

**Does Ethnicity Influence Sweat Sodium Loss?**

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## **ABSTRACT**

### **Background**

Leading sports nutrition experts recommend customised fluid and electrolyte replacement strategies for optimal rehydration. Inadequate replacement of sodium can impair performance and health, but replacement needs are unique to an individuals' sweat sodium loss (SSL) - a product of sweat sodium concentration ( $[Na^+]_{sweat}$ ) and sweat volume (or rate). Estimating  $[Na^+]_{sweat}$  requires laboratory analysis of an individuals' sweat sample, and therefore the sports nutrition practitioner (SNP) can struggle to provide professional sports teams with customised sodium replacement advice in the field setting. Therefore research identifying groups prone to higher  $[Na^+]_{sweat}$  than others ('*saltier sweaters*') may help the SNP to do so.

Ethnicity may be a practical approach to identifying '*saltier sweaters*' within sports teams. Small field studies in the US suggest that 'White' male athletes may incur higher SSL than their 'Black' counterparts. No such research has been conducted in New Zealand (NZ) which primarily consists of athletes identifying with the Maori, Pacific or NZ European (NZE) ethnic groups. The Maori and Pacific (MP) groups can be considered one group given their shared ancestral origins, and therefore the objective of this study was to investigate whether '*saltier sweaters*' can be identified by the proxy of ethnic group in NZ. An overarching goal was to inform SNPs about the SSL of athletes identifying with the MP or NZE ethnic groups for their future practice in giving sodium replacement advice to either group of athlete.

### **Methods**

Fifty-eight highly trained adult male team-sport athletes were recruited from four cohorts within NZ and one cohort in Tonga between June and September 2011.

Participants' body mass was measured before and after a 60 minute exercise protocol on a stationary bike. Sweat patches were placed on their right scapula to enable estimates of  $[Na^+]_{sweat}$  and ultimately SSL. Environmental conditions can differ between NZ and Tonga, and therefore a difference in heat acclimatisation status was suspected within the MP group of participants (MP-ALL). Consequently data from this group were further categorised as being collected in NZ (MP-NZ) or Tonga (MP-TGA). The study results were examined with and without the MP-TGA cohort to evaluate the potential confounding effect of a disparity in acclimatisation status.

## Results

The mean  $[Na^+]_{sweat}$  of the MP-ALL group was significantly higher than the NZE group ( $37.6 \pm 19.7 \text{ mmol.L}^{-1}$  versus  $34.9 \pm 17.6 \text{ mmol.L}^{-1}$  respectively,  $p=0.003$ ). There was no evidence of a difference in SSL between the MP-ALL and NZE ethnic groups ( $31.8 \pm 19.8 \text{ mmol.h}^{-1}$  versus  $33.2 \pm 22.5 \text{ mmol.h}^{-1}$  respectively,  $p=0.871$ ). In contrast, SSL was significantly higher among the MP-NZ compared to NZE group ( $42.2 \pm 16.3 \text{ mmol.h}^{-1}$  versus  $33.2 \pm 22.5 \text{ mmol.h}^{-1}$  respectively,  $p=0.038$ ). The mean  $[Na^+]_{sweat}$  was also significantly higher among the MP-NZ group compared to NZE ( $45.1 \pm 18.7 \text{ mmol.L}^{-1}$  versus  $34.9 \pm 17.6 \text{ mmol.L}^{-1}$  respectively,  $p=0.013$ ), although the MP-NZ group appeared to exercise at a marginally lower if not similar intensity than the NZE group (based on mean heart rate data). This may have attenuated the difference in mean  $[Na^+]_{sweat}$  as a consequence of a confounding effect on mean sweat rates which were similar if not marginally higher among the MP-NZ compared to NZE group ( $0.98 \pm 0.34 \text{ L.h}^{-1}$  and  $0.89 \pm 0.33 \text{ L.h}^{-1}$  respectively).

## Discussion

The main finding of this study is that sweat sodium replacement can be individualised among athletes on the basis of ethnicity in NZ. The MP-NZ ethnic group were '*saltier*

sweaters' and overall they incurred a higher SSL than the NZE group. This means sodium replacement needs were greater among the MP-NZ group and the difference may be more pronounced in reality when/if exercise intensity is held constant between the two groups. Overall this study is important for SNPs to be aware of in NZ before encouraging rapid rehydration between morning and afternoon training sessions – particularly in hot or humid environmental conditions.

## PREFACE

This thesis marks the end of a longer than expected research journey. It has given me valuable experience in research and working with elite athletes to provide leading-edge rehydration advice. I have been tested but I have not been broken, and I reach my destination today as a stronger character owing many thanks to friends, family and colleagues who all helped me along the way.

Firstly, thank you to my supervisors - Drs Katherine Black and Rachel Brown. Your patience, guidance and encouraging comments helped me to realise my core values as a professional practitioner. Thanks also to those who helped with recruitment or data collection; In particular, Dane Baker and Sam Higgins - for getting the recruitment ball rolling in Auckland; Louise Bee and Adam Keen - for keeping it rolling in Oamaru and Dunedin; and to Matt Blair - for taking this show on the road to such a humbling team of athletes in Tonga.

To my friends and family - thank you for being there. Your support and smiles when times were tough helped me hold focus for writing, stay on track with training, and in the big scheme of things, keep everything in perspective. K.M.A. and have a nice day.

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# TABLE of CONTENTS

ABSTRACT .....	i
PREFACE .....	iv
TABLE of CONTENTS .....	v
LIST of TABLES .....	viii
LIST of FIGURES .....	ix
LIST of ABBREVIATIONS .....	x
1 INTRODUCTION.....	1
2 LITERATURE REVIEW.....	3
2.1 The Physiology of Sweating .....	3
2.2 Determinants of Sweat Sodium Concentration.....	12
2.3 Literature Review Conclusion.....	41
3 METHODOLOGY.....	42
3.1 Study Design.....	42
3.2 Participants .....	44
3.3 Exercise Protocol .....	46
3.4 Laboratory Analyses.....	51
3.5 Derived Variables .....	52
3.6 Statistical Analyses .....	54
4 RESULTS.....	56
4.1 Overview .....	56

4.2	Control Variable Candidates .....	59
4.3	Sweat Sodium Concentration by Ethnic group .....	65
4.3.1	Total Sample (MP-ALL and NZE participants) .....	65
4.3.2	NZ Sample (MP-NZ and NZE Participants) .....	67
4.4	Sweat Sodium Loss by Ethnic Group .....	69
4.4.1	Total Sample (MP-ALL and NZE Participants) .....	69
4.4.2	NZ-Based Sample (MP-NZ and NZE Participants) .....	71
4.5	Blood Sodium Concentration.....	73
5	DISCUSSION.....	74
5.1	Overview .....	74
5.2	Evaluation of Heat Acclimatisation Status .....	74
5.3	Ethnicity and Sweat Sodium Concentration or Loss.....	76
5.4	Limitations .....	82
5.5	Application to Practice.....	86
6	CONCLUSION and RESEARCH RECOMMENDATIONS .....	91
	REFERENCES.....	92
	APPENDICES .....	101
	APPENDIX 1. Consent form for participants .....	102
	APPENDIX 2. Health & demographic survey .....	103
	APPENDIX 3. 24-hour urine collection (2-day protocol) .....	105
	APPENDIX 4. 24-hour urine collection (3-day protocol) .....	106
	APPENDIX 5. Individualised feedback for participants.....	107

APPENDIX 6. Environmental conditions .....	112
APPENDIX 7. Interaction inspection– sweat sodium concentration .....	113
APPENDIX 8. Interaction inspection - sweat sodium loss .....	117
APPENDIX 9. Assessment of Data Distribution .....	121
APPENDIX 10. Control variable selection - sweat sodium concentration.....	129
APPENDIX 11 Control variable selection - sweat sodium loss.....	130
APPENDIX 12. Change in blood sodium by ethnic group .....	131
APPENDIX 13. Sodium content of recovery foods .....	132

## LIST of TABLES

Table 1. Field studies investigating sweat sodium concentration ( $[Na^+]$ <sub>sweat</sub> ) and/or sweat sodium loss (SSL) .....	7
Table 2. Laboratory studies collecting unevaporated sweat samples with whole body washdown (WBW) <sup>A</sup> .....	13
Table 3. Experimental studies investigating the influence of dietary sodium intake on sweat sodium concentration ( $[Na^+]$ <sub>sweat</sub> ) or sweat sodium loss (SSL) .....	29
Table 4. Review of studies investigating the influence of racial group on sweat sodium concentration ( $[Na^+]$ <sub>sweat</sub> ) or sweat sodium loss (SSL) .....	35
Table 5. Physical characteristics of participants (mean $\pm$ SD and range) by cohort and by ethnic group .....	45
Table 6 The sodium and potassium content of water provided to participants .....	47
Table 7 Environmental conditions during each cohort's exercise protocol .....	49
Table 8. Unadjusted sweat sodium concentration ( $[Na^+]$ <sub>sweat</sub> ) and loss (SSL) data of the total sample by ethnic group .....	56
Table 9. Pre-exercise characteristics (mean $\pm$ SD, range) of the total sample by ethnic group .....	59
Table 10. Univariable analyses of $[Na^+]$ <sub>sweat</sub> determinants – Total Sample .....	65
Table 11. Univariable analyses of $[Na^+]$ <sub>sweat</sub> determinants - NZ Sample .....	67
Table 12. Univariable analyses of sweat sodium loss (SSL) determinants - Total Sample .....	69
Table 13. Univariable analyses of sweat sodium loss (SSL) determinants - NZ Sample .....	71
Table 14. Change in mean blood sodium concentration ( $[Na^+]$ <sub>blood</sub> ) relative to sweat sodium loss (SSL) – Pukekohe cohort (n=23) .....	73
Table 15. Interaction assessment with simple linear regression – log transformed sweat sodium concentration ( $[Na^+]$ <sub>sweat</sub> ) by ethnic group (Total Sample and NZ Sample) .....	113
Table 16. Interaction assessment with simple linear regression – log transformed sweat sodium loss (SSL) by ethnic group (Total Sample and NZ Sample) .....	117
Table 17. Table showing the control variable candidates selected for multiple linear regression analyses of sweat sodium concentration – Total Sample (MP-ALL and NZE) .....	129
Table 18. Table showing the control variable candidates selected for multiple linear regression analyses of sweat sodium concentration – NZ Sample (MP-NZ and NZE) .....	129
Table 19. Table showing the control variable candidates selected for multiple linear regression analyses of sweat sodium loss – Total Sample (MP-ALL and NZE) .....	130
Table 20. Table showing the control variable candidates selected for multiple linear regression analyses of sweat sodium loss – NZ Sample (MP-NZ and NZE) .....	130
Table 21. Table showing typical recovery foods with sodium content per serve. ....	132

## LIST of FIGURES

Figure 1. The process of sodium reabsorption within activated sweat glands.....	4
Figure 2. The relationship between sweat sodium concentration ( $[Na^+]$ <sub>sweat</sub> ), sweat volume/rate, and sweat sodium loss (SSL) .....	5
Figure 3. Summary of potential confounding effects on <sup>A</sup> sweat sodium concentration ( $[Na^+]$ <sub>sweat</sub> ) and <sup>B</sup> sweat sodium loss (SSL) .....	41
Figure 4a. Overview of the study design for all five participating cohorts .....	42
Figure 4b. Outline of data collection protocol followed for each cohort .....	43
Figure 5. Outline of laboratory analyses to determine <sup>A</sup> Urinary sodium concentration ( $[Na^+]$ <sub>urine</sub> ), <sup>B</sup> Urinary potassium concentration ( $[K^+]$ <sub>urine</sub> ), <sup>C</sup> Sweat potassium concentration ( $[K^+]$ <sub>sweat</sub> ), <sup>D</sup> Sweat sodium concentration ( $[Na^+]$ <sub>sweat</sub> ) .....	51
Figure 6. Outline of calculations used to derive variables for statistical analyses.....	52
Figure 7. Sweat potassium concentration ( $[K^+]$ <sub>sweat</sub> ) data of the total sample by ethnic group .....	58
Figure 8. Body mass change (%) data of the total sample by ethnic group.....	61
Figure 9. Mean sweat rate ( $L.h^{-1}$ ) data of the total sample by ethnic group.....	62
Figure 10. Relative humidity (%) of exercise sessions by cohort.....	63
Figure 11. Environmental temperature (Temp <sub>ENVIRO</sub> ) of exercise sessions by cohort .....	63
Figure 12. Mean heart rate (HRmean) data of the total sample by ethnic group .....	64
Figure 13. Box plot showing the distribution of mean sweat sodium concentration ( $[Na^+]$ <sub>sweat</sub> ) data of the total sample by the MP-ALL and NZE ethnic groups. ....	122
Figure 14. Box plot showing the distribution of mean sweat sodium concentration ( $[Na^+]$ <sub>sweat</sub> ) data of the total sample by the MP-NZ, MP-TGA, and NZE ethnic groups. ....	122
Figure 15. Box plot showing the distribution of estimated sweat sodium loss (SSL) data of the total sample by the MP-ALL and NZE ethnic groups.....	124
Figure 16. Box plot showing the distribution of estimated sweat sodium loss (SSL) data of the total sample by the MP-NZ, MP-TGA, and NZE ethnic groups.....	124
Figure 17. Box plot showing the distribution of mean sweat potassium concentration ( $[K^+]$ <sub>sweat</sub> ) data of the total sample by the MP-ALL and NZE ethnic groups.....	126
Figure 18.. Box plot showing the distribution of mean sweat potassium concentration ( $[K^+]$ <sub>sweat</sub> ) data of the total sample by the MP-NZ, MP-TGA and NZE ethnic groups. ....	126
Figure 19. Box plot showing the distribution of mean sweat rate data of the total sample by the MP-ALL and NZE ethnic groups.....	128
Figure 20. Box plot showing the distribution of mean sweat rate data of the total sample by the MP-NZ, MP-TGA and NZE ethnic groups. ....	128
Figure 21. Relationship between the change in blood sodium concentration ( $[Na^+]$ <sub>blood</sub> ) and sweat sodium loss (SSL) for the MP-NZ and NZE ethnic groups – Pukekohe cohort (n=23) .....	131

## LIST of ABBREVIATIONS

ADH	Anti-diuretic hormone
ASG	Activated sweat gland
AVP	Arginine vasopressin
BSA	Body surface area
CFTR	Cystic fibrosis transmembrane regulator
DASH	Dietary approaches to stop hypertension
EAH	Exercise associated hyponatraemia
ECF	Extracellular fluid
ENaC	Epithelial sodium channel
HR <sub>mean</sub>	Mean heart rate
ICC	Intraclass correlation coefficient
ICF	Intracellular fluid
[K <sup>+</sup> ] <sub>sweat</sub>	Sweat potassium concentration
[K <sup>+</sup> ] <sub>urine</sub>	Urinary potassium concentration
MP	Maori and Pacific
[Na <sup>+</sup> ] <sub>blood</sub>	Blood sodium concentration
[Na <sup>+</sup> ] <sub>sweat</sub>	Sweat sodium concentration
[Na <sup>+</sup> ] <sub>urine</sub>	Urinary sodium concentration
NZ / NZE	New Zealand / New Zealand European
PABA	Para-amino benzoic acid
SGO	Sweat gland output
SNP	Sports nutrition practitioner
SSL	Sweat sodium loss
TBW	Total body water
Temp <sub>CORE</sub>	Core body temperature
Temp <sub>ENVIRO</sub>	Environmental temperature
Temp <sub>SKIN</sub>	Skin temperature
WBW	Whole body washdown

# 1 INTRODUCTION

Dietary sodium is like a chameleon to the world of nutrition. Within public health circles there is strong evidence linking high sodium intakes with hypertension and the burden of cardiovascular disease (Intersalt Cooperative Research Group, 1988; Galloway & Maughan, 1997; Luft, 1979; Sacks et al., 2001; Fowkes Godek et al., 2005; World Health Organisation, 2007). Accordingly, population guidelines are aimed at reducing sodium intake to less than 100mmol per day ( $2300\text{mg} \cdot \text{day}^{-1}$ ) to improve health outcomes (Institute of Medicine, 2005; National Health and Medical Research Council, 2006; World Health Organisation, 2007). However, trained athletes can lose this amount of sodium in their sweat during one typical training or competition session (Bergeron, 2003; Fowkes Godek et al., 2010; Kurdak et al., 2010; Maughan et al., 2004; Maughan et al., 2007; Neville et al., 2010; Palmer et al., 2010; Shirreffs et al., 2005; Stofan, 2002; Stofan et al, 2005). Sodium is the major electrolyte found in sweat, and leading sports nutrition experts recommend full sweat sodium replacement for optimal rehydration in post-exercise recovery (Sawka et al., 2007). Therefore appropriate sodium intakes for the general population can be considered inadequate for some athletes depending on their sweat sodium loss (SSL).

Assessing an athlete's SSL can be challenging for the sports nutrition practitioner (SNP). Absolute SSL is the product of sweat volume and sweat sodium concentration ( $[\text{Na}^+]_{\text{sweat}}$ ) (Institute of Medicine, 2005). Sweat volume can be estimated with relatively little expense or expertise by measuring the athlete's change in body mass after adjusting for any fluid consumed or urine produced during an exercise session - adjustments for respiratory water loss and glycogen changes are minimal and unnecessary within a timeframe of up to approximately two hours (King et al., 2008).

In contrast,  $[Na^+]_{sweat}$  is more difficult to estimate because laboratory analysis of a sweat sample is needed. Therefore the SNP cannot readily provide customised sodium replacement advice in the field setting, and identifying groups with higher  $[Na^+]_{sweat}$  compared to others ('*saltier sweaters*') may be of practical use to them.

Ethnicity may be one way of identifying '*saltier sweaters*' in New Zealand (NZ). The adult population primarily consists of the Maori, Pacific, and NZ European (NZE) ethnic groups (Statistics New Zealand, 2006). Population-based surveys in NZ differentiate the Maori and Pacific ethnic groups (University of Otago & Ministry of Health, 2011), but they can be considered as one based on their ancestry which is an important element of Maori identity (Houkamau & Sibley, 2010; UMR Research Limited, 2009). Maori are descendants of Pacific peoples who arrived in NZ during the 14<sup>th</sup> century (Irwin, 2009). Therefore the adult male sporting population in NZ predominantly consists of athletes identifying with the Maori and/or Pacific (MP) or NZE ethnic groups (Sport and Recreation New Zealand, 2008).

There are no peer reviewed studies comparing  $[Na^+]_{sweat}$  or SSL by ethnic group in NZ at present. Limited research from the US suggests that differences in  $[Na^+]_{sweat}$  and/or SSL may exist between the 'Black' and 'White' racial groups (Condon et al., 2010; Condon et al., 2007; Dill, 1983; Kopec et al., 2008; Palacios et al., 2003), but whether or not these observations extend to athletes in NZ is unclear. Such intrigue forms the foundation of the current study in which the primary objective was to investigate whether '*saltier sweaters*' can be identified by the proxy of ethnic group in NZ. The overarching goal was to inform SNPs about SSL of the MP and NZE ethnic groups of athletes for their practical application. Ultimately the findings may offer one step towards more targeted sodium replacement advice while adding to the body of global literature on  $[Na^+]_{sweat}$  and SSL.

## 2 LITERATURE REVIEW

### 2.1 The Physiology of Sweating

#### 2.1.1 Thermoregulation

Sweating facilitates thermoregulation through evaporative cooling of the skin. It begins when the mechanisms of dry heat loss cannot regulate core body temperature ( $\text{Temp}_{\text{CORE}}$ ) around approximately 37°C (Nadel, 1979; Sawka & Young, 2006). A  $\text{Temp}_{\text{CORE}}$  above normal (hyperthermia) leads to heat exhaustion which impacts on performance and if left untreated, heat stroke can ensue (Nadel, 1979; Sato et al., 1989; Sawka & Young, 2006).

Dry heat loss involves the transfer of excess heat away from the body's core. Internal heat production increases as fuel substrates are oxidised to provide energy for contracting muscles (Nadel, 1979; Saltin & Hermansen, 1966). Blood flow from the core to periphery increases and this leads to skin temperature ( $\text{Temp}_{\text{SKIN}}$ ) rising. When  $\text{Temp}_{\text{SKIN}}$  is greater than the environmental temperature ( $\text{Temp}_{\text{ENVIRO}}$ ), heat energy is dissipated away from the body through radiation (heat passing from the skin to air through heat waves) and convection (the warming of air surrounding the skin causing it to rise while being replaced by cooler air) (Nadel, 1979; Sawka & Young, 2006). In other words, heat energy transfers down a positive temperature gradient which means the body can actually gain heat through these mechanisms if  $\text{Temp}_{\text{SKIN}}$  is less than  $\text{Temp}_{\text{ENVIRO}}$ . Before this can occur, individual sweat glands found over most parts of the body are activated by the brain which enables sweating and subsequently evaporative heat loss to begin (Nadel, 1979; Sato et al., 1989; Sawka & Young, 2006; Shibasaki et al., 2006). This means sweating is important for the thermoregulation of athletes during training sessions and in competitive events.

## 2.1.2 Sweat Production

Sweating involves water, sodium and other electrolytes moving from extracellular fluid (ECF) into the secretory coils of activated sweat glands (ASG). An isotonic precursor fluid forms in the ASG at that stage (Sato & Dobson, 1970; Cage & Dobson, 1965; Quinton, 2007). As the precursor fluid moves toward the skin for secretion, sodium and other electrolytes are reabsorbed back into the ECF (Quinton, 2007). Sodium ions pass through epithelial sodium channels (ENaC) to follow chloride ions which are reabsorbed through the cystic fibrosis transmembrane regulator (CFTR) (Figure 1.). The final  $[Na^+]$ <sub>sweat</sub> typically ranges between 10-70mmol.L<sup>-1</sup> which is hypotonic relative to ECF (Sawka et al., 2007).

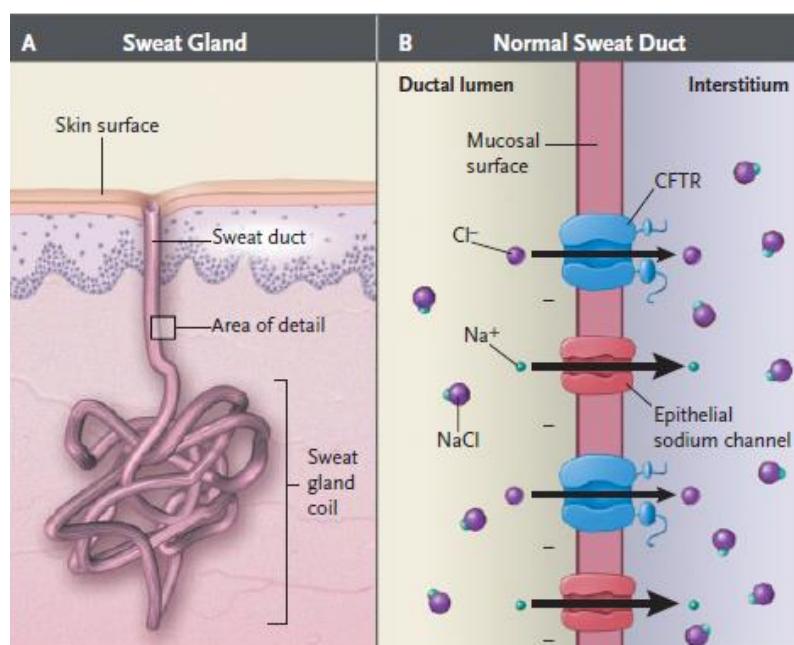


Figure 1. The process of sodium reabsorption within activated sweat glands (adapted with permission from Rowe et al. 2005. Copyright Massachusetts Medical Society).

### Blood sodium concentration and sweat sodium loss

The ECF consists of plasma (blood) and interstitial fluid (Institute of Medicine, 2005). Approximately 95% of total body sodium stores are found within these fluid compartments, and blood sodium concentration ( $[Na^+]$ <sub>blood</sub>) typically ranges between 136-145mmol.L<sup>-1</sup> (Hew-Butler et al., 2008; Institute of Medicine, 2005; Popowski et al., 2001). Deviations below this range can occur during or soon after exercise in the

condition known as exercise associated hyponatraemia (EAH) (Hew-Butler et al., 2008). Symptoms of EAH can vary from nausea or bloating through to cerebral oedema or death in extreme cases. The most common cause of EAH is an excessive fluid intake relative to fluid loss causing a dilution of  $[Na^+]_{blood}$ , although EAH can also occur among those in total body water (TBW) balance (euhydrated) or negative TBW balance (hypohydrated) (Hew-Butler et al., 2008; Hew-Butler et al., 2007). Therefore SSL may play a role in the aetiology of EAH independent to, or in combination with high volumes of fluid intake (Hew-Butler et al., 2008).

### Sweat sodium loss of athletes

Sweat sodium loss is the product of a relationship between  $[Na^+]_{sweat}$  and sweat volume or rate (Figure 2.). It can be highly variable between athletes (Table 1.). On one hand,  $[Na^+]_{sweat}$  mediates the relationship between sweat volume and SSL (Baron & Kenny, 1986). For instance, SSL will be greater among individuals with raised  $[Na^+]_{sweat}$  compared to those sweating the same volume over a given timeframe but with a lower  $[Na^+]_{sweat}$  (Table 1.). However,  $[Na^+]_{sweat}$  depends on sweat rate similar to how nutrient absorption relates to transit time in the gut (Baron & Kenny, 1986; Eichner, 2008; Frazier et al., 2004; Roy et al., 1991). For example,  $[Na^+]_{sweat}$  increases linearly with sweat rate as the time available for sodium reabsorption from precursor fluid is reduced (Buono et al., 2007; Buono et al., 2008; Quinton, 2007; Sato & Dobson, 1970).

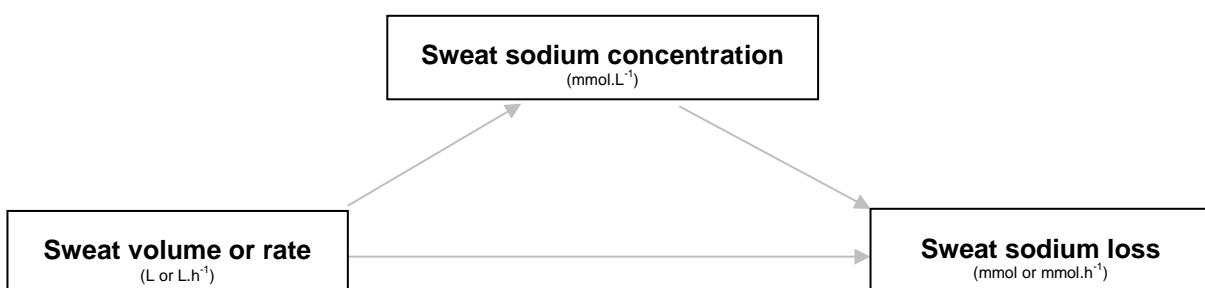


Figure 2. The relationship between sweat sodium concentration ( $[Na^+]_{sweat}$ ), sweat volume/rate, and sweat sodium loss (SSL) - assuming the absence of confounding effects.

A positive linear relationship between sweat rate and  $[Na^+]_{sweat}$  is generally only seen under controlled laboratory conditions rather than during field observations (Allan & Wilson, 1971; Buono et al., 2007; Buono et al., 2008; Cage & Dobson, 1965; Costill, 1977; Hamouti et al., 2010a; Inoue et al., 1998; Maughan et al., 2004; Maughan et al., 2005; Sato & Dobson, 1970; Sato et al., 1989; Shamsuddin et al., 2005; Verde, 1982; Yoshida et al., 2006). This is probably because laboratory studies can examine the impact of altering sweat rate on  $[Na^+]_{sweat}$  within individuals (Table 2.) whereas field studies compare  $[Na^+]_{sweat}$  between individuals sweating at variable rates (Table 1.). In other words field studies are more exposed to confounding effects on  $[Na^+]_{sweat}$  and SSL given the practical realities of their observational study design.

The current study aims to investigate whether ethnicity influences  $[Na^+]_{sweat}$  and/or SSL among NZ athletes. To isolate any such influence, key determinants of  $[Na^+]_{sweat}$  such as sweat rate are important to identify so they can be controlled for during data collection or alternatively measured and their potential for confounding effects on results considered afterwards with statistical analysis. Therefore the purpose of this literature review was to identify and explore potential determinants of  $[Na^+]_{sweat}$  and/or SSL in guiding the design of this observational field study.

**Table 1.** Field studies investigating sweat sodium concentration ( $[Na^+]$ <sub>sweat</sub>) and/or sweat sodium loss (SSL)

Reference	Aim	Participants	Protocol	Environment	Sweat volume or rate	$[Na^+]$ <sub>sweat</sub> <sup>A</sup>	SSL <sup>B</sup>	$[K^+]$ <sub>sweat</sub> <sup>C</sup>
Bergeron, 2003	Not specified	<b>Total:</b> (mean±SE) n=17 heat acclimated tennis players with a history of heat cramping $22.2\pm1.9$ years, $81.0\pm1.3$ Kg	<b>Exercise session:</b> Tennis singles match play <b>Sweat collection:</b> Regional sweat patch (forearm). Unspecified sweat volume/sweat rate	<b>Temp<sub>ENVIRO</sub></b> <sup>D</sup> : (mean±SE) $31.9\pm0.5$ °C <b>Relative Humidity:</b> (mean±SE) $55\pm2\%$	Mean±SE: $2.6\pm0.1$ L.h <sup>-1</sup>	Mean±SE: $44.5\pm3.5$ mmol.L <sup>-1</sup>	Mean±SE: $118.1\pm11.0$ mmol.h <sup>-1</sup>	Not reported
Fowkes Godek et al., 2010	To compare sweat rate, $[Na^+]$ <sub>sweat</sub> <sup>A</sup> , and SSL <sup>B</sup> in 3 groups of American Football players differing by body surface area (BSA).	<b>Large (Lge):</b> n=14 BSA <sup>E</sup> = $2.63\pm0.15$ m <sup>2</sup> <b>Medium (Med):</b> n=12 BSA <sup>E</sup> = $2.37\pm0.07$ m <sup>2</sup> ( $p<0.010$ vs, Lge and vs. Sml) <b>Small (Sml):</b> n=18 BSA <sup>E</sup> = $2.14\pm0.09$ m <sup>2</sup> ( $p<0.010$ vs, Lge and vs. Med)	<b>Exercise session:</b> 135 minutes of usual morning training AND 135 minutes of usual afternoon training sessions <b>Sweat collection:</b> Regional sweat patch (forearm) collected during the first 40 minutes of each training session (unadjusted for whole body sweat sodium concentration). Body mass change (adjusted for fluid intake and urine loss)	<b>Temp<sub>ENVIRO</sub></b> <sup>E</sup> : $25.9\pm1.9$ °C <b>Relative Humidity:</b> Not specified	<b>Total</b> <sup>E</sup> : (n=44) $1.84\pm0.64$ L.h <sup>-1</sup> ( $0.43\text{--}3.16$ L.h <sup>-1</sup> ) <sup>F</sup> <b>Lge</b> <sup>E</sup> : $2.25\pm0.68$ L.h <sup>-1</sup> ( $p>0.050$ vs. Med) ( $p<0.010$ vs. Sml) <b>Med</b> <sup>E</sup> : $1.98\pm0.48$ L.h <sup>-1</sup> ( $p<0.05$ vs Sml) <b>Sml</b> <sup>E</sup> : $1.4\pm0.45$ L.h <sup>-1</sup>	<b>Total</b> <sup>F</sup> : n=44 $15\text{--}99$ mmol.L <sup>-1</sup> ( $p=0.860$ between groups in ANOVA. Post hoc analysis not performed between groups because $p>0.050$ ) <b>Lge</b> <sup>E</sup> : $53\pm25$ mmol.L <sup>-1</sup> <b>Med</b> <sup>E</sup> : $48\pm23$ mmol.L <sup>-1</sup> <b>Sml</b> <sup>E</sup> : $50\pm16$ mmol.L <sup>-1</sup>	<b>Total</b> <sup>F</sup> : n=44 $22.5\text{--}291$ mmol.h <sup>-1</sup> ( $p=0.060$ between groups in ANOVA. Post hoc analysis not performed between groups because $p>0.050$ ) <b>Lge</b> <sup>E</sup> : $121\pm8$ mmol.h <sup>-1</sup> <b>Med</b> <sup>E</sup> : $96\pm5$ mmol.h <sup>-1</sup> <b>Sml</b> <sup>E</sup> : $73\pm4$ mmol.h <sup>-1</sup>	Not reported
Hamouti et al., 2010a	To identify in which indoor team sports professional players achieve fluid and electrolyte deficits that could potentially affect performance	<b>Total sample:</b> n=43 male athletes (mean±SD) <b>Soccer:</b> (n=9) BSA= $1.9\pm0.1$ m <sup>2</sup> ( $p<0.05$ vs other teams) <b>Basketball:</b> (n=11) BSA= $2.3\pm0.2$ m <sup>2</sup> <b>Volleyball:</b> (n=10) BSA= $2.3\pm0.1$ m <sup>2</sup> <b>Handball:</b> (n=13) BSA= $2.4\pm0.1$	<b>Exercise Session:</b> Usual mid-season morning training session (between 1000-1200hr and lasting $83\pm9$ min <sup>E</sup> ). <i>ad libitum</i> fluid intake during 3-4 scheduled breaks. <b>Sweat Collection:</b> Regional sweat patch (forearm) Body mass change (nude adjusted for fluid intake and urine loss only) SSL = sweat rate x reg. $[Na^+]$ <sub>sweat</sub> (adjusted for whole body)	<b>Temp<sub>ENVIRO</sub></b> <sup>E</sup> : $21\pm2$ °C <b>Relative Humidity</b> <sup>E</sup> : $32\pm7\%$	<b>Total sample:</b> (mean±SD) $1.4\pm0.3$ L.h <sup>-1</sup> <b>Soccer mean:</b> $1.8\pm0.7$ L.h <sup>-1</sup> ( $p=0.02$ vs volley. & vs. handball) <b>Basketball:</b> $\approx1.5\pm0.4$ L.h <sup>-1</sup> <b>Volleyball:</b> $1.2\pm0.3$ L.h <sup>-1</sup> <b>Handball:</b> $1.1\pm0.3$ L.h <sup>-1</sup>	<b>Total sample:</b> mean±SE $62.1\pm4.9$ mmol.L <sup>-1</sup> ( $p>0.05$ across teams) No correlation with sweat rate ( $p>0.05$ )	<b>Total sample:</b> (mean±SD) $1200\pm200$ mg ( $p>0.05$ across teams) <b>Soccer:</b> $1900\pm1100$ mg ( $1100\text{--}4400$ mg) <sup>F</sup> <b>Basketball:</b> $1900\pm500$ mg ( $1300\text{--}2800$ mg) <sup>F</sup> <b>Volleyball:</b> $1400\pm1000$ mg ( $400\text{--}3500$ mg) <sup>F</sup> <b>Handball:</b> $1500\pm600$ mg ( $600\text{--}2600$ mg) <sup>F</sup>	<b>Total sample:</b> (mean±SD) $4.3\pm0.3$ mmol.L <sup>-1</sup> ( $p>0.05$ across teams)

<sup>A</sup>Sweat sodium concentration, <sup>B</sup>Sweat sodium loss, <sup>C</sup>Sweat potassium concentration, <sup>D</sup>Environmental temperature, <sup>E</sup>Mean±SD or mean±SE unspecified, <sup>F</sup>Range (Table 1. continues on next page)

Table 1 (continued).

