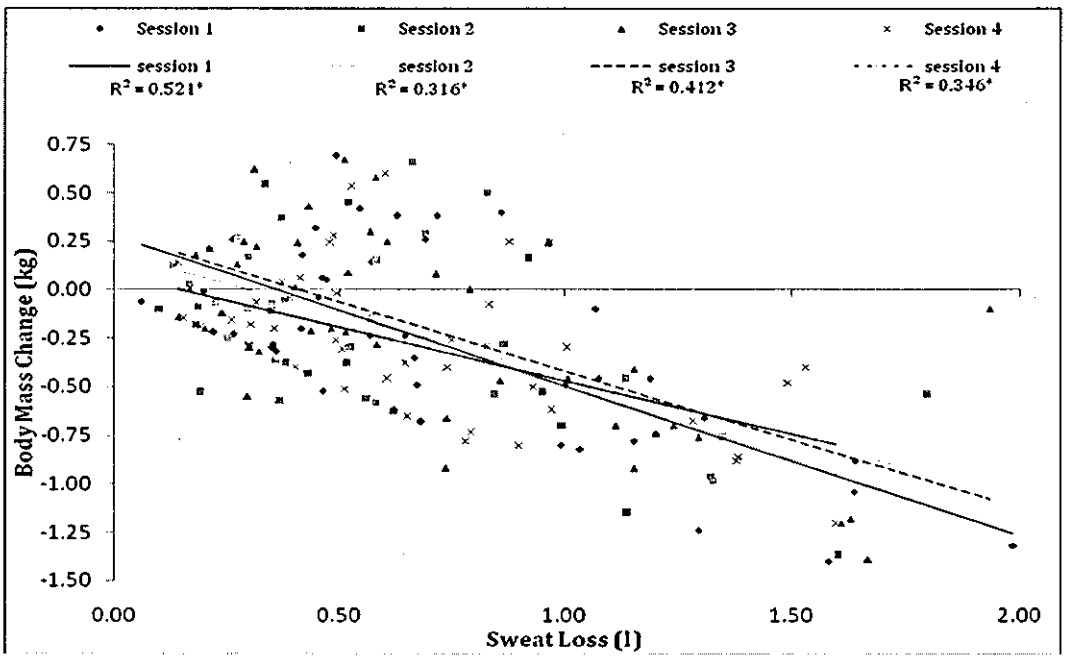


Figure 4.3.2. Sweat Losses (l) During Session 1 and the 95 % Confidence Intervals for Each Subject.



**indicates a significant (p<0.05) correlation between body mass change (kg) and sweat loss (l).*

Figure 4.3.3. The Relationship Between Body Mass Change (kg) and Sweat Loss(l) for the Four Training Sessions.

Fluid Intake

For the four training sessions mean ± SD fluid intake was 0.35±0.27 litres. The mean±SD for the difference between the first training session and the other three training sessions was 0.29±0.26 litres. There were no significant difference for the group mean between the first training session and the other four training sessions (p=0.803). Individual differences between the first training session and the other training sessions with respect to fluid intake are shown in Figure 3.3.4. The 95% confidence intervals for the group between the training sessions are shown in Table 3.3.3.

The largest individual difference for fluid intake from the first training session compared to any of the other training sessions was 0.73 litres (0.00 litres was ingested during the first training session and then in a subsequent session, this individual consumed 0.73 litres of fluid). Individual 95 % confidence intervals resulted in mean ± SD 0.26±0.23 litres, median (range) 0.22 (0.00-0.94) litres.

There was a significant correlation between the first training session and the second training session ($p<0.01$, $r^2=0.706$), between the first training session and the third training session ($p<0.01$, $r^2=0.639$), and between the first training session and the fourth ($p<0.01$, $r^2=0.717$). As shown in Figure 3.3.5., fluid intake was correlated with sweat loss for session 1 ($p<0.001$ $r^2=0.436$), session 2 ($p<0.001$ $r^2=0.336$), session 3 ($p=0.006$, $r^2=0.162$) and session 4 ($p<0.001$, $r^2=0.407$).

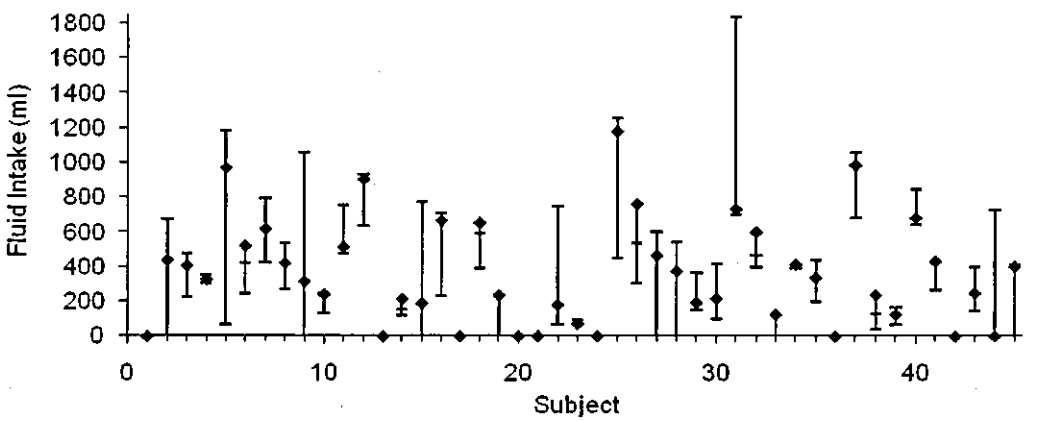
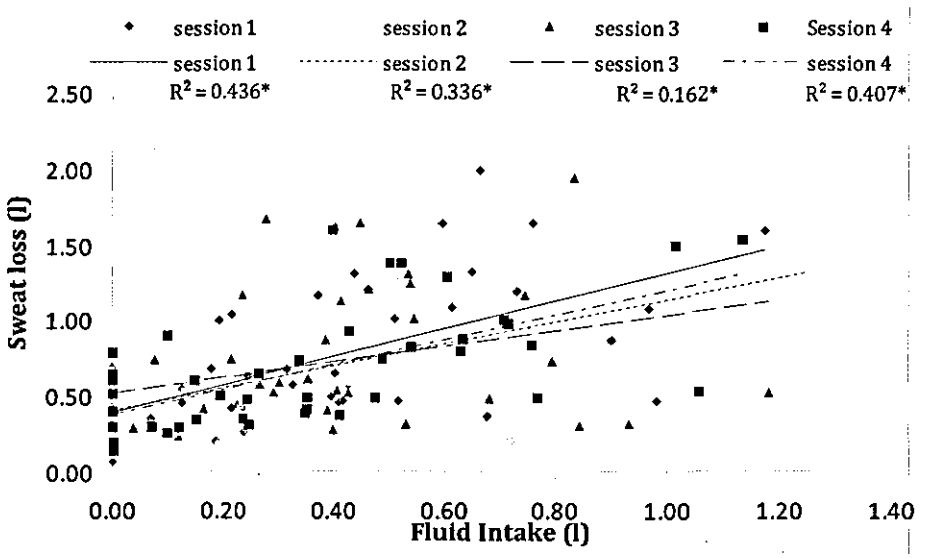


Figure 4.3.4. Fluid Intake Volumes (l) Between Training Session 1 and the Maximum and Minimum in the Other Sessions.

Table 4.3.3. Mean, Standard Deviation, Median, Minimum and Maximum and 95 % Confidence Intervals for the Four Training Sessions with Respect to Fluid Intake (l).

	Mean	SD	Median	Minimum	Maximum	95 % Confidence Interval
	(l)	(l)	(l)	(l)	(l)	(l)
Session 1	0.35	0.30	0.33	0.00	1.18	
Session 2	0.33	0.32	0.30	0.00	1.25	0.49
Session 3	0.38	0.37	0.36	0.00	1.84	0.54
Session 4	0.32	0.31	0.25	0.00	1.13	0.51



**Denotes a significant correlation (p<0.05) between fluid intake (l) and sweat losses (l).*

Figure 4.3.5. Correlation between Fluid Intake (l) and Sweat Loss (l) During the Four Training Sessions.

Body mass change

Body mass at the start of session 1 and session 4 was not significantly different (p=0.706). The mean ± SD absolute body mass change for the group during the four training sessions was -0.23±0.41 kg. Expressed relative to body mass, this is 0.29±0.66 %. Mean ± SD group values for body mass change during each of the training sessions are shown in Table 4.3.4.

The 95 % confidence intervals for each subject for the relative change in body mass between the first training session and sessions 2, 3 and 4 resulted in mean±SD 0.65±0.45 % median (range) 0.49 (0.10-1.73) %.

The largest variation from the relative change in body mass which occurred in the first training session was 1.36 % (% body mass change during the first session was -0.02 % and the largest % change in body mass was +1.34 %).

Table 4.3.4. Mean, Standard Deviation, Median, Range during Each Session and 95 % Confidence Intervals for % the First Session Body Mass Change.

	Mean	SD	Median	Minimum	Maximum	95 % Confidence
	(%)	(%)	(%)	(%)	(%)	(%)
Session 1	-0.30	0.69	-0.36	-0.02	1.19	
Session 2	-0.27	0.69	-0.37	-0.05	1.36	0.62
Session 3	-0.21	0.73	-0.29	-0.01	1.35	0.57
Session 4	-0.37	0.50	-0.42	-0.04	0.92	0.79

Sweat sodium concentration

The mean ± SD sweat sodium concentration for the group during the four training sessions was 54±16 mmol.l^{-1}, with mean sweat sodium concentrations for each session being shown in Table 4.3.5. 95 % confidence intervals for sweat sodium concentration between the training sessions for

the group are shown in Table 4.3.5. the greatest 95 % confidence interval between the first training session and any of the other training sessions was 22 mmol.l^{-1}.

When individual differences between the first training session and the subsequent training sessions were calculated the mean ± SD difference in sweat sodium concentrations was 12±8 mmol.l^{-1} and the median (range) was 11 (3-28) mmol.l $^{-1}$. The greatest individual difference in sweat sodium concentration betwee session 1 and any of the other 3 training sessions was 28 mmol.l^{-1} (range 32 mmol.l^{-1} during the first training session and 60 mmol.l^{-1} for the highest concentration). Figure 4.3.6. shows the individual differences for sweat sodium concentrations between session 1 and the other three training sessions. The largest 95 % confidence interval for an individual subject was 31 mmol.l^{-1}. The mean of the individual 95 ' confidence intervals was 14± 7 mmol.l^{-1} and the median (range) was 28 (2-31) mmol.l^{-1}.

There was a significant correlation between the first training session and session two ($p<0.01$, $r^2=0.585$), session one and session three ($p=0.01$, $r^2=0.463$) and between session one and session four ($p=0.02$, $r^2=0.446$) with respect to sweat sodium concentrations.

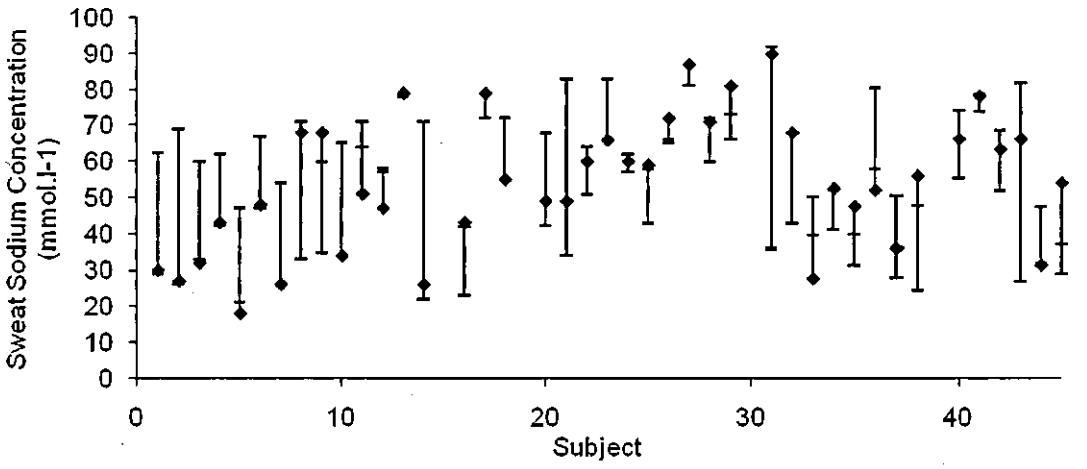


Figure 4.3.6. Individual Difference Between Training Session 1 and the Maximum and Minimum from the other Three Sessions for Sweat Sodium Concentration (mmol.l^{-1}).

Table 4.3.5. Mean, Standard Deviation, Median, Minimum and Maximum And 95 % Confidence Intervals with Respect to Sweat Sodium Concentration (mmol.l^{-1}) during the Four Training Sessions.

	Mean	SD	Median	Min	Max	95 % Confidence
	(mmol.l^{-1})	(mmol.l^{-1})	(mmol.l^{-1})	(mmol.l^{-1})	(mmol.l^{-1})	(mmol.l^{-1})
Session 1	55	17	53	24	90	
Session 2	52	16	51	21	83	14
Session 3	56	16	55	21	92	22
Session 4	51	16	47	22	85	19

If it is assumed that all sodium losses were in the form of sodium chloride (NaCl), then sodium chloride losses can be calculated. Therefore mean±SD NaCl losses were 2.4±1.7 grams and salt (NaCl) losses for each session were 2.4±1.9, 2.0±1.7, and 2.3±1.7 and 1.6±1.4 g for sessions 1, 2, 3 and 4 respectively. Again, 95% confidence intervals for the group between the sessions for NaCl are shown in Table 4.3.6.

The largest range in NaCl loss for an individual between the first session and any of the other 3 training sessions was 1.4 g (3.4 g during session 1 and a lower salt loss of 1.0 g in a subsequent session). Individual difference with respect to NaCl loss between the first training session and the subsequent sessions are shown in Figure 4.3.7. When the individual range between the first training session and the subsequent training sessions was calculated the mean ± SD was 0.6±0.4 g and the median (range) for the range was 0.5 (0.0-1.4). Individual 95 % confidence intervals for each subject are shown in Figure 4.3.8. alongside NaCl loss during the first training session. The mean ± SD of the individual 95 % confidence intervals was 0.6 ± 0.5 g, median (range) 0.6 (0.1-1.5) g.

There was a significant correlation between session one and session two ($p<0.01$, $r=0.700$), between session one and session two ($p<0.01$, $r^2=-0.643$) and between session one and session four ($p<0.01$, $r^2=0.550$). There was a significant correlation between sweat sodium concentration and sweat chloride concentration ($p=0.004$, $r^2= 0.222$) but there was no significant correlation between sweat sodium concentration and sweat potassium concentration ($p=0.703$, $r^2= 0.039$).

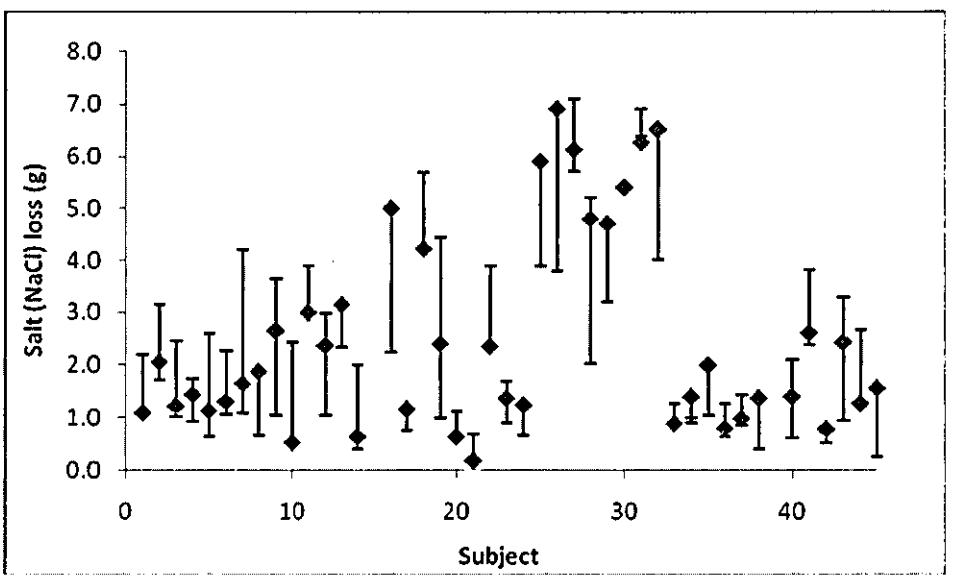


Figure 4.3.7. Calculated Sweat Salt (NaCl) Loss During Session 1 and the Maximum and Lowest Salt Losses (g).

Table 4.3.6. Mean, Standard Deviation, Median, Minimum and Maximum and 95 % Confidence Intervals with Respect to Sweat NaCl Loss (g) during the Training Sessions.

	Mean	SD	Median	Minimum	Maximum	95 % Confidence
	(g)	(g)	(g)	(g)	(g)	(g)
Session 1	2.4	1.9	1.6	0.2	6.9	
Session 2	2.0	1.7	1.7	0.3	7.5	1.0
Session 3	2.3	1.7	1.3	0.5	5.7	1.1
Session 4	1.6	1.4	1.2	0.4	6.6	0.8

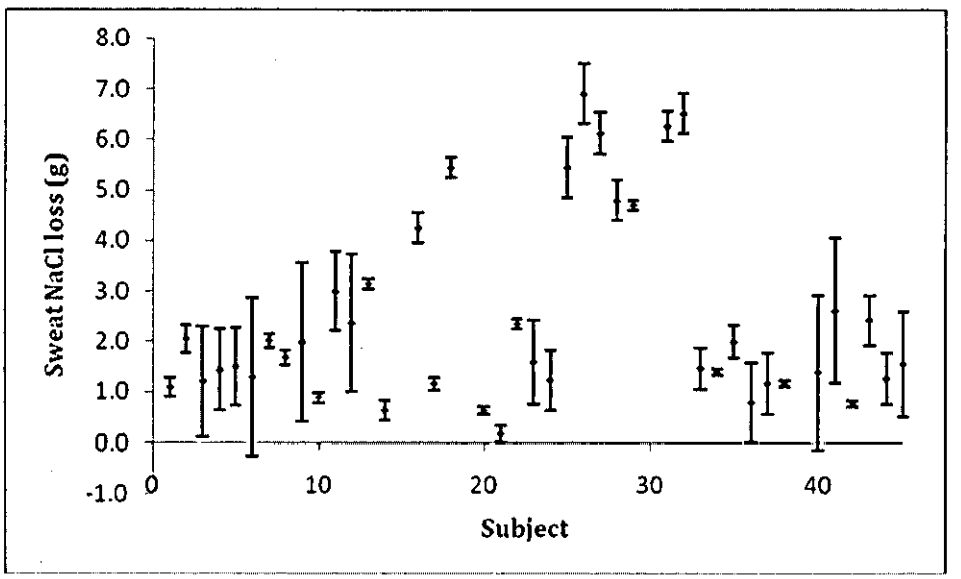


Figure 4.3.8 Calculated Sweat Sodium Chloride Losses (g) from the First Training Session with 95 % Confidence Intervals.

Sweat Potassium and Chloride Concentrations

The mean ± SD sweat potassium and sweat chloride concentrations for the group during the four training sessions were 5.7±1.3 mmol.l^{-1} and 50±16 mmol.l^{-1} respectively.

The largest individual difference in sweat potassium concentration between the first training session and any of the other training sessions was 3.1 mmol.l^{-1} (range 5.2-8.4 mmol.l^{-1}). 95 % confidence intervals with respect to sweat potassium concentration were 1.8 mmol.l^{-1} between the first session and session two, 2.9 mmol.l^{-1} between session one and three and was 3.0 mmol.l^{-1} between the first and fourth training session.

With respect to sweat chloride concentration, the 95 % confidence intervals with respect to sweat chloride concentration were 17 mmol.l^{-1} between session one and two, 18 mmol.l^{-1} between the first and third session and 21 mmol.l^{-1} between the first training session and the fourth training session. The largest difference between the sessions for an individual was 21 mmol.l^{-1} (range 40-61 mmol.l^{-1}).

Pre-training Urine Osmolality

Overall, pre-training urine osmolality was 607±233 mosmol.kg^{-1}. The mean values for session 1, 2, 3 and 4 were 571±317, 591±259, 565±279 and 646±304 mosmol.kg^{-1}. The 95% confidence intervals for the group between the training sessions are shown in Table 3.3.7.

The greatest difference in urine osmolality for a single individual between training session 1 and any of the other sessions was 703 mosmol.kg^{-1} (1089 mosmol.kg^{-1} before the first training session, and 386 mosmol.kg^{-1} in a subsequent training session). The individual difference between session 1 and the other 3 training sessions is shown in Figure 4.3.7. the mean±SD was 402±224 mosmol.kg^{-1}; the median (range) was 375 (4-924) mosmol.kg^{-1}. The individual 95 % confidence intervals resulted in a mean ± SD of 372±191 mosmol.kg^{-1} and a median (range) of 357 (70-794) mosmol.kg^{-1}. The results do show that some participants were hypohydrated prior to their exercise session, despite this they were allowed to exercise as the study was concerned with their normal behaviour during exercise. There was a significant correlation between pre-training urine osmolality and fluid intake ($p=0.037$, $r^2=0.081$).

Table 4.3.7. Mean, Standard Deviation, Median, Minimum and 95 % Confidence Intervals with Respect to Pre-training Urine Osmolality (mosmol.kg^{-1}) before the Four Training Sessions.

	Mean	SD	Median	Min	Max	95 % Confidence Interval
	(mosmol.kg^{-1})	(mosmol.kg^{-1})	(mosmol.kg^{-1})	(mosmol.kg^{-1})	(mosmol.kg^{-1})	(mosmol.kg^{-1})
Session 1	571	317	609	118	1089	
Session 2	591	259	593	96	1093	590
Session 3	565	279	597	85	1090	630
Session 4	646	304	711	95	1140	679

Heart Rate and training time

Mean ± SD for each session with respect to heart rate was 142±20, 143±18, 144±23 and 141±27 beats.mi for sessions 1, 2, 3 and 4 respectively. There was no significant difference between the exercise sessions v respect to heart rate (p= 0.988).

Mean±SD training time was 77±24 minutes. There was no significant difference for the group between the 4 training sessions (p=0.638). Despite this one subject had a difference in training time of 93 minutes (162 minutes in the first session and 69 minutes in the fourth session for this individual).

4.4. DISCUSSION

The first session was used as the session to which all subsequent sessions would be compared as this session most closely resembles single testing sessions i.e. participants are unfamiliar with testing and therefore will not adapt behaviour according to the testing. This study shows that there is no significant difference with respect to sweat loss, fluid intake, or sweat sodium losses between four training sessions for participants undertaking recreational type exercise. Further to this the 95% confidence intervals demonstrate that the results obtained from the first session are reproducible and are within a range which is nutritionally not significant. Therefore it is argued that single testing session results are reliable indicators of an individual's sweat and salt losses during normal training. Although the 95 % confidence intervals with respect to pre-training urine osmolality were large more than 50 % of participants were either euhydrated before all the training sessions or hypohydrated before all training sessions.

Sweat losses

Sweat losses in this study are low compared to those previously reported in a review of the literature amongst able-bodied sportsmen and women by Broad et al. (1996). This lower sweat loss is likely due to the number of participants in this study who exercised at very low intensities for short periods of time. Previous studies concerning sweat loss have mainly focused on elite or professional sportsmen and women during their training or while in competition. However, the low sweat loss observed in the present study may more accurately reflect the sweat losses of athletes with a disability when considering that sweat losses have generally been reported to be lower for disabled people when compared to their able-bodied counterparts. Burnham et al. (1998) found sweat rates in wheelchair basketball to be 0.9 $l.h^{-1}$, values which are comparable with results from the present study. There is a large variation of 1.49 litres, with a range of sweat loss of 0.06-1.55 litres, a finding which is not unexpected given that sweat loss is affected by so many factors including training status, acclimation, type of clothing, genetics, age, training intensity and duration (Benardot, 2006; Godek et al., 2005). Indeed it is common to see sweat losses varying by 1.5 litres between football players undertaking the same training session (Maughan et al., 2005; Shirreffs et al., 2005). Variable sweat losses were expected in this study, since every participant in this study undertook a different training session in a variety of environmental conditions. It is acknowledge that this variability in sweat losses were expected and this variability may contribute to the non-significant differences between the training

sessions for the group. The mean ± SD difference for the group between session 1 and the other sessions was 0.23±0.15 litres. This difference is lower than the findings of Stofan et al. (2001); whose study found in a group of American football players had mean sweat rates of 0.99±0.44 litres during a morning training session, and 1.80±1.48 litres during an evening training session. Therefore the difference in sweat loss between the two training sessions in the study by Stofan et al. (2001) is approximately 0.5 litres. As the absolute sweat losses are lower in the present study this may reflect the lower absolute difference between the sessions in the present study.

A body water deficit of 2 % of body mass marks the level at which dehydration can significantly affect performance (Casa et al., 2006; Cheuvront et al. 2003). The aim of most fluid intake strategies during exercise should be to replace sweat losses by consuming a minimum amount of fluid to ensure that body mass losses do not exceed 2 % of pre-exercise (euhydrated) body mass. Conversely, fluid intake should not be ingested in an excessive amount which exceeds sweat losses ultimately resulting in a gain of body mass (Casa et al., 2006).

In order for a single testing session to be a useful tool for designing hydration strategies, it must be possible for an individual to determine their sweat losses to within 2 % of pre-training body mass. The 95% confidence intervals for each subject in this study were low, with the largest 95 % confidence interval being 0.69 litres (sweat loss in the first session was 0.86 litres). Although this variability in sweat loss is large 80 % its impact on hydration as a difference in body mass change is low. The largest individual 95 % confidence interval relative to body mass represented 0.81 %. Therefore for the participants in this study future sweat losses could be predicted to within 0.81 % of initial body mass or, to within 0.69 litres by using the values obtained in the first training session. A 0.81 % decrease in body mass is within the 2 % level recommended by the American College of Sports Medicine as the greatest level of % body mass loss during exercise (Casa et al., 2006). It is also within the 1-2 % body mass decrease which Coyle (2004) believed that endurance athletes (exercise lasting more than 90 minutes) training or competing in temperate environments (20-21°C) can tolerate without a significant effect on performance. Despite this acceptable margin for dehydration, it is noted that even at 1 % of body mass loss due to water loss there is an effect on the thermoregulatory responses to exercise (Greenleaf, 1992). Although some authors suggest that a small decrease in body mass could aid performance especially in sports which require body mass to be moved against gravity, hypohydration would result in a decrease in body mass without a loss of power (Maughan and Shirreffs, 2004; Coyle 2004).

If sweat losses are lower in a future training session than during the testing session, and if an athlete is advised to consume fluid at a rate which matches the sweat losses in the testing session, then an increase in body mass of 0.81 % could potentially occur. As a result, the athlete would be carrying extra mass during exercise and the body would act to excrete the excess fluid, thus any period of hyperhydration will be short (Freund et al., 1995). However, it may last for the duration of the exercise session, because exercise results in a decreased glomerular filtration rate therefore urine excretion is decreased (ACSM, 2007). Therefore, during exercise which requires body mass to be moved against gravity, the increase in body mass could result in a decreased power output per kilogram of body mass and decrease performance (Coyle, 2004). However, there is no data to support this theory (Coyle, 2004). Other research would suggest that time to exhaustion during a treadmill run in the heat (49°C) is increased when hyperhydrated by 2 litres, although this improved performance was not significant (17.3 minutes versus 16.9 minutes) (Blyth and Burt, 1961). Hyperhydration may delay the onset of hypohydration and its benefits may lie in this effect. Therefore, hyperhydration may or may not improve performance and the effects of a 0.81% gain in body mass are likely to be minimal as this increase is relatively small. This becomes even more salient when considering that the potential 0.81 % gain represents a similar change in body mass to the normal daily fluctuation in body mass which was approximately 0.66 % (0.51±0.20 kg) amongst 65 males (body mass 77.15±10.80 kg) (Cheuvront et al., 2004).

Additional support bolstering the reliability of a single testing session in determining sweat losses across subsequent sessions can be gleaned by comparing the mean±SD range between the sessions for each subject with the values shown for the reproducibility of the whole body wash down technique. In a more controlled setting, Shirreffs and Maughan (1997) tested the reproducibility of a whole body wash down technique for measuring sweat loss under controlled laboratory conditions. Shirreffs and Maughan (1997) controlled the environmental temperature, relative humidity, exercise type, exercise intensity (60 % $\dot{V}O_2$ peak) and exercise duration during each of the training sessions. Additionally, diet for 24 hours prior to testing was controlled. Shirreffs and Maughan (1997) found the mean range for sweat loss was 0.312 litres, with one individual's sweat loss varying by 0.480 litres between the testing sessions. Despite the lack of control placed on the training sessions in the present studies, the mean ± SD range of sweat loss between the four training sessions was 0.38±0.30 litres, gives credence to the reproducibility of this type of testing session with respect to sweat loss. Even when comparing

the largest range for an individual (0.69 litres) between the first training session and the other three sessions in the present study, the value is only slightly greater than the largest range seen for the four participants tested by Shirreffs and Maughan (1997). This larger maximum range in the present study probably reflects the lack of controls placed on the participants during this study. However, as the mean (range) was similar to acceptable values for reproducibility with regard to sweat losses in a controlled laboratory setting, it can be concluded that a single testing session is a reliable indicator of future sweat losses during training.

Despite the lack of controls imposed on the participants during this study, it emerged from conversations with the participants post training that they had trained at a similar intensity (heart rate was 142±20, 143±18, 144±23 and 141±27 beats.min^{-1} for sessions 1,2,3 and 4 respectively). There were no significant differences with respect to training time ($p=0.638$). Due to the short time frame between each training session, it is also unlikely that changes in fitness levels would have altered to such an extent as to have any affect on the sweat loss of an individual between the training sessions. None of the participants in the study were undergoing any heat acclimatisation during the period of the study and is therefore unlikely that acclimatisation would have altered sweat losses. It was also observed qualitatively by the investigator that the participants tended to train in similar clothing for each session.

All of the data collected during this study suggest that sweat losses can be determined within 1 % of body mass from a single training session. One percent of body mass is a level of hypohydration which can be tolerated by athletes (Casa et al., 2006) and is considered to be an important benchmark level when determining hydration strategies. However, for athletes undertaking prolonged activities or repeated exercise bouts e.g. wheelchair tennis a difference of 0.81 % or 0.69 litres may become significant when sweat losses are large and physical activity last for a prolonged period of time as a chronic effect would occur as dehydration cumulates.

Fluid Intake

The 95% confidence intervals between the first training session and the other three sessions for fluid intake covered 0.59 litres. This confidence interval is similar to the confidence intervals for the sweat losses and reflecting a possible relationship between fluid intake and sweat losses. These finding are further illustrated by the significant correlation between sweat loss and fluid intake. Given that fluid intakes are under conscious control it is this is a variable which can be manipulated in order to enhance drinking strategies during training to promote the optimal level of hydration during training and hopefully improve performance. The variability of fluid intakes shows that the majority of participants appear to be habitually consume the same volume of fluid during each training session. This supports the views of Burke and Hawley (1997) who suggest that habit plays a role in the volume of fluid consumed during training. Despite this habitual intake Barr (1999) believes athletes are able to learn new drinking strategies by either increasing or decreasing intake to improve performance. Therefore, athletes who are required to change their drinking strategies based on results of a single training session can be encouraged to drink more or less. Since variability is low, these behaviours can be planned in to the training session e.g. increasing or decreasing the number of breaks for drinking.

The correlations between sweat losses and fluid intakes tend to suggest that when sweat losses increase, fluid intake does as well. This may be important as it suggests that if athletes are able to adapt their fluid consumption depending on sweat losses they will therefore be able to minimise any hypohydration which may occur during training. This finding has not been seen amongst other field studies investigating the fluid balance (Maughan et al., 2005; Shirreffs et al., 2005). The reason for the significant finding in the present study, compared to the lack of a relationship in the studies by Maughan et al. (2005) and Shirreffs et al. (2005), reflects the larger number of participants in the present study (n=45). There is also a larger variability of subject characteristics in the present study when compared to subject numbers (n= 17) and the homogeneity of the football training undertaken in the study by Maughan et al. (2005) and Shirreffs et al. (2005) in which 26 male football players undertook the same training session. Further, the between subject variability is greater in the present study (sweat loss 0.13-2.58 litres; fluid intake 0.00-1.86 litres) when compared to the range seen by Maughan et al (2005) (sweat loss 1.06-2.65 litres; fluid intake 0.044- 0.951 litres), or by Shirreffs et al. (2005) (sweat loss 1.67-3.14 litres; fluid intake 0.24-1.72 litres). Previous studies all result in a mean body mass loss which is within 2 % of pre-training body mass. As fluid balance is a

combination of sweat loss and fluid intake and this correlation shows that athletes are able to regulate their fluid intakes to match differences in sweat losses.

A limitation to the use of mass to determine fluid intake involves the type of drink consumed. While the assumption that 1 litre of fluid is equal to 1 kg of fluid, is true for water, it is not the case for all drinks. Therefore one litre of a carbohydrate electrolyte drink does not have a mass of 1 kg as the specific gravity of such a drink is 1.02 it will increase body mass by 1.02 kg not the assumed 1.00 kg (Maughan et al., 2007). However, variability in fluid intake in the present study is 0.59 litres and a 0.02 difference is unlikely to have a large effect on the results. Further, it is cautioned that the ingestion of carbohydrate may also affect the relative oxidation of carbohydrate having implications on body mass changes.

Sweat Sodium Concentrations

The sweat sodium concentrations observed in each of the sessions is slightly higher than the mean values reported for soccer training. Sweat sodium concentrations for soccer players averaged 49±12 mmol.l^{-1}, 42±13 mmol.l^{-1} and 30±19 mmol.l^{-1} (Maughan et al., 2004); Maughan et al., 2005; Shirreffs et al., 2005). While mean sodium concentration for each of the sessions in this study is slightly lower than that found by Godek et al. (2006) in professional ice hockey players (60.9 mmol.l^{-1}) during training, some individual values were higher than the typical 20-80 mmol.l^{-1} concentration seen for sweat sodium (Maughan and Murray, 2000). However, values as high as 100 mmol.l^{-1} have previously been reported by Stofan et al. (2001) amongst male collegiate American Football players. Shirreffs and Maughan (1997) reported mean sweat sodium concentrations of 75.3 mmol.l^{-1}, with a standard deviation of 21.9 mmol.l^{-1} when comparing the regional sweat loss and whole body sweat loss, so the values found in some individuals in the present study are not without precedence. The significant correlation between sweat sodium and sweat chloride concentration is similar to findings by Maughan and colleagues (2004). This probably reflects the presence of sodium and chloride in large quantities in the extracellular space and the loss of these two electrolytes as sodium chloride. It may also represent the association between sweat rates and sweat sodium and sweat chloride concentration shown by Allan and Wilson (1971). The lack of correlation between sweat sodium concentration and sweat potassium concentration may reflect the low quantities of potassium in the extracellular fluid and the fact that it is unrelated to sweat rate (Allan and Wilson, 1971). Maughan and colleagues (2004) also found that there was no significant correlation between sweat sodium and sweat potassium concentration.

The mean 95 % confidence interval of a subject showed that sweat sodium concentration could be predicted within 14 mmol.l^{-1}, the range for sweat sodium concentrations is 20-80 mmol.l^{-1}, making the 95 % confidence intervals relatively small. However, the mean ±SD coefficient of variation for each individual with respect to sweat sodium concentration between the four training sessions is low (4.6±1.6 %). This compares to a 3.3 % correlation coefficient for sweat sodium concentrations produced by the whole body wash down technique described by Shirreffs and Maughan (1997). The higher value in the present study represents the lower levels of control placed on the participants in this study. Amongst the five participants tested to assess the reproducibility of the whole body sweat collection technique by Shirreffs and Maughan (1997), the mean range in sweat sodium concentrations was 22 mmol.l^{-1}, with one subject having sweat sodium concentrations covering 30 mmol.l^{-1}. In the present study,

the mean range of sweat sodium concentrations was 12±8 mmol.l^{-1}. The largest range in sweat sodium concentration for a subject in this study is 28 mmol.l^{-1}. The range of values seen in the present study is therefore similar to those seen by Shirreffs and Maughan (1997).

Given that sweat sodium is related to sweat rate (Allan and Wilson, 1971), it is possible that the variability of the sweat sodium concentrations is in part due to the variability of sweat rates. Further to this, an athlete would need to know their total sodium losses and would replace these losses mainly in the form of NaCl. The results show that sweat sodium concentrations and sweat chloride concentrations were similar (54 ±16 mmol.l^{-1} and 50 ±16 mmol.l^{-1}) it can be seen that there is a close relationship between sodium and chloride losses. Assuming that all of the sodium losses were in the form of NaCl, and then these losses can be calculated. Individual 95% confidence intervals for total NaCl loss indicate a single testing session will determine the NaCl losses of future training sessions to within 0.6 g. The largest individual 95 % confidence interval was 1.5 g and the greatest individual difference between session 1 and any of the other sessions with respect to NaCl loss is 1.4 g. These differences are similar to those found for a group of American Football players who had not previously suffered from heat associated cramp, found by Stofan et al. (2005).

The correlation between session 1 and the other three sessions with respect to NaCl loss shows that if an individual has a high sweat salt loss during the first training session then this is likely to occur in all sessions. This will mean that athletes with large sweat NaCl losses can be identified from a single session and the team nutritionist can be alerted. These athletes can then be advised and further monitoring can take place if needed. The mean 95 % confidence interval for each subject in the present study represent 10 % of the British Government's target intake of 6 g.d^{-1} (Food Standard Agency, 2006) and only 5 % of the average reported intake for males in the UK, which averages 11 g.d^{-1} (Henderson et al., 2003) and represents 8 % of the average daily salt intake of females in the UK which is reported to be 8 g.d $^{-1}$ (Henderson et al., 2003). As all sodium losses do not need to be replaced during the training session (Maughan, 2001), a difference of 0.6 g will be replaced with a normal diet following training and would probably not significantly change advice given to an athlete.

A potential source of error for using regional collection sites to analyse for whole body sweat sodium losses tends to result in an overestimation of losses by 30-40 % (Maughan and Shirreffs, 1997; Patterson et al., 2000; Shirreffs et al., 2005). As described

in Chapter 2: Literature Review for this thesis, theories surrounding the overestimation generally focus on an increased sweat rate occurring in the microclimate underneath the sweat patch. This will then have an impact on the sweat electrolyte concentrations in the sample collected (Sato and Dobson, 1970). Despite this, there is a strong relationship between whole body and regional sweat collection methods and it is concluded that the sweat patches are a suitable tool for collecting sweat samples in the field, where whole body wash down techniques are impractical (Patterson et al., 2000).The individual 95 % confidence intervals with respect to total salt loss suggest that a testing salt loss during a single training session provides a reproducible indication of future losses.

Sweat Potassium and Chloride Concentrations

Sweat chloride and sweat potassium concentrations were also within the typical range of 20-60 mmol.l^{-1} and 2-8 mmol.l^{-1} respectively (Maughan and Murray, 2000).

The individual mean difference between session 1 and the other 3 sessions for sweat potassium concentration was 3.1 mmol.l^{-1} and the largest individual range was 6.6 mmol.l^{-1}. When these ranges are compared to the normal range for sweat potassium concentration, which is 2-8 mmol.l^{-1} (Maughan and Murray, 2000) a potential difference between training sessions of 3.1 mmol.l^{-1} is large and covers 50 % of the normal physiological range. However, due to the low absolute sweat potassium losses it is unlikely that differences in sweat potassium concentration will have any significant effect on performance or health. This is especially true if a commercial carbohydrate electrolyte drink is consumed. With every 100 ml of drink consumed 0.3 mmol of potassium will be replaced or 1.5 mmol per 500 ml bottle (which represents half of the potential difference in sweat potassium concentrations). The dietary reference values for potassium are 2 g.d^{-1} (Brouns, 2002) therefore a healthy diet would be sufficient to replace these potassium losses.

Pre-Training Urine Osmolality

Pre-training urine osmolalities in this study show that individuals tend to be well hydrated before undertaking exercise 607±233 mosmol.kg^{-1}. The mean pre-training urine osmolality was similar to the mean urine osmolality of first pass of the day found by Shirreffs and Maughan (1998). The difference in mean osmolalities for the group between the 4 sessions was only 66 mosmol.kg^{-1} demonstrating that the pre-training urine osmolalities between the sessions for the group was small, which is something

that has also been seen in adolescent tennis players by Bergeron et al. (2006). Yet, it is cautioned that this may mask individual differences.

There was a significant although not strong correlation between pre-training urine osmolality and fluid intake. Armstrong and colleagues, (1997) found that when participants were dehydrated (-3.6±0.2 % initial body mass) prior to exercise and then allowed to drink ad libitum they consumed more water than when they started exercise euhydrated. It was proposed that the mechanism for this was an elevated plasma osmolality and an active thirst drive would increase water intake (Armstrong et al. 1997). It is possible that the increased urine osmolality representing hypohydration results in an increased perception of thirst and thus an increased drive to drink, resulting in the correlation between pre-training urine osmolality and fluid intake. This is in contrast to findings of Maughan et al. (2004) on soccer players during training. The difference may be due to the larger number of subjects in the present study.

