Dietary Sodium Intake, Sweat Sodium, Salt Appetite and Exercise

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ABSTRACT

Background: Dietary sodium intake plays an important role in the regulation of fluid and electrolyte homeostasis within the human body. Both a high sweat sodium concentration and sweat rate can induce considerable sweat sodium losses during exercise. Excessive sodium loss is an important factor involved in the aetiology of exercise associated hyponatremia (EAH); a plasma sodium concentration <135mmol.L^{-1} during and/or after prolonged exercise, with reported prevalence rates higher amongst females than males. Salt appetite is described as a behavioural state that arises in response to physiological sodium deficiency in humans. During periods of body sodium depletion, this regulatory mechanism drives an individual to seek dietary sources of salt to support optimal regulation of sodium homeostasis.

Objective: Limited research has investigated the relationship between habitual dietary sodium intake and sweat sodium concentration during moderate intensity exercise. Given that females are at greater risk of EAH, the aim of this study was to investigate the association between dietary sodium intakes and sweat sodium losses amongst exercising females. A secondary aim was to assess the influence of whole body sweat sodium losses on acute salt appetite.

Methods: In a cross sectional study, fourteen recreationally active females participated in two exercise trials, separated by one week. In the 24 hours preceding each trial participants' completed a 24-hour urine collection and a 24-hour weighed food record to enable estimation of urinary sodium excretion and dietary sodium intake respectively. Participants' cycled in temperate laboratory conditions; 21°C (±1.1°C) and 87% (±6.5%) humidity for four intervals of ten minutes cycling, separated by a five

minute rest period. Blood and urine indices as well as subjective feelings of salt cravings were measured prior to and following exercise. Finally, a dietary behavior questionnaire (DBQ) regarding dietary salt intake was completed; for which a higher total score was reflective of a greater sodium intake. Sweat patches were placed prior to exercise on four regional body sites for estimation of sweat sodium concentration and whole body sweat sodium loss. Following exercise, the ad libitum addition of salt to oven-baked potato fries was measured.

Results: The mean (SD) whole body sweat sodium concentration during exercise was 46.9 (15.4) $mmol.L^{-1}$. A significant positive association was found between twenty-four hour urinary sodium excretion, the gold standard measure of dietary sodium intake, and whole body sweat sodium concentration during exercise ($p=0.026$). Total scores from the DBQ were significantly inversely associated with whole body sweat sodium losses during exercise ($p=0.012$). There was no significant association between whole body sweat sodium concentration or sweat sodium losses and acute salt cravings, nor the addition of salt to food post-exercise ($p>0.05$).

Conclusion: A greater acute dietary sodium intake is associated with an increase in whole body sweat sodium concentration during moderate intensity cycling exercise. The concentration of sodium in sweat or volume of whole body sweat sodium losses during exercise however do not appear to influence acute salt appetite in females; a finding to support the notion that sodium appetite may be an unconscious behavioural response to an acute period of body sodium depletion.

PREFACE

This research project was a part of the larger study: Resting and Exercising Sodium Tests. This thesis was supervised by Dr. Katherine Black, from the Department of Human Nutrition at the University of Otago.

Under supervision, the candidate was responsible for the following:

- Participant recruitment and eligibility screening
- Communication with participants and provision of appropriate equipment prior to laboratory trials
- Administration of questionnaires and fingerprick blood samples during laboratory trials
- Data collection and data entry
- Laboratory analysis of sweat, urine and blood samples
- Compilation of results including statistical analysis
- Interpretation of results and drawing study conclusions

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This thesis marks the end of an enjoyable five months of research, which concludes my two years of postgraduate Dietetic study.

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TABLE of CONTENTS

LIST of TABLES

LIST of FIGURES

LIST of ABBREVIATIONS

American College of Sports Medicine	ACSM
Antidiuretic hormone	ADH
Coefficient of variation	CV
Central nervous system	CNS
Day	d
Degrees Celsius	°C
Dietary behaviour questionnaire	DBQ
Exercise associated hyponatremia	EAH
Extracellular fluid	ECF
Gram	g
Hour	hr
Intracellular fluid	ICF
Kilogram	kg
Litre	L
Metre	m
Milligram	mg
Millilitre	mL
Millimetre	mm
Rate of perceived exertion	RPE
Resting and Exercising Sodium Tests	REST
Revolutions per minute	RPM
Standard Deviation	SD

1 INTRODUCTION

The role of dietary sodium in the maintenance and restoration of sodium homeostasis is critical to achieving optimal fluid and electrolyte balance amongst athletes and recreationally active individuals alike. The major source of sodium in the diet is in the form of sodium chloride, otherwise known as salt (NaCl), used in cooking, processing and seasoning (Valentine, 2007). Contemporary diets high in sodium are an important risk factor for cardiovascular diseases and a global reduction in salt intake is recommended for the reduction and treatment of hypertension (World Health Organisation, 2003, 2011). For exercisers who may lose a considerable volume of sodium through sweat however, the relationship between dietary salt intake, sweat sodium loss and sodium homeostasis has received less research attention.

During exercise, a high sweat sodium concentration and large volume of sweat loss can induce substantial whole body sodium losses (Shirreffs and Maughan, 1997). Sodium is the most abundant electrolyte in the extracellular fluid (ECF) and plays an important role in the regulation of plasma osmolality within the human body (Thibodeau and Patton, 2007). The tight knit relationship between fluid and sodium balance demonstrates the importance of optimal fluid and electrolyte replacement during exercise and/or recovery. Sodium intake may be supported through a physiological salt appetite that arises in response to body sodium deficiency (Geerling and Loewy, 2008). Research has postulated that this motivated behavioural response may be unconsciously regulated to preserve total body sodium within the normal physiological range (Geerling and Loewy, 2008; Leshem, 2009). During periods of sodium depletion, humans have shown to exhibit an increased palatability to and preference for salty foods; which will be reviewed in section 2.1.2 of the literature review (Beauchamp et

al., 1990; Huggins et al., 1992; Leshem et al., 1999; Takamata et al., 1994; Wald and Leshem, 2003).

Excessive fluid consumption relative to sweat rate is the primary cause mediating the development of exercise associated hyponatremia (EAH); a blood electrolyte disorder characterised by a plasma sodium concentration <135mmol.L^{-1} during or after prolonged exercise (Hew-Butler et al., 2008). An excessive loss of sodium through sweat however is also thought to be an important factor involved in the etiology of EAH (Hew-Butler et al., 2008; Montain et al., 2006). Following the publication of the first American College of Sports Medicine (ACSM) hydration guidelines in 1975, global prevalence rates of EAH have significantly increased amongst athletes (Noakes, 2010). Given the generally smaller stature and longer race time, female athletes are more likely to develop EAH during endurance events than males (Hew-Butler et al., 2008).

Importantly, the concentration of sodium in thermal sweat may be influenced by sodium intake, sweat rate and heat acclimation status of an individual (Allsopp et al., 1998). There is little evidence that currently exists to demonstrate the relationship between dietary sodium intake and sweat sodium losses during exercise, with even less research investigating salt appetite amongst females. The influence of habitual sodium intake on sweat sodium losses and consequently females' acute salt appetite therefore forms the foundation of this observational study. Hence, the significance and findings of this study may contribute to the body of evidence regarding sodium intake and exercise-induced sodium depletion to ultimately reduce risk of EAH.

2 LITERATURE REVIEW

2.1 Mechanisms of fluid and sodium homeostasis

The regulation of fluid and electrolyte balance within the human body is so closely controlled that the balance and/or imbalance of one is likely to impact on the other. A close understanding of both fluid and sodium homeostasis is fundamental to improving exercise performance; therefore this review will outline the regulation of fluid and sodium balance and the guidelines that exist to promote this.

2.1.1 Water and sodium balance

Within the human body, neural and hormonal mechanisms tightly regulate water intake and excretion to adjust the concentration of solutes in the ECF (Geerling and Loewy, 2008; Thibodeau and Patton, 2007). During exercise, fluid losses through sweat decrease plasma volume (hypovolemia), increasing blood solute concentration and osmotic pressure. Fluid migration from the intracellular fluid (ICF) to the ECF occurs in order to maintain normal solute concentrations. This physiological response is detected by the volume-depleted osmoreceptors of the hypothalamus and a neural message is initiated to induce thirst and water ingestion to restore blood volume (Luetkemeier et al., 1997; Thibodeau and Patton, 2007). In addition to the redistribution of fluid, antidiuretic hormone (ADH), or vasopressin, is secreted from the pituitary gland in response to hypovolemia. ADH is responsible for regulation of ECF concentrations and initiates its action through increasing water resorption into blood in the kidney tubules (Thibodeau and Patton, 2007). Following endurance activity, during which increased body water is lost through sweat, athletes have been found to have detectable levels of ADH; suggesting that the secretion of ADH is unable to be suppressed during prolonged exercise (Siegel et al., 2007). In order to maintain plasma

volume during exercise, this conservation of body water is critical to prevent performance decrements associated with dehydration. However, when the volume of sodium lost through sweat is large, inappropriately elevated levels of ADH and increased water resorption can result in a dilution of plasma sodium known as hyponatremia; discussed further in section 2.3.

Mechanisms of electrolyte balance are closely linked to fluid balance predominately through adjustments in water and/or sodium excretion (Geerling and Loewy, 2008; Thibodeau and Patton, 2007). Sodium, the most abundant electrolyte in the ECF, has the greatest influence on ECF osmolality and therefore regulation of the ECF volume. In response to body sodium deprivation, a steroid hormone named aldosterone is secreted into the blood from the adrenal gland. Aldosterone serves to regulate the resorption of sodium into blood in the kidney tubules and also stimulate sodium appetite through the central nervous system (CNS) (Geerling and Loewy, 2008; Thibodeau and Patton, 2007). This process of sodium resorption causes increased water retention to restore the ECF volume. When sweat losses become appreciable, such as with elevated environmental temperatures or prolonged high intensity exercise, resultant feelings of thirst may often lead to replacement of lost water but not sodium. Failure to adequately replace sodium lost in sweat can compromise adequate rehydration, as the ECF volume is directly proportional to the total body content of sodium. Low sodium rehydration solutions may often result in diuresis, therefore restoration of sodium balance is a prerequisite to establish euhydration; a normal body water content (Geerling and Loewy, 2008; Shirreffs et al., 2004; Shirreffs et al., 1996; Valentine, 2007).