Reference	Aim	Participants	Protocol	Environment	Sweat volume or rate	$[Na^+]$ <sub>sweat</sub> <sup>A</sup>	SSL <sup>B</sup>	$[K^+]$ <sub>sweat</sub> <sup>C</sup>
Kurdak et al., 2010	To describe sweating responses of football players during match play in a warm environment.	<b>Total sample:</b> n=22 male  $20\pm2$ years <sup>E</sup> $1.76\pm0.02$ m <sup>E</sup> $68\pm7$ Kg <sup>E</sup>	<b>Exercise Session:</b> Three competitive FIFA regulation 90 min matches during summer 2008  <b>Sweat Collection:</b> Regional sweat patches (forearm, chest, back, thigh) – removed after the first half of play.  Body mass change (underwear only, adjusted for fluid intake and urine loss only)  SSL = sweat rate x reg. $[Na^+]$ <sub>sweat</sub> (unadjusted for whole body)	<b>Temp<sub>ENVIRO</sub>:</b> (mean±SD) 34.3±0.6°C  <b>Relative Humidity:</b> (mean±SD) 64±2%	(mean±SD)  <b>Group 1:</b> $3.1\pm0.6$ L (2.2-3.8L) <sup>F</sup>  <b>Group 2:</b> $3.1\pm0.5$ L (2.4-3.9L) <sup>F</sup>	(mean±SD)  <b>Group one:</b> $43\pm11$ mmol.L <sup>-1</sup> (27-59mmol.L <sup>-1</sup> ) <sup>F</sup>  <b>Group two:</b> $46\pm8$ mmol.L <sup>-1</sup> (25-53mmol.L <sup>-1</sup> ) <sup>F</sup> No difference between groups ( $p>0.437$ )	(mean±SD)  <b>Group one:</b> $137\pm50$ mmol (87-219mmol) <sup>F</sup>  <b>Group two:</b> $140\pm26$ mmol (97-189mmol) <sup>F</sup>	(mean±SD)  <b>Group one:</b> $3.5\pm0.5$ mmol.L <sup>-1</sup> (2.9-4.0mmol.L <sup>-1</sup> ) <sup>F</sup>  <b>Group two:</b> $3.5\pm0.3$ mmol.L <sup>-1</sup> (3.2-3.9mmol.L <sup>-1</sup> ) <sup>F</sup>
Maughan et al., 2004	To measure fluid balance during a preseason training session in the first team squad of an English Premier League football team.	<b>Total:</b> n=24 (mean±SD)  $27\pm4$ years (19-32y) <sup>F</sup> ,  $1.81\pm0.04$ m (1.70-1.90m) <sup>F</sup> ,  $79.4\pm4.7$ Kg (68.9-88.0Kg) <sup>F</sup>  $24.3\pm1.4$ Kg.m <sup>-2</sup> (20.6-26.8Kg.m <sup>-2</sup> ) <sup>F</sup>	<b>Exercise Session:</b> 90 minute training session  <b>Sweat Collection:</b> As per Kurdak et al. (2010)	<b>Temp<sub>ENVIRO</sub>:</b> 24-29°C  <b>Relative Humidity:</b> 46-64%	(mean±SD)  $2.03\pm0.41$ L (1.39-2.83L) <sup>F</sup>	(mean±SD)  $49\pm12$ mmol.L <sup>-1</sup>	$99\pm24$ mmol <sup>E</sup> (53-133mmol) <sup>F</sup>	(mean±SD) $6.0\pm1.3$ mmol.L <sup>-1</sup>
Maughan et al., 2005	To collect descriptive data on sweat loss and fluid intake in soccer players training in a cool environment.	<b>Total sample:</b> n=17 (mean±SD)  $24\pm4$ years, $1.80\pm0.07$ m $78.1\pm6.8$ Kg	<b>Exercise Session:</b> Usual training session from approximately 1030-1210hours  <b>Sweat Collection:</b> As per Kurdak et al. (2010) except regional patches remained in situ for entire match	<b>Temp<sub>ENVIRO</sub>:</b> $5.1\pm0.7$ °C  <b>Relative Humidity:</b> 81±6%	<b>Volume</b> (mean±SD) $1.69\pm0.45$ L (1.06-2.65L) <sup>F</sup>  <b>Rate</b> (mean±SD) $1.13\pm0.30$ L.h <sup>-1</sup> (0.71-1.77L.h <sup>-1</sup> ) <sup>F</sup>	(mean±SD)  $42.5\pm13$ mmol.L <sup>-1</sup> (16-66mmol.L <sup>-1</sup> ) <sup>F</sup>	(mean±SD)  $73\pm31$ mmol (29-121mmol) <sup>F</sup>	(mean±SD) $7.1\pm2.8$ mmol.L <sup>-1</sup> (3.4-14.3mmol.L <sup>-1</sup> ) <sup>F</sup>

<sup>A</sup>Sweat sodium concentration, <sup>B</sup>Sweat sodium loss, <sup>C</sup>Sweat potassium concentration, <sup>D</sup>Environmental temperature, <sup>E</sup>Mean±SD or mean±SE unspecified, <sup>F</sup>Range (Table 1. continues on next page)

Table 1 (continued).

Reference	Aim	Participants	Protocol	Environment	Sweat volume or rate	$[Na^+]$ <sub>sweat</sub> <sup>A</sup>	SSL <sup>B</sup>	$[K^+]$ <sub>sweat</sub> <sup>C</sup>
Maughan et al., 2007	To assess fluid balance and electrolyte losses in the members of 2 teams engaged in a competitive football match	<b>Total sample:</b> n=29 (mean±SD)  <b>Team A:</b> (n=9) 24±6years, 179±3cm, 79.51±7.51Kg, 24.9±1.9Kg.m <sup>-2</sup>  <b>Team B:</b> (n=11) 18±1years, 181±5cm, 76.83±6.72Kg, 23.5±1.8Kg.m <sup>-2</sup>  <b>Reserves:</b> (n=9) 17±0years, 181±2cm, 72.27±5.08Kg, 23.0±1.6Kg.m <sup>-2</sup>	<b>Exercise session:</b> Reserve grade match of the English Premier League. Reserves received nil game time.  <b>Sweat Collection:</b> As per Kurdak et al. (2010) except regional patches remained in situ for entire match	<b>Temp<sub>ENVIRO</sub></b> <sup>F</sup> : 6-8°C  <b>Relative Humidity</b> <sup>F</sup> : 50-60%	(mean±SD)	(mean±SD)	(mean±SD)	(mean±SD)
Neville et al., 2010	To assess the thermoregulatory responses to elite professional big-boat yacht racing, according to crew position and up and downwind sailing.	<b>Total:</b> n=32 male (2 America's Cup crews) (mean±SD) 36±7years, 92.3±11.9Kg, 1.84±0.06cm, 2.17±0.17m <sup>2</sup>	<b>Exercise Session:</b> 100 minutes of racing.  <b>Sweat Collection:</b> 4 regional patches (chest, scapula, forearm, thigh) remained in situ for entire race	(mean±SD)	(mean±SD)	(mean±SD)	(mean±SD)	(mean±SD)
Newell et al., 2008	To investigate fluid and electrolyte balance in elite gaelic football players.	<b>Total:</b> n=20 male 18-36years <sup>F</sup> 81.4±4.8Kg, 1.009±0.006g.mL <sup>-1</sup> (1.002-1.027g.mL <sup>-1</sup> ) <sup>F</sup>	<b>Exercise Session:</b> Usual 80min training session.  <b>Sweat Collection:</b> Regional sweat collection (upper arm, back, chest, thigh) for 20 minutes.  Body mass change (adjusted for fluid intake and urine loss only)  SSL = sweat rate x reg. $[Na^+]$ <sub>sweat</sub> (unadjusted for whole body)	<b>Temp<sub>ENVIRO</sub></b> : 16-18°C  <b>Relative Humidity:</b> 82-88%	(mean±SD)	(mean±SD)	(mean±SD)	Not reported
Pahnke et al., 2010	To determine if changes in serum sodium concentration are related to fluid balance as well as SSL in ultra-distance triathletes	<b>Total sample:</b> n=46 Endurance trained athletes. (mean±SD)  <b>Male:</b> n=26 44.5±11.4years, 77.0±10.3Kg <b>Female:</b> n=20 41.8±9.9years 61.8±5.6Kg	<b>Exercise Session:</b> 30 minute stationary cycling at 70-75% of self-reported maximum heart rate.  <b>Sweat Collection:</b> Regional sweat patches (forearm and scapula). Body mass change adjusted for fluid intake and sweat contained in clothing. SSL calculation not specified	<b>Temp<sub>ENVIRO</sub></b> : 28.3±1.2°C  <b>Relative Humidity:</b> 65.6±3.8%	(mean±SD)	(mean±SD)	(mean±SD)	Not reported

<sup>A</sup>Sweat sodium concentration, <sup>B</sup>Sweat sodium loss, <sup>C</sup>Sweat potassium concentration, <sup>D</sup>Environmental temperature, <sup>E</sup>Mean±SD or mean±SE unspecified, <sup>F</sup>Range (Table 1. continues on next page)

Table 1 (continued).

Reference	Aim	Participants	Protocol	Environment	Sweat volume or rate	$[Na^+]$ <sub>sweat</sub> <sup>A</sup>	SSL <sup>B</sup>	$[K^+]$ <sub>sweat</sub> <sup>C</sup>
Palmer et al., 2010	To evaluate the repeatability of hydration and sweat measures taken during ice hockey practices with players drinking only water; and to determine whether having a carbohydrate (CHO)/electrolyte solution to drink impacted on these measures	<b>Male:</b> n=18 Elite ice hockey players (mean±SE) 17.6±0.3years, 83.0±1.7Kg 182.9±1.4cm	<b>Exercise Session:</b> 3 observations of regular training (1.58(0.07)hours) over a 6 week period  <b>Sweat Collection:</b> Regional sweat patch (forehead) removed after 30 minutes.  Body mass change adjusting for fluid intake and urine loss only.  SSL = sweat rate x reg. $[Na^+]$ <sub>sweat</sub> (unadjusted for whole body)	<b>Temp<sub>ENVIRO</sub>:</b> 11.4±0.8°C  <b>Relative Humidity:</b> 52±3%	(mean±SE)	(mean±SE)	(mean±SE)	Not reported
Shirreffs et al., 2005	To assess individual sweat and solute loss during a professional football pre-season training session.	<b>Total Sample:</b> n=26 (mean±SD) 26±4years, 1.81±0.05cm, 77±5Kg  Sweat collection sub-sample (n=7)	<b>Exercise Session:</b> One usual training session (2nd of day) from approximately 1930-2100hours.  <b>Sweat Collection:</b> Regional (chest, forearm, back, thigh) collection from the sub sample during the first 15-30 minutes of training session.  Body mass change adjusted for fluid intake and urine loss	<b>Temp<sub>ENVIRO</sub>:</b> 32±3°C  <b>Relative Humidity:</b> 20±5%	(mean±SD)	(mean±SD)	(mean±SD)	(mean±SD)

<sup>A</sup>Sweat sodium concentration, <sup>B</sup>Sweat sodium loss, <sup>C</sup>Sweat potassium concentration, <sup>D</sup>Environmental temperature, <sup>E</sup>Mean±SD or mean±SE unspecified, <sup>F</sup>Range (Table 1. continues on next page)

Table 1 (continued).

Reference	Aim	Participants	Protocol	Environment	Sweat volume or rate	$[Na^+]$ <sub>sweat</sub> <sup>A</sup>	SSL <sup>B</sup>	$[K^+]$ <sub>sweat</sub> <sup>C</sup>
Stofan et al., 2005	To determine if footballers with a history of heat cramps have elevated fluid or sodium losses during 2 per day training.	<b>Total sample:</b> n=10 (size and race matched) <b>Crampers:</b> n=5 <b>Controls:</b> n=5	<b>Exercise session:</b> Pre-season training camp in Summer 2002. Morning and evening sessions = 2.5 hours each  <b>Sweat Collection:</b> Regional (forearm) sweat patch in situ for first 30 minutes of each session  Body mass change adjusted for fluid intake, urine loss, and ankle strapping.  SSL = sweat rate x reg. $[Na^+]$ <sub>sweat</sub> (unadjusted for whole body)	<b>Temp<sub>ENVIRO</sub>:</b> <b>Morning:</b> 22.7-26.0°C <b>Evening:</b> 30.8-28.2°C  <b>Relative Humidity:</b> <b>Morning:</b> 72-93% <b>Evening:</b> 51-72%	(mean±SD)	(mean±SD)	(mean±SD)	Not reported
Stofan et al., 2002	To measure sweat loss and fluid intake in 16 NFL players and sweat sodium concentration in a subset of the players (n = 13) during three practices in different US regions.	Total sample (n=16) sub sample (n=13) (mean±SD)	<b>Exercise session:</b> Pre-season training session in 3 different areas in the US lasting approximately 2 hours.  <b>Sweat Collection:</b> Regional arm bag collection  Body mass change adjusted for fluid intake, urine loss.  SSL = sweat rate x reg. $[Na^+]$ <sub>sweat</sub> (unadjusted for whole body)	<b>Temp<sub>ENVIRO</sub>:</b> 12-31°C  <b>Relative Humidity:</b> Not reported	<b>Range<sup>E</sup>:</b> 1.3-5.2L.h <sup>-1</sup>	<b>Range<sup>E</sup>:</b> 22-101mmol.L <sup>-1</sup>	<b>Range<sup>E</sup>:</b> 0.8-9.9g	Not reported

<sup>A</sup>Sweat sodium concentration, <sup>B</sup>Sweat sodium loss, <sup>C</sup>Sweat potassium concentration, <sup>D</sup>Environmental temperature, <sup>E</sup>Mean±SD or mean±SE unspecified, <sup>F</sup>Range

## 2.2 Determinants of Sweat Sodium Concentration

### 2.2.1 Sweat Sample Collection

#### Whole body washdown

Whole body washdown (WBW) is currently considered a gold standard for collecting sweat samples in research. Prior to a study by Shirreffs and Maughan (1997), methods of WBW were questionable in terms of completeness, and therefore Shirreffs and Maughan (1997) designed an apparatus to standardise data collection and reduce the risk of sampling error. All unevaporated sweat produced during a specified exercise protocol is captured within this apparatus and a total body sweat sample can be extracted and analysed for  $[Na^+]_{sweat}$ . Subsequently SSL can be calculated if sweat volume (body mass change) data has been collected. Five participants were instructed to repeat an exercise protocol four times (with two days between each trial) under the same exercise and environmental conditions they were originally exposed to in achieving a body mass loss of approximately 2% (Table 2.). The mean range for within-participant variation of  $[Na^+]_{sweat}$  was 22mmol (506mg), but in one of the four trials, the participants incurred either a body mass loss above or below the target of 2% which may explain such variance. For example, the intraclass correlation coefficient (ICC) indicates that approximately 80% ( $R=0.800$ ) of the variance was between participants rather than within them (Shirreffs and Maughan, 1997). Consequently this WBW technique has been adopted for use in more recent laboratory studies measuring  $[Na^+]_{sweat}$  and SSL (Table 2.).

Whole body washdown is unsuitable for collecting sweat samples from sports teams in field research. The method requires participants to exercise on a stationary bicycle inside a plastic chamber which is generally (but not always) under controlled

Table 2. Laboratory studies collecting unevaporated sweat samples with whole body washdown (WBW)<sup>A</sup>

Reference	Aim	Participants	Protocol	Environment	Sweat Volume / Sweat Rate	[Na <sup>+</sup> ] <sub>sweat</sub> <sup>B</sup>	[K <sup>+</sup> ] <sub>sweat</sub> <sup>C</sup>
Baker et al., 2009	Simultaneous WBW and regional collections from exercising athletes to generate regression equations for predicting WBW sweat [Na <sup>+</sup> ] and [K <sup>+</sup> ] from single- and five-site REG sweat patch	<b>Total:</b> n=25 mean±SD  <b>Comparison study:</b> 10 male, 10 female 37±10years, 72±11Kg, 1.85m <sup>2</sup>  <b>Repeatability study:</b> 5 male 34±7years, 73±9Kg	<b>Exercise:</b> 90 min stationary bicycle at 75% maximum heart rate  <b>Sweat Collection:</b> <b>WBW:</b> Shirreffs and Maughan (1997) <b>Reg:</b> Absorbent patches – forehead, chest, scapula, forearm, thigh  <b>Repeatability study:</b> Exercise repeated 3 times by participants with ≥1 and <2 weeks between each trial. Within-participant hydration status confirmed by baseline body mass and USG. Heat acclimatisation status was inconsistent between participants but consistent within-participants.	<b>Temp<sub>ENVIRO</sub>:</b> 30°C  <b>Relative Humidity:</b> 44%	Mean±SD (range)  <b>Whole body sweat rate:</b> Males (n=10): 1.0±0.2L.h <sup>-1</sup> (0.7-1.2L.h <sup>-1</sup> )  Nude body mass change adjusted for fluid intake, urine loss, respiratory water loss, and substrate oxidation.	Mean±SD (range)  <b>[Na<sup>+</sup>]<sub>sweat Whole body:</sub></b> 41±19mmol.L <sup>-1</sup> (14-88mmol.L <sup>-1</sup> ) P<0.05 vs. all reg. sites except thigh  <b>[Na<sup>+</sup>]<sub>sweat Regional:</sub></b> Forehead: 72±28mmol.L <sup>-1</sup> Chest: 66±33mmol.L <sup>-1</sup> Scapula: 64±27mmol.L <sup>-1</sup> Forearm: 52±32mmol.L <sup>-1</sup> Thigh: 39±25mmol.L <sup>-1</sup>  Mean of 5 sites: 59±27mmol.L <sup>-1</sup> (21-127mmol.L <sup>-1</sup> )	[K <sup>+</sup> ] <sub>sweat Whole body:</sub> 3.6±0.7mmol.L <sup>-1</sup> (2.5-4.9mmol.L <sup>-1</sup> )  [K <sup>+</sup> ] <sub>sweat Regional:</sub> Forehead: 4.6±1.2mmol.L <sup>-1</sup> Chest: 3.8±0.7mmol.L <sup>-1</sup> Scapula: 4.0±0.7mmol.L <sup>-1</sup> Forearm: 4.8±1.0mmol.L <sup>-1</sup> Thigh: 5.0±0.9mmol.L <sup>-1</sup>  Mean of 5 sites: 4.4±0.7mmol.L <sup>-1</sup> (3.1-5.8mmol.L <sup>-1</sup> )
Patterson et al 2000	To assess regional variations in sweat composition and compare with whole body composition.	<b>Total:</b> n=10 (male) Mean±SD  22.1±4.6years 76.4±6.9Kg	<b>Exercise:</b> 90 min stationary bicycle at 45.5(10)% peak aerobic power.  <b>Sweat Collection:</b> <b>WBW:</b> Shirreffs and Maughan , 1997 <b>Reg:</b> Absorbent patches – forehead, chest, scapula, abdomen, lower back, upper arm, forearm, hand, thigh, calf, foot	<b>Temp<sub>ENVIRO</sub>:</b> 20°C  <b>Relative Humidity:</b> 50%	Body mass change (unspecified if nude or clothed). Adjusted for fluid intake and urine loss only.  <b>Whole body:</b> 0.7±0.1mg.cm <sup>-2</sup> .min <sup>-1</sup>	Mean±SD  <b>[Na<sup>+</sup>]<sub>sweat Whole body:</sub></b> 24.1±15.0mmol.L <sup>-1</sup>  <b>[Na<sup>+</sup>]<sub>sweat Regional:</sub> (mmol.L<sup>-1</sup>) Forehead: 56.7±28.9 Chest: 47.6±25.7 Scapula: 41.1±24.8 Forearm: 42.2±25.8</b>	Mean±SD  [K <sup>+</sup> ] <sub>sweat Whole body:</sub> 3.25±0.62mmol.L <sup>-1</sup>  [K <sup>+</sup> ] <sub>sweat Reg.: (mmol.L<sup>-1</sup>) Forehead: 4.5±1.6 Chest: 3.8±1.2 Scapula: 3.5±1.2 Forearm: 5.9±1.3</sub>
Shirreffs & Maughan, 1997	To describe an improved method for collection of whole body sweat from exercising individuals with whole body washdown (WBW) and compare with a regional (back) collection method	<b>Total:</b> n=7 Healthy young adult men (n=5) and women (n=2) unspecified physical characteristics.  <b>Recovery Trial:</b> n=4  <b>Repeatability Trial:</b> n=5  <b>Normal Values trial:</b> n=7  <b>Comparison trial:</b> n=5	<b>Exercise:</b> 5-minute bouts at ≈60% VO <sub>2max</sub> with 5-minute rest intervals to achieve ≈2% body mass loss  <b>Recovery:</b> 8 trials of the washdown procedure after a solution of NaCl and K <sup>+</sup> was poured over participants at rest to assess completeness of sweat electrolyte recovery  <b>Repeatability:</b> 4 repeated exercise trials per participant under the same conditions to assess reproducibility of results. Assessed with the mean within-participant range of [Na <sup>+</sup> ] <sub>sweat</sub> and [K <sup>+</sup> ] <sub>sweat</sub> , and ICC <sup>E</sup>  <b>Normal Values:</b> To determine a range of 'normal' values for sweat [Na <sup>+</sup> ] and [K <sup>+</sup> ] of participants  <b>WBW vs. Regional:</b> 4 repeated exercise trials per participant. Sweat [Na <sup>+</sup> ] and [K <sup>+</sup> ] collected from WBW vs. Regional (back)	<b>Temp<sub>ENVIRO</sub>:</b> Recovery: 20°C Others: 34°C  <b>Relative Humidity:</b> ≈60-70%	<b>Volume Recovery:</b> mean% ± SD (CV) = 102±2, (3.3%)  Nude body mass change adjusted for respiratory water loss and substrate oxidation. Fluid intake adjustment and urine loss not required or specified.	<b>Na<sup>+</sup> Recovery:</b> mean% ± SD (and CV) = 99±2, (1.8%)  <b>Repeatability:</b> Mean within-participant range (ICC): Na <sup>+</sup> =22mmol (R=0.800)  <b>Normal:</b> Mean±SD, ( range) Na <sup>+</sup> =50.8±16.5, (35.2-81.0mmol.L <sup>-1</sup> )  <b>WBW vs. Regional:</b> Mean±SDmmol.L <sup>-1</sup> WBW = 51.6±18.3 Reg = 75.3±21.9	<b>K<sup>+</sup> Recovery:</b> Mean % ± SD (and CV) = 98±9, (6.4%)  <b>Repeatability:</b> Mean within-participant range (ICC): K <sup>+</sup> =3.3mmol (R=0.951)  <b>Normal:</b> Mean±SD, ( range) K <sup>+</sup> =4.8±1.6, (2.7-6.8mmol.L <sup>-1</sup> )  <b>WBW vs. regional:</b> Mean± SDmmol.L <sup>-1</sup> WBW = 5.6±1.8) Reg = 3.7±2.2

<sup>A</sup>Whole body washdown (Shirreffs & Maughan, 1997),<sup>B</sup>Sweat sodium concentration,<sup>C</sup>Sweat potassium concentration,<sup>D</sup>Environmental temperature,<sup>E</sup>Intracorrelation coefficient

environmental conditions (Pahnke et al., 2010; Shirreffs & Maughan, 1997). Only one participant can enter the chamber and perform exercise at any one time, and all participants are burdened by the need to clean their body and rinse with deionised water before entering the exercise chamber which must be disassembled, cleaned, rinsed with deionised water and reassembled between each participant to reduce the risk of sample cross-contamination (Shirreffs & Maughan, 1997). Such time- and resource-intensive requirements can restrict the number of participants able to have sweat samples collected in a timely manner.

#### *Regional sweat sample collection*

Regional sweat sample collections offer a practical alternative to WBW in field research. They can be captured from single or multiple sites of skin by applying sweat absorbent patches to the area chosen such as the chest or scapula, or by placing a polyethylene bag over the hand and arm of participants' before an exercise protocol (Bergeron, 2003; Fowkes Godek et al., 2010; Hamouti et al., 2010b; Henkin et al., 2010; Kurdak et al., 2010; Maughan et al., 2007; Newell et al., 2008; Pahnke et al., 2010; Palacios et al., 2003; Palmer et al., 2010; Stofan, 2002). Participant burden is reduced compared to WBW, and athletes can generally perform their usual training or competition session without major disruption. However, careful consideration is needed to avoid overestimating the whole body  $[Na^+]_{sweat}$  and/or SSL.

Regional  $[Na^+]_{sweat}$  can become falsely elevated when occlusive sweat collection techniques such as the arm bag are used. With this particular method, the local area of covered skin remains wet during episodes of sweating which can cause sodium, potassium and chloride ions to leach from the outer layer of skin known as the stratum corneum (Weschler, 2008). In contrast, samples collected with absorbent patches can keep the stratum corneum relatively dry by drawing sweat away from the skin as it is secreted. Leaching is still possible with absorbent patches but this can be

assessed by measuring sweat potassium concentration ( $[K^+]_{sweat}$ ). Leaching can be excluded when  $[K^+]_{sweat}$  is within a normal physiological range of 2.5-6.8mmol.L<sup>-1</sup> as determined from WBW sweat sample collection (Baker et al., 2009; Patterson et al., 2000; Shirreffs & Maughan, 1997).

An individual's  $[K^+]_{sweat}$  remains relatively constant regardless of their sweat rate. This is in stark contrast to the linear relationship between regional  $[Na^+]_{sweat}$  and sweat rate which differs by region across the body (Allan & Wilson, 1971; Baker et al., 2009; Buono et al., 2007; Buono et al., 2008; Costill, 1977; Inoue et al., 1998; Kondo et al., 1998 ; Patterson et al., 2000; Sato & Dobson, 1970; Sato et al., 1989; Shirreffs & Maughan, 1997). Comparisons between regional and WBW sweat collection methods consistently show that whole body  $[Na^+]_{sweat}$  is significantly lower than the regional  $[Na^+]_{sweat}$  of individual sites such as the chest, forearm, forehead, scapula, and the mean of these sites (Table 2.). As each individual's sweat rate increases, sodium reabsorption from precursor fluid decreases which means their  $[Na^+]_{sweat}$  increases (Buono et al., 2008; Inoue et al., 1998; Quinton, 2007). Therefore regional  $[Na^+]_{sweat}$  should ideally be adjusted to reflect an estimate of whole body  $[Na^+]_{sweat}$  before being used to estimate an individual's whole body SSL.

Adjusting regional  $[Na^+]_{sweat}$  to a whole body estimate is possible with calculations developed by Baker et al. (2009). The authors collected sweat samples from 10 male and 10 female participants performing a standardised exercise protocol for 90 minutes under controlled environmental conditions (Table 2). Sweat samples were collected from each participant with the WBW method of Shirreffs & Maughan (1997), and with absorbent patches from multiple regional skin sites for subsequent comparisons of  $[Na^+]_{sweat}$ . The respective mean regional  $[Na^+]_{sweat}$  of the scapula, forearm, chest, forehead and thigh (and the mean of these five sites combined) were significantly correlated with the mean of whole body  $[Na^+]_{sweat}$  and there was no

evidence of sex having an influence on these results (Table 2.). Therefore the study verified that regional  $[Na^+]_{sweat}$  can be adjusted to more accurately estimate whole body  $[Na^+]_{sweat}$  when data is collected in similar exercise and environmental conditions.

#### Application to field study design

Collection of sweat samples can contribute to the variance in  $[Na^+]_{sweat}$  and SSL reported in the literature (Table 1.). The cleaning and drying of a participant's skin site before making a regional collection can reduce the risk of sample contamination from sodium already present on the skin. Applying an absorbent patch rather than attaching an arm bag to collect regional sweat samples can lower the risk of leaching (Weschler, 2008), but all patches should be placed immediately in airtight storage to avoid any evaporation of water which would increase the  $[Na^+]_{sweat}$ . Some existing field studies have adjusted regional  $[Na^+]_{sweat}$  to whole body  $[Na^+]_{sweat}$  (Hamouti et al., 2010a), but more often this has not been done for unspecified reasons (Fowkes Godek et al., 2010; Henkin et al., 2010; Palmer et al., 2010). In some cases this is because the research was conducted before valid adjustment equations were available (Baker et al., 2009; Maughan et al., 2004; Maughan et al., 2005; Maughan et al., 2007; Newell et al., 2008; Stofan et al., 2005; Shirreffs et al., 2005). However a standardised sweat sample collection protocol is indicated for use in future field studies to minimise the potential for confounding effects on  $[Na^+]_{sweat}$  results.

#### **2.2.2 Sweat Rate**

The literature clearly shows a relationship exists between sweat rate and  $[Na^+]_{sweat}$  (Allan & Wilson, 1971; Baker et al., 2009; Buono et al., 2007; Cage & Dobson, 1965; Kondo et al., 1998; Sato et al., 1989; Shirreffs & Maughan, 1997). It is also well known that sweat rate can be highly variable within and between individuals which can be explained by environmental factors such as Temp<sub>ENVIRO</sub>, relative humidity and

wind speed, and factors unique to individual athletes such as body surface area (BSA) and their respective intensity of exercise (Cheuvront, 2004; Fowkes Godek et al, 2005; Galloway & Maughan, 1997; Kondo et al., 1998; Neville et al., 2010; Nielson, 1996). Therefore all of these variables can be considered determinants of  $[Na^+]_{sweat}$  and/or SSL through their influence on sweat rate.

#### Environmental conditions

Evaporative cooling is the only heat loss mechanism possible when the gradient between  $Temp_{SKIN}$  and  $Temp_{ENVIRO}$  becomes negligible ( $Temp_{SKIN} \approx Temp_{ENVIRO}$ ) and negative ( $Temp_{SKIN} < Temp_{ENVIRO}$ ) (Nadel, 1979). Under controlled conditions, Galloway and Maughan (1997) studied eight healthy male volunteers (25±2 years, 72.1±3.5Kg, and 176±3cm) over four exercise trials on stationary bicycles. Each trial was separated by one to two weeks - on each occasion the participants exercised until they could no longer continue or maintain a minimum of 60 revolutions per minute on the bicycle. The participants were described as moderately active but not specifically trained (mean  $VO_{2max}$  of  $4.01\pm0.17L.min^{-1}$ ). The mean sweat rates were significantly different between all of the four trials in  $Temp_{ENVIRO}$  of  $3.6\pm0.3^\circ C$  ( $0.55L.h^{-1}$ ),  $10.5\pm0.5^\circ C$  ( $0.65L.h^{-1}$ ),  $20.6\pm0.2^\circ C$  ( $0.78L.h^{-1}$ ),  $30.5\pm0.2^\circ C$  ( $1.15L.h^{-1}$ ) respectively (Galloway & Maughan, 1997). In other words this study demonstrated that incremental increases in  $Temp_{ENVIRO}$  corresponded to increased rates of sweating when relative humidity was held constant ( $70\pm2\%$ ).

Relative humidity determines the efficiency of evaporative cooling in a given environment. It describes the moisture content of that environment - increases indicate the environment is moving toward moisture saturation which means the efficiency of evaporative cooling is subsiding (Nielson et al., 1997; Nielson et al., 1993). Sweat simply drips from the skin as the body attempts to thermoregulate which can initially increase sweat rate but ultimately can lead to earlier development

of hyperthermia and termination of exercise compared to more favourable environmental conditions (Nielson et al., 1997; Shapiro et al., 1982). The maximum evaporation rate of an athlete can be calculated when the Temp<sub>ENVIRO</sub>, BSA, and rate of metabolic heat production are known (Shapiro et al., 1982; Nielson, 1996).

### Individual characteristics

The BSA of an athlete can influence their sweat rate. In a field study comparing the mean sweat rate of 10 American Footballers with the mean sweat rate of five cross country runners with a significantly lower mean BSA ( $2.4\pm0.2\text{m}^2$  versus  $1.9\pm0.2\text{m}^2$  respectively,  $p<0.001$ ), Fowkes Godek et al. (2005) observed a significantly higher sweat rate among the Footballers compared to runners ( $2.14\pm0.53\text{L.h}^{-1}$  versus  $1.77\pm0.4\text{L.h}^{-1}$ ,  $p<0.01$ ). After adjusting for BSA, the difference disappeared (Fowkes Godek et al., 2005). This finding demonstrates that BSA can influence sweat rate, although the intensity of exercise was not necessarily constant between the runners and Football players.

Exercise intensity influences metabolic heat production. Internal heat production increases as fuel substrates are oxidised for providing energy to contracting skeletal muscles (Saltin & Hermansen, 1966). Muscle contraction is approximately 20% efficient meaning that 80% of the energy produced by contracting muscles is released into the circulatory system as heat (Kenney, 1998). Using a sample of nine young adult males of similar height, weight and fitness level in controlled environmental conditions ( $28.3\pm0.2^\circ\text{C}$  and  $42.6\pm2.4\%$  relative humidity), Kondo et al. (1998) demonstrated that increasing the relative intensity of exercise from low (35%  $\text{VO}_{2\text{max}}$ ) to moderate (50%  $\text{VO}_{2\text{max}}$ ), and from moderate to high (50% to 65%  $\text{VO}_{2\text{max}}$ ) in a series of 30 minute stationary bicycle exercise sessions separated by at least one day could significantly increase sweat rate (from  $0.39\pm0.03\text{mg.cm}^{-2}$  to  $0.71\pm0.06\text{mg.cm}^{-2}$ , and from  $0.71\pm0.06\text{mg.cm}^{-2}$  to  $1.07\pm0.08\text{mg.cm}^{-2}$  respectively,

$p<0.05$ ). The authors attributed this finding to a greater number of activated sweat glands (ASG) and increased sweat gland output (SGO) from each ASG (Kondo et al., 1998). Therefore athletes with a high BSA working at comparable intensity to athletes with lower BSA will be predisposed to higher sweat rates and higher  $[Na^+]_{sweat}$  assuming all other determinants of  $[Na^+]_{sweat}$  are held constant (Kondo et al., 1998; Fowkes Godek et al., 2005; Sato & Dobson, 1970).

#### *Application to field study design*

An exercise protocol controlling for BSA and exercise intensity may not be practical in a field observation of team sport athletes. In the laboratory setting, participants can be matched for these variables and their sweat rate controlled (Buono et al., 2007; Buono et al., 2008). However, BSA and exercise intensity can be expected to differ markedly within a sports team considering the physical requirements of certain positions within that team (Fowkes Godek et al., 2005). For example a prop forward would be expected to have a larger BSA compared to a scrum half in Rugby Union.

Measuring the change in body mass can accurately estimate sweat volume over an exercise session (Sawka et al., 2007). Sweat rate reflects an individual's sweat volume or change in TBW status relative to time. Adjusting for fluid intake or losses other than from sweat (i.e. urine) helps to estimate more closely the fluid lost from sweat (King et al., 2008). Nude measurements are ideal, while minimal clothing is preferable to wearing multiple layers including socks which can trap secreted sweat and therefore underestimate body mass changes. Towel drying off excess sweat before the post-exercise measurement also helps to avoid underestimating sweat loss (Oppiger & Bartok, 2002). Adjusting for water losses from respiration and water gains from substrate metabolism is recommended (King et al., 2008), but many field and some laboratory studies choose not to under the assumption that losses of this nature are relatively minor and may actually be counter-balanced in terms of TBW

change (Sawka et al., 2007; Buono et al., 2007; Hamouti et al., 2010a; Henkin et al., 2010; Institute of Medicine, 2005; Kurdak et al., 2010; Maughan et al., 2005; Shirreffs et al., 2005; Patterson et al., 2000). An estimation of sweat rate is completed by dividing the absolute estimate of sweat volume into the duration of exercise and multiplying by 60 minutes.

### **2.2.3 Heat Acclimatisation**

Acclimatisation describes a series of physiological adaptations to improve thermoregulatory control during exercise in the heat (Sawka & Young, 2006). Acclimation describes the same adaptations achieved through artificial means such as an environmental chamber (Kirby & Convertino, 1986; Saat et al., 2005; Smiles & Robinson, 1971). Both methods confer an earlier onset of sweating, a higher sweat rate, and lower  $[Na^+]_{sweat}$  for a given exercise intensity compared to the non-acclimatized/acclimated state (Buono et al., 2007; Kirby & Convertino, 1986; Sawka & Young, 2006; Smiles & Robinson, 1971). These adaptations effectively promote sodium retention within the ECF which accommodates increased plasma volume whilst maintaining  $[Na^+]_{blood}$  (Sawka & Young, 2006). An increased plasma volume leads to reduced heart rate, lower cardiovascular strain, and ultimately prolonged exercise tolerance time in hot and humid conditions (Chinevere et al., 2008; Kirby & Convertino, 1986; Nielson et al., 1993; Nielson et al., 1997; Smiles & Robinson, 1971). Therefore comparisons of  $[Na^+]_{sweat}$  or SSL between groups or individuals who differ in heat acclimatisation status can be limited by this confounding effect.

#### *Application to field study design*

Comparing heat acclimatisation status between individuals or groups can be a difficult task. Given that acclimatisation develops in stages, there is no single marker which can verify when the full effects are achieved. Studies under controlled conditions show this can occur after approximately 14 days of consistent training in

the heat (Sawka & Young, 2006). Reduced  $[Na^+]_{sweat}$  can occur within 10 days and an increased sweat rate can develop between seven and 14 days (Armstrong, 2000). These effects begin to subside after one week without exercise in the heat and will have largely disappeared after approximately three weeks (Sawka & Young, 2006). Therefore all individuals having consistently trained in hot and humid conditions within these timeframes can be considered acclimatised to local conditions. However, this assumption can be compromised by confounding effects on  $[Na^+]_{sweat}$  such as exercise intensity (via sweat rate), hydration status or dietary sodium intake.

#### **2.2.4 Hydration Status**

Hypohydration is associated with reductions in sweat rate as the body attempts to preserve TBW (Cheuvront, 2004; Sawka & Young, 2006; Shibasaki et al., 2003). A positive linear association between sweat rate and  $[Na^+]_{sweat}$  has been observed under controlled conditions (Allan & Wilson, 1971; Baker et al., 2009; Cage & Dobson, 1965; Sato et al., 1989; Shirreffs & Maughan, 1997). By this logic and assuming all other determinants of  $[Na^+]_{sweat}$  are constant, athletes beginning exercise hypohydrated may be predisposed to lower  $[Na^+]_{sweat}$  during the session compared to euhydrated individuals. However, no such comparison has been made to date. Cheung and McClellan (1998a; 1998b) have designed studies to include hypohydrated and euhydrated participants at baseline, but  $[Na^+]_{sweat}$  was not a focus for their research. The effect of progressive dehydration on  $[Na^+]_{sweat}$  during exercise has been investigated, but participants in those particular studies followed instruction to ensure they were euhydrated at baseline (Brown et al., 2011; Montain et al., 2007; Morgan et al., 2004).