When considering the individual range of urine osmolality for the individual participants, it is evident that they cover a wide range 402 mosmol.kg^{-1}. The 95 % confidence intervals also produce a large range of 679 mosmol.kg^{-1} and the normal range of urine osmolalities spans 1200 mosmol.kg^{-1}. These 95% confidence intervals show that the use of pre-training urine osmolalities is not a reproducible indicator of future pre-training hydration status. The largest individual variation for urine osmolality was 924 mosmol.kg^{-1} (range 1089 mosmol.kg^{-1} before the first training session and 165 mosmol.kg^{-1} before the second training session). Such a large variation probably reflects differences in the volumes of fluid consumed prior to testing and may reflect access to fluids. This large variation in urine osmolality is only slightly greater than that seen by Shirreffs and Maughan (1998), where the first urine sample of the day ranged between 429-1014 momol.kg^{-1} when participants undertook their normal activities and consumed foods and fluids without any restriction. However, if a urine osmolality of 700 mosmol.kg^{-1} is used to indicate a level of euhydration i.e. below 700 mosmol.kg^{-1} represents euhydration (Shirreffs and Maughan, 1998), then 22 of the participants were euhydrated according to their pre-training urine osmolality prior to all four training sessions, with 7 of the participants being hypohydrated prior to the commencement of training before all four training sessions. This shows that for the majority of participants, there was some consistency concerning their pre-training hydration with 65 % of the participants being consistently euhydrated or hypohydrated prior to their training. Therefore it may be that the use of urine osmolality to provide an

indication of hydration status is valid in determining whether an athlete is euhydrated or hypohydrated, but not reproducible in terms of absolute values of osmolality. As the relevance of exact values for urine osmolality to an athlete is likely to be minimal, they need to know if they are adequately hydrated or not. Reasons for the large variability seen amongst the participants possibly include training at different times of day and following different activities for example some of the participants were students and so were unable to consume any fluid prior to their training, whereas other sessions occurred when the participants were able to consume fluid ad libitum. Further to this, a vast majority of the participants in this study were students studying Sport and Exercise Nutrition and were therefore aware of the assessment of pre-training hydration by urine colour and the importance of being adequately hydrated prior to exercise. Having urinated into the jug before their first training session they were able to tell whether they were adequately hydrated or not and then this may have affected future fluid intakes prior to training. This may have also affected their fluid intakes during the training session and may have contributed to the significant correlation between pre-training urine osmolality and fluid intake in the subsequent exercise session.

Limitations

All participants were requested to urinate into a container, so that this water loss could be incorporated into the data to calculate sweat losses. It is of course possible that some participants may not have collected all their urine since this was done in private, the only validation of that all urine was collected is the participants word. Further information regarding the activities of participants prior to each testing session may have helped to explain the wide range of urine osmolalities. If participants came for testing immediately after lecturers they may not have had the same access to fluid as on days when they had been at home prior to testing. This may also have had an effect on their sweat losses prior to the testing sessions. There is always the possibility that not all the fluid in the subject's containers was consumed and that some spillages could have occurred despite the fact that participants were questioned about spillages and told to only drink from their containers).

The use of regional sweat patches to determine sweat electrolyte concentration has previously been criticised for overestimating sodium and chloride concentrations (Patterson et al., 2000; Shirreffs et al., 2007). This overestimation is in the region of 30-40 % (Shirreffs et al., 2007) but, despite this, in mild conditions the use of regional collection sites is a valid measure of sweat electrolyte composition (Patterson et al.,

2000). Further, the use of a whole body sweat collection to determine sweat electrolyte losses would not be feasible in an applied sport setting.

4.5. Conclusion

This study illustrates that the sweat testing of athletes over a single training session is a useful tool in determining water and electrolyte losses over the acute term. Therefore, one off testing is a useful tool for planning nutritional and fluid strategies for athletes. This study means that the data collected in Chapter 5 represents the typical sweat loss volumes and sweat electrolyte concentrations of the athletes tested. This is especially true when the low sweat losses and mean age of the participants in this study are related to the findings amongst athletes with a disability.

This study also adds a degree of reliability to the regional collection of sweat losses in the field and provides descriptive data concerning non-elite participants undertaking recreational exercise. Further research determining the effect of feedback to the athletes on future drinking strategies and practices may be of benefit in optimising single session sweat testing.

Chapter 5: Sweat Rate and Sweat Electrolyte Composition of Athletes with a Disability During a Training Session.

5.1. INTRODUCTION

Exercise can result in large rises in core temperature which can be associated with reduced exercise performance. The sweating mechanism helps attenuate these rises (Maughan, 2001). Although the evaporation of sweat aids with thermoregulation it also means that there is a loss of water and electrolytes which can lead to dehydration and electrolyte depletion both of which may affect performance. In able-bodied sport it has been demonstrated that even moderate amounts of dehydration can result in decreased performance in subsequent endurance running exercise (Armstrong et al., 1985) and reduced intermittent running exercise (Maxwell et al., 1999). More severe dehydration can result in health problems (Kirkendall, 1993). In contrast to hypohydration is hyponatraemia, a condition which occurs when serum sodium concentration drops below 135 $mmol.l^{-1}$ (Hsieh et al., 2002). Hyponatraemia is increasingly being reported in collapsed athletes (Hsieh, 2004). Theories regarding its aetiology include the loss of large amounts of sodium in sweat during prolonged exercise (Hiller, 1989) and the excessive intake of hypotonic fluids which can cause hypovolaemic or dilutional hyponatraemia (Noakes et al., 1985). Hsieh et al. (2002) suggests that hyponatraemia can be prevented in athletes if appropriate fluid intake strategies are adopted.

There is a vast amount of published research concerning the sweat quantity and sweat electrolyte composition of athletes without a disability. The research on able-bodied athletes has been undertaken in both field and laboratory settings. This field based research has taken place in both training and competitive situations, has covered a wide variety of sports, in different climatic conditions and used male and female participants from a wide range of ages as shown in Table 4.1.1., reviews of the sweat losses of team sports has been covered by Burke and Hawley (1997) and Reher and Burke (1996).

Sweat rates within team sports vary between players depending on the intensity of the exercise, which is associated with playing position, an individual's fitness and state of acclimatisation (Burke, 1997; Maughan, 2001).

In a review of sweat rates for basketball players, the mean male sweat rate for able-bodied basketball players was 1.5 $l.h^{-1}$ (Burke and Hawley, 1997), although sweat rates as low as 0.330 ± 0.155 $l.h^{-1}$ have been reported (Burke and Hawley, 1997). Sweat losses for able-bodied rugby union have been reported to be 1.6-2.2 $l.h^{-1}$ (Reher and

Burke, 1996). There is less information regarding the sweat losses of sprint athletes. A summary of previous research is shown in Table 4.1.1.

There is very little information concerning the quantity and composition of sweat loss and the volume of fluid intake of athletes with a disability in either athletic training or during competitive activities. The majority of existing published research that has taken place amongst this population has been performed in controlled laboratory settings (Price and Campbell, 1997; Price and Campbell, 2003; Petrofsky, 1992). As discussed in Chapter 2 controlled laboratory based studies lack external validity as they do not reflect the actual activity patterns of athletes in training or in competition (Atkinson and Nevill, 2001). Further, of the published research concerning sweat losses of disability athletes there is no information to date regarding the sweat electrolyte losses through sweat of athletes with a disability.

Sweating also results in the loss of electrolytes. The major electrolytes in sweat are sodium and chloride, usually occurring in concentrations of 20-80 $mmol.l^{-1}$ and 20-60 $mmol.l^{-1}$ respectively (Maughan, 2001). Although these are the generally accepted ranges for sweat sodium and sweat chloride concentrations there have been some reports in the literature of values which lie outside these values. Godek et al. (2006) reported mean±SD values for sweat sodium concentration of 60.9±31.0 $mmol.l^{-1}$ during training and 67.4±20.5 $mmol.l^{-1}$ during ice hockey matches. This indicates that some individuals had sweat sodium concentrations as high as 91.9 $mmol.l^{-1}$ and 87.9 $mmol.l^{-1}$ during training and matches respectively. Stofan et al. (2001) reported sweat sodium concentrations of 100 $mmol.l^{-1}$ in one cramp prone subject during 60 minutes of cycling in the heat. Sweat electrolyte concentrations are effected by numerous factors including sweat rate (for sodium and chloride but not in the case of potassium concentration) (Cage and Dobson, 1965), the state of acclimatisation, differences in diet and biological differences may also effect the sweat sodium and sweat chloride concentrations (Maughan, 2001). The loss of electrolytes, especially sodium, has been linked to the aetiology of muscle cramp (Bergeron, 1996; Stofan, 2003). Sweat electrolyte losses need to be replaced between training sessions for the complete restoration of body fluid balance (Takamata et al., 1994; Shirreffs, 2000). At present, there is no data regarding the sweat electrolyte content for athletes with a disability.

There has been an increase in the number of participants at events such as the Paralympic Games. This is reflected in participation rates at the Games which have risen from 400 athletes in Rome 1960 to 3806 athletes at Athens 2004 (International

Paralympic Committee, 2006a). It has been known since the 1960's that individuals with a spinal cord injury have problems thermoregulating due to a decreased ability to sweat (Randell et al., 1966). This impaired thermoregulatory response is dependent on the extent and level of the spinal cord lesion, with individuals who have suffered a complete lesion being unable to produce any sweat below the level of the lesion. This is due to the loss of sympathetic regulation following a spinal cord injury (Theisen and Vanlandewijck, 2002). The loss of sympathetic activation results in an inability to sweat below the level of the level of the lesion either during passive heating (Randell et al., 1966) or during exercise (Price and Campbell, 1999). During 45 minutes of arm cranking exercise in the heat (35°C and 70% relative humidity) participants without a disability lost 718 g of sweat whereas those with spinal cord injury at T2-T6, T7-T9 and T10-T12 only lost 172, 335 and 425 g respectively (Hopman, 1994). From these results Hopman (1994) concluded that there is a linear relationship between sweat produced and the skin surface area above the lesion, reflecting the area available for active sweat regulation. However, the studies presented in this paragraph were all performed in the laboratory setting where workload and duration are determined by the investigator. Normal activities associated with training are more self selecting in the determination of intensity.

It is important to note that individuals with a spinal cord injuries only make up a portion of the competitors in disability sport. Other disabilities include cerebral palsy, spina bifida, amputations, blindness or any other form of physical disability that does not fit into any of the other categories (les Autres). There is even less information regarding the sweat volume or sweat composition for athletes with these disabilities. It was suggested by Maltais et al. (2004) that individuals with cerebral palsy may rely more on dry heat exchange than able-bodied individuals. If this is the case, then it is possible that athletes with cerebral palsy have a different sweat loss compared to able-bodied athletes. Since sweat composition is effected by sweat rate (Cage and Dobson, 1965), any alteration in sweat rates may result in sweat sodium and sweat chloride concentrations which are different from those seen in able-bodied populations. Petrofsky (1992) suggested that sweat rates on the sensate skin of those with a spinal cord injury were approximately six times greater than those of able-bodied controls. Given that sweat sodium and sweat chloride concentration increase with sweat rate, it is possible that the sweat concentrations of these two electrolytes are higher than seen amongst able-bodied athletes (Allan and Wilson, 1971). At present, nutritional and drinking strategies for athletes with a disability are based on the data available from

able-bodied sport. However, if differences in sweat rates and sweat electrolyte composition do exist, then these guidelines are inappropriate for athletes with a disability.

When fluid intakes have been ad libitum tetraplegics during exercise have been shown to be greater during exercise in warm environments than for able-bodied participants. This could be due to an attempt by the tetraplegics to attenuate feelings of becoming hot (Price and Campbell, 2003).

Three of the Paralympic sports which participated in this study are wheelchair basketball, wheelchair rugby and sprint athletics. These sports cover both wheelchair sports and non-wheelchair sports. Wheelchair basketball is based on the able-bodied version of basketball, in that it is played on the same court and follows similar rules. It can be described as a multi-sprint sport and it has been reported that players cover approximately 5 km over the 40 minutes of the game (Goosey-Tolfrey, 2005). Coutts (1992) found that 10 % of playing time consisted of high intensity intermittent efforts. Despite this in recent years wheelchair basketball training has become more aerobic based (Goosey-Tolfrey, 2005). Wheelchair rugby originated as a sport for tetraplegics who were unable to play wheelchair basketball due to the extent of their injuries. It is played on an indoor basketball court and consists of two teams of four players playing four quarters each lasting eight minutes each. The aim the game is to score the most number of points by crossing the back line between two cones. Paralympic athletics is similar to its able-bodied equivalent but races are classified into different levels of disability meaning there could be several races/ field events of the same discipline. Disability sports all have a classification system which is the method for fair competition to occur. This classification system organises players/ athletes into classes defined by the degree of function presented by the disability (International Paralympic Committee, 2008). These classes are determined by various criteria in different situations and may include a physical and technical assessment and observation in and out of competition. The classes are defined by each sport and form part of the sport rules (International Paralympic Committee, 2008).

In Paralympic team sports, players are given a number of points depending on their ability to perform certain sports related tasks. The teams are then allowed to have a maximum number of points on court at one time. In athletics athletes in the same impairment group with a similar range of mobility compete against each other.

This study's primary aim was to collect descriptive data about the sweat rates and sweat composition of athletes with a disability during training. The study concentrated on wheelchair rugby, wheelchair basketball and sprint athletics.

5.2. METHOD

Participants were recruited by contacting their respective team coaches either in person, via e-mail or by telephone. Measurements were made from an international wheelchair rugby, international sprint athletics squad, a club level wheelchair basketball team and international wheelchair basketball team. All measurements were taken during normal training sessions. Ethical approval was obtained from the Research Ethics Committee of Loughborough University. After the study had been explained, the following athletes gave written informed consent to participate in this study.

- 17 male and 1 female international wheelchair rugby players,
- 9 male and 1 female members of an international sprint team for athletes with a disability training squad,
- 4 male and 4 female recreational wheelchair basketball players
- 6 male international wheelchair basketball players

Full details of participant information are described in Table 5.2.1. All classifications in each respective sport were represented - wheelchair rugby players were classified from 0.5-3.5 points according to the International Wheelchair and Amputee Society (IWAS) classification system and the athletes were classified as according to the International Paralympic Committee Athletics Code for classification and wheelchair basketball players were classified as 1.0,1.5, 2.0,2.5,3.0 and 4.5 according to the International Wheelchair Basketball Federation (IWBF) classification system. Disabilities included both neurological impairments- (spinal cord injury, spina bifida, cerebral palsy and polio) and musculoskeletal injury – (amputation).

Table 5.2.1: Participant Characteristics- Participant Numbers (Male and Female), Classification and Mean±SD Body Mass of the International Wheelchair Rugby Squad, Sprint Athletics Training Squad, Club Level Wheelchair Basketball Team and International Wheelchair Basketball Squad.

Team	Numbers (Males,	Classes	Disability	Mean±SD Pre
International wheelchair Rugby	18 (17 male, 1 female)	1 class 0.5	1 SCI-C5 complete	73.30±12.26
		1 class 1.0	1 SCI- C5/6 complete	
		4 class 1.5	4 SCI-1=C6 complete 3=C6/7 complete,	
		5 class 2.0	5 SCI-3=C6/7 incomplete,2=C6/7 complete	
		4 class 2.5	4 SCI- 2=C6/7 complete, 2= C7 complete	
		1 class 3.0	1 SCI- C5 incomplete	
		2 class 3.5	2 SCI- 2 C7 incomplete	
Athletics	8 (7 male, 1 female)	4 T36	4 CP	68.30±9.69
		2 T37	2 CP	
		1 T38	1 CP	
		1 T42	1 Amputee	
Club Basketball	8 (4 male, 4 female)	4 class 1.0	4 SCI- 2=T4, 1= T4/T5, 1=T5 all complete	80.36± 26.75
		1 class 2.5	1 Spina Bifida	
		2 class 3.5	2 CP	
		1 class 4.0	1 Amputee	
International Wheelchair Basketball	6 male	1 class 1.0	1 SCI- T5	79.52± 13.88
		1 class 1.5	1 SCI- T5/6	
		1 class 2.0	1 SCI- T8	
		1 class 2.5	1 Spina Bifida	
		1 class 3.0	1 Polio	
		1 class 4.5	1 Amputee	
Total	40 (34 male, 6 female)			76.74±17.58

Training sessions

International Wheelchair Rugby

The participants in this study were all training at a British Paralympic wheelchair rugby training camp. The training session from which the data were obtained was a morning training session commencing at 10 am following a team breakfast. On the day of the session, players had already participated in a swim session, which was part of the training camp. The training session occurred mid season and so most of the players had played in a tournament 2 days prior to the data collection.

The atmospheric conditions in the hall were a temperate 18.8±0.8°C and 48.5±4.2% relative humidity. Unfortunately due to the nature of the injuries (lack of sensory feedback) in this group heart rate monitors became dislodge and were not repositioned. Therefore there is no heart rate data for this group.

Training consisted of a warm up, passing skills and defensive/ attacking play, match simulation followed by as many laps of a circuit as possible in 12 minutes and a cool down with stretching. Training lasted 120 minutes.

International Sprint Athletics

The participants in this study were all participating in a U.K. Athletics training camp for athletes with a disability and were all competing for places on the 2008 British Paralympic team. The athletics training session lasted 110 minutes during which athletes participated in a warm up before splitting into smaller groups to practice their individual events, all of which were sprint events. Training took place indoors and the atmospheric temperature was 17.9±0.8°C with a relative humidity of 37±4%. Average heart rates for the session were 114±9 beats.min^{-1} (range 104-133 beats.min^{-1}).

Club level wheelchair basketball

The participants in this group were undertaking wheelchair basketball on a weekly basis and at the time of the study were not members of any Paralympic squad. Data was collected from two training sessions separated by one week; participants were different in each session.

The training sessions lasted 114 minutes in the first week (session 1) and 75 minutes for the second week (session 2). Environmental conditions for session 1 were 22.0±0.8°C and 52.5±3.0%. The conditions for session 2 were atmospheric temperature

18.8± 0.7 °C with a relative humidity of 42.1±1.6%. Both training sessions consisted of skills practice and match play. There were 2 males 2 female players present at session 1 during which mean heart rate during was 129±19 beats.min^{-1} (range 112-162 beats.min^{-1}). In session 2 there were 2 males and 2 females, mean heart rate during this session was 134±25 beats.min-1 (range 114-161 beats.min^{-1}).

International Wheelchair Basketball

All the data was collected during a single training session during which all training took place indoors. The training session was part of a training camp for the British wheelchair basketball squad and all the participants were all members of this squad. The training sessions both took place in the morning (10 am – 12 pm) following breakfast and formed part of a 3 day residential training camp. The session from which data was taken lasted 118 minutes. The mean ± SD atmospheric temperature for the session was 21.0 ±1.2 °C with a relative humidity of 26.0±2.6%. During training players warmed up by practising shooting and generally moving around the hall. This was followed by high intensity sprinting, ball skills and finished with match play games. Mean ± SD heart rate during this session was 125±26 beats.min^{-1} (range 71-186 beats.min^{-1}).

The actual testing protocol was similar for each of the teams (the only exception being the positioning of the gauze patches to obtain sweat samples and measurement of body mass with clothing or minimal clothing). Upon arrival at the training session, participants were first asked to empty their bladders/ catheter bags and to provide a urine sample, which was obtained by the participants/ team physiotherapists (for some individuals who were catheterised and who had limited hand function) in private. This was used to measure hydration status via urine osmolality as described by Shirreffs (2000).

Once the players had emptied their bladders, their body mass was measured and recorded, their drinks bottles were weighed and sweat patches were positioned. These measures were obtained by a team of researchers in any order to minimise the time required with the squad prior to the training session.

Body mass was measured on a balance (AE Adam CFW-150, Milton Keynes, UK) adapted with a chair to allow players to transfer. Body mass was measured to the nearest 20 g. The international wheelchair rugby and international wheelchair basketball players were weighed in their training trousers (lightweight tracksuit bottoms) and shoes (trainers). This was to protect the dignity of any player who may be

catheterised and again to minimise the time taken before training to obtain the measures, thus reducing the impact on the training session. The club level wheelchair basketball players and the track athletes were weighed in their underwear. After training players towelled them-selves dry and were reweighed in the same clothing as pre training. Any player who was catheterised was weighed with their bag before and after training. Players using prosthetic limbs were weighed with these in place before and after training, again to limit the time taken to weigh the participants, and to minimise any disruption to the players' preparation for the training session. However, sweat trapped in clothing represents ~ 0.2 % of total sweat losses (Cheuvront et al., 2002).

Polar Team Heart rate monitors (Polar Electro Oy, Finland) were positioned, activated and remained in place throughout training to record heart rate every 5 seconds.

Any drinks containers that the players had were weighed and the contents recorded. To ensure players only drank from their drinks container, containers were labelled and players were instructed to only drink from their labelled containers and asked to use the fluid in these containers for drinking only. Players were monitored during training to ensure they only drank from their containers and that they did not use their drinks to pour over their heads in an attempt to cool themselves down. Any additional fluid required during training was also weighed before the players consumed it. At the end of training all drinks containers were reweighed.

If any participant needed to urinate during training they were asked to collect it in a urine container which was subsequently weighed. Sweat losses and sweat rates were calculated from these measures.

Sweat samples were collected during the training session by absorbent 2.5*4 cm gauze patches with an adhesive waterproof cover 5*7 cm (3M Health Care, Loughborough, UK) applied to the skin surface on the right hand side of the body. All patches were placed on the right hand side of the body. These patches were positioned at three sites at the shoulder, nape of the neck and chest for the wheelchair rugby players; on the shoulder, chest, forearm and thigh for the sprint athletes; on the chest, shoulder, lower abdomen and triceps on the club level wheelchair basketball players and on the forearm, chest and shoulder for the international wheelchair basketball players. The patches were positioned in areas where it was thought that sweat production would be sufficient to provide an adequate sample. Despite patches

previously remaining in place during contact sports such as rugby, it was noticed that following the testing of the wheelchair basketball players it was noticed that the majority of patches on the forearm became detached and therefore contaminated. Following this, it was decided that this site was not acceptable for wheelchair athletes, hence the alteration in positioning of the patches between the international players and the club level wheelchair basketball players.

Patches were positioned before the start of training, after the skin site had been appropriately cleaned and dried and remained in place during the training session. Patches were removed at the end of training sessions with tweezers and placed in sealed storage tubes, of known weights.

Storage tubes and patches were then reweighed and the volume of sweat on each patch was calculated. Sweat volume was calculated by subtracted the pre training weight of the universal tube plus the patch from the weight of the tube plus the patch following the training session. Deionised water was then added to the universal tube, and the tubes were reweighed and mixed before the sweat samples were analysed for sodium concentration and potassium concentration using flame photometry (Corning, 410, New York, USA). Chloride concentration was also measured (Jenway PCLM 3, Essex, UK).

The urine samples were analysed for osmolality via freezing point depression (Gonotec Osmomat 030, YSI, Farnborough, UK).

Statistical analysis

Data was collected from 44 athletes. Due to the nature of the sports involved, resulting in sweat samples becoming contaminated as patches became detached from the skin surface and the impaired sweat loss in some of these athletes sweat samples were not collected at some sites in some of the individuals. Therefore there was some missing data from the sweat concentration calculations. Sweat electrolyte losses for athletes were calculated using the arithmetical mean using 14 participants.

Firstly data were analysed to determine the normative distribution using the Kolmogorov-Smirnov test. Where data were normally distributed, regional differences were determined using a one-way ANOVA and Tukey's post hoc test. Where data were not normally distributed differences in regional electrolyte concentrations were determined by Kruskal-Wallis followed by a Mann-Whitney test. Relationships between variables were determined using spearman's rho correlation analysis. All data which

were not normally distributed is presented as median (range), normative data is provided as mean ± SD. Changes during training pre to post measures were analysed using a paired t –test. Differences between teams and between disabilities were determined using a Krustal-Wallis followed by a Mann-Whitney test. Differences between those with a spinal cord injury and those with a different disability were analysed using a Mann-Whitney.

5.3. RESULTS

Table 5.3.1: Mean, Standard Deviation, Values for Pre Training Body Mass (kg), Pre Training Urine Osmolality (Mosmol.kg^{-1}), Change in Body Mass From Pre To Post Training (kg), Body Mass Change (%), Fluid Intake (l), Sweat Losses(l) and Sweat Rates(l.h^{-1}).

Team	Pre Training Body Mass	Urine Osmolality	Body Mass Change	% Body Mass Change	Fluid Intake	Sweat Loss	Sweat Rate
	(kg)	*(mosmol.kg^{-1})*	*(kg)*	*(%)*	*(l)*	*(l)*	*(l.h^{-1})*
			Overall				
Mean	74.73	502	0.05	0.09	0.84	0.68	0.40
SD	17.16	244	0.69	0.81	0.49	0.66	0.47
			Wheelchair Rugby				
Mean	73.77	403	0.31	0.45	0.80	0.34	0.25
SD	12.47	148	0.34	0.48	0.24	0.27	0.20
			International Sprint Athletics				
Mean	66.08	511	-0.27	-0.41	0.52	0.70	0.30
SD	7.54	307	0.24	0.36	0.19	0.25	0.20
			Club wheelchair basketball				
Mean	80.77	626	0.01	0.02	0.96	0.95	0.62
SD	26.98	245	1.21	1.27	0.74	1.08	0.87
			International wheelchair Basketball				
Mean	79.52	618	-0.05	0.04	1.37	1.19	0.60
SD	13.88	282	0.35	0.41	0.46	0.53	0.27

Pre-Training hydration - Urine Osmolality

The urine osmolalities for each team and overall values are shown in Table 5.3.1., mean urine osmolality for the group was 502 mosmol.kg^{-1} (range 111-1111 mosmol.kg^{-1}). Pre training osmolality did not differ significantly between the playing classes (p=0.074). However, there was a significant difference between the teams with respect to urine osmolality (p=0.029). The difference seems to lie between the wheelchair rugby team and the club wheelchair basketball team (p=0.03) and between the wheelchair rugby team and the international wheelchair basketball team (p=0.069). There was no significant difference between the type of injury and the pre-training urine osmolality (p=0.155).

Wheelchair Rugby

It is worth noting that all players had catheter bags. The overall mean urine osmolality was 403± 148 mosmol.kg^{-1} (range 193 -643 mosmol.kg^{-1}). Urine osmolalities were not significantly related to playing class (p=0.804). The pre-training urine osmolality was not significantly correlated with the volume of fluid consumed during the subsequent training session (r^2 =0.024, p=0.551).

International Sprint Athletics

Mean urine osmolality was 511±307 mosmol.kg^{-1} (range 146- 1111 mosmol.kg^{-1}). 1 of the athletes started training slightly dehydrated with urine osmolalities greater than 700 mosmol.kg^{-1}. There was a significant correlation between pre training urine osmolality and the volume of fluid consumed during training (r^2=0.555, p=0.013) but fluid consumption was not significantly correlated to the amount of sweat lost (r^2=0.218, p=0.243).

Club Wheelchair Basketball

Mean urine osmolality was 626±245 mosmol.kg^{-1} (range 111-865 mosmol.kg^{-1}). 1 of the players started training slightly dehydrated with urine osmolalities greater than 700 mosmol.kg^{-1}. Again pre training hydration measure via osmolality was not significantly correlated to the volume of fluid consumed during the training session (r^2=0.250, p= 0.253) nor was fluid intake correlated to the subsequent sweat loss during training (r^2=0.250, p=0.117).

International Wheelchair Basketball

1 player started training mildly hypo hydrated as determined by a urine osmolality of 925 mosmol.kg^{-1}. Mean urine osmolality was 618 ± 282 mosmol.kg^{-1} (range 181-925 mosmol.kg^{-1}). There was no relationship between initial hydration and sweat rate (r^2= 0.157, p=0.483). Pre-exercise urine osmolality was not correlated with fluid intake (r^2= 0.013, p=0.831) or with player classification (r^2=0.267, p=0.294).

Fluid Balance

Overall

Pre training body mass was not significantly different between the disability groups (p=0.294) nor was there a significant difference between those with and without a spinal cord injury (p=0.521). There was no significant difference between the teams with respect to pre-training body mass (p=0.298).

The body mass changes, fluid consumption and sweat rates for each training session are shown in Table 5.3.1. There was a mean gain in body mass by the club level wheelchair basketball squad and the international wheelchair rugby squad but body mass decreased with the international sprint athletics squad and decreased slightly in the international wheelchair basketball squad. Despite this there was no significant difference between the teams with respect to percent body mass change (p=0.177). Overall there was a slight increase in body mass 0.05±0.69 kg (range -3.16-+1.83 kg) or expressed as a percent of body mass a slight increase of 0.09±0.81 % (range -3.09-+1.75 %). This appears to be a large variation in body mass change. There were no significant differences between the playing classes with respect to body mass change during training when expressed as either an absolute value (p=0.823) or expressed relative to initial body mass (p=0. 673).

There was no significant difference between the different disability groups with respect to % body mass change (p=0.141). Further to this there was no significant difference in the % body mass change between those who had a spinal cord injury and those with a different type of disability (p=0.076). The fluid balance of the athletes according to their disability is shown in Figure 5.3.1.

Sweat losses were [median (range)] 0.58 litres (0.01-4.02) which meant sweat rates were 0.35 $l.h^{-1}$ (0.00-3.22 $l.h^{-1}$). Again neither of these factors were significantly different between the playing classes (p= 0.515 and p=0.905 respectively). There was no significant difference between the sweat rates of each disability type (p=0.109). Nor was there a significant difference in the sweat rates between those who had a spinal cord injury and those without a spinal cord injury (p=0.182).

Fluid intake was not significantly different between the playing classes (p=0.070), with the median (range) fluid intake being 0.74 litres (0.19-2.48 litres). There was no significant difference for fluid intake between the different disability types (p=0.057)

nor between those with a spinal cord injury and those without a spinal cord injury (p=0.095).

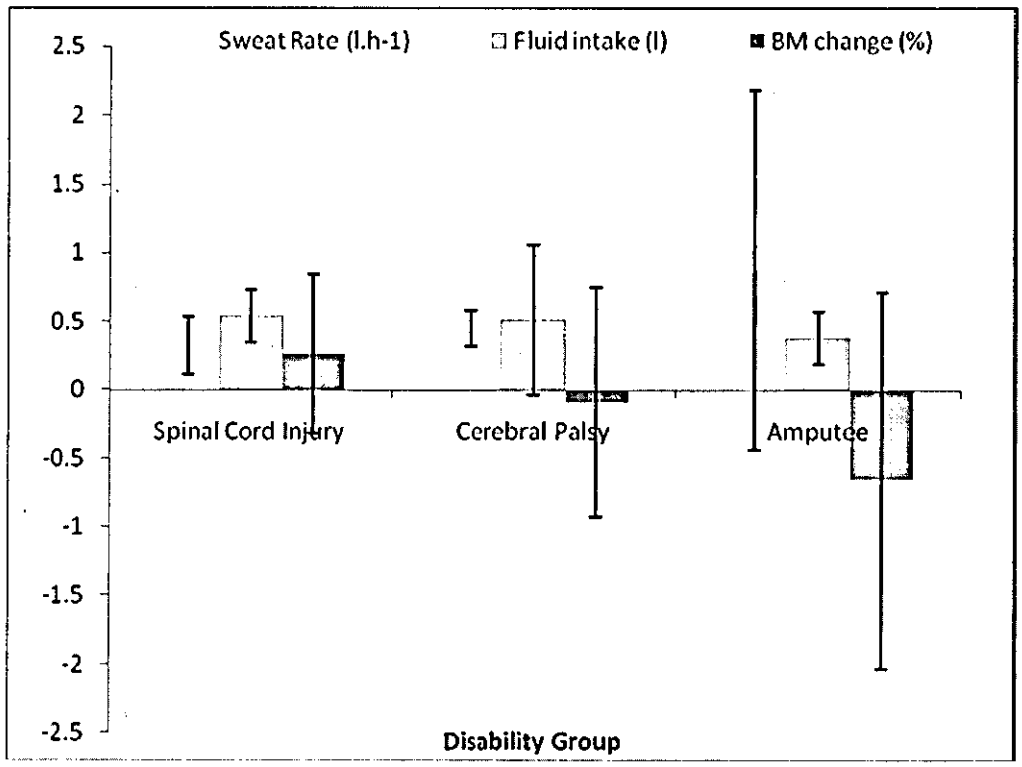


Figure 5.3.1. Mean ±Standard Deviation for Sweat Rates ($l.h^{-1}$), Fluid Intake (l) and Relative Body Mass Change (%) for the Spinal Cord Injured, Cerebral Palsy and Amputee Athletes.

Across the teams, there was a significant difference with respect to fluid intake (p=0.002). It appears that the significant difference with respect to fluid intake was due to the difference between the wheelchair rugby squad and the international basketball squad (p=0.045) and between the athletics squad and the international basketball (p=0.003) and recreational basketball clubs (p=0.023). Sweat loss was also significantly different between the teams (p=0.002) with a significant difference between the wheelchair rugby squad and the international basketball squad (p=0.022) and the recreational wheelchair basketball squad (p=0.022). There was no significant difference with respect to sweat rate (p=0.158), change in body mass either absolute (p=0.092) nor relative (p=0.117) was significantly different between the teams.

Wheelchair Rugby

Only 4 of the players had a body mass decrease during the training session this meant that there was a mean increase in body mass during the training session of 0.31±0.34 kg (range -0.34 - +1.75 kg) equating to a 0.45±0.48% increase in body mass during the training session. The change in body mass during the training session was not correlated to playing classification (r^2=0.062, p=0.392). This change in body mass was the result of a fluid intake during training of 0.80±0.24 litres (range 0.40-1.37 litres) combined with a sweat loss of 0.34±0.27 (range 0.01-1.04 litres). It is evident from the results that some individuals lost no sweat. These sweat losses equated to sweat rates of 0.25±0.20 $l.h^{-1}$ (range 0.00-0.77$l.h^{-1}$). Sweat loss and the volume of fluid consumed during the training session were not significantly correlated (r^2=0.042, p=0.431). Sweat loss was significantly correlated with playing class (r^2=0.468, p=0.004) However the volume of fluid consumed was not significantly correlated to playing class (r^2=0.073, p=0.313).

International Sprint Athletics

All but one of the athletes lost body mass during the training session. This equated to a mean body mass change for the squad of -0.27±0.24 kg (range -0.59-+0.10 kg), which as a percent of body mass equates to -0.41±0.36% (range -0.81-+0.15%) change. The change in body mass was not correlated to racing classification (r^2=0.04, p=0.574).

During the training session, the athletes consumed 0.52±0.19 litres (range 0.19-0.75 litres) of fluid. Fluid intake was not significantly correlated to racing classification (r^2=0.330, p=0.630).

Sweat losses during the training session were 0.68±0.24 litres (range 0.39-1.26 litres), this meant that sweat rates for the training session were 0.39±0.13 $l.h^{-1}$ (range 0.21-0.69 $l.h^{-1}$). Sweat losses were not significantly correlated to racing classification (r^2=0.13, p=0.33).

There was no significant difference between the sweat rates of the sprint athletics training squad and the wheelchair team (p=0.829).

Club wheelchair basketball

Three of the players lost body mass during the training session, resulting in a mean change in body mass of 0.01±1.21 kg (range -3.16-+1.83 kg) or 0.02±1.27 % (range -3.09-+1.73%) as a percent from pre-training body mass.

The fluid intake for the squad was 0.96±0.74 litres (range 0.24-2.48 litres).

Sweat losses for the team were median (range) 0.95 litres (range 0.19-4.02 litres). This meant that sweat rates were median (range) 0.37 $l.h^{-1}$ (0.15-3.22 $l.h^{-1}$).

International wheelchair basketball

Details of body mass change, sweat loss, sweat rate and fluid intake are shown in Table 4.3.1. Initial body mass of the participants was 79.52±13.88 kg (range 60.63-90.90 kg) Post -training body mass was 79.54±13.84 kg, this was not a significant change in body mass from pre-training to post training ($p>0.05$). Three players gained weight during the training session and there was a decrease in body mass for three players. Mean % body mass change was +0.03±0.42% (range -0.41-+0.68%) and was not affected by classification ($r^2=0.00$, $p=0.608$).

Mean fluid intake was 1.37±0.46 litres (range 0.74- 1.88 litres). Fluid volume consumed was not related to sweat loss ($r^2= 0.206$, $p=0.369$). The volume of fluid consumed related to player classification ($r^2= 0.039$, $P=0.848$) either. The lowest classification player, 1.0 (spinal cord injury at T5-6), drank the most fluid.

Sweat losses were 1.19±0.53 litres (range 0.72-1.86 litres). Sweat losses were not related to classification ($r^2=0.196$, $p=0.271$). The mean sweat rate was 0.603±0.268 $l.h^{-1}$ (range 0.37-0.95 $l.h^{-1}$). Sweat rate was greatest in the class 2.0 and 4.5 players (spinal cord injury and amputee). The lowest sweat rate was seen in the player classified as 1.5, who had an incomplete spinal cord lesion in the lower thoracic region.

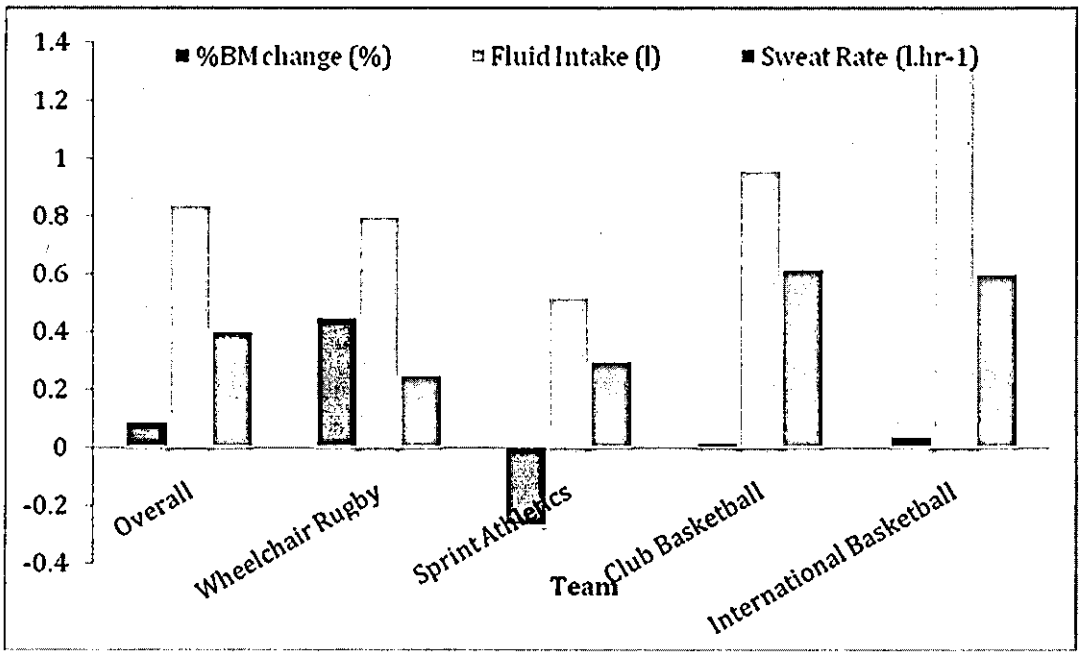


Figure 5.3.2: Mean % Body Mass Change, Fluid Intake (l) and Sweat Rates ($l.h^{-1}$) for the Wheelchair Rugby Squad, International Wheelchair Basketball, Club Wheelchair Basketball and International Sprint Athletics Squad during Training.

Table 5.3.2. Sweat Rates ($l.h^{-1}$) And Sweat Losses Relative to Body Mass (%) between the Disability Groups.

Disability	*Mean Sweat Rate*	*Standard Deviation*	*Median Sweat Rate*	*Sweat Rate Range*
	($l.h^{-1}$)	*($l.h^{-1}$)*	*($l.h^{-1}$)*	*($l.h^{-1}$)*
Spinal Cord injury	*0.32*	*0.21*	*0.32*	*0.01-0.95*
Cerebral Palsy	*0.45*	*0.13*	*0.39*	*0.31-0.69*
Amputees	*0.87*	*1.31*	*0.33*	*0.15-3.22*
Polio	*0.21*	-	-	-
Spina Bifida	*0.39*		*0.39*	*0.36-0.42*
Disability	*Mean Sweat Loss Relative to Body Mass*	*Standard Deviation*	*Median Sweat Loss Relative to Body Mass*	*Sweat Loss Relative to Body Mass Range*
	(%)	*(%)*	*(%)*	*(%)*
Spinal Cord injury	*0.67*	*0.55*	*0.56*	*0.01-2.01*
Cerebral Palsy	*1.10*	*0.36*	*1.00*	*0.58-1.73*
Amputees	*1.28*	*1.50*	*0.71*	*0.23-3.93*
Polio	*1.01*	-	-	-
Spina Bifida	*0.68*		*0.68*	*0.44-0.92*

Table 5.3.2. shows the sweat rates for the different disabilities of the participants. There was no significant difference between the sweat rates of those who had a spinal cord injury and those who had a different type of disability (p= 0.452). However, excluding the individual with Polio as there was only one subject with this disability, those with a spinal cord injury had the lowest mean and median sweat rate. There was no significant

difference between any of the groups with respect to sweat rate p=0.788. When expressed relative to body mass there was a significant difference between the sweat loss as a % body mass for those with a spinal cord injury compared to those without a spinal cord injury (p=0.028).

Table 5.3.3. Rates of Fluid Intake ($l.h^{-1}$) For the Different Disability Groups.

Disability	*Mean Fluid intake*	*Standard Deviation*	*Median Fluid Intake*	*Fluid Intake Range*
	($l.h^{-1}$)	*($l.h^{-1}$)*	*($l.h^{-1}$)*	*($l.h^{-1}$)*
Spinal Cord injury	*0.54*	*0.19*	*0.55*	*0.22-1.01*
Cerebral Palsy	*0.51*	*0.55*	*0.37*	*0.11-1.99*
Amputees	*0.38*	*0.19*	*0.32*	*0.19-0.69*
Polio	*0.57*	-	-	-
Spina Bifida	*0.80*		*0.80*	*0.36-1.08*

There was no significant difference between the different disability groups with respect to the rate of fluid intake (p=0.125). There was no significant difference between the fluid intake rates of those with a spinal cord injury and those with a different type of disability (p=0.085).

