

This item was submitted to Loughborough's Institutional Repository (<u>https://dspace.lboro.ac.uk/</u>) by the author and is made available under the following Creative Commons Licence conditions.



For the full text of this licence, please go to: http://creativecommons.org/licenses/by-nc-nd/2.5/

# The Effects of Exercise on Sodium Balance in Humans

By

Thomas Darrell Love

A Doctoral Thesis

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

November 2010

© By T.D. Love 2010

#### Abstract

During exercise water and electrolytes are lost in sweat. There is a large variation in both sweat rate and sweat composition and as a consequence sweat electrolyte loss can be large, especially for sodium, the primary cation in sweat. The loss of large amounts of sodium in sweat has been linked with hyponatraemia and muscle cramps. Sodium intake is encouraged in some athletes and in some exercise situations, which is in direct contrast to guidelines aimed at the general population aimed at reducing average sodium intakes to 2.4g of sodium per day (6g salt/day).

Dietary sodium intakes have been determined by numerous methods, including weighed dietary records and 24h urine collections. As dietary sodium intake in excess of basal requirement is primarily excreted in the urine in non-sweating individuals, and the basal requirement for sodium is small, 24h urine collections can provide an accurate estimate of dietary sodium intake. In Chapter 3, 24h urinary sodium excretion was measured in eighteen subjects on 4 separate occasions. Subjects consumed their normal diet with the exception of a 5g creatine supplement and 500ml of water, which was part of a separate investigation. The relationship between urine sodium excretion in each 24h collection period was weak, but on average males excreted  $200 \pm 48$ mmol of sodium per day and females excreted  $157 \pm 33$ mmol of sodium per day, which is equivalent to 4.6g and 3.6g of sodium, respectively. This is in excess of the current recommended intake.

In chapter 4, the variation in sodium excretion was determined in eight subjects who consumed the same diet for 5 consecutive days. Despite the similar intake of sodium each day, a day to day variation in sodium excretion of 13% was still observed. This was not related to either sodium intake or potassium intake.

In chapter 5, nine subjects consumed their normal diet for 5 consecutive days but weighed and recorded all food and drink consumed. During this period, 24h urine samples were also collected. No strenuous exercise was permitted apart from an exercise task on day 4. This involved intermittent cycling in the heat until 2% body mass (BM) was lost. Sweat was collected from four absorbent patches placed on the back, chest, forearm and thigh. Sweat sodium concentration was adjusted to account for the 35% over-estimation using this regional collection method. Subjects lost  $1.51 \pm 0.19$ L of sweat and  $66 \pm 16$ mmol (range 32 - 86mmol) of sodium. There was no difference in sodium balance between each 24h period due to a significant decrease in urine sodium excretion on the day of exercise (day 4).

In chapter 6, the effect of prior exercise on sweat composition during a second exercise bout completed later that same day was determined. Eight healthy males cycled for 40 minutes in the heat on one or two occasions. A period of 5h elapsed between exercise bouts when two exercise sessions were performed. Sweat was collected using a whole body washdown method and by 4 absorbent patches placed on the back, chest, forearm and thigh. The main finding was that prior exercise did not affect sweat rate or sweat sodium, potassium and chloride concentrations in the second exercise bout when using the whole body washdown method.

Chapter 7 determined the effects of two exercise sessions completed on the same day on electrolyte balance. Nine subjects followed their normal dietary behaviour but weighed and recorded all food and drink consumed during 5 consecutive days. During this period 24h urine samples were also collected. No strenuous exercise was permitted during this period apart from two exercise tasks on day 4. During exercise sweat was collected using a whole body washdown technique. Sweat rate and sweat sodium, potassium and chloride concentrations during the second exercise bout were found to be similar to the first exercise bout. Subjects lost 2.64L (range 1.80 - 3.48L) of sweat and  $138 \pm 106$ mmol of sodium (range 32 - 287mmol). Sodium balance was not significantly affected on the day of exercise (Day 4) and the day following exercise (day 5), indicating significant sodium conservation by the kidney. In contrast, no change in sodium intake was observed.

In chapter 8, the effect of skimmed milk and a sports drink in restoring fluid balance was examined following exercise-induced dehydration. Seven physically active males cycled intermittently in the heat until 2% BM was lost. During a 1h rehydration period a sports drink (23mmol Na<sup>+</sup>/L) or skimmed milk (32mmol Na<sup>+</sup>/L) was consumed in a volume equivalent to 150% of BM loss. Fluid balance at the end of the 3h recovery period tended to be more positive when milk was consumed. Despite this, no difference in exercise capacity in the heat was observed.

This thesis shows that exercise did not increase sodium intake, but this may be due to the already high dietary sodium intake of individuals. Sodium balance was maintained in the majority of individuals due to a significant conservation of sodium by the kidneys. When sweat sodium losses are large, urine sodium conservation may not be sufficient to prevent a negative sodium balance. When no food is consumed in the acute period post-exercise, the higher sodium content of skimmed milk than a sports drink may be partly responsible for the increased retention of the ingested fluid. But this did not enhance subsequent performance in the heat.

Key words: Fluid balance, electrolyte balance, hydration, sodium, potassium, sweat, exercise

### Acknowledgements

There are many people that I would like to thank, although it will be hard to convey my true appreciation for all their help and assistance throughout my time at Loughborough University in such a small section of this thesis, but I will give it a go.

Firstly, I would like to thank my supervisor Professor Ron Maughan. His limitless knowledge, eye for detail and patience have helped me tremendously with my research. The opportunities given outside of the laboratory setting have encouraged me to continue working in the area of exercise physiology and for which I am extremely grateful.

Thanks must also go to Dr Susan Shirreffs and Dr Phil Watson for their assistance in the laboratory and with blood sampling in Chapter 8 of this thesis. Your advice and guidance have been invaluable.

I have had the fortune to work with numerous research students, all of whom have been extremely helpful and made my time at Loughborough enjoyable. I would therefore like to thank Dr Jason Lee, Dr Gethin Evans, Dr Javad Fallah, Mrs Normah Jusoh, Dr Ian Rollo, Ms Emma Clapp, Ms Ruth Hobson, Mr Lewis James, Mr George Aphamis and Dr Katherine Black. Special thanks must go to Ms. Ruth Hobson, Mr Lewis James, Mr George Aphamis and Dr Katherine Black. Their constant self-less assistance in and around the laboratory throughout my time at Loughborough was unremitting and despite the numerous thank-you's I gave; I don't think I'll ever be able to say thank-you enough. I would also like to apologise to my housemates; Dr Katherine Black, Ms. Marie Dannhaeuser and Dr Colm Fitzgerald for my poor washing-up skills throughout my stay in Loughborough!

Thanks also to my family for their unwavering support throughout my university career.

Special thanks must also go to Dr Helen Peterkin, for being there.

Lastly, it would all not have been possible without the time and effort of the volunteers that participated in the studies in this thesis. They did so for no reward, just in the name of science. I applaud you.

# **Publications**

The results of chapter 8 of this thesis have been published in the following journal:

Watson P, Love TD, Maughan RJ and Shirreffs SM (2008) A comparison of the effects of milk and a carbohydrate-electrolyte drink on the restoration of fluid balance and exercise capacity in a hot, humid environment. European Journal of Applied Physiology 104: 633-642.

# Table of contents

| Abstract              | i     |
|-----------------------|-------|
| Acknowledgements      | iv    |
| Publications          | v     |
| Table of contents     | vi    |
| List of tables        | xiv   |
| List of figures       | xviii |
| List of abbreviations | xxii  |
|                       |       |

| Chapter 1 | Introduction                                 | 1  |
|-----------|--|----|
| 1.1       | Sodium in the human body - Overview          | 2  |
| 1.2       | Body water                                   | 2  |
| 1.3       | Regulation of fluid balance                  | 3  |
| 1.4       | Dietary electrolyte intake                   | 5  |
| 1.5       | Body electrolyte losses                      | 6  |
| 1.6       | Sweat gland structure and function           | 7  |
| 1.7       | Sweat electrolyte losses in sport            | 7  |
| 1.8       | Factors affecting sweat composition          | 12 |
|           | 1.8.1 Sweat collection techniques            | 12 |
|           | 1.8.2 Method of sweat stimulation            | 14 |
|           | 1.8.3 Sweat rate                             | 15 |
|           | 1.8.4 Heat acclimation                       | 15 |
|           | 1.8.5 Training                               | 16 |
|           | 1.8.6 Hydration                              | 17 |
|           | 1.8.7 Age                                    | 18 |
|           | 1.8.8 Gender                                 | 19 |
|           | 1.8.9 Diet                                   | 21 |
| 1.9       | Sodium deficiency                            | 21 |
| 1.10      | Effects of dehydration on performance        | 22 |
| 1.11      | Fluid and electrolyte intake before exercise | 23 |
| 1.12      | Fluid and electrolyte intake during exercise | 25 |
|           | 1.12.1 Sodium intake and performance         | 26 |
|           | 1.12.2 Hyponatraemia                         | 26 |
|           | 1.12.3 Cramp                                 | 28 |

|           | 1.12.4 Drink palatability                    | 30 |
|-----------|--|----|
| 1.13      | Fluid and electrolyte intake after exercise  | 30 |
|           | 1.13.1 Effect of drink composition           | 30 |
|           | 1.13.2 Effect of drink volume                | 32 |
|           | 1.13.3 Effect of food ingestion              | 32 |
| 1.14      | Aims of this thesis                          | 33 |
|           |  |    |
| Chapter 2 | General methods                              | 34 |
| 2.1       | Introduction                                 | 35 |
| 2.2       | Ethical approval                             | 35 |
| 2.3       | Subjects                                     | 35 |
| 2.4       | Preliminary trials                           | 35 |
|           | 2.4.1 Familiarisation                        | 35 |
|           | 2.4.2 Anthropometry                          | 35 |
| 2.5       | Pre-trial standardisation                    | 36 |
| 2.6       | Urine collection and analysis                | 36 |
|           | 2.6.1 24h urine collection                   | 36 |
|           | 2.6.2 Laboratory-based urine collection      | 36 |
|           | 2.6.3 Urine analysis                         | 37 |
| 2.7       | Sweat collection and analysis                | 37 |
|           | 2.7.1 Regional collection                    | 37 |
|           | 2.7.2 Whole body sweat collection            | 38 |
| 2.8       | Dietary analysis                             | 39 |
| 2.9       | Blood collection, sampling and analysis      | 40 |
| 2.10      | Coefficients of variation                    | 40 |
| 2.11      | Statistical analysis                         | 41 |
|           |  |    |
| Chapter 3 | The daily variation in electrolyte excretion | 42 |
| 3.1       | Introduction                                 | 43 |
| 3.2       | Method                                       | 44 |
|           | 3.2.1 Subjects                               | 44 |
|           | 3.2.2 Experimental protocol                  | 44 |
|           | 3.2.3 Urine collection                       | 44 |
|           | 3.2.4 Dietary intake                         | 45 |