In the controlled crossover experiment by Morgan et al. (2004), eight healthy young adult males received either regular hydration to replace pre-determined sweat losses (euhydration group) or no hydration (dehydration group) during a stationary bicycle

exercise lasting for two hours (relative intensity of  $39.5\pm1.6\%$   $\text{VO}_{2\text{max}}$ ). Hypohydration was confirmed among the dehydration group compared to euhydration group given that mean heart rate,  $\text{Temp}_{\text{SKIN}}$ ,  $\text{Temp}_{\text{CORE}}$ , and  $[\text{Na}^+]_{\text{blood}}$  all increased significantly as expected (Cheuvront, 2004; Morgan et al., 2004; Sawka & Young, 2006; Shibasaki et al., 2003). Surprisingly though, mean sweat rate was similar between the dehydration and euhydration groups ( $0.94\pm0.06\text{L.h}^{-1}$  versus  $0.89\pm0.08\text{L.h}^{-1}$  respectively,  $p=0.310$ ), and  $[\text{Na}^+]_{\text{sweat}}$  was significantly higher among the dehydration compared to euhydration group ( $91.1\pm6.8\text{mmol.L}^{-1}$  versus  $81.1\pm5.9\text{mmol.L}^{-1}$ ,  $p=0.04$ ). Morgan et al. (2004) suggest this may be because of a significantly higher  $[\text{Na}^+]_{\text{blood}}$  among the dehydrated compared to euhydrated participants ( $142\pm1\text{mmol.L}^{-1}$  versus  $139\pm1\text{mmol.L}^{-1}$ ,  $p<0.05$ ) which would explain a higher sodium concentration of the precursor fluid given it is isotonic in relation to  $[\text{Na}^+]_{\text{blood}}$  (Sato & Dobson, 1970; Cage & Dobson, 1965; Quinton, 2007). This has since been indirectly verified by Brown et al. (2011).

Brown et al. (2011) designed an exercise protocol to induce approximately 3% body mass loss (with 20 minute exercise bouts at 50% relative intensity and 5 minute recovery periods but rehydration disallowed) among 18 participants aged 18-40 years of similar body size and fitness level. Unfortunately the participants'  $[\text{Na}^+]_{\text{sweat}}$  after each 20 minute exercise bout was not reported as it was not a primary outcome of interest to the authors, but they did report an observation of  $[\text{Na}^+]_{\text{sweat}}$  increasing with progressive dehydration (Brown et al., 2011). In other words, dehydration which is considered by experts as significant during an exercise protocol (Sawka et al., 2007) can confound  $[\text{Na}^+]_{\text{sweat}}$  and SSL results. However, Montain et al. (2007) demonstrated that  $[\text{Na}^+]_{\text{sweat}}$  can remain relatively constant over sustained periods of sweating when significant dehydration is prevented. Seven heat acclimated participants (six males, one female) completed five bouts of 60 minute treadmill

exercise ( $1.56 \text{ m.s}^{-1}$ , 2% grade) with 20 minutes rest between each bout in conditions of  $27^\circ\text{C}$  and 40% relative humidity. Sweat samples were collected from participants with a pouch attached to their upper back during exercise bouts one (10-70 minutes), three (170-230 minutes), and five (330-390 minutes). The  $[\text{Na}^+]_{\text{sweat}}$  of participants remained similar for all three exercise bouts ( $874 \pm 485 \mu\text{g.mL}^{-1}$ ,  $888 \pm 568 \mu\text{g.mL}^{-1}$ , and  $828 \pm 471 \mu\text{g.mL}^{-1}$  respectively,  $p>0.05$ ) when dehydration in excess of 2% initial body mass was prevented by allowing *ad libitum* fluid intake throughout each session (Montain et al., 2007).

#### Application to field study design

Current literature suggests that pre-exercise hydration status may have a confounding effect on  $[\text{Na}^+]_{\text{sweat}}$  and SSL. Such an effect would be difficult to assess because differences in baseline hydration status may be sustained, corrected or exacerbated during an exercise protocol depending on the amount of fluid consumed by participants (Montain et al., 2007; Brown et al., 2011; Morgan et al., 2004). Furthermore, controlling hydration status before and during a field observation may be unrealistic. Therefore monitoring this variable at baseline and after the exercise protocol is indicated for consideration of it having a confounding effect on the participant's  $[\text{Na}^+]_{\text{sweat}}$  and/or SSL during exercise. However, hydration status is a complicated variable to measure.

The dynamic nature of fluid balance means there is no set value defining euhydration – this fluctuates within a small range as fluid is lost (dehydration) and gained (rehydration) through activities of daily living (Greenleaf, 1992). For healthy euhydrated populations at rest, most fluid is lost in urine while rehydration is achieved through food and fluid intake. In other words, dehydration and rehydration generally do not occur concurrently. The mechanisms of sodium and fluid balance

are regularly in action to preserve fluid and electrolyte balance instead (Institute of Medicine, 2005).

Sweating is the main form of dehydration during exercise. Under normal circumstances,  $[Na^+]_{blood}$  progressively increases because more water is lost in sweat than electrolytes (Baker et al., 2008; Popowski et al., 2001; Shibasaki et al., 2006). Through osmosis, the increased  $[Na^+]_{blood}$  draws fluid from the intracellular fluid (ICF) to equalise solute concentration between the ECF and ICF compartments (Institute of Medicine, 2005). A reduced ICF volume is detected in the brain which stimulates secretion of arginine vasopressin (AVP) otherwise known as anti-diuretic hormone (ADH). Arginine vasopressin increases water reabsorption in the kidneys causing urine volume to decrease and become more concentrated with solute (Robertson et al., 1976). This cascade of events enables hydration status to be assessed directly through TBW changes, or by the monitoring of blood or urinary biomarkers associated with restoring euhydration. However, not all methods are appropriate for assessing hydration status in a cross-sectional field study.

Total body water is an accurate indicator of hydration status when repeat measures can be made. Deuterium oxide dilution is considered the gold standard for collecting such data (Schoeller et al., 1980). Depending on body composition, it can measure TBW in humans to an accuracy of 200mL (Halliday & Miller, 1977). However, the method is expensive and given the time resource required, it is more suitable for use in laboratory settings or extravagant field research rather than everyday field observations. Bioelectrical impedance is an alternative method to deuterium oxide dilution for measuring TBW (Kushner & Schoellar, 1986). Ideally participants will have fasted for at least four hours and will be euhydrated prior to TBW measurements being taken for optimal accuracy and precision (Oppliger & Bartok, 2002). These prerequisites defeat the purpose of assessing an athletes' natural

hydration status on any given day. Measuring change in body mass can accurately estimate an athlete's change in TBW (Baker et al., 2009). Current consensus for optimal hydration practice during activity is to aim for <1-2% body mass loss which demonstrates that sports nutrition experts consider body mass change to be a reliable indicator of TBW change (Sawka et al., 2007). However, like all methods of hydration assessment with TBW, body mass change is unsuitable for spot assessment in the pre-exercise period unless repeat measurements are possible.

Blood biomarkers are suitable for spot measures of pre-exercise hydration status. Measures include  $[Na^+]$ <sub>blood</sub>, osmolarity, haematocrit, haemoglobin, and even the fluid and sodium regulating hormones aldosterone and AVP can be indicative of hydration status (Cheuvront & Sawka, 2005). However, collecting blood samples from participants requires specially trained staff, and specialised equipment for analysis is needed for some measures such as plasma osmolarity - this can be expensive and cause delays to the delivery of results (Oppliger & Bartok, 2002). However, approximately 80-90% of plasma osmolality is due to sodium content, and this means  $[Na^+]$ <sub>blood</sub> can be measured as an alternative to plasma osmolarity (Greenleaf, 1992). Results are determined immediately after a finger-prick blood sample is obtained and analysed with a portable instrument such as an I-Stat Point of Care device (Abbott Laboratories, USA). Even so, assessing hydration status with blood biomarkers can be considered invasive to study participants compared to urinary biomarkers.

Urinary analyses provide practical measures of pre-exercise hydration status in the field. They are inexpensive, provide immediate feedback to athletes or researchers, and require minimal expertise (Oppliger & Bartok, 2002). The common markers include urine colour, osmolality, urine specific gravity (USG) and 24-hour volume. Osmolality refers to the concentration of all dissolved particles or waste products in a standard volume of urine, and USG refers to the density or mass per volume of a

urine sample in relation to pure water (Armstrong, 2000). Armstrong et al. (1998) investigated the validity and sensitivity of urine colour, osmolality and USG by comparing these measures against changes in TBW (as determined by change in body mass) among nine highly trained males of similar physical characteristics over the course of a dehydration and rehydration exercise protocol. The authors found that changes in the urinary biomarkers reflected TBW loss and changes in blood biomarkers during dehydration (Armstrong et al., 1998). They concluded urinary analyses as reliable for measuring pre-exercise hydration status in field research.

Urine specific gravity is an ideal urinary marker of hydration status in field research. Alternatives such as urine volume burden athletes to collect urine for 24 hours which can affect the completeness of samples (Oppliger & Bartok, 2002). Urine osmolality is accurate compared to blood markers and TBW change (Armstrong et al., 1994; Armstrong et al., 1998), but requires time, money and a trained technician all of which can be impractical in the field setting (Oppliger & Bartok, 2002). Similarly, urine colour is a valid measure of hydration status (Armstrong et al., 1994; Armstrong et al., 1998), but as this is a subjective assessment, inter- and intra-investigator errors are possible. To the contrary, USG is less time and resource-intensive. It provides an immediate, valid, and objective measure of pre-exercise hydration status to the investigator (Armstrong et al., 2008; Armstrong et al., 1994; Armstrong et al., 1998; Oppliger & Bartok, 2002). The currently agreed on threshold of euhydration for USG assessment is  $\leq 1.020\text{g.mL}^{-1}$  (Sawka et al., 2007). Even so, misclassification of hydration status can occur when using this threshold (Hamouti et al., 2010a).

Urine specific gravity is highly dependent on urine osmolality. This means USG can be influenced by dietary protein intake or muscle mass (Oppliger et al., 2005). In a study of nine male rugby players and nine male endurance runners, Hamouti et al. (2010b) found a positive correlation between muscle mass and metabolites of protein

in urine ( $p=0.04$ ), and between urinary protein metabolites and USG ( $p<0.001$ ). Muscle mass was significantly higher in the rugby players compared to the runners ( $42\pm6$ Kg versus  $32\pm3$ Kg respectively,  $p<0.001$ ). The rugby players were more likely than the runners to be classified as hypohydrated according to USG (56% versus 11%,  $p=0.03$ ) despite all study participants being classified as euhydrated with blood biomarkers. In other words, muscular athletes such as rugby players can be falsely identified as hypohydrated which is a limitation to acknowledge. Likewise, if large volumes of fluid are consumed by hypohydrated individuals before they provide a urine sample, an increased production of dilute urine can occur as the body is unable to equilibrate the water within the ICF and ECF body water compartments which means false negative results implying euhydration are possible (Oppliger & Bartok, 2002; Popowski et al., 2001; Kovacs et al., 2002). Therefore the behaviour is difficult to control and can only be acknowledged as a limitation of assessing hydration status with USG.

Urine specific gravity can be measured with refractometry, hydrometry or reagent strips. Stuempfle and Drury (2003) investigated the reliability and validity of the three methods within a sample of 21 healthy young adult members of a high performance wrestling team (all of similar physical characteristics). Study participants' urine samples were measured twice with each of the three methods (total of 6 measurements per sample) to determine the intra-investigator repeatability of each method which was then compared between investigators. The results derived from refractometry were used as the controls for comparing hydrometry and reagent strips against. All urine samples were collected from participants at the same time of day after refraining from exercise for 24 hours (Stuempfle & Drury, 2003). Intraclass correlation coefficients indicated that measurements derived from refractometry and hydrometry were consistent when tested twice by the same investigator ( $R=0.998$

and  $R=0.987$  respectively) but results from reagent strips were considered inconsistent within each tester ( $R=0.854$ ) (Stuempfle & Drury, 2003). Hydrometry and reagent strip measurements produced significantly higher mean estimates of USG compared to refractometry ( $1.018\pm0.006$  and  $1.017\pm0.007$  versus  $1.015\pm0.006\text{g.mL}^{-1}$  respectively,  $P<0.05$ ), therefore the chances of false positive results were greater with hydrometry or reagent strips compared to refractometry (Stuempfle & Drury, 2003).

The USG of urine samples represent an athletes' hydration status at the time the sample was collected. When there is a delay between sample collection and exercise, the urine sample may no longer reflect a participants' TBW status directly before the exercise. For example, in a field study observing  $[\text{Na}^+]_{\text{sweat}}$  of elite indoor sports athletes, Hamouti et al. (2010a) noted that individuals classified as hypohydrated on waking (based on USG of first morning void) may have corrected their negative TBW balance with fluid consumption before participating in their study later in the day. An appropriate standardised action would be to collect urine samples for USG analysis directly before exercise.

### **2.2.5 Dietary Sodium Intake**

Dietary sodium intake can influence  $[\text{Na}^+]_{\text{sweat}}$  and SSL. Over the past 30 years, laboratory studies have consistently shown that restricting sodium intakes to less than the current upper limit of 100mmol per day ( $2300\text{mg.day}^{-1}$ ) can significantly reduce  $[\text{Na}^+]_{\text{sweat}}$  or SSL compared to higher intakes (Table 3.). However, the impact is less clear when dietary sodium intakes are above 100mmol per day (Allsopp et al., 1998; Armstrong et al., 1985).

In a crossover experimental design, Armstrong et al. (1985) compared the mean SSL of participants following a daily sodium intake of 399mmol (approximately 9000mg)

Table 3. Experimental studies investigating the influence of dietary sodium intake on sweat sodium concentration ( $[Na^+]_{sweat}$ ) or sweat sodium loss (SSL)

Reference	Aim	Design	Participants	Protocol	Environment	Sweat Volume / Sweat Rate	$[Na^+]_{sweat}^A$	SSL <sup>B</sup>
Allsopp et al., 1998	To investigate the effect of manipulating dietary sodium intake on sweat sodium secretion during a heat acclimation protocol	Controlled experimental	<b>Total</b> Male: n=25 (unacclimatised) 18-40y 64-102Kg 20-34Kg.m <sup>-2</sup>	<b>Acclimation / Exercise:</b> Confined to environmental chamber for a 3 day control period followed by 5 day heat acclimation period with 60min light exercise protocol each day  <b>Dietary Sodium Conditions:</b> High (HNa) 348.4±0.8mmol.d <sup>-1</sup> (n=7)  Moderate (MNa) 174.1±0.6mmol.d <sup>-1</sup> (n=9)  Low (LNa) 66.3mmol.d <sup>-1</sup> (n=9)  <b>Sweat Collection:</b> WBW (Collins, 1971) on days 4 (baseline) and 8 (post acclimation)	<b>Temp<sub>ENVIRO</sub><sup>C</sup>:</b> Control: 25°C  <b>Relative Humidity:</b> Control and Acclimation: 40%	Not calculated from body mass losses because participants were free-living and had ad libitum access to fluid intake	Not assessed because of missing sweat volume data.	Determined by WBW (Collins, 1971)  <b>Day 4 (baseline):</b> Mean±SE HNa: 78.8±10.3mmol MNa: 63.8±8.0mmol LNa: 53.5±8.5mmol  p>0.05 between groups  <b>Day 8 (heat acclimated):</b> Mean±SE HNa: 52.6±6.2mmol MNa: 39.1±4.0mmol LNa: 24.8±2.1mmol  LNa < MNa and HNa (p<0.05)  Day 8 < day 4 for all 3 groups (p<0.01)
Armstrong et al., 1985	To investigate the effect of manipulating dietary sodium intake on sweat sodium secretion during a heat acclimation protocol.	Randomised crossover with 24.1±1.7 days between treatments.	<b>Total</b> n=9 mean±SE (unacclimatised & untrained)  Male: n=9 24.7±1.6y 177.2±1.9cm 71.9±3.3Kg 3.70±0.20L.min <sup>-1</sup>	<b>Acclimation / Exercise:</b> 8 day acclimation with 90min / day treadmill exercise (constant speed) in the environmental chamber. Free living outside of treadmill session.  <b>Sodium Diet:</b> High = 399mmol.day <sup>-1</sup> Low = 98mmol.day <sup>-1</sup>  <b>Sweat Collection:</b> WBW (Vellar, 1968) on sessions 1 and 8.	<b>Temp<sub>ENVIRO</sub>:</b> mean±SE 40.1±0.1°C  <b>Relative Humidity:</b> mean±SE 23.5±0.4%	<b>Protocol:</b> Nude body mass change adjusted for fluid intake and respiratory loss.  <b>Findings (mean±SE):</b> Baseline: High= 530±40mL.m <sup>-2</sup> .h <sup>-1</sup> Control=500±60mL.m <sup>-2</sup> .h <sup>-1</sup>  Day 8: High= 550±30mL.m <sup>-2</sup> .h <sup>-1</sup> Control=510±30mL.m <sup>-2</sup> .h <sup>-1</sup>	Not specified	mean±SE  <b>Baseline:</b> High= 61.2±7.9mmol.90min <sup>-1</sup> Control=63.2±7.4mmol.90min <sup>-1</sup> (p>0.05)  <b>Day 8:</b> High= 86.5±16.3mmol.90min <sup>-1</sup> Control= 37.8±3.2mmol.90min <sup>-1</sup> (p<0.05)
Hargreaves et al 1989	To examine the effect of sodium restriction on physiological responses to combined exercise and heat stress	Randomised crossover double-blind trial.	<b>Total:</b> n=8 Mean±SE (unacclimatised)  Male: n=8 23.4±1.1years 4.05±0.24L.min <sup>-1</sup>	<b>Acclimation / Exercise:</b> 60min at ~60% VO <sub>2max</sub> on stationary bicycle in environmental chamber after following the prescribed sodium diet for 2 weeks. Fluid intake was withheld during the session.  <b>Sodium Diet:</b> Control = 150mmol.day <sup>-1</sup> Low = 50mmol.day <sup>-1</sup>  <b>Sweat Collection:</b> WBW (Vellar, 1968)	<b>Temp<sub>ENVIRO</sub>:</b> 35°C  <b>Relative Humidity:</b> 25%	<b>Protocol:</b> Body mass change adjusted for respiratory and metabolic water loss  <b>Findings (mean±SE):</b> Control = 1.15±0.11L.h <sup>-1</sup> Low = 1.12±0.09L.h <sup>-1</sup>	Mean±SE  Control: 49.7±4.3mmol.L <sup>-1</sup>  Low: 38.4±5.2mmol.L <sup>-1</sup>  Control > Low (p<0.01)	Mean±SE  Control: 54.8±2.9mmol.h <sup>-1</sup>  Low: 40.6±3.6mmol.h <sup>-1</sup>  Control > Low (p<0.001)

<sup>A</sup>Sweat sodium concentration, <sup>B</sup>Sweat sodium loss, <sup>C</sup>Environmental temperature

with the mean SSL of those consuming 98mmol (approximately 2300mg) over eight days of heat acclimation. Whole body sweat samples were collected from nine untrained and unacclimated adult males over a 90-minute walking exercise protocol (Table 3.). After heat acclimation, there was a significant difference in the mean SSL between the high and low dietary sodium intakes ( $86.5 \pm 16.3$ mmol versus  $37.8 \pm 3.2$ mmol,  $p < 0.050$ ). Of particular interest, the mean SSL of the high sodium intake group significantly increased from baseline ( $61.2 \pm 7.9$ mmol to  $86.5 \pm 16.3$ mmol,  $p < 0.050$ ) whereas SSL significantly decreased among those consuming the lower sodium intake of 98mmol or approximately 2300mg ( $63.2 \pm 7.4$ mmol to  $37.8 \pm 3.2$ mmol,  $p < 0.050$ ). Unfortunately no further intakes of sodium above 100mmol were investigated by Armstrong et al. (1985) and a critical dietary sodium intake at which SSL began to increase is unclear.

Allsopp et al. (1998) have since provided further insight into the influence of dietary sodium intakes on SSL. They prescribed daily sodium intakes of  $348.4 \pm 0.8$ mmol (approximately 8000mg),  $174.1 \pm 0.6$ mmol (approximately 4000mg) and  $66.3 \text{mmol} \cdot \text{day}^{-1}$  (approximately 1500mg) for participants to follow for a period of eight days of heat acclimation (Table 3.). Participants assigned to the highest sodium intake of approximately 8000mg incurred a similar SSL compared to those prescribed approximately 4000mg before and after the heat acclimation protocol of the study ( $78.8 \pm 10.3$ mmol versus  $63.8 \pm 8.0$ mmol at baseline respectively,  $p > 0.050$  and  $52.6 \pm 6.2$ mmol versus  $39.1 \pm 4.0$ mmol after heat acclimation respectively,  $p > 0.050$ ). Following the acclimation period, the SSL of both groups consuming relatively high sodium intakes (approximately  $8000\text{mg} \cdot \text{day}^{-1}$  and  $4000\text{mg} \cdot \text{day}^{-1}$ ) were significantly higher ( $p < 0.010$ ) than participants following a restricted intake of approximately  $1500\text{mg} \cdot \text{day}^{-1}$  (Table 3.). However, all sweat samples were collected from participants of varying body mass size and unspecified training status which means

there was a potential for confounding of  $[Na^+]_{sweat}$  and SSL results attributable to differences in sweat rate. Furthermore, the sweat samples were not collected during a specific exercise protocol, and the WBW method was questionable in regard to the completeness of capturing all unevaporated sweat compared to the WBW method of Shirreffs and Maughan (1997). Regardless, the results suggest a critical dietary sodium intake influencing SSL among this sample appeared to be somewhere between approximately 1500mg and 4000mg per day which is more specific (but remains very broad) compared to the research of Armstrong et al. (1985).

#### *Application to field study design*

The literature suggests that dietary sodium intake can cause a confounding effect on SSL investigations (Table 3.). Whether this research holds true for trained individuals is unknown. Controlling dietary sodium intakes can be impractical in the context of field research, and this means estimating participants' dietary sodium intake may be a worthwhile alternative to manage the potential for confounding with statistical analyses. However, dietary sodium intakes are highly variable and multiple days of assessment are recommended for greatest accuracy of individual estimates (Basiotis et al., 1987; Liu, 1979). Estimates can be derived from urinary analysis or from dietary recall, diet records or food frequency questionnaire, although multiple days of collections from individuals are not always possible in field research. Therefore selecting the most appropriate method for a single assessment of dietary sodium intake is an important decision given the strengths and weaknesses associated with each method.

Twenty-four hour urine collection is considered the gold standard for estimating the dietary sodium intake of a population (Pan-American Health Organisation, 2010). Approximately 90-95% of total sodium intake is excreted in urine under normal conditions each day, and this means 24-hour urinary sodium excretion (24-hour urine

volume multiplied by urinary sodium concentration ( $[Na^+]_{urine}$ ) can be considered a proxy measure of dietary sodium intake (Holbrook et al., 1984; Institute of Medicine, 2005; Pietinen, 1982). It has been used to estimate dietary sodium intake in many large studies across the globe (Intersalt Cooperative Research Group, 1988; Pan-American Health Organisation, 2010; Sacks et al., 2001), although participants are burdened by the need to collect their urine for a full 24-hour period. This means collections may be incomplete for reasons including forgetfulness or inconvenience. In addition, urine collection periods coinciding with acute episodes of sodium losses (such as exercise-induced SSL) can lead to dietary sodium intake being underestimated unless it is quantified and used to adjust for the calculated 24-hour urinary sodium loss. Therefore the strengths of 24-hour urinary sodium for population estimates of dietary sodium intake are not necessarily accurate at the individual level. Para-amino benzoic acid (PABA) can validate urine collection completeness. It is entirely excreted in urine which means the percentage of a known dose recovered in the participant's sample can inform the researcher how compliant each participant was for urine collection (Bingham & Cummings, 1983; National Centre for Social Research, 2008). However, participants must consume one PABA tablet at each of the three main meals over 24 hours of urine collection which is not always practical or recommended due to extra compliance burden (Pan-American Health Organisation, 2010). Therefore, studies have assessed completeness by careful questioning of participants about the possibility of missed specimens (Intersalt Cooperative Research Group, 1988; Liu, 1979). This practice relies on truthful responders otherwise the sodium intake may be underestimated.

Spot urine collection is a pragmatic alternative to 24-hour urine collection for estimating dietary sodium intake. The method aims to combine the reliability of 24-hour urinary sodium analysis whilst reducing participant burden with the convenience

of a spot urine collection (Kawasaki et al., 1993; Mann & Gerber, 2010; Pan-American Health Organisation, 2010). Full 24-hour urinary sodium excretion can be estimated from the spot urine sample with high correlation to actual 24-hour urinary sodium if an actual or estimated value of 24-hour urinary creatinine excretion is available (Kawasaki et al., 1993; Mann & Gerber, 2010; Pan-American Health Organisation, 2010). Exercise and high protein diets increase urinary creatinine excretion, and this means spot urine samples from groups such as athletes may result in the underestimation of urinary sodium excretion with the 24-hour excretion estimation formula (Institute of Medicine, 2005). Furthermore, the time of day at which the sample is collected can influence the correlation with actual 24-hour urinary sodium excretion (Mann & Gerber, 2010; Kawasaki et al., 1993).

Dietary recall, food records and FFQ are alternatives to urinary analyses for estimating dietary sodium intake. They are less preferable because of recall bias, and difficulties in quantifying discretionary salt intake or the sodium content of foods produced at home and commercially. In other words food composition data on sodium intake are unreliable. In fact, research shows estimates of dietary sodium intake derived from dietary assessment underestimate sodium intake when compared to urinary analysis overseas and in NZ (Espeland, 2001; McLean et al., 2011). Therefore the literature suggests that urinary sodium analysis is most preferable for collecting dietary sodium intake data in a field study.

## **2.2.6 Ethnicity**

Definitions of ‘ethnicity’ and ‘race’ are important to understand in this area of research. Ethnicity is a measure of cultural affiliation which describes the ethnic group or groups an individual feels they belong to (Statistics New Zealand, 2005). The groups can consist of people who share some or all of the following characteristics: a common proper name; one or more elements of common culture

which need not be specified but may include religion, custom, or language; a unique community of interests, feelings and actions; a shared sense of common origins or ancestry, or a common geographic origin (Smith, 1986). ‘Race’ on the other hand, is more specific and defined as a “biological indicator” (Statistics New Zealand, 2005). Strictly speaking, this means racial groups are differentiated by genetics or biology which is one of many reasons an individual may identify with an ethnic group. At present, there are no peer reviewed studies comparing  $[Na^+]$ <sub>sweat</sub> or SSL between athletes by ethnic group in NZ. The adult population of sportsmen predominantly consists of those identifying with the MP or NZE ethnic groups (Sport and Recreation New Zealand, 2008). The notion that differences in  $[Na^+]$ <sub>sweat</sub> or SSL may exist between these two ethnic groups is based on US research differentiating participants by racial group (Table 4).

Dating back to 1983, Dill et al. concluded that  $[Na^+]$ <sub>sweat</sub> was similar when compared between the ‘Black’ and ‘White’ racial groups of participants in their field study. Sweat samples were collected from the hands of 31 ‘White’ men aged from 30 to 88 years and 21 ‘Black’ men aged from 16 to 61 years. The participants wore rubber gloves and walked outdoors in desert heat for one hour on three separate occasions to induce sweating responses (Table 4.). However, the occlusive sweat collection method may have led to false elevations of  $[Na^+]$ <sub>sweat</sub> among an unknown number of participants considering the possibility that leaching of electrolytes occurred (Weschler, 2008), and the study was not focussed on collecting sweat samples specifically from highly trained male athletes. In fact, the exercise protocol was of relatively low intensity which is unlikely to replicate or simulate the exercise performances of athletes in training or competition settings. Therefore the study designed by Dill et al. (1983) was not necessarily applicable to ‘Black’ and ‘White’ adult male athletes in the US.

Table 4. Review of studies investigating the influence of racial group on sweat sodium concentration ( $[Na^+]$ <sub>sweat</sub>) or sweat sodium loss (SSL)

Reference	Aim	Design	Participants	Protocol	Environment	Sweat Volume / Sweat Rate	$[Na^+]$ <sub>sweat</sub> <sup>A</sup>	SSL <sup>B</sup>	$[K^+]$ <sub>sweat</sub> <sup>C</sup>
Condon et al. 2007	To collect sweat samples from 'Black' and 'White' players and make racial comparisons in $[Na^+]$ <sub>sweat</sub> .	Observational cohort	<b>Total sample:</b> Professional American Football players (n=31) <b>Racial Groups:</b> 'Black' (n=21) 'White' (n=10)	<b>Exercise session:</b> Three consecutive days of 'two-per-day' pre-season practices at the end of week one in camp <b>Data Collection:</b> Regional (upper forearm) with absorbent patches.	<b>Temp<sub>ENVIRO</sub></b> <sup>D</sup> : 78.4±2.4 to 84±3.6°F <b>Relative Humidity:</b> Not reported	Not reported	<b>Racial Groups:</b> <sup>E</sup> 'Black' 52±21 mmol.L <sup>-1</sup> (22-98 mmol.L <sup>-1</sup> ) <sup>F</sup>  'White' 45±28 mmol.L <sup>-1</sup> (20-79mmol.L <sup>-1</sup> ) <sup>F</sup> p>0.05	Not reported	Not reported
Condon et al. 2010	To compare sweat rate, $[Na^+]$ <sub>sweat</sub> and SSL among 'Black' and 'White' American Football players	Observational cohort	<b>Total sample:</b> One Professional team of American Football players matched by BSA (n=36) <b>Racial Groups:</b> 'Black' (n=18) 'White' (n=18)	<b>Exercise session:</b> Two consecutive training camps in 2007 and 2008 <b>Data Collection:</b> Regional (upper forearm) with absorbent patches.  Body mass change adjusted for fluid intake and urine loss divided by practice duration  Sweat rate x regional $[Na^+]$ <sub>sweat</sub> (unadjusted for whole body $[Na^+]$ <sub>sweat</sub> )	<b>Temp<sub>ENVIRO</sub>:</b> Not reported <b>Relative Humidity:</b> Not reported	'Black' <sup>E</sup> 1.56±0.40L.h <sup>-1</sup>  'White' <sup>E</sup> 1.97±0.60L.h <sup>-1</sup> P<0.030	'Black' <sup>E</sup> 52±21mmol.L <sup>-1</sup> (18-85mmol.L <sup>-1</sup> ) <sup>F</sup>  'White' <sup>E</sup> 47±25mmol.L <sup>-1</sup> (23-99mmol.L <sup>-1</sup> ) <sup>F</sup> P=0.500	'Black' <sup>E</sup> 1850±880mg.h <sup>-1</sup> (range unreported)  'White' <sup>E</sup> 2260±1690mg.h <sup>-1</sup> (range unreported) P>0.050	Not reported
Dill et al. 1983	To investigate sweat sodium concentration among 'Black' and 'White' free-living adults.	Observational cohort	<b>Total sample:</b> Male and female residents of Southern Nevada recruited from jogging groups led by one of the study authors (n=110) <b>Racial Groups:</b> 'Black' men (n=31) 'White' men (n=21)	<b>Exercise session:</b> Three one-hour walks on an outdoor track at approximately 40% predetermined $VO_{2\max}$ by walking at one of three speeds during the Summers of 1979 ('White' participants) and 1980 ('Black' participants).  <b>Data Collection:</b> Regional (hand) by wearing a rubber glove whilst exercising.  Nude body mass change after each one-hour walk, adjusted for respiratory fluid loss	<b>Temp<sub>ENVIRO</sub>:</b> 32-44°C <b>Relative Humidity:</b> Not reported	mean±SD  <b>80m.min<sup>-1</sup>:</b> 'Black' men 878±189mL  'White' men 928±237mL P>0.050  <b>100m.min<sup>-1</sup>:</b> 'Black' men 1292±416mL  'White' men 1008±240mL P<0.001  <b>120m.min<sup>-1</sup>:</b> 'Black' men 1236±254mL  'White' men 1353±348mL P<0.001	mean±SD  <b>80m.min<sup>-1</sup>:</b> 'Black' men 36±29mmol.L <sup>-1</sup>  'White' men 31±14mmol.L <sup>-1</sup> P value not reported  <b>100m.min<sup>-1</sup>:</b> 'Black' men 37±12mmol.L <sup>-1</sup>  'White' men 28±14mmol.L <sup>-1</sup> P value not reported  <b>120m.min<sup>-1</sup>:</b> 'Black' men 21±8mmol.L <sup>-1</sup>  'White' men 31±15mmol.L <sup>-1</sup> P value not reported	Not reported	mean±SD  <b>80m.min<sup>-1</sup>:</b> 'Black' men 9.8±6.0mmol.L <sup>-1</sup>  'White' men 8.2±5.0mmol.L <sup>-1</sup> P value not reported  <b>100m.min<sup>-1</sup>:</b> 'Black' men 13.8±4.9mmol.L <sup>-1</sup>  'White' men 7.6±3.8mmol.L <sup>-1</sup> P value not reported  <b>120m.min<sup>-1</sup>:</b> 'Black' men 12.7±7.8mmol.L <sup>-1</sup>  'White' men 7.8±4.5mmol.L <sup>-1</sup> P value not reported

<sup>A</sup>Sweat sodium concentration, <sup>B</sup>Sweat sodium loss, <sup>C</sup>Sweat potassium concentration, <sup>D</sup>Environmental temperature, <sup>E</sup>mean±SD or mean±SE unspecified, <sup>F</sup>Range. (Table 4. continues on next page)

Table 4 (continued).

Reference	Aim	Design	Participants	Protocol	Environment	Sweat Volume / Sweat Rate	$[Na^+]$ <sub>sweat</sub> <sup>A</sup>	SSL <sup>B</sup>	$[K^+]$ <sub>sweat</sub> <sup>C</sup>
Kopec et al., 2008	To compare $[Na^+]$ <sub>sweat</sub> and SSL among 'Black' and 'White' American Football players	Observational cohort	<p><b>Total sample:</b> Convenience sample of professional and tertiary grade American Football players matched by BSA (n=64)</p> <p><b>Racial Groups:</b> 'Black' (n=32) 'White' (n=32)</p>	<p><b>Exercise session:</b> Pre-season 'two-per-day' practice at the end of week one in each respective squads' camp</p> <p><b>Data Collection:</b> Regional (upper forearm) with absorbent patches.</p> <p>SSL calculated "using simultaneous sweat rate data collected in another study."</p>	<p><b>Temp<sub>ENVIRO</sub>:</b> <i>Morning:</i> <math>77.1 \pm 4.4^\circ F</math> <i>Afternoon:</i> <math>82.4 \pm 3.2^\circ F</math></p> <p>p&gt;0.050</p> <p><b>Relative Humidity:</b> Not reported</p>	Not reported	<p>'Black'<sup>E</sup> <math>49 \pm 19 \text{ mmol.L}^{-1}</math> (<math>15-85 \text{ mmol.L}^{-1}</math>)<sup>F</sup></p> <p>'White'<sup>E</sup> <math>44 \pm 22 \text{ mmol.L}^{-1}</math> (<math>13-99 \text{ mmol.L}^{-1}</math>)<sup>F</sup></p> <p>P=0.430</p>	<p>'Black'<sup>E</sup> <math>1780 \pm 796 \text{ mg.h}^{-1}</math> (<math>517-3670 \text{ mg.h}^{-1}</math>)<sup>F</sup></p> <p>'White'<sup>E</sup> <math>2075 \pm 1444 \text{ mg.h}^{-1}</math> (<math>353-3880 \text{ mg.h}^{-1}</math>)<sup>F</sup></p> <p>P=0.340</p>	Not measured or reported
Palacios et al., 2003	To compare SSL between 'Black' and 'White' girls	Experimental	<p><b>Total sample:</b> Adolescent females (11-15 years) participating in a controlled crossover metabolic study in 1999 (n=36)</p> <p><b>Racial Groups:</b> 'Black' (n=22) 'White' (n=14)</p>	<p><b>Exercise session:</b> 30 minute exercise at 50% maximal heart rate (calculated by subtracting 220 from age) in a temperature controlled room.</p> <p><b>Data Collection:</b> Regional with polyethylene bag over each arm</p> <p>Nude body mass change adjusted for respiratory fluid loss. Adjusting for fluid intake and urine loss not required.</p>	<p><b>Temp<sub>ENVIRO</sub>:</b> <math>27-28^\circ C</math></p> <p><b>Relative Humidity:</b> 33-37%</p>	Not reported	<p>Mean<math>\pm</math>SE 'Black' <math>130 \pm 114 \text{ mmol.L}^{-1}</math></p> <p>'White' <math>151 \pm 236 \text{ mmol.L}^{-1}</math></p> <p>P&lt;0.050</p>	<p>Mean<math>\pm</math>SE 'Black' <math>6.6 \pm 3.6 \text{ mg.h}^{-1}</math></p> <p>'White' <math>10.2 \pm 5.6 \text{ mg.h}^{-1}</math></p> <p>P&lt;0.050</p>	<p>Mean<math>\pm</math>SE 'Black' <math>68 \pm 8 \text{ mmol.L}^{-1}</math></p> <p>'White' <math>48 \pm 7 \text{ mmol.L}^{-1}</math></p> <p>P&gt;0.050</p>

<sup>A</sup>Sweat sodium concentration,<sup>B</sup>Sweat sodium loss,<sup>C</sup>Sweat potassium concentration,<sup>D</sup>Environmental temperature,<sup>E</sup>mean $\pm$ SD or<sup>F</sup>mean $\pm$ SE unspecified,<sup>F</sup>Range

Palacios et al. (2003) compared regional SSL between the ‘Black’ and ‘White’ racial groups under controlled conditions. They recruited 22 ‘Black’ and 14 ‘White’ adolescent females into their laboratory study which involved a 30 minute exercise protocol after participants had followed a dietary sodium intake of approximately 4000mg per day for three weeks (Table 4.). The mean  $[Na^+]_{sweat}$  was slightly but not significantly higher among the ‘White’ compared to ‘Black’ racial group of participants ( $151.0 \pm 235.9 \text{ mmol.L}^{-1}$  versus  $130.4 \pm 114.0 \text{ mmol.L}^{-1}$ ,  $p>0.05$ ), although an exact p-value was not reported so it is impossible to know if the difference was approaching significance or not. Mean arm sweat volume was significantly higher among the ‘White’ compared to ‘Black’ participants ( $347 \pm 53 \text{ mL}$  versus  $179 \pm 44 \text{ mL}$ ,  $p<0.05$ ), and ultimately SSL was significantly higher among the ‘White’ compared to ‘Black’ participants ( $5.1 \pm 2.8 \text{ mg}$  versus  $3.3 \pm 1.8 \text{ mg}$ ,  $p<0.05$ ). However, sweat samples were collected with occlusive arm bags which probably led to false elevations of  $[Na^+]_{sweat}$  among an unknown number of study participants. For example, the mean  $[K^+]_{sweat}$  was well above a normal physiological range which suggests that leaching of electrolytes may have occurred in some sweat samples (Baker et al., 2009; Palacios et al., 2003; Patterson et al., 2000; Weschler, 2008). The study participants were also untrained adolescent females which means they were predisposed to lower sweat rates than adults given that the thermoregulatory response is immature in adolescence (Meyer et al., 1992). In addition, untrained individuals generate less metabolic heat than trained individuals (Mora-Rodriguez et al., 2010); and females generally have lower BSA than males (Fowkes Godek et al., 2005). Therefore the results of Palacios et al. (2003) are difficult to extend to populations of adult male athletes for comparison. However, they do at least provide a contrast to field observations of the ‘Black’ and ‘White’ racial groups of adult athletes (Condon et al., 2010; Condon et al., 2007; Kopec et al., 2008; Palacios et al., 2003).