2.1.2 Salt appetite

In humans, salt is generally regarded as highly palatable. Contemporary diets high in salt contribute to an excessive daily sodium intake; a modifiable risk factor associated with hypertension, commonly known as high blood pressure (World Health Organisation, 2003, 2011). Sodium appetite, also known as salt appetite, is described by Geerling and Loewy (2008) as the behavioural drive to ingest salt. This behavioural state arises in response to physiological sodium deficiency and like thirst, it is vital for restoring ECF volume to maintain fluid equilibrium in the body (Geerling and Loewy, 2008; Valentine, 2007). Despite this response, it is important to note that research has postulated that humans do not necessarily develop a 'hunger' for salt but instead demonstrate a preference of salt concentration in food (Beauchamp et al., 1990; Leshem, 2009; Leshem et al., 1999). Given that dietary sodium is most often consumed in the form of salt, both sodium appetite and salt appetite have been used interchangeably within prior research. In this thesis, this behavioural response will from now on be referred to as salt appetite.

A study by Leshem and colleagues (1999) investigated the influence of exercise on preferred salt concentrations in male students. One hour of exercise training resulted in an immediate increase in salt preference by over 50 percent; tested through the ad libitum addition of salt to soup within 30 minutes of terminating exercise. No changes in salt preference occurred amongst controls (no exercise). Notably, preferred salt concentrations amongst exercisers remained elevated twelve hours after exercise; highlighting the potential influence of salt appetite during periods of body sodium depletion. The observed results of this study may not be attributed to differences in lifestyle behaviours between exercisers and controls, as no differences in urinary

sodium concentration or discretionary salt intake were reported at baseline. Similar results were also demonstrated by Takamata et al. (1994) who reported increased salt appetite following exercise-induced sodium depletion. After eight 30-minute bouts of cycling exercise, palatability ratings to hypertonic salt solutions were highly correlated with plasma sodium levels throughout a 23-hour rehydration period. Subjective ratings of thirst, albeit not salt cravings, were obtained post-exercise. Interestingly, in this study increased salt palatability may be attributed to elevated plasma aldosterone levels at six hours post-exercise; leaving future research to investigate whether increased salt palatability may contribute to conscious discretionary salt intake post-exercise.

The vast majority of evidence supports the notion that the taste of salt becomes more attractive to humans as body sodium levels diminish (Beauchamp et al., 1990; Huggins et al., 1992; Leshem et al., 1999; Morris et al., 2008; Takamata et al., 1994). During periods of sodium deficiency, taste plays a central role in identifying food sources of salt to restore sodium balance (Morris et al., 2008). Beauchamp and colleagues (1990) reported an increased preference for salty foods in students following a very low sodium diet (112mmol over ten days). In contrast to exercise-induced sodium depletion, experimental sodium loss in this study was accomplished through administration of dietary sodium restrictions and diuretics. Although substantial body sodium depletion did not alter participants' salt sensitivity, this physiological state did influence their acute judgements of the palatability of salt taste in food; determined by way of a salty food desirability questionnaire. An earlier study by Beauchamp et al. (1987) also found that during a fourteen week period of dietary sodium restriction ($70mmol.d^{-1}$), discretionary table salt use increased to compensate for approximately 20 percent of the sodium reduction. It is interesting to note however that although no

changes in salt taste preferences were observed, the use of table salt reportedly increased in response to a decreased palatability of low sodium foods. Results therefore draw attention to the premise that the conscious addition of table salt may not reflect a behavioural response to physiological sodium depletion, but instead contribute to an increased palatability of food.

A contrary position was taken by Huggins et al. (1992) who investigated the influence of salt supplementation on salt preferences and discretionary salt use. In comparison to placebo, two weeks of salt supplementation (120mmol.d^{-1}) resulted in a significant reduction in the preferred level of salt added to unsalted tomato juice amongst healthy volunteers. More recently, Cosgrove and Black (2013) employed a 72 kilometre cycling time trial protocol and examined the influence of salt supplementation during exercise on acute salt appetite. Despite no difference in subjective salt cravings following exercise, placebo athletes consumed on average 275mg more sodium in a post-exercise meal than those in the sodium supplementation intervention ($p=0.047$). These findings align with earlier results from Wald et al. (2003), who reported that untasted salt supplements paired with a drink of root beer post-exercise conditioned a taste preference in direct proportion to the amount of sweat lost during exercise. Amongst the student exercisers who were unaware of salt supplementation, high levels of sweat loss induced a greater salt preference than low levels of sweating. However, in this study neither sweat sodium concentration nor whole body sweat sodium losses were measured to quantify the volume of sodium lost through sweat during exercise. Further investigation into the influence of whole body sweat sodium losses on acute salt appetite is warranted to explore potential conditioned flavour preferences and salt cravings post-exercise.

Existing evidence clearly demonstrates the influence of body sodium depletion on salt appetite. Previous studies have suggested however that the behavioural response of salt appetite following a period of salt deprivation may require many hours to show an effect (Geerling and Loewy, 2008; Takamata et al., 1994). Leshem et al. (1999) also drew attention to this idea, as they acknowledged that the rapidity of the increase in participants' salt preferences post-exercise were unexpected. Nevertheless, increased salt preference post-exercise is likely related to the state of mild sodium deprivation incurred and/or sympathetic activation of the hormones of sodium retention, namely aldosterone (Geerling and Loewy, 2008; Leshem et al., 1999). As acknowledged by Cosgrove and Black (2013), salt appetite may be generated without physical cravings in the mouth; however, the role of fluid replacement in the restoration of electrolyte homeostasis must also not be overlooked. Given considerable inter-individual variability in salt intake, controlling sodium intakes in laboratory settings can be impractical. Alternatively, estimations of dietary sodium intake may support investigation into the relationship between habitual salt intake, sweat sodium losses and acute salt appetite.

2.2 Development of the Hydration Guidelines

Over the past 40 years, hydration guidelines have alternated between the extremes of no fluid ingestion to aggressive patterns of fluid consumption during exercise (Beltrami et al., 2008). Given the relationship between fluid and sodium balance within the body, both of these strategies may impact on plasma sodium concentration. With limited information available regarding sodium balance however, less attention is paid to electrolyte regulation. This has contributed to a global increase in sodium related health problems amongst athletes, namely EAH; discussed further in section 2.3. The

following review of the hydration guidelines helps to understand the increased prevalence of EAH.

Historically, fluid restriction was a commonly accepted and enforced practice amongst endurance exercisers who were advised to ignore sensations of thirst during exercise (Kay and Marino, 2000). In contrast to this paradigm of fluid restriction, 1975 saw the publication of the first internationally recognised ACSM hydration guidelines, advising athletes to ingest fluids frequently during competition (American College of Sports Medicine, 1975). Race sponsors were advised to provide water stations at every 3–4 kilometres of an endurance race to prevent performance decrements associated with significant body water deficit.

Following an increase in the prevalence of thermoregulation injuries, in 1987 the ACSM released a subsequent position stand advising that frequent fluid consumption will reduce the risk of heat injury (American College of Sports Medicine, 1987). The frequency of water stations was increased to every 2–3 kilometres and it was recommended that athletes consumed fluid regularly to minimise dehydration. No mention however was made of the implications that this may have on plasma sodium concentrations or sodium balance. In 1996, an updated ACSM position stand was published which stated that during exercise fluid replacement should equal fluid loss (Convertino et al., 1996). In this publication electrolytes were mentioned for the first time, which is interesting given the interplay between hydration and electrolyte concentrations within the body. Notably, in both position stands athletes were advised to start drinking early and at regular intervals; promoting the widespread attitude of

aggressive hydration (600 – 1200mL.hr^{-1}) during endurance exercise (Convertino et al., 1996).

Dissemination of the latter advice promoted hyperhydration amongst athletes and a global increase in EAH (Noakes, 2010). Fluid consumption in excess of sweat rate is the primary factor involved in the aetiology of EAH, highlighting the importance of fluid and electrolytes when studying human fluid balance. Increases in the prevalence of over-drinking and EAH prompted a reformed ACSM position statement in 2007 (Sawka et al., 2007). This most recent position statement demonstrates significant developments in scientific literature as the authors utilised a strength of evidence taxonomy to recommend fluid replacement strategies based on rate of sweat loss. In recognition of inter-individual variability in total sweat losses, the ACSM recommends individualised fluid patterns of no more than 400 – 800mL.hr^{-1} to prevent excessive dehydration (>2% body weight loss) and changes in electrolyte balance (Sawka et al., 2007). During endurance exercise, it is recommended that athletes consume beverages containing 20 – 30mmol.L^{-1} of sodium, dependant on the specific exercise task and environmental conditions (Sawka et al., 2007). However, minimal evidence exists to demonstrate the influence of sweat sodium concentrations on the depletion of plasma sodium. As a result, this recommended beverage concentration lies at the lower end of typical sweat sodium concentrations that may range between 20 – 80mmol.L^{-1} (Maughan and Shirreffs, 1998). Further research is therefore warranted to contribute evidence upon which to base recommendations regarding sweat sodium loss and sodium intake during and/or following exercise.

2.3 Hyponatremia

EAH is a blood electrolyte disorder defined by a plasma sodium concentration <135mmol.L^{-1} during or up to 24 hours after prolonged exercise (Hew-Butler et al., 2008). The primary causative factor involved in the pathogenesis of EAH is an excessive fluid intake during exertion and/or recovery (Montain et al., 2006; Sawka et al., 2007). However, excessive sodium losses through sweat, heat or exercise stress and impaired renal function are additional causative factors that can contribute to the development of EAH; although very little research has investigated these factors on plasma sodium concentration (Hew-Butler et al., 2008; Montain et al., 2001; Rosner, 2009). EAH is a condition that is more frequently observed amongst female athletes than males (Almond et al., 2005; Hew-Butler et al., 2008; Speedy et al., 1999; Wagner et al., 2012). Speedy et al. (1999) reported that 45 percent of female race finishers in the NZ Ironman triathlon developed EAH compared with 14 percent of male race finishers; a result that may be attributed to smaller body stature (lesser body water) and a longer race time (Rosner and Kirven, 2007; Speedy, Noakes, Kimber, et al., 2001; Wagner et al., 2012). Research investigating sodium balance and risk of EAH amongst females during exercise of a shorter duration is however limited.

In humans, normal plasma sodium concentrations range between 135 – 146mmol.L^{-1} (Morris et al., 2008). Mild hyponatremia (plasma sodium 130 – 135mmol.L^{-1}) is often asymptomatic however symptoms of weakness, confusion, dizziness and nausea may develop (Montain et al., 2001; Murray and Eichner, 2004). These symptoms are similar to dehydration and may severely compromise exercise performance (Rosner, 2009). Symptomatic hyponatremia (plasma sodium <125mmol.L^{-1}) has been observed during prolonged exercise, where the risk of symptoms is associated with the rate of decline in

plasma sodium and length of time an individual is hyponatremic (Sawka and Young, 2006; Speedy, Noakes, and Schneider, 2001; Valentine, 2007). Manifestations of more severe EAH include seizures, cerebral oedema, dilutional encephalopathy and in extreme cases, coma or death (Hew-Butler et al., 2008; Murray and Eichner, 2004).