\_\_\_\_

|           | 3.2.5  | Physical activity                                  | 45 |
|-----------|--------|--|----|
|           | 3.2.6  | Sample analysis                                    | 45 |
|           | 3.2.7  | Statistical analysis                               | 45 |
| 3.3       | Result | ts   | 46 |
|           | 3.3.1  | Subject characteristics and body mass changes      | 46 |
|           | 3.3.2  | Duration of urine collection                       | 46 |
|           | 3.3.3  | Urine volume & electrolyte excretion               | 46 |
|           | 3.3.4  | Relationship in the excretion of urine parameters  |    |
|           |        | between each 24h collection                        | 49 |
|           | 3.3.5  | Relationship between urine volume and urine        |    |
|           |        | electrolyte excretion                              | 49 |
|           | 3.3.6  | Relationship between urine sodium and potassium    |    |
|           |        | excretion  | 50 |
| 3.4       | Discu  | ssion  | 50 |
|           | 3.4.1  | Body mass  | 50 |
|           | 3.4.2  | Sodium   | 51 |
|           | 3.4.3  | Potassium  | 53 |
|           | 3.4.4  | Conclusion   | 54 |
|           |        |  |    |
| Chapter 4 | The v  | ariation in water and electrolyte excretion whilst |    |
|           | consu  | ming the same diet                                 | 55 |
| 4.1       | Introd | uction   | 56 |
| 4.2       | Metho  | od   | 57 |
|           | 4.2.1  | Subjects   | 57 |
|           | 4.2.2  | Experimental protocol                              | 57 |
|           | 4.2.3  | Dietary monitoring                                 | 57 |
|           | 4.2.4  | Urine collection                                   | 58 |
|           | 4.2.5  | Physical activity                                  | 58 |
|           | 4.2.6  | Sample analysis                                    | 58 |
|           | 4.2.7  | Statistical analysis                               | 58 |
| 4.3       | Result | ts   | 59 |
|           | 4.3.1  | Body mass  | 59 |
|           | 4.3.2  | Duration of urine collection                       | 59 |
|           | 4.3.3  | Completeness of urine collection                   | 59 |

|           | 4.3.4   | Dietary intake and urine excretion                          | 60 |
|-----------|---------|---|----|
|           | 4.3.5   | Relationship between energy intake & electrolyte intake     | 64 |
|           | 4.3.6   | Relationship between electrolyte intake & electrolyte       |    |
|           |         | excretion   | 64 |
|           | 4.3.7   | Electrolyte balance   | 66 |
|           | 4.3.8   | Relationship between oscillations in electrolyte excretion  |    |
|           |         | and electrolyte intake                                      | 70 |
|           | 4.3.9   | Relationship between oscillations in sodium and chloride    |    |
|           |         | excretion with potassium intake and the relationship betwee | en |
|           |         | oscillations in potassium excretion with sodium intake      | 71 |
|           | 4.3.10  | Hydration status  | 72 |
|           | 4.3.11  | Relationship between sodium, potassium and chloride         |    |
|           |         | excretion   | 72 |
|           | 4.3.12  | Relationship between urine volume and urine electrolyte     |    |
|           |         | excretion   | 72 |
| 4.4       | Discus  | sion  | 73 |
|           | 4.4.1   | Body mass   | 73 |
|           | 4.4.2   | Sodium and chloride   | 73 |
|           | 4.4.3   | Potassium   | 77 |
|           | 4.4.4   | Conclusion  | 78 |
| Chapter 5 | The ef  | fect of exercise on electrolyte balance                     | 79 |
| 5.1       | Introdu | action  | 80 |
| 5.2       | Metho   | d   | 81 |
|           | 5.2.1   | Subjects  | 81 |
|           | 5.2.2   | Experimental protocol                                       | 81 |
|           | 5.2.3   | Dietary monitoring  | 82 |
|           | 5.2.4   | Urine collection  | 82 |
|           | 5.2.5   | Physical activity   | 82 |
|           | 5.2.6   | Sample analysis   | 83 |
|           | 5.2.7   | Statistical analysis  | 83 |
| 5.3       | Result  | 8   | 83 |
|           | 5.3.1   | Exercise  | 83 |
|           | 5.3.2   | Estimated whole body sweat composition & loss               | 84 |

|           | 5.3.3   | Sweat composition at regional collection sites          | 84  |
|-----------|---------|---|-----|
|           | 5.3.4   | Relationship between sweat rate and sweat composition   | 84  |
|           | 5.3.5   | Hydration status  | 85  |
|           | 5.3.6   | Body mass   | 85  |
|           | 5.3.7   | Duration of urine collection                            | 85  |
|           | 5.3.8   | Dietary intake and urine excretion                      | 86  |
|           | 5.3.9   | Electrolyte balance                                     | 90  |
|           | 5.3.10  | Relationship between electrolyte intake & electrolyte   |     |
|           |         | excretion   | 91  |
|           | 5.3.11  | Relationship between energy intake & electrolyte intake | 91  |
|           | 5.3.12  | Relationship between electrolyte intake & sweat         |     |
|           |         | electrolyte loss  | 92  |
| 5.4       | Discus  | ssion   | 92  |
|           | 5.4.1   | Sodium and chloride                                     | 93  |
|           | 5.4.2   | Potassium   | 96  |
|           | 5.4.3   | Hydration status  | 97  |
|           | 5.4.4   | Conclusion  | 98  |
| Chapter 6 | The ef  | ffect of prior exercise on sweat composition and loss   | 99  |
| 6.1       | Introdu | uction  | 100 |
| 6.2       | Metho   | d   | 101 |
|           | 6.2.1   | Subjects  | 101 |
|           | 6.2.2   | Pre-trial standardisation                               | 101 |
|           | 6.2.3   | Experimental protocol                                   | 102 |
|           | 6.2.4   | Sample analysis   | 103 |
|           | 6.2.5   | Statistical analysis                                    | 103 |
| 6.3       | Result  | S   | 104 |
|           | 6.3.1   | Exercise  | 104 |
|           | 6.3.2   | Whole body sweat collection                             | 105 |
|           | 6.3.3   | Regional sweat collection                               | 106 |
|           | 6.3.4   | Sweat composition at regional collection sites          | 107 |
|           | 6.3.5   | Relationship between sweat rate and sweat composition   | 108 |
|           | 6.3.6   | Relationship between regional & whole body washdown     |     |
|           |         | techniques  | 109 |
|           |         |   |     |

|           | 6.3.7   | Dietary intake  | 109 |
|-----------|---------|---|-----|
|           | 6.3.8   | Hydration status and urine variables                      | 111 |
| 6.4       | Discus  | ssion   | 111 |
|           |         |   |     |
| Chapter 7 | The ef  | fect of two exercise sessions on electrolyte balance      | 118 |
| 7.1       | Introdu | uction  | 119 |
| 7.2       | Metho   | d   | 120 |
|           | 7.2.1   | Subjects  | 120 |
|           | 7.2.2   | Experimental protocol                                     | 120 |
|           | 7.2.3   | Dietary monitoring  | 121 |
|           | 7.2.4   | Urine collection  | 121 |
|           | 7.2.5   | Physical activity   | 121 |
|           | 7.2.6   | Sample analysis   | 122 |
|           | 7.2.7   | Statistical analysis                                      | 122 |
| 7.3       | Result  | S   | 123 |
|           | 7.3.1   | Exercise  | 123 |
|           | 7.3.2   | Relationship between sweat rate and sweat composition     | 123 |
|           | 7.3.3   | Hydration status  | 123 |
|           | 7.3.4   | Body mass   | 124 |
|           | 7.3.5   | Duration of urine collection                              | 124 |
|           | 7.3.6   | Completeness of urine collection                          | 124 |
|           | 7.3.7   | Dietary intake and urine excretion                        | 125 |
|           | 7.3.8   | Electrolyte balance                                       | 129 |
|           | 7.3.9   | Relationship between electrolyte intake and electrolyte   |     |
|           |         | excretion   | 130 |
|           | 7.3.10  | Relationship between energy intake and electrolyte intake | 130 |
|           | 7.3.11  | Relationship between electrolyte intake and sweat         |     |
|           |         | electrolyte loss  | 131 |
| 7.4       | Discus  | ssion   | 131 |
|           | 7.4.1   | Sodium  | 132 |
|           | 7.4.2   | Potassium   | 134 |
|           | 7.4.3   | Hydration status  | 135 |
|           | 7.4.4   | Conclusion  | 136 |

| Chapter 8   | The effects of two commercially available drinks in |     |
|-------------|---|-----|
|             | restoring fluid balance and on subsequent exercise  |     |
|             | performance in the heat                             | 137 |
| 8.1         | Introduction  | 138 |
| 8.2         | Method  | 140 |
|             | 8.2.1 Subjects                                      | 140 |
|             | 8.2.2 Preliminary trials                            | 140 |
|             | 8.2.3 Pre-trial standardisation                     | 141 |
|             | 8.2.3 Experimental protocol                         | 141 |
|             | 8.2.4 Sample analysis                               | 144 |
|             | 8.2.5 Statistical analysis                          | 144 |
| 8.3         | Results   | 144 |
|             | 8.3.1 Pre-exercise hydration status                 | 144 |
|             | 8.3.2 Urine volume                                  | 145 |
|             | 8.3.3 Percentage of drink retained                  | 146 |
|             | 8.3.4 Urine osmolality                              | 147 |
|             | 8.3.5 Net fluid balance                             | 148 |
|             | 8.3.6 Sweat composition                             | 149 |
|             | 8.3.7 Electrolyte balance                           | 149 |
|             | 8.3.8 Serum electrolyte concentration               | 152 |
|             | 8.3.9 Serum osmolality                              | 153 |
|             | 8.3.10 Plasma volume                                | 154 |
|             | 8.3.11 Blood glucose                                | 155 |
|             | 8.3.12 Subjective feelings and drink palatability   | 155 |
|             | 8.3.13 Exercise capacity test                       | 157 |
| 8.4         | Discussion  | 159 |
| Chanter 0   | Conoral discussion                                  | 166 |
| 9 1         |   | 167 |
| 9.1         | Dietary sodium intake                               | 168 |
| 9.2         | Effect of evercise on urine sodium everation        | 100 |
| 9.3<br>0.4  | Effect of everyise on dietary sodium intake         | 171 |
| 9.4<br>0.5  | Effect of everyise on sodium balance                | 172 |
| 9.3.<br>0.6 | Effect of everying on potentium balance             | 174 |
| 9.0         | Effect of exercise on potassium balance             | 1/4 |

| 9.7        | Effect of exer | cise on hydration status           | 176 |
|------------|----------------|------------------------------------|-----|
| 9.8        | Conclusions    |                                    | 178 |
|            |                |                                    |     |
| References |                |                                    | 181 |
|            |                |                                    |     |
| Appendices |                |                                    |     |
|            | Appendix A     | Subjective Feelings Questionnaire  | 218 |
|            | Appendix B     | Subjective Drink Questionnaire     | 220 |
|            | Appendix C     | Rating of Perceived Exertion Scale | 222 |
|            | Appendix D     | Rating of Thermal Sensation Scale  | 224 |

## List of tables

- Table 1.1Distribution of fluid between body compartments of a 70kg male.<br/>(Source: Institute of Medicine, 2004).
- Table 1.2Sweat, plasma and intracellular water electrolyte concentrations (mmol/L)<br/>(Source: Maughan & Nadel, 2000).
- Table 1.3Sweat sodium and potassium losses during various sports.
- Table 2.1Mean, standard deviation and coefficient of variation of duplicate samples<br/>using the analytical procedures administered during experimental chapters<br/>in this thesis.
- Table 3.1Physical characteristics of individuals.Values are mean  $\pm$  SD. \*denotes<br/>significant difference between males and females (P<0.05).</th>
- Table 3.2The duration of each day's urine collection. Values are median (range).
- Table 3.3 The within-individual CV (%) in urine excretion for day 1 (from trial SPIKED and trial CONTROL), day 5 (from trial SPIKED and trial CONTROL), trial SPIKED (day 1 and 5), trial CONTROL (day 1 and 5) and over all days.
- Table 3.4The relationship of urine volume or urine electrolyte excretion between<br/>each 24h period. Values are correlation coefficients. \*denotes significant<br/>relationship (P<0.05).</th>
- Table 3.5The relationship between urine volume and urine electrolyte excretion.<br/>Values are correlation coefficients. \*denotes significant relationship<br/>(P<0.05).</th>
- Table 3.6The relationship between urine sodium and urine potassium excretion.<br/>Values are correlation coefficients. \*denotes significant relationship<br/>(P<0.05).</th>
- Table 4.1BM on the first (day 1) and last day (day 6) of the study period. Values are<br/>median (range). \* denotes significantly different from day 1 (P<0.05).</th>
- Table 4.2The duration of each day's urine collection. Values are median (range).
- Table 4.3 Urine creatinine excretion during each 24h period. Values are mean  $\pm$  SD.
- Table 4.4Urine volume during each 24h collection period and the average over all<br/>days (5 Day Average) and days 2, 3, 4 and 5 (4 Day Average). Values are<br/>mean  $\pm$  SD.
- Table 4.5Dietary nutrient intake during each 24h period and the average over all days<br/>(5 Day Average) and days 2, 3, 4 and 5 (4 Day Average). Values are mean<br/> $\pm$  SD.