Condon et al. (2007) were first to observe that differences in  $[Na^+]_{sweat}$  may exist between the 'Black' and 'White' racial groups of adult male athletes. They compared the mean  $[Na^+]_{sweat}$  of 21 'Black' professional American Footballers to 10 of their 'White' counterparts during the first week of a preseason training camp (Table 4.). Sweat samples were collected from participants with absorbent patches attached to the upper forearm (Condon et al., 2007). The mean  $[Na^+]_{sweat}$  was slightly but not significantly higher among the 'Black' compared to 'White' group of participants ( $52 \pm 21 \text{ mmol.L}^{-1}$  versus  $45 \pm 28 \text{ mmol.L}^{-1}$ ,  $p > 0.050$ ). However, the study was limited by a small number of 'White' participants and an exact p-value was not reported so it is impossible to know if the slight difference in mean  $[Na^+]_{sweat}$  between the two groups was approaching significance or not (Condon et al., 2007). This is important because the finding was in complete contrast to Palacios et al. (2003) who observed the 'White' racial group to have a slightly but not significantly higher  $[Na^+]_{sweat}$  than the 'Black' group in their laboratory study (Table 4.). Unfortunately Condon et al. (2007) either did not capture nor did not report sweat rate data from their study participants and SSL could not be determined nor compared with the results observed by Palacios et al. (2003).

In a later field study by Kopec et al. (2008), 32 'White' adult male athletes from two grades of American Football (professional and tertiary) incurred a slightly but not significantly higher mean SSL than their 'Black' counterparts ( $2075 \pm 1444 \text{ mg.h}^{-1}$  versus  $1780 \pm 796 \text{ mg.h}^{-1}$ ,  $p = 0.340$ ). Kopec et al. (2008) did not report the participants' respective mean sweat volume or sweat rate, but the 'Black' group produced sweat with a similar  $[Na^+]_{sweat}$  compared to 'White' ( $49 \pm 19 \text{ mmol.L}^{-1}$  versus  $44 \pm 22 \text{ mmol.L}^{-1}$ ,  $p = 0.430$ ). The difference in SSL was not statistically significant, although it may have been clinically significant in terms of sweat sodium replacement needs. Observations were made during the second week of each respective squad's preseason training,

and sweat samples were collected with absorbent patches applied to participants' right upper forearm (Table 4.). However, there was potential for a difference in pre-season training protocol between the two grades of players which may have influenced exercise intensity levels during data collection (Kopec et al., 2008). Differences in exercise intensity can influence sweat rate (Kondo et al., 1998). Therefore a difference in sweat rates possibly due to differences in exercise intensity between the 'Black' and 'White' racial groups may have confounded the  $[Na^+]_{sweat}$  and SSL comparisons.

Most recently, Condon et al. (2010) observed a slightly but not significantly higher mean SSL among 18 'White' compared to 18 'Black' adult male athletes ( $2260 \pm 1690 \text{mg.h}^{-1}$  versus  $1850 \pm 880 \text{mg.h}^{-1}$ ,  $p>0.050$ ). An exact p-value was not reported so whether this difference was approaching statistical significance is unknown (Condon et al., 2010). The 'Black' group of athletes did produce sweat with a similar  $[Na^+]_{sweat}$  as the 'White' group ( $52 \pm 21 \text{mmol.L}^{-1}$  or approximately  $1200 \pm 480 \text{mg.L}^{-1}$  versus  $47 \pm 25 \text{mmol.L}^{-1}$  or approximately  $1080 \pm 575 \text{mg.L}^{-1}$ ,  $p=0.500$ ), but the mean sweat rate of the 'Black' group was significantly lower than 'White' ( $1.6 \pm 0.4 \text{L.h}^{-1}$  versus  $2.0 \pm 0.6 \text{L.h}^{-1}$ ,  $p<0.030$ ). The participants were recruited from one professional American Football team over the course of two preseason training camps in 2007 and 2008, and they were matched for BSA which is a determinant of sweat rate (Condon et al., 2010). In keeping with earlier observations, regional sweat samples were collected with absorbent patches attached to the right upper forearm of each participant (Condon et al., 2010; Condon et al., 2007; Kopec et al., 2008). Therefore this particular field study supports the experimental finding of the 'White' racial group incurring greater SSL than 'Black' (Palacios et al., 2003), while also corroborating a trend observed in field studies for which 'Black' athletes sweated lower volumes of slightly more sodium concentrated sweat than 'White' athletes.

(Condon et al., 2010; Condon et al., 2007; Kopec et al., 2008). Whether or not these observations extend to male athletes of the MP and NZE ethnic groups in NZ is yet to be investigated.

An individual's ethnic or racial group is a subjective or self-perceived identifier. Census data collected in the US asks that individuals self-identify with as little or as many racial groups they feel a belonging to at the time of responding (Humes et al., 2011). Similarly, Census data collected in NZ prompts individual's to self-identify with the ethnic rather than racial group(s) they feel they belong to (Statistics New Zealand, 2005). Ultimately individuals identifying with any ethnic or racial group may not share the same genetic or biological traits as other members within that group which may have implications in the current study.

## 2.3 Literature Review Conclusion

Sweat sodium loss is the product of  $[Na^+]_{sweat}$  and sweat volume (absolute) or rate (relative). This can differ markedly within and between individuals depending on many circumstances or conditions (Figure 3.). Overseas evidence suggests that differences in  $[Na^+]_{sweat}$  or SSL may exist between racial groups of male athletes. Whether or not these observations extend to male athletes of the MP and NZE ethnic groups in NZ is yet to be investigated. The literature informs us that  $[Na^+]_{sweat}$  can be influenced by an athlete's BSA, hydration status, dietary sodium intake, and heat acclimatisation status prior to exercise. In addition, environmental conditions and the athlete's exercise intensity and sweat rate during exercise can influence their  $[Na^+]_{sweat}$  and/or SSL results. All of these variables are important to measure and consider for potential confounding effects when designing a field study investigating whether 'saltier sweaters' can be identified by ethnic group in NZ. However, individuals identifying with a particular ethnic group may not share the same genetic or biological traits as other members in that group and this may impact on the interpretation of results in the current study.

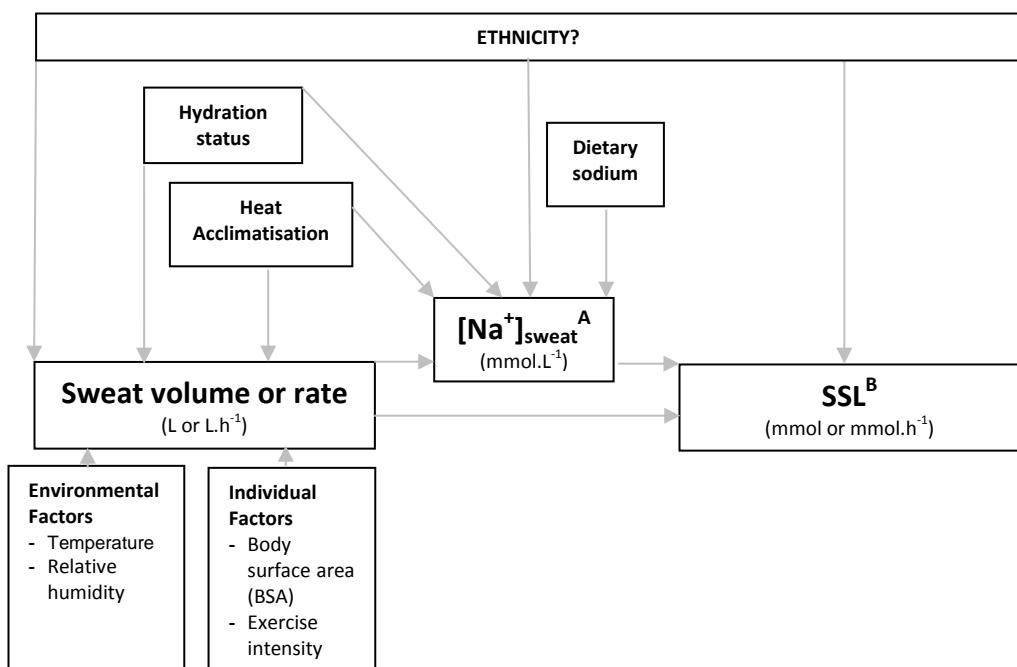


Figure 3. Summary of potential confounding effects on <sup>A</sup>sweat sodium concentration ( $[Na^+]_{sweat}$ ) and <sup>B</sup>sweat sodium loss (SSL)

### **3 METHODOLOGY**

#### **3.1 Study Design**

This observational cross sectional study was conducted between June and September 2011. Five cohorts of high performance male team-sport athletes received invitations to participate – they were either national squads of NZ or Tonga, or regional squads from within NZ. Participation involved three phases over two to three days depending on each cohort's availability to attend recruitment, complete a 24-hour urine collection, and complete an exercise protocol (Figure 4a).

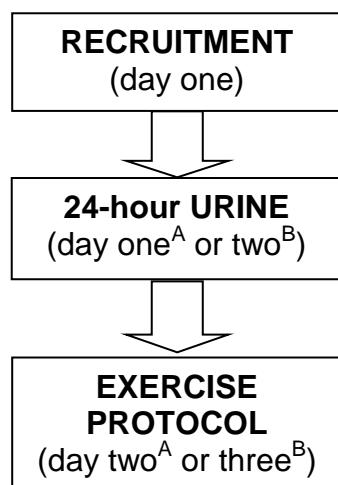


Figure 4a. Overview of the study design for all five participating cohorts  
(<sup>A</sup>Dunedin, <sup>A</sup>Oamaru, <sup>A</sup>Pukekohe, <sup>A</sup>Tonga, <sup>B</sup>Albany)

Recruitment involved prospective participants giving informed written consent to enter the study on the first day of their cohort's involvement (Appendix 1.). They completed a self-administered health and demographics questionnaire which captured the ethnic group or groups each individual identified with at that particular time (Appendix 2.). Written and verbal instructions were then given to each individual informing them when to begin and finish a 24-hour urine collection (Appendices 3. and 4.). The urine collections were planned to commence after the participants' first void on waking the following morning (day two of participation) and completed after their first void on waking on day three of participation. However, only one cohort was

able to commit to a three-day period of participation, and therefore the collections began immediately after recruitment for the cohorts in Dunedin, Oamaru, Pukekohe, and Tonga. Completion of their urine collections coincided with arriving at a local gym for the third phase of the study - the exercise protocol which involved a series of measurements taken before, during and after a 60-minute exercise routine on stationary bicycles (Figure 4b.).

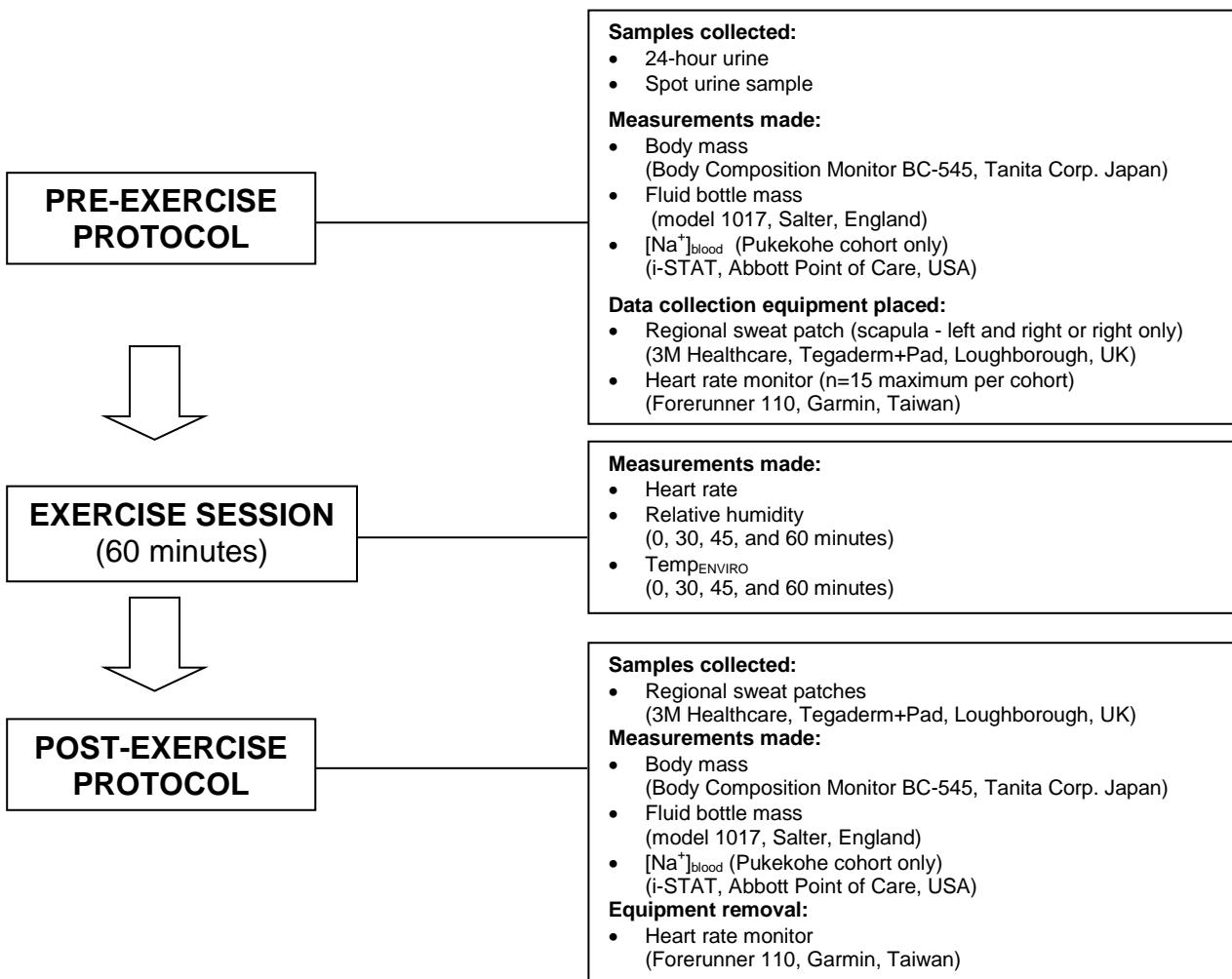


Figure 4b. Outline of data collection protocol followed for each cohort

The study design was reviewed and approved by the University of Otago Human Ethics Committee in April 2011, and all participants received individualised feedback on their hydration and sodium balance results within one month of their participation (Appendix 5.).

### **3.2 Participants**

The total study sample consisted of 58 participants. Sixty-one prospective participants entered recruitment from one hockey squad ( $n=14$ ), one cricket squad ( $n=8$ ), and three rugby union or rugby sevens squads ( $n=39$ ). To be eligible for the study, participants were required to be: male; aged 18-65 years; identifying with the Maori, Pacific and/or NZE ethnic group; and physically active but without health problems which may have been exacerbated through participation. Three prospective participants did not meet the inclusion criteria due to their respective ethnic group and their data were excluded from statistical analyses.

Sweat samples were collected from all MP participants (MP-ALL) either in NZ or Tonga. In contrast, the samples from NZE participants were collected exclusively in NZ. Records show that environmental conditions in Tonga can differ substantially to NZ at the time of year when data collection was planned (Appendix 6.), and for this reason a difference in heat acclimatisation status was suspected within the MP-ALL group of participants, and also between the MP-ALL and NZE ethnic groups. A question to assess training history was therefore added to the health and demographics questionnaire prior to recruiting participants in Tonga which enabled the likelihood of participants in Tonga being acclimatised to local conditions to be considered with statistical analyses. To do so, the MP-ALL group was further categorised as MP-NZ or MP-TGA. Their physical characteristics are shown in Table 5. by cohort and by their respective ethnic group. Ultimately the study results were examined with and without data from the MP-TGA group.

**Table 5. Physical characteristics of participants (mean $\pm$ SD and range) by cohort and by ethnic group**

	COHORT									
	<b>Hockey</b> (n=11)		<b>Rugby Union</b> (n=23)		<b>Rugby Union</b> (n=7)		<b>Rugby Sevens</b> (n=9)		<b>Cricket</b> (n=8)	
	MP-NZ (n=0)	NZE (n=11)	MP-NZ (n=14)	NZE (n=9)	MP-NZ (n=6)	NZE (n=1)	MP-TGA (n=9)	NZE (n=0)	MP-NZ (n=0)	NZE (n=8)
Location	Albany, NZ		Pukekohe, NZ		Oamaru, NZ		Nuku'Alofa, Tonga		Dunedin, NZ	
Date of exercise protocol	30 <sup>th</sup> June 2011		5 <sup>th</sup> July, 2011		15 <sup>th</sup> August, 2011		2 <sup>nd</sup> September, 2011		22 <sup>nd</sup> September, 2011	
Age (years)	-	23 $\pm$ 4 (19-31)	25 $\pm$ 5 (19-33)	22 $\pm$ 2 (20-24)	26 $\pm$ 6 (19-33)	26	23 $\pm$ 4 (17-28)	-	-	21 $\pm$ 3 (18-26)
Height (m)	-	-	1.82 $\pm$ 0.10 (1.59-1.94)	1.90 $\pm$ 0.07 (1.77-1.96)	1.81 $\pm$ 0.06 (1.75-1.89)	1.80	1.83 $\pm$ 0.06 (1.72-1.90)	-	-	1.84 $\pm$ 0.09 (1.68-1.93)
Weight (Kg)	-	82.1 $\pm$ 6.6 (72.4-95.8)	101.0 $\pm$ 12.5 (77.6-124.1)	100.2 $\pm$ 9.9 (84.8-112.9)	100.9 $\pm$ 15.6 (81.8-120.4)	93.1	92.8 $\pm$ 8.7 (78.7-110.0)	-	-	85.8 $\pm$ 10.5 (69.0-101.7)

### **3.3 Exercise Protocol**

#### **3.3.1 Pre Exercise**

##### Twenty-four hour urine collection

The participants' 24-hour urine collections were aimed at estimating dietary sodium intake in the 24 hours preceding their exercise protocol. Participants' returned their collection on arrival to the gym for the exercise protocol. All returned collections were weighed on a set of electronic scales (model 1017, Salter, England) to determine total urine volume after adjusting for the weight of the collection container and assuming 1g of urine was equal to 1mL (Lenter, 1981). An approximate 20mL sample of each 24-hour urine collection was retained and stored in a chiller-bag for sending back to the laboratory in Dunedin for analysis of  $[Na^+]$ <sub>urine</sub>.

##### Spot urine

Spot urine samples were collected for estimating each participant's pre-exercise hydration status. All participants were asked to empty their bladders and collect a pre-exercise mid-stream spot urine sample. These samples were stored in sealed containers within the chiller-bag alongside the 24-hour urine samples. The USG of each spot urine sample was measured later that day with a hand held refractometer (Atago Uricon-N, Japan).

##### Regional sweat patch placement

Participants reported to a trained researcher for placement of regional sweat patches. Their left and right scapula skin regions were thoroughly cleaned with deionised water before drying. One sweat absorbent patch (3M Healthcare, Tegaderm+Pad, Loughborough, UK) was placed on either or both shoulder blades depending on time constraints. The right-hand scapula was chosen in the instances where placing only one sweat patch was possible.

### Body mass and fluid bottle mass

While wearing shorts only, each participant's pre-exercise body mass was measured on a set of body mass scales (Tanita Body Composition Monitor BC-545, Tanita Corporation Japan). After this measurement, the NZ-based participants received one uniquely identifiable 750mL water bottle to consume *ad libitum* throughout the exercise session (Table 6.). They were also advised that more bottles were available on demand throughout the exercise, but instructed to only drink their assigned water from the time of receiving it until their post-exercise body mass had been measured. The participants were also instructed to refrain from using the water as a mouth rinse then spitting it out. All fluid bottles were weighed before being assigned to each player so their individual fluid intake could be calculated and adjusted for after the exercise in line with similar field studies (Maughan et al., 2004; Maughan et al., 2005). Water bottles were not provided to the MP-TGA participants because of logistical constraints. These participants arrived at the gym for testing without personal fluid bottles and received no hydration during the exercise protocol. The coaching staff reported this as common practice among the MP-TGA cohort.

Table 6 The sodium and potassium content of water provided to participants

	COHORT				
	Hockey (Albany, n=11)	Rugby Union (Pukekohe, n=23)	Rugby Union (Oamaru, n=7)	Rugby Sevens (Nuku'Alofa, n=9)	Cricket (Dunedin, n=8)
<b>Water company</b>	Waiwera Water (waiwera.co.nz)		Signature Range, Spring Water		Signature Range, Spring Water
<b>Sodium content (mg.L<sup>-1</sup>)</b>	1.6		8.9	-	8.9
<b>Potassium content (mg.L<sup>-1</sup>)</b>	2.6		3.9	-	3.9

### Blood sodium

One squad of rugby union players (n=23) was selected as a sub-sample for monitoring  $[Na^+]_{blood}$  before and after their exercise session. A finger prick blood sample was drawn from each participant by researchers during the pre-exercise protocol. Participants' fingers were cleaned with alcohol wipes before being punctured. A blood sample was then collected into a capillary tube and  $[Na^+]_{blood}$  was determined with an i-STAT Point of care analyser (Abbott Point of Care, USA). The i-STAT CG8+ cartridges have a co-efficient of variation of 0.59% for blood sodium and have been used previously for sports nutrition research providing valid measures of sodium concentration (Erickson & Wilding, 1993).

#### **3.3.2 Exercise Session**

Six exercise sessions lasting 60 minutes were hosted indoors by four fitness gyms in NZ and one in Tonga. They were completed between 0600-1100 hours in the morning or between 1500-2000 hours in the afternoon or evening (local time). Each of these exercise sessions were the only training session of the day for all cohorts other than the rugby union squad in Pukekohe. In that case, it was their second training session of the day. There had been approximately four hours for rest and recovery after that cohort's morning training session of skills and drills.

The exercise sessions were standardised as much as possible across all five cohorts. Despite being led by a different gym instructor on each occasion, the instructors encouraged genuine effort from participants throughout the entire session which began with a low intensity warm up for ten minutes followed by a mixture of high intensity sprint intervals, moderate intensity aerobic intervals, and low intensity recovery intervals for 40 minutes. All sessions ended with a 10 minute low intensity warm down followed by stretching after dismount. A regimen of variable intensity exercise was planned to promote higher sweat rates compared to one of constant

intensity (Mora-Rodriguez et al., 2008), and therefore to minimise the risk of insufficient sweat collection from participants.

### Exercise intensity

Participants' exercise intensity was measured with mean heart rate data ( $\text{HR}_{\text{mean}}$ ). Heart rate monitors (Forerunner 110, Garmin, Taiwan) were provided to a random selection of up to 15 participants per cohort during the pre-exercise period. These participants were asked to begin recording their heart rate from the outset through to completion of the exercise session. All heart rate monitors were collected in the post-exercise protocol and data was uploaded to the [www.mygarminconnect.com](http://www.mygarminconnect.com) database.

### Environmental conditions

Relative humidity and  $\text{Temp}_{\text{ENVIRO}}$  were monitored with a portable weather station (WS9623 Wireless 868MHz, La Crosse Technology, France). Measurements were made at baseline, 30 minutes, 45 minutes and at the conclusion of each session. The mean relative humidity and mean  $\text{Temp}_{\text{ENVIRO}}$  for each cohort was calculated from these measures (Table 7.). Cooling fans remained off at all times to minimise the influence of wind velocity on sweat rate,  $[\text{Na}^+]_{\text{sweat}}$ , and SSL (Cheuvront, 2004; Neville et al., 2010).

Table 7 Environmental conditions during each cohort's exercise protocol

	<b>Cohort</b>				
	Hockey 30 <sup>th</sup> June (MP-ALL=0, NZE=11)	Rugby Union 5 <sup>th</sup> July (MP-NZ=14, NZE=9)	Rugby Union 15 <sup>th</sup> August (MP-NZ=6, NZE=1)	Rugby Sevens 2 <sup>nd</sup> September (MP-TGA=9, NZE=0)	Cricket 22 <sup>nd</sup> September (MP-ALL=0, NZE=8)
<b>Location</b>	Albany, NZ	Pukekohe, NZ	Oamaru, NZ	Nuku'Alofa, Tonga	Dunedin, NZ
<b>Time (hours)</b>	1000-1100	1500-1600	1845-1945	0600-0800	1600-1700
<b>Temp<sub>ENVIRO</sub> (°C)</b> Mean±SD (range)	15.3±0.8 (14.3-16.1)	16.5±0.4 (16.0-16.9)	7.6±0.3 (7.2-7.9)	23.2±0.3 (22.8-23.7)	18.6±0.3 (18.4-18.9)
<b>Relative humidity (%)</b> Mean±SD (range)	59±1 (57-60)	64±1 (63-64)	62±2 (59-63)	73±1 (72-73)	52±3 (50-55)

### **3.3.3 Post-Exercise**

#### *Regional sweat patch removal*

Participant's towel-dried after the exercise if necessary to remove any excess sweat. The absorbent sweat patches were removed by the same researcher who had originally placed them using a pair of plastic tweezers whilst wearing rubber gloves to minimise risk of sample contamination. Once removed, the patches were individually placed in sterile air tight tubes and transported back to the laboratory in Dunedin for sweat extraction and electrolyte composition analysis.

#### *Body mass and fluid bottle mass*

Post-exercise body mass was measured with the same set of body mass scales used at baseline (Tanita Body Composition Monitor BC-545, Tanita Corporation Japan). Participants again wore shorts only. After the body mass measurements were completed, all used fluid bottles were gathered and re-weighed on the same set of electronic kitchen scales used for baseline measurements (model 1017, Salter, England).

### 3.4 Laboratory Analyses

All sweat and urine samples were returned to Dunedin and refrigerated until laboratory analysis. Sweat was extracted by a trained researcher wearing sterile rubber gloves. All sweat and urine samples were then pipetted into solutions containing cesium with either potassium or sodium, as appropriate, at dilution factors of 1:200. Samples were analysed to determine 24-hour  $[Na^+]_{urine}$  or urinary potassium concentration ( $[K^+]_{urine}$ ), and  $[Na^+]_{sweat}$  or  $[K^+]_{sweat}$  with flame atomic absorption spectroscopy (analytikjena ContrAA 700, Jena, Germany). The samples were analysed in duplicate where enough urine or sweat was available (Figure 5.). In cases of minimal sweat volume, samples were diluted with deionised water to aid the sweat extraction process and a correction factor later applied to the analysed result. The sweat samples were examined for signs of leaching of electrolytes by contrasting  $[K^+]_{sweat}$  against values observed within the normal physiological range of 2.5-6.8mmol.L<sup>-1</sup> as determined from WBW sweat sample collection (Baker et al., 2009; Patterson et al., 2000; Shirreffs & Maughan, 1997).

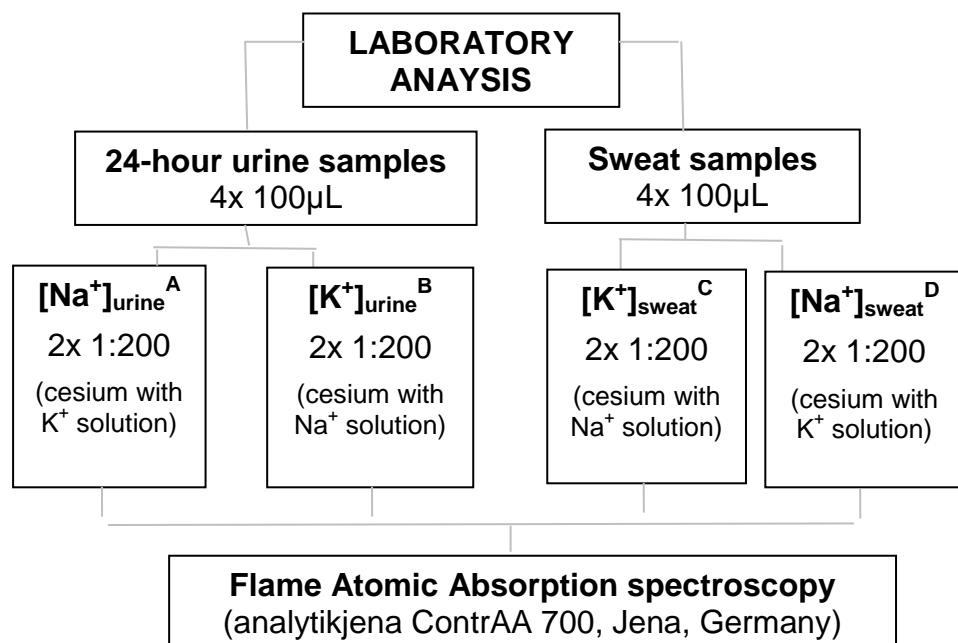


Figure 5. Outline of laboratory analyses to determine <sup>A</sup>Urinary sodium concentration ( $[Na^+]_{urine}$ ), <sup>B</sup>Urinary potassium concentration ( $[K^+]_{urine}$ ), <sup>C</sup>Sweat potassium concentration ( $[K^+]_{sweat}$ ), <sup>D</sup>Sweat sodium concentration ( $[Na^+]_{sweat}$ )

### 3.5 Derived Variables

Figure 6. shows the formulae used to calculate variables identified *a priori* as important to consider for confounding effects in the current study.

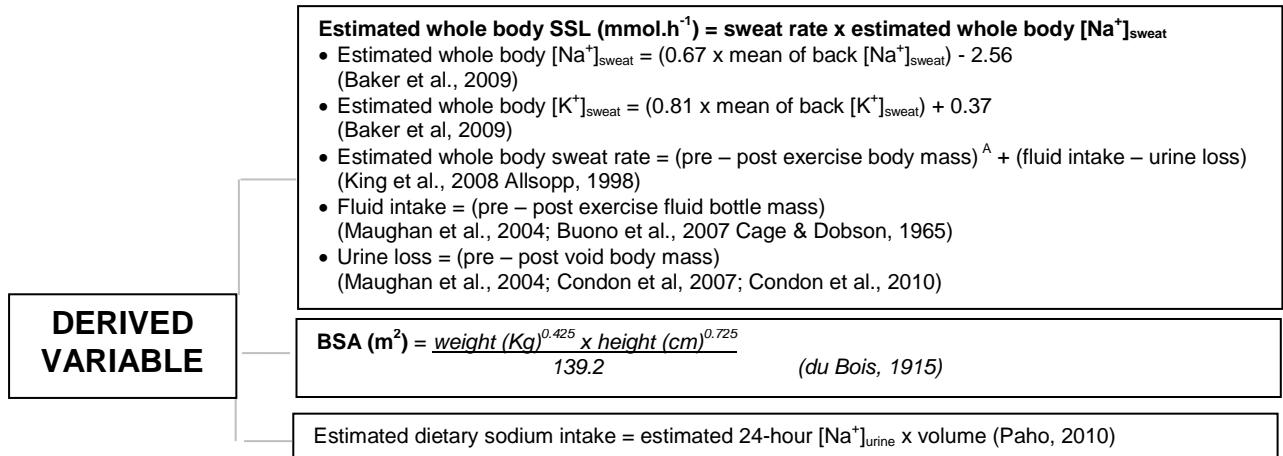


Figure 6. Outline of calculations used to derive variables for statistical analyses

#### 3.5.1 Estimated Whole Body Sweat Sodium Loss

Each participant's whole body SSL was estimated by multiplying their whole body  $[\text{Na}^+]_{\text{sweat}}$  by sweat rate. Whole body  $[\text{Na}^+]_{\text{sweat}}$  was adjusted from regional  $[\text{Na}^+]_{\text{sweat}}$  (Baker et al., 2009), and sweat rate was estimated by subtracting post-exercise body mass from pre-exercise body mass (body mass change). All estimates of sweat rate were adjusted for fluid intake and urine loss during the 60 minute exercise routine (King et al., 2008). Fluid intake was estimated by subtracting each participant's post-exercise fluid bottle mass from the pre-exercise bottle mass. A 1g reduction was assumed to equal 1mL of water consumed (Lenter, 1981). Two participants passed urine during the exercise routine – both men were weighed before and after voiding on the body mass scales used at baseline (Tanita Body Composition Monitor BC-545, Tanita Corporation Japan). In line with similar research, metabolic water production was assumed to be balanced by respiratory water loss and was not considered in estimating sweat rate (Fowkes Godek et al., 2010; Hamouti et al., 2010a; Henkin et al., 2010; Kurdak et al., 2010; Maughan et al., 2004).

### **3.5.2 Pre Exercise Body Surface Area**

Pre-exercise BSA was estimated for each subject with an equation by du bois and du bois (1915).

$$\text{BSA (m}^2\text{)} = \frac{\text{weight (Kg}}{139.2}^{0.425} \times \text{height (cm}}{139.2}^{0.725}$$

The participants' height was either: self-reported in the health and demographic survey, made available by the squad's SNP, or player profiles were accessed online for the information at a later date. However, height data from the Albany cohort was unavailable through any of these means and therefore their BSA data could not be calculated for that particular cohort.

### **3.5.3 Estimated Dietary Sodium**

Dietary sodium intake was estimated by multiplying each participant's total 24-hour urine volume by their  $[\text{Na}^+]$ <sub>urine</sub> determined in laboratory analysis.

## 3.6 Statistical Analyses

### 3.6.1 Multiple Linear Regression

The  $[Na^+]$ <sub>sweat</sub> and SSL results of this study were compared by ethnic group with multiple linear regression analyses. A thorough review of the literature had identified that  $[Na^+]$ <sub>sweat</sub> and SSL both depend on a multitude of factors which makes isolating one factor in particular difficult unless all others can be held constant during data collection. Such control was not practically possible across all cohorts in this observational study, and therefore regression analyses were indicated for the purpose of variable screening (Myers, 1990; Neter et al., 1996). In other words, ethnic group was isolated as a dependent variable of  $[Na^+]$ <sub>sweat</sub> and SSL after adjusting for important confounding effects (control variables). The regression models comparing  $[Na^+]$ <sub>sweat</sub> and SSL were restricted to five input variables respectively based on the widely accepted guideline that the number of participants per input variable should be no greater than 10 to avoid unstable comparisons or large standard error in analyses relative to the sample size (Myers; 1990 Neter et al., 1996; Hosmer & Lemeshow, 2000). Ethnic group was the primary variable of interest, and therefore four control variables were identified with the purposeful selection method of Hosmer & Lemeshow (2000).

Purposeful selection is a systematic process of selecting control variables for regression analyses. The method is equally as effective as other means of selecting control variables such as forward, backward, and stepwise selection (Bursac et al., 2008). To begin, the individual effect size ( $R^2$ ) of each control variable candidate was evaluated with simple linear regression. An effect was considered important when  $p<0.250$  because setting lower values such as  $p<0.050$  can fail to detect some confounding effects at that stage of analysis (Bursac et al., 2008; Hosmer & Lemeshow, 2000). Univariable regression models were then generated by ethnic

group for each control variable candidate and visually inspected for interactions (Appendices 7. and 8.). A traditional value of  $p<0.050$  was set to test for significant correlations at that stage to ensure only meaningful interactions would be considered for selection as control variable (Hosmer & Lemeshow, 2000). All of the univariable models generated up to this point were checked for normal distribution of residuals, and outcome variables of  $[Na^+]_{sweat}$  or SSL were log transformed and model diagnostics repeated if any assumptions of normality were violated (Kirkwood & Sterne, 2003). Finally the control variables were selected in order of greatest effect size (influence or confounding effect) on  $[Na^+]_{sweat}$  and SSL results respectively, and/or whether a significant interaction existed between the ethnic groups. However, the suspected difference in heat acclimatisation status between the MP-NZ, MP-TGA, and NZE ethnic groups of participants could not be considered in this manner because a unique marker of heat acclimatisation status is non-existent (Sawka & Young, 2006). Therefore descriptive statistics of  $[Na^+]_{sweat}$ , SSL results were presented with and without the MP-TGA cohort of participants (mean $\pm$ SD; range) to enable any impact of their results within the MP-ALL ethnic group to be considered before proceeding to regression analyses. Disparities between the MP-TGA and MP-NZ groups and the control variable candidates were assumed to be (albeit not necessarily) due to a difference in heat acclimatisation status.