Sweat Electrolytes

International Rugby

Due to the low sweat losses of the wheelchair rugby players sweat potassium and sweat sodium concentrations could only be determined for 2 of the players. A class 2.5 player had a sweat sodium concentration of 36 mmol.l^{-1} and a sweat potassium concentration of 5.1 mmol.l^{-1}(from the sample taken from the neck) and a 3.5 class player had sweat sodium concentrations of 62, 49, 75 mmol.l^{-1} from the shoulder, chest and neck sites. His sweat potassium concentrations were 5.6, 6.2 and 5.7 mmol.l^{-1} again from the shoulder, chest and neck sites respectively. This meant his mean sweat sodium concentration was 61±13 mmol.l^{-1} and mean sweat potassium concentration was 5.9±0.3 mmol.l^{-1}. This meant that the salt loss during training for the class 2.5 player was 0.4 g and for the class 3.5 player was 2.1 g.

International sprint athletes

Again some athletes did not produce enough sweat at all the site for enough sweat to be collected to be analysed for sweat electrolyte concentrations. Data relates to 6 athletes and full details of sweat electrolytes from these 8 athletes are described in Table 5.3.2. Mean sweat sodium concentration was 46±16 mmol.l^{-1} (range 30-65 mmol.l^{-1}). This indicates that the mean salt loss was 1.7±0.6 g (range 1.1-2.5 g). Mean sweat potassium concentration was 3.9±2.8 mmol.l^{-1} (range 3.7-8.2 mmol.l^{-1}). There was insufficient sweat for sweat chloride concentrations to be determined.

Table 5.3.4. Shows the Sweat Sodium Concentrations (mmol.l^{-1}), Sweat Salt Loss (g) And the Sweat Potassium Concentrations by Each Classification for the International Sprint Athletics Squad during the Training Session

Classification	Sodium (mmol.l^{-1})	Salt loss (g)	Potassium (mmol.l^{-1})
T13	49	1.1	6.9
T36	32	1.2	8.2
T36	31	1.4	3.9
T37	30	2.2	5.0
T38	66	2.5	3.7
T42	35	1.2	4.9
Mean	44	1.6	5.5
SD	16	0.6	1.6

Recreational Wheelchair Basketball

Again due to the small quantity of sweat produced values for sweat electrolytes could only be determined for 3 of the players. A class 1.0 player had a sweat sodium concentration of 66 mmol.l^{-1}, sweat chloride concentration of 53 mmol.l^{-1} and a sweat potassium concentration of 6.4 mmol.l^{-1}. This meant there was a loss of 0.7g of sodium and 1.81 g of salt. Sweat chloride values were also determined for a class 2.5 player of 38 mmol.l^{-1} on the upper arm.

International basketball

The mean sweat sodium concentration for the team was 64±13 mmol.l^{-1} (range 42-80 mmol.l^{-1}). Sweat sodium concentration was not affected by classification ($p>0.05$, $r^2= 0.01$) and neither was it related to sweat loss ($r^2=0.589$, $p>0.05$). Total sodium losses were 1.6±0.5g (range 1.2-2.5g). Total sodium losses were not correlated to classification ($r^2= 0.622$, $p>0.05$). There was a significant correlation between total sodium loss and sweat losses ($r^2=0.742$, $p<0.05$). Mean salt (NaCl) loss was 4.1±1.2 g (range 3.1-6.4 g).

Total salt losses were not related to classification (r^2=0.446, p>0.05). The mean sweat chloride concentration was 66±13 mmol.l^{-1} (range 51-79 mmol.l^{-1}). Sweat chloride concentration was also unaffected by classification (r^2= 0.023, p>0.05). Sweat chloride concentrations are shown in Table 5.3.5. The mean sweat potassium concentration was 5.0±2.4 mmol.l^{-1} (range 1.0-8.8 mmol.l^{-1}). Sweat potassium concentrations were not correlated to classification of the players (r^2=0.225, p>0.05). Sweat Potassium concentrations are shown in Table 5.3.5.

Table 5.3.5. Showing Sweat Sodium Concentration (mmol.l^{-1}), Sweat Potassium Concentration (mmol.l^{-1}) and Sweat Chloride (mmol.l^{-1}) Concentration from an International Wheelchair Basketball Training Session.

Classification	Sodium	Salt loss	Potassium	Chloride
	(mmol.l^{-1})	(g)	(mmol.l^{-1})	(mmol.l^{-1})
1.0	56	3.3	3.0	69
1.5	80	3.6	4.7	62
2.0	42	4.0	5.6	51
2.5	70	3.8	1.0	73
3.0	75	3.3	9.6	52
4.5	59	5.6	6.1	79
Mean	64	3.9	4.8	66
SD	14	0.9	2.5	15

5.4. Discussion

Urine Osmolality

Shirreffs and Maughan (1998) found that mean urine osmolalities for individuals in a euhydrated state was 675±232 mosmol.kg^{-1} and mean urine osmolalities for individuals who were hypohydrated was 924±99 mosmolmosmol.kg^{-1}. The first urine passed each morning was obtained from 11 subjects over 5 who were undertaking normal activities and drinking ad-libitum. A second group of 10 subjects who had been hypohydrated by cycling the previous day and who were then been restricted from replacing these water losses, were assessed as being hypohydrated. Shirreffs and Maughan (1998) suggested that a urine osmolality of 700 mOsmol.kg^{-1} indicated a level of hypohydration. The results indicate that 12 of the 44 participants in the 4 squads had a pre training urine osmolality above 675 mosmol.kg^{-1}. Further to this, none of the wheelchair rugby players had a urine osmolality above 675 mosmol.kg^{-1}. These results indicate that the majority of the athletes/players and all of the wheelchair rugby players were euhydrated before the start of their respective training sessions. Given that all training sessions took place in a temperate environment it is unlikely that the mild hypohydration that was present in some individuals will lead to heat illness.

When considering mean urine osmolality values for each group and collectively they are lower than those previously reported amongst able-bodied footballers in these studies in which mean values have been reported as 872±177 mosmol.kg^{-1} (Maughan et al., 2005) This may be due to a greater knowledge of fluid requirements amongst the disabled athletes especially those with a spinal cord injury who are encouraged to drink sufficient fluids in an attempt to decrease the risk of urinary tract infection. This is highlighted by the fact that none of the rugby players had a pre training urine osmolality above 675 mosmol.kg^{-1} (the wheelchair rugby players all had SCI). It could also be linked to a lower sweat loss in previous training sessions implying that it is easier for the athletes with a disability to rehydrate between training sessions.

Fluid Balance

Body mass Change

Mental functioning can be impaired by a 2% decrease in body mass due to hypohydration (Burke, 1997) and is associated with impaired occupational and athletic

performance (Kay and Marino, 2000). Only one of the participants in this study lost more than 2% of their initial body mass, and the 3% loss in body mass that they achieved may have affected performance. This subject was an amputee and a sweat rate that was much higher than was seen by the other participants in this study. It has been noted in able-bodied sports that sweat rates can cover a large range even when participants are undertaking the same activities (Maughan et al., 2005). The overall change in body mass for all the participants together is minimal and would have very little if any effect on hydration status or performance. No measures of performance were taken in this study so the impacts of such a decrease in body mass cannot be concluded. Body mass gain during exercise is not associated with improved performance or with an improved thermoregulatory response to exercise (Maughan, 2001). Nor does hyperhydration affect heart rate response to exercise (Latzka and Sawka, 2000). Drinking too much during training usually results in excess fluid being excreted (Maughan, 2001; Freund et al., 1984). Gastrointestinal discomfort can develop if too much fluid is consumed (Robinson et al., 1995). Noakes (2003) also cautions against athletes drinking excess amounts of fluid during exercise. It is not necessary to replace all sweat losses and that a small degree of dehydration is tolerable without any adverse effects on performance (Noakes, 2002). This information was ascertained for able-bodied athletes and its relevance to athletes with a disability who are catheterised and may have impaired feelings of gastrointestinal discomfort is unclear. Amongst the wheelchair rugby players there was a mean± SD 0.45± 0.34 % increase in body mass. This contrasts to a body mass loss seen in able-bodied football training (Maughan et al., 2004; Maughan et al., 2005; Shirreffs et al., 2005). For players with impaired bladder control, the inability to control the urge to urinate will cause interruptions to training sessions and matches, although the effects of different hydration levels amongst athletes with a disability are unknown. The large increase in body mass seen amongst the wheelchair rugby squad may be explained by the suggestions of Price and Campbell (2003) which suggest that individuals with an SCI consume fluid during exercise in an attempt to attenuate the increased thermal strain. It is believed that consuming fluids may provide a psychological benefit to those with a SCI.

Overall body mass is slightly higher than values previously reported for paraplegic athletes by Price and Campbell (1999), but the international wheelchair basketball squad which was comprised of paraplegic athletes is similar. The wheelchair rugby team which contained only tetraplegics had a higher body mass than has previously been reported by Cardús and colleagues, (1985). Increases in body mass amongst

wheelchair athletes have previously been reported, a 0.2 kg gain in body mass being recorded during wheelchair basketball matches a 0.2 kg in which players were allowed to drink ad-libitum (Burnham et al., 1998). In this study, only the wheelchair rugby squad had a mean increase in body mass of this magnitude.

Sweat Rates

The sweat rates of athletes with and without a disability are influenced by many factors including environmental temperature and relative humidity (Maughan, 2001), exercise intensity (Greenhaff and Clough, 1989), fitness level (Baum et al., 1976), acclimatisation status (Maughan and Shirreffs, 2004), body mass (Maughan and Burke, 2002) and the clothing being worn (Maughan, 2001). Even if standard conditions are applied there is a large variation within sweat rate (Maughan, 2001). Further factors that could affect the sweat rate of athletes with a disability include the severity of the disability, type of disability, playing chair -differences in back height could have resulted in varying degrees of insulation from the chair thus affecting the thermal strain and sweat response (Price and Campbell, 2003). Even amongst those with the same disability, differences in the amount of sweat that they are able to produce can be seen. For example, amongst the SCI population the level and completeness of the spinal cord lesion will affect the sweat rate. Since there is a loss of sympathetic stimulation of the sweat glands below the level of the lesion the sweating capacity of the insensate skin is impaired (Randell et al., 1966; Yaggie et al., 2002).

Large variations in sweat rates have been reported in previous work conducted on able-bodied sports men, with Shirreffs et al. (2005) and Maughan et al. (2005) reporting a range of sweat rates between 1.67-3.14 $l.h^{-1}$ and 1.06-2.65 $l.h^{-1}$ respectively during football training sessions.

The sweat rate of the wheelchair rugby squad (0.25 $l.h^{-1}$) is low when compared to those seen previously in able-bodied forms of rugby (Rehrer and Burke, 1996). This is explained by the findings of Randell (1966) who found that only 25-30% of the sweat glands could be activated in people with a high level (cervical region) spinal cord injury. The wheelchair rugby players also had the lowest sweat rates in the study. This likely reflects the fact that they all had SCI, and that their injuries were at a high level. Therefore this group probably had the lowest surface area with the ability to secrete sweat. When considering that sweat rate was correlated to classification amongst this squad it further highlights that the higher and more severe the injury, the lower the sweat rate. This is in line with findings of Price and Campbell (2003).

The mean sweat rate for both wheelchair basketball squads was less than half of the mean sweat rates of 1.5 $l.h^{-1}$ reported for male basketball players without a disability (Burke and Hawley, 1997). Despite the mean sweat rate of a basketball player without a disability being 1.5 $l.h^{-1}$, sweat rates as low as 0.680 $l.h^{-1}$ have been recorded in junior elite stand up basketball (Broad, 1996). The sweat rates seen in this study for the wheelchair basketball players is slightly lower than the 0.9 $l.h^{-1}$ seen during an international wheelchair basketball match by Burnham et al. (1998). This is possibly because matches were played at a higher intensity to the training sessions in this study. Therefore the mean sweat rates from the 2 squads of wheelchair basketball players in this study were at the lower end of the sweat rate range for basketball players without a disability.

When comparing the sweat rates of able-bodied sports such as rugby or basketball, which require full body movement, to wheelchair basketball or rugby, which utilise mainly the upper body musculature, the differences in thermoregulatory response between lower and upper body exercise need to be considered. It is known that when performing either upper body or lower body exercise at the same relative intensity, the upper body exercise will result in a smaller thermoregulatory response due to the lower metabolic heat production associated with upper body exercise (Sawka et al., 1984). This means that even for players without a spinal cord injury, there is likely to be a smaller thermoregulatory response and therefore a smaller sweat loss than seen for their able-bodied counterparts when assuming that both sports are being played at the same relative intensity. Price (2006) in a review of the literature showed that sweat rates for those with a spinal cord injury were approximately half the values seen for able-bodied athletes. However, when the sweat rates of those competing in the whole body sprint athletic type events was compared to the wheelchair athletes involved in basketball and rugby there was no significant difference in the sweat losses. Therefore the lower sweat rates seen in the athletes with a disability compared to previous results on able-bodied athletes may be due to a reduced exercise capacity that may be present in some athletes with a disability due to their reduced muscle mass/ decreased neurological control of their muscle mass. It may also be the result of the long breaks that were taken during the athletics training session. This means that the absolute exercise intensities of those athletes are lower than their able-bodied counterparts, even though they are working at the same relative intensity (Maltais et al., 2004). A reduced sweat rate amongst cerebral palsy participants was seen during walking exercise by Maltais et al., (2004) found that despite higher rectal temperatures in the

group of children with cerebral palsy sweat loss was the same as that of the able-bodied matched controls thus concluding children with cerebral palsy might rely more on dry heat exchange than on evaporative sweat loss. Amputees have a smaller surface area for sweat loss. Given that body mass and body surface area both affect sweat rates (Maughan and Burke, 2004), it would be expected that this group would have a lower sweat rate. Therefore even in sports which require the whole body to be moved, there are factors which would result in a lower sweat rate amongst athletes with a disability. This makes it difficult to compare the sweat rates of the current studies to previous studies involving players of able-bodied sports.

There was however a significant difference between the participants with a spinal cord injury and those without in respect to sweat loss when sweat losses were expressed relative to body mass. Havenith et al. (1995) found that nearly half (~47 %) of the differences in sweat loss between participants could be attributed to differences in body mass, with heavier participants excreting more sweat.

The present study showed a significant correlation between the sweat losses and playing classification for the wheelchair rugby squad. This probably reflects the difference in the level of lesion which partially determines classification. Previous studies have shown a linear relationship between sweat loss and the level of spinal cord injury. Price and Campbell, (2003) found that C5-C8 tetraplegics lost less sweat (310 ml) during 60 minutes of wheelchair ergonometry in 31.5°C heat than low level paraplegics (below T7) who lost 690 ml of sweat during the same exercise protocol. This may be due to a reduced capacity for the sweat glands below the lesion to produce sweat or alternatively it may reflect a reduction in metabolic heat production with higher level spinal cord lesions due to a smaller muscle mass being utilised, resulting in a smaller rise in core temperature and therefore a smaller thermoregulatory response.

Despite the reduced capacity to produce sweat amongst those with a spinal cord injury there was no significant difference in the sweat rates of the participants with a spinal cord injury and those who had a different type of disability. This may be due to the different training sessions that those with a spinal cord injury and those without participated in, with the majority of the participants with a spinal cord injury participating in wheelchair rugby. Even within a training session players may be working at different absolute and relative exercise intensities throughout the training session. Sweat rates amongst able-bodied football players have shown wide variation even when they are participating in the same training session (Maughan et al., 2004).

As the effect of hypohydration and hyperhydration with respect to both health and performance have not been studied in detail amongst athletes with a disability it is difficult to suggest appropriate fluid intakes. Further work looking at changes in core temperature may provide some information to help determine changes in metabolic heat production amongst this group.

Fluid Intake

As with the study by Maughan et al. (2004) fluid intake was not correlated with either pre-exercise urine osmolality or sweat losses.

The volume of was fluid consumed was also unrelated to classification. This varies from the findings of Price and Campbell (2003) who found that with free access to water during exercise, those with tetraplegia consume more fluid despite lower sweat losses. Price and Campbell (2003) believed the thermal strain was higher amongst those with the highest SCI and they therefore consumed more fluid in an attempt to decrease the thermal strain. However, in the present study there was no significant difference between the rate of fluid intake amongst those who had a spinal cord injury and those with a different type of disability. This is more in line with the findings between those with a lower level spinal cord injury and the able-bodied participants in the study by Price and Campbell (2003). This probably reflects the fact that the participants in this study undertook different training sessions where access to fluids may have been more freely available during some training sessions than others.

There was a significant difference between the international wheelchair basketball squad and the recreational basketball squad with respect to fluid intake. This may be due to a different understanding of fluid intake requirements between the two teams. The international side are likely to have received information regarding hydration from team nutritionists. It may also reflect access to fluids which will have differed between the two teams. This may also be the reason for the significant difference seen between the international sprint athletes and the international wheelchair basketball team.

During exercise body mass losses should be limited to no more than 2 % decrease of initial body mass and prevent excessive body mass increases (ACSM, 2008). Therefore when an athlete has determined their sweat losses for a training session they can then determine future losses (as shown by the previous Chapter) and formulate drinking strategies with the appropriate drink volumes. This is the case for able-bodied

athletes but the effect of hypohydration or hyper hydration for disabled athletes is not known.

Sweat Electrolytes

Even though the sites from which the sweat samples were obtained was different from the sites used in previous research (Maughan et al., 2004) Patterson and colleagues (2000) showed that individual sites can be used to calculate whole body sweat electrolyte losses. The sweat sodium concentrations for the teams are within the range seen in individuals without a disability, 20-80 $mmol.l^{-1}$ (Maughan and Burke, 2004). Sweat sodium concentrations were not related to sweat rate. This is in line with similar studies conducted on soccer players during their training sessions in which sweat sodium concentrations were not correlated to sweat rates (Maughan et al., 2005). However as shown in the previous chapter when sweat sodium losses are determined from a single session this value can be used to determine future sweat sodium losses.

Sweat sodium concentration was not significantly related with classification, although this may be due to the low subject numbers. The changes in total sodium loss likely reflect sweat losses.

Salt losses from training need to be replaced. Although replacement during training may not be a requirement, it is necessary during the recovery period (Maughan, 2001). The salt losses for the team were lower than the mean salt intake for the UK male population of 11.0 $g.day^{-1}$, calculated from urinary sodium, and 85 % of men in the UK consume more than 6 grams of salt per day (Henderson et al., 2003). Due to low sweat rates no player exceeded a 6 g salt (NaCl) loss during training. The highest loss was 5.6 g, and although no dietary analysis was performed, it is likely that their normal diet would contain sufficient salt to replace this loss. As this training session formed part of a training camp, where multiple training session were taking place through out the day, players whose total salt losses were at the higher end of the range need to ensure they consume sufficient salt in preparation for the next training session.

The mean sweat chloride concentration was within the range seen in athletes without a disability (20-60 $mmol.l^{-1}$). Sweat chloride concentrations have been reported as high as 70.4 $mmol.l^{-1}$ from a single site (Shirreffs and Maughan, 1997). One participant in this study had a mean sweat chloride concentration (79 $mmol.l^{-1}$) with concentrations at the chest of 80 $mmol.l^{-1}$ and of 78 $mmol.l^{-1}$ at the shoulder. Patterson et al. (2000) found mean sweat chloride concentrations at the forehead of 53.8±28.6 $mmol.l^{-1}$

indicating that some participants had values as high as 80 mmol.l^{-1}. The high sweat chloride concentration may be due to the sample being contaminated. Contamination may have occurred due to inappropriate cleaning of the skin prior to training, or from the patch becoming temporally partially detached from the skin allowing the evaporation of some water. However as the sweat sodium values were not elevated above the normal physiological range it is unlikely that the patch became contaminated.

The mean sweat potassium concentration for the group was the same as previously found in participants without a disability (Shirreffs and Maughan, 1997). Only one sample was higher than what is considered the normal concentration range of 4-8 mmol.l^{-1}, but Patterson et al. (2000) also found sweat potassium concentrations greater than 8 mmol.l^{-1}.

Heart Rate

The mean heart rate found during the training sessions for the teams was similar to post match heart rates found by Burnham et al. (1998) of 120 beats.min^{-1}. Changes in players' heart rates during training reflect the intermittent nature of the training sessions. When using heart rate as a measure of exercise intensity in a group of SCI athletes, it needs to be remembered that individuals with a spinal cord lesion above T6 may have a decrease in cardiac performance during exercise as they may have partly disturbed cardiac sympathetic innervation allowing vagal activity to overrule and so limit their maximal heart rate (Theisen and Vanlandewijck, 2002). This phenomenon may affect the heart rate data of athletes with an SCI. Alternatively ratings of perceived exertion could be used to measure exercise intensity as this has been shown to be a valid method of monitoring exercise intensity (Dunbar et al., 1992). This method would have been inappropriate during this study as it would have meant asking the players during the training session and this would have caused disruption to the training session and may have altered drinking behaviour by reducing the time available to obtain a drink.

Limitations.

The heterogeneity of the participants in this study and the differences between the sports means that the results cannot be generalised to a wider disabled population. As players were weighed in minimal clothing, rather than weighed for nude body mass some sweat may have been trapped in the trousers and shoes of the players. Respiratory water losses and metabolic mass loss were also not measured or factored into the calculation of sweat losses. However, these losses are small and represent only a small error (Cheuvront et al., 2002). Even when trapped sweat was not accounted for total sweat losses could still be closely estimated (Cheuvront et al., 2002). Unfortunately it was not possible to record nude body mass of some participants due to constraints put in place by the coaches of the teams.

All of the training sessions took place under different environmental conditions and therefore differences in sweat rates may have been affected by environmental temperature. However, in able-bodied soccer Maughan et al. (2005) shown that sweat rates (1.82 l in the warm 1.69 l in the cold conditions) are similar in warm (25 °C) and cold (5 °C) environments. This is due to differences in the amount and type of clothing worn (Maughan et al., 2005). Differences in environmental temperature may affect fluid intake volumes. Soccer players training in warm conditions tend to drink greater quantities of fluid than those training in cool conditions (Maughan et al., 2005).

The use of heart rate as a measure of exercise intensity may not be applicable to those with a SCI at or above T6 due to interruptions in the vagal tone, which results in an altered heart rate response and maximal heart rates are lower than seen for able-bodied participants. Further to this during exercise cardiac drift can occur, this can elevate heart rate by 18 beats.min^{-1} for the same power output even if the subject remains euhydrated throughout training (Jeukendrup and Dieman, 1998). Therefore it may appear that participants are working at a higher exercise intensity than they were actually performing.

As mentioned in Chapter 1 and Chapter 2 of this thesis, not all body mass losses are due to sweat losses and there is a gain of body water from substrate metabolism, for athletes with a disability this may be significant and an indication of these losses and gains is described below:

Respiratory water loss

Taking the average heart rate of the participants in each sport to be 114 beats.min^{-1} for athletics (60 % maximum heart rate), 134 beats.min^{-1} for the club wheelchair basketball squad (70 % maximum heart rate) and 125 beats.min^{-1} (70 % maximum heart rate) for the international basketball squad then an estimation of % $\dot{V}O_2$ can be calculated, using the calculations of Swain et al. (1994). In this calculation

'% Maximum heart rate= (0.643 * % $\dot{V}O_2$ max) +37'

Swain et al. (1994)

This corresponds to a % $\dot{V}O_2$ peak of 35 % $\dot{V}O_2$ peak for the athletics group and 50 % for the 2 wheelchair basketball groups. Amongst international wheelchair basketball players $\dot{V}O_2$ peak = 2.65 l.min^{-1} which was the mean $\dot{V}O_2$ peak found by Goosey-Tolfrey (2005). Therefore $\dot{V}O_2$ values could be estimated to be 0.93 l.min^{-1} for the athletics group and 1.33 l.min^{-1} for both wheelchair basketball groups. The mean temperature and relative humidity during the training sessions was 17.5°C and 48 %. This equates to a water vapour Pressure of 8.4 mmHg. Therefore using the equation of Mitchell et al. (1972) whereby

Respiratory water loss= 0.019 * $\dot{V}O_2$ (l.min^{-1}) (44-Water Vapour Pressure)

Then Respiratory water losses for the training sessions would equate to a loss of 0.63 g.min^{-1} for the athletics squad and 0.90 g.min^{-1} for the 2 wheelchair basketball squads. Therefore over a the period of the training sessions (110 minutes for international sprint athletics, 114 minutes for the club basketball and 118 for the international basketball players) respiratory water losses would be 69, 103 and 106 g respectively. This is of course a very rough estimation as the absolute $\dot{V}O_2$ is based on values previously published for a different group of athletes. Further, the calculation of $\dot{V}O_2$ peak from heart rate responses does not take into account cardiac drift, which would artificially elevate heart rate. As mentioned in the Literature Review at low absolute $\dot{V}O_2$ values the equation overestimates the rate of respiratory water loss and this can be by 15-20 % (Mitchell et al., 1972). An overestimation by 20 % would mean an overestimation by 14 g for the international sprint athletics, 21 g for the club basketball players and the international wheelchair basketball players. Also, the relationship between O_2 uptake and respiratory water loss is not linear above 70 % $\dot{V}O_2$ max but the deviation is minimal up

to workloads of 80 % $\dot{V}O_2$ max within 0.25 g.min^{-1} compared to direct measurements of humidity in the expired air, Mitchell et al. (1972). A further complication for those individuals who have a spinal cord injury ventilatory rate may be altered; this would therefore affect the respiratory water losses. However, as yet studies concerning the respiratory rates (increase in the number of breaths per minute) of individuals with a spinal cord injury have only been conducted in response to heat exposure (Wilsmore et al., 2006). This increase in ventilatory response was almost twice that of able-bodied subjects and higher amongst those with the highest lesion levels (Wilsmore et al., 2006). It is theorised that this may be an adaptation to aid cooling, if this is the case then the increase in thermal stress associated with exercise may have a similar effect to passive heating amongst those with a spinal cord injury. This increase in breathing frequency may decrease the amount of water lost per breath (McFadden, 1988) however, the greater number of breaths taken in total may result in an increase in respiratory water losses.

Calculation of body mass change due to substrate metabolism

When substrates are oxidised, carbon dioxide is produced and this is then lost from the body via expiration, water is also produced however this remains within the body. Some of these by products obtain their oxygen from the atmospheric air, therefore both the amount of oxygen from the atmospheric air; the loss of carbon dioxide in expired air and the production of water need to be included when determining body mass changes and hydration changes during exercise. The water and mass changes from the oxygenation of carbohydrate can be determined from the stoichiometric equation:

$$\longrightarrow C_6H_{12}O_6 + 6O_2 \qquad 6CO_2 + 6H_2O$$

At 35 % $\dot{V}O_2$ peak 50 % of the energy demands are met from carbohydrate sources and the remaining 50 % from fat sources (Maughan et al., 2007). At 50 % $\dot{V}O_2$ max 60 % of the energy demand comes from carbohydrate and 40 % from fat (Maughan et al., 2007). This is obviously ignoring any protein metabolism that might occur although protein oxidation is likely to be minimal, even during endurance events protein does not contribute more than 5-6 % of the total energy turnover (Lemon, 1991). It has also been shown that obese individuals the relative contribution of fat is higher than amongst lean individuals, it is known that individuals with a spinal cord injury tend to have a greater relative fat mass than able-bodied participants. It has been reported that even physically active individuals with a spinal cord injury have a greater

proportion of fat mass than their able-bodied counterparts (Konica, 1997). Diet and aerobic fitness will also affect the relative contributions of fat and carbohydrate for substrate metabolism (Hargreaves et al., 2004). As no expired air samples were taken from the participants during training it is not possible to accurately determine the exact proportion of energy that was derived from carbohydrate or from fat. The mean body mass of the athletes was 66 kg and was 81 kg for the club level wheelchair basketball players and 80 kg for the international wheelchair basketball players. According to Ainsworth et al. (2000) the metabolic equivalent for running is 10 MET and for wheelchair basket ball is 6.5 MET. This would equate to an energy requirement of 1210 kcals for the athletics group and 1000 kcals for the club level wheelchair basketball squad and 1035 kcals for the international wheelchair basketball squad during their training session. However, the actual energy requirements of the players will depend on the type of injury, the efficiency with which they propel the wheelchair, playing position, training intensity and the type of wheelchair therefore the energy requirement will vary from player to player. Further the MET system is derived from able-bodied populations and due to the loss of muscle mass in those with a spinal cord injury, the likelihood is that the use of the MET system to determine energy requirements for those with a spinal cord injury will result in an overestimation. However, if an energy requirement of 1210 kcals is assumed for athletics and 1000 is assumed for the wheelchair basketball squads then that would equate to 610 kcals (149 g) from carbohydrate and 610 kcals (66 g) from fat for the athletics and 600 kcals (146g) from carbohydrate and 400 kcals (43 g) from fat for the wheelchair basketball players. 1 g of carbohydrate oxidation results in a net mass loss of 0.4 g. The net body mass gain from 1 g of fat oxidation is 0.13 g. Therefore during the their training the athletes will have lost 60 g of body mass due to carbohydrate oxidation but will have gained 9 g of body mass from fat oxidation. This results in a net mass loss of 51 g from sources other than sweat loss. For the wheelchair basketball squads the net mass gain from carbohydrate oxidation is likely to be 58 g and the body mass gain from fat oxidation is likely to be 6 g. This is therefore a net body mass loss for the wheelchair basketball players of 52 g from substrate oxidation.

However, when carbohydrate and fat are oxidised for energy, water is also released for each gram of carbohydrate 0.6 g of water are released and for every gram of fat oxidised 1.13 g of water are released. Therefore the water released due to substrate metabolism for the athletic group would be 164 g (89.4 g from carbohydrate oxidation and 74.6 g from fat oxidation). For the wheelchair basketball squad the water

released due to substrate metabolism would be 87.6 g from carbohydrate sources and 48.6 g from fat oxidation, so total water release due to substrate metabolism is 136.2 g.

Therefore overall from substrate oxidation the athletes would lose 51 g of body mass unrelated to water losses and gain a further 164 g of water from substrate oxidation. This would mean that the athletes could lose 215 g of body mass without a decrease in body water. For the wheelchair basketball players they lost 52 g of body mass from substrate oxidation and gained 136 g of water therefore they could have a decrease in body mass of 188 g with no alteration in body water occurring. As muscle atrophy is associated with spinal cord injuries then differences in metabolic rate will be present and so this calculation is likely to be incorrect for such groups.

Water release from glycogen stores

Glycogen is stored in the muscles with water when glycogen is utilised this water is released. This release of water means that its loss from body water will not result in hypohydration (Maughan et al., 2007). During exercise some glycogen will be utilised from muscle stores and some from liver glycogen stores. It is likely that liver glycogen is stored with less water than is associated with muscle glycogen (Maughan et al., 2001). If it is assumed that for every gram of glycogen that is utilised 3 g of free water are released then during the two hour training session the release of water from glycogen stores will be 555 ml. Therefore 555 ml of water can be lost from the body without it effecting osmolalities/causing hypohydration.

Mass of sweat

Due to the solute content of sweat the assumption that 1 g of sweat is equal to 1 ml is not true. 1 ml of sweat actually has a mass of 1.001-1.008 g depending on the composition of the sweat sample. However, such a small difference in mass is too small to be detected by the measurement of participant's body mass.

"Sweat loss = body mass loss + ingested fluid – substrate oxidation + metabolic water – respiratory water loss"

Maughan et al. (2007)

"Body Water loss = body mass loss- substrate oxidation – metabolic water- water stored with glycogen + urine and faecal losses"

Maughan et al. (2007)

Table 5.4.1. Calculated Respiratory Water Losses (g), Net Change in Body Mass Due to Substrate Oxidation (g), Body Water Gain from Metabolic Substrate Oxidation (g) and Released from Glycogen Stores (g) for the Mean Athlete, Club and International Wheelchair Basketball Player during Training.

	Athletics	Club Basketball	International Basketball
	(g)	(g)	(g)
Respiratory water loss	69	103	106
Substrate oxidation-net body mass change	-51	-52	-52
Metabolic Water gain	164	136	136
Water stored with glycogen	555	555	555
Total change BM without loss of body water	770	743	743
Sweat loss	700	950	1190
Potential change without decrease in body water as % of sweat loss	110 %	78 %	62 %

If the above assumptions were correct then it is possible that the changes in body mass that were seen and attributed to sweat loss and therefore as an indication of dehydration have been overestimated. The values calculated are substantial. Therefore, it may be the case that % dehydration during training amongst these athletes is even smaller than seen for body mass changes alone. Further research into these non-eccrine

water losses and sources of water gain during exercise in the disabled population is needed in order for a better understanding of the fluid balance of athletes with a disability. As the lower active muscle mass of those with a spinal cord injury is likely to mean a lower metabolic rate. The relative proportions of body mass which are lean mass and fat mass is different between those with a spinal cord injury and able-bodied participants. Those with a spinal cord injury have a greater relative fat mass than able-bodied individuals (Kocina, 1997). There are potential differences in ventilatory rates between able-bodied athletes and those with a spinal cord injury which would affect respiratory water losses (Wilsmore et al., 2006). Therefore the above data is likely to be flawed. Any overestimation of sweat losses will also result in an overestimation of sweat electrolyte losses as the concentrations of the electrolytes in the samples obtained are multiplied by the sweat losses to determine absolute electrolyte loss. Therefore, if an overestimation of sweat loss occurs an overestimation of sweat electrolyte loss will also occur.

5.5. Conclusion

The heterogeneity of participants in this study means that it is difficult to tell whether the sweat response of disabled athletes differs between the classifications. The results may be due to individual differences. Due to the numerous factors that can effect sweat loss and therefore subsequent nutritional requirements (with respect to water and salt) in the disabled athletic population it is best to advise individuals based on their specific sweat losses.

Overall it does appear that sweat rates are lower for athletes with a disability compared to values reported for able-bodied athletes during training (Burke and Hawley, 1997). Despite this lower sweat rate fluid intakes are similar to those reported for able-bodied athletes (Burke and Hawley, 1997). Therefore body mass tends to increase during training. Sweat electrolyte composition with respect to sodium, chloride and potassium is within the physiological range.

It needs to be considered whether either a player's performance, or health, will suffer detrimental effects due to their over drinking. In able-bodied, weight bearing, sports an increase in body mass has been shown to be detrimental to performance. Up to a 2% reduction in body mass due to sweating in able-bodied athletes is thought to have no detrimental effects on performance or health. However, no studies have looked at the effect of different hydration statuses in a group of athletes with a disability. The reason for the players drinking during training has been assumed due to an increase in

core temperature during the training session and subjective feelings of getting hot. Studies looking at the feelings and thermal comfort of these athletes need to be undertaken with individuals with a spinal cord injury, especially those with a high level spinal cord injury so that a better understanding of the reasons for their drinking behaviour can be ascertained. If those with a spinal cord injury drink large volumes of fluid during training does this then have implications for the periods when they are not exercising i.e. is fluid intake low throughout the rest of the day?

Chapter 6: Total Body Water and Water Turnover Rates of a Wheelchair Rugby Squad during an International Tournament

6.1 INTRODUCTION

The previous Chapter found that those athletes with a spinal cord injury consumed vast quantities of fluid during the training session. This Chapter is designed to determine the daily fluid intake of athletes with a spinal cord injury.

It is known that following a spinal cord lesion there muscle atrophy occurs (Hagerman et al., 2006; Kocina, 1997; Drummond et al., 2008). In some cases this atrophy is accompanied by functional impairments which limit the ability to train muscle groups even though they are still innervated by intact nerve flow from the spinal cord e.g. if the biceps are no longer functioning this may affect the ability of the individual to train the triceps (Kocina, 1997). These factors mean that individuals who have a spinal cord lesion have a relatively lower lean mass compared to healthy individuals, especially healthy able-bodied athletes (Kocina, 1997). Due to the higher proportion water in lean mass compared to fat mass, a loss of lean mass is associated with a lowered total body water relative to body mass. However the body water content of individuals with a spinal cord injury undertaking regular exercise is unknown.

Poor hydration has been linked with an increased risk of urinary tract infection (Manz and Wentz, 2005). A SCI puts individuals at greater risk of a urinary tract infection (Waites et al., 1993) they are therefore encouraged to maintain adequate levels of hydration. On the other hand in those with spinal injuries above T6 there is a risk of autonomic dysreflexic attack if too much water is consumed (Schmid et al., 2001). This makes water balance amongst this group highly important.

Sodium citrate is a supplement (an alkalising agent), which has been shown to improve performance during high intensity activities lasting between 2 and 4 minutes to improve performance amongst able-bodied cyclists (McNaughton and Cedaro, 1991). As the active transport of sodium and glucose plays a role in the absorption of water from the gastrointestinal tract supplementation with sodium citrate may affect the water turnover rates, 24 hour urine volumes and 24 hour sodium losses. Sodium citrate is a supplement that is regularly taken by the wheelchair rugby squad in an attempt to aid performance, as urinary sodium concentrations are to be measured in this Chapter it is important to note that some players consumed a sodium supplement.

There have been studies performed on able-bodied athletes – cyclists, American footballers and runners, which have investigated their total body water and water

turnover rates compared to sedentary able-bodied control participants (Stofan et al., 2007; Leiper et al., 1996; Leiper et al., 2001). The main differences between the physically active and the sedentary groups have been attributed to a greater non-renal loss amongst the athletic groups, probably due to an increased sweat loss in the athletes. However, very few individuals with a spinal cord lesion are able to sweat below the level of the lesion. In some high level lesions (tetraplegics) this means that they are unable to sweat on any part of their body except their heads (Hopman et al., 1992; Hopman et al., 1993). This means that sweat losses are low amongst this group of athletes (the reasons for this are described in Chapter 4). Amongst participants with a spinal cord injury those with the higher level injuries (C5/C6-C7/C8) consumed the greatest volumes of fluid (0.764±0.342 l versus 0.472±0.252 l for high paraplegics, T1-T6, and 0.381±0.251 l for low paraplegics, below T7) but had the lowest sweat rates (0.31±0.42 l for tetraplegics, 0.71±0.33 l for high paraplegics, 0.69±0.53 l for low paraplegics) (Price and Campbell, 2003). The effects of a smaller sweat loss during exercise and the possible increase in fluid consumption on water turnover rate and upon non-renal losses have not previously been studied.

Body water can be determined by use of a non radioactive isotope – deuterium oxide (heavy water, D_20). Deuterium oxide is neither metabolised nor produced by the body therefore if a known quantity is consumed the total body water and the rate of water turnover can be determined by the initial dilution and the rate of dilution over a period of time. Measuring the urine volume produced each day, allows non-renal losses can be calculated.

As the previous Chapter showed high fluid intakes of wheelchair rugby players. The aim of this study was to describe the 24 hour fluid balance of wheelchair rugby players with a spinal cord injury during a period of competition.

6.2. Method

Ethical approval from Loughborough University was amended through the ethical advisory committee at Loughborough to allow samples to be collected in the U.S.A. Eleven male wheelchair rugby players (all of whom were members of the Canadian international squad) volunteered and provided written informed consent to participate in this study. All participants completed a health screening questionnaire and self-reported to be clear from urinary tract infections during the period of the study. Physical

characteristics are described in Table 6.2.1. All participants had a spinal cord injury at the T4 level or above and reported their playing classification as obtained by the International Wheelchair Rugby Federation. The study took place during a training camp in Florida, U.S.A. in January 2008, mean environmental temperature outside was 27± 5 °C and relative humidity was 68±15 %. During the study players participated in a wheelchair rugby tournament, the temperature at the tournament was 21.0±3.3 °C with a relative humidity of 22±10 %. Temperatures were taken hourly from 07:00-22:00 h for outdoor temperature and from the time of arrival ~ 09:00 h until the time of departure ~ 16:00 h for the indoor competition venue. Players had been at the training camp for at least a month prior to the study and so were acclimatised to the conditions.

Before retiring to bed on Day 0 players emptied their catheter bags, the mass of this urine volume was measured and a sample retained from each player. Participants then consumed an absolute known mass of deuterium oxide mean±SD 10.0013±0.0032 g (99.9 atom%; Sigma, London, U.K.). Following ingestion the deuterium container (a 20 ml tube) was filled with a fluid of the subject's choice and again consumed by the participant, this was repeated several times to ensure all deuterium was drunk. For the following 6 days, players were provided with labelled 24 hour urine containers into which they emptied all the contents of their catheter bags each day. Every morning the mass of the urine container was measured and recorded (this value was used to calculate 24 hour urine volumes, after correction for the mass of the empty container). Approximately 20 ml from each 24 hour container was retained for later analysis. During the study participants were free to consume their normal diet and participate in their daily activities (which were recorded by the investigator). On Day 1 of the study all participants took part in 2 wheelchair rugby matches, on Days 2,3 and 4 participants undertook no formal exercise, Day 5 consisted of 2 wheelchair rugby matches at a tournament and on Day 6 participants undertook 3 wheelchair rugby matches as part of the tournament. Participants were free to consume any food or fluid they required.

Table 6.2.1: Showing the Physical Characteristics- Body Mass (kg), Age (y), Spinal Cord Injury Level and Wheelchair Rugby Playing Classification of Participants.

Player	Body Mass	Age	Injury	Playing Classification
	(kg)	(y)	(SCI level)	
1	77.3	34	C4	0.5
2	63.6	19	C5	1.0
3	65.9	25	C6	2.0
4	59.6	25	C5-6	1.0
5	66.0	40	C6-7	2.0
6	90.9	41	C6-7	2.0
7	59.1	31	C6-7	2.0
8	91.0	32	C6-7	3.5
9	64.0	28	C7	1.5
10	77.0	27	C7-C8	3.0
11	63.6	31	T4	3.0
Mean	***70.7***	***30***		
SD	***11.6***	***7***		
Median	***65.9***	***31***		
Min	***59.1***	***19***		
Max	***91.0***	***41***		

All urine samples were measured for deuterium oxide concentration by infra red spectrometry using the methods described by Lukaski and Johnsonn (1985) and described in the general methods section. The urine sample prior to the ingestion of deuterium oxide were used to determine baseline concentrations of deuterium; no player had any significant levels of deuterium in their urine at baseline. All the urine collected during the night of deuterium ingestion was weighed in the morning and a sample of this urine was used to determine total body water (TBW) using the formula of Schloerb et al. (1950) after correction for baseline levels. With the assumption that body water remained stable throughout the period of the study the rate of decline of ^{2}H concentration in the subsequent morning samples was used to estimate daily water

turnover rate (Lifson and McClintock, 1966). The difference between the daily water turnover and 24 hour urine volume was calculated and used to determine daily non-renal losses. All urine samples were also measured for sodium and potassium concentration by flame photometry (Corning, 410, New York, USA), chloride concentration was determined by coulometric titration (Jenway PCLM 3, Essex, UK), and osmolality via freezing point depression (Gonotec Osmomat 030, YSI, Farnborough, UK). During the study 5 of the players consumed a sodium citrate supplementation of 0.1 $g.kg^{-1}$ of body mass during days 1-3 and 6 of the players consumed this supplementation on days 4-6. The sodium citrate was dissolved into the players drink and consumed with breakfast.