| Table 4.6 | The within-individual CV (%) in nutrient intake and urine electrolyte excretion over all experimental days (5 Day), days 2, 3, 4 & 5 (4 Day), days 3, 4 & 5 (3 Day) and days 4 & 5 (2 Day)   |
|-----------|--|
| Table 4.7 | Urine Osmolality (mosmol/kg) of the first void from each day. Values are mean $\pm$ SD.  |
| Table 4.8 | Correlation coefficients between electrolytes excreted in the urine. *denotes significant relationship (P<0.05).   |
| Table 4.9 | Correlation coefficients between urine volume and urine electrolytes during each 24h period and the average over all days and 4 days (days 2, 3, 4, 5).  |
| Table 5.1 | Estimated whole-body sweat electrolyte concentrations ([electrolyte]) and total electrolyte losses during exercise.  |
| Table 5.2 | Sweat electrolyte concentrations ([electrolyte]) at each regional collection site. <sup>a</sup> denotes significantly different to the forearm, <sup>b</sup> denotes significantly different from the thigh.   |
| Table 5.3 | The relationship between whole body sweat rate and regional sweat composition at each collection site and estimated whole body (WB) sweat composition. Values are correlation coefficients.  |
| Table 5.4 | Osmolality of the first void of each day. Values are mean $\pm$ SD and (range).  |
| Table 5.5 | Duration of each day's urine collection. Values are median (range).  |
| Table 5.6 | Dietary nutrient intake during each 24h period and the average over all days (5 Day Average) and days 1, 2 and 3 (3 Day Average). Values are mean $\pm$ SD. * denotes significantly different from day 4.  |
| Table 5.7 | Urine volume during each 24h collection period and the average over all days (5 Day Average) and days 1, 2 and 3 (3 Day Average). Values are mean $\pm$ SD.  |
| Table 5.8 | The CV (%) in nutrient intake and urine excretion over all experimental days (All Days) and days 1, 2 and 3 (3 Days).  |
| Table 5.9 | The relationship between dietary water intake and urine volume, dietary sodium intake and urine sodium, dietary potassium intake and urine potassium and dietary chloride intake and urine chloride excretion in each 24h period, the 3 Day Average (days 1, 2, 3) and the 5 day Average (all experimental days). Values are correlation coefficients. *denotes significant relationship (P<0.05). |

Table 5.10The relationship between dietary energy intake (kcal) and dietary electrolyte<br/>intake (mmol) during each 24h period, the 3 Day Average (days 1, 2, 3) and<br/>5 Day Average (days 1, 2, 3, 4, 5). Values are correlation coefficients.<br/>\*denotes a significant relationship (P<0.05).</th>

- Table 5.11 The relationship between dietary electrolyte intake (mmol) and estimated whole body sweat electrolyte loss (mmol). Dietary values are the means of days 1, 2 and 3 (3 Day Average), day 3 and day 4. Values are correlation coefficients.
- Table 6.1Ambient temperature and humidity inside (Bag) and outside (Room) of the<br/>large polyethylene bag and the workload during each exercise trial
- Table 6.2Sweat loss and % dehydration during exercise.
- Table 6.3Sweat electrolyte composition from the regional sweat collection technique<br/>and whole body washdown technique. Values are mean  $\pm$  SD and median<br/>(range). \* denotes significant difference between techniques.
- Table 6.4Energy, carbohydrate, protein, fat and electrolyte (sodium, potassium,<br/>chloride) content of breakfast, lunch and the total consumed during the<br/>study period.
- Table 6.5Pre-exercise urine osmolality and body mass. Values are mean  $\pm$  SD or<br/>median (range).
- Table 7.1Whole body sweat loss, sweat electrolyte concentrations ([electrolyte]) and<br/>total electrolyte losses during AM and PM and cumulative losses for both<br/>exercise sessions (Total).
- Table 7.2 Osmolality of the first void of each day. Values are mean  $\pm$  SD and (range).
- Table 7.3Duration of each day's urine collection. Values are median (range).
- Table 7.4Urine creatinine excretion (mg) during each 24h period. Values are mean ±<br/>SD.
- Table 7.5Urine volume during each 24h collection period and the average over all<br/>days (5 Day Average) and days 1, 2 and 3 (3 Day Average). Values are<br/>mean  $\pm$  SD.
- Table 7.6Dietary nutrient intake during each 24h period and the average over all days<br/>(5 Day Average) and days 1, 2 and 3 (3 Day Average). Values are mean ±<br/>SD. \* denotes significantly different from day 4.
- Table 7.7The CV (%) in nutrient intake and urine excretion over all experimental<br/>days (All Days) and days 1, 2 and 3 (3 Days).
- Table 7.8 The relationship between dietary water intake and urine volume and dietary electrolyte intake and urine electrolyte excretion in each 24h period, the 3 day average (days 1, 2, 3) and the 5 day average (all 5 days). Values are correlation coefficients. \*denotes significant relationship (P<0.05).
- Table 7.9 The correlation coefficients between dietary energy intake (kcal) and dietary electrolyte intake (mmol) during each 24h period, the 3 Day Average (days 1, 2, 3) and 5 Day Average (days 1, 2, 3, 4, 5). \*denotes significant relationship (P<0.05).

- Table 7.10The relationship between dietary electrolyte intake (mmol) and whole body<br/>sweat electrolyte loss (mmol). Dietary values are the means of days 1, 2<br/>and 3 (3 Day Average), day 3 and day 4. Values are correlation<br/>coefficients.
- Table 8.1Composition of test drinks. Electrolyte concentrations and osmolality are<br/>expressed as mean  $\pm$  SD. \* denotes values obtained from drink labels
- Table 8.2Pre-exercise body mass (kg), urine and serum osmolality (mosmol/kg).<br/>Values are mean  $\pm$  SD.
- Table 8.3Cumulative urine volume (mL) produced during the recovery period. Pre<br/>and post-exercise time points have been omitted from calculations. Values<br/>are mean  $\pm$  SD. <sup>a</sup> denotes trial CHO-E significantly different from trial M<br/>(P<0.05).</th>
- Table 8.4Percentage of drink retained (%). Values are mean  $\pm$  SD. <sup>a</sup> denotes trial<br/>CHO-E significantly different from trial M (P<0.05).</th>
- Table 8.5Total sweat electrolyte losses (mmol) and electrolyte intake (mmol) from<br/>test drinks. Values are mean  $\pm$  SD. <sup>a</sup> denotes trial M significantly different<br/>from trial CHO-E (P<0.05).</th>
- Table 8.6Cumulative urinary electrolyte excretion (mmol). Pre and post-exercise time<br/>points have been omitted from calculations. Values are mean  $\pm$  SD. <sup>a</sup><br/>denotes trial CHO-E significantly different from trial M (P<0.05).</th>
- Table 8.7Serum electrolyte concentrations (mmol/L) over the duration of the<br/>experiment. Values are mean  $\pm$  SD. <sup>a</sup> denotes trial CHO-E significantly<br/>different from trial M (P<0.05), <sup>b</sup> denotes trial CHO-E significantly<br/>different from pre-exercise value (P <0.05), <sup>c</sup> denotes trial M significantly<br/>different from pre-exercise value (P <0.05).</th>
- Table 8.8Heart rate (HR) (bpm), mean skin temperature ( $T_{sk}$ ; °C), rectal temperature<br/>( $T_{re}$ ; °C), thermal sensation (TS) and rating of perceived exertion (RPE)<br/>during exercise to exhaustion. <sup>a</sup> denotes trial CHO-E significantly different<br/>from trial M (P<0.05).</th>

### List of figures

- Figure 3.1 Schematic of the study period. 24h urine collection (U), first void of urine (AM) and creatine ingestion (Cr). Each 24h urine collection started the morning of one day and was terminated the morning on the following day, to account for the lag in electrolyte excretion.
- Figure 3.2 Urine Volume (A), sodium (C) and potassium (E) excreted during each 24h collection period. Gender comparisons are shown for urine volume (B), sodium (D) and potassium (F) during each 24h collection period. \*denotes significant difference between males and females (P<0.05).
- Figure 4.1 Schematic of the study period. Body mass (BM), dietary (D) and urine (U) collections. Each 24h urine collection started on the morning of one day and was terminated on the morning of the following day.
- Figure 4.2 Dietary intake and urine excretion of sodium (A), potassium (B) and chloride (C) during each 24h period. All Values are mean ± SD.
- Figure 4.3 The relationship between (A) dietary sodium intake and urine sodium excretion, (B) dietary potassium intake and urine potassium excretion, (C) dietary chloride intake and urine chloride excretion. Values represent the average electrolyte intake and excretion over all 5 experimental days.
- Figure 4.4 Sodium, potassium and chloride balance during the 5-day period. Values are calculated from dietary and urinary analysis.
- Figure 4.5 Variation in sodium balance from the mean sodium balance of individuals calculated from days 2, 3, 4 and 5. (A) Subject 1, (B) Subject 2, (C) Subject 3, (D) Subject 4, (E) Subject 5, (F) Subject 6, (G) Subject 7, (H) Subject 8.
- Figure 4.6 Variation in potassium balance from the mean potassium balance of individuals calculated from days 2, 3, 4 and 5. (A) Subject 1, (B) Subject 2, (C) Subject 3, (D) Subject 4, (E) Subject 5, (F) Subject 6, (G) Subject 7, (H) Subject 8.
- Figure 4.7 Variation in chloride balance from the mean chloride balance of individuals calculated from days 2, 3, 4 and 5. (A) Subject 1, (B) Subject 2, (C) Subject 3, (D) Subject 4, (E) Subject 5, (F) Subject 6, (G) Subject 7, (H) Subject 8.
- Figure 4.8 The relationship between (A) oscillations in sodium excretion and sodium intake; (B) oscillations in potassium balance and potassium intake; (C) oscillations in chloride balance and chloride intake. Electrolyte intake is based on the 5-Day average and oscillations in electrolyte excretion on the 4-Day average.
- Figure 4.9 The relationship between potassium intake and (A) oscillations in sodium excretion, (B) oscillations in chloride excretion and (C) between sodium intake and oscillations in potassium excretion. Electrolyte intake is based on the 5-Day average and oscillations in electrolyte balance are based on the 4-Day average.

- Figure 5.1 Schematic of the study period. Body mass (BM), dietary collection period (D), 24h urine collection (U) and exercise (Ex).
- Figure 5.2 Dietary intake and urine excretion of sodium (A), potassium (B) and chloride (C) during each 24h period. All Values are mean  $\pm$  SD. \* denotes significant difference between dietary intake and urine excretion.
- Figure 5.3 Sodium (A), potassium (B) and chloride (C) balance over each 24h period. Values are calculated from urinary electrolyte excretion, dietary electrolyte intake and sweat electrolyte loss. Values are mean  $\pm$  SD.
- Figure 6.1 Schematic of schedule for both (A) SINGLE and (B) MULTIPLE exercise sessions. Body mass (BM), urine collection (U), breakfast (B), lunch (L) and whole body washdown procedure (WBW).
- Figure 6.2 Sweat electrolyte composition (A) and total sweat electrolyte losses (B) obtained from whole body washdown method. <sup>a</sup> denotes significantly different from trial MULTI-AM, <sup>b</sup> denotes significantly different from MULTI-PM.
- Figure 6.3 Sweat electrolyte composition (A) and total sweat electrolyte losses (B) obtained from regional collection methods. <sup>a</sup> denotes significantly different from trial MULTI-AM.
- Figure 6.4 Regional (back, chest, forearm and thigh) sweat electrolyte concentrations. <sup>a</sup> denotes significantly different from the back, <sup>b</sup> denotes significantly different from the chest, <sup>c</sup> denotes significantly different from the arm, <sup>d</sup> denotes significantly different from the thigh (P<0.05).
- Figure 6.5 The relationship between whole body sweat rate and sweat electrolyte concentrations obtained from the regional (A, C, E) and whole body (B, D, F) sweat collection techniques.
- Figure 6.6 Fluid intakes during the morning, afternoon and entire study period. \* denotes significant difference between trials (P<0.05).
- Figure 7.1 Schematic of the study period. Body mass (BM), dietary collection period (D), 24h urine collection (U) and whole body washdown procedure (WBW). Each 24h urine collection started the morning of one day and was terminated on the morning the following day.
- Figure 7.2 Dietary intake and urine excretion of sodium (A), potassium (B) and chloride (C) during each 24h period. All Values are mean ± SD. \* denotes significant difference between dietary intake and urine excretion. <sup>a</sup> denotes urine electrolyte excretion significantly different from day 1, <sup>b</sup> denotes urine electrolyte excretion significantly different from day 3.
- Figure 7.3 Sodium (A), Potassium (B) and Chloride (C) balance over each 24h period. Values are calculated from urinary excretion, dietary intake and sweat loss. Values are mean  $\pm$  SD. <sup>a</sup> denotes significantly different from day 1 (P<0.05)