### **3.6.2 Sample Size**

A sample size of 54 participants was calculated as necessary to compare  $[Na^+]_{sweat}$  between two independent groups with 80% power of detecting a difference of  $10\text{mmol.L}^{-1}$  at the two-sided 0.05 level. A standard deviation of  $13\text{mmol.L}^{-1}$  was assumed based on similar observational field research (Maughan et al., 2004; Stofan et al., 2005). All statistical analyses were performed with Stata version 11.1 (StataCorp, USA).

## 4 RESULTS

### 4.1 Overview

Fifty-eight adult male athletes participated in this study. Their data were collected from four cohorts within NZ (49 participants) and one within Tonga (9 participants).

Table 8. displays the  $[Na^+]_{sweat}$  and SSL results of the MP-ALL (n=29), MP-NZ (n=20), MP-TGA (n=9), and NZE (n=29) ethnic groups respectively (mean $\pm$ SD; range). All comparisons between the ethnic groups were unadjusted for confounding effects at this preliminary stage of analyses.

Table 8. Unadjusted sweat sodium concentration ( $[Na^+]_{sweat}$ ) and loss (SSL) data of the total sample by ethnic group

SWEAT SODIUM-	ETHNIC GROUP			
	MP-ALL (n=29)	MP-NZ (n=20)	MP-TGA (n=9)	NZE (n=29)
Concentration (mmol.L <sup>-1</sup> )	37.6 $\pm$ 19.7 (11.2, 79.1)	45.1 $\pm$ 18.7 (16.3, 79.1)	23.4 $\pm$ 13.0 (11.2, 55.4)	34.9 $\pm$ 17.6 (5.8, 70.6)
Loss (mmol.h <sup>-1</sup> )	31.8 $\pm$ 19.8 (6.7, 79.1)	42.2 $\pm$ 16.3 (14.7, 79.1)	12.0 $\pm$ 5.2 (6.7, 22.1)	33.2 $\pm$ 22.5 (4.4, 87.9)

#### 4.1.1 Sweat Sodium Concentration

Table 8. shows there was a wide range of  $[Na^+]_{sweat}$  results within each ethnic group. The mean of the MP-ALL group appeared to be similar to the NZE group (37.6 $\pm$ 19.7(11.2, 79.1)mmol.L<sup>-1</sup> and 34.9 $\pm$ 17.6(5.8, 70.6)mmol.L<sup>-1</sup> respectively). A difference appeared to exist between the MP-NZ and NZE groups (45.1 $\pm$ 18.7(16.3, 79.1)mmol.L<sup>-1</sup> and 34.9 $\pm$ 17.6(5.8, 70.6)mmol.L<sup>-1</sup> respectively), and an even greater difference appeared between the MP-TGA and MP-NZ groups (23.4 $\pm$ 13.0(11.2, 55.4)mmol.L<sup>-1</sup> and 45.1 $\pm$ 18.7(16.3, 79.1)mmol.L<sup>-1</sup> respectively). The  $[Na^+]_{sweat}$  results were positively skewed for the MP-ALL and MP-TGA groups but more normally distributed for the MP-NZ and NZE groups (Appendix 9.). One outlier was detected

among the MP-TGA group ( $55.4\text{mmol.L}^{-1}$ ) and this was retained for statistical analysis. Outliers were absent among the MP-NZ and NZE groups.

#### **4.1.2 Sweat Sodium Loss**

Table 8. shows there was also a wide range of SSL results within each ethnic group. The mean SSL of the MP-ALL group appeared similar to the NZE group ( $31.8\pm19.8(6.7, 79.1)\text{mmol.h}^{-1}$  and  $33.2\pm22.5(4.4, 87.9)\text{mmol.h}^{-1}$  respectively). A difference appeared to exist between the MP-NZ and NZE groups and their respective SSL ( $42.2\pm16.3(14.7, 79.1)\text{mmol.h}^{-1}$  and  $33.2\pm22.5(4.4, 87.9)\text{mmol.h}^{-1}$ ), and a greater disparity appeared between the MP-TGA and MP-NZ groups respectively ( $12.0\pm5.2(6.7, 22.1)\text{mmol.h}^{-1}$  versus  $42.2\pm16.3(14.7, 79.1)\text{mmol.h}^{-1}$ ). The SSL results were positively skewed for the MP-NZ and NZE groups but more normally distributed for the MP-ALL and MP-TGA groups (Appendix 9.). Outliers were absent among all groups.

#### **4.1.3 Quality Control – Sweat Sample Collection**

Figure 7. shows  $[K^+]_{\text{sweat}}$  data for the total sample differentiated by the MP-ALL ( $n=15$ ), MP-NZ ( $n=8$ ), MP-TGA ( $n=7$ ), and NZE ( $n=24$ ) ethnic groups. The data were positively skewed for each of the ethnic groups (Appendix 9.). Individual values ranged between  $2.0\text{-}9.1\text{mmol.L}^{-1}$  among the MP-ALL group,  $2.0\text{-}4.2\text{mmol.L}^{-1}$  among the MP-NZ group,  $4.0\text{-}9.1\text{ mmol.L}^{-1}$  among the MP-TGA group, and  $1.9\text{-}5.4\text{mmol.L}^{-1}$  among the NZE group. There were two outliers within the NZE group ( $4.5\text{mmol.L}^{-1}$  and  $5.4\text{mmol.L}^{-1}$ ) but they were retained for statistical analyses because such values are within a physiological range confirming sample quality (Baker et al., 2009; Weschler, 2008). Three of the MP-ALL participants returned  $[K^+]_{\text{sweat}}$  samples above this range suggesting electrolyte leaching had occurred (Baker et al., 2009; Weschler, 2008). Their data was excluded from regression analyses in due course.

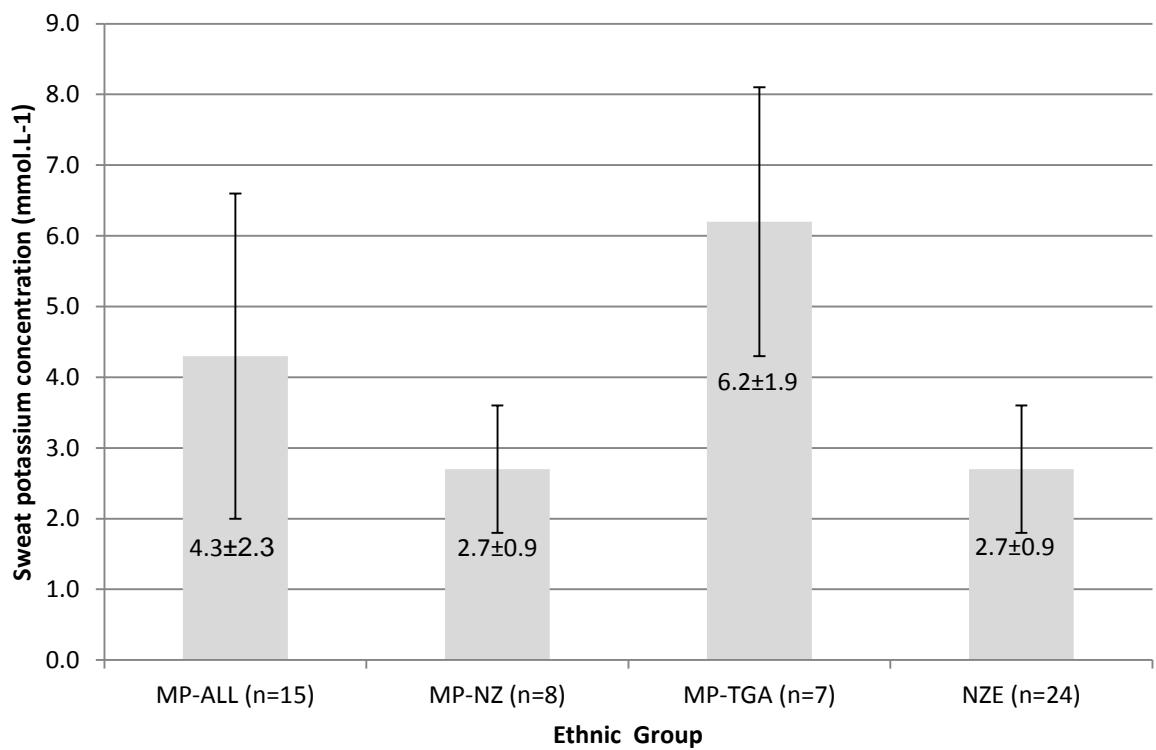


Figure 7. Sweat potassium concentration ( $[K^+]_{\text{sweat}}$ ) data of the total sample by ethnic group

## 4.2 Control Variable Candidates

The pre-exercise control variable candidates for the total sample are shown in Table 9. by ethnic group (MP-ALL=29, MP-NZ=20, MP-TGA=9, and NZE=29) Pertinent data collected during the participating cohorts' respective exercise session are shown in Figures 8. to 12.

Table 9. Pre-exercise characteristics (mean $\pm$ SD, range) of the total sample by ethnic group

	ETHNIC GROUP			
	MP-ALL (n=29) <sup>A</sup>	MP-NZ (n=20) <sup>A</sup>	MP-TGA (n=9) <sup>A</sup>	NZE (n=29) <sup>A</sup>
<b>Body mass</b> (Kg)	98.4 $\pm$ 12.3 (77.6, 124.1)	101.0 $\pm$ 13.0 (77.6, 124.1)	92.7 $\pm$ 8.7 (78.7, 110.0)	89.1 $\pm$ 11.6 (69.0, 112.9)
<b>Height<sup>B</sup></b> (cm)	182 $\pm$ 8 (159, 194)	181 $\pm$ 9 (159, 194)	183 $\pm$ 6 (172, 190)	186 $\pm$ 8 (168, 196)
<b>Body surface area<sup>B</sup></b> (m <sup>2</sup> )	2.18 $\pm$ 0.13 (1.93, 2.39)	2.20 $\pm$ 0.14 (1.93, 2.39)	2.15 $\pm$ 0.12 (1.95, 2.34)	2.17 $\pm$ 0.18 (1.78, 2.46)
<b>Urine specific gravity</b> (g.mL <sup>-1</sup> )	1.024 $\pm$ 0.007 (1.005, 1.037)	1.024 $\pm$ 0.008 (1.005, 1.035)	1.025 $\pm$ 0.007 (1.014, 1.037)	1.016 $\pm$ 0.009 (1.004, 1.032)
<b>Dietary sodium<sup>C</sup></b> (mg.day <sup>-1</sup> )	2120 $\pm$ 1320 (210, 4850)	2430 $\pm$ 1510 (210, 4850)	1570 $\pm$ 700 (490, 2600)	3970 $\pm$ 1250 (1540, 7370)

<sup>A</sup>Unless otherwise stated,

<sup>B</sup>n=22 (MP-ALL), n=13 (MP-NZ), n=16 (NZE),

<sup>C</sup>n=22 (MP-ALL), n=14 (MP-NZ), n=8 (MP-TGA) n=20 (NZE),

### 4.2.1 Body Surface Area

The mean body mass of MP-ALL participants appeared to be higher than the NZE group (98.4 $\pm$ 12.3Kg versus 89.1 $\pm$ 11.6Kg). There was a greater disparity in mean body mass observed when contrasting between the MP-NZ and NZE groups (101.0 $\pm$ 13.0Kg versus 89.1 $\pm$ 11.6Kg respectively), whereas the mean body mass of the MP-TGA group (92.7 $\pm$ 8.7Kg) fell between the MP-NZ and NZE groups. The mean height of the MP-All, MP-NZ, MP-TGA and NZE groups all appeared to be similar (182 $\pm$ 8cm, 181 $\pm$ 9cm, 183 $\pm$ 6cm and 186 $\pm$ 8cm respectively), as did the mean BSA of each respective group (2.18 $\pm$ 0.13m<sup>2</sup>, 2.20 $\pm$ 0.14m<sup>2</sup>, 2.15 $\pm$ 0.12m<sup>2</sup>, and 2.17 $\pm$ 0.18m<sup>2</sup>).

#### **4.2.2 Hydration Status**

Mean USG of the MP-ALL group appeared to be higher than the NZE group ( $1.024 \pm 0.007 \text{ g.mL}^{-1}$  versus  $1.016 \pm 0.009 \text{ g.mL}^{-1}$  respectively). It also appeared higher among the MP-NZ and MP-TGA groups compared to the NZE group ( $1.024 \pm 0.008 \text{ g.mL}^{-1}$  and  $1.025 \pm 0.007 \text{ g.mL}^{-1}$  versus  $1.016 \pm 0.009 \text{ g.mL}^{-1}$ ).

#### **4.2.3 Dietary Sodium**

The mean estimate of dietary sodium intake was lower among the MP-ALL compared to the NZE ethnic group ( $2120 \pm 1320 \text{ mg}$  versus  $3970 \pm 1250 \text{ mg}$ ). It also appeared to be lower among the MP-NZ compared to NZE group ( $2430 \pm 1510 \text{ mg}$  versus  $3970 \pm 1250 \text{ mg}$ ). The lowest mean estimate of dietary sodium intake was derived from the MP-TGA group ( $1570 \pm 700 \text{ mg}$ ),

#### 4.2.4 Body Mass Change

Figure 8. shows the mean body mass change of MP-ALL participants appeared to be similar to the NZE group ( $-0.15\pm0.50\%$  versus  $-0.02\pm0.48\%$  respectively). Figure 8. also shows a greater change in body mass occurred among the MP-TGA compared to MP-NZ and NZE groups respectively ( $-0.59\pm0.17\%$  versus  $0.08\pm0.45\%$  and  $-0.02\pm0.48\%$ ). A positive mean body mass change indicates more fluid was consumed than lost in sweat by some individuals within the MP-NZ and NZE groups.

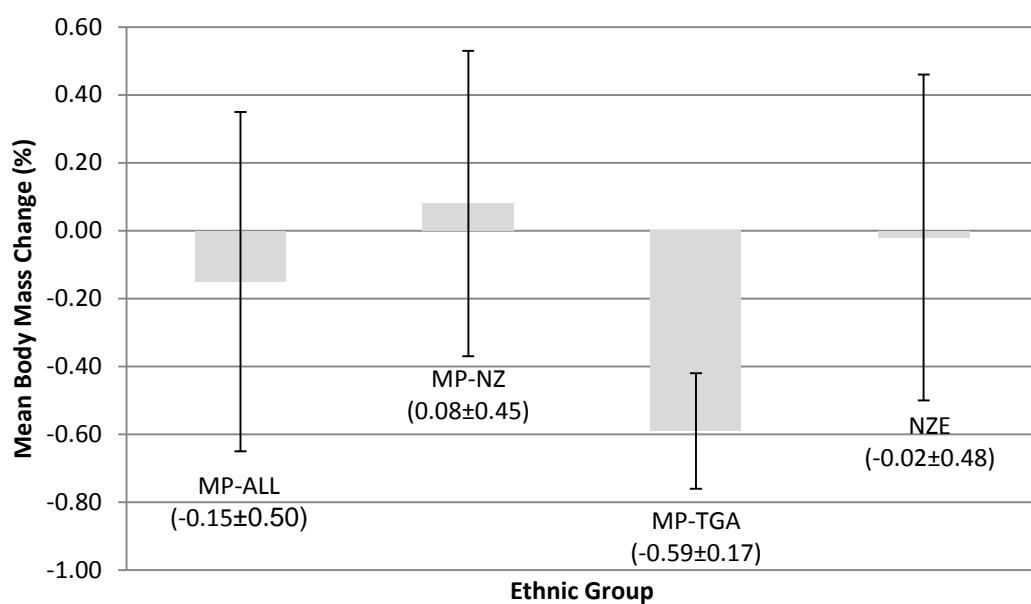


Figure 8. Body mass change (%) data of the total sample by ethnic group

#### 4.2.5 Sweat Rate

Figure 9. shows mean sweat rate data of the total sample by the MP-ALL, MP-NZ, MP-TGA and NZE ethnic groups. There was a wide range of results within each group although mean sweat rate of the MP-TGA group ( $0.54\pm0.14(0.40, 0.80)L.h^{-1}$ ) was remarkably lower than all other groups. The mean sweat rate of the MP-ALL group appeared to be similar, if not marginally lower than the NZE group ( $0.84\pm0.36(0.40, 1.91)L.h^{-1}$  and  $0.89\pm0.33(0.35, 1.99)L.h^{-1}$  respectively), whereas it appeared similar, if not marginally higher, among MP-NZ participants compared to the NZE group ( $0.98\pm0.34(0.57, 1.91)L.h^{-1}$  and  $0.89\pm0.33(0.35, 1.99)L.h^{-1}$  respectively). The data were positively skewed for the MP-ALL, MP-NZ and NZE ethnic groups (Appendix 9). One outlier existed among the MP-ALL group ( $1.91L.h^{-1}$ ) and two among the NZE group ( $1.46L.h^{-1}$  and  $1.99L.h^{-1}$ ). Two outliers were observed among the MP-NZ group ( $1.55L.h^{-1}$  and  $1.91L.h^{-1}$ ). All outliers were retained for statistical analyses.

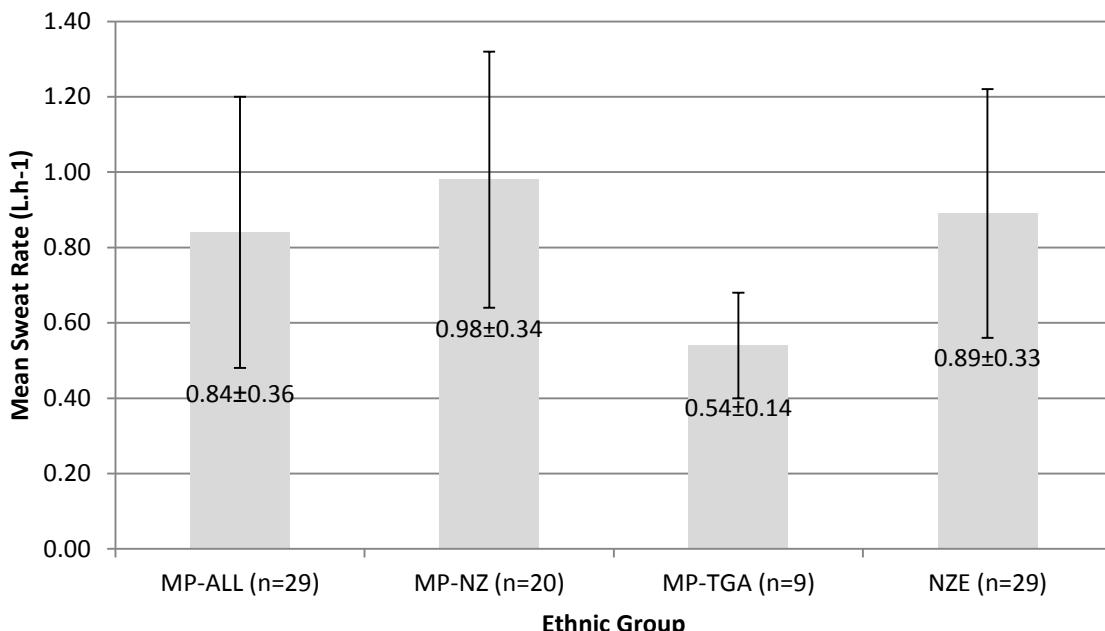


Figure 9. Mean sweat rate ( $L.h^{-1}$ ) data of the total sample by ethnic group

#### 4.2.6 Environmental Conditions

Figure 10. shows that mean relative humidity measures across all exercise sessions ranged between 52% (Dunedin) and 64% (Pukekohe) within NZ, and between 72-73% in Nuku'Alofa, Tonga. Figure 11. shows that mean Temp<sub>ENVIRO</sub> ranged across all cohorts from 7.6°C (Oamaru, NZ) to 23.2°C (Nuku'Alofa, Tonga), and from 7.6°C (Oamaru) to 18.6°C (Dunedin) exclusively within NZ.

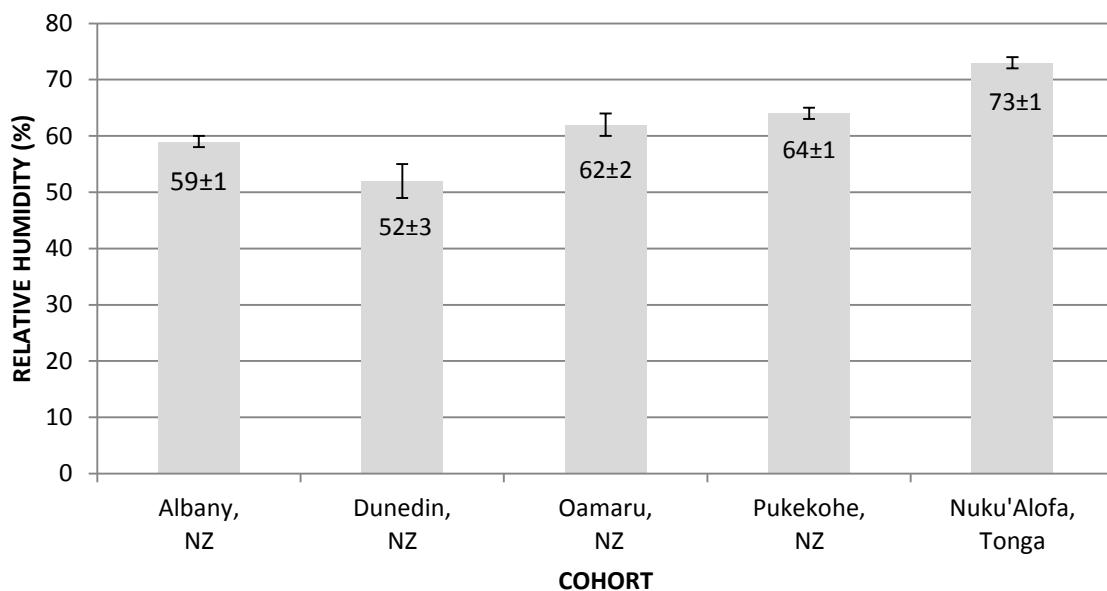


Figure 5. Relative humidity (%) of exercise sessions by cohort.

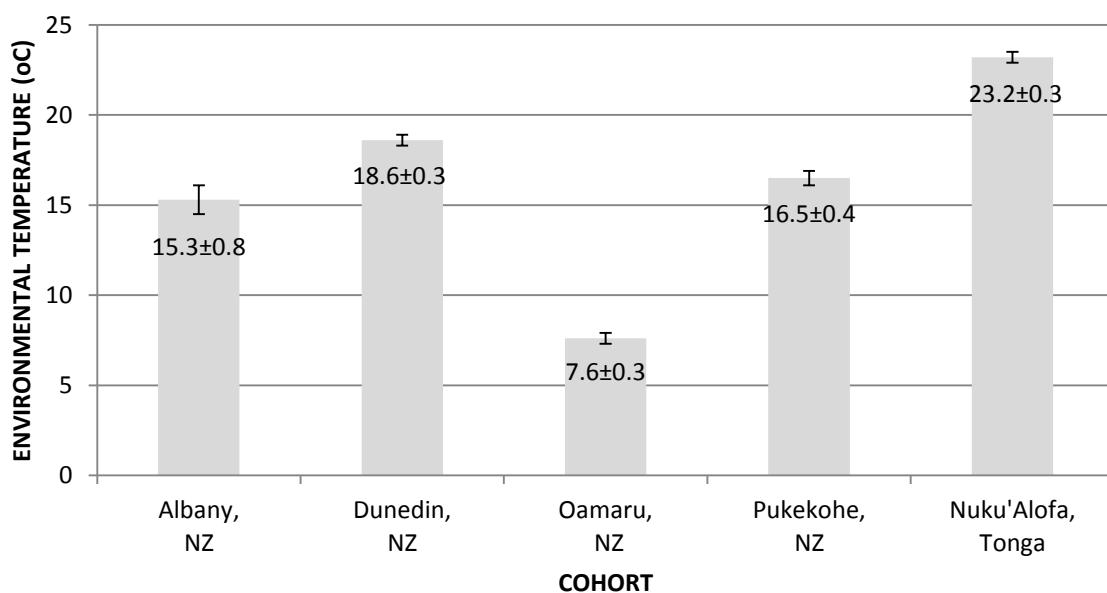


Figure 6. Environmental temperature (Temp<sub>ENVIRO</sub>) of exercise sessions by cohort.

#### 4.2.7 Exercise Intensity

Figure 12. shows  $\text{HR}_{\text{mean}}$  data for the total sample by the MP-ALL (n=15), MP-NZ (n=11), MP-TGA (n=4), and NZE (n=23) ethnic groups. Despite variance within each group,  $\text{HR}_{\text{mean}}$  appeared to be slightly higher among the NZE group compared to the MP-NZ and MP-TGA groups respectively ( $143 \pm 15$ (114, 167)bpm versus  $125 \pm 16$ (87, 142)bpm and  $104 \pm 17$ (88, 128)bpm). The  $\text{HR}_{\text{mean}}$  also appeared marginally higher if not similar among the MP-NZ group compared to MP-TGA ( $125 \pm 16$ (87, 142)bpm versus  $104 \pm 17$ (88, 128)bpm).

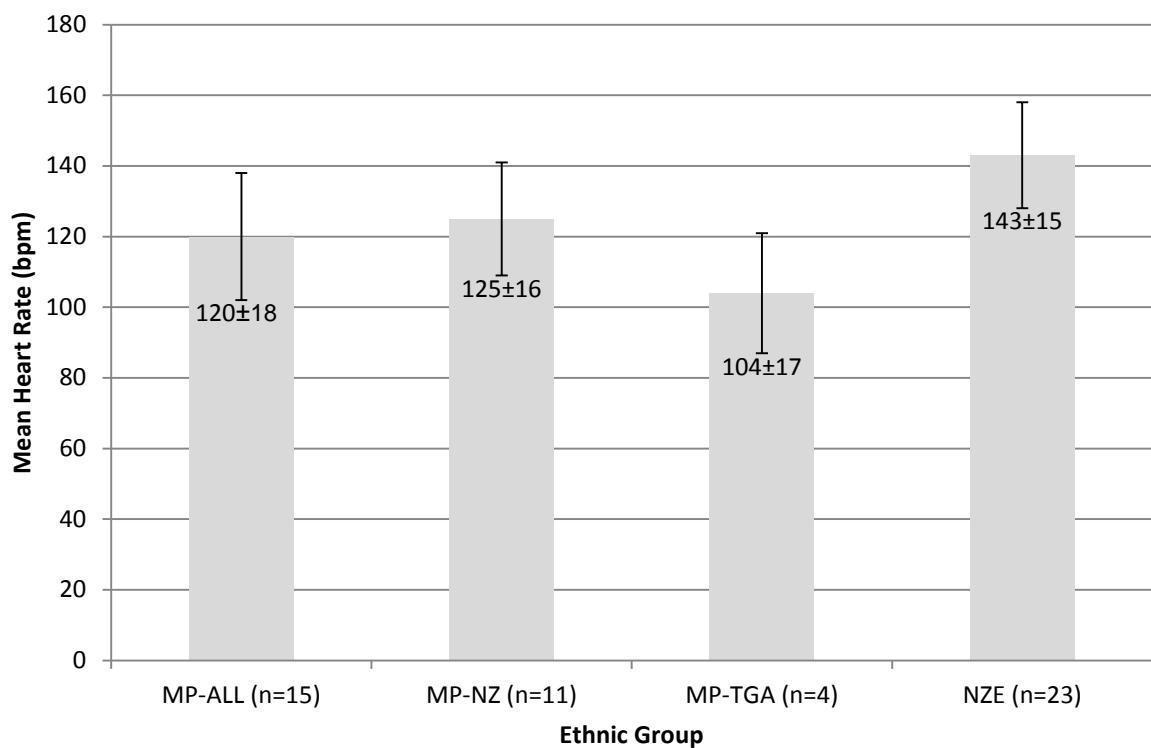


Figure 7. Mean heart rate ( $\text{HR}_{\text{mean}}$ ) data of the total sample by ethnic group

## 4.3 Sweat Sodium Concentration by Ethnic group

### 4.3.1 Total Sample (MP-ALL and NZE participants)

Table 10. shows the individual influence ( $R^2$ ) of ethnic group and all control variable candidates on the log transformed mean  $[Na^+]_{sweat}$  for the total sample (MP-ALL and NZE ethnic groups combined).

Table 10. Univariable analyses of  $[Na^+]_{sweat}$  determinants – Total Sample

	Total Sample (n=58) <sup>A</sup>	
	Effect Size ( $R^2$ )	p value
Ethnic group	0.005	0.604
Sweat rate <sup>B</sup>	0.044	0.128
- Environmental temperature	0.145	0.005
- Relative humidity	0.041	0.143
- Mean heart rate <sup>C</sup>	0.003	0.753
- Body surface area <sup>C</sup>	0.007	0.653
Urine specific gravity	0.005	0.609
Estimated dietary sodium intake <sup>D</sup>	0.003	0.738

<sup>A</sup>Unless otherwise stated, <sup>B</sup>n=56, <sup>C</sup>n=38, <sup>D</sup>n=42

Ethnic group as a variable did not have an important influence on the log transformed mean  $[Na^+]_{sweat}$  ( $R^2=0.005$ ,  $p=0.604$ ). Likewise, BSA, USG, estimated dietary sodium, and  $HR_{mean}$  all failed to explain any significant variance ( $R^2=0.007$ ,  $p=0.653$ ;  $R^2=0.005$ ,  $p=0.609$ ;  $R^2=0.003$ ,  $p=0.738$ ;  $R^2=0.003$ ,  $p=0.753$  respectively). Interactions appeared to exist between the MP-ALL and NZE groups regarding the log transformed estimates of  $[Na^+]_{sweat}$  and dietary sodium intake or USG (Appendix 7.). All of these correlations failed to reach significance when formally tested ( $p>0.05$ ).

Sweat rate as a variable explained approximately 4% of variance in the log transformed estimate of  $[Na^+]_{sweat}$  ( $R^2=0.044$ ,  $p=0.128$ ). The Temp<sub>ENVIRO</sub> variable individually explained approximately 15% of the variance ( $R^2=0.145$ ,  $p=0.005$ ), and relative humidity explained approximately 4% ( $R^2=0.041$ ,  $p=0.143$ ). There also appeared to be an interaction between the MP-ALL and NZE ethnic groups for sweat rate and log transformed mean  $[Na^+]_{sweat}$  (Appendix 7.). A linear relationship was approaching significance for the NZE group ( $y=2.833+0.636x$ ,  $p=0.064$ ) but not for the MP-ALL group ( $y=3.392+0.113x$ ,  $p=0.719$ ).

The control variable candidates selected for further analyses were: sweat rate, Temp<sub>ENVIRO</sub>; relative humidity; and an interaction term for ethnic group and sweat rate (Appendix 10.). After adjusting for these confounding effects with multiple linear regression, there was evidence of a significant difference in the log transformed mean  $[Na^+]_{sweat}$  between the MP-ALL and NZE ethnic groups ( $\beta_1=-1.41$ ,  $p=0.003$ ). Back transforming this difference translated to the mean  $[Na^+]_{sweat}$  being  $0.2 \text{ mmol.L}^{-1}$  lower among the NZE group for every unit increase in  $[Na^+]_{sweat}$  among the MP-ALL group (95% CI: 0.1, 0.6).

#### 4.3.2 NZ Sample (MP-NZ and NZE Participants)

Table 11. shows the individual influence ( $R^2$ ) of ethnic group and each of the control variable candidates on the log transformed mean  $[Na^+]_{sweat}$  exclusively for the NZ-sample (MP-NZ and NZE ethnic groups combined).

Table 11. Univariable analyses of  $[Na^+]_{sweat}$  determinants - NZ Sample

	NZ Sample (n=49) <sup>A</sup>	Effect Size ( $R^2$ )	p value
Ethnic group	0.074	0.070	
Sweat rate <sup>B</sup>	0.015	0.427	
- Environmental temperature	0.065	0.092	
- Relative humidity	0.004	0.675	
- Mean heart rate <sup>C</sup>	0.012	0.541	
- Body surface area <sup>D</sup>	0.006	0.697	
Urine specific gravity	0.047	0.154	
Estimated dietary sodium intake <sup>C</sup>	0.041	0.281	

<sup>A</sup>Unless otherwise stated, <sup>B</sup>n=47, <sup>C</sup>n=34, <sup>D</sup>n=29

Ethnic group as a variable explained approximately 7% of variance in the log transformed mean  $[Na^+]_{sweat}$  among the NZ-based sample ( $R^2=0.074$ ,  $p=0.070$ ). The mean USG of this sample explained approximately 5% of variance ( $R^2=0.047$ ,  $p=0.154$ ), but the respective means of BSA, estimated dietary sodium, relative humidity and  $HR_{mean}$  all failed to have an important individual influence on the log transformed mean of  $[Na^+]_{sweat}$  ( $R^2=0.006$ ,  $p=0.697$ ;  $R^2=0.041$ ,  $p=0.281$ ;  $R^2=0.004$ ,  $p=0.675$ ; and  $R^2=0.012$ ,  $p=0.541$  respectively).

The mean sweat rate did not individually explain any variance in the log transformed mean  $[Na^+]_{sweat}$  for the NZ-based sample of MP-NZ and NZE participants ( $R^2=0.015$ ,  $p=0.427$ ). The mean  $Temp_{ENVIRO}$  explained approximately 7% of the variance

( $R^2=0.065$ ,  $p=0.092$ ), but relative humidity and  $HR_{mean}$  did not have important individual effects on the variance of the log transformed estimate of  $[Na^+]_{sweat}$  ( $R^2=0.004$ ,  $p=0.675$ ; and  $R^2=0.012$ ,  $p=0.541$  respectively). An interaction appeared to exist between the MP-NZ and NZE ethnic groups for their respective sweat rate and log transformed mean  $[Na^+]_{sweat}$  (Appendix 7.). A negative linear relationship was approaching significance for the MP-NZ group ( $y=4.341-0.623x$ ,  $p=0.056$ ) whereas a positive linear relationship was approaching significance for the NZE group ( $y=2.833+0.636x$ ,  $p=0.064$ ). No further interactions were observed.

The control variable candidates selected for further analyses were:  $Temp_{ENVIRO}$ ; USG; and an interaction term for ethnic group and sweat rate (Appendix 10.). This interaction term means sweat rate was included as an individual control variable in addition to the interaction term. After adjusting for the confounding effects with multiple linear regression, there was evidence of a significant difference in the log transformed estimates of  $[Na^+]_{sweat}$  between the MP-NZ and NZE groups ( $\beta_1= -1.32$ ,  $p=0.013$ ). Back transforming this translated to the mean  $[Na^+]_{sweat}$  being  $0.3 \text{ mmol.L}^{-1}$  lower among the NZE group for every unit increase in  $[Na^+]_{sweat}$  among the MP-NZ group (95% CI: 0.1, 0.7).

## 4.4 Sweat Sodium Loss by Ethnic Group

### 4.4.1 Total Sample (MP-ALL and NZE Participants)

Table 12. shows the individual influence ( $R^2$ ) of ethnic group and each of the control variable candidates on the log transformed mean SSL for the total sample (MP-ALL and NZE ethnic groups combined).

Table 12. Univariable analyses of sweat sodium loss (SSL) determinants - Total Sample

	Total Sample (n=58) <sup>A</sup>	
	Effect Size ( $R^2$ )	p value
Ethnic group	<0.001	0.978
Sweat rate <sup>B</sup>	0.410	<0.001
- Environmental temperature	0.210	0.001
- Relative humidity	0.099	0.020
- Mean heart rate <sup>C</sup>	0.009	0.583
- Body surface area <sup>C</sup>	0.014	0.501
Sweat sodium concentration <sup>D</sup>	0.670	<0.001
- Urine specific gravity	0.002	0.732
- Estimated dietary sodium <sup>E</sup>	0.015	0.471

<sup>A</sup>unless otherwise stated, <sup>B</sup>n=56, <sup>C</sup>n=38, <sup>D</sup>n=54, <sup>E</sup>n=42

Ethnic group as a variable did not have an important influence on the log transformed estimate of SSL ( $R^2 <0.001$ ,  $p=0.978$ ). Likewise, BSA, USG and estimated dietary sodium all failed to explain any significant variance ( $R^2=0.014$ ,  $p=0.501$ ;  $R^2=0.002$ ,  $p=0.732$ ; and  $R^2=0.015$ ,  $p=0.471$  respectively). There appeared to be an interaction between the MP-ALL and NZE ethnic groups regarding their respective estimated dietary sodium and log transformed mean SSL, and also regarding their USG and log transformed mean SSL (Appendix 8.), but there was no evidence that any of these correlations were significant when formally tested (i.e.  $p>0.05$ ). Mean heart rate failed

to make an important contribution toward the variance in the log transformed estimate of SSL ( $R^2=0.009$ ,  $p=0.583$ ), and there was no evidence of an interaction between the MP-ALL and NZE groups for this variable (Appendix 8).