2.3.1 The development of exercise associated hyponatremia

Oral fluid consumption

Hyperhydration, a body water content in excess of normal fluctuations, occurs as a result of ingestion of hypotonic fluids in excess of sweat, urine and insensible losses (Shirreffs, 2003). During exercise, fluid replacement may be driven by thirst or behaviours in line with recommendations to avoid dehydration. This notion is supported by the rare incidence of EAH during the period when athletes were advised against fluid replacement during exercise (Rosner, 2009). Following the publication of the 1987 and 1996 ACSM position stands that advocated aggressive fluid replacement, the reported incidence of EAH began to rise globally; highlighting the close link between hydration and sodium balance (Noakes, 2010; Sawka et al., 2007).

In addition to endurance events, consumption of fluids at a rate greater than sweat loss has been observed amongst elite rugby players during resistance exercise (Cosgrove et al., 2014). Moreover, McLean (2012) recently reported a significant correlation between fluid ingestion and dilution of plasma sodium amongst male athletes following one hour of cycling exercise. Although Rosner et al. (2009) acknowledge that the current rate of fluid ingestion suggested by the ACSM (400 – 800mL.hr^{-1}) is well below the levels associated with development of dilutional hyponatremia (up to 1.5L.hr^{-1}), yet above that associated with exercise-induced dehydration; hyponatremia has been reported in those who ingest fluid at rates below 1.5L.hr^{-1} (McLean, 2012).

The influence of optimal sodium intakes to support the preservation of fluid and electrolyte balance during exercise and/or recovery however remains to be demonstrated.

Sweat sodium loss

Existing research demonstrates that athletes who excrete relatively salty sweat can complete endurance exercise both dehydrated and hyponatremic (Montain et al., 2006). Surprisingly, observational studies have reported cases of EAH amongst endurance athletes with only modest fluid intakes during exercise (300 – 700mL.hr^{-1}) (Black et al., 2014; Speedy, Noakes, and Schneider, 2001; Stuempfle, 2010). In light of this, excessive sodium losses through sweat play an important role in the pathogenesis of EAH (Hew-Butler et al., 2008; Montain et al., 2006; Montain et al., 2001; Rosner, 2009).

The concentration of sodium in thermal sweat is extremely variable between individuals, with typical values between 20 – 80mmol.L^{-1} (Maughan and Shirreffs, 1998). Importantly, sweat solute content is strongly influenced by diet, sweat rate and heat acclimitisation state of an individual; variables of which exhibit great inter-individual variation (Allsopp et al., 1998; Buono et al., 2007; Robinson and Robinson, 1954). Total whole body sweat sodium losses are the product of both sweat sodium concentration and sweat rate. Sweat rate increases in proportion to work rate, specifically exercise intensity and duration, and increments in environmental heat stress (Buono et al., 2007; Shirreffs et al., 2005). Amongst elite soccer players, Shirreffs et al. (2005) reported sweat rates between 0.99 – 1.93L.hr^{-1} and sweat sodium concentrations of a range between 15.5 – 66.3mmol.L^{-1}. Notably, the inherent demands of exercise

intensity and differences in body composition influence the variability observed in sweat sodium losses during exercise (Godek et al., 2005; Shirreffs et al., 2005). Greater sweat rates combined with the behavioural condition of excessive fluid consumption during exercise can significantly increase the potential of negative sodium balance and the development of EAH (Sawka and Young, 2006).

Marked inter-individual variations in sweat sodium losses render the volume of sodium intake to reduce risk of EAH unclear. Cosgrove and Black (2013), were one of the first to examine the role of salt supplementation (700mg.hr^{-1}) in the prevention of EAH. In this study, salt supplementation did not result in significantly higher sweat sodium losses between groups (placebo 27.1mmol.hr^{-1}; salt 40.8mmol.hr^{-1}; P=0.29). However, after controlling for dietary sodium intake, Cosgrove and Black (2013) suggest that acute sodium intakes may influence sweat sodium concentration and therefore whole body sweat sodium losses during exercise. These results hold importance at present as no definitive evidence exists to demonstrate a relationship between dietary sodium intake and sweat sodium losses during moderate intensity exercise.

2.4 Sodium indices

2.4.1 Urinary sodium excretion

For the estimation of dietary sodium intake, 24-hour urine collection is considered the gold standard (Land et al., 2014). This method of urinary analysis represents at least 90 percent of the sodium ingested at the time of collection and captures all sources of sodium intake including processed foods, medications and table salt use (Ji et al., 2012). Twenty-four hour urinary sodium excretion; calculated by multiplying 24-hour urine volume (L.d^{-1}) by 24-hour urinary sodium concentration (mmol.L^{-1}), can

therefore be used as a proxy measure of dietary sodium intake (Land et al., 2014; Pietinen, 1982). In the application of 24-hour urinary analysis to laboratory studies, potential limitations include an inaccuracy of completeness via under or over-collections and a high participant burden to collect all urine produced.

Early research by Holbrook et al. (1984) employed urinary sodium analysis, specifically 24-hour and 7-day samples, to assess daily nutrient intakes over one year. Urinary sodium excretion was significantly correlated with dietary sodium intake, albeit lower than intake. Alternative methods of analysis such as spot or overnight urine samples have been previously utilised, however whether these methods prove as reliable in estimating individual sodium intakes remains uncertain (Brown et al., 2013; Ji et al., 2012; Micheli and Rosa, 2003). Based on current literature to date, for the purpose of estimating 24-hour urinary sodium excretion and assessing nutrient intake, 24-hour urine collections are recommended (World Health Organisation, 2011).

2.4.2 Dietary sodium intake

Diet records, dietary recall or food frequency questionnaires are an alternative method to urinary analyses for the estimation of dietary sodium intake. However, as a result of widespread availability of processed foods and salt use 24-hour dietary record methods may be less reliable (Holbrook et al., 1984; Micheli and Rosa, 2003; Walker, 1996). Due to marked variability in dietary sodium intakes, multiple days of dietary assessment are recommended for greatest accuracy (Basiotis et al., 1987).

Food composition data that is collected in nutrition surveys is often associated with dietary analysis software. This may be used for estimations of sodium intake when

accurate data on the salt content of local foods are available (Pietinen, 1982). In the application of dietary assessment to research, the use of 24-hour dietary records for the estimation of individual sodium intakes may prove challenging. Quantifying salt intake based on the sodium content of commercial foods or food prepared at home alongside potential error during dietary documentation can limit the reliability of sodium intake estimations in comparison to urine collections (Micheli and Rosa, 2003; Pietinen, 1982). Selecting the most appropriate method for single assessment of dietary sodium intake is therefore an important consideration given the inherent strengths and weaknesses associated with 24-hour dietary records.

2.5 Conclusions

The majority of scientific evidence demonstrates the influence of optimal fluid and electrolyte balance on exercise performance and recovery. Importantly, restoration of fluid and electrolyte homeostasis is largely governed by exercise protocol, environmental conditions and individual physiological responses to exercise; specifically sweat rate and sweat sodium concentration. During periods of physiological sodium depletion; salt appetite, as exhibited through increased salt palatability, plays an important role in the behavioural drive to seek dietary sources of salt. Whether habitual dietary sodium intakes may influence whole body sweat sodium losses and therefore salt appetite however, remains to be investigated. Given the current body of literature, this study aims to investigate the association between dietary sodium intake, sweat sodium losses and acute salt appetite within the female population.

3 OBJECTIVE STATEMENT

A high sweat sodium concentration and large volume of sweat loss influence the physiological state of exercise-induced sodium depletion; an important risk factor involved in the etiology of EAH (Hew-Butler et al., 2008; Rosner, 2009; Rosner and Kirven, 2007). To our knowledge, there have been no studies to date that have investigated the relationship between dietary sodium intake and sweat sodium concentration during moderate intensity exercise. The majority of literature does however demonstrate increased salt appetite during periods of body sodium depletion (Leshem et al., 1999; Takamata et al., 1994; Wald and Leshem, 2003). Research investigating the influence of whole body sweat sodium losses on acute salt appetite is warranted to explore conditioned salt preferences and support optimal restoration of fluid and sodium balance post-exercise. Whether an increased salt appetite and/or salt preference may contribute to conscious discretionary salt intake following exercise remains unclear, as research investigating females' salt appetite and intake is limited. Given that this population is at greater risk of EAH, this is an important gap in scientific literature. Therefore, the primary aim of this study is to investigate the relationship between dietary sodium intake and whole body sweat sodium losses during exercise. Secondly, this study aims to investigate the influence of sweat sodium losses on acute salt appetite amongst the exercising female population.

4 METHODS

4.1 Study Design

This cross sectional study is part of the larger REST study: Resting and Exercising Sodium Tests, that aims to determine the reliability and validity of sweat sodium testing methods and the applicability of their use to athletes. This thesis focuses on the relationship between dietary sodium intake, whole body sweat sodium losses and salt appetite amongst recreationally active females.

This study received ethical approval from the University of Otago Human Ethics Committee (Health) in June 2014 and was conducted between July and September 2014. Participants were recruited via word of mouth, email and social media in Dunedin, New Zealand. They were informed of study procedures and any associated risks and gave informed written consent to participate (appendix A).

In order to reduce the chance of error in results, participants completed two trials, separated by at least seven days, in the Human Nutrition clinic at the University of Otago. At the completion of the study, all participants received a $10 grocery voucher to cover travel and parking expenses incurred during the study.

4.2 Participants

In the larger REST study, a sample size of 30 participants will provide a power of 0.80 at a p-level of less than 0.05 to detect a moderate association between testing variables, and account for a dropout rate of 10%. Due to time restraints in the present study however, we recruited fourteen female participants aged 19 – 34 years. They were recreationally active and reported to be healthy and free from injury before the exercise

testing sessions. Participants were excluded if they had cardiovascular disease, hypertension, blood disorders or were taking blood pressure medication. Participants were also ineligible if they had any sweat rate or sweat sodium related problem such as cystic fibrosis or hyperhidrosis, or they did not meet the inclusion criteria as outlined in the study protocol.