Statistical Analysis

All data were tested by a Shapiro Wilk test for normal distribution. Where data were normally distributed it is provided as mean±SD. Data that was not normally distributed the median (range) is provided. Data that were not normatively distributed were tested using a Friedman test to measure for significant differences. To ascertain between which days the difference laid a Wilcoxon signed-rank test with a Bonferronni correction was used. In all cases the initial a level of $p<0.05$ was used for significance, corrections for post hoc tests meant that the significance level was $p<0.05$/number of comparisons performed. Correlations between water turnover rate and playing classification were calculated using a Spearman's rank test.

6.3. Results

The median 24 hour urine volume was 1.53 litres (range 0.26-5.77 litres). The median (range) 24 hour urine volumes for the players during each day of the study are shown in Figure 6.3.1.

Figure 6.3.1 shows that median urine volumes tended to be greater on match days than non-match days, however, due to the small number of participants in this study and the large variation between the participants meant that this trend was not significant ($p>0.05$).

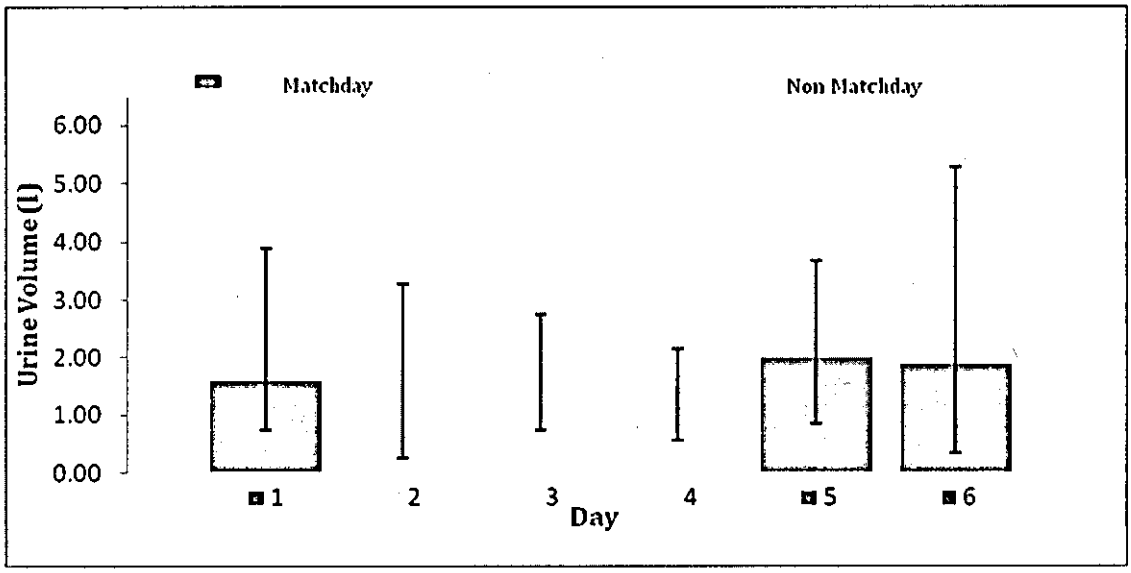


Figure 6.3.1: Median (Range) 24 Hour Urine Volumes (l) For a Group of Wheelchair Rugby Players on Match Days and Non Match Days during an International Tournament.

All participants had a day to day variation in urine volume of over 1 litre. Expressed relative to body mass median 24 hour urine volume was 22.7 ml.kg.d^{-1} (range 10.6-90.2 ml.kg.d^{-1}). The results showed that 4.6% of TBW was lost via urinary output per day (range 2.1-16.8 %).

With sodium citrate supplementation 24 hour urine volumes were 1.7 (0.3-5.8) litres and without supplementation they were 1.4 (0.3-4.0) litres. There was no significant difference between the days with supplementation and the days with no supplementation, although the trend towards higher urinary volumes with sodium citrate supplementation neared significance (p=0.052).

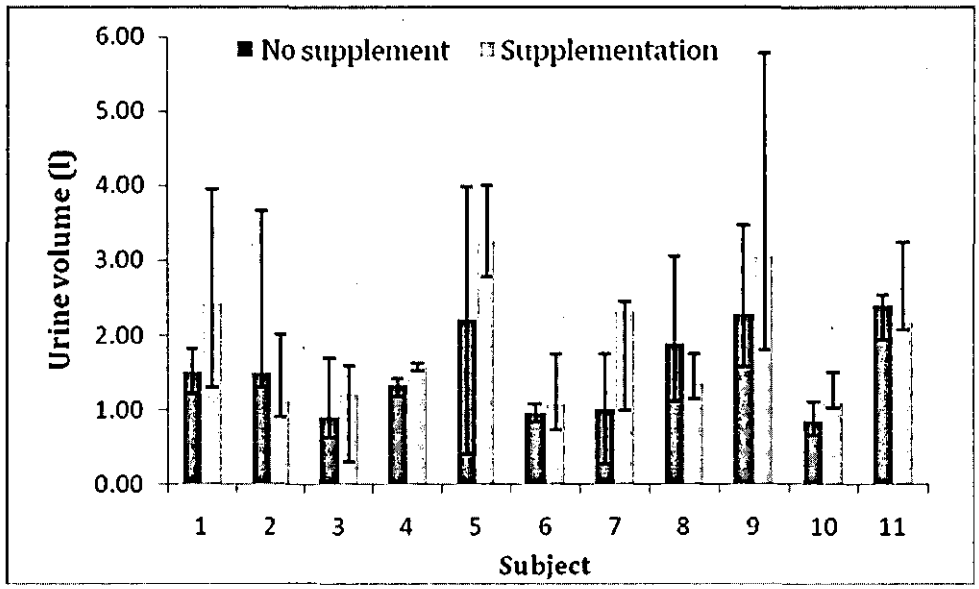


Figure 6.3.2.: Median (Range) 24 Hour Urine Volume (l) for Each Subject with and Without Sodium Citrate Supplements

Total Body Water (TBW)

Median total body water for the squad was 33.8 litres (range 24.6- 50.8 litres). When expressed as a percent of body mass, TBW made up (median) 53.11 % of the players body mass (range 36.88-61.83 %). Total body water was significantly correlated to body mass (r^2=0.629, p=0.0385). There was a significant relationship between playing classification and the % of body mass that was water, as shown by Figure 6.3.3. This was significant (p=0.012, r^2=0.524).

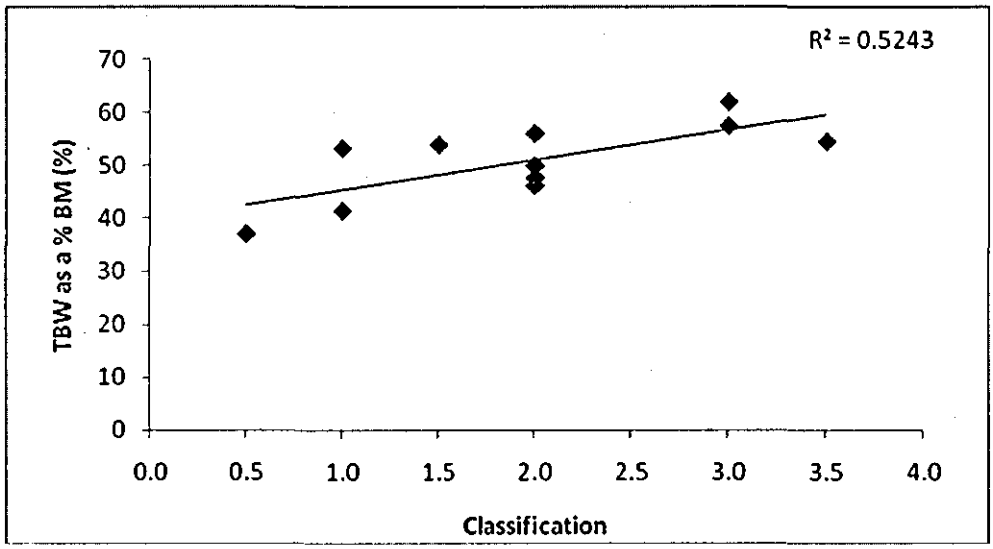


Figure 6.3.3: Correlation between TBW as a % Body Mass and Playing Classification for a Squad of Wheelchair Rugby Players.

Water turnover rate (WTR)

The median water turnover rate for the squad was 2.23 $l.d^{-1}$ (range 1.04-4.23 $litres.day^{-1}$). Expressed relative to body mass median daily water turnover rate was 27.5 $ml.kg.d^{-1}$ (range 8.02-100.78 $ml.kg.d^{-1}$). Expressed relative to TBW the median (range) water turnover per day was 5.6% of total body water (0.7-18.7%). During the days when the participants ingested sodium citrate water turnover rates were 2.32 (0.81-6.45) $l.d^{-1}$. There was no significant difference between match days and non-match days with respect to water turnover rate although there was a tendency for higher water turnover rates on match days ($p=0.088$). However, when the players were not ingesting the sodium citrate water turnover rates were 1.75 (0.23-4.21) $l.d^{-1}$. The difference between days consuming sodium citrate and days not consuming sodium citrate were not significantly different ($p=0.729$). Water turnover rate was significantly correlated to daily urine volume ($r^2=0.777$, $p=0.005$).

The difference between water turnover rates and 24 hour urine volumes meant that the median non-renal loss for the squad was 0.39 litres (range 0.10-3.35 litres). Non-renal losses were not significantly correlated with playing classification ($r^2=-0.264$, $p<0.05$). The relationship between TBW, WTR and non-renal loss is shown in Figure 6.3.4.

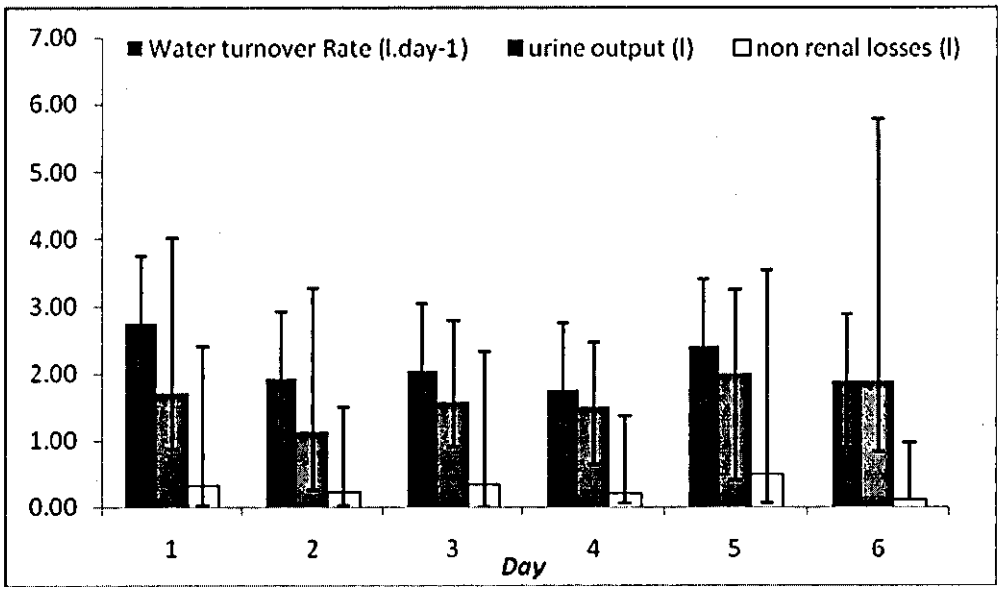


Figure 6.3.4. The Median (Range) Daily Water Turnover Rate ($l.d^{-1}$), Daily 24 Hour Urine Volume (l) and Daily Non-Renal Losses (l) of a Wheelchair Rugby Squad.

Urinary Electrolytes

Mean 24 hour urine sodium concentration was 115±17 $mmol.l^{-1}$ (range 40-200 $mmol.l^{-1}$) therefore mean 24 hour sodium losses via urine were 191±98 $mmol.24h^{-1}$ or 4.4±2.3 g (0.7-10.1 g) and so mean salt loss via the urine was 11.2±5.8 g (range 1.8-31.8 g).

Whilst taking sodium citrate median (range) 24 hour urinary sodium losses were 5.08 (0.71-9.56) g. When not consuming the sodium citrate supplementation median (range) urinary sodium losses per 24 hours were 3.14 (0.80-12.50) g. This was not a significant difference between supplementation and no supplementation p=0.086.

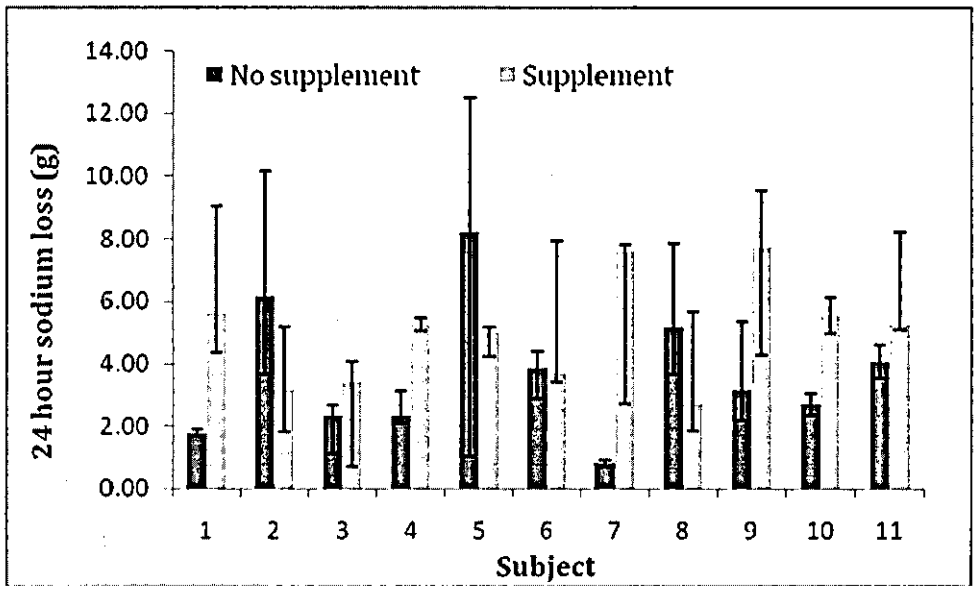


Figure 6.3.5. Median (Range) 24 Hour Urinary Sodium Losses (g) with Sodium Citrate Supplementation and Without Sodium Citrate Supplementation.

24 hour urinary sodium concentrations were not significantly correlated with TBW, when expressed either as an absolute value or relative to body mass ($p>0.05$, $r^2=0.003$). 24 hour urinary sodium losses were not significantly correlated with urine osmolality ($p=0.218$, $r^2= 0.03$). However, 24 hour urinary sodium loss was correlated to 24 hour urine volume ($p<0.01$, $r^2=0.513$) and water turnover rate ($p<0.001$, $r^2= 0.282$).

Mean urine potassium concentration was 41±6 mmol.l^{-1} (range 8-89 mmol.l^{-1}) this meant that 24 hour potassium loss was 83±59 mmol or 1.6±1.1 g. 24 hour urine potassium concentrations was not correlated to absolute or relative TBW ($p>0.05$, $r^2=0.003$; $p>0.05$, $r^2=-0.032$), nor was it correlated to playing class ($p>0.05$, $r^2=0.041$).

Mean 24 hour urinary chloride concentration was 68±16 mmol.l^{-1} (range 10-159 mmol.l^{-1}). Again 24 hour urinary chloride concentration was not significantly correlated with absolute or relative TBW or playing class ($p=0.832$, $r^2=0.005$; $p=0.555$, $r^2=0.04$; $p=0.956$, $r^2=0.000$).

Urine Osmolality

The 24 hour urine samples showed that mean urine osmolality was 478±73 mosmol.kg^{-1}. With only 4 of the samples possessing a urine osmolality greater than 700 mosmol.kg^{-1}, all 4 of these values were reported in the same player.

With sodium citrate supplementation urine osmolality was 479 (175-1104) mosmol.kg^{-1} whereas during the days with no sodium citrate supplementation urine osmolality was 460 (232-946) mosmol.kg^{-1}. This difference between days with supplementation and no supplementation was not significantly different ($p>0.05$).

Urine osmolality was not correlated to absolute TBW ($p= 0.631$, $r^2=0.026$), or TBW relative to body mass ($p= 0.401$, $r^2= 0.079$), neither was it significantly correlated to water turnover rate ($p=0.306$, $r^2=0.019$), nor was it significantly correlated with urine volume ($p=0.068$, $r^2=0.055$).

6.4. Discussion

This is the first study undertaken to look at the fluid balance of tetraplegics during sporting activities. Following a spinal cord injury muscle atrophy occurs below the level of the injury this is because this region is no longer innervated by the spinal cord, thus the amount of lean mass decreases (Kocina, 1997). All the participants in this study had some form of impairment in their arm function. The functional impairment and the resultant decrease in muscle mass from the spinal cord injury on functional capacity will be reflected by the playing classification of the participants. Players with a lower playing classification will have the greatest degree of functional impairment.

As shown in Figure 6.3.3. there appears to be a positive relationship between the percent of body mass which is water and playing classification, this would imply that those players with a greater functional capacity (lower lesion level) also have a higher percent of lean muscle mass. Even though this group of rugby players were quite homogenous all were tetraplegics, and all had a complete lesion there is still a marked change in total body water. This probably reflects the relative amount of muscle mass that is still innervated by the spinal cord. Higher level lesions will result in a greater proportion of the muscle mass which cannot be innervated. This would lead to a greater relative loss of muscle mass, as muscles which cannot be innervated become atrophied. Amongst able-bodied participants there is a tendency for total body water relative to body mass to be lower amongst sedentary populations than athletes reflecting the differences in lean mass (Leiper and Maughan, 2004). Values for TBW relative to body

mass for able-bodied swimmers have been reported as 20.1±6.3 % compared to age matched controls which had relative TBW of 17.5±1.4 % (Leiper and Maughan, 2004).

The absolute TBW values amongst the wheelchair rugby players in this study [33.8 litres (range 24.6-50.8 litres)] are similar to mean values previously seen amongst tetraplegics (34.9±7.4 litres). Values are slightly lower than paraplegic men (39.0±4.6 litres) (Cardús et al., 1984). These values are lower than those found by Leiper et al. (2001) for active trained cyclists, where median values of 54.3 litres (43.2-62.3 litres) were found, and their control participants for whom median TBW was 41.4 litres (35.8- 49.0 litres). Therefore absolute TBW of wheelchair rugby players is similar to that previously reported for Tetraplegics (Cardús et al., 1984) but lower than values for paraplegics and able-bodied groups (Cardús et al., 1984; Leiper et al., 2001). This would reflect the decreased muscle function and muscle atrophy that occurs following a SCI.

As with absolute total body water, when total body water is expressed relative to body mass the median values seen for the wheelchair rugby players [53.1 % (range 36.9-61.8 %)] were lower than those seen for able-bodied cyclists (Leiper et al., 2001). However, relative total body water values tend to be lower amongst well trained sports men than individuals who are recreationally active or sedentary (Leiper et al., 2001; Battistini et al., 1994; Shimamoto and Komiya, 2003). However, comparing the values against previous findings for healthy males shows that the % of body mass that is water in the wheelchair rugby players is smaller than the mean values (mean±SD 61.8±3.5 %) seen by Schloerb et al. (1950). Comparing the values to the data of Cardús et al. (1984) showed that the wheelchair rugby players had lower total body water relative to body mass than paraplegics (63 %) and tetraplegics (58 %) who were in the hospital rehabilitation phase following spinal cord injury. This is probably due to the longer time since injury in the present study as muscle atrophy becomes greater with time (Castro et al., 1999).

Water Turnover Rate and Non-Renal losses

Leiper and Maughan (2004) reported greater water turnover rates amongst swimmers than amongst aged matched controls. This faster WTR was due to the swimmers ingesting a larger volume of water during the study and was unrelated to a higher exercise induced sweat volume loss. The median daily WTR for the wheelchair rugby squad is similar to values previously reported for sedentary controls by Leiper et al. (2001) and Leiper and Maughan (2004). Leiper et al. (2001) found median non-renal losses to be 0.4 litres.day^{-1} (0.3-1.5 litres.day^{-1}) and Leiper and Maughan (2004) found mean±SD non-renal losses to be 0.9±1.3 litres.day^{-1}, in the present study median non-

renal losses were lower 0.2 litres.day^{-1} (0.1-3.5 litres.day^{-1}). It is known that following a spinal cord injury there is a decreased capacity for sweat production below the level of the lesion. This would account for the lower median values for non-renal losses seen in this study and for the very low values which are similar to the lowest values seen for elderly participants (Leiper et al., 2005). This reduction in sweat production is more pronounced when non-renal losses for the current study are compared to non-renal losses of able-bodied athletes. Mean values of 2.7±1.4 litres.day^{-1} have been found in swimmers by Leiper and Maughan, (2004) and median values of 1.4 litres.day^{-1} (range 1.1-3.0 litres.day^{-1}) in cyclists (Leiper et al. 2001). Comparing the values of non-renal losses for cyclists with the wheelchair rugby players shows that the median non-renal losses of the cyclists was over a litre greater than the median value for the wheelchair rugby players. When the non-renal losses are expressed relative to body mass or to TBW the values are lower for the wheelchair rugby players, than those seen in either able-bodied swimmers or cyclists (Leiper and Maughan, 2004; Leiper et al., 2001). Expressed relative to both body mass and TBW the non-renal losses are smaller in the wheelchair rugby players than reported for sedentary able body participants (Leiper and Maughan, 2004; Leiper et al., 2001). The values seen for the wheelchair rugby players are similar to values seen in elderly participants (over 75 years old) (Leiper et al., 2005). The low values seen in the elderly were attributed to an age related decrease in transdermal and respiratory loss and a decreased sweat production (Leiper et al., 2005). It is likely that the sweat loss in the elderly was greater than the rate of sweat loss in some of the wheelchair rugby players. Therefore, the low non-renal losses seen for the wheelchair rugby players in the present study may represent an inability to produce sweat on the insensate skin. Indicating that similar water turnover rates between able-bodied participants and those with a spinal cord injury is due to a larger fluid intake amongst those with a spinal cord injury. If the water turnover rate was due to a large fluid intake then it would be expected that urine volumes would be high.

Urine Volumes

Median daily urinary volume for the wheelchair rugby players is higher than the mean urinary loses seen by Leiper and Maughan (2004) for active swimmers (0.92±0.4 l.d^{-1}) but lower than the median values seen by Leiper et al. (2001) in trained cyclists. Again when expressed relative to body mass the median daily urinary volume of the wheelchair rugby players is higher than values previously reported for able-bodied swimmers (Leiper et al., 2001) but lower than values seen in trained cyclists (Leiper and Maughan 2004). As already mentioned there may be a large volume of fluid drunk

during exercise by this group of individuals in an attempt to attenuate the thermal strain (Price and Campbell, 2003). In cool environments the metabolic heat produced from upper body exercise is not great enough to induce a thermoregulatory strain. When exercising in the heat, individuals with a spinal cord injury gain heat from the environment. As they are unable to thermoregulate efficiently and therefore there is an increased thermoregulatory strain amongst this group (Petrofsky, 1992). Therefore under the conditions of the study it is possible that fluid was consumed in an attempt to reduce the thermoregulatory strain. Also it is possible that the raised levels of activity (two -three matches per day) could increase energy intake (food) and water intake. This occurs because there is a relationship between energy intake and water intake. Food intake would increase to meet the energy demands of exercise, and with larger volumes of food in the gastrointestinal tract fluid intake may be enhanced (Leiper and Maughan, 2004).

A significant aspect of health care for individuals with a spinal cord injury and an indwelling catheter is the constant consumption of sufficient quantities of fluid to promote urinary output and minimise the risk of urinary tract infection (Sand et al., 1973). Although there is no clear evidence that increasing fluid consumption above euhydration levels reduces the risk of urinary tract infections compared to euhydration (Manz and Wentz, 2005).

It is possible that fluid intakes were elevated in this study due to the warm environmental conditions throughout the period of the study. As the participants felt hot because of the climate they increased their fluid consumption to try to feel cooler. This may have been a factor in the non-significant difference between match days and non-match days with respect to water turnover rate or 24 hour urine volume.

24 hour urinary electrolyte losses

24 hour urine collection and analysis for electrolytes is believed to be one of the best methods for determining electrolyte intake in free living individuals (Schachter et al., 1980). Intakes can be determined in healthy individuals as sodium intakes match losses. If large sweat losses occur then this may affect the results due to sodium losses in the sweat. This is due to an underreporting and errors associated with dietary analysis of intakes. Previous studies have shown a good correlation between the analysis of sodium intake through dietary analysis of weighed food intakes and urinary excretion analysis for sodium and potassium (Schachter et al., 1980). The values for 24 hour urinary sodium excretion by the wheelchair rugby players' are slightly higher than those previously reported for healthy

males in New Zealand (177 -184 mmol.d^{-1}) (Schachter et al., 1980), but lower than values reported for a group of individuals with a slightly elevated blood pressure who excreted 238.4±82.2 mmol.d^{-1} (Martin et al., 1990). Although these values are higher than reported in other studies where 24 hour urinary sodium losses have been reported as 122±12 mmol.d^{-1} and 113(48) mmol.d^{-1} (Leiba et al., 2005; Caggiula et al., 1985). The higher values seen in the rugby players may be related to the fact that some players consumed sodium citrate as a supplement on some of the match days. As 24 urinary sodium excretion is correlated to intake and in the majority of this subject group it is assumed that sweat sodium losses will be very small it is possible to estimate their sodium and salt intakes. The results from this study suggest that the wheelchair rugby players consumed 11.2±5.8 g.d^{-1} of salt; this is assuming that all sodium losses are in the form of NaCl (salt) but this is not the case and therefore this value is probably an overestimate. This amount of salt intake is well above the government recommended salt intake of 6 g.d^{-1} (Food Standards Agency, 2006). Even though it could be argued that salt intakes greater than 6 g may be required in athletes with high sweat sodium losses, but generally, in this group sweat sodium losses will be minimal. There is however, an impaired ability to produce dilute urine even when large volumes of urine are being produced (Wall et al., 1993). If large amounts of sodium are being lost in the urine, it is possible that individuals with a spinal cord injury require a large amount of sodium in their diet.

Although urine osmolality was not significantly correlated with urine sodium concentration the p value was 0.068, this is close to significance and may reflect a low participant number. As urine sodium concentrations (160 mmol.l^{-1}) and urine osmolalities (1000 mosmol.kg^{-1}) had a wide range which will have affected the statistical analysis. Although sodium is present in the largest quantities in the urine other electrolytes are present (Siener and Hesse, 2002). The main component of urine is water; this will therefore have an effect on the urine osmolality. These other constituents of urine will all affect osmolality. This is because urine osmolality is determined by solute particle concentrations (Armstrong et al 1998).

Effect of Sodium Citrate

It can be seen from Figure 6.3.6. the higher urinary sodium losses came on the days when sodium citrate was ingested. Only 3 of the 11 players had a lower 24 hour sodium excretion in the days when their diet was being supplemented by sodium citrate. The variability in sodium loss means that the dietary intake of sodium was probably greater than the volume ingested with supplementation and therefore diet is likely to have a

greater effect on 24 hour sodium losses. As discussed in the General Methods Chapter dietary intake was not controlled as a descriptive account of normal habits was required. The higher 24 hour urine volumes and larger water turnover rates with supplementation indicate that on these days the players consumed more fluids.

6.5. Conclusions

There was no significant difference between the match days and non-match days with respect to 24 hour urine volume or water turnover rate. However, these two variables were high considering the non-renal losses of this group, suggesting high fluid intakes on each day of the study. This may have been due to the warm environmental conditions and a study in a cooler climate may be beneficial.

The results of the present study indicate that total body water is related to playing classification in wheelchair rugby players. This reflects the greater lean body mass present in the higher level classifications due to their ability to train and utilise a greater percent of their muscle mass than those lower classification players who have lost control of a greater proportion of their bodies. Non-renal losses in some members of the squad were very low and this probably reflects the inability to produce sweat below the lesion.

Chapter 7: Water Turnover Rate of Individuals with a Spinal Cord Injury.

7.1. INTRODUCTION

As discussed in Chapter 2: Literature Review following a spinal cord injury (SCI) there are several physiological changes, which affect the cardiovascular, hormonal and thermoregulatory responses of an individual with a spinal cord injury (Theisen and Vanderlandwijck, 2002). Some of these changes result in an altered urinary excretion pattern compared to the pattern seen amongst a healthy able-bodied population (Kilinic et al., 1999). The diurnal pattern for urine volume seen in able-bodied participants has been shown to be absent in those with a spinal cord injury (Kilinic et al., 1999). If a constant urine volume is produced throughout 24 hours then this may affect fluid intakes. This may mean the water turnover rates of individuals with a spinal cord injury and healthy able-bodied individuals are different. Previous research has shown that hospitalised patients who have had a spinal cord injury for at least thee months had a lower total body water than healthy able-bodied participants, which was believed to reflect a decrease in muscle mass (Cardús et al., 1984). However, the total body water of those with a spinal cord injury after they have been discharged from hospital is unknown.

One of the thermoregulatory impairments that occur following a spinal cord injury is the inability to sweat below the level of the lesion (Randell et al., 1966). This is due to a lack of innervation of the sweat glands to produce sweat. The reduced capacity to sweat could affect water turnover rates by reducing non-renal losses due to the decreased sweat loss and secondly via higher fluid intakes than able-bodied individuals in an attempt to attenuate any increase in core temperature which cannot be attenuated via the normal methods of sweat evaporation or blood redistribution to cool the body. This theory is based on the findings of Price and Campbell (2003) who found individuals with tetraplegia drank more fluid during exercise tests than the able-bodied group despite lower sweat losses amongst those with a spinal cord injury. However, the effect of a spinal cord injury on water turnover rates during normal daily living is unknown.

In the previous Chapter the total body water and water turnover rates of a group of tetraplegics was researched. The pathophysiology of tetraplegics is different to those who have a lower level spinal cord injury. Further to this the participants in the previous study were all taking part in a competitive tournament at the time of the study and were living away from their normal homes. Also, the environmental conditions in the previous Chapter may have had an effect on the drinking behaviour of the participants. Therefore this study was undertaken to determine the total body water

and water turnover rate of those with paraplegia who were living in their own homes, with cooler climatic conditions and undertaking their normal activities. Additionally as previous research has shown that urine volume did not alter between day and night for individuals with spinal cord injury participants (Kilinic et al., 1999). In the present study, participants were asked to record the time and volume at each urination/ emptying of their catheter bag. By collecting individual samples from each urination/ emptying their catheter bag a better understanding of the state of hydration status throughout the 24 hours can be ascertained.

Therefore the aim of this study was to provide a descriptive pattern of fluid balance amongst those with a spinal cord injury in a cool climate and who were not involved in competitive sport. It also aims to provide some information on the pattern of urine excretion in those with a spinal cord injury.

7.2. Method

Severn (5 male and 2 female) adult participants volunteered to participate in the study. Four of the participants (2 male and 2 female) were British international wheelchair basketball players and the other three participants were recreationally active with handcycling. Full subject characteristics are shown in Table 7.2.1. All participants provided written informed consent before commencing the study. All participants completed a health screening questionnaire and self reported in writing and verbally that they were clear from urinary tract infections during the period of the study. All participants had a spinal cord injury.

Table 7.2.1: Individual Characteristics for the Participants Including Gender, Body Mass (kg), Age (y), Level of Lesion, Time since Injury (y)

Subject	Gender	Body mass	Age	Lesion level	Time since injury
		(kg)	(y)		(y)
1	Male	79.4	27	T4 complete	3
2	Male	72.0	53	T7 incomplete	14
3	Male	79.8	29	T8 complete	11
4	Male	82.4	32	L1 incomplete	17
5	Female	62.1	43	T4/T5 complete	20
6	Female	74.1	37	T8 incomplete	22
7	Male	78.9	45	T5/6 incomplete	26
Mean		75.5	38		16
Standard Deviation		6.9	9		8

Before retiring to bed on Day 0, participants emptied their bladders/catheter bags, into a measuring jug, measuring and recording the volume of the urine passed. Participants then retained a urine sample of approximately 20 ml. This sample was used to determine baseline levels of deuterium oxide.

The participants then consumed mean ± SD 9.8653±0.4859 g (99.9 atom%, Sigma, London, U.K.) of deuterium oxide, making a note of the time. To ensure that all the deuterium was consumed, participants were asked to rinse the container several times and to drink the fluid with each rinse.

After consuming the deuterium oxide, each time a subject needed to empty their bladder/catheter bags they noted the time, measured the urine volume, and retained a sample of approximately 20 ml. During the study participants continued to urinate at time points, consistent with their typical urination patterns. During the period of the

study participants were free to consume their normal diet and continue with their normal activities. Dietary and physical activity controls were not put in place since a descriptive pattern of the daily habits was required, as discussed later, in the General Discussion Chapter.

At the end of the study, all urine samples were collected and analysed for sodium and potassium concentration (flame photometry, Corning 410, New York, USA), chloride concentration (coulometric titration, Jenway PCLM 3, Essex, UK) and osmolality via freezing point depression (Gonotec Osmomat 030, YSI, Farnborough, UK).

Participants were asked to empty their bladders/ catheter bags at the same time each morning in order to complete a full 24 hour urine collection. This sample was used to determine deuterium concentration by infra red spectrometry (Miran-1a, The Foxboro Company, Connecticut, USA). The methods for determining deuterium concentration are described in Chapter 2; and are based on the methods described by Lukaski and Johnsonn (1985). The first urine sample produced by participants following the ingestion of deuterium oxide was used to determine total body water. Samples produced to complete the 24 hour urine collection were used to calculate water turnover rate. Non-renal losses were calculated by subtracting the 24 hour urinary volume from the water turnover rate of each day.

Statistical Analysis

Data were analysed for normality of distribution using a Shapiro Wilk test. Data that was normally distributed is presented as mean±SD, and when data were not normally distributed, results are provided as median (range). Correlations for non-parametric data were determined using a spearman's rho correlation. Significance was set at a level of $p<0.05$.

7.3. Results

As shown in Table 7.3.1., median (range) total body water was 33.5 (24.8-45.0) litres. Expressed relative to body mass, this was 45.2 (34.9-58.0) %. Absolute values for water turnover rate were median (range) 3.1 (1.4-8.4) litres, when expressed relative to body mass and total body water, water turnover rate equated to 44 (17-125) $ml.kg.d^{-1}$, and 9.4 (3.3-21.2) %. Absolute water turnover rates were significantly correlated to non-renal losses ($p<0.001$, $r^2=0.607$).

Non-renal losses were 1.2 (0.2-3.8) $l.d^{-1}$ and expressed relative to body mass and relative to total body water, were 11.3 (2.3-47.4) $ml.kg.d^{-1}$ and 2.3 (0.6-12.1) %.

Table 7.3.1: Median, Minimum and Maximum Values For Absolute Total Body Water (TBW) (l), TBW Relative to Body Mass ($ml.kg.d^{-1}$), Absolute Water Turnover Rate (WTR) (l), Water Turnover Rate Relative to Body Mass ($ml.kg.d^{-1}$), Water Turnover Rate Relative to Body Mass (%), Absolute Non-Renal Losses (l), Non-Renal Losses Relative to Body Mass ($ml.kg.d^{-1}$), Non-Renal Losses Relative to Total Body Water (%).

	TBW	TBW%BM	WTR	WTR:BM	WTR%TBW	Non-renal	NR:BM	NR% W
	(l)	(%)	(l)	($ml.kg.d^{-1}$)	(%)	($l.d^{-1}$)	($ml.kg.d^{-1}$)	(%
Median	33.5	45.2	3.1	33.5	9.4	1.2	11.3	2.3
Min	24.8	39.7	1.4	7.7	3.3	0.2	2.3	0.(
Max	45.0	58.0	8.4	83.5	21.2	3.8	47.4	12.

Median 24 hour urinary volume was 2.1 (0.9-6.2) $l.d^{-1}$. 24 hour urine volume was not correlated to water turnover rate ($p=0.086$, $r^2= 0.128$) or to non-renal losses ($p=0.877$, $r^2= 0.001$). Urine volume was significantly correlated to sodium loss (g) ($p<0.001$, $r^2=0.378$). The median (range) urine volume produced between 08:00 and 19:59 was 1.45 (0.23-3.88) litres and the median (range) urine volume produced between the hours of 20:00 to 07:59 was 1.40 (0.25-3.03) litres. There was not a significant difference between the urine volumes produced during the day and the volumes produced at night ($p>0.05$). The median (range) urine volume produced between 08:00 and 21:59 was 1.71 (0.5-4.26) litres, but the median (range) urine volume produced between 22:00 and 07:59 was 0.93 (0.07-3.11) litres. This was a significant difference between night and day ($p=0.016$). When the urinary output rate was calculated for 08:00 and 21:59 it was 122 (32-304) $ml.min^{-1}$ and the urinary output rate between the hours of 22:00 and 07:59 was 93 (7-311) $ml.min^{-1}$. This was not a significant difference ($p=0.898$).

Median (range) urinary sodium concentration was 26 (15-167) $mmol.l^{-1}$, meaning that 24 hour urinary sodium losses were 3.0 (0.4-7.8) $g.d^{-1}$. Urinary sodium concentration was not significantly correlated with urine osmolality ($p=0.115$, $r^2= 0.013$).

As shown in Table 7.3.2., median (range) urinary potassium concentration was 47 (13-138) $mmol.l^{-1}$ and urinary chloride concentration was 43 (8-164) $mmol.l^{-1}$. Urine chloride concentration was significantly correlated to urine sodium concentration ($p<0.010$, $r^2=0.080$).

Table 7.3.2. Median, Minimum and Maximum Values for Urinary Sodium Concentration ($mmol.l^{-1}$), 24 Hour Sodium Loss (g), Urinary Potassium Concentration ($mmol.l^{-1}$) and Urinary Chloride Concentration ($mmol.l^{-1}$)

	Sodium concentration	24 hour urinary sodium loss	Potassium concentration	Chloride concentration
	($mmol.l^{-1}$)	(g)	($mmol.l^{-1}$)	($mmol.l^{-1}$)
Median	26	3.0	47	43
Minimum	15	1.1	13	8
Maximum	167	7.8	138	164

Median (range) urine osmolality was 295(118-934) $mosmol.kg^{-1}$ and for the waking sample, median (range) urine osmolality was 336 (134-703) $mosmol.kg^{-1}$. These values are shown in Table 6.3.3. There was a significant negative correlation between urine osmolality and urine volume ($p<0.01$, $r^2=0.253$).

Table 7.3.3: Median, Minimum and Maximum Values with Respect to Urine Osmolality (mosmol.kg^{-1}), Osmolality of the First Pass of Each Morning (mosmol.kg^{-1}) and 24 Hour Urine Volume (l).

	Urine Osmolality	Urine Osmolality of the first sample each day	Urine Volume
	(mosmol.kg^{-1})	(mosmol.kg^{-1})	(l)
Median	295	336	2.1
Minimum	118	134	0.9
Maximum	934	703	6.2

7.4. DISCUSSION

Total body water

The absolute total body water for this group was lower than previously reported for healthy, sedentary, able-bodied males (Battistini et al., 1994). Even taking into account the female participants values in this study the values were still lower than mean values reported for a group of male and female participants (5 males and 4 females) (Schoeller et al., 1982). When expressed relative to body mass, the values are lower than those reported by Leiper et al. (2001) for endurance cyclists and sedentary male control participants. This indicates that there is a lower lean mass amongst those with a SCI than findings discussing able-bodied, male, participants. This supports findings by Kocina (1997). Who concluded in a review of the literature that even physically active individuals with a spinal cord injury have a higher fat mass relative to body mass (16-24 % for men and 24-32% for females). These values are higher than the values reported in able-bodied participants. Individuals with a spinal cord injury who are sedentary, have an even greater percent of body mass which is fat (> 25 % for male spinal cord injured, and > 32 % for female's with a spinal cord injury) (Kocina, 1997). This increase in relative fat mass is associated with a decrease in total body water. The decrease in body water was approximately 15% for a group of paraplegics and tetraplegics who had been injured for at least three months compared to healthy able-bodied participants (Cardús et al., 1984). The results of this study seem to show a lower total body water when compared to values previously

reported for able-bodied participants. This is illustrated when comparing values from spinal cord injured participants in this study with the values seen by Schloerb and colleagues (1950) where relative to body mass, total body water was 61.8 % for 17 healthy, able-bodied, males aged 18-29 years and whose mean body mass was 71.8 kg. Amongst 11 female participants of the same age with a mean body mass of 58.2 kg, relative total body water was 51.9 %. The median total body water relative to body mass in this study is 45.2 %. This is 27 % lower than the values seen in able-bodied male participants and 13 % lower than the values for healthy able-bodied, female participants. Research on able-bodied participants has demonstrated that females have lower total body water relative to body mass compared to male participants (Schloerb et al. 1950). This lower relative total body water reflects a greater % body fat in female participants, due to the lower water content of fat mass. The total body water relative to body mass is lower than found in Chapter 6, which is likely due to the inclusion of female participants.