Sweat rate and  $[Na^+]_{sweat}$  independently explained approximately 41% and 67% of variance in the log transformed estimate of SSL within the total sample ( $R^2=0.408$ ,  $p<0.001$  and  $R^2=0.670$ ,  $p<0.001$  respectively). An interaction between the MP-ALL and NZE ethnic groups regarding sweat rate and log transformed estimates of SSL was observed (Appendix 8.). Positive correlations were confirmed among the MP-ALL and NZE groups when formally tested ( $y=2.219+1.205x$ ,  $p=0.001$  and  $y=1.741+1.666x$ ,  $p<0.001$  respectively). An interaction between the MP-ALL and NZE groups was observed in regard to the mean  $[Na^+]_{sweat}$  and log transformed mean SSL of both ethnic groups (Appendix 8.). A more positive correlation was confirmed among the NZE group compared to MP-ALL when tested for significance ( $y=1.848+0.040x$ ,  $p<0.001$  and  $y=2.149+0.029x$ ,  $p<0.001$  respectively).

Environmental temperature as an independent variable explained approximately 21% of variance in the log transformed estimate of SSL ( $R^2=0.210$ ,  $p=0.001$ ). Relative humidity independently explained approximately 10% of the variance ( $R^2=0.099$ ,  $p=0.020$ ). Both environmental variables were negatively correlated with the log transformed estimate of SSL among the MP-ALL group ( $y=4.722-0.089x$ ,  $p<0.001$  and  $y=11.752-0.128x$ ,  $p<0.001$  respectively), but there was no evidence of a relationship for the NZE group (Appendix 8.). Therefore the control variable candidates selected for further analyses were: sweat rate,  $[Na^+]_{sweat}$ ,  $Temp_{ENVIRO}$  and relative humidity (Appendix 11.). After adjusting for these confounding effects with multiple linear regression, there was no evidence of a difference in the log transformed mean SSL between the MP-ALL and NZE groups ( $\beta_1= -0.01$ ,  $p=0.871$ ).

#### 4.4.2 NZ-Based Sample (MP-NZ and NZE Participants)

Table 13. shows the individual influence ( $R^2$ ) of ethnic group and all control variable candidates on the log transformed mean SSL for the exclusive NZ-Based sample (MP-NZ and NZE ethnic groups combined).

Table 13. Univariable analyses of sweat sodium loss (SSL) determinants - NZ Sample

Predictor	NZ Sample (n=49) <sup>A</sup>	Effect Size ( $R^2$ )	p value
Ethnic group	0.087	0.049	
Sweat rate	0.316 <sup>B</sup>	<0.001	
- Environmental temperature	0.040	0.190	
- Relative humidity	0.011	0.488	
- Mean heart rate	0.015 <sup>C</sup>	0.497	
- Body surface area	0.013 <sup>D</sup>	0.584	
Sweat sodium concentration	0.650 <sup>E</sup>	<0.001	
- Urine specific gravity	0.065	0.090	
- Dietary sodium	0.059 <sup>C</sup>	0.198	

<sup>A</sup>unless otherwise stated, <sup>B</sup>n=47, <sup>C</sup>n=34, <sup>D</sup>n=29, <sup>E</sup>n=45

Ethnic group as a variable independently explained approximately 9% of variance in the log transformed mean SSL ( $R^2=0.087$ ,  $p=0.049$ ). Urine specific gravity independently explained approximately 7% of the variance ( $R^2=0.065$ ,  $p=0.090$ ), and dietary sodium explained approximately 6% ( $R^2=0.059$ ,  $p=0.198$ ). Mean body surface area and heart rate both failed to make important individual contributions towards the variance in the log transformed estimate of SSL ( $R^2=0.015$ ,  $p=0.497$  and  $R^2=0.013$ ,  $p=0.584$  respectively), and interactions between the MP-NZ and NZE groups were absent for these variables (Appendix 8).

Sweat rate and  $[Na^+]_{sweat}$  independently explained approximately 32% and 65% of variance in the log transformed estimate of SSL for the NZ Sample ( $R^2=0.316$ ,  $p<0.001$  and  $R^2=0.650$ ,  $p<0.001$  respectively). An interaction between the MP-NZ and NZE ethnic groups regarding their respective estimates of sweat rate and log transformed mean SSL was evident (Appendix 8.). There was a positive correlation among the NZE ethnic group ( $y=1.741+1.666x$ ,  $p<0.001$ ) but not among the MP-NZ group ( $y=3.381+0.266x$ ,  $p=0.355$ ). An interaction between the MP-NZ and NZE groups was also observed regarding their respective mean  $[Na^+]_{sweat}$  and log transformed estimate of SSL (Appendix 8.). A more positive correlation was confirmed among the NZE group compared to MP-NZ group when formally tested for significance ( $y=1.849+0.040x$ ,  $p<0.001$  and  $y=2.965+0.016x$ ,  $p=0.002$  respectively).

Environmental temperature explained approximately 4% of variance in the log transformed mean SSL ( $R^2=0.040$ ,  $p=0.190$ ). There were no correlations between Temp<sub>ENVIRO</sub> and the log transformed mean SSL for either of the MP-NZ or NZE groups (Appendix 8.). Relative humidity made an unimportant contribution towards the variance in the log transformed estimate of SSL ( $R^2=0.011$ ,  $p=0.488$ ), and there were no correlations observed between relative humidity and the log transformed mean SSL for the MP-NZ or NZE groups (Appendix 8.).

The control variable candidates selected for further analyses were sweat rate,  $[Na^+]_{sweat}$ , and interaction terms for ethnic group and sweat rate, and for ethnic group and  $[Na^+]_{sweat}$  (Appendix 11.). After adjusting for these confounding effects with multiple linear regression, there was evidence of a significant difference in the log transformed mean SSL between the MP-NZ and NZE groups ( $\beta_1= -0.61$ ,  $p=0.038$ ). Back transforming this translated to mean SSL approximately  $0.5\text{mmol.h}^{-1}$  lower among the NZE group for every unit increase among the MP-NZ group (95% CI:0.3,1.0).

## 4.5 Blood Sodium Concentration

Table 14. shows  $[Na^+]$ <sub>blood</sub> and SSL data captured from the Pukekohe cohort during their one hour exercise protocol. Results are presented as the MP-NZ and NZE ethnic groups combined (n=21) and differentiated (MP-NZ=12 and NZE=9). The mean (SD, range)  $[Na^+]$ <sub>blood</sub> of this subsample fell from  $141\pm2(138, 146)$  mmol.L<sup>-1</sup> before exercise to  $139\pm2(134, 143)$  mmol.L<sup>-1</sup> immediately after exercise. There was no evidence of a difference in baseline  $[Na^+]$ <sub>blood</sub> between the MP-NZ and NZE groups ( $141\pm2$  mmol.L<sup>-1</sup> versus  $140\pm1$  mmol.L<sup>-1</sup> respectively, p=0.821), nor was there a difference in post-exercise  $[Na^+]$ <sub>blood</sub> between the MP-NZ and NZE groups ( $139\pm2$  mmol.L<sup>-1</sup> versus  $138\pm2$  mmol.L<sup>-1</sup> respectively, p=0.345). The lowest recorded post-exercise  $[Na^+]$ <sub>blood</sub> of 134 mmol.L<sup>-1</sup> was measured in one out of the nine NZE participants and this was below the asymptomatic threshold for EAH of 135 mmol.L<sup>-1</sup> (Montain et al., 2001). There was no difference in mean SSL between the MP-NZ and NZE groups nor was there evidence of correlations between SSL and  $[Na^+]$ <sub>blood</sub> for either of the ethnic groups (Appendix 12.).

Table 14. Change in mean blood sodium concentration ( $[Na^+]$ <sub>blood</sub>) relative to sweat sodium loss (SSL) – Pukekohe cohort (n=23)

	Ethnic Group			<b>p value<sup>A</sup></b>
	Combined (n=21) Mean±SD (Range)	MP-NZ (n=12) Mean±SD (Range)	NZE (n=9) Mean±SD (Range)	
Pre-exercise $[Na^+]$ <sub>blood</sub> (mmol.L <sup>-1</sup> )	$140\pm2$ (138, 146)	$141\pm2$ (138, 146)	$140\pm1$ (138, 142)	0.821
Post-exercise $[Na^+]$ <sub>blood</sub> (mmol.L <sup>-1</sup> )	$139\pm2$ (134, 143)	$139\pm2$ (137, 143)	$138\pm2$ (134, 140)	0.345
$[Na^+]$ <sub>blood</sub> change (mmol.L <sup>-1</sup> )	$2\pm2$ (-2, 6)	$2\pm2$ (-2, 6)	$3\pm1$ (1, 5)	0.138
Sweat Sodium Loss (mmol.h <sup>-1</sup> )	$35.3\pm19.3$ (6.2, 73.3)	$40.0\pm14.5$ (14.5, 58.3)	$28.8\pm24.0$ (6.2, 73.3)	0.117

<sup>A</sup>non-parametric test (Mann-Whitney),

## **5 DISCUSSION**

### **5.1 Overview**

The goal of this study was to provide SNPs with information for guiding appropriate rehydration practice among elite athletes in NZ. The primary objective was to investigate whether ‘*saltier sweaters*’ could be identified by the proxy of ethnicity among a sample of highly trained male athletes identifying with the MP and/or NZE ethnic groups. Data were collected from the MP-ALL group of participants in NZ (MP-NZ) and Tonga (MP-TGA) whereas data from the NZE group were collected in NZ. The entire MP-TGA group had been training consistently in Tonga for at least two weeks prior to their participation which is sufficient time for heat acclimatisation to occur among them if not already established (Sawka & Young, 2006; Kirby & Convertino, 1986). Heat acclimatisation describes a series of physiological adaptations which include an earlier onset of sweating, increased sweat rate, reduced  $[Na^+]_{sweat}$  and ultimately lower SSL compared to the non-heat acclimatised state (Buono et al., 2007; Sawka & Young, 2006; Kirby & Convertino, 1986; Smiles & Robinson, 1971). In other words the suspected difference in heat acclimatisation status within the MP-ALL group had potential to confound comparisons of  $[Na^+]_{sweat}$  and SSL with the NZE group. Therefore evaluating the data with and without the MP-TGA group was of primary importance for the validity of any conclusions in this study.

### **5.2 Evaluation of Heat Acclimatisation Status**

Sweat sodium concentration was significantly higher among the MP-ALL compared to NZE group. Conversely, the mean sweat rate of the MP-ALL group was slightly but not significantly lower than the NZE group and overall the mean SSL of both groups were similar. In contrast, mean SSL of the MP-NZ group was significantly higher than the NZE group. The mean  $[Na^+]_{sweat}$  of the MP-NZ group was significantly higher than

the NZE ethnic group, and the MP-NZ group were also sweating at a marginally (but not significantly) higher rate than the NZE group. Therefore the MP-TGA group appear to attenuate a difference in SSL results when their data were combined with the MP-NZ group and results of the MP-ALL group were compared with the NZE group. In other words a confounding effect occurred within the MP-ALL group but this may not necessarily be due to a difference in heat acclimatisation status.

The mean sweat rate of the MP-TGA group was remarkably lower than the MP-NZ group ( $0.54\pm0.14\text{L.h}^{-1}$  versus  $0.98\pm0.34\text{L.h}^{-1}$ ). Although untested for statistical significance, this observation was unexpected if a true difference in heat acclimatisation status existed between the two groups. In fact assuming all other predictors of sweat rate such as BSA and exercise intensity were constant (Fowkes Godek et al., 2005; Kondo et al., 1998), a higher sweat rate would be seen among the MP-TGA group given their training history and exposure to higher Temp<sub>ENVIRO</sub> and relative humidity compared to the MP-NZ group (Sawka & Young, 2006). Mean BSA measures were similar between the MP-NZ and MP-TGA groups, but exercise intensity actually appeared to be lower among the MP-TGA group. This may suggest that MP-TGA generated less metabolic heat during their exercise protocol compared to the MP-NZ group which would explain a lower sweat rate given their need to dissipate excess internal heat would be lower than the MP-NZ group (Saltin & Hermansen, 1966). Therefore the confounding effect of MP-TGA participants within the MP-ALL group was possibly caused by a disparity in exercise intensity combined with or independent to a difference in heat acclimatisation status. In any case the discussion from hereon will focus on results from the NZ-based sample to remove any confounding effects introduced by the MP-TGA group of participants,

### **5.3 Ethnicity and Sweat Sodium Concentration or Loss**

The MP-NZ ethnic group in this study can be considered ‘*saltier sweaters*’ than their NZE counterparts. After adjusting for important confounding effects (sweat rate, Temp<sub>ENVIRO</sub>, and pre-exercise hydration status), the mean [Na<sup>+</sup>]<sub>sweat</sub> of the MP-NZ group was significantly higher than the NZE group. When the [Na<sup>+</sup>]<sub>sweat</sub> data of these two groups were considered with corresponding sweat volume data, SSL was also significantly higher among the MP-NZ compared to NZE group. These are novel observations which add to overseas research investigating differences in [Na<sup>+</sup>]<sub>sweat</sub> and/or SSL by racial group (Condon et al., 2007; Condon et al., 2010; Kopec et al., 2008; Palacios et al., 2003).

#### **5.3.1 Overseas Research**

Palacios et al. (2003) found that SSL was significantly higher among 14 ‘White’ compared to 22 ‘Black’ participants in their laboratory study involving a 30 minute exercise protocol of light intensity. Their finding resembles the current study whereby SSL was significantly different between the two ethnic groups of MP-NZ and NZE participants studied. However, there were key points of difference between the two studies when considering [Na<sup>+</sup>]<sub>sweat</sub> and sweat rate data. For example, Palacios et al. (2003) reported no evidence of a difference in mean [Na<sup>+</sup>]<sub>sweat</sub> among the ‘White’ and ‘Black’ racial groups, whereas [Na<sup>+</sup>]<sub>sweat</sub> was significantly higher among the MP-NZ group compared to NZE in the current study. Palacios et al. (2003) found that the ‘White’ group of participants were sweating at a significantly higher rate than the ‘Black’ group which may have confounded the SSL results given that sweat rate is linearly related to [Na<sup>+</sup>]<sub>sweat</sub> (Allan & Wilson, 1971; Cage & Dobson, 1965; Baker et al., 2009; Buono et al., 2007). In contrast, sweat rates were similar between the MP-NZ and NZE ethnic groups in the current study and this would mitigate any such confounding effect. Palacios et al. (2003) also recruited untrained adolescent female

participants into their study which means the results are difficult to extend to populations of trained or professional adult male athletes for comparison such as the current study or other international field studies (Condon et al., 2010).

Condon et al. (2010) observed a similar mean SSL among 18 'White' compared to 18 'Black' participants recruited from one professional squad of adult male American Football players. Both racial groups produced sweat with a similar  $[Na^+]_{sweat}$  although Condon et al. (2010) observed that mean sweat rate of the 'Black' group was significantly lower than the 'White' group. The study participants were matched for BSA but differences in exercise intensity were not considered despite this being an important determinant of sweat rate,  $[Na^+]_{sweat}$  and ultimately SSL (Condon et al., 2010; Fowkes Godek et al., 2005; Kondo et al., 1998). Even so, the authors (Condon et al., 2010) verified earlier comparisons of  $[Na^+]_{sweat}$  and/or SSL made between the 'Black' and 'White' racial groups in US field studies whereby  $[Na^+]_{sweat}$  and/or SSL results were similar if not clinically different between the 'Black' and 'White' racial groups (Condon et al., 2007; Condon et al., 2010; Kopec et al., 2008). However, all of those field studies suffered a recurring flaw in study design – a lack of control for exercise intensity.

Exercise intensity was measured with  $HR_{mean}$  data in the current study. The MP-NZ group appeared to perform their exercise protocol at a marginally lower if not similar intensity than the NZE group. Although untested for statistical significance, this slight difference was intriguing given the mean sweat rate of the MP-NZ group was marginally higher if not similar to the NZE group. In other words, an underlying mechanism explaining the difference in  $[Na^+]_{sweat}$  and/or SSL between the MP-NZ and NZE ethnic groups may exist.

### 5.3.2 Possible Mechanisms

It is unclear whether one particular ethnic group in NZ shares the  $[Na^+]_{sweat}$  and SSL characteristics of the ‘Black’ or ‘White’ racial groups in the US (Condon et al., 2007; Condon et al., 2010; Kopec et al., 2008; Palacios et al., 2003). This was not a goal of the study to investigate. An individuals’ ethnic or racial identity reflects their perceived sense of belonging to either group depending on where in the world their data was collected (Smith, 1991). Census data in the US asks that individuals identify with as little or as many racial groups they feel a belonging to ((Humes et al., 2011). Census data in NZ explicitly asks individuals to self-identify with ethnic rather than racial group(s) (Statistics New Zealand, 2005). Ancestry is an important reason for individuals to identify with the Maori or Pacific ethnic groups in NZ (Houkamau & Sibley, 2010), and those identifying with the ‘Black’ racial group in the US may be doing so to embrace their ancestry (Smith, 1991; Sellars et al., 1998; Cross, 1978). Thus, the mechanisms which may be involved in differences or similarities between  $[Na^+]_{sweat}$  and SSL by racial group based on their ancestral origins may help to explain the significant difference observed between the MP-NZ and NZE ethnic groups in the current study.

Maori are descendants of Polynesians. They travelled from Tahiti to NZ in waka or canoe via other Pacific Islands during the 13<sup>th</sup> century (Irwin et al., 2009). The origins of the ‘Black’ racial group in the US date back to the 18<sup>th</sup> century when ‘slaves’ endured a long boat journey from the African continent to the Americas (Curtin, 1992). Death was more likely among those suffering acute sodium losses in sweat or vomit during this voyage (Curtin, 1992). Researchers have since suggested that the ‘Black’ racial group adapted through natural selection to retain sodium, and they believe these adaptations have evolved into the present day to explain racial differences in sodium homeostasis including SSL (Brown et al., 2011; Burt et al.,

1995; Condon et al., 2010; Curtin, 1992; Palacios et al., 2003; Pratt et al., 2002; Pratt et al., 1989; Pratt et al., 1993). For example, the ‘Black’ racial group has consistently been observed with higher circulating levels of plasma aldosterone than ‘White’ (Pratt et al., 1989; Pratt et al., 1993). Aldosterone acts on ENaC within the kidneys to increase sodium reabsorption for  $[Na^+]_{blood}$  homeostasis (Rossier et al., 2002; Institute of Medicine, 2005). It may have a similar effect in sweat glands where ENaC facilitate the reabsorption of sodium from precursor fluid during sweat production (Brown et al., 2011; Morgan et al., 2004).

Epithelial sodium channel activity in the sweat gland is regulated by the CFTR. Individual CFTR facilitate reabsorption of chloride ions from precursor fluid which in turn attracts sodium to follow through the ENaC (Quinton, 2007; Rowe et al., 2005). This means the reabsorption of chloride and subsequently sodium decreases in cases of CFTR dysfunction (i.e. cystic fibrosis), thereby increasing  $[Na^+]_{sweat}$  regardless of normal functioning ENaC (Garty & Palmer, 1997; Quinton, 2007; Rowe et al., 2005). Therefore an ethnic difference in aldosterone and any subsequent effect on ENaC within the sweat gland is unlikely to be a major cause of difference in  $[Na^+]_{sweat}$  between the MP-NZ and NZE ethnic groups, and it was not a priority to measure in the current study.

Estimated dietary sodium intake was significantly lower among the MP-NZ compared to NZE group. This may suggest that the MP-NZ group would have higher circulating levels of aldosterone to promote sodium reabsorption in the kidneys (Institute of Medicine, 2005), and theoretically this would lead to increased sodium reabsorption through ENaC, lower  $[Na^+]_{sweat}$  and ultimately lower SSL compared to the NZE group (Allsopp et al., 1998). In fact the opposite was observed and SSL was significantly higher among the MP-NZ group compared to NZE. Therefore a difference in the

prevalence of CFTR dysfunction within each ethnic group may be more important to consider as a mechanism.

The literature suggests that defective CFTR are more common among the ‘White’ compared to ‘Black’ racial group of individuals (Eichner, 2008). While this may explain a higher  $[Na^+]_{sweat}$  of ‘White’ compared to ‘Black’ participants in the laboratory study by Palacios et al. (2003), it becomes an anomaly when considering that the ‘Black’ and ‘White’ racial groups were consistently observed with similar  $[Na^+]_{sweat}$  and SSL in field studies of professional adult male athletes in the US (Condon et al., 2010; Condon et al., 2007; Kopce et al., 2008). Perhaps the sample size of each field studies was too small to detect a difference, or maybe the prevalence of CFTR dysfunction is not actually a major determinant of  $[Na^+]_{sweat}$  and/or SSL. Regardless, it may be that the actual number of CFTR present within each sweat gland is the cause instead. For example, Brown et al. (2011) found among six healthy individuals without any known CFTR defects but with  $[Na^+]_{sweat}$  considered high ( $>70\text{mmol.L}^{-1}$ ), that these individuals had significantly less CFTR present in their sweat glands compared to six healthy individuals also with no known dysfunctional CFTR but with  $[Na^+]_{sweat}$  considered normal ( $<60\text{mmol.L}^{-1}$ ). The study was small and participants were not differentiated by ethnic/racial group, but it introduces an interesting idea that the significant differences in  $[Na^+]_{sweat}$  and SSL observed in the current study may relate to ethnic differences in CFTR abundance within the individuals’ sweat gland.

The differences in  $[Na^+]_{sweat}$  and SSL may also relate to thermoregulation. The MP-NZ group were sweating at a marginally higher if not similar rate as the NZE group in this study despite exercising at a lower if not similar intensity. In other words, the MP-NZ group appeared to rely more on evaporative cooling for thermoregulation than the NZE group, and this may relate to a lower  $Temp_{CORE}$  threshold, superior physical fitness, or it may even be an effect of heat acclimatisation and evolution (Buono &

Sjoholm, 1988; Condon et al., 2010; Nadel et al., 1971; Nadel et al., 1974; Sato & Sato, 1983; Shibasaki et al., 2006). If exercise intensity was matched between the MP-NZ and NZE groups, then the metabolic heat production and sweat rate of the MP-NZ group could be expected to increase (Saltin & Hermansen, 1966). Consequently a greater difference in  $[Na^+]_{sweat}$  may have been observed given the plethora of research showing a positive linear relationship between sweat rate and  $[Na^+]_{sweat}$  (Allan & Wilson, 1971; Baker et al., 2009; Cage & Dobson, 1965; Smiles & Robinson, 1971). In other words, the significantly higher  $[Na^+]_{sweat}$  among the MP-NZ compared to the NZE group in this study may be more pronounced in reality. However, Temp<sub>CORE</sub> of participants was not measured in the current study and such a conclusion cannot be drawn from evidence-informed speculation.

## 5.4 Limitations

### 5.4.1 Statistical Analyses

Comparing  $[Na^+]_{sweat}$  and/or SSL between two ethnic groups is difficult to achieve with an observational study design. Given the constraints of collecting data from five separate cohorts of athletes across NZ and in Tonga, the potential for confounding effects on  $[Na^+]_{sweat}$  and ultimately SSL results was ever-present in the current study. The factors identified *a priori* as potential confounders of  $[Na^+]_{sweat}$  and/or SSL could not be held constant during data collection (e.g. pre-exercise hydration status, dietary sodium intake, BSA, and exercise intensity), and therefore inferential statistics with methods such as the student-t test were meaningless. For example, a significant difference in SSL may have been due to an ethnic difference in at least one of these confounding effects rather than a true difference in SSL. Therefore regression analyses were indicated as an appropriate method of statistical analyses. In theory, this would enable ethnic group to be isolated as a variable of interest by adjusting for confounding effects which may have occurred during data collection (Myers, 1990). However, some of these confounding effects went unadjusted given the sheer number of them relative to a sample size of 58 and consequent risk of introducing error to the analyses (Myers, 1990; Neter et al., 1996; Hosmer & Lemeshow, 2000).

Environmental temperature, pre-exercise hydration status (USG), and estimated dietary sodium were all identified as having individually influenced SSL results within the NZ-based sample with purposeful selection (Hosmer & Lemeshow, 2000). They were not included as control variables given that other candidate variables (i.e. interactions between ethnic group and sweat rate or between ethnic group and sweat sodium concentration) were found to have had greater confounding effects on results. Therefore the significant difference in SSL observed in the current study may be partially related to confounding effects rather than a true effect of ethnic group.

alone. This is impossible to quantify given the limitations associated with collecting data on ethnic group and some of the control variable candidates before and during each cohort's respective exercise protocol.

#### **5.4.2 Ethnic group**

Participants in this study self-identified with the ethnic group or groups they felt they belonged to at the time of recruitment. Identifying with a particular ethnic group can be for objective reasons such as birth place or origins of ancestry, and/or subjective reasons such as the sharing of a common culture including traditions, feelings and actions (Statistics New Zealand, 2005). This means individuals self-identifying with the MP-NZ or NZE ethnic groups in this study may not have shared the same genetic or biological traits as other members within that group.

#### **5.4.3 Dietary sodium**

Estimates of dietary sodium intake for the MP-NZ and NZE ethnic groups were probably underestimated. They were derived from 24-hour urine collection which is the current gold standard for estimating dietary sodium intake (Pan-American Health Organisation, 2010), although daily sodium intakes can be highly variable within individuals and multiple days of assessment are recommended for greater accuracy among large samples (Basiotis et al., 1987; Liu, 1979). However, this was not possible in the current study given the observational cross-sectional design. Only two of the four NZ-based cohorts were able to commit to even making one 24-hour collection because of time constraints. This means 24-hour urine data were missing from nine athletes of NZE ethnicity (31% of all NZE participants) and six athletes of the MP-NZ ethnic group (30% of all MP-NZ participants). Furthermore, one of these cohorts underwent a regular squad training session on the morning of their scheduled exercise protocol. Sweat samples were not captured from this particular cohort during their regular morning training session and consequently each

participant's SSL could not be quantified nor considered in estimating their 24-hour dietary sodium intakes (consisting of 100% of all MP-NZ and 45% of all NZE athletes who provided 24-hour urine collections in this study).

It is possible that dietary sodium intakes of both ethnic groups were similar in the current study. In a population estimate obtained from a subsample of 3315 participants in the 2008/9 NZANS, estimated dietary sodium intakes (based on single spot urine samples) among NZ men aged 19-24 and 25-44 years of all ethnicities were  $190.5 \pm 86.0 \text{ mmol} \cdot \text{day}^{-1}$  ( $4382 \pm 1978 \text{ mg} \cdot \text{day}^{-1}$ ) and  $184.3 \pm 84.8 \text{ mmol} \cdot \text{day}^{-1}$  ( $4239 \pm 1950 \text{ mg} \cdot \text{day}^{-1}$ ) respectively (McLean et al., 2011). There was no evidence found for a statistically significant difference in these estimates by ethnic group which are similar to those of NZE participants in the current study (McLean et al, 2011). On such grounds it is assumed that estimated dietary sodium intakes were also similar between the MP-NZ and NZE groups in the current study.

#### **5.4.4 Fitness Level**

The study participants were from a variety of sporting codes (cricket, hockey and rugby union or sevens). Given the limited time for collecting data from each cohort, their actual fitness levels were not formally tested with validated methods such as the Yo-Yo Intermittent Recovery Test (Krustrup et al., 2003). This is important to acknowledge because physically trained individuals have larger sweat glands which are more sensitive to stimulation compared to untrained individuals – much the same as heat acclimatised compared to non-acclimatised individuals (Buono et al., 2007; Buono et al., 2008; Nadel et al., 1971; Nadel et al., 1974; Sato & Sato, 1983). All of the squads were in the mid-late pre-season period of their respective competition season and were assumed to be of equal physical fitness. Therefore the potential for a confounding effect on SSL as a consequence of variable fitness levels exists and further investigation would be needed to control or adjust for this factor.

#### **5.4.5 Body Surface Area**

The mean body mass of the MP-NZ group appeared to be greater than the NZE group. Means of height were similar between both groups as were their calculated mean BSA (du Bois, 1915). However, height data were missing from seven of the 20 (35%) MP-NZ participants, and 13 of the 29 (45%) NZE participants. Therefore the accuracy of BSA data considered in current analyses may be questionable which may impact on the interpretation of  $[Na^+]_{sweat}$  data by ethnic group given that BSA can influence sweat rate (Godek et al., 2005), and sweat rate can influence  $[Na^+]_{sweat}$  and SSL (Buono et al., 2007; Buono et al., 2008).

#### **5.4.6 Exercise intensity**

Exercise intensity was measured with  $HR_{mean}$  data. Measuring intensity of exercise with the gold standard of  $\%VO_{2max}$  was not practicably possible given the resource constraints of this project. A linear relationship between heart rate and  $VO_2$  at submaximal exercise intensity has been reported, but an individual's  $HR_{mean}$  depends on environmental factors such as relative humidity and  $Temp_{ENVIRO}$ , and physiological factors such as the individual's hydration status (Achten & Jeukendrup, 2003). These conditions were not held constant across all cohorts during data collection in the current study as already discussed, and they were not necessarily adjusted for in the subsequent statistical analyses. Furthermore,  $HR_{mean}$  data were collected from sub-samples within each cohort rather than from all study participants. Therefore the true means of exercise intensity for the MP-NZ and NZE ethnic groups are unknown, and this may have impacted on how the  $HR_{mean}$  data was interpreted in this study. For example,  $HR_{mean}$  was not included as a control variable in analyses of SSL results even though it could not be controlled across all cohorts (despite every effort to do so). Therefore the potential for a confounding effect on sweat rate,  $[Na^+]_{sweat}$ , and SSL results cannot be discounted at the conclusion of this study.

## **5.5 Application to Practice**

Full replacement of SSL is fundamental for optimal rehydration during and after exercise (Sawka et al., 2007). Determining the SSL of individual athletes can be difficult in the field even when laboratory analysis of sweat samples can be arranged - results of sweat sample analyses are unavailable immediately after sample collection. The current consensus of sports nutrition experts from overseas is that sweat sodium replacement can be achieved after exercise with the athletes' usual food and fluid pattern (Sawka et al., 2007). However, this opinion is not based on evidence derived from the MP-NZ or NZE ethnic groups. Furthermore, it assumes situations where rapid rehydration is not indicated (Sawka et al., 2007). Therefore the current study provides important information to SNPs in NZ and perhaps Australia for guiding their sweat sodium replacement advice to professional athletes routinely training twice per day (i.e. when rapid rehydration is indicated).

### **5.5.1 Sweat Sodium Replacement and Habitual Dietary Sodium Intake**

Sweat sodium replacement needs are determined by an athlete's absolute SSL. The mean estimates of SSL among the MP-NZ and NZE participants in the current study were  $42.2 \pm 16.3 \text{ mmol.h}^{-1}$  and  $33.2 \pm 22.5 \text{ mmol.h}^{-1}$  respectively. The mean estimate of dietary sodium intake for the 24-hours preceding the study exercise protocol was lower among the MP-NZ compared to NZE group ( $2430 \pm 1510 \text{ mg}$  versus  $3970 \pm 1250 \text{ mg}$ ), although this was probably underestimated as previously discussed. Even so, it was more than double the mean estimate of SSL incurred by the MP-NZ group during the exercise protocol. This means it is likely that both ethnic groups were able to achieve full replacement after the 60 minute exercise protocol by following their habitual dietary patterns (assuming rapid rehydration was unnecessary). In other words this study provides evidence specific to NZ in support of overseas expert consensus that full replacement can be achieved through habitual

food and fluid intake behaviour of athletes when there is no need for rapid rehydration (Sawka et al., 2007). An example of practical recovery foods and fluids for the participants to achieve full sweat sodium replacement include a low fat ham and cheese sandwich, one full 750mL bottle of commercial rehydration fluid, and a small can of creamed rice (Appendix 13.). However, this quantity of food or volume of fluid may cause gastro-intestinal tolerance issues when time for digestion is restricted between training sessions. Therefore the SNP may like to consider other means of achieving full sodium replacement such as fortifying foods with table salt.

### **5.5.2 Sweat Sodium Replacement and Rapid Rehydration**

Rapid rehydration is often indicated among high performance athletes in NZ. They regularly engage in ‘twice per day’ training sessions, especially during pre-season periods. In such circumstances, time for recovery can be limited, and this is one reason why professional athletes begin afternoon training sessions in a hypohydrated state overseas (Godek et al., 2010). Rapid rehydration entails a fluid intake of approximately 150% sweat volume concurrent with full sweat sodium replacement (Sawka et al., 2007). However, an athlete’s usual food and fluid intake behaviour after their morning session but before the afternoon session may not achieve full sodium replacement without carefully planned dietary advice or intervention (Sawka et al., 2007). Therefore the risk of developing EAH may be increased when attempting to rehydrate rapidly without full or aggressive sweat sodium replacement.

Exercise associated hyponatraemia is a potentially fatal condition characterised by the dilution of  $[Na^+]_{blood}$  (Hew-Butler et al., 2008). It can be clinically diagnosed when  $[Na^+]_{blood}$  falls below 135mmol.L<sup>-1</sup>, and this can occur during or up to 24 hours after exercise (Hew-Butler, 2008; Montain et al., 2001). The most common causes of EAH are fluid intakes in excess of total fluid loss, excessive sodium loss, or a combination of both factors (Hew-Butler et al., 2008; Montain et al., 2001; Speedy et al., 2000).

Mild symptoms include bloating, nausea, vomiting, or headache whereas more severe symptoms include cerebral or respiratory oedema leading to seizure, coma or death in extreme cases (Hew-Butler et al., 2008). Symptoms generally do not appear until  $[Na^+]_{blood}$  falls below 130mmol.L<sup>-1</sup> which is why some groups consider this value to be diagnostic rather than 135mmol.L<sup>-1</sup> (Hew-Butler et al., 2008; Montain et al., 2006). However, a reduction in  $[Na^+]_{blood}$  equivalent to 7-10% over the 24 hour post-exercise period can produce symptoms of EAH despite  $[Na^+]_{blood}$  remaining above 130mmol.L<sup>-1</sup> (Hew-Butler et al., 2008; Montain et al., 2006).

The current study demonstrates how easily EAH can develop in athletes. The  $[Na^+]_{blood}$  of participants was monitored before and after exercise in the Pukekohe cohort. The mean  $[Na^+]_{blood}$  of this cohort fell by  $2\pm2$ mmol.L<sup>-1</sup> from a baseline measure of  $141\pm2$ mmol.L<sup>-1</sup>. Participants were unable to match their mean estimate of SSL during the exercise protocol given the low sodium content of water consumed *ad libitum*. This is one reason which would contribute to the observed dilution of  $[Na^+]_{blood}$  (Hew-Butler et al., 2008). Furthermore, participants' mean intake of fluid outweighed mean sweat volume which is the main reason for diluted  $[Na^+]_{blood}$  during or after exercise (Hew-Butler et al., 2008). Alarmingly, one out of nine (11%) NZE participants in this sub-sample developed asymptomatic EAH when his  $[Na^+]_{blood}$  fell from 139mmol.L<sup>-1</sup> at baseline to 134mmol.L<sup>-1</sup> directly after the 60 minute protocol (Montain et al., 2001). Given the shorter duration of the study exercise protocol in relation to usual team training sessions, this highlights why full sweat sodium replacement advice is important for athletes to achieve after exercise for safe and appropriate rehydration or in other words to lower their risk of developing EAH.

### **5.5.3 Sweat Sodium Replacement and Population Guidelines**

The current study demonstrates how dietary sodium needs of athletes can exceed a daily intake considered appropriate for the general population. Guidelines for the NZ

population and across the globe aim to limit dietary sodium intake to 100mmol (2300mg) per day (World Health Organisation, 2007; Institute of Medicine, 2005; National Health and Medical Research Council, 2006). The mean SSL of MP-NZ participants' in the current study was more than one third of this daily target ( $971 \pm 374$ mg), and this was sustained after only one hour of exercise. Strictly speaking, adhering to the population guidelines of an appropriate sodium intake would require the study participants to follow a low sodium diet for the remainder of the day after following sweat sodium replacement guidelines for safe and appropriate rehydration (Institute of Medicine, 2005; National Health and Medical Research Council, 2006; Sawka et al., 2007; World Health Organisation, 2007). The dietary approaches to stop hypertension (DASH) diet is widely recognised as a low sodium dietary pattern providing approximately 2300mg of sodium each day with an energy intake of approximately  $2100\text{Kcal}.\text{day}^{-1}$  (Sacks et al., 2001; Svetkey et al., 1999). However, higher energy intakes would be more appropriate for participants in the current study, and this corresponds to higher dietary sodium intakes (Sacks et al., 2001; Svetkey et al., 1999). Therefore it would be extremely difficult and inappropriate for the study participants (and probably athletes in general) to restrict their dietary sodium to an upper limit of 2300mg per day based on their unique sweat sodium replacement needs – especially among those training several times per day and where rapid rehydration may be indicated. Fortunately this fact is recognised already and athletes are specifically excluded from the population guidelines on dietary sodium intake.

#### **5.5.4 Application to Practice - Summary**

Sweat sodium replacement needs are determined by an athlete's absolute SSL. This is the product of  $[\text{Na}^+]$ <sub>sweat</sub> and sweat volume. In the current study, the mean sweat volumes of the MP-NZ and NZE ethnic groups were similar if not marginally higher

among the MP-NZ group, but mean  $[Na^+]_{sweat}$  was significantly higher among the MP-NZ compared to NZE participants. Ultimately the MP-NZ ethnic group incurred a significantly higher mean SSL than the NZE group which translated to an increased sodium replacement requirement of approximately 200mg over and above the NZE group for every hour of exercise-induced sweat loss (assuming similar training conditions). The results of this study suggest that both ethnic groups of participants could achieve full sodium replacement by consuming a combination of foods and/or fluids commonly on offer to adult male athletes in NZ during their recovery phase (Appendix 13.). However, the SNP may wish to advise that MP-NZ athletes consume dietary sodium more aggressively than their NZE counterparts during recovery to achieve safe and appropriate rehydration in hot and humid conditions or situations where large sweat losses have occurred. Liberal use of the salt shaker on foods could be encouraged as this additional source of sodium.