- Figure 8.1 Schematic of the study period. Drink consumption (D), blood (B), urine (U), questionnaire (Q) and expired air (GAS) collection and body mass (BM), ratings of perceived exertion (RPE), thermal comfort (TC), heart rate (HR), skin temperature ( $T_{sk}$ ) and rectal temperature ( $T_{re}$ ) measurements.
- Figure 8.2 Urine volume (mL) over the duration of the experiment. The pre-exercise sample has been omitted. Points are mean  $\pm$  SD. <sup>b</sup> denotes trial CHO-E significantly different (P<0.05) from post-exercise. <sup>c</sup> denotes trial M significantly different (P<0.05) from post-exercise.
- Figure 8.3 Urine osmolality over the duration of the experiment. <sup>a</sup> denotes CHO-E significantly different from M (P<0.05). <sup>c</sup> denotes trial M significantly different (P<0.05) from pre-exercise.
- Figure 8.4 Whole body net fluid balance over the duration of the experiment. <sup>a</sup> denotes CHO-E significantly different from M (P<0.05). <sup>b</sup> denotes trial CHO-E significantly different (P <0.05) from pre-exercise. <sup>c</sup> denotes trial M significantly different (P <0.05) from pre-exercise.
- Figure 8.5 Whole body net sodium (A), potassium (B) and chloride (C) balance over the duration of the experiment. <sup>a</sup> denotes trial M significantly different from trial CHO-E (P <0.05),<sup>b</sup> denotes trial CHO-E significantly different from pre-exercise (P <0.05),<sup>c</sup> denotes trial M significantly different from pre-exercise (P <0.05).
- Figure 8.6 Serum osmolality (mosmol/kg) over the duration of the experiment. Values are mean  $\pm$  SD. <sup>a</sup> denotes trial CHO-E significantly different from trial M (P<0.05), <sup>b</sup> denotes trial CHO-E significantly different from pre-exercise value (P <0.05), <sup>c</sup> denotes trial M significantly different from pre-exercise value (P <0.05).
- Figure 8.7 Changes in plasma volume over the duration of the experiment relative to the post-exercise time point. Values are mean  $\pm$  SD. <sup>b</sup> denotes trial CHO-E significantly different from post-exercise value (P <0.05), <sup>c</sup> denotes trial M significantly different from post-exercise value (P <0.05).
- Figure 8.8 Blood glucose (mmol/L) over the duration of the experiment. Values are mean  $\pm$  SD. <sup>a</sup> denotes trial CHO-E significantly different from trial M (P<0.05), <sup>b</sup> denotes trial CHO-E significantly different from pre-exercise (P <0.05), <sup>c</sup> denotes trial M significantly different from pre-exercise (P <0.05).
- Figure 8.9 Perceived drink characteristics (A). Values are mean  $\pm$  SD or median where appropriate. <sup>a</sup> denotes CHO-E significantly different from M (P<0.05). Subjective feelings of thirst (B), fullness (C), bloatedness (D), hunger (E), mouth feel (F), tiredness (G), alertness (H), ability to concentrate (I) and headache (J). Values are mean  $\pm$  SD. <sup>a</sup> denotes CHO-E significantly different from M (P<0.05).
- Figure 8.10 Exercise time to exhaustion for each individual on trial CHO-E and trial M.

Figure 9.1 The percentage contribution of food types to average daily sodium intake (Henderson et al, 2003).

# List of abbreviations

| []                   | concentration                               |
|----------------------|---|
| ANF                  | Atrial natriuretic factor                   |
| ANOVA                | analysis of variance                        |
| bpm                  | beats per minute                            |
| BM                   | body mass                                   |
| BMI                  | body mass index                             |
| °C                   | degrees Celsius                             |
| СНО-Е                | carbohydrate-electrolyte                    |
| Cl                   | chloride                                    |
| CV                   | coefficient of variation                    |
| d                    | day   |
| g                    | grams                                       |
| h                    | hour  |
| HR                   | heart rate                                  |
| $K^+$                | potassium                                   |
| K <sub>2</sub> EDTA  | potassium ethylenediamine tetra acetic acid |
| kg                   | kilogram                                    |
| L                    | litre                                       |
| min                  | minute                                      |
| mL                   | millilitre                                  |
| mmol                 | millimole                                   |
| mmol/L               | millimole per litre                         |
| mosmol/kg            | milliosmole per kilogram                    |
| Na <sup>+</sup>      | sodium                                      |
| RPE                  | rating of perceived exertion                |
| RH                   | relative humidity                           |
| RER                  | respiratory exchange ratio                  |
| rpm                  | revolutions per minute                      |
| SD                   | standard deviation                          |
| VO <sub>2</sub> peak | peak oxygen uptake                          |
| VO <sub>2</sub> max  | maximal oxygen uptake                       |
| W                    | watts                                       |
| У                    | years                                       |

# Chapter 1

# General introduction

## 1.1 Sodium in the human body - Overview

The adult human body (70kg) contains approximately 4000mmol of sodium (Forbes & Lewis, 1956), but will vary between individuals due to differences in body composition. The analytical method employed will also affect the values obtained as isotopic dilution methods underestimate total body sodium levels (Forbes & Perly, 1951) compared to those obtained using dissection techniques. Nevertheless, total body sodium remains relatively constant throughout adult life (Ellis et al, 1976). Sodium is primarily located in the extracellular fluid compartment and in bone (Edelman et al, 1954). The sodium content of bone is reported to be ~1500-2000mmol for a 70kg adult (Edelman et al, 1954). Approximately 45% of bone sodium is exchangeable (Edelman et al, 1954), with the remaining 55% forming a non-exchangeable sodium pool. In total approximately 70% (~2870mmol) of the sodium within the body is exchangeable (Hubbard et al, 1990).

Sodium plays an integral part in the determination of the membrane potential of cells and the active transport of substances across cell membranes but as the predominant cation in the extracellular fluid compartment it plays a pertinent role in fluid balance and this will be the focus of the present thesis.

# **1.2** Body water

Water accounts for between 50–70% of an individual's body mass (BM). This is largely dictated by an individual's body composition as muscle comprises 70-80% water and adipose tissue approximately 10% water (ACSM, 2007). For the average 70kg male, total body water is approximately 42 litres (L) (Sawka et al, 2005; Institute of Medicine, 2004). Body water is distributed between the intracellular and extracellular fluid compartments, which account for about 65% (28L) and 35% (14L) of total body water, respectively. The extracellular fluid compartment is further sub-divided into interstitial fluid (11L) and plasma (3L) (Table 1.1; Institute of Medicine, 2004).

**Table 1.1**Distribution of fluid between body compartments of a 70kg male.<br/>(Source: Institute of Medicine, 2004).

| Fluid Compartment               | Volume (litres) |
|---------------------------------|-----------------|
| Total Body Water                | 42              |
| Intracellular Fluid Compartment | 28              |
| Extracellular Fluid Compartment | 14              |
| Of which Interstitial Fluid     | 11              |
| Of which Plasma                 | 3               |

Despite the large quantities of water present within the human body, relatively small deficits (~2% BM) in fluid balance have been reported to affect health, cognition and athletic performance (Institute of Medicine, 2004; ACSM, 2007). Therefore the daily variation in body water is tightly regulated, fluctuating at rest by approximately 0.22% in temperate and 0.48% in warm environments (Sawka et al, 2005). Euhydration is defined as the normal body water content which takes into account these daily fluctuations. Hyperhydration and hypohydration refer to a body water content above or below normal body water content, respectively (ACSM, 2007).

Water is lost from the body via respiration, the skin (insensible cutaneous losses and sweating), in tears, urine and in faeces. The water lost during respiration is due to the humidification of the inspired air and consequently the magnitude of water loss is influenced by environmental temperature, humidity and by ventilation volume. This avenue of water loss typically accounts for 320mL/day (Maughan & Nadel, 2000). Insensible cutaneous water loss is also influenced by the humidity and temperature of the environment, but is typically associated with a water loss of approximately 530mL/day. The loss of water in faeces is small (eg 100mL/day), but the loss of water in urine (eg 1400mL/day) is usually much greater than other avenues of water loss. As will be discussed later, sweat losses are highly variable both between and within individuals as sweat losses are dictated by environmental conditions, the clothing worn, the duration and intensity of exercise and many host factors. Sweat rates typically range between 0.29litres/hour (L/h) and 2.60L/h during exercise, (ACSM, 2007) but for some individuals, sweat rates can exceed 3L/h. Sweating can therefore also represent a large avenue of water loss and poses a great threat to the maintenance of fluid balance. To balance these losses, water is replenished through food and fluid intake and as a by-product of metabolism. The amount of water available as a by-product of metabolism is similar in magnitude to that lost during respiration (eg ~300mL). Water intake through drinking is influenced by a multitude of factors including drink temperature, flavour, texture, availability, social factors and by thirst (Passe, 2001).

#### **1.3 Regulation of fluid balance**

Thirst is an adequate stimulator of fluid intake under resting conditions as fluid balance is maintained from day to day by the consumption of normal food and fluid intake (Greenleaf, 1992; Casa et al, 2000). But, thirst is not a sensitive indicator of hydration

status during exercise as individuals allowed ad libitum access to fluids typically do not replace all the sweat losses incurred during exercise (Greenleaf, 1992). However, some individuals can also overdrink (Hew-Butler et al, 2008). Thirst is stimulated by hypovolaemia and hyperosmolality due to the resultant increase in the circulating hormones, vasopressin, aldosterone and angiotensin II (Greenleaf, 1992). Changes in plasma volume and plasma osmolality are also implicated in the control of urine production.

Plasma osmolality is maintained within a narrow range (280-292 mosmol/kg) by osmoreceptors located in the supra-optic and paraventricular areas of the anterior hypothalamus (Lote, 1994). A small increase in plasma osmolality will stimulate the release of vasopressin from the posterior pituitary gland into the circulation. For every 1mosmol/kg rise in plasma osmolality there is a 0.41pmol/L increase in plasma vasopressin concentration, provided plasma osmolality is above the threshold for vasopressin release (~285mosmol/kg) (Baylis, 1987). Vasopressin increases the permeability of the collecting ducts in the kidney, allowing water to be reabsorbed which results in the formation of a more concentrated urine. Vasopressin secretion is also stimulated by a decrease in plasma vasopressin concentration is seen (Baylis, 1987).

The renin-angiotensin system can also influence fluid and electrolyte balance. Renin is an enzyme that is synthesised and stored in the juxtaglomerular apparatus of the kidney (Lote, 1994). It is released into the circulation when the body sodium content and consequently blood volume decline. In response to a fall in blood volume, baroreceptors trigger an increased sympathetic nerve activity to the arterioles of the kidney which stimulates the release of renin from granular cells. A decrease in renal perfusion pressure and a decline in the delivery of sodium to the macula densa can further stimulate the release of renin (Lote, 1994). Subsequently renin acts on the protein, angiotensinogen, causing a cascade effect and conversion to Angiotensin II. Angiotensin II has a number of actions including the stimulation of thirst, vasopressin release, increased proximal tubular sodium reabsorption, vasoconstriction and the release of the hormone aldosterone from the zona glomerulosa of the adrenal cortex. Aldosterone increases distal tubular sodium reabsorption and through osmosis can increase the reabsorption of water when in the

presence of vasopressin. In this way the body can respond to situations of fluid and electrolyte loss.