4.3 Experimental trials

4.3.1 Pre-test protocol

Twenty-four hour urine collection

In the 24 hours prior to each exercise trial, participants were asked to complete a 24-hour urine collection to enable estimation of urinary sodium excretion ($mg.d^{-1}$). Urine collections commenced after the first void on the day preceding the trial and were complete when participants arrived with their collections at the testing clinic. During this time all urine produced was collected into a five litre urine collection container. Urine collection equipment was provided three days prior to each trial.

Twenty-four hour diet record

Prior to their first trial, participants also completed a weighed diet record for 24 hours for the estimation of dietary sodium intake ($mg.d^{-1}$). They were asked to record as detailed measurements as possible of all food and fluid consumed, using weight or accurate portion size measures. Detailed 24-hour dietary records were recorded in a booklet provided with the urine collection equipment (appendix B). Diet records were handed in on arrival at the first trial. Each diet was typed electronically and emailed back to participants with instructions to replicate this prior to their subsequent trial, in order to standardise pre-trial dietary intakes. Dietary sodium intakes were analysed using the dietary analysis software Kai-culator (version 1.11a).

4.3.2 Pre-exercise measures

Each exercise trial was completed between 0630 – 0930 hours. On the day of testing participants arrived at the clinic following an overnight fast, with the exception of 500mL of water two hours before the trial to ensure euhydration.

Subjective questionnaires

Participants were first seated and asked to complete one pre-exercise subjective questionnaire regarding thirst and salt appetite (appendix C). This consisted of fourteen questions that asked them to rate feelings of thirst, mouth comfort and salt appetite on a 100mm visual analogue scale, anchored at each end by "not at all" and "extremely". Although not a validated tool, this subjective questionnaire has been previously utilised in research to determine feelings of thirst and salt appetite prior to and following exercise (Cosgrove and Black, 2013).

At the first trial, participants also completed a dietary behaviour questionnaire (DBQ) which comprised 31 questions to characterise habitual behaviours regarding salt intake (appendix D). Participants were asked to indicate answers on a four point scale that ranged from frequently to never. This DBQ questionnaire has not been validated for use in New Zealand, however has been previously employed in overseas research to characterise dietary behaviours regarding salt intake (Walker, 1996).

Blood sample

After participants had been seated at rest for fifteen minutes, a baseline finger-prick blood sample was obtained. Initially the finger was cleaned with an alcohol swab before being punctured with a BD Microtainer Lancet (BD Microtainer Contact-Activated Lancet, Plymouth, United Kingdom). Blood samples (approximately 2mL)

were collected into a labeled eppendorf tube and placed into a chilled container for laboratory analysis of plasma sodium concentration ($mmol.L^{-1}$).

Regional sweat patch placement

One square (8x8cm) absorbent sweat patch (3M Healthcare, Tegaderm+Pad, Loughborough, UK) was applied on the upper back, chest, forearm and mid-thigh on the right hand side of the body. Prior to patch positioning, skin regions were cleaned with deionised water and dried with a clean, electrolyte-free gauze swab. The patches remained in place during exercise to collect sweat as it was produced on the skin surface.

Body composition analysis

Participants then changed into pre-weighed exercise clothing (Wiltshire Fusion, Electronic Kitchen Scale, Auckland, NZ). At the first trial, body composition, including body mass, was measured on a set of electronic bio-electrical impedance (BIA) scales to the nearest 0.1 kilogram (Wedderburn BC-418, Tanita, Tokyo, Japan). Body mass only was obtained on these scales at the second trial. The dry weight of the clothing was subtracted from participants' body mass to determine their nude weight.

4.3.3 Exercise protocol

Exercise Intensity

Participants cycled on a stationary cycle ergometer (Mark III, Monark 815 Ergometer, Sweden). Before exercise, a wet heart rate monitor (Garmin Forerunner 110, Taiwan) was fitted around the chest. Participants started cycling at a fixed workload equivalent to two watts per kilogram of body mass, at a cadence of at least sixty revolutions per minute (RPM). This is a moderate intensity for a regular exerciser however was

adjusted to meet the ability of each participant if necessary. Each exercise session consisted of four intervals of ten minutes cycling, separated by a five minute rest period. At the end of each ten minute interval heart rate was recorded to monitor work rate.

Environmental conditions

During exercise, the room temperature was maintained at a mean (SD) temperature of 21^{o}C (1.1^{o}C) and 87% (6.5%) relative humidity to induce sweat production. Air temperature and relative humidity were recorded at the beginning of each ten minute interval (Endeavour Weather Station, Auckland, New Zealand).

4.3.4 Post-exercise protocol

Blood sample

At the conclusion of exercise, participants remained on the bike and a blood sample was obtained in a similar manner to pre-exercise (BD Microtainer Contact-Activated Lancet, Plymouth, United Kingdom) (section 4.3.2). Labeled eppendorf tubes were placed into a chilled container for laboratory analysis of plasma sodium concentration (mmol.L^{-1}).

Body mass

The heart rate monitor was removed and body mass was obtained (Wedderburn BC-418, Tanita, Tokyo, Japan). Participants' exercise clothing was weighed and this weight was subtracted from their post-exercise body mass to account for the sweat retained in clothing (Wiltshire Fusion, Electronic Kitchen Scale, Auckland, NZ). The change in body mass pre- to post-exercise was used to calculate sweat rate.

Regional sweat patch removal

Sterile tweezers that had been rinsed in deionised water were used to remove sweat patches. The sweat patches were placed in labeled sterile containers, sealed and stored in the freezer at -20°C before laboratory sweat extraction and analysis of sweat sodium concentration ($mmol.L^{-1}$).

Provision of food and drink

Participants were then invited into the metabolic kitchen and asked to complete a post-exercise subjective thirst and salt appetite questionnaire (100mm visual analogue scale), the same questionnaire as pre-exercise (Appendix C). Following this, they were provided with 200g (frozen weight) of straight cut, low sodium (28mg per 100g), oven baked potato fries (Pams Products Ltd, Auckland, NZ). A pre-weighed salt-shaker (Cerebos iodised table salt, NZ) was also offered for ad libitum consumption. After the meal the salt-shaker was re-weighed and the difference in weight was used to calculate the volume of salt consumed (to the nearest 100mg) (PB 801, Watson Victor Ltd, NZ).

4.4 Laboratory Analyses

4.4.1 Urine analysis

Each five litre 24-hour urine collection container was weighed on a set of electronic scales (Selectronic 2200, Salter Electronic, England) and adjusted for the weight of the container to determine the total 24-hour urine volume ($L.d^{-1}$). It was assumed that 1g of urine was equal to 1mL. Approximately 20mL of each collection was transferred with a disposable pipette into a labeled and sterile sealed tube. Samples were frozen at -20°C before laboratory analysis of sodium concentration ($mmol.L^{-1}$).

Twenty-four hour urine samples were defrosted before analysis of 24-hour urinary sodium concentration ($mmol.L^{-1}$) . This was performed using the Cobas c311 (Roche Hitachi, Tokyo, Japan) via the Ion Selective Electrode (ISE) technique, with a coefficient of variation of 0.9%. To reduce the effect of evaporation only 30 samples were analysed at once. Two controls containing a reference solution were also included in each run to measure any error. The Cobas c311 was calibrated before each analysis to enhance the accuracy of results.

4.4.2 Blood analysis

On the day of exercise testing, finger-prick blood samples were centrifuged for ten minutes at 5000 RPM (Mini Spin Plus, Global Science). Approximately 1mL of plasma was pipetted into a labeled and sterile eppendorf tube. Pre- and post-exercise plasma samples were stored in the freezer at -20°C before laboratory analysis of plasma sodium concentration ($mmol.L^{-1}$). Samples were later defrosted and sodium concentration analysis was performed using the Cobas c311 (Roche Hitachi, Tokyo, Japan) via the ISE technique, with a coefficient of variation of 1.0%. For the 34 samples with insufficient plasma volume for analysis, this data was excluded.

4.4.3 Sweat analysis

Sweat patches collected from the four regional body sites were removed from the freezer to defrost before laboratory analysis of sodium concentration ($mmol.L^{-1}$). Firstly, the containers were weighed and 1.5mL of deionised water was added to the sweat patches to aid sweat extraction. These were weighed again and left to absorb for one hour. All containers were then vortexed and 100 microlitres of sweat was extracted and transferred into a clear, labeled cuvette. Cuvettes were analysed for sodium concentration using the Cobas c311 (Roche Hitachi, Tokyo, Japan) via the ISE

technique, with a coefficient of variation of 0.5%. Similarly to urine analysis (section 4.4.1), only fourteen samples were analysed at once to reduce the effect of evaporation and an increase in sodium concentration. Two control samples of an 80mmol.L^{-1} sodium solution were also analysed in each run to assess the precision of measurements. In order to determine the sodium concentration in each respective patch, a dilution factor was multiplied by the sodium concentration obtained from Cobas c311 analysis.

To account for variation in regional site sweat sodium concentrations, all four sites were adjusted to a mean whole body sweat sodium concentration estimate using the equation;

Whole body sweat sodium concentration = 28.2% chest + 28.2% scapula + 11.3% forearm + 32.3% thigh

(Patterson et al., 2000).

In order to estimate whole body sweat sodium loss during exercise, sweat rate (estimated from body mass change during exercise) was multiplied by participant's mean whole body sweat sodium concentration.

4.4.4 Estimated sodium intake

Urinary sodium excretion (mmol.d^{-1}) was estimated by multiplying 24-hour urine volume (L.d^{-1}) by 24-hour urinary sodium concentration (mmol.L^{-1}). Urinary sodium excretion (mmol.d^{-1}) was then multiplied by 23 in order to convert from mmol.d^{-1} to mg.d^{-1} to provide an estimation of dietary sodium intake.

Twenty-four hour diet records were analysed for dietary sodium intake ($mg.d^{-1}$) using Kai-culator (version 1.11a); the dietary analysis software developed in the department of Human Nutrition, University of Otago (Department of Human Nutrition). The food composition database includes versions of FOODfiles from Plant and Food Research Ltd and recipes calculated for the 2008/09 New Zealand Adult Nutrition Survey. Where Kai-culator did not contain particular food items, appropriate substitutions were made for alternative foods with a similar nutritional profile.

4.4.5 Subjective questionnaires

As described in section 4.3.2, subjective questionnaires were used pre- and post-exercise to measure thirst and salt appetite on a 100mm visual analogue scale (appendix C). Scores for salt cravings (mm) were measured and recorded to determine participants' salt cravings prior to and following exercise.

The DBQ completed at trial one consisted of 31 questions that reflect dietary behaviours associated with a low or high salt intake (appendix D). For this questionnaire, individual answers for questions 1 – 26 that ranged from frequently to never were assigned a numerical four point likert scale score (3 – 0). This scale was reversed for negative questions (0 – -3) and a total score was calculated for each participant (Walker, 1996). The highest score possible was +48 and the lowest was -30. A higher total score was reflective of a greater habitual sodium intake.