## 6 CONCLUSION and RESEARCH RECOMMENDATIONS

This observational cross-sectional field study provides evidence that a sample of highly trained male athletes identifying with the Maori or Pacific ethnic groups can be considered ‘*saltier sweaters*’ than their NZE counterparts. Collectively considered as one, the MP-NZ group also incurred a greater SSL than the NZE participants. Therefore these results suggest sweat sodium replacement strategies can be customised on the basis of ethnicity in NZ, although an elegantly designed laboratory study may be needed to verify this speculative conclusion. In other words, a laboratory study which controls for potential confounding effects of exercise intensity, hydration status (pre- and during-exercise), dietary sodium intake, Temp<sub>ENVIRO</sub> and relative humidity. A heat acclimation protocol would be needed to manage any differences in heat acclimatisation status between participants, and measuring Temp<sub>CORE</sub> of the MP-NZ and NZE ethnic groups to investigate whether a true difference in thermoregulation exists. Lastly, quantifying and comparing the number of CFTR present within the sweat glands of participants may give valuable insight into explaining a difference in [Na<sup>+</sup>]<sub>sweat</sub> and/or SSL by ethnic group under these conditions.

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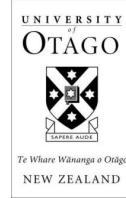
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## **APPENDICES**

# APPENDIX 1. Consent form for participants

11/070  
27 April 2011



## Sweat sodium concentrations among male athletes of Maori/Pacific Island or New Zealand European ethnicity – CONSENT FORM FOR PARTICIPANTS

I have read the Information Sheet concerning this project and understand what it is about. All my questions have been answered to my satisfaction. I understand that I am free to request further information at any stage.

I know that:-

1. My participation in the project is entirely voluntary;
2. I am free to withdraw from the project at any time without any disadvantage;
3. Personal identifying information (such as the questionnaire with my name, date of birth and ethnicity) will be destroyed at the conclusion of the project but any raw data on which the results of the project depend will be retained in secure storage for at least five years;
4. If I am randomly selected to provide a finger prick blood sample before and after training, I may experience short term and minor discomfort. If bruising occurs, this should disappear within one day;
5. I am entitled to my individual results and those of the entire project;
6. The results of the project may be published in a scientific journal and they will be available in the University of Otago Library (Dunedin, New Zealand). Every attempt will be made to preserve my anonymity;
7. At the end of the study, I consent to any remaining samples being disposed of using:

Standard disposal methods, OR;

Disposed with appropriate karakia, OR;

Returned to me.

I agree to take part in this project.

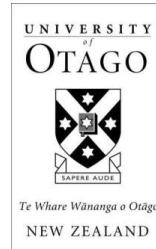
.....  
Signature of participant  
Name of participant

.....  
Date

This study has been approved by the University of Otago Human Ethics Committee. If you have any concerns about the ethical conduct of the research you may contact the Committee through the Human Ethics Committee Administrator (ph 03 479 8256). Any issues you raise will be treated in confidence and investigated and you will be informed of the outcome.

## APPENDIX 2. Health & demographic survey

11/070  
27 April 2011



### Sweat sodium concentrations among male athletes of Maori/Pacific Island or New Zealand European ethnicity - HEALTH & DEMOGRAPHIC SURVEY -

#### Demographic Information:

Name: .....

Date of birth (dd / mm / yyyy): .....

Which ethnic group(s) do you belong to?

*Mark the space or spaces which apply to you*

- New Zealand European
- Maori
- Samoan
- Cook Islands Maori
- Tongan
- Niuean
- Tokelauan
- Fijian
- Other Pacific Peoples -  
*please specify.....*
- Chinese
- Indian
- Other (such as Dutch, Japanese).  
*please State.....*

**Health Survey:**

It is important that volunteers participating in research studies are currently in good health. This is to ensure (i) their own continuing well-being and (ii) to avoid the possibility of individual health issues confounding study outcomes.

**Please complete this brief questionnaire to confirm fitness to participate:**

**1. At present**, do you have any health problem for which you are (circle as appropriate):

- |   |            |            |
|---|------------|------------|
| a. on medication, prescribed or otherwise                             | <b>Yes</b> | <b>/No</b> |
| b. taking any sports supplements                                      | <b>Yes</b> | <b>/No</b> |
| c. taking any non-steroidal anti-inflammatory e.g. ibuprofen, aspirin | <b>Yes</b> | <b>/No</b> |

If **YES** to either question 1a, b, or c, please list below:

.....  
.....

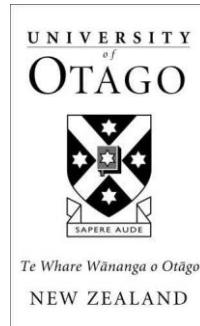
**2. Have you ever had any of the following (circle as appropriate)?**

- |  |            |            |
|--|------------|------------|
| a. Eczema  | <b>Yes</b> | <b>/No</b> |
| b. Allergy to elastoplasts                           | <b>Yes</b> | <b>/No</b> |
| c. Allergy to any food, medication or other compound | <b>Yes</b> | <b>/No</b> |
| d. A blood disorder                                  | <b>Yes</b> | <b>/No</b> |
| e. Diabetes  | <b>Yes</b> | <b>/No</b> |
| f. Digestive problems                                | <b>Yes</b> | <b>/No</b> |
| g. Heart problems                                    | <b>Yes</b> | <b>/No</b> |
| h. Disturbance of vision                             | <b>Yes</b> | <b>/No</b> |
| i. Thyroid problems                                  | <b>Yes</b> | <b>/No</b> |
| j. Kidney or liver problems                          | <b>Yes</b> | <b>/No</b> |
| k. Muscle cramps                                     | <b>Yes</b> | <b>/No</b> |

**If YES to either question 2a-2k, please describe briefly if you wish (e.g. to confirm problem was/is short-lived, insignificant or well controlled)**

## **APPENDIX 3. 24-hour urine collection (2-day protocol)**

11/070  
27 April 2011



**Sweat sodium concentrations among male athletes of Maori/Pacific Island or New Zealand European ethnicity –**

### **24-HOUR URINE COLLECTION (written guidance)**

- 1) Your 24 hour urine collection period begins now.
- 2) For each time you empty your bladder over the next 24 hour period, use the large container provided to collect all urine
- 3) Return your 24-hour urine collection to the researchers at the check in station on arriving to training

## **APPENDIX 4. 24-hour urine collection (3-day protocol)**

11/070  
27 April 2011



**Sweat sodium concentrations among male athletes of Maori/Pacific Island or New Zealand European ethnicity –**

### **24-HOUR URINE COLLECTION (written guidance)**

- 1) On the morning your 24 hour urine collection begins, empty your bladder on waking and discard this initial urine sample. Note the time (e.g. 7.00 a.m.).
- 2) For each time you empty your bladder over the next 24 hour period, use the large container provided to collect all urine
- 3) Repeat these steps up to and including the first emptying of your bladder tomorrow morning
- 4) Record the time of your final sample (the first emptying of your bladder on waking)
- 5) Return your 24-hour urine collection to the researchers at the check in station on arriving to training

## **APPENDIX 5. Individualised feedback for participants**

**Player:**

**Date:** 2<sup>nd</sup> September 2011

**Training Session:** 60 minute stationary bike exercise (average air temperature = 23.2°C, average relative humidity = 72.5%)

### HYDRATION STATUS – before training

**Measured by:** Urine colour score

<b>Guideline:</b>  Aim to begin all sessions well hydrated <ul style="list-style-type: none"> <li>• Urine colour scores of 6 or under represent good hydration</li> <li>• Urine scores of 7 or above indicate dehydration</li> </ul>	<b>24h urine Score</b>	<b>Pre-training urine score</b>	<b>Comments:</b>
	7	7	Results suggest you were dehydrated over the full day and before the training session. Being well hydrated is important for optimal performance.  You may like to monitor your hydration status further (see chart provided).

### HYDRATION PRACTICE – during training

**Assessed from your:**

- % body mass change
- % fluid replacement of sweat loss

**Guideline:**

Aim for good hydration by drinking enough during each session to prevent:

- Significant fluid loss (losing more than 1-2% body mass)
- Fluid overload (gaining any body mass)

<b>body mass before</b>	<b>body mass after</b>	<b>body mass change</b>	<b>fluid intake</b>	<b>urine volume</b>	<b>sweat volume</b>	<b>% fluid replacement</b>
97.2Kg	96.6Kg	LOST 0.6%	0mL	0mL	600mL	0%

**Comments:**

Results may seem to suggest you prevented significant fluid loss during the session. **BUT** no fluid was consumed. This is not an ideal hydration practice. It means you were unable to replace any of the fluid lost during the session. As you were probably dehydrated before starting, the extra fluid loss would suggest you became more dehydrated. Dehydration can limit your performance.

Aim to begin all sessions well hydrated. Drink enough during the session to prevent significant fluid loss. Remember to replace the losses which you are unable to prevent. To calculate, weigh yourself before & after each session. A 1Kg body mass loss = 1L fluid loss (see chart provided).

**Tips:**

Start rehydrating as soon as each session ends. Drinking coconuts are an excellent rehydration fluid choice. Water is a better choice than soda. Drinking with food can help the body absorb more fluid. Sipping on a drink can be better than taking a large volume all at once. Drink a bit more than your fluid losses to help make sure you are well hydrated for the next session.

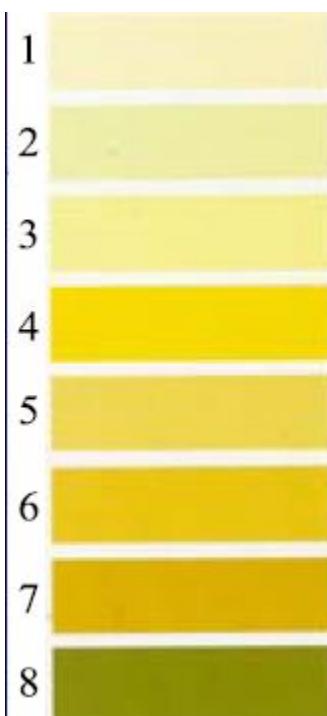
### SWEAT SODIUM LOSS – during training

**Estimated from:** sweat patch sample and sweat volume

<b>Guideline:</b>  The usual range is 10-80 mmol.L <sup>-1</sup> Values higher than this have been seen	<b>Sweat sodium concentration</b>	<b>Total sweat sodium loss</b>	<b>Comments:</b>
	11.2mmol.L <sup>-1</sup>	154mg	Sweat sodium concentration was in the usual range. 1 drinking coconut would replace this.

## HYDRATION MONITORING

### Urine Colour Chart:



Adapted from Armstrong LE. (2000). *Performing in Extreme Environments*. Champaign. Human Kinetics.

### Estimating Fluid Loss:

DATE	PRE EXERCISE WEIGHT (Kg)	POST EXERCISE WEIGHT to AVOID <sup>A</sup> (0.98 x pre exercise weight)	ACTUAL POST EXERCISE WEIGHT (Kg)	ESTIMATED FLUID LOSS (unadjusted) <sup>B</sup>
		≤		
		≤		

<sup>A</sup>Assuming you are well hydrated when measuring pre exercise weight

<sup>B</sup>Unadjusted means that any fluid intake and urine loss during the session was not considered. To adjust for these, measure your fluid intake and any urine loss during the session if you can. Add the fluid intake value to the unadjusted estimated fluid loss value and subtract urine volume

**Taimi Fakamalohisino:**

Fakamalohisino 'aka pasikala tu'uma'u he miniti 'e 60 ('Avalisi e ngaahi fua - mafana e 'ea = 23.2 , hauhau e 'ea = 72.5 %)

**Tu'unga e lahi e vai 'i he sino – kimu'a he fakamalohi sino**

**Fua'aki 'a e:** Fika e lanu 'o e tu'uofi

<b>Fakahinohino pe founiga ke ngaue'aki</b>  Mahu'inga ke lahi fe'unga 'a e vai ho sino kumu'a pea toki kamata 'a e fakamalohisino  <ul style="list-style-type: none"> <li>• Fika 6 pe ma'ulalo ange ai – faka'ilonga 'oku lahi fe'unga 'a e vai 'i ho sino</li> <li>• Fika 7 pe lahi ange he 7 – faka'ilonga ia 'oku maha pe 'ikai lahi fe'unga 'a e vai 'i ho sino</li> </ul>	<b>Fika e lanu 'o e tu'uofi he houa 'e 24</b>  <b>7</b>	<b>Fika e lanu 'o e tu'uofi kimu'a pea toki fai e fakamalohisino</b>  <b>7</b>	<b>Fakamatala</b>
			<p>'Oku 'ilonga na'e maha pe 'ikai fe'unga 'a e vai ho sino he 'aho pea mo e kimu'a 'a e fakamalohisino. 'Oku matua'aki mahu'inga 'aupito ke fe'unga 'a e vai ho sino ki ho'o fakamalohisino, tokoni ia ke ma'u e ola 'oku lelei.</p> <p>'E malava ke ke leva'l mo tala 'a e tu'unga 'oku I ai 'a e vai ho sino 'aki ho'o vakai ki he saati 'oku 'oatu</p>

**Tu'unga 'o e vai ho sino - lolotoga e fakamalohi sino**

**Vakai'i 'eni 'aki 'a e ngaahi liliu hange koe :**

- Mamafa ho sino (peseti)
- Vai ke fetongi 'aki e pupuha (%)

**Fakahinohino pe founiga ke ngaue'aki**

Mahu'inga ke lahi fe'unga ho'o inu lolotonga e va'inga – tokoni 'eni ke fakasi'isi'i :

- Vai 'oku mole mei he sino (peseti 'e 1 – 2 ho mamafa)
- Tatanaki e vai ho sino (tupu ho mamafa)

Mamafa ho sino 'I he kamata	Mamafa ho sino 'I he osi	Kehekehe ho mamafa	Lahi ho'o Inu u	Lahi ho'o tu'uofi	Lahi ho pupuha	Vai ke fetongi'aki e vai kuo mole mei ho sino
97.2Kg	96.6Kg	LOST 0.6%	0mL	0mL	600mL	0%

**Fakamatala:**

Koe ola ko 'eni – 'e malava pe ke tau pehe na'e 'ikai ke ke pupuha'ia pe lahi ha vai na'e mole he fakamalohisino. KA 'oku mahino pe na'e 'ikai ke ke inu. 'Oku 'ikai ko ha founiga lelei e 'eni, he taumaia te ke lava 'o fetongi 'a e vai mo e pupuha na'a ke ngaue'aki he fakamalohisino. 'Okapau na'e to lalo e vai ia ho sino kimu'a he fakamalohisino, 'e toe 'asili ai pe ke maha e vai, pea te ne uesia foki 'a e tu'unga mo e lelei ho'o va'inga.

**'Oku mahu'inga ke:**

Lahi fe'unga e vai ho sino he va'inga kotoa pe. Lahi fe'uga ho'o inu lolotonga e fakamalohisino. Tokanga ke ke inu ke fetongi 'a e vai 'oku mole he pupuha. Fua e lahi e vai 'oku mole mei he sino – fua ho mamafa kimu'a pea mo e 'osi 'a e fakamalohisino pe va'inga. Holo 'aki e mamafa ha kilo 'e taha – koe mole ia lita vai 'e taha mei he sino. Vakai ki he saati mo e fakamatala 'oku 'oatu

**Ngaahi me'e 'e tokoni atu ka koe:**

Mahu'inga ke ke inu ma'u pe he tuku 'a e va'inga. 'Oku kau 'a e niu mata he inu lelei 'aupito ke fakafetongi e vai mo ho pupuha. Inu e vai 'ata'ata - lelei ange ia he kapa inu mo e inu kasa. Tokoni ho'o inu fakataha mo ho'o kai ('umaki) ki hono tanaki e vai 'i ho sino. Lelei ange ho'o inu mammalie 'i ha'o fakaholo'l faka'angataha ho inu. Mahu'inga ke lahi ange ho'o inu he vai 'I he pupuha 'oku mole 'I he va'inga, lelei ia ki ho'o mo'ui, mo fakapapau'i 'oku lahi fe'unga 'a e vai 'I ho sino 'o

*mateuteu ki he va'inga hoko.*

### **Mole 'a e masima – fakamalohisino**

Fakafuofua'i mei he lahi 'o e pupuha

Fakahinohino pe founiga ke ngaue'aki	Lahi 'o e masima mo e pupuha	Lahi 'o e masima ne mole	Fakamatala:
Fakafuofua ki he vaha'a 'o e mililita 'e 10 – 80 (mmol.L <sup>-1</sup> ). 'Oku fa'a ai pe e ngaahi pupuha ia 'e ni'ihi 'oku lahi ange ia he fika ko 'eni.	11.2mmol.L <sup>-1</sup>	154mg	'Oku sai pe 'a e lahi 'o e masima 'I ho sino. <i>Ko e ngaahi mole kotoa mei ho sino I ho'o pupuha'ia 'e malava 'o fakafoki kotoa 'I ho'o inu 'a e fo'l niu mata pe 'e taha.</i>

## APPENDIX 6. Environmental conditions

### Relative Humidity (%)

	Monthly Mean (%)			
	June	July	August	September
<b>New Zealand</b>				
Auckland (Albany & Pukekohe)	83	83	82	80
Dunedin	77	76	73	71
Oamaru	82	81	79	73
<b>Tonga</b>				
Nuku'Alofa	77	75	75	74

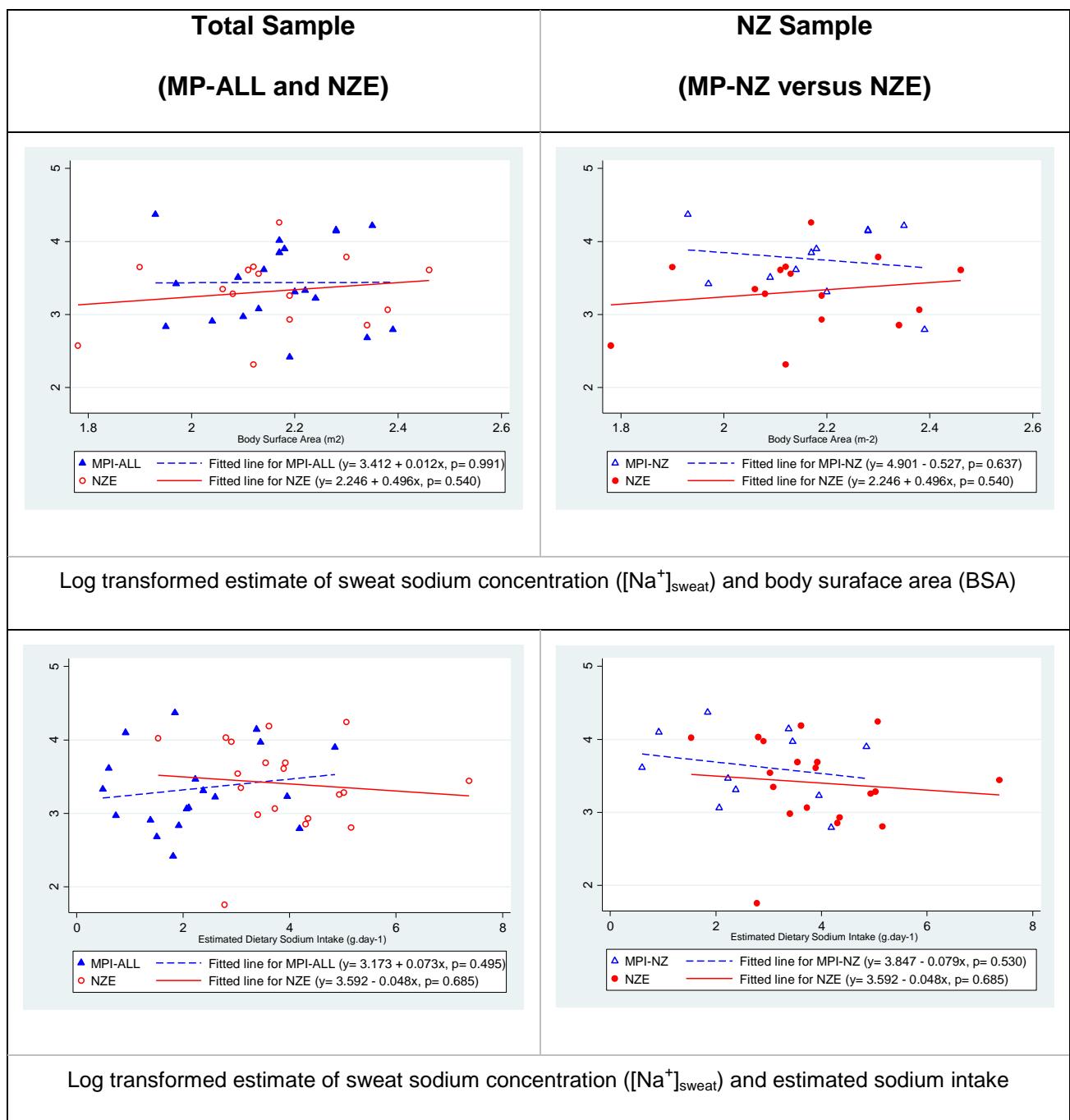
### Environmental Temperature (°C)

	Monthly Mean (°C)			
	June	July	August	September
<b>New Zealand</b>				
Auckland (Albany & Pukekohe)	12	11	11	12
Dunedin	6	5	6	8
Oamaru	5	5	6	8
<b>Tonga</b>				
Nuku'Alofa	21	21	21	21

<http://www.weatherbase.com> (29<sup>th</sup> June, 2012)

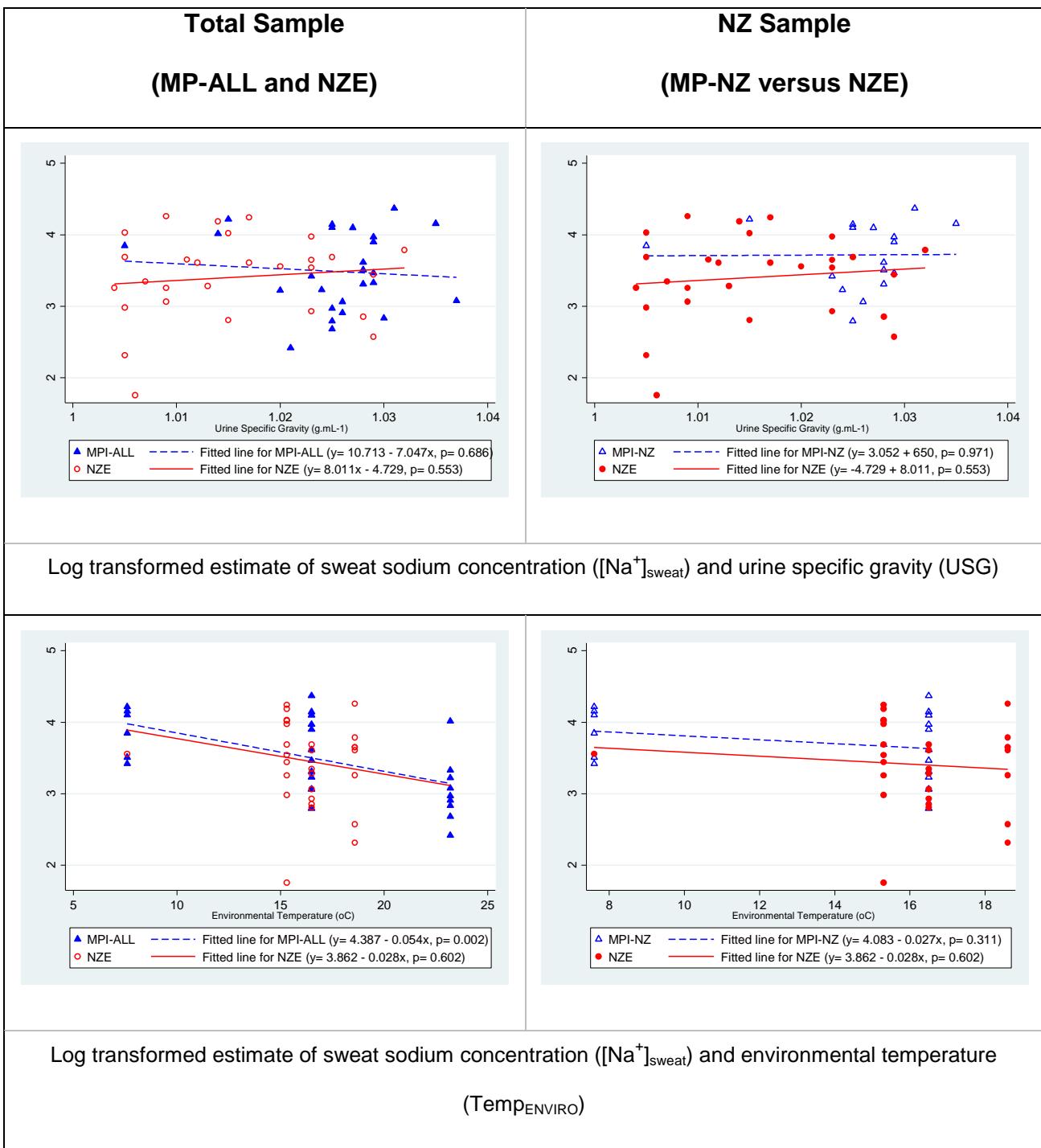
## APPENDIX 7. Interaction inspection– sweat sodium concentration

Table 15. Interaction assessment with simple linear regression – log transformed sweat sodium concentration ( $[Na^+]_{sweat}$ ) by ethnic group (Total Sample and NZ Sample)



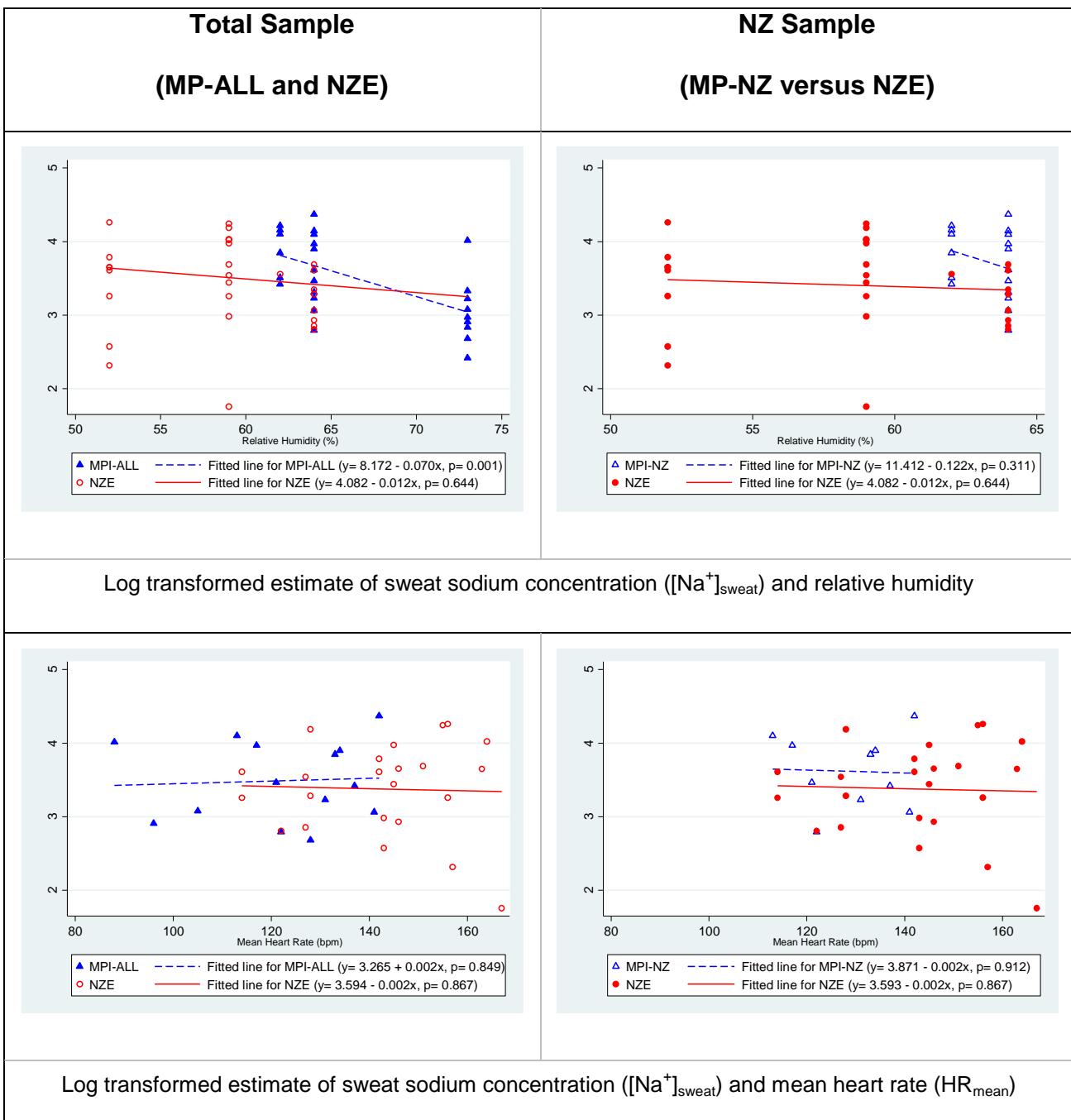
*Continued on next page*

Table 15 (continued).



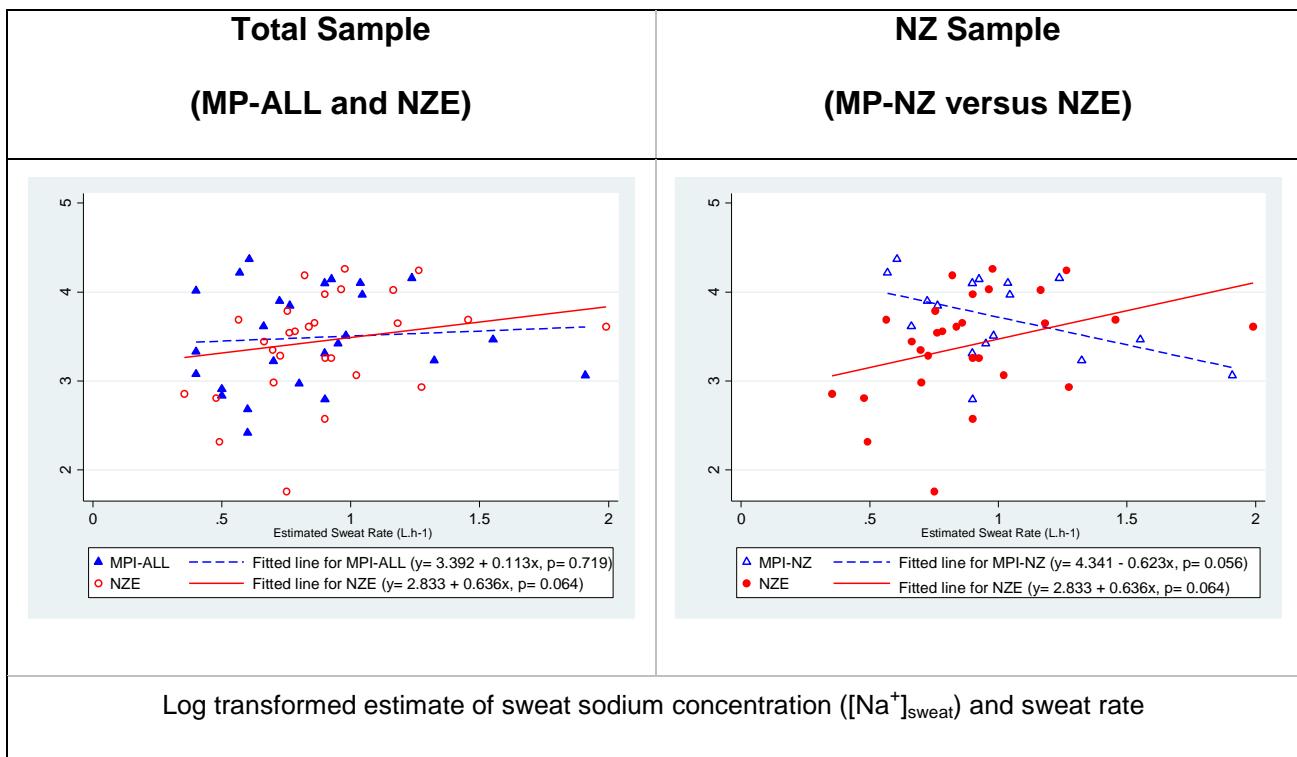
*Continued on next page*

Table 15 (continued)



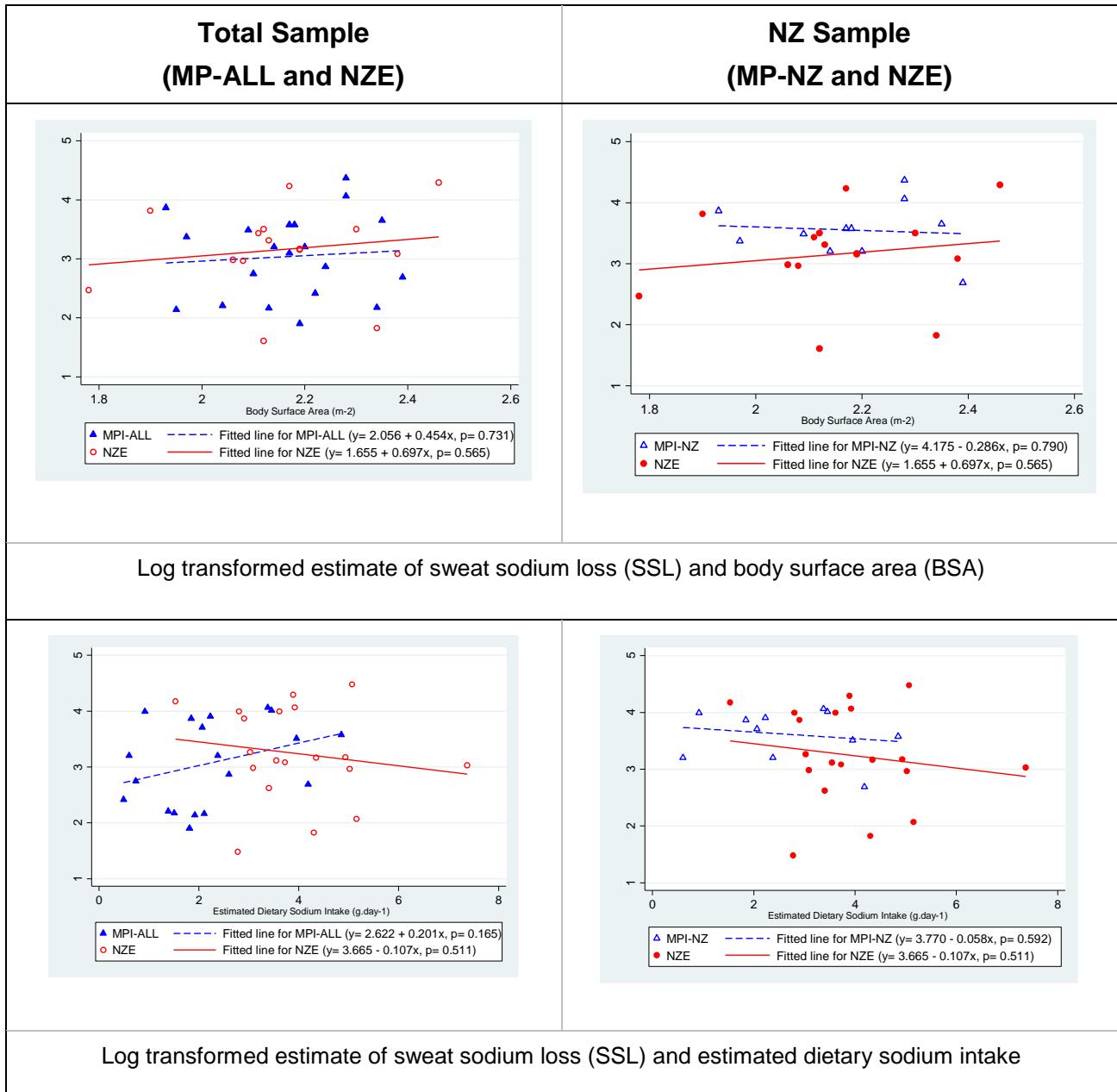
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Table 15 (continued)