The difference in total body water between participants in this study and able-bodied participants of Schloerb et al. (1950) is greater than the 10-15 % difference recorded by Cardús et al. (1984). This greater difference may be reflective of the longer period since injury for participants taking part in the present study. As time since injury increases so does the loss of muscle mass (Castro et al. 1999). When comparing the results of participants in this study to those with a spinal cord injury in the previous study of Cardús and colleagues (1984), there is likely to be a decrease in relative lean mass. Although, it is acknowledgedtotal body water is dependent on many factors and can vary amongst any population. The lower total body water relative to body mass seen in individuals with a spinal cord injury has been attributed to a decrease in the intracellular fluid volume. This decrease in intracellular volume has been attributed to a loss of cell mass, a result of muscle atrophy occuring below the level of the lesion (Cardús et al., 1984).

Water turnover rate

The water turnover rate of participants with spinal cord injuries [3.1 (1.4-8.4) $l.d^{-1}$] was similar to values previously reported amongst able-bodied participants including cyclists, swimmers and sedentary participants reported by Leiper and Maughan (2004) and Leiper et al. (2001). Water turnover rates for these groups ranged between 2.1-4.9 $l.d^{-1}$. When expressed relative to body mass, the results of the present study [33.5 (7.7-83.5) $ml.kg.d^{-1}$] are generally lower than for healthy able-bodied participants in a study by Fusch et al. (1996) (45±7 $ml.kg.d^{-1}$). The participants in the study by Fusch et al. (1996) had similar

characteristics to this study in that it used 11 males and 2 females aged 30±5 years and a mean body mass of 73.5±10.3 kg. Despite the trend for lower water turnover rates in the present study, it is evident that when considering the range of values amongst those with a spinal cord injury, that some of the participants had a higher water turnover than was reported amongst able-bodied participants (Fusch et al., 1998). The highest values for water turnover rates amongst those with a spinal cord lesion is only slightly lower than values reported for able-bodied American football varsity players who were training twice a day in the heat (19.3-25.6°C) reported by Stofan et al. (2007) of 88±13 ml.kg.d^{-1}. There is a large variation in water turnover rates in this study, which range from values lower than those for sedentary able-bodied participants to values which are nearly as high as those for athletes during a period of heavy training in the heat. This demonstrates just how varied water turnover rates can be. Amongst the participants in this study, some of the variability in water turnover rate will be due to the level of the lesion and the effect that has on sweat losses. Secondly, the activity levels of the individuals in this study were not controlled for and differences in water turnover rate will be affected due to differences in sweat losses, differences in respiratory water losses, and metabolic water production due to exercise. The highest value of water turnover rates amongst individuals with a spinal cord injury likely reflects the high fluid intakes consumed by some of the participants in this study.

24 hour urine losses

Since non-renal losses were low, it is expected that 24 hour urinary losses will be higher than seen amongst able-bodied participants. 24 hour urinary losses were higher than values reported for active and sedentary able-bodied participants (Leiper and Maughan, 2004, Leiper et al., 2001). In the present study, one subject urinated more than 6 litres per day for each day of the study. This indicates that fluid intakes amongst individuals with a spinal cord injury are similar, or higher, to the intakes reported in healthy, able-bodied, individuals. However, non-renal losses were lower amongst participants with a spinal cord injury than those previously reported for able-bodied participants (Leiper et al., 2001; Leiper and Maughan, 2004). Water must be lost via some route, and it is excreted as urine. The large variation in 24 hour urine volumes observed in this study tend to suggest that the high variability for group with respect to water turnover rate was due to variations in fluid consumption. Despite the involvement of participants with lower lesion levels, and cooler conditions, 24 hour urine volumes were higher than those seen for the wheelchair rugby players in the previous Chapter. This may reflect a difference in attitude towards fluid intake amongst the participant groups (British versus Canadian). It may also represent a greater access to fluid amongst the

participants in this study since participants were at home and they had a larger degree of freedom when compared to the wheelchair rugby players for whom the only drinks available were those provided to them by team managers and support staff.

Non-renal losses

Non-renal losses in this study were lower compared to values previously reported in able-bodied participants (Leiper and Maughan, 2004). This probably represents the lack of sweating below the level of the lesion in individuals with a spinal cord injury. The impact that lesion level can have on non-renal losses is demondtarted by the variation of non-renal losses between participants in this study, with differences covering a range of 3.6 litres. Non-renal losses were greater than observed in Chapter 6, despite the cooler conditions and the lower activity levels of the participants in this Chapter. This may be due to the lower lesion levels of participants in this Chapter, providing them with a greater area for sweat loss. Lesion level affects sweat losses, but so too does activity level, training status and genetic factors. While it is important to consider non-renal contributions to sweat loss, one must also acknowledge the impact of non-renal losses such as respiration and faecal losses; but it is not possible to tell from the methods used in this study which route the non-renal losses occurred. Physical activity levels were not strictly controlled in this Chapter, but participants did confirm verbally that they had not undertaken any exercise during the period of the study. Sweat losses due to physical activity would have contributed to non-renal losses and thus water turnover rates.

Fluid intakes in this group may be high in an attempt to attenuate any increases in core temperature that cannot be controlled for by the usual sweat or blood redistribution responses (Price and Campbell, 2003). However, temperatures during this study were not particularly hot, and since metabolic heat production for upper body exercise being performed by persons with an SCI is likely to be low due to the small muscle mass that is able to produce work, increases in core temperature are unlikely to be big. Indeed, Price and Campbell, (1999) report core temperature increases of 0.6±0.3 ^{0}C during arm cranking exercise in cool conditions (21.5±1.7^{0}C and 49.0±7.8 % relative humidity) (Price and Campbell, 1999). Additionally, the consumption of large volumes of fluid may be a habit adopted by individuals with a spinal cord injury in attempt to decrease the risk of urinary tract infections. Although all participants were clear from urinary tract infections at the time of the study, it was known via conversations with the participants that some had previously suffered from urinary tract infections. Despite the

fact that there is no existing evidence which supports the notion that increasing fluid intake above levels which maintain euhydration decreases the risk of urinary tract infection, high fluid intakes are often recommended for individuals with urinary tract infections (Manz and Wentz, 2005).

Night and Day

The non significant difference between urine volumes produced between 08:00 and 19:59 urine volume produced between the hours of 20:00 to 07:59, supports findings by Kilinic et al. (1999) who found no significant differences between the volume of urine produced between the hours of 08:00 to 22:00 and between 22:00 and 08:00 amongst a spinal cord injured group. The participants in the study by Kilinic et al. (1999) were in a hospital setting at the time of the study, making it easier to monitor and control the times at which they were lying and sitting. In the present study, participants were asked to undertake normal activity patterns and unfortunately no recording of their sleep patterns was taken. As such, there is possibility that during some of the hours which have been classed as night, participants may have been awake and during the hours classed as day they may have been supine. It is possible to get an indication of the time participants went to bed on the first night of the study since they drank the deuterium and noted the time that it was consumed, an act which was intended to be done before retiring to bed. It is also possible to determine the time at which participants rose on the first morning of the study since this time was used as the cut off time for the completion of the 24 hour urine samples, with a urine sample from their catheter bag or bladder being obtained at this time each morning. Due to alterations in daily patterns especially between weekdays and weekends it is impossible to conclude with absolute certainty that these times relate to the actual time participants went to bed or got up in the morning, but it is inferenced these times approximated to 22:00 until 08:00. If these times are used to determine urine volumes at night and during the day, then there is a significant difference between the night and day urine volume. Using these time points means that there is a 4 hour time difference and these volumes need to be corrected. If the urine flow rate ($ml.h^{-1}$) is used, then there is no significant difference between night and day. Reasons for the absence of a diurnal pattern of urinary losses has been linked to alterations in vasopressin responses. Blood measurements have shown that the lack of a diurnal urinary excretion pattern amongst individuals with a spinal cord injury is due to an altered vasopressin response, which is similar during the day and at night (Kilinic et al. 1999). A theory for the lack of diurnal pattern of vasopressin is due to differences in blood redistribution which occurs from

an erect to lying position between day and night. Whilst in an erect position during the day, blood pools in the lower limbs, and at night in the lying position, blood is able to return to circulation thereby increasing blood volume. Thus, there is no increase in vasopressin release. This redistribution is also visible in able-bodied individuals but it is greater in those with a spinal cord injury, having a more pronounced effect on plasma hormone levels (Wall et al., 1993). It should be noted that there were individual variations in the differences between urine output at night and during the day, with some participants showing a similar pattern of urine output to able-bodied patterns.

Urine Osmolality

Urine osmolalities recorded in this study indicate that the participants were euhydrated throughout the study, with the exception of one urine sample, which had an osmolality greater than 700 mosmol.kg^{-1}. The osmolality of the first urination of the day was well below values reported by Shirreffs and Maughan (1998) for euhydrated able-bodied participants. Again, this may reflect a fluid consumption which is greater than is required to maintain euhydration. This is especially for participants who had urine osmolalities which were only slightly greater than 100 mosmol.kg^{-1}, indicative of the most dilute form of urine. The median urine osmolality in this study of 295 mosmol.kg^{-1} is lower than the mean ±SD 556±50 mosmol.kg^{-1} baseline urine osmolality values for 14 tetraplegics (C4-C7 injuries) following an overnight (8-10 hours) fluid restriction (Wall et al., 1993). Possible reasons for the lower urine osmolalities in the present study when compared to findings by Wall et al. (1993) could be that urine osmolalities were slightly elevated in the previous study due to the overnight fast and fluid restriction. Additionally, the freezing point of urine may have been affected by participants' consumption of 10 g of deuterium oxide, having a potential to alter osmolality. Maughan et al. (2004) added 12 g of deuterium to solutions ranging in osmolality from 321 to 628 mosmol.kg^{-1}. The addition of the deuterium resulted in a decrease in osmolality of ~10-15 % (278-558 mosmol.kg^{-1}). However, in this study only 10 g of deuterium was consumed which represents a small proportion (less than 10%) of any urine volume and 0.03 % of the median total body water.

It is evident when considering the range in urinary sodium concentrations, that in some individuals sodium load was low. Urinary sodium concentrations for individuals with a spinal cord injury have previously been reported to be 46±4 mmol.l^{-1} for tetraplegics, and 52±11 mmol.l^{-1} for paraplegics (Claus-Walker et al., 1982). The values seen in the present study were lower than this. However, it is not really possible to compare the two studies

due to dietary changes that have occurred in the twenty five years between the two studies, especially with the high profile government campaign to reduce salt intakes over recent years. This again would reflect a high consumption of water, which needs to be excreted, resulting in dilute urine. These urine concentrations combined, with the urine volumes, indicate that sodium loss per day was 3.0 (0.4-7.8) g. If it is assumed that all of this sodium loss was in the form of sodium chloride (NaCl) then NaCl loss can be roughly calculated to be 7.6 (2.8-19.9) g. This is quite a large range of salt losses, with some individuals excreting less than the government's recommended intake of 6 $g.d^{-1}$ (Food Standard Agency, 2006). The median value is also lower than values reported by Schachter et al. (1980). However, due to the large urine volumes recorded, it is possible that there is a greater need for sodium intake amongst this group since their urinary losses are high when compared to able-bodied individuals. Urinary potassium and urinary chloride concentrations were similar to those previously reported Leiba et al. (2005).

7.5. Conclusions

The low number of participants in this study, when combined with the large differences in subject characteristics (level and type of injury, activity levels and gender), meaning that it is not possible to draw any firm conclusions from this study. However, the results do tend to show a lower total body water both in absolute and relative terms in individuals with a spinal cord lesion when compared to previous data relating to healthy, able-bodied, participants, as previously reported in the literature (Cardús et al., 1984).

The higher non-renal losses seen in this Chapter compared to the last Chapter likely represent the differences in the level of spinal cord injury between the two groups. This is despite the cooler environmental conditions, and restriction of physical activity.

By analysing individual urine samples (samples for each bag emptying/ urination) it was possible to build a pattern of urinary excretion. There appeared to be an altered urinary excretion pattern amongst partciapnts with a spinal cord injury, since the normal diurnal pattern of excretion did not occur. Similarly, the osmolality of the samples produced at night and during the day were not significantly different (Kilinic et al., 1999). However, the low number of subjects in this study may also explain these findings as not being significantly different.

Chapter 8: Body Water Turnover of Participants at the British Transplant Games.

8.1. INTRODUCTION

Individuals with a spinal cord injury are not the only group who may have altered patterns of fluid intake when compared to the general population. It is suggested that kidney transplant recipients are also likely to display unique fluid intake patterns. Prior to kidney transplantation, individuals with end stage renal disease are placed on a strict low salt diet. This diet is rigid and restricts potassium, phosphorus, sodium and fluid intake (Durose et al., 2004). This is because they are in renal failure and therefore the kidney is unable to maintain homeostasis. Yet, generally following kidney transplantation, there are no further restrictions on a recipient's diet (National Kidney Foundation, 2008). Despite this there is no published literature regarding the fluid balance or dietary habits following kidney transplantation.

Individuals with chronic kidney failure are likely to lead an inactive lifestyle and physical performance is decreased (Johansen, 2005). The primary cause of mortality is cardiovascular disease. A major risk factor of coronary vascular disease is a sedentary lifestyle. For kidney transplant recipients, the anti-rejection drugs, steroids and calcineurin inhibitors they are placed on following transplantation increases the risk of cardiovascular disease (Gordon et al., 2005; Jindal and Zawada, 2004). Therefore, kidney transplant recipients are encouraged to return to physical activity following transplantation. Exercise attenuates the risk of coronary vascular disease and also improves graft function (Juskowa et al., 2006), decreases the adverse effects associated with corticosteroids and the deconditioning which occurs during dialysis, and generally improves overall quality of life (Gordon et al., 2005).

The 'British Transplant Games' ('the Games') were established in 1978 with the aim of encouraging transplant recipients to re-engage in physical activity. They now take place on an annual basis at various locations across the UK. Teams of transplant recipients from across the British Isles participate in events over 4 days. Events include running, swimming and archery. Some participants train intensively in preparation for the Games attempting to win and gain a place at the World Transplant Games, whereas others participate for the fun of participating at 'the Games' (Transplant Sport UK, 2008).

There are obviously physiological differences between kidney transplant recipients and healthy individuals, most notably the fact that recipients have only one kidney compared to the two in a healthy person. Whereas a person with a spinal cord injury may require higher

salt intakes than those advised for the general healthy population (as shown by Chapters 6 and 7). There was also the indication that fluid intakes are greater than the able-bodied population amongst the spinal cord injured in Chapters 5, 6 and 7. Kidney transplant recipients by contrast have, at some point, been on a strict low salt diet since they are unable to maintain sodium and fluid homeostasis whilst in renal failure. It may be that kidney transplant recipients have smaller sodium intakes than the general population. Since a single transplanted kidney will grow to a larger size than one in a pair of kidney in healthy participants, and will act in a similar manner to two kidneys. The extent to which kidney transplantation affects the rate of body water turnover is unclear. The restrictive diet that patients with renal problems are placed on prior to a transplant operation may remain with them later in life, but this is not at present clearly established. The answers to both of these questions are of some significance. In view of the drastic changes in diet seen prior to kidney transplantation, there may be some differences in the fluid balance of this population. The aim of the present study was to measure some components of water and salt balance in kidney transplant recipients taking part in the British Transplant Games.

8.2. Method

Seven kidney transplant recipients (5 male and 2 female), competing at the 2007 British Transplant Games in Edinburgh volunteered to participate in the study. One male participant was a member of the British Transplant team and competed in the running events, and 1 female participant was the swimming champion for her age group. The other participants were all competing for fun- 2 male participants competed in fishing, 1 male athlete took part in archery, and 1 male and 1 female participant took part in badminton. Ethical clearance for the study was obtained from the ethical advisory committee at Loughborough University. Prior to participating in the study all volunteers were provided with information relating to the study and had an opportunity to ask the researchers any questions regarding the study. The volunteers then provided written informed consent and completed a health screening questionnaire. Participants were free to withdraw from the study at any time without giving a reason for their withdrawal. Details of the participants in this study are described in Table 8.1.1. The Games took place at the end of July with outdoor temperatures for this period of mean ± SD 15±5 °C relative humidity was 62±7 %.

Before retiring to bed (the evening before the official start of the Games) on Day 0, participants emptied their bladders, measured the volume of urine and retained a sample of

approximately 20 ml. Participants then consumed 10.0716±0.1290 g of deuterium oxide (99.9 atom%; Sigma, London, U.K.) and noted the time. Participants were asked to rinse the bottle containing the deuterium several times and to drink each rinse, thus ensuring all the deuterium was consumed. From this point (Days 1-4) all further urinations were measured for volume, a 20 ml sample retained and the time of pass was noted. On day 5, only the first pass of the day was collected. Each morning (Day 1-5), before breakfast, participants in the study were measured for body mass (0.01 kg) (Adams CE) by the investigator. At this time, participants were asked about their activities for the day and confirmed their activities from the previous day.

All urine samples were analysed for sodium and potassium concentrations using flame photometry (Corning 480, Essex, U.K.), chloride concentration by coulometric titration (Jenway PCLM 3, Essex, UK) and osmolality by freezing point depression (Osmomat 030, Gonotec GmbH, Berlin, Germany).

Samples were analysed for deuterium content by infra red spectrometry (Miran-1a, The Foxboro Company, Connecticut, USA). The urine sample collected prior to the ingestion of deuterium oxide was analysed for deuterium concentration and the value was used to determine baseline levels of deuterium. The first urine sample of Day 1 was used to determine total body water using the methods of Schloerb et al. (1950) as described in the General Methods Chapter of this thesis. The remaining morning urine samples were used to determine the rate of water turnover as described in Chapter 2 of this thesis. Non-renal losses were calculated from the difference between water turnover rate and 24 hour urine volumes.

To ensure a complete 24 hour urine collection, all urine samples were analysed for creatinine concentration by spectrophotometry (Shimadzu UV-Visible recording spectrophotometer UV-160, Kyoto, Japan) following the methods described by Vitro Scient (Hanover, Germany) which are based on the Jaffé (1886) reaction.

Table 8.2.1. Subject Characteristics for Participants in the Study- Gender, Age (Y), Time Since Transplantation (Y And Months), Body Mass on Day 1 (kg), Body Mass on Day 5 (kg).

Subject	Gender	Age (y)	Time since transplantation (y and months)	Body Mass Day 1 (kg)	Body Mass Day 5 (kg)
1	Male	33	4 y 3 months	86.40	86.46
2	Female	45	17 y 6 months	65.56	65.64
3	Male	44	3 y 6 months	109.01	109.75
4	Male	48	7 months	78.34	80.20
5	Female	40	9 y 0 months	62.18	62.27
6	Male	55	20 y 5 months	74.06	75.67
7	Male	52	12 y 0 months	90.97	92.91
Median		45	9 y 0 months	78.34	80.20
Range		(33-55)	(7 months- 20 y 5 months)	(62.18-109.01)	(62.27-109.75)

Statistical analysis

All data was tested for normal distribution by a Shapiro-Wilk test. Data are provided as median (range). Body mass changes were normally distributed and therefore analysed by a one way repeated measures ANOVA with Tukey post hoc tests to determine if their was any significant change in body mass over the period of the study. Urine creatinine values were not normally distributed and so differences in urine creatinine values were determined by Friedman's test. Significance was set at $p<0.05$.

8.3. RESULTS

Median (range) body mass at the start of the study was 78.34 (62.18-109.01) kg, and at the end of the study, body mass was 80.2 (62.27-109.75) kg. This change in body mass was not significant, (p=0.085).

Median (range) TBW for the participants was 43.1 (33.6-67.5) litres, which means that as a percent of body mass TBW was 55.5 (40.7-66.7) %. Values for each subject are shown in Table 8.3.1.

Table 8.3.1. Median (Range) for Body Mass (kg) TBW (l), TBW as a % of BM (%), WTR ($l.d^{-1}$), WTR Relative to Body Mass ($ml.kg.d^{-1}$), WTR as a % of BM (%), Non-Renal Losses ($l.d^{-1}$) and Non-Renal Losses Relative to Body Mass ($ml.kg.d^{-1}$) and Non-Renal Losses as a % of TBW (%).

	TBW	TBW % BM	WTR	WTR:BM	WTR % TBW	Non-renal losses	Non-Renal losses : BM	Non-renal losses % TBW	24 hour Urine Volume
	(l)	(%)	(l)	($ml.kg.d^{-1}$)	(%)	(l)	($ml.kg.d^{-1}$)	(%)	(l)
Median	43.06	55.54	2.38	34.05	5.45	1.13	10.66	2.21	1.35
Min	35.55	40.68	1.16	17.03	3.33	0.38	0.82	0.76	0.67
Max	67.50	66.66	4.59	47.29	9.86	1.90	24.31	3.93	2.59

Median (range) WTR for the participants during the study was 2.4 (1.2-4.6) $l.d^{-1}$. Expressed relative to body mass WTR was 34.05 (17.03-47.29) $ml.kg.d^{-1}$, meaning that 5.5 (3.3-9.9) % of the TBW was turned over each day. The data for individual participants are shown in Table 8.3.1.

The median (range) non-renal losses for the group during the study were 1.1 (0.4-1.9) $l.d^{-1}$. When expressed relative to body mass non-renal losses were 10.7 (0.8-24.3) $ml.kg.d^{-1}$. Therefore the non-renal losses accounted for 2.2 (0.8-3.9) % of the TBW each day. The median (range) for each participant during the period of the study is shown in Table 8.3.1.

Relative to body mass 24 creatinine values were 0.7 (0.1-4.2) $mmol.kg^{-1}d^{-1}$. The difference between days for creatinine values was 0.03 (0.01-0.06) $mmol.kg^{-1}d^{-1}$. Median (range) coefficient of variation for the absolute 24 hour creatinine loss of each subject was 5 (2-9) %.

Between the days of the study; there was no significant difference in the 24 hour creatinine values (p=0.135).

Over the period of the study (4 days), the reported median (range) 24 hour urine output was 1.35 (0.67-2.59) litres. Median (range) values for each participant are shown in Table 8.3.1., and the group median (range) 24 hour urine volumes for each day are shown in Figure 8.3.1.

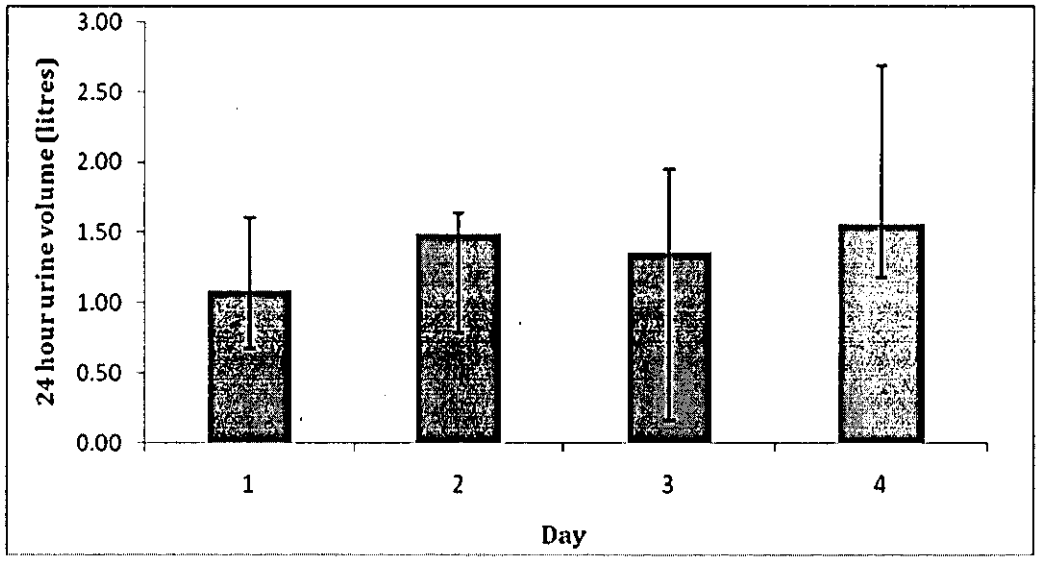


Figure 8.3.1: Median (range) 24 Hour Urine Volumes (l) for Each Day of the Study

Median (range) urine sodium concentration was 100 (62-164) mmol.l^{-1}. The median (range) urinary sodium concentrations for each participant are shown in Table 8.3.2. There was not a significant correlation between urinary sodium concentration and urine volume (p=0.63, r^2= 0.018).

Assuming that all sodium lost in the urine was in the form of sodium chloride (NaCl), it is possible to estimate approximate NaCl losses. Therefore the median (range) 24 hour NaCl loss during the study was 7.4 (2.5-18.0) grams. The median (range) daily sodium chloride data for each participant is shown in Table 8.3.2.

Median (range) urinary potassium concentrations were 64 (15-88) mmol.l^{-1}. Median (range) urinary chloride concentrations were 56 (29-145) mmol.l^{-1}. Median (range) urinary potassium and urinary chloride values for each participant are shown in Table 8.3.2.

All of the urine samples collected from the participants had osmolalities of less than 800 mosmol.kg^{-1}, with the median (range) urine osmolality being 500 (249-708) mosmol.kg^{-1}. The median (range) urine osmolality values for each participant during the study is shown in Table 8.3.2.

Table 8.3.2. Median, minimum and maximum values with respect to 24 hour urine sodium concentration (mmol.l^{-1}), 24 hour sodium chloride loss (g), 24 hour urinary potassium concentration (mmol.l^{-1}), 24 hour urinary chloride concentration (mmol.l^{-1}) and urine osmolality (mosmol.kg^{-1})

	Subject	Sodium	Sodium Chloride	Potassium	Chloride	Osmolality
		(mmol.l^{-1})	(g)	(mmol.l^{-1})	(mmol.l^{-1})	(mosmol.kg^{-1})
Median	Overall	100	7.40	64	56	500
Min	Overall	62	2.50	15	29	249
Max	Overall	164	18.00	88	145	708

8.4. Discussion

Given that body mass did not alter significantly during the period of the study, it can be assumed that TBW did not significantly change during the study period. The absolute TBW values seen in this study are similar to those seen for healthy sedentary participants of similar body mass (Leiper et al., 2001; Leiper and Maughan, 2004; Shimamoto et al., 2003). Yet, the range of values seen in the kidney transplant recipients is greater than those values reported by Leiper et al. (2001). This may reflect the tighter controls on age, gender and activity levels that were imposed by Leiper et al. (2001), whereas the present study included males and females, placing no controls on their age or activity levels. Additionally, two of the participants competed to win and the rest competed for 'fun'. When expressed relative to body mass, there is some overlap in range of values presented by Leiper et al. (2001) and the present study. The general tendency is for the participants with kidney transplantation to have a lower TBW as a percent of body mass than healthy participants. This would suggest that kidney transplant recipients have a lower lean body mass. This lower percentage of lean mass in the kidney transplant recipients likely reflects the fact that 2 of the 7 participants were female and in healthy populations, female participants generally have a lower lean mass than males (Schloerb et al., 1950). Females have a higher relative body fat compared to males and since as fat mass contains less water than the same mass of lean tissue, female

participants in this study will have lowered the values for relative total body water in this participant group (Schloerb et al., 1950). Secondly, renal failure is usually accompanied by a decrease in lean mass (Mitch, 1998), which may remain following transplantation.

The absolute median water turnover rate of this study is slower than values previously reported for athletes and sedentary participants by Leiper et al. (2001) and Shimamoto and Komiya et al. (2003) but it is similar to values seen for healthy sedentary controls reported by Leiper and Maughan (2004). This could indicate a lower fluid intake due to lower water losses. When water turnover rates are expressed relative to body mass, the kidney transplant recipients have a lower daily water turnover rate than mean values previous reported for healthy participants (Leiper and Maughan, 2004; Leiper et al., 2001; Shimamoto and Komiya, 2003). This may reflect a lower fluid intake amongst those with a kidney transplantation due to the fluid restrictions which occur prior to transplantation whilst still on dialysis. This may have a residual effect, influencing their post-surgery habitual fluid intake and lowering water turnover rates. This is further highlighted by the low 24 hour urine volumes observed in some participants.

The wide range of values seen for the kidney transplant recipients probably reflects the differences in activity levels seen between the participants and the difference in the amount of activity that each subject did from day to day. For example, one subject hiked up to the top of Arthur's seat twice one day and then spent the next day fishing.

Collection of urine samples can be inconvenient for participants, leading to a lack of compliance. 24 hour urinary creatinine excretions were relatively constant for the majority of participants and therefore can be used to determine completeness of 24 hour urine collections (Curtis and Fogel, 1970). Curtis and Fogel (1970) study showed that the coefficients of variation for a group of healthy males (n=9) and females (n=3), aged 19-48 who for the 6 days of the study were inpatients on a research ward ranged from 4-8 %. Greenblatt et al. (1976) reported coefficients of variation of 10.5-14.4 % and mean urinary creatinine excretions of 21.4-25.4 $mg.kg.^{-1}$ amongst 8 healthy males aged 23-45 years over a collection period of six consecutive days. The median (range) coefficients of variation in this study were comparable to the values seen by Curtis and Fogel (1980). Further, statistical analysis showed no significant differences for urinary creatinine excretion between days for any of the participants. These factors suggest that the reported 24 hour urine volume of the participants was complete. Generally, creatinine values for the kidney transplant recipients were lower than the quoted reference range

suggested by VitroScient for the methods used in this study. This is possibly due to a lower muscle mass amongst those with a kidney transplant which can occur during dialysis. Daily creatinine losses are directly correlated to the size of the body's creatinine pool (Lykken et al., 1980) and urinary creatinine thus correlates better with muscle mass than body mass (Narrayanan and Appleton, 1980). These low values were similar on each day of the study and may represent the low muscle mass in these participants.

The values for the kidney transplant recipients are towards the lower end of the normal range for urine output. The urine volumes reported in this study are lower than those reported by Leiper et al. (2001) but similar to values reported for sedentary participants and swimmers studied by Leiper and Maughan (2004). These low urine volumes also suggest a lower fluid intake amongst those who have received kidney transplantation than the general healthy population. Despite this, all urine samples in this study had a urine osmolality below 800 mosmol.kg^{-1}. This indicates that all the participants in the study maintained euhydration throughout the period of the study and thus throughout the duration of the British Transplant Games. The median urine osmolality in this study is lower than that found by Shirreffs and Maughan (1998) in a healthy sedentary population. It is possible, due to the monitoring of fluid that occurs after transplantation, that awareness of individual water balance is greater amongst kidney transplant recipients, making them better able to adapt their drinking strategies to maintain euhydration. As 24 hour urine volumes were at the lower end of the normal range and that urine osmolalities were low, solute losses were low for those in individuals with a kidney transplant.

Non-renal losses represent insensible losses, faecal losses, respiratory and sweat losses. The variation in non-renal losses also reflects the differences in activity levels amongst participants with non-renal losses relative to body mass varying from 2.2 ml.kg.d^{-1} to 20.7 ml.kg.d^{-1}. It is not possible to determine which proportion of non-renal losses were due to sweat losses, although sweat losses would increase with higher levels of physical activity. The non-renal losses in this study are similar to those previously reported for healthy sedentary controls (Leiper et al., 2001; Leiper and Maughan, 2004) which is the case even if the values are reported relative to body mass or as a percent of total body water. The variability in non-renal losses probably reflects differences in sweat losses since participants competed in different activities and at different intensities.

The WTR and the 24 hour urine volumes for the kidney transplant recipients were towards the lower end of the range seen in healthy sedentary participants by Leiper and Maughan (2004) but the non-renal losses were similar to sedentary healthy participants. These findings suggest fluid restrictions placed upon the participants during their dialysis may have led to a lower fluid intake following transplantation. Despite this, the participants still consumed enough fluid to maintain euhydration (assuming that the single kidney has the same solute diluting ability of a normal pair of kidneys). This possibly highlights an increased awareness of their fluid requirements.

24 hour urinary sodium values recorded for the kidney transplant recipients are lower than that found by Schachter et al. (1980). The 24 hour urinary sodium chloride values seen in this study cover a wide range from 2.5 grams to 18.0 grams. Salt lost un sweat must be added to this value. The government's target intake for NaCl is less than 6 $g.d^{-1}$ (Food Standards Agency, 2006), but the daily average salt intake of UK males is about 11g with women consuming an average of 8 $g.day^{-1}$ (Henderson et al., 2003). The data from this study suggests that after a kidney transplantation NaCl intakes are lower than those of the general healthy population, but higher than the target NaCl intake set by the government. Although the range of NaCl values seen amongst the kidney transplant recipients means that some individuals with kidney transplantation do consume less NaCl than the government's target. Suggesting that being placed on a low salt diet prior to kidney transplantation results in slightly decreased salt intake after transplantation. However, the diet of the participants throughout the Games may not be reflective of true dietary habits since all food was eaten at a cafeteria, and control over salt intake may have been altered, changing normal dietary habits may have changed.

Limitations

With only 7 participants able to participate, and a large inter-individual variability, larger participant numbers are necessary before for any definite conclusions concerning water balance and salt intake can be made. This study also covers a wide age range, however this water turnover rates were similar in a group of elderly people (69-88 years, WTR 1.5-2,2 $l.d^{-1}$) (Leiper et al. 2005) when compared to values for younger sedentary control subjects (17±2 years, WTR 1.7 $l.d^{-1}$) (Leiper and Maughan, 2004). Raman et al. (2004) showed a decrease in water turnover rate with age from 3.81±1.24 $l.d^{-1}$ in 40-49 year old, males compared to 3.55±0.92 $l.d^{-1}$

for males aged 60-69 years. Non-renal losses were also shown to decline with age 1.61± 0.79 $l.d^{-1}$ for the 40-49 year group and 1.00± 0.67 $l.d^{-1}$ for the 60-69 year group. There is also a difference for WTR and non-renal losses with respect to gender. Females have a lower WTR (3.26±0.78 $l.d^{-1}$) than for males (3.81±1.24 $l.d^{-1}$). Non-renal losses were 1.10±0.68 $l.d^{-1}$ for females which is lower than male values (1.61±0.79 $l.d^{-1}$) Raman et al. 2004).

All participants were away from home and by consuming canteen food they had less control over their salt intakes and values in this study may not represent typical values.

8.5. Conclusion

This study gives some indication of the water turnover in athletes following kidney transplantation, but it is acknowledged that behaviour patterns may be altered at an event like the Transplant Games. This makes it difficult to transfer the findings to athletes in training, or to the general kidney transplant population who engage in an active lifestyle.

The results do show that there may be a lower salt (NaCl) intake and a lower fluid intake amongst those with a kidney transplant. Despite lower fluid intakes, participants were euhydrated throughout the study suggesting an awareness of fluid needs. These differences from the general population are probably a result of the restricted diet they followed prior to transplantation. These results are also in contrast to the findings of the spinal cord injured participants (Chapter 6 and 7). Therefore, the appropriateness of the governments' campaign to reduce salt intake may not be applicable to this group.

Chapter 9: General Discussion.

9.1. General Findings

Chapter 5 showed that while the sweat losses differed for those with a spinal cord injury compared to the values for the equivalent able-bodied activities. Conversely, sweat composition - sweat sodium, chloride and potassium concentrations, did not differed from values typically reported able-bodied athletes. Further, fluid intake was greater when related to sweat loss than has been reported during able-bodied sports, resulting in a net mass gain during training. This thesis looked at fluid balance during daily activities in two groups for whom it was assumed that fluid balance may differ from the general population. Chapters 6 and 7 are the first studies to investigate the water turnover rates of individuals with a spinal cord injury. To date, no published research had attempted to describe the salt (NaCl) losses and water turnover rates of a population of kidney transplant recipients. Comparing the data obtained in this thesis with the general guidelines on fluid and salt intakes, based on the healthy, able-bodied population, highlights two groups for whom these guidelines may not be applicable. The British government aims to reduce salt intake (NaCl) to less than 6 $g.d^{-1}$. The British government's advice regarding water intake is to consume 1.2 litres of water per day (Food Standards Agency, 2008). The data collected from disabled athletes in Chapter 5, the results of the studies examining fluid balance of those with a spinal cord injury in Chapters 6 and 7 and those who have received a kidney transplant in Chapter 8 demonstrated that there are certain populations for whom fluid and salt intake guidelines determined by the government are not be applicable.

9.2. Sweat Variation Between Training Sessions And The Validity Of A Single Testing Session

Information regarding sweat losses, both in terms of water loss and salt loss, are important for athletes' performances and health. Not all sweat losses need to be replaced during the training session, as small degrees of hypohydration can be tolerated without a negative effect on performance. It has been shown that ironman triathletes are able to tolerate body mass losses of 3 % without negative effects on performance (Laursen et al., 2006) and in endurance events such as running drinking to replace sweat losses may actually impair performance due to the higher body mass that has to be carried compared to a slight level of hypohydration (<2 % body mass) (Noakes, 2007). However, the losses do need to be replaced prior to the commencement of the following training session; otherwise an accumulative effect of

hypohydration will occur (Sawka et al., 2007). If an inappropriate replacement strategy is employed, then subsequent performance may be compromised due to dehydration or hyperhydration (Sawka et al., 2001; Hargreaves and Hawley, 2003). In addition, an excessive loss of sodium in sweat may contribute to heat or exercise induced muscle cramps (Bergeron, 2003; Eichner, 2007). Factors such as diet, fitness level, genetics, environmental temperature, clothing worn, and the duration and intensity of the training session can affect sweat volume and electrolyte composition (Burke, 1997; Greenhaff and Clough, 1989; Maughan, 2001). These factors will vary from athlete to athlete, with Shirreffs and colleagues (2007) suggesting that general guidelines regarding fluid intake during training and during recovery periods have little benefit to athlete . Many studies and sporting organisations use a single training session to determine athletes' sweat volumes and sweat electrolyte losses, determining the nutritional strategies for athletes from this data. However, factors which have the potential to affect sweat losses between individuals can also affect an individual over time, thus varying across training sessions.

Currently, there is a vast quantity of existing literature regarding electrolyte composition and sweat loss volumes specific to many different groups of athletes; (football Maughan et al., 2005; Shirreffs et al., 2005, American Football, Stofan et al., 2005). This information has been used both to devise individualised hydration strategies for the participants involved in the studies, and used as baseline guides for others within the sport to determine their nutritional needs. To date, no published data examines the reliability of such one off testing. Chapter 4 illustrated that despite numerous factors affecting the volume of sweat loss and sweat electrolyte composition, there were no significant differences between four training sessions with respect to the volume of sweat loss, sweat sodium concentration, sweat chloride concentration or sweat potassium concentration. Nor was fluid intake or pre-training hydration status significantly different between sessions. This non-significant difference observed for the group may reflect the subject numbers that were used and the large variation between participants. However, when individual data is examined, there is little difference between the training sessions with respect to the volume of sweat lost, sweat electrolyte concentrations, or volume of fluid consumed between each training session. Therefore, it is concluded that the use of testing over one session is a practical and valid measure of sweat volume losses and sweat electrolyte losses. The wide variation in sweat losses between the participants shows the importance of individualising hydration strategies for athletes.

The majority of participants who took part in this study were classified as recreationally active (n=40) so it is doubtful whether these findings can be applied to elite sportsmen and women who have multiple training sessions in a single day at high intensities and long durations. Some participants (n = 5) involved in the study were international athletes and their sweat loss variability was similar to the rest of the participants. Further investigation is needed regarding the effects of multiple training sessions per day on the sweat volume and composition in elite athletes, especially when competing in the heat where sweat losses would be high. In the context of this thesis the sweat losses were similar for the participants in this study and for those in Chapter 5. Chapter 4 showed that the use of a single testing session is a reliable indicator of sweat losses which is relevant when analysing the findings in Chapter 5.

9.3. Sweat Losses Of Athletes With A Disability

One of the most variable routes of water loss is via sweating. There is substantial published literature regarding the sweat loss of able-bodied athletes, but little data exists regarding the sweat loss for athletes with a disability during training. Athletics and wheelchair basketball were both part of the 1960 Paralympics in Rome, and provide a contrast between wheelchair and non-wheelchair activities. Wheelchair rugby was first designed in the 1970s and is played by those with a more severe disability (tetraplegia) than would play wheelchair basketball (paraplegia, amputation). For disabled athletes without a spinal cord injury, sweat rates were lower than reported in the literature on able-bodied counterparts (Burke and Hawley, 1997). In wheelchair sport, upper body exercise results in lower absolute workloads when compared to sports using the whole body, or the larger muscle groups of the legs (Pivarnik et al., 1988; Pimentel et al., 1984). Therefore, it may be more applicable to include a group undertaking whole-body exercise when conducting research with disabled athletes involved in wheelchair and non-wheelchair events. The group of international sprint athletes that were tested in Chapter 5 of this thesis undertook whole body exercise but their sweat rates were lower than reported in the literature (Burke and Hawley, 1997). The lower sweat rates observed amongst the track athletes in this thesis likely represent the lower total workload that they performed. Indeed, it is noted that their training session was interspersed with long rest/ recovery periods, and large quantities of time were taken up with technical aspects of sprinting.

Although heart rate measures were taken, it is difficult to determine the relative intensities at which the wheelchair athletes were training. Heart rate gives an indication of the relative exercise intensity, but for individuals with a spinal cord injury, there is an altered heart rate and stroke volume response making interpretations of heart rate less reliable for this group (Petrofsky, 2001). Further, two of the athletes were amputees who were weighed before and after training while wearing their prosthesis. This needs to be taken into account because their whole body sweat rates are likely to be lower due to a smaller surface area for sweat production and evaporation. Body mass was measured with the athletes wearing their prosthesis due to time constraints of removing their training clothing and prosthetic in order to be weighed without the artificial limb. Although participants were weighed in training clothing before and after training, the volume of sweat trapped in the clothing is likely to be small. Cheuvront and colleagues (2002) found amongst female runners in 20-30 °C, sweat losses amounted to 1.49-3.88 litres. Of this, 0.05-0.66 kg of sweat was trapped in their clothing (t-shirt or racing singlet, shorts, socks and shoes). In cooler conditions, sweat losses were lower, 1.31-2.94 litres of which 0.03-0.46 kg of sweat was trapped in the clothing. Athletes without spinal cord lesions were weighed in minimal clothing (underwear). Only athletes with spinal cord injuries wore their full training kit when body mass was measured. Due to the nature of their injuries it is unlikely that any sweat was produced underneath the clothing due to their impaired ability to sweat below the level of the lesion, and the amount of sweat trapped in the clothing is unlikely to be larger than values seen by Cheuvront et al. (2002) who concluded that calculating sweat loss by body mass changes by accounting for fluid intake and urinary loss provided the total sweat loss to within 0.2 %.