4.5 Statistical analysis

All statistical analyses were performed using Stata version 11.2 (StataCorp LP, College Station, Texas, USA). Data is presented as means and standard deviations. Mixed model regression analysis was used to compare variable averages from the two trials, which accounted for random effects due to the repeated design. Unstandardised regression coefficients, 95% confidence intervals and p-values were calculated. For each regression the residuals were assessed for constant variance and normality. A probability value (P value) of less than 0.05 was set as the criterion for statistical significance.

5 RESULTS

Fourteen New Zealand European participants completed both laboratory trials. Baseline physical characteristics of participants are presented in table 5-1.

Table 5-1. Physical characteristics of participants: mean (SD) and range (n=14)

	Mean (SD)	**Range**
Age (years)	24.4 (4.4)	19 – 34
Height (cm)	167.4 (4.8)	159 – 178
Weight (kg)	64.2 (12.9)	47.2 – 101.2
BMI (kg/m^2)	22.8 (3.5)	18.7 – 32.2
Body fat (%)	27.8 (7.2)	15.8 – 44.4

5.1 Dietary sodium intake

The mean dietary sodium intake from 24-hour diet records (2927mg.d^{-1}) was greater than the mean 24-hour urinary sodium excretion (2205mg.d^{-1}), table 5-2. The highest sodium intake recorded from 24-hour diet records was 6848mg.d^{-1}, in comparison to the estimation from 24-hour urine which was 4668mg.d^{-1} for a different participant. The mean (SD) total DBQ score was 3 (11). The highest total DBQ score was 19, out of a highest possible score of 48. The lowest total score was -14, out of a lowest possible score of -30. A higher total score was indicative of a greater chronic dietary sodium intake.

Table 5-2. Dietary sodium intake ($mg.d^{-1}$), 24-hour urinary sodium excretion ($mg.d^{-1}$) and total dietary behaviour questionnaire scores (-30 – +48): mean (SD) and range

	Mean (SD)	**Range**
Dietary sodium intake ($mg.d^{-1}$) (24 hour diet record)	2927 (1595)	1242 – 6848
24-hour urinary sodium excretion ($mg.d^{-1}$)	2205 (946)	670 – 4668
Total DBQ score (-30 – +48)	3 (11)	-14 – +19

The total DBQ scores were significantly associated with dietary sodium intake from 24-hour diet records (B=83.516, 95% CI: 4.06, 162.97, p=0.041); with every one-point increase in total DBQ score associated with a $84mg.d^{-1}$ increase in dietary sodium intake as measured by diet records. There was no significant association between total DBQ scores and 24-hour urinary sodium excretion (B=1.91, 95% CI: -33.32, 37.14, p=0.915).

Dietary sodium intake, as measured from 24-hour diet records, was not linearly associated with 24-hour urinary sodium excretion (B=0.080, 95% CI: -0.15, 0.31, p=0.493), figure 5-1.

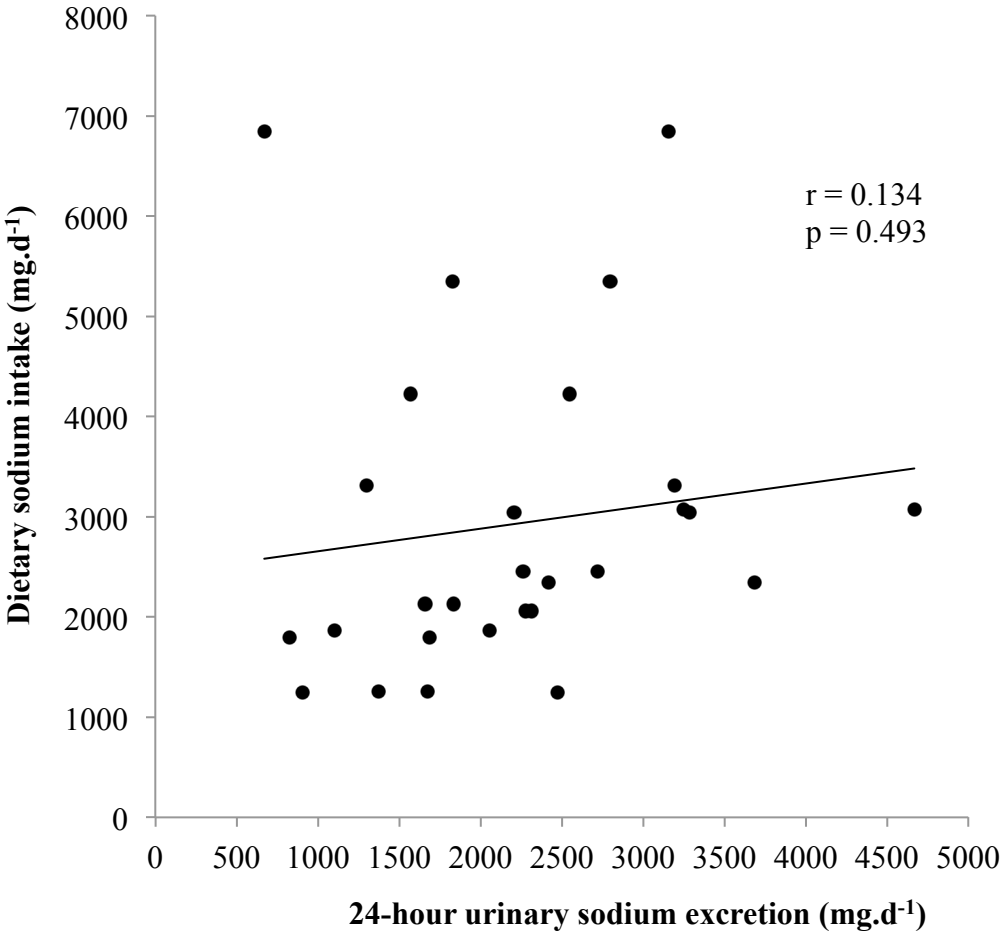

Figure 5-1. Association between 24-hour urinary sodium excretion ($mg.d^{-1}$) and dietary sodium intake ($mg.d^{-1}$)

5.2 Sweat sodium indices

The mean (SD) whole body sweat sodium concentration was 46.9 (15.4) $mmol.L^{-1}$ with a range between 20.7 – 66.9$mmol.L^{-1}$, table 5-3. The chest produced the highest sweat sodium concentration during exercise: 58.6 (18.8) $mmol.L^{-1}$ and the thigh produced the lowest: 36.8 (14.7) $mmol.L^{-1}$.

The mean whole body sweat sodium loss during exercise was 23.2 (9.8) $mmol.hr^{-1}$, with a range between 8 – 38.1$mmol.hr^{-1}$. This is equivalent to a mean (SD) sodium loss of 534 (225) $mg.hr^{-1}$, with a range between 184 – 876$mg.hr^{-1}$

Table 5-3. Regional site and whole body sweat sodium concentrations (mmol.L^{-1}) during exercise: mean (SD) and range

Sweat sodium concentration (mmol.L^{-1}):	**Mean (SD)**	**Range**
Chest	58.6 (18.8)	17.3 – 91.5
Upper back	50.6 (19.9)	22.7 – 81.0
Forearm	37.3 (13.8)	14.4 – 54.0
Thigh	36.8 (14.7)	12.0 – 62.6
Whole body	46.9 (15.4)	20.7 – 66.0

A significant positive association was found between 24-hour urinary sodium excretion and whole body sweat sodium concentration during exercise (p=0.026); every 100mg increase in urinary sodium excretion was associated with a 0.4mmol.L^{-1} increase in sweat sodium concentration, table 5-4. No association was observed between 24-hour urinary sodium excretion and whole body sweat sodium loss (p=0.802). This relationship however may be confounded by sweat rate. Indeed, when sweat rate was added into the regression model the association between 24-hour urinary sodium excretion and whole body sweat sodium loss was strengthened (B=0.002, 95% CI: -0.001, 0.005, p=0.191), although remained non-significant.

A significant inverse association was found between total DBQ score and whole body sweat sodium loss during exercise (p=0.012); for every one point increase in total DBQ score, whole body sweat sodium losses were 0.61mmol.hr^{-1} lower, table 5-4. There was no significant association between total DBQ score and whole body sweat sodium concentration during exercise (p=0.161).

No significant association was found between pre- or post-exercise salt cravings or change in salt cravings (pre- to post-exercise) and whole body sweat sodium concentration during exercise ($p>0.05$), table 5-4. Similarly, there was no significant association between salt cravings and whole body sweat sodium loss during exercise ($p>0.05$). The addition of salt to fries post-exercise was not significantly associated with whole body sweat sodium concentration ($p=0.806$) nor sweat sodium loss during exercise ($p=0.226$).

Table 5-4. Associations between whole body sweat sodium concentration ($mmol.L^{-1}$) or sweat sodium losses during exercise ($mmol.hr^{-1}$) and 24 hour urinary sodium excretion ($mg.d^{-1}$), total dietary behavior questionnaire score (-30 – +48), salt cravings (mm) and the addition of salt to food post-exercise (mg)

	Sweat sodium concentration ($mmol.L^{-1}$)			**Sweat sodium loss ($mmol.hr^{-1}$)**		
	B	**95% confidence interval**	**P value**	**B**	**95% confidence interval**	**P value**
24-hour urinary sodium excretion ($mg.d^{-1}$)	0.004	(0.00, 0.01)	0.026	0.001	(-0.01, 0.01)	0.802
DBQ score (-30 – +48)	-0.512	(-1.22, 0.20)	0.161	-0.610	(-1.09, -0.13)	0.012
Salt cravings (mm):						
Pre-exercise:	-0.226	(-0.55, 0.10)	0.176	-0.018	(-0.31, 0.27)	0.906
Post-exercise:	-0.019	(-0.23, 0.19)	0.856	0.014	(-0.20, 0.23)	0.898
Change:	0.048	(-0.14, 0.23)	0.612	0.024	(-0.19, 0.24)	0.830
Salt added to food post-exercise (mg)	-2.629	(-23.64, 18.38)	0.806	-10.541	(-27.62, 6.54)	0.226

5.3 Salt appetite

Subjective feelings of salt cravings increased by 27.4 (22.7) mm from pre- to post-exercise, table 5-5. For three participants, salt cravings decreased during exercise (range: -5.5 – 57.5mm). The mean (SD) amount of salt added to oven baked fries post-exercise was 338.5 (297.3) mg, although the least amount was 0mg and the greatest was 900mg.