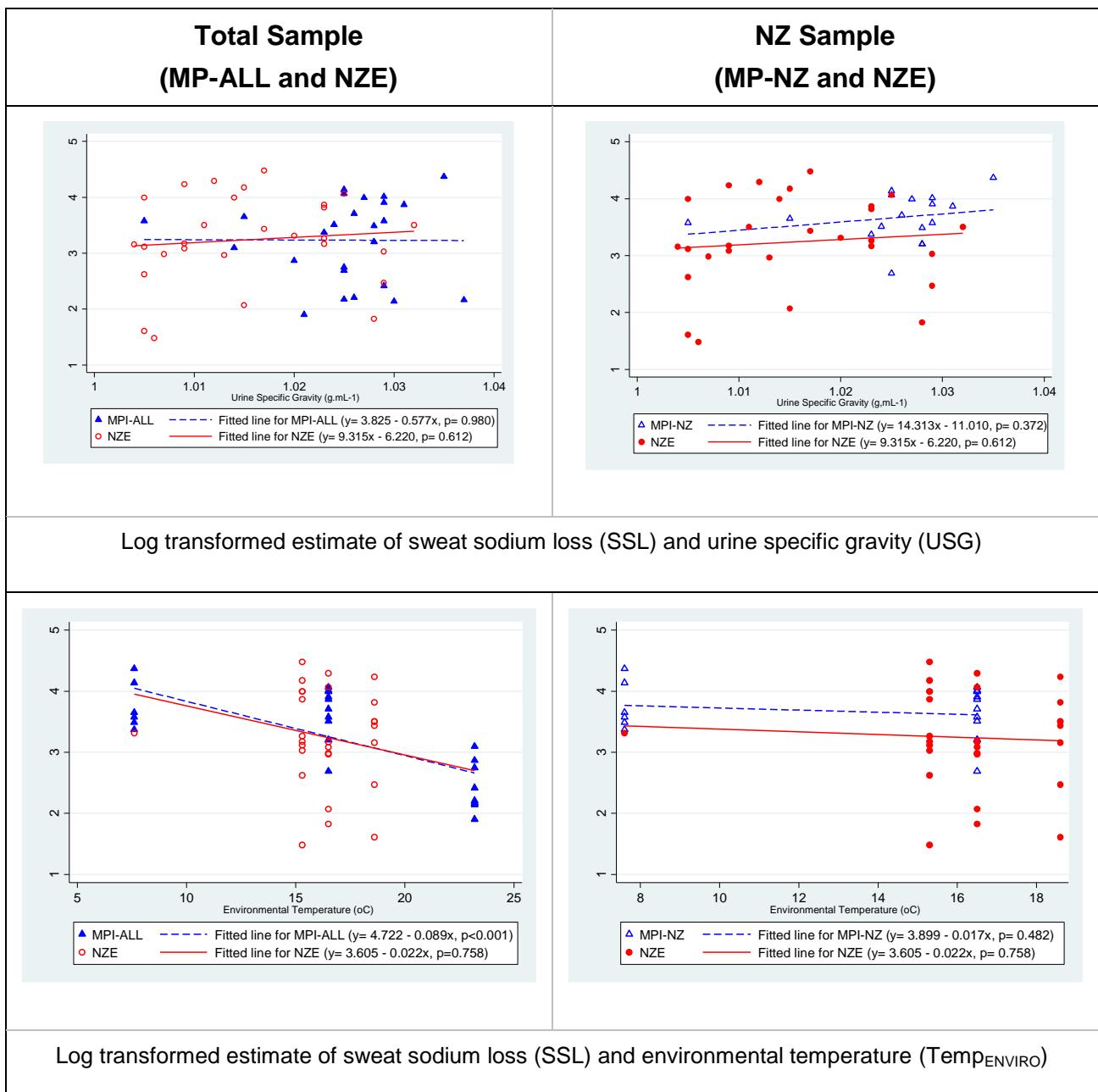
## APPENDIX 8. Interaction inspection - sweat sodium loss

Table 16. Interaction assessment with simple linear regression – log transformed sweat sodium loss (SSL) by ethnic group (Total Sample and NZ Sample)



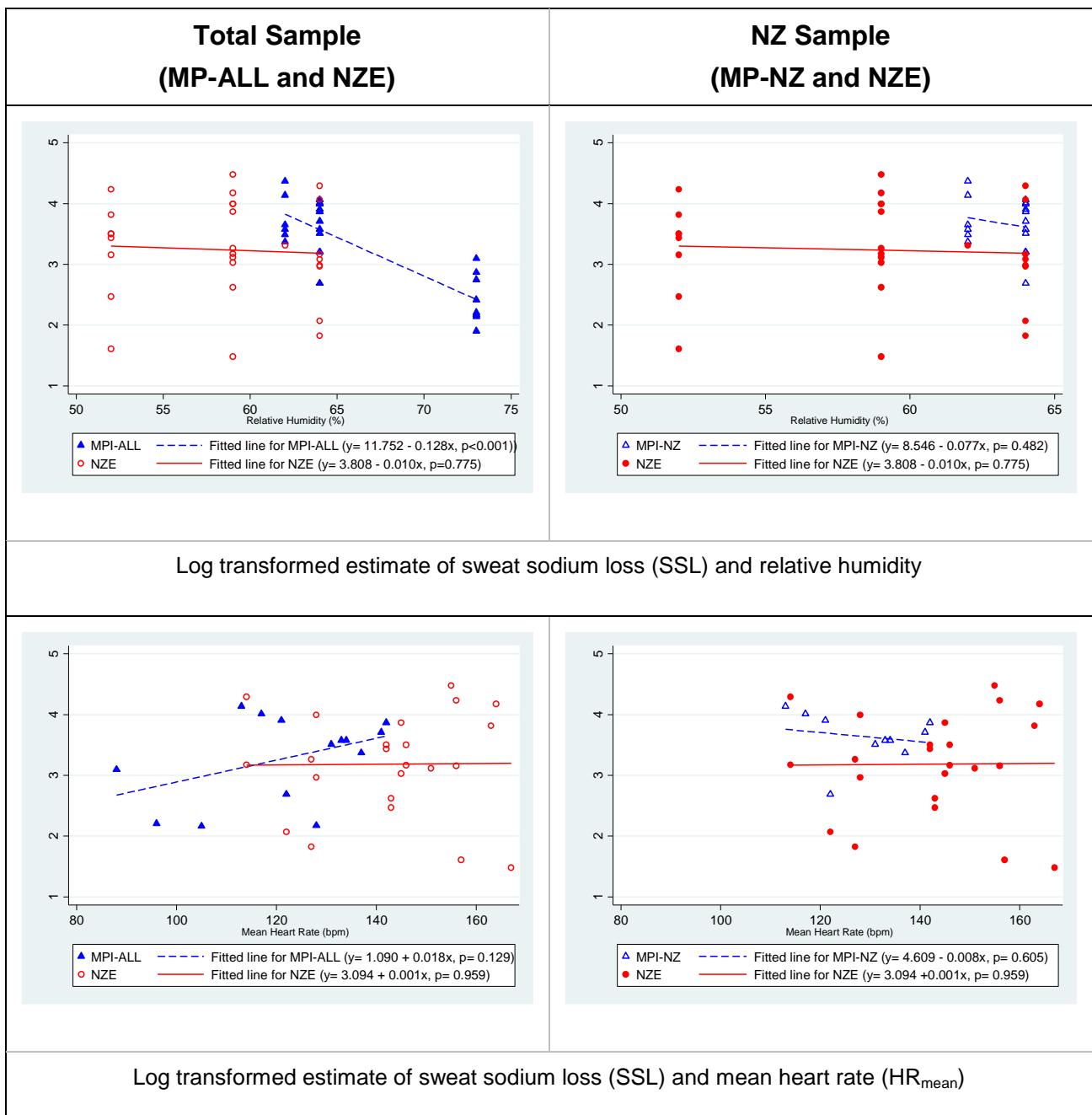
*Continued on next page*

Table 16 (Continued)..



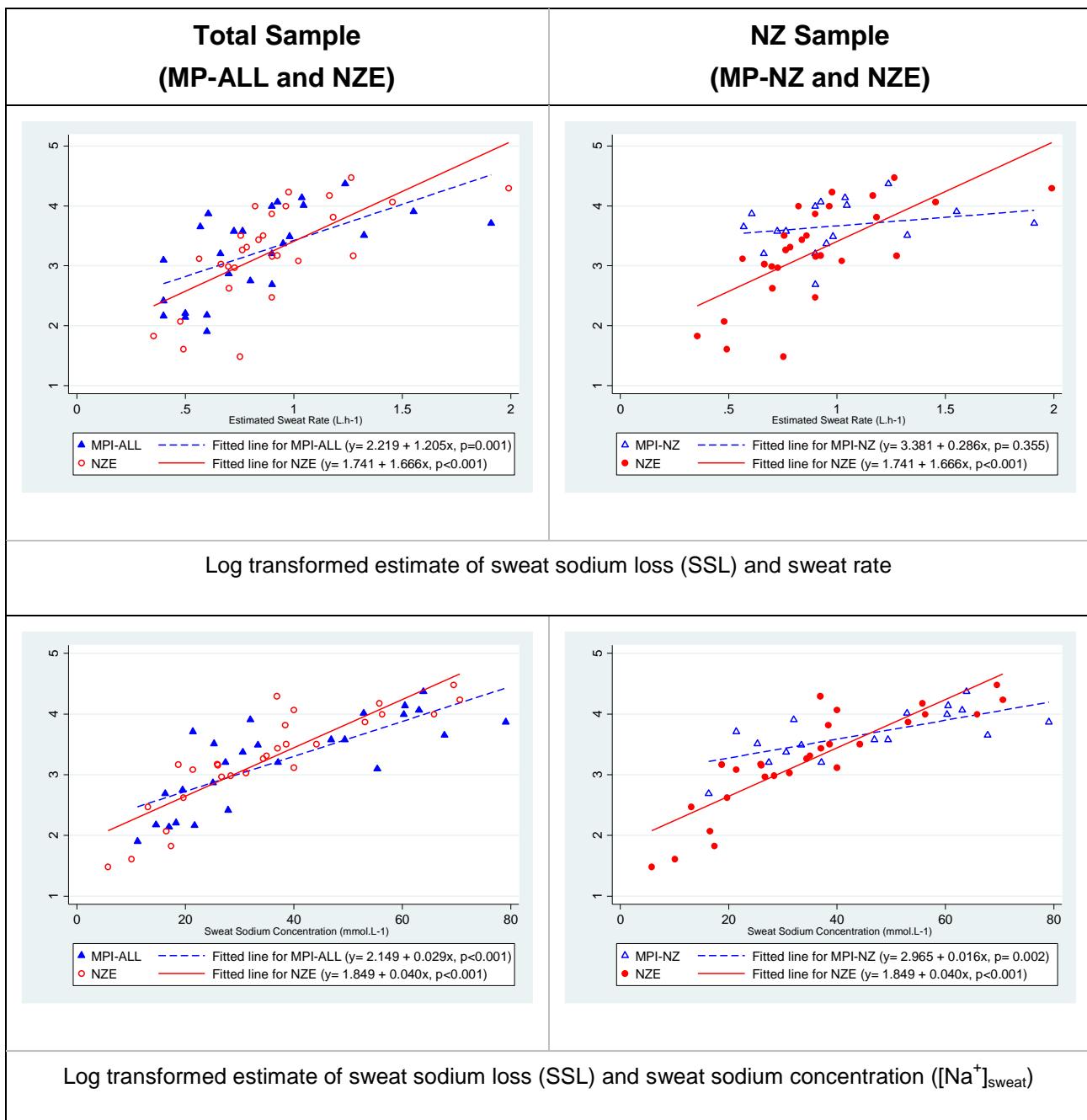
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Table 16 (Continued)..



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Table 16. (continued).



## APPENDIX 9. Assessment of Data Distribution

### Sweat Sodium Concentration

Figure 13. shows the distribution of estimated  $[Na^+]_{sweat}$  results of the total sample by the MP-ALL and NZE ethnic groups. Data from MP-ALL participants were positively skewed as evidenced by their mean  $[Na^+]_{sweat}$  being greater than their median  $[Na^+]_{sweat}$  ( $37.6 \pm 19.7 \text{ mmol.L}^{-1}$  versus  $31.3 \text{ mmol.L}^{-1}$ ). Data from NZE participants were more normally distributed as evidenced by the mean and median  $[Na^+]_{sweat}$  values being similar ( $34.9 \pm 17.6 \text{ mmol.L}^{-1}$  and  $34.7 \text{ mmol.L}^{-1}$  respectively).

Figure 14. shows the distribution of estimated  $[Na^+]_{sweat}$  results of the total sample by the MP-NZ, MP-TGA and NZE ethnic groups. The mean and median  $[Na^+]_{sweat}$  of the MP-NZ ( $45.1 \pm 18.7 \text{ mmol.L}^{-1}$  and  $34.9 \pm 17.6 \text{ mmol.L}^{-1}$  respectively) and NZE participants ( $46.9 \text{ mmol.L}^{-1}$  and  $34.7 \text{ mmol.L}^{-1}$  respectively) were similar. Data from MP-TGA participants were positively skewed as evidenced by a mean  $[Na^+]_{sweat}$  appearing to be greater than the median ( $23.4 \pm 13.0 \text{ mmol.L}^{-1}$  versus  $19.5 \text{ mmol.L}^{-1}$ ).

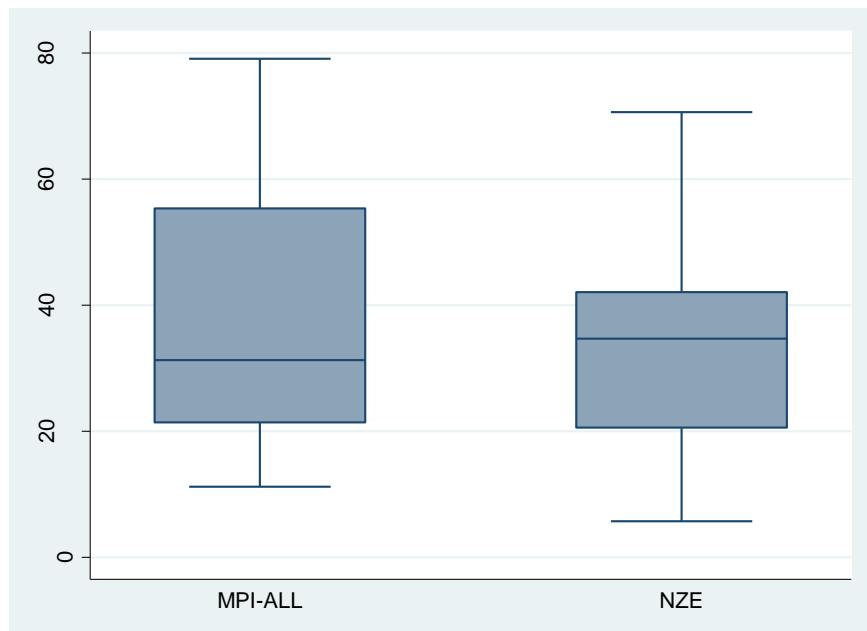


Figure 8. Box plot showing the distribution of mean sweat sodium concentration ( $[Na^+]_{sweat}$ ) data of the total sample by the MP-ALL and NZE ethnic groups.

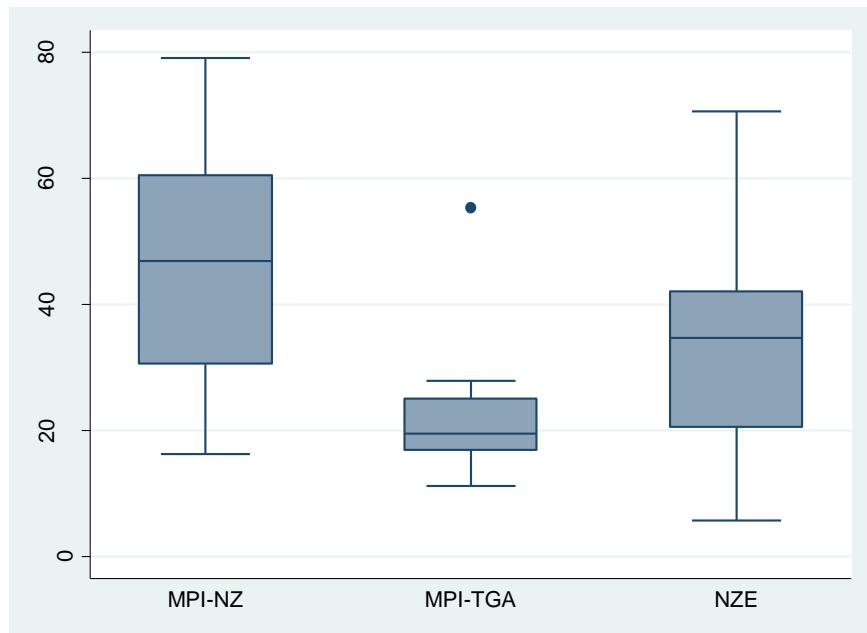


Figure 9. Box plot showing the distribution of mean sweat sodium concentration ( $[Na^+]_{sweat}$ ) data of the total sample by the MP-NZ, MP-TGA, and NZE ethnic groups.

## **Sweat Sodium Loss**

Figure 15. shows the distribution of estimated SSL results of the total sample by the MP-ALL and NZE ethnic groups. Data from NZE participants were positively skewed as evidenced by the mean SSL of this group being greater than their median SSL ( $33.2\pm22.5\text{mmol.h}^{-1}$  and  $25.1\text{mmol.h}^{-1}$ ). Data from MP-ALL participants were normally distributed as evidenced by their mean and median SSL values being similar ( $31.8\pm19.8\text{mmol.h}^{-1}$  and  $31.0\text{mmol.h}^{-1}$ ). Figure 16. shows the distribution of estimated SSL results of the total sample by the MP-NZ, MP-TGA and NZE ethnic groups. Data from all groups were positively skewed.

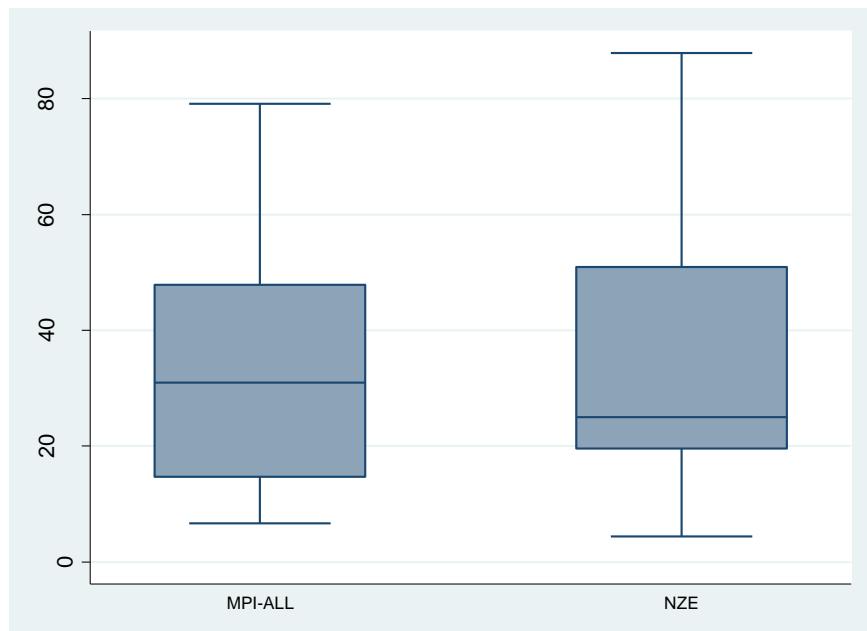


Figure 10. Box plot showing the distribution of estimated sweat sodium loss (SSL) data of the total sample by the MP-ALL and NZE ethnic groups

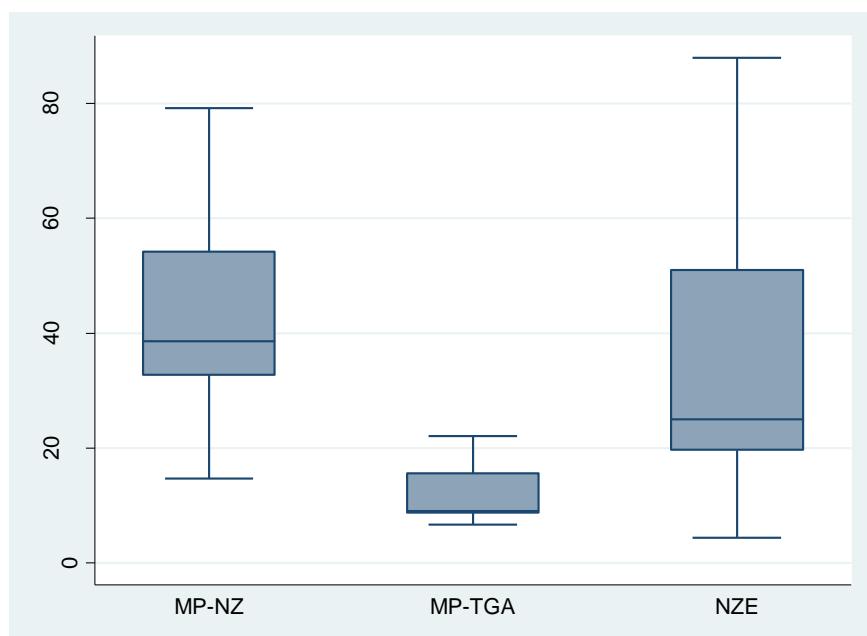


Figure 11. Box plot showing the distribution of estimated sweat sodium loss (SSL) data of the total sample by the MP-NZ, MP-TGA, and NZE ethnic groups

## Sweat Potassium Concentration

Figure 17. shows the distribution of  $[K^+]_{\text{sweat}}$  data from the total sample by the MP-ALL and NZE ethnic groups. Data from the MP-ALL and NZE participants were positively skewed as evidenced by their mean  $[K^+]_{\text{sweat}}$  being greater than their respective medians ( $4.3 \pm 2.3 \text{ mmol.L}^{-1}$  versus  $4.0 \text{ mmol.L}^{-1}$  and  $2.7 \pm 0.9 \text{ mmol.L}^{-1}$  versus  $2.3 \text{ mmol.L}^{-1}$ ). Figure 18. shows the distribution of  $[K^+]_{\text{sweat}}$  results of the total sample by the MP-NZ, MP-TGA and NZE ethnic groups. Data from all three groups were positively skewed.

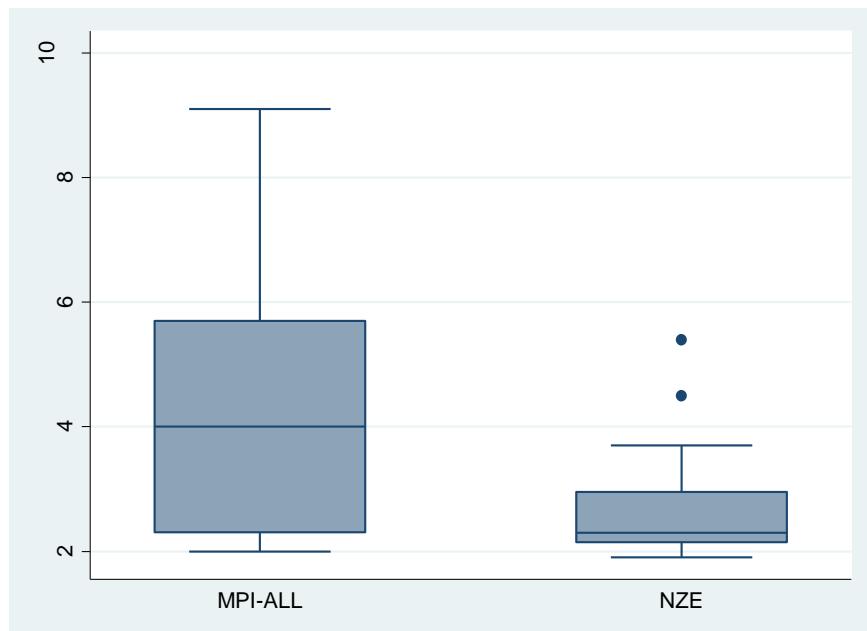


Figure 12. Box plot showing the distribution of mean sweat potassium concentration ( $[K^+]$ <sub>sweat</sub>) data of the total sample by the MP-ALL and NZE ethnic groups

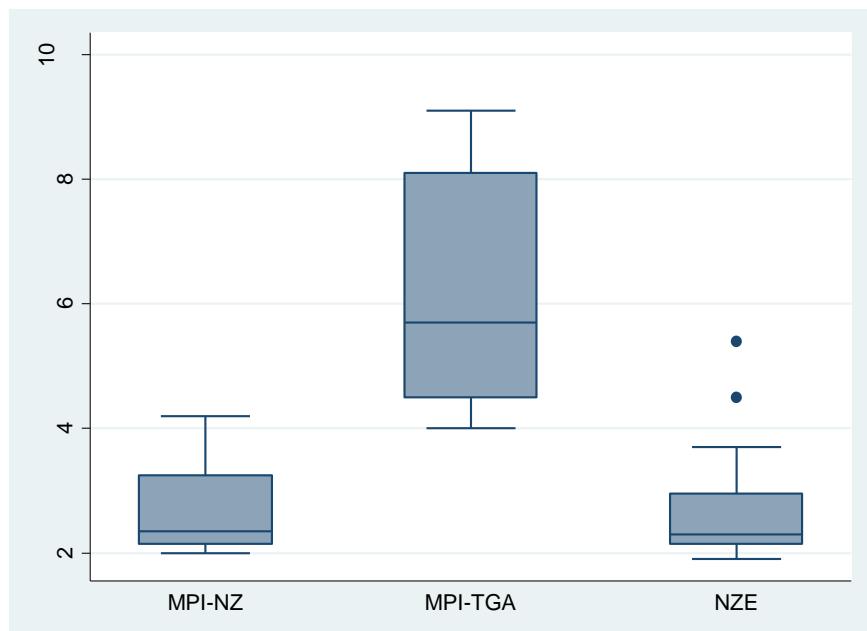


Figure 13.. Box plot showing the distribution of mean sweat potassium concentration ( $[K^+]$ <sub>sweat</sub>) data of the total sample by the MP-NZ, MP-TGA and NZE ethnic groups.

## Sweat Rate

Figures 19. and 20. show the distribution of sweat rate data of the total sample differentiated by the MP-ALL and NZE, and by the MP-NZ, MP-TGA and NZE ethnic groups respectively. Data from the MP-ALL, MP-NZ and NZE groups were positively skewed as evidenced by their respective mean sweat rates being greater than their medians ( $0.84\pm0.36\text{L.h}^{-1}$  versus  $0.76\text{L.h}^{-1}$ ,  $0.98\pm0.34\text{L.h}^{-1}$  versus  $0.91\text{L.h}^{-1}$ , and  $0.89\pm0.33\text{L.h}^{-1}$  versus  $0.84\text{L.h}^{-1}$  respectively). The mean sweat rate of MP-TGA participants appeared more normally distributed as the mean and median sweat rate values of this group were similar ( $0.54\pm0.14\text{L.h}^{-1}$  versus  $0.50\text{L.h}^{-1}$ ).

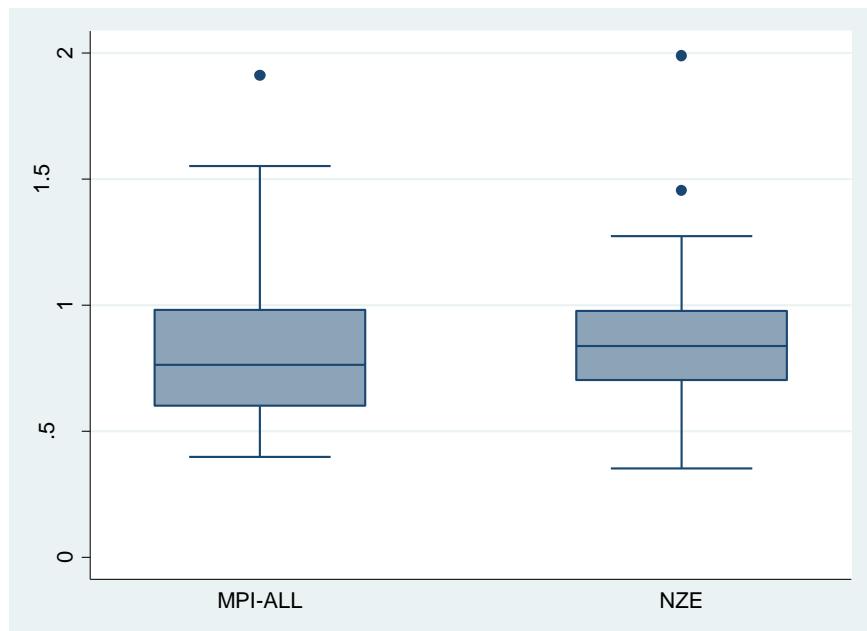


Figure 19. Box plot showing the distribution of mean sweat rate data of the total sample by the MPI-ALL and NZE ethnic groups

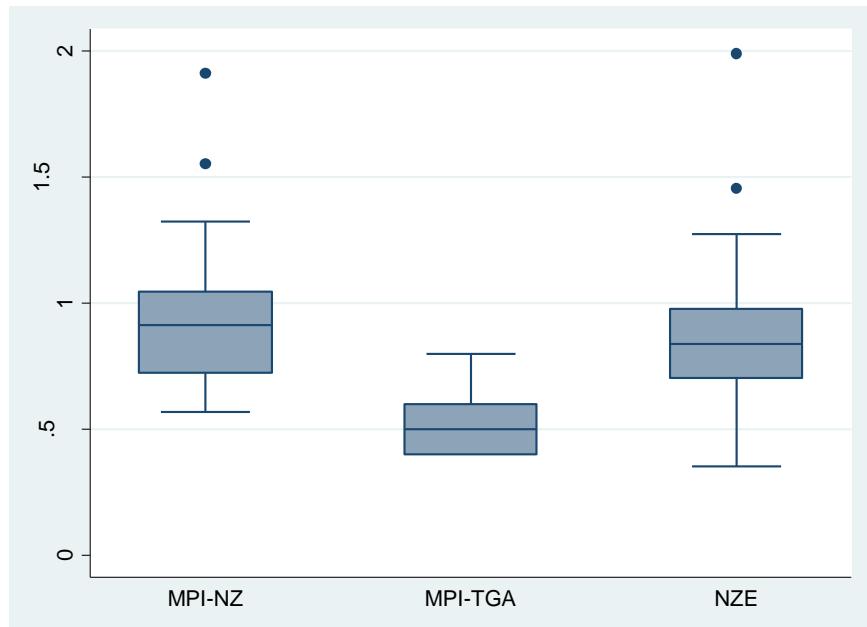


Figure 140. Box plot showing the distribution of mean sweat rate data of the total sample by the MPI-NZ, MPI-TGA and NZE ethnic groups.

## APPENDIX 10. Control variable selection - sweat sodium concentration

Control variables were selected for multiple linear regression if univariable analyses revealed an important influence exerted by the control variable candidate on the variance in  $[Na^+]_{sweat}$  ( $p<0.250$ ), and/or whether significant interactions existed by ethnic group (Table 17.).

Table 17. Table showing the control variable candidates selected for multiple linear regression analyses of sweat sodium concentration – Total Sample (MP-ALL and NZE)

Predictor	Rationale
Ethnic group	Primary predictor of interest $R^2=0.005$ , $p=0.604$
Sweat rate	$R^2=0.044$ , $p=0.128$ (i.e. $p<0.250$ )
Ethnic group and sweat rate Interaction	See Appendix 7.
Environmental temperature (Temp <sub>ENVIRO</sub> )	$R^2=0.145$ , $p=0.005$ (i.e. $p<0.250$ )
Relative humidity	$R^2=0.041$ , $p=0.143$ (i.e. $p<0.250$ )

Table 18. Table showing the control variable candidates selected for multiple linear regression analyses of sweat sodium concentration – NZ Sample (MP-NZ and NZE)

Predictor	Rationale
Ethnic group	Primary predictor of interest $R^2=0.074$ , $p=0.070$ (i.e. $p<0.250$ )
Sweat rate	See Appendix 7.
Ethnic group and sweat rate interaction	See Appendix 7.
Environmental temperature (Temp <sub>ENVIRO</sub> )	$R^2=0.065$ , $p=0.092$ (i.e. $p<0.250$ )
Urine specific gravity (USG)	$R^2=0.047$ , $p=0.154$ (i.e. $p<0.250$ )

## APPENDIX 11 Control variable selection - sweat sodium loss

Control variables were selected for multiple linear regression if univariable analyses found an effect of the predictor on the variance in SSL respectively ( $p<0.250$ ), and/or interactions by ethnic group were evident on visual inspection of their potential relationships with SSL (Appendix 8.).

Table 19. Table showing the control variable candidates selected for multiple linear regression analyses of sweat sodium loss – Total Sample (MP-ALL and NZE)

Predictor	Rationale
Ethnic group	Primary predictor of interest $R^2<0.001$ , $p=0.978$
Sweat rate	$R^2=0.410$ , $p<0.001$ ( $p<0.250$ )
Sweat sodium concentration ( $[Na^+]_{sweat}$ )	$R^2=0.670$ , $p<0.001$ ( $p<0.250$ )
Environmental temperature (Temp <sub>ENVIRO</sub> )	$R^2=0.210$ , $p=0.001$ ( $p<0.250$ )
Relative humidity	$R^2=0.099$ , $p=0.020$ ( $p<0.250$ )

Table 20. Table showing the control variable candidates selected for multiple linear regression analyses of sweat sodium loss – NZ Sample (MP-NZ and NZE)

Predictor	Rationale
Ethnic group	Primary predictor of interest $R^2=0.087$ , $p=0.049$ ( $p<0.250$ )
Sweat rate	$R^2=0.316$ , $p<0.001$ ( $p<0.250$ )
Sweat sodium concentration ( $[Na^+]_{sweat}$ )	$R^2=0.650$ , $p<0.001$ ( $p<0.250$ )
Ethnic group and sweat rate interaction	See Appendix 8.
Ethnic group and $[Na^+]_{sweat}$ interaction	See Appendix 8.

## APPENDIX 12. Change in blood sodium by ethnic group

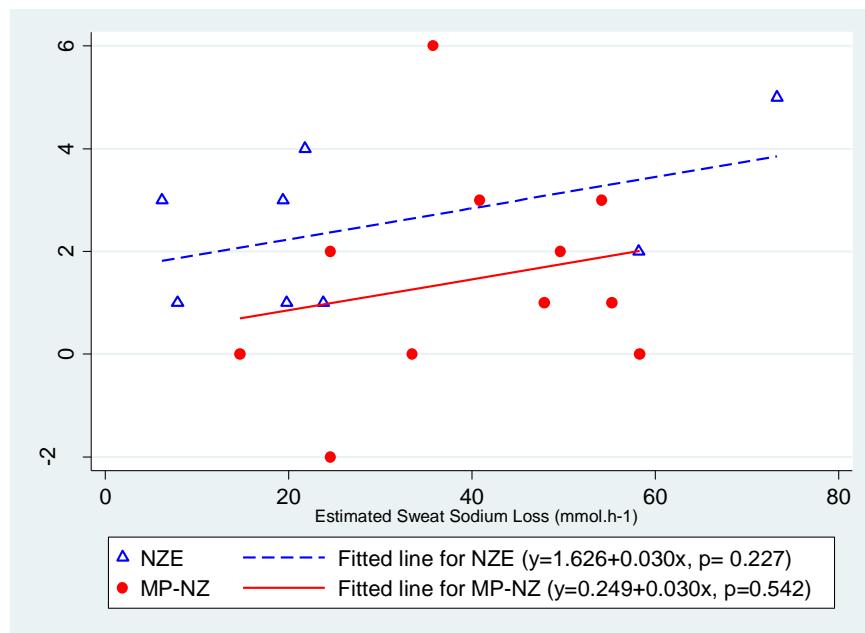


Figure 151. Relationship between the change in blood sodium concentration ( $[Na^+]$ blood) and sweat sodium loss (SSL) for the MP-NZ and NZE ethnic groups – Pukekohe cohort (n=23)

## APPENDIX 13. Sodium content of recovery foods

Table 21. Table showing typical recovery foods with sodium content per serve.

Food	Serving size	Sodium per serve (mg)
Creamed rice <sup>A</sup>	1x 220g can (vanilla)	130mg
Sandwich <sup>B</sup> – low fat ham, cheese and salad	2x slices wholegrain toast, 1x 2cm cube edam 1x 16g slice ham	(303mg) (61mg) <u>(244mg)</u> 608mg
Liquid breakfast <sup>C</sup>	350mL carton (choc ice)	210mg
Chicken noodle soup <sup>B</sup>	1 cup	590mg
Low fat yoghurt <sup>D</sup>	1x 150g potte (strawberry)	33mg
Commercial rehydration fluid <sup>E</sup>	1x 750mL bottle	209mg
Drinking coconut <sup>F</sup>	1x whole coconut	216mg
Flavoured milk <sup>G</sup>	1x 600mL bottle (chocolate)	240mg

<sup>A</sup>Aunt Betty's Vanilla Flavoured Creamy Rice

<sup>B</sup>Food composition tables (Ministry of Health, 2009)

<sup>C</sup><http://www.sanitarium.co.nz/products/breakfast/up-and-go/up-and-go-choc-ice>

accessed Thursday 29th November 2012

<sup>D</sup><http://www.freshnfruity.co.nz/fnf-nips.pdf> accessed Thursday 29th November 2012

<sup>E</sup><http://www.livepositively.co.nz/Page/YourHealth/nutrition-comparison-tool> accessed Thursday 29th November 2012

<sup>F</sup>[http://www.nal.usda.gov/fnic/foodcomp/cgi-bin/list\\_nut\\_edit.pl](http://www.nal.usda.gov/fnic/foodcomp/cgi-bin/list_nut_edit.pl) accessed Thursday 29th November 2012

<sup>G</sup><http://www.primo.co.nz/flavours> accessed Thursday 29th November 2012