The mean sweat sodium concentrations were 53±17 mmol.l^{-1} (range 30-80 mmol.l^{-1}) for the wheelchair rugby, wheelchair basketball and athletics teams. This value is within the normal range reported as reference values (20-80 mmol.l^{-1}) and comparable with previous concentrations reported for similar tests done with able-bodied athletes. As discussed in the General Introduction, there is some evidence that there can be an overestimation with the use of sweat patches to determine whole body sweat electrolyte losses when compared to whole body wash down techniques (Shirreffs et al., 2007; Patterson et al., 2000). Which is believed to be between 30-40 % (Shirreffs et al., 2007; Patterson et al., 2000). If the median overestimation value is taken, then sweat sodium concentrations for the wheelchair rugby, wheelchair basketball and athletics squads would be 46 ±12 mmol.l^{-1}.

Again, sweat potassium concentration range was 1.0-9.2 mmol.l^{-1} for the wheelchair rugby, basketball and athletic squads respectively. Some of these values were outside the quoted reference range for sweat potassium concentration (2-8 mmol.l^{-1}), but they are comparable to previous data from able-bodied participants. Maughan and colleagues (2005) found values as low as 1.4 mmol.l^{-1}, and as high as 8.6 mmol.l^{-1}.

Seventeen of the twenty one participants with a spinal cord injury had an increase in body mass following their respective training sessions, comparable to a body mass increase during the training session in one of the fifteen participants who did not have a spinal cord injury. This could be attributed to a lower sweat loss accompanied by a higher fluid intake. This supports previous findings by Price and Campbell., (2003), who found that ad libitum fluid consumption during an exercise test was higher for those with a spinal cord injury than for able-bodied participants, despite lower sweat losses. At present, the reasons for such high fluid volume intakes have not been researched, although Price and Campbell (2003) believed the high volume of fluid consumed was an attempt to alleviate feelings of being too hot. Observations during some of the training sessions tend to support this theory. However, no ratings of perceived thermal stress were recorded during this study and it is impossible to draw any substantial conclusions. It is possible that due to the higher incidence of urinary tract infection amongst those with a spinal cord injury larger volumes of fluid are habitually consumed in an attempt to reduce the risk of suffering an infection (Sand et al., 1983). Three of the four groups tested for this thesis were Paralympic athletes and it assumed they will have received nutritional advice at some point in their careers. They may have been instructed to consume more fluid by their doctor in an attempt to attenuate an increased risk of a urinary tract infection, or from a team nutritionist.

Further research comparing the core temperature of athletes with a disability, especially those with a spinal cord injury, to feelings of thirst and thermal comfort may help to explain the fluid intake patterns amongst this group. It is noted that the traditional method of using rectal temperature as a measure of core temperature of individuals with a spinal cord injury may not be valid. Since Gass and colleagues (1988) found a 0.7°C difference between oesophageal temperature and rectal temperature at the end of exercise in physically trained paraplegic males, this difference was attributed to blood flow distribution. It is suggested that ingestible core temperature sensors would provide valid readings and allow for core temperatures to be measured in the field. It is also true that the traditional calculation for

skin temperature does not accurately reflect the heat storage for individuals with a spinal cord injury due to the differences in heat storage and blood redistribution between individuals who have a spinal cord injury and their able-bodied counterparts.

9.4. Total Body Water And Spinal Cord Injury

Chapters 6 and 7 are the first studies to determine total body water by deuterium oxide dilution in spinal cord injured populations. The results of these Chapters determined that the total body water was lower relative to body mass for individuals with a spinal cord injury than values previously reported for healthy, able-bodied, participants (Schloerb et al., 1950). This was true whether the individuals with the spinal cord injury were physically active or not, and whether the values were compared to values for athletes or sedentary able-bodied participants (Leiper et al., 2001; Leiper and Maughan, 2004; Fusch et al., 1998; Battistini et al., 1994). This finding is supported by the research of Cardús et al. (1984) who found that total body water relative to body mass was approximately 15 % lower in a group of individuals who had acquired a spinal cord lesion at least three months prior to the study, but who were still in the hospital setting, when compared to healthy, able-bodied, participants. This lower total body water has been attributed to a decrease in intracellular water, although due to the nature of the studies undertaken in this thesis intracellular and extracellular water were not measured. The loss of intracellular fluid has been attributed to a loss of muscle cells and a decrease in the volume of water within the cells below the level of the lesion (Cardús et al., 1984; Cardús et al., 1985). Additionally, body fat contains less water than lean tissue and a large relative fat mass will result in lower relative total body water content (Sawka, 1992). Since individuals with a spinal cord lesion generally have a higher percent body fat compared to a healthy able-bodied individual, they are more likely to have a lower total body content relative to body mass (Konica, 1997). Increases in relative body fat to body mass occur because of a decrease in lean tissue, and because of lower levels of activity that are seen in this population (Konica, 1997).

The lower non-renal losses in Chapters 6 and 7 are in line with previous research showing decreased whole bodied sweat losses in those with a spinal cord injury. The calculated non-renal losses were lower for those with a spinal cord injury than values previously reported for sedentary healthy, able-bodied participants (Raman et al., 2004). Although the methods used in this thesis do not make it possible to determine the proportion of non-renal losses which were due to changes in sweat loss. Previous studies using able-bodied participants

have attributed differences in non-renal losses to differences in sweat losses between athletes and sedentary participants (Leiper et al., 2001).

Despite these lower non-renal losses, water turnover rates were similar to values reported for able-bodied participants (Leiper et al., 2001). Therefore, the 24 hour urine volumes were higher amongst those with a spinal cord injury than values reported amongst able-bodied populations. These findings suggest that individuals with a spinal cord injury tend to consume similar or greater volumes of fluid than their able-bodied counterparts. It is not clear as to why this is the case and further research is required in this area to determine the reasons for such volumes of fluid intake. It is suggested that it may be an attempt to attenuate the increased risk of urinary tract infection (Sand et al., 1983). Since urine volumes are high, sodium losses are also high and the dietary sodium requirements of individuals with a spinal cord injury may be different to those of an able-bodied individual upon whom the dietary reference values are based (Food Standards Agency, 2006). This suggests that dietary recommendations specifically for the spinal cord injured community may need to be devised.

9.5. Total Body Water And Kidney Transplantation

A further group for whom fluid intakes may differ from those of the general public is those who have received a kidney transplantation. This is also a group for whom there is little published information regarding dietary habits. In Chapter 8, the total body water of individuals who had received a kidney transplant was determined. Findings were similar to values reported for healthy, able-bodied, sedentary participants (Leiper et al., 2001; Leiper and Maughan, 2004; Shimamoto and Komiya, 2003). Although the participants were participating at the British Transplant Games, some would not be classed as active and could be classified as obese with a BMI of 30. Fat mass contains less water than lean mass and obese individuals would be expected to have a lower total body water relative to body mass (Sawka, 1992).

Non-renal losses covered a wide range amongst this group of participants reflecting the different levels of activity amongst the group. However, the majority of the participants had similar non-renal losses to those reported for healthy sedentary participants or athletes (Leiper et al., 2001).

The data from daily urine creatinine relative to body mass was similar for each day of the study. This proved that full 24 hour urine collections were obtained from the participants and

values calculated for non-renal losses and for 24 hour sodium losses can be considered to be relatively accurate. Findings of low urine osmolality values combined with lower 24 hour urine values suggests that daily urinary solute losses were low. The major solute of the urine is sodium, and this study tends to indicate that following kidney transplantation, individuals tend to continue with a lower salt diet than the general population (Henderson et al., 2003). This could be due to habituation that occurred during time spent on dialysis when they were required to reduce their intake of salt and protein or, it may be a conscious effort to consume a low salt diet, especially when considering studies linking high sodium intakes with increased risk of stroke and other cardiovascular disease. Conversations with individuals at the British Transplant Games, indicate that it was a combination of the two. For some, the habit of eating foods without added salt began whilst on dialysis and following transplantation, individuals become accustomed to the taste of food without salt. This combined with the knowledge that people should cut back on their salt intake, may encourage individuals with a transplant to continue leaving additional salt out of their diets Following their transplantation.

Further research into the dietary intakes of individuals following transplantation and their dietary beliefs would be of interest. Unfortunately in this study, the number of years post transplantation differed greatly between the participants. Further research looking at different time points following a kidney transplant operation and their dietary intake and sodium losses would be of interest. There is some research which has shown lower sweat sodium concentrations in kidney transplant recipients than in healthy participants, but this study placed all participants on a low salt diet containing 80-120 mmol of sodium for at least a week before testing (Woch et al., 1993). The mean ±S.D. of the time since transplantation was 11±3 months, meaning that the participants in the study had not had their transplanted kidney for long and had potentially been on a restricted diet a year before the study (Woch et al., 1993). Further research to determine the sweat sodium losses of this group of individuals after consuming their regular diet may be of interest. In a sport and physical activity context, this information is of importance since the World Transplant Games are to be held on the Gold Coast, Australia in 2009 where temperatures are usually around 5-20 °C and relative humidity is 40-60 % during August. These conditions combined with sporting competitions could result in substantial sweat losses.

9.6 Thesis Limitations

The main limitation of the studies in this thesis is the lack of subject numbers, especially when considering that the studies were attempting to determine factors that can be altered by many different variables amongst a homogenous population. In addition to the low subject numbers, the subject populations were not very homogenous. This was due to the difficulty in recruiting participants with a spinal cord injury and kidney transplantation recipients and factors such as age, gender, fitness level, body mass and time since injury/ transplantation were not controlled. A descriptive understanding of the daily fluid balance of individuals with either a spinal cord injury or kidney transplant was desired, and the studies were designed to have minimal interference with the participants' daily living. Deuterium oxide and its use to measure body water turnover rates allows an understanding of fluid balance, but it does not allow to distinguish between water consumed in the form of liquid or as food. This could have been determined by asking the participants to complete a weighed food intake diary for the period of the study. However, the use of such food diaries has been criticised since participants tend to either misreport their intakes or they change their dietary habits during the period of recording (Hill and Davies, 2001). The underreporting of energy intake is detected by the use of doubly labelled water which provides investigators a method for determining energy balance by determining energy expenditure. Goran et al. (1992) reported a 21 % difference over a 3 day study period between self assessed energy intake by way of a food record and the energy expenditure measured by doubly labelled water in healthy able-bodied participants. Similar findings were found by Martin et al. (1996), who found a 20 % difference between a 7 day weighed recorded food intake and energy expenditure calculated by use of doubly labelled water in healthy, able-bodied, participants. Amongst athletes, the discrepancy between self reported food intake and energy expenditure also exists. Haggarty et al. (1988) studied 4 elite female endurance runners during the 21 days, during which time participants completed a 21 day weighed food intake diary. The difference between food diary analysis and the energy expenditure determined by doubly labelled water was 34 %.

Physical activity was not accounted or measured in detail during the period of the study. This could have been achieved by having participants wear heart rate monitors to continuously measure heart rates, but due to the long duration of the studies (up to a week), there would have been insufficient memory on a heart rate monitor to record this. Participants undertook the study away from the laboratory and physical contact with the investigator occured only at

the start and at the end of the study period, making it impossible to obtain heart rate data, since monitor memory is not large enough to capture data over such an extended period of time . For Chapter 6, participants were all tetraplegics and therefore the heart rate response of this group would have differed from the general population and making the interpretation of findings difficult. Attempts were made to observe the physical activity levels of these participants by recording the number of matches and training sessions they undertook on each day of the study. In Chapter 8, participants self reported their physical activity levels daily to the investigator. As some participants in Chapter 8 were competing in swimming events and the use of heart rate monitors was deemed inappropriate. It is possible to measure energy expenditure with the use of doubly labelled water, but the primary focus of the study was to determine fluid balance, and the additional expense of doubly labelled water did not seem appropriate. In Chapter 7, participants were asked not to undertake any form of physical activity throughout the study period, and they self reported that they had not undertaken any physical activity.

Matched controls were not used throughout this thesis given the difficulties associated with matching participants' characteristics. Matching for body mass would have resulted in differences in lean mass and fat mass (Kocina, 1997). Matching activity levels would also have been difficult due to differences between upper body and lower body exercise (Sawka et al., 1989), a factor which would have been present even if no physical activity took place (general locomotion). Thus true matches could not have been obtained.

All of the studies were field based, which permitted very little control over confounding factors making it extremely difficult to determine if differences were due to disability or transplantation, or other factors. However, due to the nature of the participants required for these studies it was logistically difficult to recruit the numbers required.

9.7. Overall Conclusion

During exercise, athletes with a disability tend to have lower sweat rates than their able-bodied counterparts. This finding is not unique, with previous research reporting lower sweat rates amongst wheelchair basketball players than has been reported for able-bodied basketball players. The lower sweat rate observed amongst disabled athletes is likely due to a lower workload during the training sessions. For those with amputations and spinal cord injury's a smaller area of the body is available for sweating. A novel finding of this chapter

was the sweat electrolyte composition of the athletes with a disability. Chapter 5 was the first study to determine the sweat electrolyte losses of disabled athletes, it found that sweat sodium, potassium and chloride concentrations were within the normal physiological range.

Total body water and water turnover rates of those with a spinal cord injury are different to the able-bodied population. The lower total body water relative to body mass represents a greater percent body fat. Previous research by Cardús et al. (1984) also found lower total body water values relative to body mass amongst paraplegics and tetraplegics. The main difference between the study by Cardús et al. (1984) and the study in this thesis is the time since injury. In the study by Cardús et al. (1984), the participants were still in hospital inpatient care; which is not reflective of their normal daily habits. The present study looked at participants who had been discharged from hospital; therefore the time since injury was longer for the present study. Further to this, Chapter 6 investigated tetraplegics who were competing in elite level sport. Previous work by Leiper et al. (2001) has shown that chronic physical activity can have an influence on total body water since because total body water is representative of the relative amounts of fat and lean tissue present in the body. Chapter 8 was the first investigation into the total body water of kidney transplant recipients. Interestingly the kidney transplant recipients had similar total body water to the wheelchair rugby players, which may be due to the higher % body fat and inactive lifestyle of some of the kidney transplant recipients. Figure 9.7.1. shows the relative total body water seen in wheelchair rugby players (Chapter 6), the spinal cord injured participants in Chapter 7 and the kidney transplant recipients compared to values seen for healthy able-bodied athletes and sedentary controls.

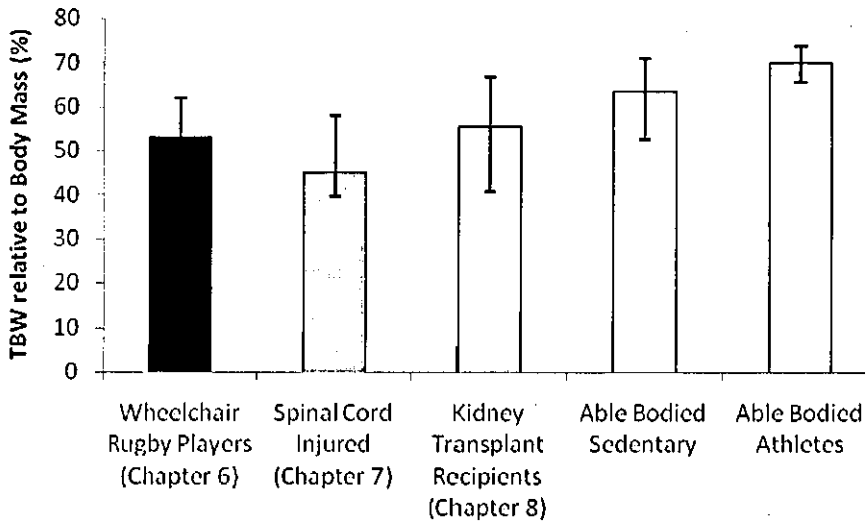


Figure 9.7.1. Total Body Water Values Relative to Body Mass (%) for Participants with a Spinal Cord Injury (Chapters 6 and 7), Kidney Transplant Recipients (Chapter 8) and Previously Published Values for Able-bodied Cyclists and Their Sedentary Controls (Leiper et al. 2001).

The studies in Chapter 6 and 7 were the first to investigate water turnover rates of individuals with a spinal cord injury, and Chapter 8 was the first investigation using this technique amongst kidney transplant recipients. Although water turnover rates were similar to the able-bodied population, for three groups the routes by which body water are lost appear to be different. Water turnover rates for Chapters 6 and 7 are shown in Figure 9.7.2., along with values previously published for able-bodied athletes and sedentary, able-bodied, participants.

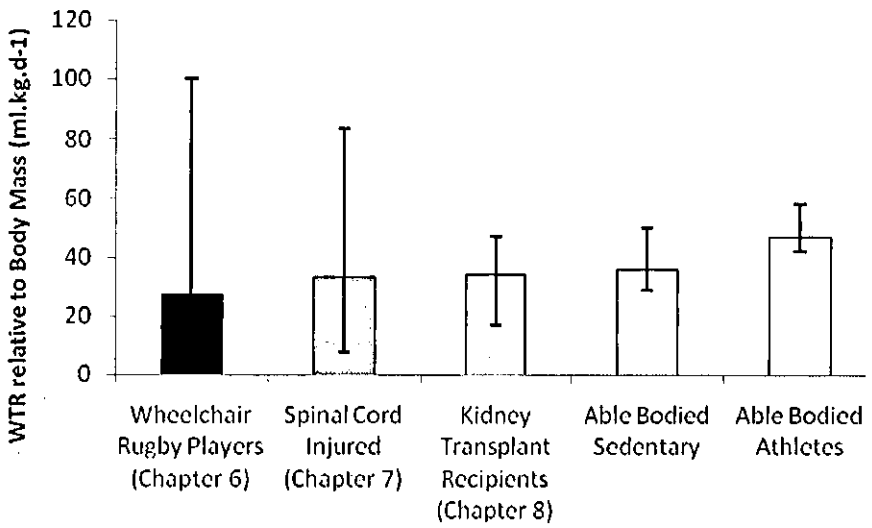


Figure 9.7.2. Water Turnover Rates (ml.kg.d-1) Relative to Body Mass for Wheelchair Rugby Players (Chapter 6), Spinal Cord Injured Participants (Chapter 6), Kidney Transplant Recipients (Chapter 8) and Previously Published Data from Able-bodied Endurance Cyclists and their Sedentary Controls (Leiper et al. 2001).

The three studies contained within this thesis concerned with daily non-renal losses, show large variations in non-renal losses within the groups studied. For all groups, non-renal losses are lower than previously reported for able-bodied athletes (Leiper et al., 2001). Although the use of deuterium cannot distinguish between the different routes for non-renal losses, it is likely that the smaller non-renal losses seen amongst tetraplegics are due to their impaired ability to sweat below the level of the lesion. The elevated levels of non-renal losses reported in Chapter 7 may be due to larger increases in core temperature driving sweat losses. Figure 9.7.3 shows the non-renal losses for the participants in Chapter 6 and 7, along with previously published values for sedentary able-bodied participants and able-bodied athletes.

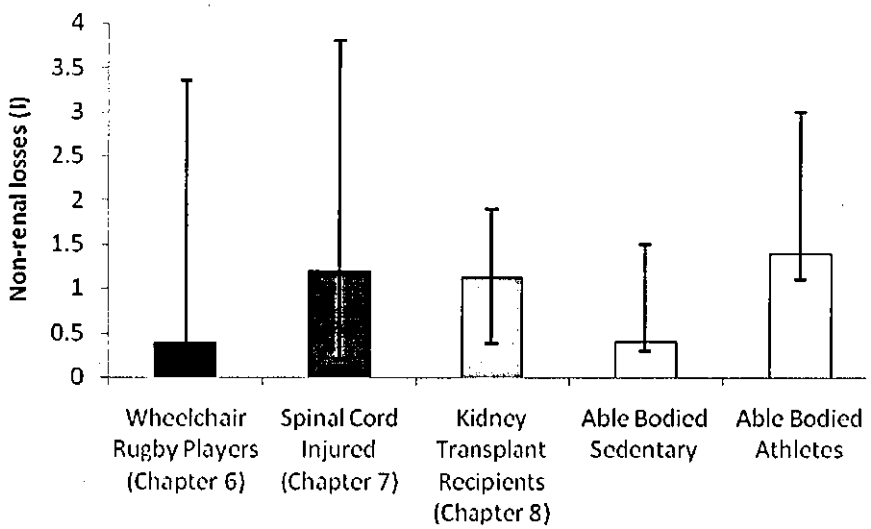


Figure 9.7.3. Non-Renal Losses (l) of Wheelchair Rugby Players (Chapter 6), Spinal Cord Injured (Chapter 7), Kidney Transplant Recipients (Chapter 8) and Previously Published Data by Leiper et al. (2001) on Able-bodied Endurance Cyclists and Sedentary Controls.

Compared to previously reported data for able-bodied participants, urinary losses for individuals with a spinal cord injury cover a similar range with the greatest values being almost double the values reported for able-bodied subjects. Median values for urine volume were similar between the spinal cord injured and able-bodied subjects. This indicates that fluid intakes between those with a spinal cord injury and healthy able-bodied participants are similar. However, as the body looses less water through non-renal routes in individuals with a spinal cord injury, the body acts to excrete this water via urination. The values for the kidney transplant recipients are lowest of all the groups possibly reflecting the tight control over fluid intakes initiated whilst in renal failure. Figure 9.7.4 shows the 24 hour urinary losses of the participants in Chapters 6 and 7, along with values previously reported for able-bodied athletes and sedentary able-bodied participants. These studies were the first to investigate the water turnover rates of individuals with a spinal cord injury.

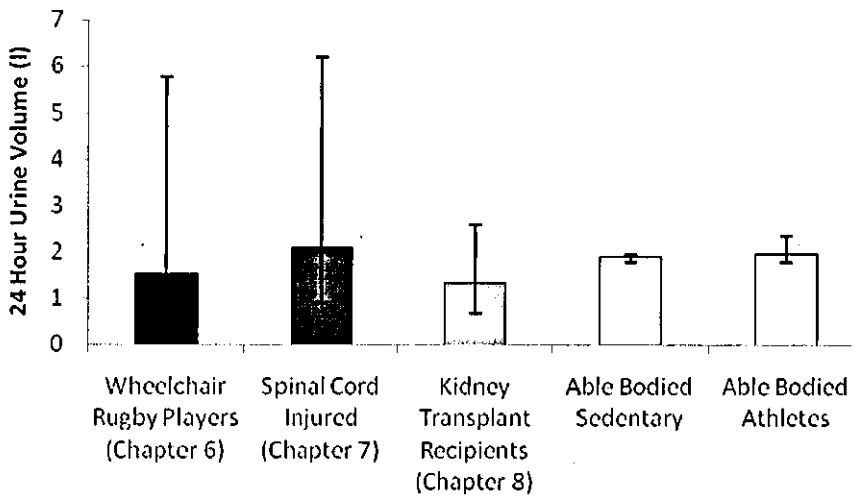


Figure 9.7.4. 24 H Urine Volumes (l) of Wheelchair Rugby Players (Chapter 6), Spinal Cord Injured (Chapter 7), Kidney Transplant Recipients (Chapter 8) and Previously Published Data by Leiper et al. (2001) on Able-bodied Endurance Cyclists and Sedentary Controls.

When looking at the urinary sodium losses and converting this to salt (NaCl) losses, it can be seen from Figure 9.7.5. that 24 hour urinary NaCl losses are higher for those individuals with a spinal cord injury compared to those seen in a recent survey by the Food Standards Agency (2008) undertaken with healthy able-bodied individuals. Individuals with tetraplegia (wheelchair rugby players) had the highest urinary salt losses. It is possible that government targets to reduce salt intake to 6 g per day are not appropriate for those with a spinal cord injury. Especially when considering urine volumes are typically high for this group and there is an inability to dilute urine to values in able-bodied participants (Wall et al., 1993). A combination of these two factors would result in a large volume of salt (NaCl) being lost daily in the urine, making daily NaCl intake requirements higher than for the sedentary able-bodied population. Conversely, analysis of urinary sodium losses showed that individuals who had received a kidney transplantation had the lowest urinary salt losses. These finding indicate that the restrictive diet that individuals are placed on prior to kidney transplantation is maintained to some extent following organ transplantation.

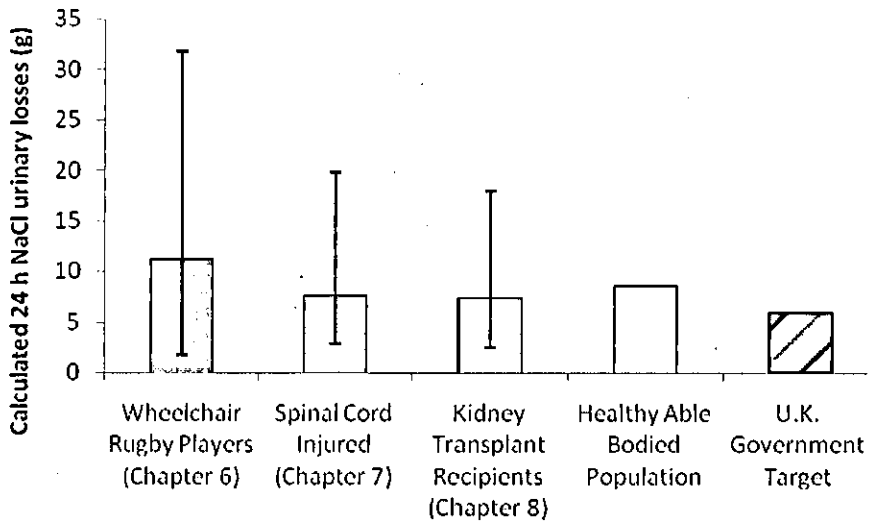


Figure 9.7.5. Urinary NaCl Losses (g) for Wheelchair Rugby Players (Chapter 6), Spinal Cord Injured Participants (Chapter 7), Kidney Transplant Recipients (Chapter 8) and Healthy Able-bodied Participants Studied by the FSA (2008) and the U.K. Governments Maximum Target Intake (FSA, 2008).

References

Abel, T., Schneider, S., Platen, P. and Struder, H. K. (2005): Performance diagnostics in handbiking during competition. *Spinal Cord 1, 1-6.*

Adachi, T., Miyazaki, M., Horiuchi, S., and Igawa, S. (2006): Comparison of water turnover rates between collegiate soft tennis players and age-matched sedentary undergraduates. *Korean Journal of Physiology and Pharmacology* **10**, 3-8.

Adams, W., Fox, R., Fry, A. and MacDonald, I. (1975): Thermoregulation during marathon running in cool, moderate, and hot environments. *Journal of Applied Physiology* **38**, 1030-7.

Adler, S. M. and Verbalis, J. G. (2006): Disorders of body water homeostasis in critical illness. *Endocrinology and Metabolism Clinics of North America* **35**, 873.

Adolph, E.F. (1947): Heat exchanges, sweat formation, and water turnover. In: *Physiology of Man in the Desert*, E.F. Adolph et al. (Eds.). New York: Interscience, 33-43. In: Leiper, J.B. and Maughan, R.J. (2004): Comparison of water turnover rates in young swimmers in training and age-matched non-training individuals. *International Journal of Sport Nutrition and Exercise Metabolism* **14**, 357.

Ainsworth, B.E., Haskell, W.L., Whitt, M.C., Irwin, M.L., Swartz, A.M. and Strath, S.J. (2000): Compendium of physical activities: An update of activity codes and MET intensities. *Medicine and Science Sports and Exercise* **32**, S498-504.

Allan, J. and Wilson, C. (1971): Influence of acclimatization on sweat sodium concentration. *Journal of Applied Physiology* **30**, 708-712.

Allsopp, A. J., Sutherland, R., Wood, P. and Wooton, S. A. (1998): The effect of sodium balance on sweat sodium secretion and plasma aldosterone concentration. *European Journal of Applied Physiology* **78**, 516-521.

Al-Salman, J., Kemp, D. and Randall, D. (2002): Hyponatremia. *Western Journal of Medicine* **176**, 173-176.

American College of Sports Medicine ACSM (2005): American College of Sports Medicine (ACSM) Offers guidance to athletes on preventing hyponatremia and dehydration during upcoming races. Retrieved June 2008.

http://www.acsm.org/Content/ContentFolders/NewsReleases/2005/ACSM_Offers_Guidance_to_Athletes_on_Preventing_Hyponatremia_and_Dehydration_during_Upcoming_Races.htm

Anderson R.J., Chung H.M., Kluge R. and Schrier R.W. (1985): Hyponatremia: A prospective analysis of its epidemiology and pathogenic role of vasopressin. *Annals of Internal Medicine* **102**, 64-68.

anderson, R., Bushinsky, D., Lindheimer, M., Bourdeau, J., Berns, A. and Garella, S. (1986): Hospital-Associated Hyponatremia. *Kidney International* **29**, 1237-1247.

Antunes-Rodrigues, J., Castro, M. D., Elias, L. L. K., Valenca, M. M. and McCann, S. M. (2004): Neuroendocrine control of body fluid metabolism. *Physiological Reviews* **84**, 169.

Araki, T., Tsiyino, A., Matsushito, K., Toda, Y .and Umeno, K. (1981): Effect of physical training on exercise-induced sweating in women. *Journal of Applied Physiology* **51**, 1526-1532.

Arieff A. L., Llach, F., and Massry, S. G. (1976): Neurological manifestations and morbidity of hyponatremia: Correlation with brain water and electrolytes. *Medicine* **55**, 121-129.

Armstrong, L. E. (2005): Hydration assessment techniques. *Nutrition Reviews 63, S40-S54.*

Armstrong, L. E. (2000): *Performing In Extreme Environments* (1st ed.). Human Kinetics, Leeds, U.K.

Armstrong, L. E., Costill, D. L. and Fink, W. J., (1985): Influence of diuretic-induced dehydration on competitive running performance. *Medicine and Science in Sports and Exercise* **17**, 456–461.

Armstrong, L. E., Maresh, C. M., Castellani, J. W., Bergeron, M. F. and Kenefick, R. W. (1994): Urinary indices of hydration status. *International Journal of Sport Nutrition* **4**, 265-279.

Armstrong, L. E., Kenefick, R. W., Castellani, J. W., Riebe, D., Kavouras, S. A., Kuznicki, J. T. and Maresh, C. M . (1997): Bioimpedance spectroscopy technique: Intra- extracellular and total body water. *Medicine and Science in Sports and Exercise* **29**, 1657-1663.

Armstrong, L. E., Maresh, C. A., Gabaree, C.V., Hoffman, J. R., Kavouras, S.A., Kenefick, R.W., Casellani, J. W. and Ahlquist, L. E. (1997): Thermal and circulatory responses during exercise:

Effects of hypohydrationm dehydration, and water intake. *Journal of Applied Physiology* **82**, 2028-2035.

Armstrong, L. E., Soto, J. A. Hacker, F.T., Casam D.J., Kavouras, S.A. and Maresh, C.M. (1998): Urinary indices during dehydration, exercise, and rehydration. *International Journal of Sports Nutrition* **8**, 345-355.

Astrand P.O. and Rodahl K. (1986): *Textbook of Work Physiology.* p 619. McGraw-Hill International, Singapore.

Atkinson, G. and Nevill, A. (2001): Selected issues in the design and analysis of sports performance research. *Journal of Sports Science*, **19**, 811-827.

Backx, K., and Palmer, G. (2003): Effects of dietary carbohydrate on cycling performance on repeated days. *Journal of Sports Sciences* **21**, 310-311.

Backx, K., Van Someren, K. A. and Palmer, G. S. (2003): One hour cycling performance is not affected by ingested fluid volume. *International Journal of Sport Nutrition and Exercise Metabolism* **13**, 333-342.

Barfield, J. P., Malone, L. A., Collins, J. M., and Ruble, S. B. (2005): Disability type influences heart rate response during power wheelchair sport. *Medicine Science Sports and Exercise* **37**, 718-723.

Barr, S. I. (1999): Effects of dehydration on exercise performance. *Canadian Journal of Applied Physiology* **24**, 164-172.

Barrueto, R., Mager, M., and Bass, D. (1959): Measurement of rates of excretion of sweat solutes under physiological conditions. *Journal of Applied Physiology* **14**, 435-438.

Barsch, P., Johannsen, B. N., and Leppaluoto, J. (2003): *Textbook of sports medicine. Basic science and clinical aspects of sports injury and physical activity.* Blackwell Sciences Ltd. Oxford, U.K.

Bartok, C., Schoeller, D. A., Sullivan, J. C., Clark, R. R., and Landry, G. L. (2004): Hydration testing in collegiate wrestlers undergoing hypertonic dehydration. *Medicine and Science in Sports and Exercise* **36**, 510-517.

Battistini, N., Virgili, F., and Bedogni, G. (1994): Relative expansion of extracellular water in elite male athletes compared to recreational sportsmen. *Annals of Human Biology* **21**, 609-612.

Battistini, N., Virgili, F., Severi, S. and Brambilla, P. (1995): Relative expansion of extracellular water in obese vs. normal children. *Journal of Applied Physiology* **79**, 609-612.

Baum, E., Bruck, K., and Schwennicke, H. P. (1976): Adaptive modifications in the thermoregulatory system of long-distance runners. *Journal of Applied Physiology* **40**, 404-410.

Beliner, R.W., B., Levinsky, N. G., Davidson, D. G. and Eden M., (1958): Dilution and concentration of the urine and the action of antidiuretic hormone. *American Journal of Medicine* **24**, 730-744.

Benardot, D. (2006): *Advanced Sports Nutrition*. Human Kinetics, Europe Ltd. Leeds, U.K.

Bergeron, M. F. (1996): Heat cramps during tennis: a case report. *International Journal of Sport Nutrition* **6**, 62–68.

Bergeron, M. F. (2003): Heat cramps: fluid and electrolyte challenges during tennis in the heat. *Journal of Science and Medicine in Sport* **6**, 19-27.

Bergeron, M. F., McLeod, K. S. and Coyle, J. F. (2007): Core body temperature during competition in the heat: National boys' 14s junior tennis championships. *British Journal of Sports Medicine* **41**, 779-783.

Bergeron, M., Waller, J. and Marinik, E. (2006): Voluntary fluid intake and core temperature responses in adolescent tennis players: Sports beverage versus water. *British Journal of Sports Medicine* **40**, 406-410.

Bhambhani, Y. (2002): Physiology of wheelchair racing in athletes with spinal cord injury. *Sports Medicine* **32**, 23-51.

Blyth, C. S and Burt J. J. (1961): Effect of water balance on ability to perform in high ambient temperatures. *Research Quarterly* **32**, 301-307.

Boot, C. R. L., Binkhorst, R. A. and Hopman, M. T. E. (2006): Body temperature responses in spinal cord injured individuals during exercise in the cold and heat. *International Journal of Sports Medicine* **27**, 599-604.

Boulant, J. (1981): Hypothalamic mechanisms in thermoregulation. *Federation Proceedings* **40**, 2843-50.

Brenner, B. M., Ballermann, B. J., Gunning, M.E. and Zeidel, M. L. (1990): Diverse biological actions of Atrial Natriuretic Peptide. *Physiology Review* **70**, 665-699.

Broad, E. (1996): Fluid requirements of team sport players. *Sports Coach* **18**, 20-23.

Broad, E. M., and Burke, L. M. (1996): Body weight changes and voluntary fluid intakes during training and competition sessions in team. *International Journal of Sport Nutrition* **6**, 307-320.

Brouns, F. (1991): Heat - sweat - dehydration - rehydration: A praxis oriented approach. *Journal of Sports Science* **9**, 143-152.

Brouns, F. (2002): *Essentials of Sports Nutrition 2nd Edition.* John Wiley and Sons, New Jersey, U.S.A.

Buchholz, A. C., and Bugaresti, J. M. (2005): A review of body mass index and waist circumference as markers of obesity and coronary heart disease risk in persons with chronic spinal cord injury. *Spinal Cord* **43**, *513-518.*

Buchsbaum, M. and Harris, E. K. (1971): Diurnal variation in serum and urine electrolytes. *Journal of Applied Physiology* **30**, 27-35.

Bulbulian, R., Johnson, R., Gruber, J. and Darabos, B. (1987): Body composition in paraplegic male athletes. *Medicine Science Sports and Exercise* **19**, 195-201.

Buono, M. J. (2000): Limb vs trunk sweat gland recruitment patterns during exercise in humans. *Journal of Thermal Biology,* **25**, 263-266.

Buono, M. J. and Connolly, K. P. (1992): Increases in sweat rate during exercise; gland recruitment versus output per gland. *Journal of Thermal Biology* **17**, 267-270.

Buono, M. J. and Maupin, C. J. (2003): Relationship between sweat gland recruitment and esophageal temperature during exercise-induced hyperthermia. *Journal of Thermal Biology* **28**, 381-384.

Buono, M. J. and Sjoholm, H. T. (1988): Effect of physical training on peripheral sweat production. *Journal of Applied Physiology* **65**, 811-814.

Buono, M. and Wall, A. (2000): Effect of hypohydration on core temperature during exercise in temperate and hot environments. *Pfluegers Archive/European Journal of Physiology* **440**, 476-480.

Burke, L. M. (1997): Fluid balance during team sports. *Journal of Sports Sciences,* **15**, 287-295.

Burke, L. M. (2005): Fluid balance testing for elite athletes: An interview with Dr. Susan Shirreffs. *International Journal of Sport Nutrition and Exercise Metabolism* **15**, 323-327.

Burke, L. M. and Hawley, J. A. (1997): Fluid balance in team sports: Guidelines for optimal practices. *Sports Medicine* **24**, 38-54.

Burnham, R., Tuchak, C., Laskin, J. and Steadward, R. (1998): Heart rate, thermoregulation and fluid balance among elite wheelchair basketball players. *Palestria* **14**, *29-31.*

Burr, R. and Nuseibeh, I. (1993): Blockage of indwelling urinary catheters: The roles of urinary composition, the catheter, medication and diet. *Paraplegia* **31**, 234-41.

Byers, F. (1979): Extraction and measurement of deuterium-oxide at tracer levels in biological-fluids. *Analytical Biochemistry* **98**, 208-213.

Byrne, C., Lee, J. K. W., Chew, S. A. N., Lim, C. L. and Tan, E. Y. M. (2006): Continuous thermoregulatory responses to mass-participation distance running in heat. *Medicine Science Sports and Exercise* **38**, 803-810.

Cage, G. and Dobson, R. (1965): Sodium secretion and reabsorption in human eccrine sweat gland. *Journal of Clinical Investigation* **44**, 1270-1276.

Caggiula, A., Wing, R., Nowalk, M., Milas, N., Lee, S. and Langford, H. (1985): The measurement of sodium and potassium intake. *American Journal of Clinical Nutrition* **42**, 391-8.

Caldwell, J. E., Ahonen, E. and Nousiainen, U. (1984): Differential effects of sauna-, diuretic-, and exercise- induced hypohydration. *Journal of Applied Physiology* **57**, 1018-1023.

Campbell, I. G., Williams, C. and Lakomy, H. K. A. (2002): Physiological responses of endurance- trained male wheelchair athletes to a 10-kilometer treadmill time trial. *Adapted Physical Activity Quarterly* **19**, 496-508.

Cardús, D. and McTaggart, W. (1984): Total body water and its distribution in men with spinal cord injury. *Archives of Physical Medicine and Rehabilitation* **65**, 509-12.

Cardús, D., and McTaggart, W. (1985): Body sodium and potassium in men with spinal cord injury. *Archives of Physical Medicine and Rehabilitation* **66**, 156-159.

Casa, D. J., Shannon, M. B., Ganio, M. S., Brown, M., Yeargin, S. W., Blowers, J. A., Glaviano, N. R., Huggins, R. A. and Armstrong, L. E. (2007): Validity of Devices That Assess Body Temperature During Outdoor Exercise in the Heat. *Journal of Athletic Training* **42**, 333–342

Casa, D. J., Clarkson, P.M., and Roberts, W. O. (2006): American college of Sports Medicine: Roundtable on Hydration and Physical Activity: Consensus Statements. http://www.acsm.org/Content/NavigationMenu/Research/Roundtables_Specialty_Conf/Past Roundtables/Hydration_Consensus_2005.pdf

Castro, M., Apple, D., Hillegass, E., and Dudley, G. (1999): Influence of complete spinal cord injury on skeletal muscle cross-sectional area within the first 6 months of injury. *European Journal of Applied Physiology* **80**, 373-378.

Chen, Y., Roseman, J., Devivo, M. and Funkhouser, E. (2002): Does fluid amount and choice influence urinary stone formation in persons with spinal cord injury? *Archives of Physical Medicine and Rehabilitation* **83**, 1002-8.

Cheung, S. S. and McLellan, T. M. (1998): Heat acclimation, aerobic fitness, and hydration effects on tolerance during uncompensable heat stress. *Journal of Applied Physiology* **84**, 1731.

Cheuvront, S. N., Carter, R., Haynes, E. M. and Sawka, M. N. (2006): No effect of moderate hypohydration or hyperthermia on anaerobic exercise performance. *Medicine Science Sports and Exercise* **38**, 1093-1097.

Cheuvront, S. N., Carter, R., Montain, S. J. and Sawka, M. N. (2004): Daily body mass variability and stability in active men undergoing exercise-heat stress. *International Journal of Sport Nutrition Exercise and Metabolism* **14**, *532-540.*

Cheuvront, S. N. and Haymes, E. M. (2001): Thermoregulation and marathon running. *Sports Medicine* **31**, 743-762.

Cheuvront, S. N., Haymes, E. M. and Sawka, M. N. (2002): Comparison of sweat loss estimates for women during prolonged high-intensity running. *Medicine and Science in Sport and Exercise* **34**, 1344-1350.

Cheuvront, S. N., Carter, R. and Sawka, M. N. (2003): Fluid balance and endurance exercise performance. *Current Sports Medicine Reports* **2**, 202-208.