Table 5-5. Salt cravings (mm) and the addition of salt to food post-exercise (mg): mean (SD) and range.

	Mean (SD)	**Range**
Salt cravings (mm):		
Pre-exercise	15.8 (18.4)	0 – 65
Post-exercise	43.1 (23.6)	6.5 – 70.5
Change (pre to post exercise)	27.4 (22.7)	-5.5 – 57.5
Salt added to food post-exercise (mg)	338.5 (297.3)	0 – 900

Neither pre-exercise salt cravings nor the addition of salt to fries post exercise were significantly associated with 24-hour urinary sodium excretion ($p=0.449$ and $p=0.716$ respectively), table 5-6.

Table 5-6. Associations between 24-hour urinary sodium excretion ($mg.d^{-1}$) and salt cravings (mm) or salt addition to food post-exercise (mg)

	24-hour urinary sodium excretion ($mg.d^{-1}$)		
	B	**95% confidence interval**	**P value**
Salt cravings (mm): pre-exercise	-0.002	(-0.006, 0.003)	0.449
Salt addition to food (mg)	-0.000	(-0.000, 0.000)	0.716

There was no significant association between post-exercise salt cravings and the addition of salt to fries post-exercise (B=0.004, 95% CI: -0.000, 0.007, p=0.078). Nor was there a significant association between the change in salt cravings (pre- to post-exercise) and the addition of salt to fries post-exercise (B=0.000, 95% CI: -0.003, 0.004, p=0.694).

5.4 Plasma sodium

Unfortunately, sufficient plasma volume for the analysis of plasma sodium concentration was not obtained pre- and post-exercise at each trial for all participants. Plasma sodium data was therefore unanalysable as there remained insufficient measurements for regression analysis. Nevertheless, from the available data the mean (SD) plasma sodium concentration pre-exercise (n=6) was 138.3 (2.8) $mmol.L^{-1}$, with a range between 137 – 142$mmol.L^{-1}$. Post-exercise, the mean (SD) plasma sodium concentration (n=5) was 139.0 (2.4) $mmol.L^{-1}$, with a range between 135 – 143 $mmol.L^{-1}$.

6 DISCUSSION

The findings of this study demonstrate that acute dietary sodium intakes, determined by way of 24-hour urinary sodium excretion, are associated with whole body sweat sodium concentration during cycling exercise. Secondly, whole body sweat sodium losses during exercise do not appear to influence females' acute salt cravings, nor the addition of salt to food post-exercise.

6.1 Dietary sodium intake and sweat sodium loss

A greater 24-hour urinary sodium excretion was significantly associated with a higher whole body sweat sodium concentration during exercise. Although only a slight increase in sweat sodium concentration ($0.4 mmol.L^{-1}$) was observed for every 100mg increase in urinary sodium excretion, this is a novel finding given the lack of research investigating the relationship between habitual dietary sodium intakes and sweat sodium concentration in females. However, future research is needed to establish the clinical relevance of this finding. In light of previous research which has shown that daily variations in sodium intake can effect sodium losses in urine and sweat, this result importantly reflects the regulation of bodily sodium homeostasis, as described in section 2.1.1 (Robinson and Robinson, 1954).

Surprisingly, there was no significant association between 24-hour urinary sodium excretion and whole body sweat sodium losses during exercise. However, when the regression analysis was adjusted for sweat rate this relationship was strengthened; suggesting that external variables such as state of fitness, genetics or heat acclimation may confound this relationship (Buono et al., 2007; Maughan et al., 2005). Whole body sweat sodium losses are considerably influenced by sweat rate, which in turn increases

in proportion to exercise intensity and environmental heat stress (Buono et al., 2007; Shirreffs et al., 2005). Although participants in our study were recreationally active, their level of physical fitness was not quantified at baseline. A defined fitness and heat acclimatisation status may have therefore influenced sweat sodium losses. Nevertheless, this finding is similar to results from Cosgrove and Black (2013) who reported no significant difference in sweat sodium losses between salt supplementation and placebo ($p=0.29$). Differences in methodology between studies must be acknowledged however, with particular regard to salt supplementation during exercise. In comparison to one hour of interval style training, Cosgrove and Black (2013) employed an outdoor 72 kilometre time trial with nine trained cyclists and reported much higher mean sweat sodium losses (placebo, 27.1mmol.hr^{-1} and salt, 40.8mmol.hr^{-1}) than in the present study (23.2mmol.hr^{-1}). Given such large variability in sweat sodium losses, it is likely that a small sample size plays a role in this non-significant finding. Future research employing prolonged high intensity exercise is warranted to induce greater sweat sodium losses and provide further insight into the influence of dietary sodium intakes on the volume of sodium lost through sweat.

In light of a more chronic measure of salt intake, a higher total score from the DBQ was significantly associated with a reduction in whole body sweat sodium loss. Although, with every one point increase in total DBQ score, sweat sodium losses decreased by only 0.61mmol.hr^{-1}; a clinically insignificant volume given the observed range between 8 – 38.1mmol.hr^{-1}. To our knowledge this is the first study to investigate the relationship between chronic dietary sodium intakes and sweat sodium losses. As the DBQ in this study was designed for use in American populations, particular questions may not provide a valid indicator of habitual salt intake within the New Zealand

population. Supplementary research utilising a more specific measure of chronic dietary sodium intakes may therefore be warranted to improve the precision of sodium intake estimations for future investigation.

Furthermore, no association was observed between the two measures of dietary sodium intake; 24-hour diet records and 24-hour urinary sodium excretion. This finding contrasts with the majority of literature which suggests that 24-hour diet records are consistent with sodium intake as measured by 24-hour urine; a method recognised as less susceptible to error during data collection and analysis (Holbrook et al., 1984; Micheli and Rosa, 2003; Walker, 1996). Considerable differences in sample sizes may explain this observed disparity as Micheli et al. (2003) and Walker et al. (1996) studied 188 children and 41 adults respectively, in contrast to the 14 participants in this study. Additionally, differences in dietary sodium intake estimations may be attributed to errors in 24-hour dietary recording and/or analysis, or missed urine collections during the 24-hours preceding each trial; noteworthy limitations that are associated with the use of these methods (Land et al., 2014; Pietinen, 1982). As portrayed in figure 4-1, it is also possible that outlying data could reflect changes in participant's diets during the data collection period, or dissimilar dietary replication in the 24-hours preceding each trial. As outliers were not adjusted for during data analysis, this may also serve to confound the relationship.

6.2 Salt appetite

A second aim of this study was to investigate the effect of sweat sodium losses on acute salt appetite. Interestingly, participants with a higher whole body sweat sodium concentration or sweat sodium loss during exercise did not experience greater acute salt cravings nor add more salt to fries following exercise. This finding contrasts the work

of Wald et al. (2003), who postulate that conditioning of salt preference post-exercise may be related to the volume of sweat loss; suggesting a relationship between sweat sodium losses and increased salt palatability (Leshem, 2009). Results from the present study may however be in part explained by a low mean (SD) volume of sweat sodium loss during exercise (23.3 (9.8) $mmol.hr^{-1}$); equivalent to 534$mg.hr^{-1}$ of sodium. In consideration of post-exercise recovery foods and/or fluids, this volume of sodium would equate to approximately two slices of wholegrain bread with cheese, 750mL of a commercial sports drink and 100g of canned tuna or one large bowl of cereal and milk (Sivakumaran et al., 2013). Following exercise of one-hour duration, the volume of sweat sodium losses observed in this study may be appropriately replaced through the consumption of recovery snacks. However, following exercise of a longer duration where sweat sodium losses may be more appreciable, optimal restoration of body sodium could prove more difficult in the absence of conscious salt cravings.

In addition, it must be acknowledged that results portray an overall mean increase in salt cravings of 24mm pre- to post-exercise; a change in salt appetite that may likely increase further with greater sweat sodium losses. This increase in salt appetite may be attributed to hypovolemia, as a result of fluid and sodium loss through sweat, and sympathetic activation of the hormones that stimulate sodium appetite in humans (Geerling and Loewy, 2008; Leshem et al., 1999). This finding complements results from Leshem et al. (1999) who reported an overall increase in the mean amount of salt added to soup following one hour of exercise. Although the authors did not specifically measure salt cravings, a baseline measure of the addition of salt to fries in our study would have enabled direct comparison of changes in salt appetite following moderate intensity exercise. Interestingly, Takamata et al. (1994) also reported an increase in salt

appetite following cycling exercise through the use of subjective palatability ratings to salt solutions. The difference between palatability ratings to pre-salted solutions and measures of salt cravings however, highlights a potential dissimilarity between preferences for salt in food and/or fluid and cravings for table salt itself. This idea has been previously explained by Leshem et al. (1999) who postulate that humans may show more of a preference for salt concentration when consuming food, rather than a specific hunger for salt; an idea that may explain the insignificant association between whole body sweat sodium losses and post-exercise salt cravings in the present study.

Following on, post-exercise salt cravings were not associated with the addition of salt to fries after exercise. This suggests that greater salt cravings may not influence the conscious addition of salt to food during an acute period of body sodium depletion. However, although this relationship was non-significant, it was in fact a strong positive association ($p=0.078$); for which a larger sample size may serve to strengthen. In consideration of the behavioural association between saltshakers and food, the use of table salt may actually represent learned behaviours or habit, rather than characterise salt preferences with resulting sensory attributes of food (Mittelmark and Sternberg, 1985). The majority of participants were health conscious individuals, who may have chosen to abstain from the use of salt despite reporting increased salt cravings post-exercise. Alternatively, the addition of salt could instead arise from previous sensory associations with more palatable salted fries; an association between flavour and learned exposure that may account for a lot of human salt intake (Leshem, 2009).

6.3 Strengths and limitations of the study

A number of strengths of this cross sectional study render the results applicable to exercising females. Primarily, this study is the first of its kind to investigate the

influence of both acute and chronic measures of dietary sodium intake on sweat sodium losses during one hour of exercise. The use of two identical exercise trials served to reduce the chance of error in results, as statistical analyses were adjusted for duplicate measurements of urine and sweat indices. In addition, a laboratory based study design also enabled control of factors such as ambient temperature, relative humidity and exercise intensity; variables which have a major influence on the sweating response (Maughan et al., 2005).

Whilst utilising such methods was advantageous, limitations of this study do limit the wider interpretation of results. Although 24-hour urine collections are considered gold standard for the estimation of sodium intake, daily sodium intakes exhibit considerable intra-individual variability. Multiple days of urinary and/or dietary assessment are therefore recommended for greatest accuracy (Basiotis et al., 1987; Land et al., 2014). Due to time restraints and a high participant burden however, multiple days of assessment were not possible. Twenty-four hour urinary sodium excretion was therefore appropriately employed as the primary measure of dietary sodium intake.