There are also systems that respond to fluid excess. Atrial natriuretic factor (ANF) is a hormone that is released by cells of the heart atria (cardiocytes) in response to a stretching of the atrial walls caused by elevated blood pressure and blood volume (Lote, 1994). ANF inhibits sodium reabsorption, inhibits the secretion of aldosterone and renin and causes vasodilation of afferent arterioles which increases glomerular filtration rate. This results in the excretion of water and sodium.

#### **1.4** Dietary electrolyte intake

Dietary surveys report a wide range of sodium intakes (Gregory et al, 1990; Henderson et al, 2003). In a recent UK-based survey (Henderson et al, 2003), the average dietary sodium and potassium intakes according to a 7-day weighed food diary were  $144 \pm 44$ mmol and  $86 \pm 17$ mmol for males and  $100 \pm 30$ mmol and  $68 \pm 19$ mmol for females. There are several methods of assessing sodium intake in addition to weighed food records which include: food diaries, 24h recalls, food frequency questionnaires, duplicate portion analysis and urine collections. Each method is associated with problems (Bingham, 1987), but most notable is the inability to accurately assess the discretionary salt use of an individual (Caggiula et al, 1985; Clark & Mossholder, 1986; Henderson et al, 2003). Although 73% of individuals added salt at the table or during cooking (Henderson et al, 2003), discretionary salt typically contributes 15-20% to total sodium intake. The primary contributor to sodium intake is manufactured or processed foods (65-70%) with the remainder (15%) being found naturally in some food items (SACN, 2003).

As the amount of ingested sodium above basal requirements is excreted primarily in the urine, 24h urinary sodium excretion can provide a good estimate of sodium intake in nonsweating individuals (Holbrook et al, 1984; Taseveska et al, 2006). Although potassium losses in faeces are greater than sodium, amounting to between 5–15mmol/day, urine potassium excretion can also provide a good estimate of potassium intake (Holbrook et al, 1984; Taseveska et al, 2006). According to the collection of a single 24h urine sample, sodium and potassium intakes of the British population were  $187 \pm 86$ mmol and  $81 \pm$ 33mmol for males and  $138 \pm 66$ mmol and  $67 \pm 30$ mmol for females (Henderson et al, 2003). The discrepancy between weighed food intakes and urine collections was attributed to the discretionary salt intake of individuals. Whilst urine electrolyte excretion can provide an objective measure of dietary electrolyte intake, one-off 24h urine collections have been criticised by some (Sowers & Stumbo, 1986; Liu & Stamler, 1984; Caggiula et al, 1985; Dyer et al, 1997) although not all (Kesteloot & Joossens, 1990), as a measure of an individual's habitual intake due to the large day to day variation in electrolyte excretion.

#### **1.5 Body electrolyte losses**

Whilst urine provides the primary avenue for sodium loss in many individuals, other avenues of water loss are also generally associated with the loss of electrolytes, although in most cases this is minimal. The loss of water during respiration is accompanied by no significant loss of electrolytes (Comar & Bronner, 1960) and faecal sodium losses amount to only between 0.8 – 8.2mmol/d (Cummings et al, 1976; Dole et al, 1950; Arn & Reimer, 1950). Electrolytes can also be lost via the skin, but insensible losses of sodium (0.09 to 2.59mmol/day), potassium (0.08 to 2.69mmol/day) and chloride (0.29 to 1.71mmol/day) are small (Dahl et al, 1955). In contrast, the loss of water and minerals through sweating can be large in some situations or in some individuals.

Typically during exercise there is some increase in core body temperature. Whilst this may be beneficial for reducing muscle stiffness and the viscosity of blood and synovial fluid (Bishop, 2003), at high temperatures there are well documented detrimental effects to health and performance (Gonzalez-Alonso et al, 1999; Nielsen et al, 1993). Sweating is the normal physiological response to an elevation in body temperature and acts in concert with other mechanisms of heat loss to help maintain homeostasis. As a consequence of sweating, both water and minerals are lost in sweat. Sweat contains many components including sodium, potassium, calcium, magnesium, chloride, bicarbonate, phosphate and sulphate (Table 1.2).

|             | •         |           |                     |
|-------------|-----------|-----------|---------------------|
|             | Sweat     | Plasma    | Intracellular Water |
|             | (mmol/L)  | (mmol/L)  | (mmol/L)            |
| Sodium      | 20 - 80   | 130 - 155 | 10                  |
| Potassium   | 4 - 8     | 3.2 - 5.5 | 150                 |
| Chloride    | 20 - 60   | 96 - 110  | 8                   |
| Calcium     | 0 - 1     | 2.1 - 2.9 | 0                   |
| Magnesium   | < 0.2     | 0.7 - 1.5 | 15                  |
| Bicarbonate | 0 - 35    | 23 - 28   | 10                  |
| Phosphate   | 0.1 - 0.2 | 0.7 - 1.6 | 65                  |
| Sulphate    | 0.1 - 2.0 | 0.3 - 0.9 | 10                  |

Table 1.2Sweat, plasma and intracellular water electrolyte concentrations (mmol/L)<br/>(Source: Maughan & Nadel, 2000).

#### **1.6** Sweat gland structure and function

Three distinguishable types of sweat gland have been identified in humans; the apocrine, apoeccrine and eccrine sweat glands. Apocrine glands produce a milky, protein-rich fluid only intermittently which is considered un-important to fluid and electrolyte balance (Sato et al, 1987). The apoeccrine glands vary in size between and within individuals but tend to be larger than eccrine glands when fully developed (Sato et al, 1987). Morphologically similar to the eccrine sweat gland, apoeccrine glands are capable of producing large amounts of clear fluid, which although more prolific than that from the eccrine sweat gland, is similar in composition (Sato & Sato, 1987). However, because apoeccrine glands are restricted to the axillae regions of the body, it is eccrine sweat glands that have the largest role in fluid and electrolyte excretion and thermoregulation (Sato et al, 1987). As a consequence, studies have focussed on eccrine sweat glands. Approximately 2-3million eccrine sweat glands are located over the body surface, which vary in density and size depending on the body region (Sato & Sato, 1983). The eccrine gland can be separated into two distinct sections, the secretory coil and the reabsorptive duct. Located in the dermis, the secretory coil is composed of 3 cell types (myoepithelial cells, clear or agranular cells and dark or granular cells). The clear cells exhibit high Na-K ATPase activity and mitochondrial density, suggesting they are the primary site of fluid and mineral excretion. The dark cells are involved in the secretion of glycoproteins (Yanagawa et al, 1986), but relatively little information is known regarding their other roles. Myoepithelial cells are located in the outer layer of the secretory coil and provide structural support to the secretory epithelium (Sato et al, 1979). The sweat duct is composed of 2 cell types; the luminal cells provide support for the duct and house both sodium and chloride channels which allow the basal ductal cells, which contain a large number of mitochondria, to carry out sodium reabsorption.

### **1.7** Sweat electrolyte losses in sport

Despite the reabsorption of sodium as it traverses through the sweat duct, sodium remains the predominant electrolyte present in sweat (Table 1.2). Table 1.3 shows the sweat and electrolyte losses of individuals participating in a range of sports. Maughan et al (2004) monitored sweat and electrolyte losses in 24 professional football players training for 90 minutes in an ambient temperature of 24-29°C. Players on average lost 2.03L of sweat and 99mmol (2.3g) of sodium. Shirreffs et al (2005) reported average sweat losses of 2.19L and sweat sodium losses of 67mmol (1.5g) during a 90 minute training session completed

in an ambient temperature of 32°C. This was the second of two exercise sessions that the professional footballers completed on the same day. Stofan et al (2005) determined sweat electrolyte losses of American Football players during two-a-day exercise sessions. Players performed exercise in full pads in an ambient temperature of between 22-30°C. Sweat losses during both the morning (2.5h) and evening (2.5h) practice were similar, amounting to approximately 7.94L and 452mmol (10.4g) of sodium for a group of individuals prone to muscle cramps for the entire day.

The loss of large volumes of sweat is not restricted solely to exercise in warm conditions. Several studies have shown sweat and electrolyte losses to be substantial even when exercise takes place in cool conditions (Maughan et al, 2005; 2007; Palmer & Spriet, 2008). Maughan et al (2005) reported the sweat loss of 17 professional footballers during a 90 minute training session in cool conditions (5°C, 81% RH). Players lost on average 1.69L of sweat and 73mmol (~1.7g) of sodium. Palmer & Spriet (2008) reported ice hockey players, training in full protective clothing to also lose substantial amounts of sweat (1.8L/h) and sodium (98mmol or 2.26g). This was largely attributed to the additional clothing worn (Palmer & Spriet, 2008; Maughan et al, 2005).

A consistent finding is the wide variation in sweat sodium losses between individuals. For some individuals training sessions that evoke large sweat losses may predispose them to an increased risk of muscle cramps (Stofan et al, 2005), hyponatraemia (Montain et al, 2006) or leave them in negative sodium balance (Godek et al, 2005).

| Study                  | Sport  | Conditions                      | Situation                | Method            | Sweat Loss   | Sodium Loss                                   | Potassium Loss                    |
|------------------------|--|---------------------------------|--------------------------|-------------------|--|---|-----------------------------------|
|                        |  |                                 | Football                 |                   |  |   |                                   |
| Maughan et al (2004)   | 24 Professional<br>Football Players              | 24-29°C                         | Training<br>(90 minutes) | Sweat Patches     | $2.03 \pm 0.41L$<br>(1.39 - 2.83L)                           | 99 ± 24mmol<br>(53 − 133mmol)                 | N/A                               |
| Maughan et al (2005)   | 17 Professional<br>Football Players              | $5.1 \pm 0.7^{\circ}$ C         | Training<br>(90 minutes) | Sweat Patches     | $1.69 \pm 0.45L$<br>(1.06 - 2.65L)                           | $73 \pm 31$ mmol (29 - 121 mmol)              | 7.1 ± 2.8mmol<br>(3.4 – 14.3mmol) |
| Shirreffs et al (2005) | 26 Professional<br>Football Players              | $32 \pm 3^{\circ}\mathrm{C}$    | Training<br>(90 minutes) | Sweat Patches     | $2.19 \pm 0.37L$<br>(1.67 - 3.14L)                           | $67 \pm 37$ mmol<br>( $26 - 129$ mmol)        | $8 \pm 2$ mmol<br>(5-12mmol)      |
| Maughan et al (2007)   | 20 Professional<br>Football Players              | D∘8-9                           | Match<br>(90 minutes)    | Sweat Patches     | $1.68 \pm 0.40L$<br>(0.82 - 2.27L)                           | $104 \pm 35$ mmol                             | $10 \pm 3$ mmol                   |
| Kilding et al (2009)   | 13 International<br>Football Players<br>(female) | $14.1 \pm 0.7^{\circ}C$         | Training<br>(90 minutes) | Sweat Patches     | $0.73 \pm 0.27L$   | $32 \pm 14$ mmol                              | $4.5 \pm 5.9$ mmol                |
| Kilding et al (2009)   | 13 International<br>Football Players<br>(Female) | $6.2 \pm 1.3^{\circ}\mathrm{C}$ | Training<br>(90 minutes) | Sweat Patches     | $0.66 \pm 0.27L$   | $35 \pm 13$ mmol                              | $3.5 \pm 1.6$ mmol                |
| Mao et al (2001)       | 13 Football<br>Players                           | 32-37°C                         | Training<br>(60 minutes) | Plastic Collector | N/A  | $82 \pm 62$ mmol<br>(5 - 255mmol)             | $6 \pm 1$ mmol ( $1 - 19$ mmol)   |
| Shirreffs & Maughan    | 92 Football                                      | J∘2C <sup>-</sup> 26            | Training                 | Sureat Datches    | $\frac{\text{Fasting}}{1.41 \pm 0.36L}$ $(0.88 - 2.69L)$     | <u>Fasting</u><br>29 ± 18mmol<br>(9 − 89mmol) | ₹/N                               |
| (2008)                 | Players  |                                 | (60-70 minutes)          |                   | $\frac{\text{Non-Fasting}}{1.61 \pm 0.51L}$ $(0.74 - 2.65L)$ | Non-Fasting<br>28 ± 16mmol<br>(7 - 78mmol)    |                                   |

Table 1.3

Sweat sodium and potassium losses during various sports.