Cheuvront, S. N., and Sawka, M. N. (2005): Hydration assessment of athletes. *Sports Science Exchange* **18**(2), 1. accessed online http://:www.gssiweb.org Retrieved 09/01, 2006,

Chia-Hhung, K., Ho, Y. J., Changlai, S. P and Ding, H.J. (1999): Gastric emptying in spinal cord injured patients. *Digestive Diseases and Sciences* **44**, 1512-1515.

Christensen, B., Olsson, B., Knepper, M., Maunsbach, A. and Nielsen, S. (1997): Cellular and subcellular localization of aquaporin-2 and aquaporin-3 in human kidney. *Journal of the American Society of Nephrology* **8**, A0072-A0072.

Clark, J., Jelbart, M., Rischbieth, H., Strayer, J., Chatterton, B. and Schultz, C. (2007): Physiological effects of lower extremity functional electrical stimulation in early spinal cord injury: Lack of efficacy to prevent bone loss. *Spinal Cord* **45**, 78-85.

Claus-Walker, J. L., Campos, R.J. and Carter, R. E. (1972): Longitudinal analyses of daily excretory rhythm in men with tetraplegia due to cervical spinal cord transaction. *Paraplegia* **10**, 142-152.

Claus-Walker, J. L., Carter, R. E. and Di Ferrante, N.M. (1978): Immediate endocrine and metabolic consequences of traumatic quadriplegia in young women. *Paraplegia* **15**, 202-208.

Claus- Walker, J., Carter, R., Lipscomb, H. and Vallbona, C. (1969): Daily rhythms of electrolytes and aldosterone excretion in men with cervical spinal cord section. *Journal of Clinical Endocrinology and Metabolism* **29**, 300.

Claus-Walker, J., Spencer, W., Carter, R. and Halstead L. (1977): Electrolytes and the renin-angiotensin-aldosterone axis in traumatic quadriplegia. *Archives of Physical Medicine and Rehabilitation* **58**, 283-286.

Claus-Walker, J. and Halstead, L. S. (1981): Metabolic and endocrine changes in spinal cord injury: I. The nervous system before and after transection of the spinal cord. *Archives of Physical Medicine and Rehabilitation* **62**, 595-601.

Claus-Walker J, and Halstead LS. (1982): Metabolic and endocrine changes in spinal cord injury: 11 (section 2) partial decentralization of the autonomic nervous system. *Archives of Physical Medicine and Rehabilitation* **63**, 632-638.

Claydon, V. E., Hol, A. T., Eng, J. J. and Krassioukov, A. V. (2006): Cardiovascular responses and post exercise hypotension after arm cycling exercise in participants with spinal cord injury. *Archive of Physical Medicine and Rehabilitation* **87**, 1106-1114.

Costill, D., Coté, R. and Fink, W. (1976): Muscle water and electrolytes following varied levels of dehydration in man. *Journal of Applied Physiology* **40**, 6-11.

Coutts, K. D. (1992): Dynamics of wheelchair basketball. *Medicine and Science in Sport and Exercise* **24**, 231-234.

Coutts, K. D. R. and McKenzie, E. C. (1983): Maximal exercise responses of tetraplegics and paraplegics. *Journal of Applied Physiology: Respiratory, Environmental and Exercise Physiology* **55**, 479-482.

Coutts, K. D. R. and McKenzie, E. C. (1985): Submaximal exercise responses of tetraplegics and paraplegics. *Journal of Applied Physiology* **59**, 237-241.

Cowan, P., Hathaway, D., Cashion, A. and Gaber, A. (2005): Exercise improves functional capacity and cardiac autonomic function in recent and long-term kidney and kidney-pancreas transplant recipients. *American Journal of Transplantation* **5**, 466-467.

Cowan, P., Hathaway, D., Gaber, A., Kreider, R., Wicks, M. and Soberman, J. (2000): Exercise improves cardiac autonomic function and functional capacity in kidney and kidney-pancreas transplant recipients. *Transplantation* **69**, S326-S326.

Coyle, E. F. (2004): Fluid and fuel intake during exercise. *Journal of Sports Science* **22**, 39-55.

Curtis, G. and Fogel, M. (1970): Creatinine Excretion: Diurnal variation and variability of whole and part-day measures. *Psychosomatic Medicine* **32**, 337-350.

Danovitch, G. M. (2004): *Handbook of Kidney Transplantation 4th edition,* Lippincott Williams and Wilkins, Philadelphia, U.S.A.

Datz, F. L., Christian, P.E. and Moore, J. (1987): Gender-related differences in gastric emptying. *Journal of Nuclear Medicine* **28,** 1204-1207.

Davis J. M., Lamb, Burgess, W. A. and Bartoli, W. P. (1987): Accumulation of deuterium oxide in body fluids after ingestion of D2O-labelled beverages *Journal of Applied Physiology* **63,** 2060-2066.

Davis, J. A., Vodak, P., Wilmore, J. H., Vodak, J. and Kurtz, P. (1976): Anaerobic threshold and maximal aerobic power for three modes of exercise. *Journal of Applied Physiology* **4**, 544.

Demanet, J., Bonnyns, M., Bleiberg, H. and Stevens, C. (1971): Coma due to water intoxication in beer drinkers. *Lancet* **2**, 1115-1117.

Dennis, S. C. and Noakes, T. D. (1999): Advantages of a smaller body mass in humans when distance-running in warm, humid conditions. *European Journal of Applied Physiology* **79**, 280-284.

Di Bona G. F. (1977): Neural regulation of renal tubular sodium reabsorption. *American Journal of Physiology* **233**, F73-F81.

Diezi, J., Michoud, P., Aceves, J. and Giebisch, G. (1973): Micropuncture study of electrolyte transport across papillary collecting duct of the rat. *American Journal of Physiology* **224**, 623-34.

Doty, S., and Seagrave, R. (2000): Human water, sodium, and calcium regulation during space flight and exercise. *Acta Astronautica* **46**, 591-604.

Drinkwater, B. L., Denton, J. E., Kupprat, I. C., Talag, T. S., and Horvath, S. M. (1976): Aerobic power as a factor in women' s response to work in hot environments. *Journal of Applied Physiology* **41**, 815-821.

Drummond, M., Glynn, E., Lujan, H., Dicarlo, S. and Rasmussen, B. (2008): Gene and protein expression associated with protein synthesis and breakdown in paraplegic skeletal muscle. *Muscle and Nerve* **37**, 505-513.

Dunbar, C.C., Robertson, R.J., Baun, R., Blandin, M. F., Metz, K., Burdett, R. and Goss, F. L. (1992): The validity of regulating exercise intensity by Ratings of Perceived Exertion. *Medicine and Science in Sport and Exercise* **24**, 94-99.

Dunn, F., Brennan, T., Nelson, A. and Robertso, G.L. (1973): Role of blood osmolality and volume in regulating vasopressin secretion in rat. *Journal of Clinical Investigation* **52**, 3212-3219.

Durose, C. L., Holdsworth, M., Watson, V. and Przygrodzka, F. (2004): Knowledge of dietary restrictions and the medical consequences of non-compliance by patients on haemodialysis are not predictive of dietary compliance. *Journal of the American Dietetic Association* **104**, 35.

Durstine, J. L. and Moore, G. E. (2002): *ACSM's Exercise Management for Persons With Chronic Diseases and Disabilities. (2nd ed.).* Human Kinetics. Leeds, U.K.

Edhem, I. and Harrison, S. C. W. (2006): Renal scarring in spinal cord injury: A progressive process? *Spinal Cord* **44**, 170-173.

Edwards, A. M. and Clark, N. A. (2006): Thermoregulatory observations in soccer match play: Professional and recreational level applications using an intestinal pill system to measure core temperature. *British Journal of Sports Medicine* **40**, 133-138.

Eichner, E. R. (2007): The role of sodium in 'heat cramping.' *Sports Medicine* **37**, 368-370.

Falk, B. (1998): Effects of thermal stress during rest and exercise. *Sports Medicine* **25**, 221-240.

Falk, B., Bar- Or, O. and MacDougall, J.D. (1991): Aldosterone and prolactin response to exercise in the heat in circumpubertal boys. *Journal of Applied Physiology* **71**, 1741-1745.

Fitzgerald, P. I., Sedlock, D.A. and Knowlton, R.G. (1990): Circulatory and thermal adjustments to prolonged exercise in paraplegic women. *Medicine and Science in Sports and Exercise* **22**, 629-635.

Fitzsimons, J. T. (1976): The physiological basis of thirst. *Kidney International* **10**, 3–11.

Fjeld, C. R., Brown, K. H., Schoeller, D. A. and International Society for Research in Human Milk and Lactation. (1988): Validation of the deuterium oxide method for measuring average daily milk intake in infants. *Human Lactation* **48**, 511-514.

Food Standards Agency (FSA) (2006): *An assessment of dietary sodium levels among adults (aged 19-64) in the general population, based on analysis of dietary sodium in 24 hour urine samples.* Retrieved 01/05/08. http://www.foodstandards.gov.uk/multimedia/pdfs/englandsodiumreport.pdf

Food Standards Agency (FSA) (2006*): An assessment of dietary sodium levels among adults (aged 19-64) in the general population, based on analysis of dietary sodium in 24 hour urine samples.* Retrieved 01/05/08. http://www.foodstandards.gov.uk/multimedia/pdfs/englandsodiumreport.pdf

Food Standards Agency (FSA) (2008): *Impact assessment of the Revised Salt Reduction.* Retrieved 25/10/2008. http://www.foodstandards.gov.uk/

Fortney, S. M. and Vroman, N.B. (1985): Exercise, performance and temperature control: Temperature regulation during exercise and implications for sports performance and training. *Sports Medicine* **2**, 8-20.

Fowkes, G. S., Bartolozzi, A. R., Burkholder, R., Sugarman, E. and Dorshimer, G. (2006): Core temperature and percentage of dehydration in professional football linemen and backs during preseason practices. *Journal of Athletic Training* **17**, 8-14.

Fowkes G, S., Bartolozzi, A. R., Burkholder, R. and Sugarman, E. (2005): Core temperature and hydration status in NFL linemen and backs during pre-season practices. (abstract). *Journal of Athletic Training* **40**, S48-S49.

Freund, P. R., Brengelmann, G. L., Rowell, L. B. and Halar, E. (1984): Attenuated skin blood flow response to hyperthermia in paraplegic men. *Journal of Applied Physiology* **56**, 1104-1109.

Freund, B. J., Montain, S. J., Young, A. J., Sawka, M. N., DeLuca, J. P. and Pandolf, K. B. (1995): Glycerol hyperhydration: Hormonal, renal, and vascular fluid responses. *Journal of Applied Physiology* **79**, 2069-2077.

Freund, B. J., Shizuru, E. M., Hashiro, G. M. and Claybaugh, J. R. (1991): Hormonal, electrolyte, and renal responses to exercise are intensity dependent. *Journal of Applied Physiology* **70**, 900-906.

Fried, L. F. and Palevsky, P.M. (1997): Hyponaetremia and hypernaetremia. *Medicine Clinical North America* **81**, 525-609.

Fusch, C. (1998): Physical fitness influences water turnover and body water changes during trekking. *Medicine and Science in Sports* **30**, 704-708.

Fusch, C., Hungerland, E., Scharrer, B. and Moeller, H. (1993): Water turnover of healthy children measured by deuterated water elimination. *European Journal of Paediatrics* **152**, 110-114.

Fusch, C., Gfrorer, W., Koch, C., Thomas, A., Grunert, A. and Moeller, H. (1996): Water turnover and body composition during long-term exposure to high altitude (4,900-7,600 m). *Journal of Applied Physiology* **80**, 1118-1125.

Gant, N., Williams, C., King, J. and Hodge, B. J. (2004): Thermoregulatory responses to exercise: Relative versus absolute intensity. *Journal of Sports Sciences* **22**, 1083-1090.

Gass, G. C. and Camp, E. M. (1987): Effects of prolonged exercise in highly trained traumatic paraplegic men. *Journal of Applied Physiology* **63**, 1846-1852.

Gass, E. M. and Camp, E. M. (1998): Rectal and esophageal temperatures during upper- and lower-body exercise. *European Journal of Applied Physiology and Occupational Physiology* **78**, 38-42.

Gass, E. M., Gass, G. C. and Pitetti, K. (2002): Thermoregulation responses to exercise and warm water immersion in physically trained men with tetraplegia. *Spinal Cord* **40**, 474-480.

Gass, G. C., Gass, E. M., Nadel, E. R., Gwinn, T. H. and Engel, P. (1988): Rectal and rectal vs. esophageal temperatures in paraplegic men during prolonged exercise. *Journal of Applied Physiology* **64**, 2265-2271.

Geelen, G., Keil, L., Kravik, S., Wade, C., Thrasher, T. and Barnes, P. (1984): Inhibition of plasma vasopressin after drinking in dehydrated humans. *American Journal of Physiology* **247**, R968-R971.

George, C., Wells, C. and Dugan, N. (1988): Validity of hydrodensitometry for determination of body composition in spinal injured participants. *Human Biology* **60**, 771-80.

Gerner, H.J., Engel, P., Gass, G. C., Gass, E. M., Hannich, T. and Feldmann, G. (1992): The effects of sauna on Tetraplegic and Paraplegic subjects. *Paraplegia* **30,** 410-419.

Giangregorio, L. and McCartney, N. (2006): Bone loss and muscle atrophy in spinal cord injury: Epidemiology, fracture prediction, and rehabilitation strategies. *Journal of Spinal Cord Medicine* **29**, 489-500.

Gibson, K. L. (2003): Caring for a patient who lives with a spinal cord injury. *Nursing* **33**, 36-42.

Godek, S. F., Bartolozzi, A. R. and Godek, J. J. (2005): Sweat rate and fluid turnover in American football players compared with runners in a hot and humid environment. *British Journal of Sports Medicine* **39**, 205-211.

Godek, S., Fowkes, Bartolozzi, A. R., Burkholder, R., Sugarman, E. and Dorshimer, G. (2006): Core temperature and percentage of dehydration in professional football linemen and backs during preseason practices. *Journal of Athletic Training* **41**, 8.

Goldman, M. B, Luchins, D. J. and Robertson, G. L. (1988): Mechanisms of altered water metabolism in psychotic patients with polydipsia and hyponatraemia. *New England Journal of Medicine* **318**, 397-403.

Gondim, F., Alencar, H., Rodrigues, C., Da Graca, J., dos Santos, A. and Rola, F. (1999): Complete cervical or thoracic spinal cord transections delay gastric emptying and gastrointestinal transit of liquid in awake rats. *Spinal Cord* **37**,793.

Goosey- Tolfrey, V. L. (2005): Physiological profiles of elite wheelchair basketball players in preparation for the 2000 Paralympic games. *Adapted Physical Activity Quarterly* **22**, 57-66.

Goosey- Tolfrey, V. L., Diaper, N.J., Crosland, J. and Tolfrey, K. (2008): Fluid intake during wheelchair exercise in the heat: Effects of localized cooling garments. *International Journal of Sports Physiology and Performance* **3**, 145-156.

Goran, M. I., Poehlman, E.T., Danforth, E. and Nair, K.S. (1992): Comparison of body composition methods on obese individuals. *Basic Life Sciences* **60,** 85-86.

Gordon, E., Prohaska, T., Siminoff, L., Minich, P. and Sehgal, A. (2005): Needed: Tailored exercise regimens for kidney transplant recipients. *American Journal of Kidney Diseases* **45**, *769-774.*

Green, J. M., Pritchett, R. C., Crews, T. R., McLester, J. R. and Tucker, D. C. (2004): Sweat lactate response between males with high and low aerobic fitness. *European Journal of Applied Physiology* **91**, 1-6.

Green, J. M., Pritchett, R. C., Tucker, D. C., Crews, T. R. and McLester, J. R. (2004): Sweat lactate response during cycling at 30C and 18C WBGT. *Journal of Sports Sciences* **22**, 321-327.

Greenhaff, P. L. and Clough, P. J. (1989): Predictors of sweat loss in man during prolonged exercise. *European Journal of Applied Physiology* **58**, 348-352.

Greenblatt, D., Ransil, B., Harmatz, J., Smith, T., Duhme, D. and Koch-Weser, J. (1976): Variability of 24-hour urinary creatinine excretion by normal participants. *Journal of Clinical Pharmacology* **16**, 321-328.

Greenleaf, J. E. (1992): Problem: thirst, drinking behaviour, and involuntary dehydration. *Medicine Science Sports and Exercise* **24**, 645–656.

Greenleaf, J. E, Brock, P. J., Keil, L.C. and Morse, J. T. (1983): Drinking and water balance during exercise and heat acclimation. *Journal of Applied Physiology* **54**, 414–419.

Greenleaf, J., Geelen, G., Keil, L., Kravik, S., Wade, C. and Thrasher, T. (1984): Effects of drinking on plasma vasopressin, renin, and aldosterone in dehydrated humans. *Federation Proceedings* **43**, 716-716.

Greenleaf, J., Morse, J., Barnes, P., Silver, J. and Keil, L. (1983): Hypervolemia and plasma vasopressin response during water immersion in men. *Journal of Applied Physiology* **55**, 1688-93.

Greenleaf, J. E. and Sargent, F. I. I. (1965): Voluntary dehydration in man. *Journal of Applied Physiology* **20**, 719–724.

Groothuis, J. T., Boot, C. R. L., Houtman, S., Van Langen, H. and Hopman, M. T. E. (2005): Leg vascular resistance increases during head-up tilt in paraplegics. *European Journal of Applied Physiology* **94**, 408-414.

Guttmann, L., Silver, J. and Wyndham, C. (1958): Thermoregulation in spinal man. *Journal Of Physiology* **142**, 406-419.

Hagobian, T. A., Jacobs, K. A., Kiratli, B. J. and Friedlander, A. L. (2004): Foot cooling reduces exercise- induced hyperthermia in men with spinal cord injury. *Medicine Science Sports and Exercise* **36**, 411-417.

Hagerman, F., Jacobs, P., Backus, D. and Dudley, G. (2006): Exercise responses and adaptations in rowers and spinal cord injury individuals. *Medicine Science Sports and Exercise* **38**, 958-62.

Haggarty, P., McGaw, B.A., Maughan, R.J. and Fenn, C. (1988): Energy-expenditure of elite female athletes measured by doubly-labelled water method. *Proceedings of the Nutrition Society*, **47**, A35

Hall, J. F. and Klemm, F. K. (1963): Insensible weight loss of clothed resting subjects in comfort zone temperatures. *Journal of Applied Physiology*. **18**, 1188-1192.

Hamill, W. H. and Freudenberg, W. (1935): Quantitative isotopic exchange reactions in the carbohydrate group. *Journal of the American Chemistry Society* **57**, 1427-1430.

Hargreaves, M. and Hawley, J. A. (2003): *Physiological Bases of Sports Performance.* McGraw-Hill New York , U.S.A.

Hargreaves, M., Hawley, J. A. and Jeukendrup, A. (2004): Pre-exercise carbohydrate and fat ingestion: Effects on metabolism and performance. *Journal of Sports Science* **22**, 31-8.

Hartung, G. H., Lally, D. A. and Blancq, R. J. (1993): Comparison of treadmill exercise testing protocols for wheelchair users. *European Journal of Applied Physiology* **66**, 362-365.

Havenith, G., Inoue, Y., Luttikholt, V. and Kenney, W. L. (1995): Age predicts cardiovascular, but not thermoregulatory, responses to humid heat stress. *European Journal of Applied Physiology and Occupational Physiology* **70**, 88-96.

Havenith, G., Luttikholt, V.G.M. and Vrijkotte, T.G.M. (1995): The relative influence of body characteristics on humid heat stress response. *European Journal of Applied Physiology and Occupational Physiology* **70**, 270-279.

Hebert, S.C., Culpepper R. M. and andreoli , T. E. (1981): NaCl transport in mouse medullary thick ascending limbs. I. functional nephron heterogeneity and ADH-stimulated NaCl co-transport. *American Journal of Physiology* **241**, F412-F431.

Hebestreit, A., Kersting, U., Basler, B., Jeschke, R. and Hebestreit, H. (2001): Exercise inhibits epithelial sodium channels in patients with cystic fibrosis. *American Journal of Respiratory Critical Care Medicine* **164**, 443-446.

Heering, P., Klein-Vehne, N. and Fehsel, K. (2004): Decreased mineralocorticoid receptor expression in blood cells of kidney transplant recipients undergoing immunosuppressive treatment: Cost efficient determination by quantitative PCR. *Journal of Clinical Pathology* **57**, 33-36.

Heering, P., Kurschat, C., Vo, D., Klein-Vehne, N., Fehsel, K. and Ivens, K. (2004): Aldosterone resistance in kidney transplantation is in part induced by a down-regulation of mineralocorticoid receptor expression. *Clinical Transplantation* **18**, 186-192.

Henane, R., Flandrois, R. and Charbonnier, J. P. (1977): Increase in sweating sensitivity by endurance conditioning in man. *Journal of Applied Physiology: Respiration, Environment, Exercise Physiology* **43**, 822-828.

Henderson, L., Irving, K., Gregory, J., Bates, C. J., Prentice, A. and Perks, J. (2003): *The national diet and nutrition survey: Adults aged 19 to 64 years. vitamin and mineral intake and urinary analytes.* http://www.food.gov.uk/multimedia/pdfs/ndnsv3.pdf

Hertzman, A. and Randall, W. (1948): Regional Differences in the Basal and Maximal Rates of Blood Flow in the Skin. *Journal of Applied Physiology* **1**, 234-241.

Hevesy, G., and Hofer, E. (1934): Elimination of water from human body. *Nature* **134**, 879.

Hill, R.J. and Davies P. S. W. (2001): Energy expenditure during 2 wk of an ultra-endurance run around Australia. *Medicine and Science in Sports and Exercise* **33**, 148-51.

Hill, R.J. and Davies P. S. W. (2001): The validity of self-reported energy intake as determined using doubly labelled water technique. *The British Journal of Nutrition* **85**, 415-430.

Hiller, W. D. B. (1989): Dehydration and hyponatremia during triathlons. *Medicine and Science in Sports and Exercise* **21**, S219-S212.

Hinton, P., Sanford, T., Davidson, M., Yakushko, O., Beck, N. (2004): Nutrient intakes and dietary behaviors of male and female collegiate athletes. *International Journal of Sport Nutrition and Exercise Metabolism* **14**, 389-405.

Hoffman, J. R. S. and Falk. H. (1995): The effect of water restriction on anaerobic power and vertical jumping height in basketball players. *International Journal of Sports Medicine* **16**, 214-218.

Hoffman, J., Maresh, C., Armstrong, L., Gabaree, C., Bergeron, M. and Kenefick, R. (1994): Effects of hydration state on plasma testosterone, cortisol and catecholamine concentrations before and during mild exercise at elevated temperature. *European Journal of Applied Physiology and Occupational Physiology* **69**, 294-300.

Hooker, S., Greenwood, J., Hatae, D., Husson, R., Matthiesen, T. and Waters, A. (1993): Oxygen-uptake and heart-rate relationship in persons with spinal-cord injury. *Medicine Science Sports and Exercise* **25**, 1115-9.

Hopman, M. T. E. (1994): Circulatory responses during arm exercise in individuals with paraplegia. *International Journal of Sports Medicine* **15**, 126-131.

Hopman, M. T. E. and Binkhorst, R.A. (1997): Spinal cord injury and exercise in the heat. *Sports Science Exchange* **10**, 1-4.

Hopman, M. T. E. and Binkhorst, R.A. (1998): Blood redistribution and circulatory responses to submaximal exercise in persons with spinal cord injury. *Scandinavian Journal of Rehabilitative Medicine,* **30**, 167-174.

Hopman, M. T. E., Groothuis, J. T., Flendrie, M., Gerritis, K. H. L. and Houtman, S. (2002): Increased vascular resistance in paralyzed legs after spinal cord injury is reversible by training. *Journal of Applied Physiology* **93**, 1966-1972.

Hopman, M. T., Houtman, S., Groothuis, J. T. and Folgering, H. T. (2004): The effect of varied fractional inspired oxygen on arm exercise performance in spinal cord injury and able-bodied persons. *Archives of Physical Medicine and Rehabilitation* **85**, 319-323.

Hopman, M. T. E., Oeseburg, B. and Binkhorst, R. A. (1992): Cardiovascular responses in paraplegic subjects during arm exercise. *European Journal of Applied Physiology* **65**, 73-78.

Hopman, M. T. E., Oeseburg, B. and Binkhorst, R. A. (1993): Cardiovascular responses in persons with paraplegia to prolonged arm exercise and thermal stress. *Medicine and Science in Sport and Exercise* **25**, 577-583.

Hopman, M., Vanasten, W. and Oeseburg, B. (1994): Blood-flow in the common femoral-artery at rest and during arm exercise in paraplegics and controls. *Journal of Physiology-London* **479**, 15P.

Hopman, M. T. E., Verheijen, P. H. E. and Binkhorst, R. A. (1993): Volume changes in the legs of paraplegic subjects during arm exercise. *Journal of Applied Physiology* **75**, 2079-2083.

Henderson, L., Irving, K., Gregory, J., Bates, C. J., Prentice, A., Perks, J., Swan, G. and Farron, M. (2003): *The national diet and nutrition survey: adults aged 19 to 64 years. Vitamin and mineral intake and urinary analytes.* The Stationary Office. London. U.K.

Hiller, W. D. B. (1989): Dehydration and hyponatremia during triathlons. *Medicine and Science in Sports and Exercise* **21**, S219-S221.

Hsieh, M., Roth, R., Davis, D. L., Larrabee, H. and Callaway, C. W. (2002): Hyponatremia in runners requiring on-site medical treatment at a single marathon. *Medicine and Science in Sports and Exercise* **34**, 185-189

Imai, M. and Kokko, J. (1976): Mechanism of sodium and chloride transport in the thin ascending limb of Henle. *Journal of Clinical Investigation* **58**, 1054-60.

Imai, M. and Kokko, J. (1974): Sodium chloride, urea, and water transport in the thin ascending limb of Henle generation of osmotic gradients by passive diffusion of solutes. *Journal of Clinical Investigation* **53**, 393-402.

Inoue, T., Nonoguchi, H. and Tomita, K. (2001): Physiological effects of vasopressin and atrial natriuretic peptide in the collecting duct. *Cardiovascular Research* **51**, 470 - 480.

Ingelstedt, S. (1956): Studies on the conditioning of air in the respiratory tract. In: Mitchell, J. W., Nadel, E. R. and Stolwijk, J. A. (1972): Respiratory weight losses during exercise. *Journal of Applied Physiology* **32**, 474-476.

IPC, International Paralympic Committee. (2006a): *Paralympic games. Retrieved 30/08, 2006, from http://www.paralympic.org/release/Main_Sections_Menu/Paralympic_Games/*

IPC, International Paralympic Committee. (2006b): *Paralympic games.* Retrieved 02/04, 2006 from http://www.paralympic.org/release/Summer_Sports/Wheelchair_Rugby/index.html

IPC, International Paralympic Committee. (2008): Classification. Retrieved 17/05/2008 from http://www.paralympic.org/release/Main_Sections_Menu/Classification/

Jacobs, P. L. and Nash, M. S. (2004): Exercise recommendations for individuals with spinal cord injury. *Sports Medicine* **34**, 727-751.

Jaffe, M. (1886): Measurement of creatinine using picric acid. *Physiological Chem*istry **10**, 391-400.

Jankowski, C. M., Sonko, B. J., Gozansky, W. S. and Kohrt, W. M.(2004): Deuterium dilution: The time course of 2h enrichment in saliva, urine, and serum. *Clinical Chemistry* **50**, 1699-1701.

Jeukendrup, A. and Van Diemen, A. (1998): Heart rate monitoring during training and competition in cyclists. *Journal of Sports Science* **16**, S91-S99.

Jindal, R. and Zawada, E. (2004): Obesity and kidney transplantation. *American Journal of Kidney Diseases* **43**, 943-952.

Johansen, K. L. (2005): Exercise and chronic kidney disease: Current recommendations. *Sports Medicine* **35**, 485.

Johnson, L. R. and Byrne, J. H.(2003): *Essential Medical Physiology.* Academic Press London, U.K.

Jones, L. M., Legge, M. and Goulding, A. (2003): Healthy body mass index values often underestimate body fat in men with a spinal cord injury. *Archives of Physical Medicine and Rehabilitation* **84** , 1068-1071.

Juskowa, J., Lewandowska, M., Bartlomiejczyk, I., Foroncewicz, B., Korabiewska, I. and Niewczas, M. (2006): Physical rehabilitation and risk of atherosclerosis after successful kidney transplantation. *Transplantation Proceedings* **38**, 157-160.

Khalili, M. A. (2004): Quantitative sports and functional classification (QSFC) for disabled people with spasticity. *British Journal of Sports Medicine* **38**, 310-313.

Kahn, T. (2003): Reset osmostat and salt and water retention in the course of severe hyponatremia *Medicine* **82**, 170-176.

Kamelhar, D., Steele, J., Schacht, R., Lowenstein, J. and Naftchi, N. (1978): Plasma-renin and serum dopamine-beta-hydroxylase during orthostatic hypotension in quadriplegic man. *Archives of Physical Medicine and Rehabilitation* **59**, 212-216.

Kay, D. and Marino, F. E. (2000): Fluid ingestion and exercise hyperthermia: Implications for performance, thermoregulation, metabolism and the development of fatigue. *Journal of Sports Sciences* **18**, 71-82.

Kenefick, R. W., Hazzard, M. P., Mahood, N. V. and Castellani, J. W. (2004): Thirst sensations and AVP responses at rest and during exercise-cold exposure. *Medicine Science Sports and Exercise* **36**, 1528-1534.

Kenny, G. P., Periard, J., Journeay, W. S., Sigal, R. J. and Reardon, F. D. (2003): Effect of exercise intensity on the post-exercise sweating threshold. *Journal of Applied Physiology* **95**, 2355-2360.

Kilinc, S., Akman, M. N., Levendoglu, F. and Ozker, R. (1999): Diurnal variation of antidiuretic hormone and urinary output in spinal cord injury. *Spinal Cord* **37**, 332-335.

Kirby, C.R. and Convertino, V.A. (1986): Plasma aldosterone and sweat sodium concentrations after exercise and heat acclimation. *Journal of Applied Physiology* **61**, 967-70.

Kirkendall, D. T. (1993): Effects of nutrition on performance in soccer. *Medicine and Science in Sports and Exercise* **25**, 1370-1374.

Kjaer, M., Keiding, S., Engfred, K., Rasmussen, K., Sonne, B. and Kirkegard, P. (1995): Glucose homeostasis during exercise in humans with a liver or kidney transplant. *American Journal of Physiology* **268**, E636-E644.

Kjaer, M., Krogsgaard, M., Magnusson, P., Engebretsen, L., Roos, H. and Takala, T. (Eds.). (2003): *Textbook of sports medicine. Basic science and clinical aspects of sports injury and physical activity. (1st ed.).* Blackwell Sciences Ltd. Oxford, U.K.

Knepper, M. (1982): Measurement of osmolality in kidney slices using vapor-pressure osmometry. *Kidney International* **21**, 653-655.

Knepper, M. (1997): Molecular physiology of urinary concentrating mechanism: Regulation of aquaporin water channels by vasopressin. *American Journal of Physiology* **272**, F3-F12.

Knepper, M. and Burg, M. (1983): Organization of Nephron Function. *American Journal of Physiology* **244**, F579-F589.

Knepper, M. and Inoue, T. (1997): Regulation of aquaporin-2 water channel trafficking by vasopressin. *Current Opinion in Cell Biology* **9**, 560-4.

Knepper, M. and Vurek, G. (1982): Urea transport in isolated, perfused cortical thick ascending limbs and proximal straight tubules from rabbits. *Kidney International* **21**, 280-280.

Kocina, P. (1997): Body composition of spinal cord injured adults. *Sports Medicine* **23**, 48-60.

Kokko, J. and Rector, F. (1972): Countercurrent multiplication system without active transport in inner medulla. *Kidney International* **2**, 214-223.

Kolka, M.A., Stephenson, L.A., Wilkerson, J. E. (1982): Erythrocyte indices during a competitive marathon. *Journal of Applied Physiology* **52**, 168-172.

Kooner, J. S., Frankel, H.L., Mirando, N., Peart,, W. S. and Matthias, C. J. (1988): Haemodynamic, hormonal and urinary responses to postural change in tetraplegic and paraplegic man. *Paraplegia* **26**, 233-237.

Kovacs, E. M. R. S. and Brouns, J. M. G. (1999): Urine color, osmolality and specific electrical conductance are not accurate measures of hydration status during post exercise rehydration. *Journal of Sports Medicine and Physical Fitness* **39**, 47-53.

Kuhlmann, U., Schwickardi, M., Trebst, R. and Lange, H. (2001): Resting metabolic rate in chronic renal failure. *Journal of Renal Nutrition* **11**, 202-206.

Kuhn, R. (1950): Functional capacity of the isolated human spinal cord. *Brain* **73**, 1-51.

Lane, H. W. (1997): Comparison of ground-based and space flight energy expenditure and water turnover in middle-aged healthy male US astronauts. *The American Journal of Clinical Nutrition* **65**, 4-12.

Laragh J. H. (1985): Atrial natriuretic hormone, the rennin-aldosterone axis and blood pressure homeostasis. *New England Journal of Medicine* **313**, 1330-1340.

Latzka, W. A. and Sawka, M. N. (2000): Hyperhydration and glycerol: Thermoregulatory effects during exercise in hot climates. *Canadian Journal of Applied Physiology* **25**, 536-545.

Latzka, W. A., Sawka, M. N., Montain, S. J., Skrinar, G. S., Fielding, R. A. and Matott, R. P. (1997): Hyperhydration: Thermoregulatory effects during compensable exercise- heat stress. *Journal of Applied Physiology* **83**, 860-866.

Laursen, P., Suriano, R., Quod, M., Lee, H., Abbiss, C. and Nosaka, K. (2006): Core temperature and hydration status during an ironman triathlon. *British Journal of Sports Medicine* **40**, 320-5.

Lee, C. R., Watkins, M. L., Patterson, J. H., Gattis, W., O'Connor, C. M. and Gheorghiade, M. (2003): Vasopressin: A new target for the treatment of heart failure. *American Heart Journal* **146**, 9-18.

Leehey, D. J., Picache, A. A. and Robertson, G. L. (1988): Hyponatraemia in quadriplegic patients. *Clinical Science* **75**, 441-444.

Leiba, A., Vald, A., Peleg, E., Shamiss, A. and Grossman, E. (2005): Does dietary recall adequately assess sodium, potassium, and calcium intake in hypertensive patients? *Nutrition* **21**, 462-466.

Leiper, J. B., Carnie, A. and Maughan, R. J. (1996): Water turnover rates in sedentary and exercising middle aged men. *British Journal of Sports Medicine* **30**, 24-26.

Leiper, J. B. and Maughan, R. J. (2004): Comparison of water turnover rates in young swimmers in training and age-matched non-training individuals. *International Journal of Sport Nutrition and Exercise Metabolism* **14**, 347-357.

Leiper, J. B., Pitsiladis, Y. and Maughan, R. J. (2001): Comparison of water turnover rates in men undertaking prolonged cycling exercise and sedentary men. *International Journal of Sports Medicine* **22**, 181-185.

Leiper, J., Seonaid, P. C., Primrose, W., Phillimore, J. and Maughan, R. (2005): A comparison of water turnover in older people in community and institutional settings. *Journal of Nutrition Health and Aging* **9**, 189-93.

Lemon, P. (1991): Effect of exercise on protein requirements. *Journal of Sports Science* **9**, 53-70.

Lentner, C. Ciba-Geigy Limited. (1981): *Geigy Scientific Tables Vol.1 Units of Measurement, Body Fluids, Composition of the Body, Nutrition.* Ciba-Geigy, Basle.

Lifson, N. and Mcclintock, R. (1966): Theory of use of turnover rates of body water for measuring energy and material balance. *Journal of Theoretical Biology* **12**, 46-74.

Lin, M., Liu, S. J. and Lim, I. T. (2005): Disorders of water imbalance. *Emergency Medicine Clinics of North America* **23**, 749.

Lohman, T. (1992): Exercise training and body composition in childhood. *Canadian Journal of Sport Science* **17**, 284-287.

Lote, C. J. (1994): *Principles of Renal Physiology 3rd edition.* Chapman and Hall, London, U.K.

Lukaski, H. and Johnson, P. (1985): A simple, inexpensive method of determining total body water using a tracer dose of D2O and infrared absorption of biological fluids. *American Journal of Clinical Nutrition* **41**, 363-370.

Lykken, G., Jacob, R., Munoz, J. and Sandstead, H. (1980): A mathematical model of creatine metabolism in normal males-comparison between theory and experiment. *American Journal of Clinical Nutrition* **33**, 2674-2685.

Magal, M., Webster, M. J., Sistrunk, L. E., Whitehead, M. T., Evans, R. K. and Boyd, J. C. (2003): Comparison of glycerol and water hydration regimens on tennis- related performance. *Medicine Science Sports and Exercise* **35**, 150-156.

Mallie, J., Ait-Djafer, Z., Saunders, C., Pierrat, A., Caira, M. V., Courroy, O., Panescu, V. and Perrin, P. (2002): Renal handling of salt and water in humans during exercise with or without hydration. *European Journal of Applied Physiology* **86**, 196-202.

Maltais, D., Unnithan, V. B., Wilk, B. and Bar-Or, O. (2004): Responses of children with cerebral palsy to arm crank exercise in the heat. *Medicine and Science Sports and Exercise* **36**, 191-197.

Manz, F. and Wentz, A. (2005): The importance of good hydration for the prevention of chronic diseases. *Nutrition Reviews* **63**, S2-S5.

Maresh, C. M., Gabaree-Boulant, C. L., Armstrong, L. E., Judelson, D. A., Hoffman, J. R. and Castellani, J. W. (2004): Effect of hydration status on thirst, drinking, and related hormonal responses during low-intensity exercise in the heat. *Journal of Applied Physiology* **97**, 39-44.

Maresh, C., Wong, B.C. and Goetz, K. L. (1985): Plasma vasopressin, renin activity, and aldosterone responses to maximal exercise in active college females. *European Journal of Applied Physiology and Occupational Physiology.* **54**, 398-403.

Marino, F. E., Kay, D. and Serwach, N. (2004): Exercise time to fatigue and the critical limiting temperature: Effect of hydration. *Journal of Thermal Biology* **29**, 21-29.

Marsh, D. and Steele, D. (1973): Mechanisms of NaCl reabsorption by hamster thin ascending limbs of henles loops. *Federation Proceedings* **32**, 71-79.

Martin, J. E., Dubbert, P. M. and Cushman, W. C. (1990): Controlled trial of aerobic exercise in hypertension. *Circulation* **81**, 1560-1567.

Mathias, C., Christensen, N., Corbett, J., Frankel, H., Goodwin, T. and Peart, W. (1975): Plasma catecholamines, plasma-renin activity and plasma aldosterone in tetraplegic man, horizontal and tilted. *Clinical Science and Molecular Medicine* **49**, 291-299.

Mathias, C and Frankel, H. (1988): Cardiovascular control in spinal man. *Annual Review of Physiology* **50**, 577-92.

Maughan, R. J. (2001): *Nutrition in Sport.* Blackwell Science. London, U.K.

Maughan, R. J. and Burke, L. M. (2004*): Sports Nutrition: Handbook of Olympic of Sports Medicine and Science.* Blackwell Publishing. London, U.K.

Maughan, R.J., Leiper, J.B. and Vist, G. E. (2004): Gastric emptying and fluid availability after ingestion of glucose and soy protein hydrolysate solutions in man. *Experimental Physiology* **89**, 101-8.

Maughan, R. J., Merson, S. J., Broad, N. P. and Shirreffs, S. M. (2004): Fluid and electrolyte intake and loss in elite soccer players during training. *International Journal of Sport Nutrition and Exercise Metabolism* **14**, 333.

Maughan, R.J. and Murray, R. (2000): *Sports Drinks: Basic Science and Practical Aspects.* CRC Press Boca Raton, U.S.A.

Maughan, R. J., and Nadel, E. R. (2001): Temperature regulation and fluid and electrolyte balance. In R. J. Maughan (Ed.), *The Encyclopaedia of Sports Medicine VII Nutrition In Sport. (1st edition ed., pp. 203-215).* Blackwell Sciences. London, U.K.

Maughan, R. and Shirreffs, S. (2004): Exercise in the heat: Challenges and opportunities. *Journal of Sports Sciences* **22**, 917-927.

Maughan, R. J. and Shirreffs, S. M. (2004): Rehydration and recovery after exercise. *Science and Sports* **28**, 234-238.

Maughan, R. J., Shirreffs, S. M. and Leiper, J. B. (2007): Errors in the estimation of hydration status from changes in body mass. *Journal of Sports Sciences* **25**, 797.

Maughan, R. J., Shirreffs, S. M., Merson, S. J. and Horswill, C. A. (2005): Fluid and electrolyte balance in elite male football (soccer) players training in a cool environment. *Journal of Sports Science* **23**, 73-79.

Maxwell, N. S., Gardner, F. and Nimmo, M.A.(1999): Intermittent running: muscle metabolism in the heat and effect of hypohydration. *Medicine and Science in Sports and Exercise* **31**, 675-683.

McCarthy, D. P. and Wilmot, C. B. (1970): Renal water and sodium conservation in acute paraplegia. *Paraplegia* **8**, 186-195.

Maynard, F. J., Bracken, M., Creasey, G., Ditunno, J. J., Donovan, W. and Ducker, T. (1997): International standards for neurological and functional classification of spinal cord injury. American spinal injury association. *Spinal Cord* **35**, 266-74.

McClean, D. E., Kearney, J. and Cawley, M. F. (1999): Environmentally responsive temperature instability in paediatric spinal cord injury. *Spinal Cord* **37**, 705-709.

McFadden, E., Strauss, L. and Nelson, J. (1998): Exercise-induced asthma - reply. *New England Journal of Medicine* **339**, 1785.

McNaughton, L. R. and Cedaro, R. (1991): Sodium Citrate ingestion and its effects on maximal anaerobic exercise of different durations. *European Journal of Applied Physiology* **64**, *36-41.*

Mendel, R., Blegen, M., Cheatham, C., Antonio, J. and Ziegenfuss, T. (2005): Effects of creatine on thermoregulatory responses while exercising in the heat. *Nutrition* **21**, 301-307.