Unfortunately, due to unforeseen blood sampling error during data collection, plasma sodium concentrations were unanalysable. Measures of whole body sweat sodium concentration and sweat sodium losses were therefore analysed to characterise exercise-induced sodium depletion. Additionally, blood levels of ADH and aldosterone were not measured in our study. Future investigation employing such indices of sodium homeostasis will provide further evidence regarding the relationship between dietary sodium intake, sweat sodium losses and risk of EAH.

Furthermore, it is likely that our small sample size reduced the statistical power to detect true associations between dietary sodium intake and sweat sodium losses. In consideration of this and together with multiple regression analyses, it is possible that positive relationships were identified that may have been due to chance. Although the inclusion of 14 participants was appropriate due to the timeframe and a high respondent burden of this study, further investigation with a larger population of recreationally active females may serve to validate results.

6.4 Conclusions and future research

In conclusion, this study portrays the association between acute dietary sodium intake and whole body sweat sodium concentration during moderate intensity exercise. Results suggest that salt appetite is not influenced by sweat sodium losses during exercise of one hour duration; a finding to reinforce the notion that salt appetite may be an unconscious behavioural response to an acute period of mild body sodium depletion.

In light of a small sample size however, results must be interpreted with caution. Given extensive inter-individual variability in both sweat composition and sweat rate, findings warrant for further investigation into whether habitual sodium intakes influence sweat sodium losses, or whether large sweat sodium losses can increase salt cravings and therefore dietary salt intake. Research employing a larger study population, longer exercise duration and measures of influential indices of sodium homeostasis will contribute further evidence upon which to base sound conclusions regarding sodium intake and sweat sodium losses to promote optimal fluid and sodium balance and reduce risk of EAH.

7 APPLICATION TO PRACTICE

Given significant inter-individual variability in sweat sodium loss, the implications of the present study are relevant to the provision of dietary advice to exercising females. With respect to the observed association between acute dietary sodium intakes and whole body sweat sodium concentration during moderate intensity exercise, results may be pertinent to both sports dietitians and female exercisers. The findings of this study draw attention to the relationship between dietary sodium intake and sweat sodium losses; for which further research in this field is warranted to confirm the association. Whether a greater dietary sodium intake and therefore a higher sweat sodium concentration may contribute to increased risk of EAH is an important consideration when addressing habitual sodium intake; posing a clear avenue for future research.

In the effort to promote optimal sodium balance during exercise and/or recovery, practitioners within the dietetic field can acknowledge that consideration must not only be given to variables that influence sweat sodium loss, such as exercise protocol and environmental conditions, but also the sodium content of the diet. Given the insignificant association found between whole body sweat sodium losses, acute salt appetite and the addition of salt to fries post-exercise, dietetic professionals can acknowledge the existence of an unconscious salt appetite that may aid the physiological regulation of body sodium during recovery. However, it must be recognised that sweat sodium losses in this study were slight and may be replaced through appropriate post-exercise recovery foods and/or fluids. As highlighted throughout this thesis, it should be noted that it can be difficult to accurately assess an individual's habitual sodium intake. The discretionary use of table salt both in cooking

and at the table is often less than common kitchen scales can measure, therefore the amount of sodium actually ingested can prove difficult to determine. Further, inaccuracies in dietary analysis software or incomplete urine collections may additionally contribute to error in estimation of habitual sodium intake. Although, in practice today, contemporary sodium intakes are considerably higher than recommended. In consideration of this and together with greater sweat sodium losses during prolonged high intensity exercise; whether the diets of exercising females may contribute to optimal replacement of sodium lost through sweat is an area for future investigation.

8 REFERENCES

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9 APPENDICES

A. Participant information sheet and consent form

B. Twenty-four hour diet record

C. Thirst and salt appetite questionnaire

D. Dietary behaviour questionnaire

Appendix A. Participant Information Sheet

Study title:	REST study: Resting and Exercising Sodium Tests. How useful are resting sweat sodium tests for athletes?	
Principal investigator:	**Name: Katherine Black** **Department of Human Nutrition** **Position: Senior Lecturer**	Contact phone number: 03 479 8358

Introduction

Thank you for showing an interest in this project. Please read this information sheet carefully. Take time to consider and, if you wish, talk with relatives or friends, before deciding whether or not to participate.
If you decide to participate we thank you. If you decide not to take part there will be no disadvantage to you and we thank you for considering our request.

What is the aim of this research project?

In athletic settings, sweat sodium concentration, could be important for both performance and health. There is a growing market in sweat testing for athletes but these methods have not been scientifically evaluated.

This study aims to determine:

1) the reliability of commercial sweat testing methods compared to whole body washdown techniques and commonly used regional sweat patches

2) the applicability of its use to athletes.

Therefore the results of this study could help inform athletes on the usefulness of commercial sweat tests.

This study will form the basis of two MDiet thesis.

Who is funding this project?

This study is funded by a University of Otago Research Grant (UORG).

Who are we seeking to participate in the project?

We are looking to recruit individuals aged 18-65 years, who train at least 3 times a week for at least one hour at each session. You should also be familiar with stationary cycling and free from injury.
However, if you have any blood disorder, cardiovascular disease, hypertension, or are taking blood pressure medication you will not be able to participate. You will also be unable to participate if you have any sweat rate or sweat sodium related problem such as cystic fibrosis or hyperhidrosis. The sweat patches we will use are similar to elasotplasts so if you have an allergy to these you should not participate.

If you participate, what will you be asked to do?

24 hours before the first trial you will weigh and record all food and drink you consume. You will then replicate this diet prior to your second and third trials. You will also collect a 24 hour urine sample in the day prior to all the trials. These will be analysed for electrolytes and hydration status. These measures may add 5 minutes to each eating/drinking occasion and urination.

Test days: You will attend the clinic following an overnight fast, with the exception of 500mL of water 2 hours before each trial.

- Upon arrival, you will be asked to provide a urine sample which will be measured for hydration status and electrolytes.
- You will then sit for 15 minutes before a baseline fingerprick blood sample is obtained this will be measured for fluid balance and electrolytes.
- Following this a sweat sample will be obtained at rest. This involves placing gel discs on your forearm, then sending a small electronic charge to the discs to stimulate sweat production.
- You will then be weighed in private so a nude body mass can be obtained. Following this you will **wash your skin with deionised water** and four small square (8*8 cm) sweat patches will be positioned. These patches will be applied on the shoulder, chest, forearm and thigh on the right hand side of your body. These patches comprise a gauze swab with a plastic adhesive backing and will remain in place during exercise and collect sweat as it is produced on the skin surface below the patch.
- A heart rate monitor will be placed around your chest, so heart rate can be monitored during the exercise protocol.
- You will then cycle on a stationary bike **(located in a sterile container)** for four blocks of 10 minutes cycling, separated by 5 minutes rest (a total of 60 minutes), at moderate intensity. During the 5 minute rest periods you will be reweighed in minimal clothing.
- At thirty minutes a sweat sample will again be collected at rest (as above).
- At the end of exercise, the sweat patches will be removed and analysed for electrolytes. **You will wash yourself with deionised water containing a tracer solution.** A fingerprick blood sample will be obtained. You will then towel dry and be weighed again (nude). The change in weight will be used to calculate sweat volume.

The first trial will be used to familiarise you with the testing procedures. During next two trials the temperature of the room for the exercise protocol will be kept at approximately 20^{o}C or 35^{o}C.

- Finally you will be asked to complete a questionnaire on your thirst and desire for salt. **You** will be provided with food and drink before you leave the clinic. The total time required for each clinic visit is 1hour 30 minutes to 2 hours.

Therefore the total time commitment is 2.5 hours per trial (clinic visit plus diet and urine collection) for three trials so 7.5 hours in total.

You will be reimbursed $10 to cover your expenses such as petrol and parking for your clinic visits.

Is there any risk of discomfort or harm from participation?

Any exercise has the potential to cause discomfort and injury, this is why we are recruiting trained participants. A heart rate monitor will continuously measure your heart rate so we can monitor how hard you are working and stop the exercise if heart rate should increase substantially. In order to reduce the risk of any complications during the testing protocol at least two researchers will be present at all times at least one will have a first aid certificate. A defibulator is located next to the clinic and a first aid kit is present in the room.

Fingerprick blood samples can cause minor discomfort and slight bruising to the finger but these will be taken by trained researchers and only two will be taken each trial (separated by at least one week). We will ensure that your hand is warm before taking the sample this will assist with blood flow.

What specimens, data or information will be collected, and how will they be used?

Tissue samples (blood, urine, sweat) will be stored in containers labeled by ID only, so you cannot be identified. They will be kept at -20^{o}C until analysis in the locked freezer room in the Department of Human Nutrition.

Upon completion of the study samples will be disposed of either by standard methods or via Karakia (Māori Prayer*)*.

What about anonymity and confidentiality?

All written records will be stored securely in Dr Katherine Black's office, as will the list connecting the ID numbers present on the raw data to the names of individual participants. Working data files containing non-identifiable information only, will be stored on the Department of Human Nutrition computer server which is password protected. Access to the files will be restricted to the study investigators. The material will be shredded ten years following publication of information in a scientific journal, as per University requirements.

If you agree to participate, can you withdraw later?

You may withdraw from participation in the project at any time and without any disadvantage to yourself.

Any questions?

If you have any questions now or in the future, please feel free to contact either:

Name: Katherine Black **Position: Senior Lecturer** **Department of Human Nutrition**	Contact phone number: (03) 479 8358

This study has been approved by the University of Otago Human Ethics Committee (Health). If you have any concerns about the ethical conduct of the research you may contact the Committee through the Human Ethics Committee Administrator (phone +64 3 479 8256 or email gary.witte@otago.ac.nz). Any issues you raise will be treated in confidence and investigated and you will be informed of the outcome.

Participant consent form

REST study: Resting and Exercising Sodium Tests. How useful are resting sweat sodium tests for athletes?

Principal Investigator: Dr Katherine Black (e-mail katherine.black@otago.ac.nz and telephone number: 03 479 8358)

CONSENT FORM FOR PARTICIPANTS

Following signature and return to the research team this form will be stored in a secure place for ten years.

Name of Participant:……..