| 1 Loss Potassium Loss | -          | lmmol N/A                       | <u>uing</u><br>pers<br>3mmol)  | ramp<br>(mmol)  | ramp<br>(muol)<br><u>ning</u><br>pers<br>formol)<br>ramp<br>ramp   | ramp<br>(mmol)<br>(ming<br>pers<br>formol)<br>ramp<br>7mmol)<br>7mmol)<br>33 ± 21 mmol | $\begin{array}{c} \mbox{ramp} \\ \mbox{(innol)} \\ \mbox{mod} \\ \mbox{finmol} \\ finmo$ | tramp<br>tramp<br>(inmol)<br>N/A<br>pers<br>formol)<br>formol) $TampTampTampersformol)33 \pm 21 mmol33 \pm 21 mmol33 \pm 21 mmol31 \pm 21 mmol31 \pm 21 mmol$   | ramp<br>(muol)<br>(inig<br>pers<br>formol)<br>ramp<br>framp<br>framp<br>frampers<br>frampers<br>frampers<br>frampers<br>frampers<br>frampers<br>frampers<br>frampers<br>frampers<br>frampers<br>frampers<br>frampers<br>frampers<br>frampers<br>frampers<br>frampers<br>frampers<br>frampers<br>frampers<br>frampers<br>frampers<br>frampers<br>frampers<br>frampers<br>frampers<br>frampers<br>frampers<br>frampers<br>frampers<br>frampers<br>frampers<br>frampers<br>frampers<br>frampers<br>frampers<br>frampers<br>frampers<br>frampers<br>frampers<br>frampers<br>frampers<br>frampers<br>frampers<br>frampers<br>frampers<br>frampers<br>frampers<br>frampers<br>frampers<br>frampers<br>frampers<br>frampers<br>frampers<br>frampers<br>frampers<br>frampers<br>frampers<br>frampers<br>frampers<br>frampers<br>frampers<br>frampers<br>frampers<br>frampers<br>frampers<br>frampers<br>frampers<br>frampers<br>frampers<br>frampers<br>frampers<br>frampers<br>frampers<br>frampers<br>frampers<br>frampers<br>frampers<br>frampers<br>frampers<br>frampers<br>frampers<br>frampers<br>frampers<br>frampers<br>frampers<br>frampers<br>frampers<br>frampers<br>frampers<br>frampers<br>frampers<br>frampers<br>frampers<br>frampers<br>frampers<br>frampers<br>frampers<br>frampers<br>frampers<br>frampers<br>frampers<br>frampers<br>frampers<br>frampers<br>frampers<br>frampers<br>frampers<br>frampers<br>frampers<br>frampers<br>frampers<br>frampers<br>frampers<br>frampers<br>frampers<br>frampers<br>frampers<br>frampers<br>frampers<br>frampers<br>frampers<br>frampers<br>frampers<br>frampers<br>frampers<br>frampers<br>frampers<br>frampers<br>frampers<br>frampers<br>frampers<br>frampers<br>frampers<br>frampers<br>frampers<br>frampers<br>frampers<br>frampers<br>frampers<br>frampers<br>frampers<br>frampers<br>frampers<br>frampers<br>frampers<br>frampers<br>frampers<br>frampers<br>frampers<br>frampers<br>frampers<br>frampers<br>frampers<br>frampers<br>frampers<br>frampers<br>frampers<br>frampers<br>frampers<br>frampers<br>frampers<br>frampers<br>frampers<br>frampers<br>frampers<br>frampers<br>frampers<br>frampers<br>frampers<br>frampers<br>frampers<br>frampers<br>frampers<br>frampers<br>frampers<br>frampers<br>frampers<br>frampers<br>frampers<br>frampers<br>frampers<br>frampers<br>frampers<br>frampers<br>frampers<br>frampers<br>frampers<br>frampers<br>frampers<br>frampers<br>frampers<br>frampers<br>frampers<br>frampers<br>frampers<br>frampers<br>frampers<br>frampers<br>frampers<br>frampers<br>frampers<br>frampers<br>frampers<br>frampers<br>frampers<br>frampers<br>frampers<br>frampers<br>frampers<br>frampers<br>frampers<br>frampers<br>frampers<br>frampers<br>frampers<br>frampers<br>frampers<br>frampers<br>frampers<br>frampers<br>frampers<br>frampers | tramp<br>tramp<br>(inmol)<br>N/A<br>pers<br>formol)<br>ramp $Tamp)Tamp)Tampol)Tampers33 \pm 21 \text{ mmol}33 \pm 21 \text{ mmol}Mon-crampers0  mmol1000  mmol24 \pm 6 \text{ mmol}N/AMax$  |
|-----------------------|------------|---------------------------------|--|---|--|--|---|---|--|---|
|                       |            | -5.2L 35 $-431m$                | $\begin{array}{ c c c c c c c c c c c c c c c c c c c$   | Evening<br>Evening<br>ers<br>Cramper<br>671 > 731 + 06m | $\left  \begin{array}{c} 1.21 \pm 2.011 \\ \text{mp} \\ \text{Mon-cran} \\ 1.35 \pm 87 \text{m} \\ 1.35 \pm 87 $ | $\begin{array}{c ccccccccccccccccccccccccccccccccccc$                                  | $\begin{array}{c ccccccccccccccccccccccccccccccccccc$   | $\begin{array}{c ccccccccccccccccccccccccccccccccccc$   | $\begin{array}{c ccccccccccccccccccccccccccccccccccc$  | $\begin{array}{c ccccccccccccccccccccccccccccccccccc$   |
| -                     |            | ag Range 1.3 –                  | $\begin{array}{c c} \hline \hline Mornin \\ \hline Crampe \\ \hline (3.79 \pm 1.5 \\ Non-crar \\ Non-crar \\ \hline (2.54 \pm 1.0 \\ \hline \end{array}$ | Crampe:<br>Crampe:                                      | (4.15 $\pm$ 0.6<br>Non-crar<br>(4.49 $\pm$ 1.4   | atch $(4.15 \pm 0.6$ $(4.49 \pm 1.4)$ $(4.49 \pm 1.4)$ $(2.9 \pm 1.6)$                 | $\begin{array}{c c} (4.15 \pm 0.6 \\ \text{Non-crar} \\ \text{Non-crar} \\ (4.49 \pm 1.4 \\ (4.49 \pm 1.6 \\ (4.49 \pm 1.6 \\ 2.9 \pm 1.6 \\ \text{only} \\ \text{only} \\ \hline 2.3 \pm 0.4 \\ 2.3 \pm 0.4 \\ \end{array}$  | $\begin{array}{c c} (4.15 \pm 0.6 \\ \text{Non-crar} \\ \text{Non-cran} \\ (4.49 \pm 1.4 \\ (4.49 \pm 1.6 \\ (4.49 \pm 1.6 \\ 2.9 \pm 1.6 \\ \text{only} \\ \text{only} \\ \hline 2.3 \pm 0.4 \\ \end{array}$ | $\begin{array}{ c c c c c c c c c c c c c c c c c c c$   | atch $\left  \begin{array}{c} (4.15 \pm \hat{0}.6 \\ \text{Non-crar} \\ \text{Non-cran} \\ (4.49 \pm 1.4 \\ (4.49 \pm 1.6 \\ 2.9 \pm 1.6 \\ \text{only} \\ \text{only} \\ \underline{2.3 \pm 0.4 } \\ 2.3 \pm 0.4 \\ \text{atch} \\ \text{atch} \\ \underline{2.6 \pm 0.6 \\ \underline{3.7 \pm 0.9 } \\ 3.7 \pm 0.9 \end{array} \right $ |
|                       | Football   | Arm Ba                          | Sweat Pe   |   |  | 2h) Sweat Pe   | 2h) Sweat Pa<br>s (forearm o  | 2h) Sweat Pe<br>s (forearm c<br>tkey  | (forearm o<br>ckey   | 2h) Sweat Pe<br>s (forearm o<br>ckey<br>Sweat Pe  |
| Situation             | American ] | r<br>raining<br>(~2h)           | Training<br>(150 minut<br>each sessio  |   |  | Training (2.2  | Training (2.2<br>in full pads   | Training (2.2<br>in full pads<br>Ice Hoo  | Training (2.2<br>in full pads<br>Ice Hoo<br>(~2h)  | Training (2.2 in full pads in full pads in full pads (2.2 in full pads (2.2 in full pads (2.2 h)       Ice Hoo       Ice Hoo       (~2h)       Game       (~3h)   |
| Conditions            |            | 12 - 31°C WBG                   | <u>Morning</u><br>22.7 – 26.0°C<br><u>Evening</u><br>28.2 – 30.8°C   |   |  | 29.4 – 32.2°C  | 29.4 – 32.2°C<br>WBGT   | 29.4 – 32.2°C<br>WBGT   | 29.4 – 32.2°C<br>WBGT<br>Training<br>12.7°C  | 29.4 – 32.2°C<br>WBGT<br>WBGT<br>12.7°C<br>12.7°C<br>Game<br>17.7°C   |
|                       |            | 16 American Football<br>Players | 10 American  |   |  | 16 Professional  | 16 Professional<br>American Football<br>Players   | 16 Professional<br>American Football<br>Players   | 16 Professional<br>American Football<br>Players<br>10 Ice Hockey   | 16 Professional<br>American Football<br>Players<br>10 Ice Hockey<br>Players   |
| Study                 |            | Stofan et al (2002)             | (2000) Io to editor  |   |  | Horswill et al   | Horswill et al<br>(2009)  | Horswill et al<br>(2009)  | Horswill et al<br>(2009)   | Horswill et al<br>(2009)<br>Godek et al (2006)  |

# Table 1.3 (continued)

| Study                       | Sport                                    | Conditions                            | Situation               | Method                    | Sweat Loss       | Sodium Loss    | Potassium Loss |
|-----------------------------|--|---------------------------------------|-------------------------|---------------------------|------------------|----------------|----------------|
|                             |  |                                       | Sailing                 |                           |                  |                |                |
| Neville et al (in<br>press) | 32 professional America's<br>Cup Sailors | $32 \pm 1^{\circ}$ C,<br>$52 \pm 5\%$ | Training<br>(150miutes) | Sweat Patches<br>(4sites) | $2.24 \pm 0.89L$ | 26 ± 17mmol/h  | N/A            |
|                             |  |                                       | Tennis                  |                           |                  |                |                |
| Bergeron (2003)             | Male Tennis Players<br>(cramp-prone)     | $31.9\pm0.5^\circ\mathrm{C}$          | Game<br>(duration N/A)  | Sweat Patch               | $2.6\pm0.1$ L/h  | 118 ± 11mmol/h | N/A            |
| Bergeron (1996)             | Male Tennis Player<br>(cramp-prone)      | 31.6°C                                | Game (1.8h)             | Sweat Patch               | 4.5L             | 162mmol        | 24.3mmol       |
| ;<br>,<br>,                 |  | :                                     |                         |                           |                  |                |                |

| Table 1.3 (continued) |
|-----------------------|
|-----------------------|

\* 1 mmol of sodium is equivalent to 22.99 mg of sodium
 \*\* 1 mmol of potassium is equivalent to 39.01 mg of potassium

#### **1.8** Factors affecting sweat composition

Electrolyte losses are governed by both the amount and composition of the sweat lost. Given that both these factors demonstrate wide variation between individuals it is unsurprising that total electrolyte losses can vary substantially. Sweat composition itself is influenced by a number of factors including the collection method, the inducing stimulus, sweat rate, heat acclimation, training status, hydration, age, gender, genetics and diet.

### 1.8.1 Sweat collection techniques

A variety of sweat collection techniques have been utilised to assess sweat composition. The use of an absorbent cotton suit allows whole body sweat to be collected (Allsopp et al, 1998; Palacios et al, 2003b), but is restricted to situations of rest rather than exercise due to the inability to quantify the amount of sweat dripping or evaporating from exposed areas of skin. Other whole body techniques include enclosing the body in a close fitting bag (Vellar et al, 1968) and variations of a washdown technique (Dill et al, 1938; Shirreffs & Maughan, 1997). In the study of Dill et al (1938), subjects washed in distilled water before walking in the heat (43°C and 10% RH). Due to the air movement over the skin and low humidity of the environment, sweat was assumed to be completely evaporated from the skin surface. After exercise, subjects washed with 5 litres of distilled water and the composition of this washdown water was analysed for electrolytes. By taking into account body weight changes during exercise, sweat electrolyte losses could be ascertained. More recently, Shirreffs & Maughan (1997) reported details of a washdown technique that is reproducible and valid during exercise, although it is limited to the laboratory setting.