Mendez, J., Prokop, E., Picon-Reategui, E., Akers, R. and Buskirk, E. (1970): Total body water by D_2O dilution using saliva samples and gas chromatography. *Journal of Applied Physiology* **28**, 354-7.

Miles, D. S., Sawka, M. N., Glaser, M. and Petrofsky, J. S. (1983): Plasma volume shifts during progressive arm and leg exercise. *Journal of Applied Physiology* **54**, 491-495.

Mitch, W. (1998): Mechanisms causing loss of lean body mass in kidney disease. *American Journal of Clinical Nutrition* **67**, 359-366.

Mitchell, J. W., Nadel, E. R. and Stolwijk, J. A. (1972): Respiratory weight losses during exercise. *Journal of Applied Physiology* **32**, 474-476.

Montain, S. J. and Coyle, E. F. (1992): Fluid ingestion during exercise increases skin blood flow independent of increases in blood volume. *Journal of Applied Physiology* **73**, 903-910.

Montain, S. J. and Coyle, E. F. (1992b): Influence of graded dehydration on hyperthermia and cardiovascular drift during exercise. *Journal of Applied Physiology* **73**, 1340-1350.

Montain, S. J., Sawka, M. N., Cadarette, B.S., Quigley, M. D. and McKay, J. M. (1994): Physiological tolerance to uncompensable heat stress: Effects of exercise intensity, protective clothing, and climate. *Journal of Applied Physiology* **77**, 216-222.

Montain, S. J. S. and Wenger, M. N. (2001): Hyponatremia associated with exercise: Risk factors and pathogenesis. *Exercise and Sport Sciences Review* **29**, 113-117.

Moran, D., Shapiro, Y., Meiri, U., Laor, A., Epstein, Y. and Horowitz, M. (1996): Exercise in the heat: Individual impacts of heat acclimation and exercise training on cardiovascular performance. *Journal of Thermal Biology* **21**, 171-181.

Morgan T. and Berliner, R.W. (1968): Permeability of the loop of Henle, vasa recta, and collecting duct to water, urea, and sodium. *American Journal of Physiology* **215**, 108-115.

Morgan, R. M., Patterson, M. J. and Nimmo, M. A. (2004): Acute effects of dehydration on sweat composition in men during prolonged exercise in the heat. *Acta Physiology Scandanavia* **182**, 37-43.

Mueller FO. (2002): *Annual survey of football injury research: 1931—2001.* National Centre for Catastrophic Sports Injuries. Chapel Hill (North Carolina), U.S.A.

Muraki, S., Yamasaki, M., Ishii, K., Kikuchi, K. and Seki, K. (1995): Effect of arm cranking exercise on skin blood flow of lower limb in people with injuries to the spinal cord. *European Journal of Applied Physiology* **71**, 28-32.

Mitch, W., (19980: Mechanisms causing loss of lean body mass in kidney disease. *American Journal of Clinical Nutrition* **67**, 359-366.

Nadel, E. R. (1979): Temperature regulation. In: Strauss, R. H.(Ed) (1979): *Sports Medicine and Physiology,* (Chap. 8, p.130-146). Saunders Philadelphia, U.S.A.

Nadel, E. R., Bullard, R.W. and Stolwijk, J. A. J., (1971): The importance of skin temperature in the regulation of sweating. *Journal of Applied Physiology* **31**, 20-87.

Nadel, E. and Nadel, J. (1973): Effect of skin wettedness on sweat gland response. *Journal of Applied Physiology* **35**, 689-694.

Nadel, E. R., Mack, G. W., Takamata, A. and Gatorade Sports Science Institute. (1992): Thermoregulation, exercise, and thirst: Interrelationships in humans. *Perspectives in Exercise Science and Sports Medicine* **6**, 225-56.

Nadel, E., Mitchell, J., Saltin, B. and Stolwijk, J. (1971): Peripheral modifications to central drive for sweating. *Journal of Applied Physiology* **31**, 828.

Nadel, E. R., Pandolf, K. B., Roberts, M. F. and Stolwijk, J. A. (1974): Mechanisms of thermal acclimation to exercise and heat. *Journal of Applied Physiology* **37**, 515-520.

Nagy, K. (1980): CO_2 Production in Animals - Analysis of potential errors in the doubly labelled water method. *American Journal of Physiology* **238**, R466-R473.

Nagy, K. A, and Costa, D. P. (1980b): Water flux in animals: analysis of potential errors in the titrated water method. *American Journal of Physiology* **238**, R454-65.

Narayanan, S. and Appleton, H. (1980): Creatinine: A review. *Clinical Chemistry* **26**, 1119-26.

National Kidney Foundation, 2008, *Living with one kidney.* Retrieved 30/05/2008. http://www.kidney.org/atoz/atozItem.cfm?id=99 Updated: 06/03/04

Nicotra, A., Asahina, M., Young, T. M. and Mathias, C. J. (2006): Heat-provoked skin vasodilatation in innervated and denervated trunk dermatomes in human spinal cord injury. *Spinal Cord* **44**, 222-226.

Nielsen B, Strange S, Chistensen N, Warberg J, and Saltin. B. (1997): Acute and adaptive responses to exercise in a warm, humid environment. Pflugers Archives **434**, 49-56.

Noakes, T. D. (1993): Fluid replacement during exercise. *Exercise and Sport Sciences Reviews* **21**, 297-330.

Noakes, T. M. (2002): IMMDA-AIMS advisory statement on guidelines for fluid replacement during marathon running. *New Studies in Athletics* **17**, 15-24.

Noakes, T. (2003): Fluid replacement during marathon running. *Clinical Journal of Sport Medicine* **13**, 309-318.

Noakes, T. D. (2003): Overconsumption of fluids by athletes. (editorial). *BMJ : British Medical Journal* **327**, 113-114.

Noakes, T.D. (2007): Hydration in the marathon. *Sports Medicine* **37**, 463-466.

Noakes, T. D., Adams, B. A., Myburgh, K. H., Greeff, C. and Lotz, T. (1988): The danger of an inadequate water intake during prolonged exercise. *European Journal of Applied Physiology and Occupational Physiology* **57**, 210-219.

Noakes, T., Goodwin, N., Rayner, B., Branken, T. and Taylor, R. (1985), Water intoxication: a possible complication during endurance exercise. *Medicine and Science in Sports and Exercise* **17**, 370–375.

Normell, L. (1974): Cutaneous thermoregulatory vasomotor response in healthy participants and paraplegic men. *Scandinavian Journal of Clinical and Laboratory Investigation* **33**, 1-44.

Nose, H., Mack G. W., Shi, X. and Nadel E. R. (1988): Role of osmolality and plasma volume during rehydration in humans. *Journal of Applied Physiology* **65**, 325–331.

Nybo, L. and Nielsen, B. (2001): Middle cerebral artery blood velocity is reduced with hyperthermia during prolonged exercise in humans. *Journal of Physiology* **534**, 279-286.

Olive, J. L., McCully, K. K. and Dudley, G. A. (2002): Blood flow response in individuals with incomplete spinal cord injuries. *Spinal Cord* **40**, 639-645.

Olive, J. L., Dudley, G. A. and McCully, K. K. (2002): Vascular Remodelling After Spinal Cord Injury. *Medicine and Science in Sports and Exercise* **35**, 901-907.

Oppliger, R.A. and Bartok, C. (2002): Hydration testing of athletes. *Sports Medicine* **32**, 959-971.

Owen, J. A. and Campbell, D. G. (1968): A comparison of plasma electrolyte and urea values in healthy patients and in hospital patients. *Clinical Chimica Acta* **22**, 611-618.

Pace, N., Kline, L., Schachman, H. K., and Harfenist, M.(1947): Studies on body composition. IV. Use of radioactive hydrogen for measurement in vivo of total body water. *Journal of Biological Chemistry* **168**, 459-469.

Pace, N. and Rathbun, E. N. (1945): Studies on body composition. III. The body water and chemically combined nitrogen content in relation to fat content. *Journal of Biological Chemistry* **158**, 685-691.

Painter, P., Hector, L., Ray, K., Lynes, L., Dibble, S. and Paul, S. (2002): A randomized trial of exercise training after renal transplantation. *Transplantation* **74**, 42-48.

Painter, P., Taylor, J., Wolcott, S., Krasnoff, J., Adey, D. and Tomlanovich, S. (2003): Exercise capacity and muscle structure in kidney recipient and twin donor. *Clinical Transplantation* **17**, 225-230.

Painter, P. L., Topp, K. S., Krasnoff, J. B., Adey, D., Strasner, A. and Tomlanovich, S. (2003): Health-related fitness and quality of life following steroid withdrawal in renal transplant recipients. *Kidney International* **63**, 2309-2316.

Palacios, C., Wigertz, K. and Weaver, C. M. (2003): Sweat mineral loss from whole body, patch and arm bag in white and black girls. *Nutrition Research* **23**, 401-411.

Parsons, T. L., Toffelmire, E. B. and King-Van Vlack, C. E. (2006): Exercise training during haemodialysis improves dialysis efficacy and physical performance. *Archive of Physical Medicine and Rehabilitation* **87**, 680-687.

Patel, G. and Balk, R. (2007): Recognition and treatment of hyponatremia in acutely ill hospitalized patients. *Clinical Therapeutics* **29**, 211-29.

Patterson, M. J., Galloway, S. D. R. and Nimmo, M. A. (2000): Variations in regional sweat composition in normal human males. *Experimental Physiology* **85**, 869-875.

Peruzzi, W. T., Shapiro, B. A., Meyer, P. R., Jr., Krumlovsky, F. and Seo, B. (1994): Hyponatremia in acute spinal cord injury. *Critical Care Medicine* **22**, 252.

Petrofsky, J. S. (1992): Thermoregulatory stress during rest and exercise in heat in patients with a spinal cord injury. *European Journal of Applied Physiology and Occupational Physiology* **64**, 503-7.

Petrofsky, J. S. (2001): Blood pressure and heart rate response to isometric exercise: the effect of spinal cord injury in humans. *European Journal of Applied Physiology* **85**, 521-526.

Pham, P. T.(2006): REVIEWS - vasopressin excess and hyponatremia. *American Journal of Kidney Diseases : The Official Journal of the National Kidney Foundation* **47**, 727.

Phillimore, J., Leiper, J., Primrose, W. R., Primrose, C. S. and Society, N. (1997): A comparison of the daily water turnover rates in independent and dependent elderly. *Proceedings-Nutrition Society of London* **57**, 32A.

Pimentel, N. A., Sawka, M. N., Billings, D.S. and Trad, L.A. (1984): Physiological responses to prolonged upper-body exercise. *Medicine Science Sports and Exercise* **16**, 360-5.

Pivarnik J., Grafner T. and Elkins E. (1988): Metabolic, thermoregulatory, and psychophysiological responses during arm and leg exercise. *Medicine Science Sports and Exercise* **20**,1-5.

Pivarnik J. M., Leeds, E. M. and Wilkerson, J. E. (1984): Effects of endurance exercise on metabolic water production and plasma volume. *Journal of Applied Physiology* **56**, 613-618.

Poortmans, J. R., Hermans, L., Vandervliet, A., Niset, G. and Godefroid, C. (1997): Renal responses to exercise in heart and kidney transplant patients. *Transplant International: Official Journal of the European Society for Organ Transplantation* **10**, 323.

Popowski, L. A., Oppliger, R. A., Lambert, G. P., Johnson, R. F. and Johnson, A. K. (2001): Blood and urinary measures of hydration status during progressive acute dehydration. *Medicine and Science in Sports and Exercise* **33**, 747-753.

Price, M. J. (2006): Thermoregulation during exercise in individuals with spinal cord injuries. *Sports Medicine* **36**, 863-879.

Price, M. J. and Campbell, I. G. (1997): Thermoregulatory responses of paraplegic and able-bodied athletes at rest and during prolonged upper body exercise and passive recovery. *European Journal of Applied Physiology* **76**, 552-560.

Price, M. J. and Campbell, I. G. (1999): Thermoregulatory responses of spinal cord injured and able-bodied athletes to prolonged upper body exercise and recovery. *Spinal Cord* **37**, 772-779.

Price, M. J. and Campbell, I. G. (2003): Effects of spinal cord lesion level upon thermoregulation during exercise in the heat. *Medicine Science Sports and Exercise* **35**, 1100-1107.

Price, M. J. and Goosey-Tolfrey, V. (2008): Heat flow of the paraplegic and able-bodied lower limb during resting heat exposure. *Journal of Thermal Biology* **33**, 255-260.

Ragnarsson, K. T. (2008): Functional electrical stimulation after spinal cord injury: Current use, therapeutic effects and future directions. *Spinal Cord* **46**, 255-274.

Ragnarsson, K., Dickey, R., Byrnes, V., Youdin, M., Kolwicz, R. and Louie, H. (1981): Stand-up devices for the spinal-cord injured patients. *Archives of Physical Medical Rehabilitation* **62**, 538-539.

Ragnarsson, K., Pollack, S., O'Daniel, W. J., Edgar, R., Petrofsky, J. and Nash, M. (1988): Clinical evaluation of computerized functional electrical stimulation after spinal cord injury: A multicenter pilot study. *Archives of Physical Medical Rehabilitation* **69**, 672-677.

Raman, A., Schoeller, D. A., Subar, A. F., Troiano, R. P., Schatzkin, A. and Harris, T.(2004): Water turnover in 458 American adults 40-79 yr of age. *American Journal of Physiology* **286**, F394-F401.

Randall, W. (1946): Quantitation and regional distribution of sweat glands in man. *Journal of Clinical Investigation* **25**, 761-767.

Randall, W. C. (1946b): Sweat gland activity and changing patterns of sweat secretion on the skin surface. *American Journal of Physiology* **147**, 391-398.

Randall, W. and Hertzman, A. (1953): Dermatomal Recruitment of Sweating. *Journal of Applied Physiology* **5**, 399-409.

Randall, W., Hertzman, A. and Ederstrom, H. (1951): Dermatomal recruitment of sweating in response to heat. *Federation Proceedings* **10**, 108-108.

Randell, W. C., Wurster, R. D. and Lewin, R. J. (1966): Responses of patients with high spinal transection to high ambient temperatures. *Journal of Applied Physiology* **21**, 985-993.

Raymond, J., Davis, G. M., Fahey, A., Climstein, M. and Sutton, J. R. (1997): Oxygen uptake and heart rate responses during arm vs combined arm/electrically stimulated leg exercise in people with paraplegia. *Spinal Cord* **35**, 680-685.

Rehrer, N.J. and Burke, L.M. (1996): Sweat losses during various sports. *Australian Journal of Nutrition and Dietetics*, **53**, S13 - S16.

Reid, I. A. (1984): Actions of angiotensin II on the brain: mechanisms and physiologic role. *American Journal of Physiology Fluid Electrolyte Physiology* **246**, F533–F543.

Ribeiro, S., Da Silva, R., de Castro, I., and Tirapegui, J. (2005): Assessment of nutritional status of active handicapped individuals. *Nutrition Research* **25**, 239-249.

Richard, R., Verdier, J., Doutreleau, S., Piquard, F., Geny, B. and Rieu, M. (2005): Exercise limitation in trained heart and kidney transplant recipients: Central and peripheral limitations. *Journal of Heart and Lung Transplantation* **24**, 1774-1780.

Robertson, G. (1987): Physiology of ADH Secretion. *Kidney International* **32**, S20-S26.

Robertson G. L., Aycinena P. and Zerbe R. L. (1982): Neurological disorders of osmoregulation. *American Journal of Medicine* **72**, 339-353.

Robertson, M., Boyajian, M., Patterson, K., and Robertson, W. (1986): Modulation of the Chloride Concentration of Human Sweat by Prolactin. *Endocrinology* **119**, 2439-2444.

Roberts, M. F., Wenger, C. B., Stolwijk, J. A. and Nadel, E. R. (1977): Skin blood flow and sweating changes following exercise training and heat acclimation. *Journal of Applied Physiology: Respiration, Environment, Exercise Physiology* **43**, 133-137.

Robinson, T. A., Hawley, J. A., Palmer, G. S., Wilson, G. R., Gray, D.A., Noakes, T. D. and Dennis, S.C. (1995): Water ingestion does not improve 1-h cycling performance in moderate ambient temperatures. *European Journal of Applied Physiology and Occupational Physiology* **71**, 153-160.

Rodrigues, C. L., Gondim, F. D. A. A., Leal, P. R. L., Camurca, F. D., Freire, C. C. F. and Santos, A. A. D. (2001): Gastric emptying and gastrointestinal transit of liquid throughout the first month after thoracic spinal cord transection in awake rats. *Digestive Diseases and Science* **46**, 1604-1609.

Rodriguez-Alonso, M., Fernandez-Garcia, B., Perez-Landaluce, J. and Terrados, N. (2003): Blood lactate and heart rate during national and international women's basketball. *Journal of Sports Medicine and Physical Fitness* **43**, 432.

Saat, M., Sirisinghe, R. G., Singh, R. and Tochihara, Y. (2005): Effects of short-term exercise in the heat on thermoregulation, blood parameters, sweat secretion and sweat composition of tropic-dwelling subjects. *Journal of Physiological Anthropology and Applied Human Sciences* **24**, 541-549.

Saga, K. (2002): Structure and function of human sweat glands studied with histochemistry and cytochemistry. *Progress in Histochemistry and Cytochemistry* **37**, 323.

Sagawa, S., Miki, K., Tajima, F., Tanaka, H., Choi, J. K., Keil, L.C., Shiraki K. and Greenleaf, J. E. (1992): Effect of dehydration on thirst and drinking during immersion in men. *Journal of Applied Physiology* **72**, 128-134.

Saltin, B. and Hermansen, l. (1966): Esophageal, rectal, and muscle temperature during exercise. *Journal of Applied Physiology* **21**, 1757-1762.

Samson, W. (1988): Central Nervous-System Actions of Atrial Natriuretic Factor. *Brain Research Bulletin* **20**, 831-837.

Sand, P., Fordyce, W. and Fowler, R. J. (1973): Fluid intake behavior in patients with spinal-cord injury: Prediction and modification. *Archives of Physical Medicine and Rehabilitation* **54**, 254-262.

Sato, K. and Dobson, R. (1970): Enzymatic basis for the active transport of sodium in the duct and secretory portion of the eccrine sweat gland. *Journal of Investigative Dermatology* **55**, 53-56.

Sawka, M. (1992): Physiological consequences of hypohydration: Exercise performance and thermoregulation. *Medicine Science Sports and Exercise* **24**, 657-670.

Sawka, M. N., Burke, L. M., Eichner, E. R., Maughan, R.J., Montain, S .J. and Stachenfeld, N. S. (2007): Exercise and Fluid Replacement. *Medicine and Science in Sports and Exercise* **39**,377-390

Sawka, M. N., Cheuvront, S. N. and Carter III, R. (2005): Human water needs. *Nutrition Reviews* **63**, S30-S39.

Sawka, M. N., Convertino, V. A., Eichner, E. R., Schneider, S. M. and Young, A. J. (2000): Blood volume: Importance and adaptations to exercise training, environmental stresses, and trauma/sickness. *Medicine and Science in Sports and Exercise* **32**, 332-348.

Sawka, M. N., Gonzalez, R. R., Drolet, L. L. and Pandolf, K. B. (1984): Heat exchange during upper- and lower-body exercise. *Journal of Applied Physiology* **57**, 1050-1054.

Sawka, M., Gonzalez, R., Young, A., Dennis, R., Valeri, C. and Pandolf, K. (1989): Control of thermoregulatory sweating during exercise in the heat. *American Journal of Physiology* **257**, R311-316.

Sawka, M. N., Latzka, W. A. and Montain, S. J. (2001): Effects of dehydration and rehydration on performance. In R. J. Maughan (Ed.), *Nutrition in sport (1st ed., pp. 216-225)*. Blackwell Science. Oxford, U.K.

Sawka, M. N., Latzka, W. A. and Pandolf, K. B. (1989): Temperature regulation during upper body exercise: Able-bodied and spinal cord injured. *Medicine and Science in Sports and Exercise* **21**, S141-S148.

Sawka, M. N. and Montain, S. J. (2000): Fluid and electrolyte supplementation for exercise heat stress. *American Journal of Clinical Nutrition* **72**, 564S-572S.

Sawka, M. N. and Pandolf, K. B. (1990): Effects of body water loss on physiological function and exercise performance. In, Gisolfi, C.V. and Lamb, D. R.(Eds.), *Fluid Homeostasis during Exercise, p1-38,* Benchmark Press. Carmel, Indiana.

Sawka, M. N., Pimentel, N. A. and Pandolf, K. B. (1984): Thermoregulatory responses to upper body exercise. *European Journal of Applied Physiology* **52**, 230-234.

Schachter, J., Harper, P., Radin, M., Caggiula, A., McDonald, R. and Diven, W. (1980): Comparison of sodium and potassium intake with excretion. *Hypertension* **2**, 695-699.

Schrier, R.W. (Editor) (1999): *Diseases of the Kidney and Urinary Tract: Clinicopathologic Foundations of Medicine (3 Volumes Set).* Lippincott, Williams and Wilkins, Philadelphia USA

Schloerb, P., Friis-Hansen, B., Edelman, I., Solomon, A. and Moore, F. (1950): The measurement of total body water in the human subject by deuterium oxide dilution; with a consideration of the dynamics of deuterium distribution. *Journal of Clinical Investigation* **29**, 1296-1310.

Schoeller, D.A. (1996): Hydrometery. In: Roche, A. F., Heymfield, S. B., Lohman, T. G., (eds). *Human Body Composition p*23-43. Human Kinetics. Champaign, U.S.A.

Schoeller, D. and Van, S. E. (1982): Measurement of energy expenditure in humans by doubly labelled water method. *Journal of Applied Physiology* **53**, 955-999.

Schoeller, D.A., Dietz, W., Van Santen, E. and Klein, P. D. (1982): Validation of saliva sampling for total body water determination by H2 18O dilution. *American Journal of Clinical Nutrition* **35**, 591–594.

Schoeller, D.A., Van Santen, E., Peterson, D. W., Dietz, W., Jaspan, J. and Klein, P. D. (1980): Total body water measurement in humans with 18O and 2H labelled water. *American Journal of Clinical Nutrition* **33**, 2686–2693.

Schoenheimer, R., and Rittenberg, D. (1935): Deuterium as an indicator in the study of intermediary metabolism. *Journal of Biological Chemistry* **111**, 163-168.

Schmid, A., Huonker, M., Barturen, J., Stahl, F., Schmidt-Trucksass, A. and Konig, D. (1998): Catecholamines, heart rate and oxygen uptake during exercise in persons with spinal cord injury. *Journal of Applied Physiology* **85**, 635-641.

Schnermann, J. (1998): Juxtaglomerular cell complex in the regulation of renal salt excretion. *American Journal of Physiology* **43**, 263-279.

Schulz, O. and Lane, H. W. (1997): Adaptation of the doubly labelled water method for subjects consuming isotopically enriched water. Journal of *Applied Physiology* **82**, 563–570.

Seeley, R.R., Stephens, T. D. and Tate, P. (2003): *Anatomy and Physiology 6th edition.* McGraw-Hill Higher Education. London, U.K.

Segal, J. L., Milne, N. and Brunnemann, S.R. (1995): Gastric emptying is impaired in patients with spinal cord injury. *The American journal of Gastroenterology : Official Publication of the National Gastroenterological Association* **90**, 466.

Siener, R. and Albrecht, H. (2002): The effect of different diets on urine and the risk of calcium oxalate crystallisation in healthy subjects. *European Urology* **42**, 289-296.

Sepahpanah, F., Burns, S. P., McKnight, B. and Yang, C. C. (2006): Role of creatinine clearance as a screening test in persons with spinal cord injury. *Archive of Physical Medicine and Rehabilitation* **87**, 524-528.

Shamsuddiin, A. K. M., Kuwahara, T., Oue, A., Nomura, C., Koga, S. and Inoue, Y. (2005): Effect of skin temperature on the ion reabsorption capacity of sweat glands during exercise in humans. *European Journal of Applied Physiology* **94**, 442-447.

Shamsuddiin, A. K. M., Yanagimoto, S., Kuwahara, T., Zhang, Y., Nomura, C. and Kondo, N. (2005): Changes in the index of sweat ion concentration with increasing sweat during passive heat stress in humans. *European Journal Applied Physiology* **94**, 292-297.

Shi, X. and Gisolfi, C. V. (1998): Fluid and carbohydrate replacement during intermittent exercise. *Sports Medicine* **25**, 157-172.

Shimamoto, H. and Komiya, S. (2000): The turnover of body water as an index of health. *Journal of Physiological Anthropology and Applied Human Science* **90**, 207–212.

Shimamoto, H. and Komiya, S. (2003): Comparison of body water turnover in endurance runners and age-matched sedentary men. *Journal of Physiological Anthropology and Applied Human Science* **22**, 311-316.

Shirreffs, S. M. (2000): Markers of hydration status. *Journal of Sports Medicine and Physical Fitness* **40**, 80-84.

Shirreffs, S. M., Aragon-Vargas, L. F., Chamorro, M., Maughan, R. J., Serratosa, L. and Zachwieja, J. J. (2005): The sweating response of elite professional soccer players to training in the heat. *International Journal of Sports Medicine* **26**, 90-95.

Shirreffs, S. M., Armstrong, L. E. and Cheuvront, S. N. (2004): Fluid and electrolyte needs for preparation and recovery from training and competition. *Journal of Sports Sciences* **22**, 57-63.

Shirreffs, S., Casa, D. and Carter, R. (2007): Fluid needs for training and competition in athletics. *Journal of Sport Sciences* **25**, 83-91.

Shirreffs, S. M. and Maughan, R. J. (1997): Whole body sweat collection in humans: An improved method with preliminary data on electrolyte content. *Journal of Applied Physiology* **82**, 336-341.

Shirreffs, S. M. and Maughan, R. J. (1998): Urine osmolality and conductivity as indices of hydration status in athletes in the heat. *Medicine and Science in Sport and Exercise* **30**, 1598-1602.

Shirreffs, S. M., and R.J. Maughan (2000): Rehydration and recovery of fluid balance after exercise. *Exercise and Sport Science Review* **28**, 27-32.

Shirreffs, S. M., Merson, S. J., Fraser, S. M. and Archer, D. T. (2004): The effects of fluid restriction on hydration status and subjective feelings in man. *British Journal of Nutrition* **91**, 951-958.

Shirreffs, S. M., Sawka, M. N. and Stone, M. (2006): Water and electrolyte needs for football training and match-play. *Journal of Sports Sciences* **24**, 699-707.

Sica, D. and Culpepper, R. (1989): Severe hyponatremia in spinal cord injury. *American Journal of Medical Science* **298**, 331-333.

Sica, D., Zawada, E., Midha, M., Stacy, W., Falls, W. and Hussey, R. (1984): Hyponatremia(H) in the cord injured patient - a neglected phenomena. *Kidney International* **25**, 40A.

Silver, J. (1991): Body composition and resting energy expenditure in long term spinal cord injury. *Paraplegia* **29**, 496-497.

Silver, J. R. (2007): Thermoregulation in tetraplegic patients. *Spinal Cord* **45**, 460.

Silver, J. R. and Doggart, J. R. (2004): Reduced sodium output following acute spinal injury. *Spinal Cord* **42**, 191-198.

Silver, J., Doggart, J. and Burr, R. (1995): The reduced urinary output after spinal cord injury: A review *Paraplegia* **33**, 721-725.

Silver, S. M., Kozlowski, S. A., Baer, J. E. and Rogers, S. J. (1995): Glycine-induced hyponatremia in the rat: A model of post-prostatectomy syndrome. *Kidney International* **47**, 262-268.

Silver, A., Montagna, W. and Versaci, A. (1964): The effect of denervation on sweat glands and meissner corpuscles of human hands. *Journal of Investigative Dermatology* **42**, 307-24.

Silver, J., Randall, W. and Guttmann, L. (1991): Spinal mediation of thermally induced sweating. *Journal of Neurology Neurosurgery Psychiatry* **54**, 297-304.

Silverthorn, D. U. (2001): *Human Physiology: An Integrated Approach.* Prentice Hall, New Jersey, U.S.A.

Singh, J., Prentice, A., Diaz, E., Coward, W., Ashford, J. and Sawyer, M. (1989): Energy expenditure of Gambian women during peak agricultural activity measured by the doubly-labelled water method. *British Journal of Nutrition* **62**, 315-29.

Solomon, A., Edelman, I. and Soloway, S. (1950): The use of the mass spectrometer to measure deuterium in body fluids. *Journal of Clinical Investigation* **29**, 1311-9.

Soni, B. M., Vaidyanthan, S., Watt, J. W. H. and Krishnan, K. R. (1994): A retrospective study of hyponatremia in tetraplegic/paraplegic patients with a review of the literature. *Paraplegia* **32**, 597.

Stein, J., Osgood, R. and Kunau, R. (1976): Direct measurement of papillary collecting duct sodium-transport in rat - evidence for heterogeneity of nephron function during ringer loading. *Journal of Clinical Investigation* **58**, 767-773.

Stephens. J. l. (1972): Concentration of Urine in a Central Core Model of Renal Counter flow System. *Kidney International* **2**, 85.

Stevenson, R. D., Roberts, C. D. and Vogtle, L. (1995): The effects of non-nutritional factors on growth in cerebral palsy. *Developmental Medicine and Child Neurology* **37**, 124.

Stofan, J., Niksich, D., Horswill, C A., Martin, R., Bream, T., Murray, R. (2001): Sweat and sodium losses in cramp-prone professional football players. *Medicine and Science in Sports and Exercise* **33**, S256

Stofan, J. R., Osterberg, K. L., Horswill, C.A., Lacambra, M. and Eichner, E. R. (2007): Daily fluid turnover during preseason training in U.S. college football. *International Journal of Sport Nutrition and Exercise Metabolism* **17**, 340-51.

Stofan, J. R., Zachweja, J. J., Horswill, C. A., Murray, R., anderson, S. A. and Eichner, E. R. (2005): Sweat and sodium losses in NCAA football players: A precursor to heat cramps? *International Journal of Sport Nutrition and Exercise Metabolism* **15**, 641-652.

Stofan, J. R., Zachwieja, J. J., Horswill, C.A. Lacambra, M. and Murray, R l. (2003): Sweat and sodium losses in NCAA Division 1 football players with a history of whole-body muscle cramping. *Medicine and Science in Sports and Exercise* **35**, S48.

Stolwijk, J. and Hardy, J. (1966): Partitional Calorimetric Studies of Responses of Man to Thermal Transients. *Journal of Applied Physiology* **21**, 967.

Stover, E. A., Zachwieja, J., Stofan, J., Murray, R. and Horswill, C. A. (2006): Consistently high urine specific gravity in adolescent American football players and the impact f an acute drinking strategy. *International Journal of Sports Medicine* **27**, 330-335.

Strejc, J. (2000): Nutrition guidelines after kidney transplantation. *Journal of Renal Nutrition* **10**, 161-167.

Swain. D. P., Abernathy, K. S., Smith, C. S., Lee, S. J. and Bunn, S. A. (1994): Target heart rates for the development of cardiorespiratory fitness. *Medicine and Science in Sports and Exercise* **26**, 112-116.

Takamata, A., Mack, G. W., Gillen C. M. and Nadel, E. R. (1994): Sodium appetite, thirst, and body fluid regulation in humans during rehydration without sodium replacement. *American Journal of Physiology - Regulatory, Integrative and Comparative Physiology* **266**, R1493-R1502.

Tam, H., Darling, R., Cheh, H. and Downey, J. (1978): Sweating response - Means of evaluating set-point theory during exercise. *Journal of Applied Physiology* **45**, 451-8.

Transplant Sport UK, 2008. *British Transplant Games.* Retrieved 30/05/2008 http://www.transplantsport.org.uk/page.asp?section=000100010004

Theisen, D. and Vanlandewijck, Y. (2002): Cardiovascular responses and thermoregulation in individuals with spinal cord injury. *European Bulletin of Adaptive Physical Activity* **1**,1-17.

Theisen, D., Vanlandewijck, Y. and Sturbois, X. (2000): Cutaneous vasomotor adjustments during arm-cranking in individuals with paraplegia. *European Journal of Applied Physiology* **83**, 539-544.

Theisen, D., Vanlandewijck, Y., Sturbois, X. and Francaux, M. (2000): Blood distribution adaptations in paraplegics during posture changes: Peripheral and central reflex response. *European Journal of Applied Physiology* **81**, 463-469.

Theisen, D., Vanlandewijck, Y., Sturbois, X. and Francaux, M. (2001): Cutaneous vascular response and thermoregulation in individuals with paraplegia during sustained arm cranking exercise. *International Journal of Sports Medicine* **22**, 97-102.

Thibonnier, M., Coles, P., Thibonnier, A. and Shoham, M. (2001): The basic and clinical pharmacology of nonpeptide vasopressin receptor antagonists. *Annual Review of Pharmacology and Toxicology* **41**, 175-202.

Thomas, J. R. and Nelson, J. K. (1996): *Research methods in physical activity.* Human Kinetics. Champaign, IL. U.S.A.

Tolfrey, K., Goosey- Tolfrey, V. L. and Campbell, I. G. (2001): Oxygen uptake-heart rate relationship in eelite wheelchair racers. *European Journal of Applied Physiology* **86**, 174-178.

Tortora, G. J. and Grabowski, S.R. (2000). *Principles of Anatomy and Physiology. 9th edition.* John Wiley and sons. New York, U.S.A.

Tse, K., Lam, M., Yip, P., Li, F., Lai, K. and Chan, T. (2004): A long-term study on hyperlipidemia in stable renal transplant recipients. *Clinical Transplantation* **18**, 274-280.

Unnithan, V. and Bogdan. B. W. (2005): The effect of upper-limb dominance on forearm sweating patterns in children and adolescents with cerebral palsy. *Pediatric Exercise Science* **17**, 182-189.

Uno, H. (1977): Sympathetic innervation of the sweat glands and piloarrector muscles of macaques and human beings. *Journal of Investigative Dermatology* **69**, 112-20.

Uno, H. and Hökfelt, T. (1975): Catecholamine-containing nerve terminals of the eccrine sweat glands of macaques. *Cellular Tissue Respiration* **158**, 1-13.

Vaidyanthan, S., Soni, B., Hughes, P. and Singh, G. (2007): Possible role of large fluid intake in delaying formation of encrustations and, thereby, prolonging working life of memokath stent for nearly 14 years in a spinal cord injury patient. *Scientific World Journal* **7**, 1663 -1669.

Valent, L. J., Dallmeijer, A. J., Houdijk, H., Janssen, T. W., Hollander, A. P. and Van Der Woude, L.H. (2007): The individual relationship between heart rates and oxygen uptake in people with tetraplegia. *Spinal Cord* **45**, 104-111.

Van Marken Lichtenbelt. W., Westerterp, K. and Wouters, L. (1994): Deuterium dilution as a method for determining total body water: Effect of test protocol and sampling time. *British Journal of Nutrition* **72**, 491-7.

Vanlandewijck, Y. C., Daly, D. J. and Theisen, D. M. (1990): Field test evaluation of aerobic, anaerobic and wheelchair basketball skill performances. *International Journal of Sports Medicine* **20**, 548-554.

Vanlandewijck, Y. C., Evaggelinou, C., Daly, D. J., Verellen, J., Van Houtte, S. and Aspeslagh, V. (2004): The relationship between functional potential and field performance in elite female basketball players. *Journal of Sports Sciences* **22**, 668-675.

Verde, T., Shephard, R. J., Corey, P. and Moore, R. (1982): Sweat composition in exercise and in heat. *Journal of Applied Physiology* **53**, 1540-1545.

Vilches, J. J., Rodriguez, F. J., Verdu, E., Valero, A. and Navarro, X. (1998): Changes in cholinergic responses of sweat glands during denervation and reinnervation. *Journal of the Autonomic Nervous System* **74**, 134-142.

Waites, K. B., Waites, K. B., Canupp, K. C. and DeVivo, M. J. (1993): Epidemiology and risk factors for urinary tract infection following spinal cord injury. *Archives of Physical Medicine and Rehabilitation* **74**, 691.

Wald, N., and Leshem, M. (2003): Salt conditions a flavour preference or aversion after exercise depending on NaCl dose and sweat loss. *Appetite* **40**, 277-284.

Wall, S. M., Han, J. S., Chou, C. L. and Knepper, M.A. (1992): Kinetics of urea and water permeability activation by vasopressin in rat terminal. *American Journal of Physiology Renal Physiology* **262**, 989-998.

Wall, B. M., Williams, H. H., Presley, D. N., Crofton, J. T., Share, L. and Cooke, C. R. (1993): Vasopressin-independent alterations in renal water excretion in quadriplegia. *American Journal of Physiology* **265**, 460-466.

Webborn, N., Price, M. J., Castle, P. C. and Goosey- Tolfrey, V. L. (2005): Effects of two cooling strategies on thermoregulatory responses of tetraplegic athletes during repeated intermittent exercise in the heat. *Journal of Applied Physiology* **98**, 2101-2107.

Wecht, J. M., Marsico, R., Weir, J. P., Spungen, A. M., Bauman, W. A. and De Meersman, R. E. (2006): Autonomic recovery from peak arm exercise in fit and unfit individuals with paraplegia. *Medicine Science Sports and Exercise* **38**, 1223-1228.

Weschler, L. B. (2005): Exercise-associated hyponatraemia: A mathematical review. *Sports Medicine* **35**, 899-922.

Westerterp, K. R. (2001): Energy and water balance at high altitude. *News in Physiological Sciences* **16**, 134-137.

Westerterp, K. R. (1999): Body composition, water turnover and energy turnover assessment with labelled water. *Proceedings- Nutrition Society of London* **58**, 945-951.

Westerterp, K. R. Wouters, L. and Van Marken Lichtenbelt, W. (1995): The Maastricht protocol for the measurement of body composition and energy expenditure with labelled water *Obesity Research* **3**,49S-57S.

Williams, H. H., Wall, B. M., Horan, J. M., Presley, D. N., Crofton, J. T., Share, L. and Cooke, C.R. (1990): Nonosmotic stimuli alter osmoregulation in patients with spinal cord injury. *Journal of Clinical Endocrinology and Metabolism* **71**, 1536-1543.

Wilsmore B. R., Cotter J.D., Bashford, G. M. and Taylor, N. A. S. (2006): Ventilatory changes in heat-stressed humans with spinal-cord injury. *Spinal Cord* **44**, 160–164.

Wittbrodt, E. T. (2003): Maintaining fluid balance and electrolyte balance during exercise. *Journal of Pharmacy Practice* **16**, 45-50.

Woch, W., Grzeszczak, W., Wiecek, A ., Szczechowskazukowska, E and Kokot, F. (1993): Do Endocrine Abnormalities in Kidney-Transplant Patients and Patients with End-Stage Chronic-Renal-Failure Influence the Electrolyte-Composition of Sweat. *Nieren-Und ochdruckkrankheiten* **22**, 9-12.

Wong, W., Sheng, H., Morkeberg, J., Kosanovich, J., Clarke, L. and Klein, P. (1989): Measurement of extracellular water volume by bromide ion chromatography. *American Journal of Clinical Nutrition* **50**, 1290-4.

Wong, W. W., Cochran, W. J., Klish, W. J., Smith, E. O., Lee, L. S. and Klein, P. D. (1988): In vivo isotope fractionation factors and the measurement of deuterium- and oxygen- 18-dilution spaces from plasma, urine, saliva, respiratory water vapor, and carbon dioxide. *American Journal of Clinical Nutrition* **47**, 1-6.

Wurster, R., Hassler, C., McCook, R. and Randall, W. (1969): Reversal in Patterns of Sweat Recruitment. *Journal of Applied Physiology* **26**, 89.

Wyss, C. R., Brengelmann, G. L., Johnson, J. M., Rowell, L. B. and Niederberger, M. (1974): Control of skin blood flow, sweating, and heart rate: Role of skin vs core temperature. *Journal of Applied Physiology* **36**, 726-733.

Yaggie, J. A., Niemi, T. J. and Buono, M. J. (2002): Adaptive sweat gland response after spinal cord injury. *Archive of Physical Medicine and Rehabilitation* **83**, 802-805.

Yamasaki, M., Kim, K. T., Choi, S. W., Muraki, S., Shiokawa, M. and Kurokawa, T. (2001): Characteristics of body heat balance of paraplegics during exercise in a hot environment. *Journal of Physiological Anthropology and Applied Human Sciences* **20**, 227-232.

Yamasaki, M., Shiokawa, M. and Muraki, S. (2000): Effect of acute heat exposure on skin blood flow of the paralyzed thigh in persons with spinal cord injury. *Spinal Cord* **38**, 224-228.

Yang, T. F., Chan, R. C., Kao, C. L., Chiu, J. W., Liu, T.J., Kao, N. T. and Kuo, T. B. J. (2002): Power spectrum analysis of heart rate variability for cerebral palsy patients. *American Journal of Physical Medicine and Rehabilitation* **81**, 350-354.

Yoshida, T., Yoshida, T., Nakai, S., Yorimoto, A., Kawabata, T. and Morimoto, T. (1995): Effect of aerobic capacity on sweat rate and fluid intake during outdoor exercise in the heat. *European Journal of Applied Physiology and Occupational Physiology* **71**, 235.

Young, A. J., Sawka, M. N., Epstein, Y., Decristofano, B. and Pandolf, K. B. (1987): Cooling different body surfaces during upper body and lower body exercise. *Journal of Applied Physiology* **63**, 1218-1223.

Zambraski E. J. (1990): Renal regulation of fluid homeostasis during exercise. In: Gisolfi, C V and Lamb, D R (eds), *Fluid homeostasis during exercise*, Carmel, Indiana, Benchmark Press.

Zetou, E., Giatsis, G., Mountaki, F. and Komninakidou, A. (2008): Body weight changes and voluntary fluid intakes of beach volleyball players during an official tournament. *Journal of Science and Medicine in Sport*, **11**, 139-145.