1. I have read the Information Sheet concerning this study and understand the aims of this research project.
2. I have had sufficient time to talk with other people of my choice about participating in the study.
3. I confirm that I meet the criteria for participation which are explained in the Information Sheet.
4. All my questions about the project have been answered to my satisfaction, and I understand that I am free to request further information at any stage.
5. I know that my participation in the project is entirely voluntary, and that I am free to withdraw from the project at any time without disadvantage.
6. I know that as a participant I will complete a health screening questionaire as well as questions on salt and fluid intakes. I will have my sweat measured at rest and during exercise, and I will provide urine and blood samples.

7. I know that the *questionnaire* will explore my dietary habits around salt and my desire to consume salt and fluid after exercise and that if the line of questioning develops in such a way that I feel hesitant or uncomfortable I may decline to answer any particular questions , and /or may withdraw from the project without disadvantage of any kind.
8. I understand the nature and size of the risks of discomfort or harm which are explained in the Information Sheet.
9. I know that when the project is completed all personal identifying information will be removed from the paper records and electronic files which represent the data from the project, and that these will be placed in secure storage and kept for at least ten years.
10. I understand that the results of the project may be published and be available in the University of Otago Library, but that I agree that any personal identifying information will remain confidential between myself and the researchers during the study, and will not appear in any spoken or written report of the study ☐
11. I know that there is no remuneration offered for this study, and that no commercial use will be made of the data.
12. I understand that the blood, urine and sweat samples will be stireed by ID only until the completion of the study at which point they will be destoyed by either standard methods ☐ or with a karakia ☐ (tick to indicate how you would like your samples disposed of).

Signature of participant: Date:

Signature and name of witness: Date:

Appendix B. Twenty-four hour dietary record

ID Code:

Food and drink diary

Food Diary

How to fill in your diary

Below is a step-by-step guide on how to fill in your food diary. Try to fill in the diary each time you have something to eat or drink rather than leave it until the end of the day so that you don't forget anything!

Step 1: When
The first thing to do is to find the right time slot in the first column of the diary (on the left) for when you ate or drank something. Then, in the column next to the time slot, write down the exact time you ate or drank something. So, for example, if you had breakfast at 7.30am, you would go to the first time slot in the diary (6am to 9am) and in the column next to it write in "7.30am".

Step 2: Where
The next column in the food diary is for you to write in where you were when you ate or drank something. This could be:

- At home – e.g. in the bedroom, at the table, in the school canteen
- Away – e.g. in the street, in the car/on a bus, at a friend's or relative's house, in a café/ restaurant (please specify McDonalds, Burger King, Meridian Mall etc.)
- At school – e.g. in the canteen/tuckshop, in the corridor, in the classroom, in the playground

Step 3: With Whom
In the next column in the food diary, please write down who you were with when you ate or drank something. For example, you might have been alone, with family or with friends.

Step 4: What
The next step in the food diary is to describe what you ate or drank, giving as much detail as you can. Include any extras like sugar and milk in your tea or cereal, butter or other spreads on your bread and sauces such as tomato sauce and mayonnaise. Do not forget to include drinking water.

If you know the cooking method used (e.g. roast, baked, boiled, fried) please write it down in this section. It would also help us if you can write down the brand name of any foods or drinks if you know it (e.g. Watties, Pams).

For breakfast cereals, as well as the brand name, please write down the name of the cereal e.g. Weetbix, Cocoa pops, Cornflakes.

For sandwiches, please describe the type of bread used, how many slices of bread were used and give details of the filling.

For salad or mixed vegetables, please describe what is in it (eg. 1 lettuce leaf, half a tomato, 6 slices of cucumber).

For pizza, please describe the topping (e.g. cheese and tomato, ham and pineapple).

Step 5: Portion size
In the next column, please write in the size of the portion of food or drink you had. For drinks you can specify glass, cup, or mug. Other descriptions include: packet (e.g. for potato chips), number (e.g. for biscuits), slice (e.g. for cake, pizza), teaspoon (e.g. for sugar), tablespoon (e.g. for tomato sauce, peas), handful (e.g. for lollies, nuts).

Step 6: Where did you get the food?
In the next column in the food diary is for you to write down where you got the food or drink from. This could be from:

- Home (food and drink, usually bought by an adult, brought into the house and stored there until eaten)
- Supermarket, dairy or other shop (food and drink bought by you for consumption outside the home)
- Restaurant/cafe (please specify the type)
- Street vendor (e.g. chip and hot dog van, ice cream van)
- Cinema kiosk/vending machine

On the first page of the diary we have filled in a whole day as an example to show you what to do.

Example:

Time slot	When	Where	With Whom	What	Portion size	Where did you get the food from
6am to 9am	8:00	In Bed	Alone	Strawberries Sugar Toast + Flora Margarine Raspberry Jam Apple Juice	5 ¼ teaspoon 1 Slice ½ teaspoon Glass	Home
9am to 12 noon	9 to 10 10:00 11:00	Football Pitch Car Watching TV	Football Team Alone Alone	E2 drink Mother Earth Baked Oaty Slice Homemade Muffin: vanilla and chocolate chip	Sports Bottle 1 1 small muffin	Home Home Home
12 noon to 2pm	12:30	Home, at table	Friends	Baked Beans Ham Cheese Toast Pams Margarine Vitafresh raspberry flavour	1 table spoon 1 slice 1 slice 1 slice 1 table spoon 1 small glass	Home

Time slot	When	Where	With Whom	What	Portion size	Where did you get the food from
2pm *to* 5pm	**2:00** **5:30**	**Lounge** **Kitchen, home**	**Family** **Alone**	**Cadbury Moro Bar** **Orange Mango Just Juice**	**60g bar** **Glass**	**Home** **Home**
5pm *to* 8pm	**7:00**	**Home, at table**	**Family**	**Chicken Breast, with herbs** **Ham (homemade)** **Cheese** **Mini roast potatoes** **Green Beans** **Orange Just Juice** **Homemade muffin: vanilla and choc chip**	**½ a breast** **1 slice** **4 cubes (about 1cm wide)** **¼ cup** **2 tbsp** **1 small glass** **1**	**Home**
8pm *to* 10pm	**8:00**	**Kitchen, home**	**Sister**	**Milk, light blue**	**Mug**	**Home**
10pm *to* 6am	-	-	-	-	-	-

Time slot	When	Where	With Whom	What	Portion size	Where did you get the food from
6am *to* 9am						
9am *to* 12 noon						
12 noon *to* 2pm						

Time slot	When	Where	With Whom	What	Portion size	Where did you get the food from
10pm to 6am						
8pm to 10pm						
5pm to 8pm						
2pm to 5pm						

Appendix C. Thirst and salt appetite questionnaire

Subject: Time:

Subjective feelings related to thirst (pre OR post exercise)

Place a vertical mark (|)on the lines below to indicate HOW YOU FEEL AT THE MOMENT.

EXAMPLE *How dry does your mouth feel now?*

Not at all dry *extremely dry*

1) How dry does your mouth feel now?

Not at all dry extremely dry

2) How irritated does your mouth feel now?

Not at all irritated extremely irritated

3) How thirsty does your mouth taste now?

Not at all thirsty extremely thirsty

4) How much do you feel like something salty now?

Not at all extreme craving

5) How hungry do you feel now?

Not at all hungry extremely hungry

6) How dry does your throat feel now?

Not at all dry extremely dry

7) How scratchy does your throat feel now?

Not at all scratchy extremely scratchy

8) How warm does your throat feel now?

Not at all warm extremely warm

9) How thirsty do you feel now?

Not at all thirsty _____ extremely thirsty

10) How pleasant would it be to drink some fluid now?

Not at all pleasant _____ extremely pleasant

11) How hungry do you feel now?

Not at all hungry _____ extremely hungry

12) How full does your stomach feel now?

Not at all full _____ extremely full

13) How alert do you feel now?

Not at all alert _____ extremely alert

14) How swollen does your tongue feel now?

Not at all swollen _____ extremely swollen

Appendix D. Dietary Behaviour Questionnaire

Subject:

REST Study - Dietary Behaviour Questionnaire

1. I add salt to my meals at the table
frequently occasionally seldom never

2. I add salt during cooking and preparation of foods
frequently occasionally seldom never

3. I eat frozen "TV" dinners, frozen entrees, pot pies and pizza
frequently occasionally seldom never

4. When cooking foods such as pasta, hot cereal, potatoes etc., I routinely add salt to the boiling water
frequently occasionally seldom never

5. When buying foods I read food labels and avoid foods high in salt, Monosodium glutamate (MSG), or other sodium containing items
frequently occasionally seldom never

6. I use salt substitutes such as Mrs Dash, Lemon pepper or herbs in place of salt
frequently occasionally seldom never

7. I use soy sauce and Worcestershire sauce when preparing foods
frequently occasionally seldom never

8. When choosing foods I look for products labeled sodium free, low sodium or reduced sodium
frequently occasionally seldom never

9. I use meat tenderizes when preparing meats
frequently occasionally seldom never

10. I season meat, fish, poultry and eggs with salt
frequently occasionally seldom never

11. I use a low sodium cookbook when preparing meals
frequently occasionally seldom never

12. I have heartburn and use baking soda or other antacids for indigestion or heartburn
frequently occasionally seldom never

13. I use baking soda toothpaste
frequently occasionally seldom never

14. I omit salt in recipes that I prepare at home
frequently occasionally seldom never

15. I add salt to foods before I taste them
frequently occasionally seldom never

16. I add salt to food after I taste them and find them lacking flavor
frequently occasionally seldom never

17. I look at the sodium content of foods before I decide which foods to buy
frequently occasionally seldom never

18. Many foods I eat taste to salty
frequently occasionally seldom never

19. In preparing recipes I follow the no added salt or low salt directions
frequently occasionally seldom never

20. If reduced salt foods were available at a reasonable price I would by them
frequently occasionally seldom never

21. I limit how much I eat of salty foods such as pickles, pretzels, ham and bacon
frequently occasionally seldom never

22. Reduced salt foods are consumed by other members in my household
frequently occasionally seldom never

23. Fresh foods such as dairy products, meat, cheese, fruits and vegetables spoil before I have a chance to prepare and eat them
frequently occasionally seldom never

24. I feel that I have a loss in taste perception
frequently occasionally seldom never

25. I feel that I have a loss in sense of smell
frequently occasionally seldom never

26. I eat meals away from home
frequently occasionally seldom never

27. In preparing meals would you most likely eat
Prepackaged foods meals prepared from fresh foods
meals prepared from canned/boxed foods

28. If eating spaghetti which would you most likely use for spaghetti sauce
Prepared mix homemade with salt homemade with low salt

29. In estimating the amount of salt I add to my food per day I would say it was
None ½tsp 1tsp 3tsp 4tsp 5+tsp

30. Does the water you drink have softeners added
Yes no

31. Do you or a family member prepare your own food
Yes no