Regional sweat collection techniques are both convenient and easy to use inside and outside the laboratory. Some studies simply scraped sweat from the skin surface (Boysen et al, 1984), but this method is hampered by the potential contamination by skin cells and from the inevitable evaporation that occurs on the skin surface leading to a concentrating effect on the sample collected (Boysen et al, 1984). The use of an enclosed bag, capsule or gauze pad (Consolazio et al, 1966; Costa et al, 1969; Dill et al, 1938, Brisson et al, 1991; Boysen et al, 1984; Hayden et al, 2004; Palacios et al, 2003a,b; Shirreffs & Maughan, 1997; Patterson et al, 2000; Ladell, 1948) eliminates the problem of sweat evaporation from the skin surface, but in doing so may alter both sweat composition and sweat rate. The use of a ventilated capsule (Barrueto et al, 1959) overcomes the problems associated with the enclosed sweat collection methods, but like all regional techniques cannot account
for the regional differences in both sweat rate and composition (Havenith et al, 2008; Costa et al, 1969; Patterson et al, 2000). Patterson et al (2000) reported large variations in sweat rate and composition between skin regions using sweat patches placed on 11 skin sites (forehead, chest, scapula, lower back, abdomen, upper arm, forearm, hand, thigh, calf and foot) during 90 minutes of exercise in temperate conditions (20°C and 50% RH). For example, sweat sodium concentrations were 57mmol/L from patches placed on the forehead but 24mmol/L from those on the foot. Costa et al (1969) used 3 sweat collection methods (arm bag, gauze pads and whole body washdown) during 40 minutes of intermittent cycling in a temperate environment (24.5°C). Comparisons made between the 3 collection techniques revealed total sweat sodium losses obtained by the gauze pads to be higher than those obtained using an enclosed bag on the arm, which in turn was higher than those obtained by the whole body collection procedure. The overestimation of sodium and/or chloride losses using regional techniques is a consistent finding (Patterson et al, 2000; Shirreffs & Maughan, 1997; Palacios et al, 2003a; Van Heyningen & Weiner, 1952; Stofan et al, 2002a; Dill et al, 1967). It would seem that the regional sweat patch technique overestimates whole body sweat sodium concentration by approximately 30-40% (Shirreffs et al, 2006).

Although the between-individual differences in sweat composition are largely attributed to differences in sweat gland function (reabsorptive and secretory capacity), the regional differences in sweating observed within an individual are primarily determined by differences in the number of active sweat glands in different regions of the body (Sato & Dobson 1970a). This could be at least partly responsible for the over-estimation of sweat sodium concentration by regional sweat collection techniques, but other factors are also postulated. The most common suggestions include an alteration in sweat composition through the restriction of evaporation, leading to a higher skin temperature and humidity at the regional collection site (Nadel et al, 1971; Robinson et al, 1950a; Weiner & van Heyningen, 1952) and/or a leaching of electrolytes from the stratum corneum (Weschler, 2008).

Robinson et al (1950a) determined the effect of skin temperature on the chloride concentration of sweat obtained from the hand and forearm. Both the left and right hand/forearm were enclosed in two separate gloves, each maintained at different temperatures during walking exercise in the heat. They reported that the hotter hand (by at

least 1.5°C) always produced a higher sweat chloride concentration. Whilst this may in part be attributed to an increased sweat rate (Nadel et al, 1971), as discussed later, it has also been attributed to a direct effect of skin temperature on the sweat gland itself (Collins & Weiner, 1962; Robinson et al 1950a; Weiner & van Heyningen, 1952). Alternatively, Weschler (2008) suggests a leaching of sodium from the stratum corneum to be a contributing factor to the elevated sweat sodium concentrations. Nevertheless, despite the overestimation, sweat sodium concentration from regional techniques correlates well with whole body values for sweat sodium concentration (Ladell, 1948; Patterson et al, 2000).

#### 1.8.2 Method of sweat stimulation

As sodium and chloride are the main electrolytes found in sweat, it is likely that the water content of sweat is derived from the extracellular fluid compartment (Costill et al, 1976; Nose et al, 1988b). Costill et al (1976) reported a decrease of 13.7% in plasma volume and 6.5% in intracellular volume in 8 men dehydrated by 5.8% BM during exercise in the heat (39.5°C and 25% RH). But, in terms of absolute fluid losses, both intracellular and extracellular compartments contributed equally. Nose et al (1988b) dehydrated subjects by 2.3% of BM through intermittent exercise in the heat (36°C and 30% RH). Fluid was lost from both intracellular and extracellular fluid compartments, but plasma water loss relative to total body water loss was approximately 60% higher than the theoretically expected values, if body water was lost proportionately from each compartment. They also reported a strong relationship between changes in extracellular fluid volume and sweat sodium concentration (r = 0.80) and changes in intracellular volume to correlate with changes in plasma osmolality. It would appear that the movement of water from the intracellular compartment compensates for the loss of plasma water in an attempt to defend plasma volume.

The method by which sweat loss is achieved may also influence the distribution of water losses from body fluid compartments. Both thermal (Kozlowski & Saltin, 1964; Melin et al, 2001; Caldwell et al, 1984) and diuretic (Caldwell et al, 1984) induced dehydration results in a greater loss of plasma volume than exercise-induced dehydration. Despite these differences, sweat composition seems little affected when sweating is evoked by thermal exposure or exercise in cool, temperate or hot conditions (Verde et al, 1982), although a tendency for lower sweat sodium and chloride concentrations has been reported during exercise compared to thermal exposure by other investigators (Kozlowski & Saltin, 1964).

## 1.8.3 Sweat rate

Sweat rate and composition are intimately linked, with the majority of investigators reporting increases in sweat sodium concentration with increases in sweat rate (Schwartz & Thaysen, 1956; Cage & Dobson, 1965; Allan & Wilson, 1971). In contrast, sweat potassium concentration is largely unaffected by sweat rate (Schwartz & Thaysen, 1956; Verde et al, 1982). The relationship between sweat sodium concentration and sweat rate is linked to the ability of the sweat duct to reabsorb sodium during the passage of sweat from the secretory coil through the sweat duct before being expelled at the skin surface. A maximal capacity for sodium reabsorption exists, above which further increases in sweat rate result in a linear increase in sweat sodium concentration (Schwartz & Thaysen, 1956; Shamsuddin et al, 2005; Buono et al, 2007).

#### 1.8.4 Heat acclimation

Heat acclimation usually (Nadel et al, 1974; Mitchell et al, 1976) but not always (Armstrong et al, 1993; Armstrong et al, 1987) results in an increase in sweat rate. An increase in sweat rate is largely dependent upon whether acclimation takes place in hothumid heat rather than a hot-dry heat. Although sweat rate can increase with acclimation, a concomitant fall in sweat sodium concentration is observed (Allan & Wilson, 1971; Buono et al, 2007), which at first appears to contradict the previous discussion. Allan & Wilson (1971) thermally stressed 3 subjects by hot-water immersion during which a weak salt solution was consumed to prevent a salt deficit. An enclosed capsule placed on the subject's scapula collected sweat. Allan & Wilson (1971) reported that at the same sweat rate, sweat sodium concentration is lower in heat-acclimated individuals, with this difference being greater at the higher sweat rates. More recently, Buono et al (2007) reported the conservation of sodium by heat acclimation is similar over a range of sweat rates. The decline in sweat sodium concentration was attributed to an increased sodium reabsorption capacity of the sweat duct after acclimation, possibly via the action of aldosterone. Some investigators report that a reduction in sweat sodium concentration with acclimation is apparent only with salt deficiency (McCance, 1938; Robinson et al, 1950b; Smiles & Robinson, 1971). This would accentuate the aldosterone response, but it has been demonstrated that a salt deficit is not necessary for this to occur (Allan & Wilson, 1971; Davies et al, 1981). Plasma aldosterone concentration is known to increase in response to exercise in the heat (Francesconi et al, 1983; Kirby & Convertino, 1986), but this increase is attenuated after acclimation (Kirby & Convertino, 1986). Nevertheless,

despite an elevated sweat rate and lower circulating aldosterone concentration, total sodium excretion was reduced by 59% after a 10 day heat acclimation protocol (Kirby & Convertino, 1986). This was attributed to an increased sensitivity of the eccrine sweat gland to aldosterone (Kirby & Convertino, 1986).

#### 1.8.5 Training

Highly-trained endurance athletes display similar characteristics (increased sweating sensitivity and decreased sweating threshold) to those of partially heat-acclimated individuals (Piwonka et al, 1965; Gisolfi & Robinson, 1969; Henane et al, 1977). Highly-trained athletes are also reported to have higher sweat rates than sedentary individuals (Henane et al, 1977; Buono & Sjoholm, 1988). Buono & Sjoholm (1988) determined the secretory activity of sweat glands in both sedentary and trained subjects in response to pilocarpine iontophoresis. Both trained males and females exhibited sweat rates greater than their sedentary counterparts. Whilst no difference in sweat gland density was apparent between the trained and untrained states, trained individuals secreted significantly more sweat per gland.

Several studies have looked at changes in thermoregulatory function before and after a physical training programme (Nadel et al, 1974; Henane et al, 1977; Gisolfi & Robinson, 1969). Gisolfi & Robinson (1969) reported a 7.3% increase in sweat rates of 5 physically active men who underwent interval training for 5h per week for 6 weeks. Similarly, Henane et al (1977) reported a 28% increase in sweat rates of sedentary men after a 3 month training programme. The difference in magnitude in sweat rate responses between the two studies may be attributable to differences in training programme duration and baseline fitness of subjects. As mentioned briefly, the purported mechanisms underlying the effects of physical training on sweat rate include an increased sweating sensitivity (Piwonka et al, 1965; Roberts et al, 1977; Shvartz et al, 1977; Nadel et al, 1974; Henane et al, 1977; Gisolfi & Robinson, 1969) and/or decreased sweating threshold temperature (Roberts et al, 1977).

Several investigators have reported sweat rate to be significantly correlated with VO<sub>2</sub>max (ml/kg/min) (Buono & Sjoholm, 1988; Henane et al, 1977; Greenhaff & Clough, 1989), but two studies that involved trained individuals reported different responses to the heat between swimmers and either runners (Piwonka et al, 1965) or skiers (Henane et al, 1977)

despite similar VO<sub>2</sub>max values. In one case the sweating response of swimmers was similar to that of sedentary individuals (Piwonka et al, 1965). Henane et al (1977) suggested that the differing levels of hyperthermia experienced during training between skiers and swimmers was fundamental to the differing sweat outputs seen between the two groups. However, Collins et al (1966) have previously suggested that the increased sweating capacity, in this case that accompanied heat acclimation, was as a result of increased sweat gland activity not elevated body temperature. Indeed, it seems that repetitive stressing of the sweat gland is fundamental to the changes reported to occur with training (Piwonka et al, 1965; Nadel et al, 1974). Although there is a large variation between individuals in the size, and in the secretory and re-absorptive capacities of the human sweat gland (Sato & Sato, 1983; Sato & Dobson, 1970a), trained individuals have larger sweat glands and therefore a greater capacity for sweating than untrained individuals, which was again attributed to the repeated stimulation of sweating during training.

In contrast to the large number of studies addressing the issue of sweat rate and training, few studies have examined differences in sweat composition between trained and untrained individuals. The study of Sato & Dobson (1970a) examined regional and individual variations in the function of the eccrine sweat gland and they reported a positive relationship between mean sweat rate and sweat sodium excretion. The slope of this relationship decreased with physical training, suggesting improved sodium reabsorption in the sweat duct. However, Kozlowski & Saltin (1964) reported no effect of training status on sweat composition.

#### 1.8.6 Hydration

A number of studies have investigated the effects of dehydration and/or water ingestion on both sweat rate and composition. Most studies report a decline in sweat rate with dehydration (Montain et al, 1995; Ellis et al, 1954; Pearcy et al, 1956; Robinson et al, 1956; Cage et al, 1970), and this appears to be in a dose response manner (Montain et al, 1995; Ellis et al, 1954; Cage et al, 1970). Sweat is hypotonic to plasma and therefore hypohydration induces a hyperosmotic hypovolaemic condition. Plasma hyperosmolality increases the temperature at which sweating begins (Fortney et al, 1984; Sawka et al, 1989; Montain et al, 1995) whilst hypovolaemia reduces sweating sensitivity (Fortney et al, 1981; Montain et al, 1995). Plasma aldosterone levels increase in response to exercise in the heat and this increase is of greater magnitude when hypohydrated (Francesconi et al, 1983; Morgan et al, 2004). However, the effects on sweat composition seem counter-intuitive. An elevated aldosterone concentration would be expected to reduce sweat sodium concentration due to its well known sodium conserving action on the sweat gland, but some studies report dehydration to increase sweat sodium and/or chloride concentrations (Morgan et al, 2004; Cage et al, 1970; Robinson et al, 1956), although others report no differences in sweat composition (Cage et al, 1970; Barnett & Maughan, 1996). Cage et al (1970) studied 14 heat-acclimated subjects exposed to the heat (43°C, 50% RH). Fluid ingestion ranged between 1 and 3L during the 90 minute exposure and appeared to lower sweat sodium concentration in 4 out of 10 subjects, but 3 subjects showed higher sweat sodium concentrations and 3 had no change. Sweat chloride and potassium concentrations also showed great variation in response to fluid ingestion. The inconsistent nature of these findings led the authors to conclude that water ingestion does not affect sweat composition in a manner that cannot be explained by a change in sweat rate. However, Robinson et al (1956) reported a higher sweat chloride concentration in subjects who demonstrated lower sweat rates compared to the hydrated state. Similarly, Morgan et al (2004) reported that when no fluid was ingested during 2h of moderate exercise in hot humid conditions (38°C, 60% RH), forearm sweat sodium and chloride concentrations increased by approximately 10mmol/L and 4mmol/L, respectively. This occurred despite no differences in sweat rate. It has been suggested that the increased sodium concentration of the primary sweat, due to the increased serum sodium concentration may have caused the elevated sweat sodium concentration in the dehydrated state. However, the increase in serum sodium concentration (3mmol/L) seemed too small in magnitude to be responsible for the increase in sweat sodium concentration (10mmol/L) (Morgan et al, 2004).

#### 1.8.7 Age

The effect of age on sweat rate has been repeatedly studied, with pre-pubertal children reported to have lower (Wagner et al, 1972; Meyer et al, 1992), or similar (Drinkwater et al, 1977) sweat rates to adults. A reduction in sweat rate has also been observed amongst elderly individuals (over the age of 60y) compared to younger adults (Davies, 1979; Tankersley et al ,1991; Armstrong & Kenney, 1993; Anderson & Kenney, 1987; Wagner et al, 1972; Kenney & Fowler, 1988). It has been suggested that the mechanism responsible for the age-related decline in sweat rate is not a difference in the number of active sweat

glands, but instead a decline in the amount of sweat secreted per sweat gland (Anderson & Kenney, 1987; Kenney & Fowler, 1988).

Meyer et al (1992) reported sweat sodium concentrations to be higher in adult men (58  $\pm$  25mmol/L) than pre-pubescent boys (aged 9y; 35  $\pm$  20mmol/L), but not pubescent boys (aged 11y; 40  $\pm$  20mmol/L) in response to cycling exercise in the heat (40°C, 20% RH). They suggested that the increased sweat sodium concentration observed in young adults was possibly the result of an increased sweat rate, although only a weak correlation between sweat rate and sweat sodium concentration was observed (r = 0.15). Lobeck & Huebner (1962) also reported an increase in sweat sodium concentration with age until the age of 20y, beyond which no further increase was observed. This increase was not related to changes in sweat rate but since plasma aldosterone concentrations were similar between pre and post pubescent boys before and after exercise (Falk et al, 1991), an alteration in the sensitivity of the sweat duct to aldosterone may occur (Meyer et al, 1992).

#### 1.8.8 Gender

Whether there are gender differences in the sweating response to exercise remains a source of contention. Field studies suggest that women generally have lower sweat rates than males (Burke & Hawley, 1997; Broad et al, 1996; Soo & Naughton, 2007; Hazelhurst & Claassen, 2006; Shirreffs et al, 2006) although these are not always statistically significant (Cox et al, 2002). Yet inconsistencies are reported in laboratory-based studies. Confounding factors contributing to the debate in the literature include the environmental conditions of the study, aerobic capacity, degree of acclimation and body composition of subjects and phase of the menstrual cycle.

Males typically have a higher aerobic capacity than females (Avellini et al, 1980a,b; Buono & Sjoholm, 1988) and because sweat rate is closely related to absolute exercise intensity (Saltin & Hermansen, 1966; Greenhaff & Clough, 1989), males would be expected to have higher sweat rates than females. Some authors report the higher sweat rates in males to persist (Avellini et al, 1980a; Frye & Kamon, 1981) although others report the gender differences in sweat rate to disappear (Avellini et al, 1980b; Buono & Sjoholm, 1988) when aerobic capacity is taken into account. Gender differences are suggested to be accounted for by the disparity in acclimation status between subjects (Fox et al, 1969). Indeed, heat acclimation eliminated the gender differences seen in the un-acclimated state during 3h of exercise at 30% VO<sub>2</sub>max in 48°C (Frye & Kamon, 1981). However, others (Avellini et al, 1980a; Wyndham et al, 1965) report contradictory findings, suggesting acclimation to accentuate the gender differences in sweat rate. This occurred despite the fact that aerobic capacity, surface area:mass, and menstrual cycle was controlled for (Avellini et al, 1980a). It is most likely that the cause of these discrepancies is the different environmental conditions of the studies.

Exposure to hot-humid conditions is consistently associated with higher sweat rates in males than females (Morimoto et al, 1967; Shapiro et al, 1980; Avellini et al, 1980a), but in hot-dry environments no significant differences between males and females have been reported (Morimoto et al, 1967; Shapiro et al, 1980; Avellini et al, 1980b). The mechanism responsible for this is linked to skin wettedness and the improved feedback present in female subjects suggestive of an enhanced sweating efficiency (Frye & Kamon, 1983).

Meyer et al (1992) compared both sweat composition and rate between genders during cycling exercise (50% VO<sub>2</sub>peak) in the heat (40°C and 20% RH). They observed men to have a higher sweat sodium concentration than women, although this reached significance only in adulthood (58  $\pm$  25mmol/L and 35  $\pm$  20mmol/L, respectively). This is in agreement with some (Lobeck & Huebner, 1962), but others have reported no differences (Brown & Dobson, 1967; Shirreffs et al, 2006) or higher sweat chloride concentrations in women (Morimoto et al, 1967).

Thermoregulatory function in eumenorrheic women varies depending on the phase of the menstrual cycle, with the hormone progesterone being largely implicated in these changes. Body temperature is elevated during the luteal phase of the cycle, which coincides with an increase in progesterone levels. Although some investigators have reported no significant differences in sweat rate between the follicular and luteal phases of the menstrual cycle (Inoue et al, 2005; Avellini et al, 1980a; Frye & Kamon, 1981, Pivarnik et al, 1992), there was a tendency for sweat rates to be higher during the luteal phase in some investigations (Avellini et al, 1980a; Garcia et al, 2006). Lieberman (1966) determined the effects of the menstrual cycle on sweat electrolyte concentration in 57 healthy females over a 4 month

period. Sweat sodium concentration followed a cyclic pattern in 50% of women, which increased during the luteal phase and then fell 3-5 days prior to menses. Similar findings have been reported by others (Palacios et al, 2003a) and it appears that these cyclic fluctuations coincide with the rise and fall of progesterone, which is known to inhibit the actions of aldosterone (Lieberman, 1966).

## 1.8.9 Diet

Studies during heat acclimatisation report sweat sodium concentration to decrease during salt deficiency and to increase during a period of salt excess (McCance, 1938; Robinson et al, 1950b; Robinson et al, 1956; Armstrong et al, 1985b; Sigal & Dobson, 1968), but Costa et al (1969) were the first to report a relationship between dietary sodium intake and sweat sodium concentration that was not dependent on the presence of a salt deficiency. Costa et al (1969) fed 12 acclimatised males a diet providing either 244 mmol (5.6g) of sodium or 148 mmol (3.4g) of sodium per day for 6 weeks. On 4 separate occasions during this 6 week period subjects exercised for 40 minutes in 24.5°C. Sweat was collected using an enclosed arm bag, 3 sweat patches positioned on the arm, back and chest, and via a whole body washdown procedure. Sweat rate was not different between diets, but all sweat collection procedures revealed sweat sodium concentration to be higher on the high sodium diet. Interestingly, the difference in sweat sodium concentration between diets was the same ratio as the differences in sodium intake between the diets. More recently Allsopp et al (1998) reported that men fed either a high-sodium diet (348mmol/d) or a moderatesodium diet (174mmol/d) for 4 consecutive days had a significantly higher sweat sodium loss (79mmol and 64mmol, respectively) than those consuming a low-sodium (66mmol/d) diet (54mmol).

### **1.9** Sodium deficiency

McCance (1936) determined the effects of salt deficiency in 4 human subjects through a combination of ingesting a low-sodium diet (40-70mg/d) and sweating. A severe salt deficiency was gradually developed over approximately 7 days and was maintained for approximately 3-4 days, before repletion over a 24h - 7 day period began. Profuse sweating was achieved by exposure to the heat for approximately 2h/d. For one individual, sweat sodium loss over the entire period of salt depletion was 18g and resulted in a net negative sodium balance of 22.5g when urine losses were taken into account. Salt deficiency was accompanied by a loss of appetite, nausea, muscle cramps, negative

nitrogen balance, excessive fatigue and general feelings of exhaustion but it is the effects on fluid balance that will be the focus of the present discussion. During the study period, water was allowed ad libitum and generally resulted in a urine volume of at least 1500mL/d. This was deemed sufficient evidence to indicate that individuals were not dehydrated during the experimental period due to inadequate fluid intake. However, during the early stages (days 1-4) of salt deficiency the loss in sodium was closely followed by a loss in body mass. The loss of approximately 3.4g (~147mmol) of sodium resulted in the loss of approximately 1kg in body mass. This is approximately the amount of sodium present in 1 litre of plasma or extracellular fluid and therefore the loss in body mass was attributed to the loss of water from the extracellular fluid compartment. After approximately 4 days the decline in body mass ceased, but abnormalities in water regulation began. At this point, despite the ingestion of large volumes of water, diuresis would not develop until many hours later. Body mass returned to normal after approximately 6 days of salt ingestion.

#### 1.10 Effects of dehydration on performance

Montain & Coyle (1992) determined the effect of different rates of fluid ingestion on cardiovascular and thermoregulatory responses during exercise. Eight cyclists, cycled at approximately 65% VO<sub>2</sub>max for 2h in a hot environment (33°C, 50% RH). In separate trials they ingested a carbohydrate-electrolyte sports drink at a rate that would result in a dehydration of 1.1%, 2.3%, 3.4% and 4.2% BM by the end of exercise. Total fluid intake on each trial was ~2380mL (large), ~1423mL (medium), ~583mL (small) and 0mL (no fluid). The elevation in rectal temperature was proportional to the degree of dehydration incurred. Rectal temperature was  $0.8^{\circ}$ C higher on the no fluid trial than when a large volume of fluid was consumed. Similarly, the increase in heart rate, and the decline in stroke volume and forearm blood flow were all proportional to the degree of dehydration incurred. Whilst there are detrimental effects to cardiovascular and thermoregulatory function with dehydration and these detrimental effects occur in a graded manner proportional to the extent of dehydration, it appears that the effects of dehydration on endurance performance depend to some extent on the environmental conditions present. There is a general consensus that when endurance exercise occurs in temperate conditions (20-21°C), a body mass loss approximately 2% BM will not significantly decrease performance (Cheuvront et al, 2003; McConell et al, 1999), but will be detrimental to

endurance performance in the heat (Cheuvront et al, 2003; Below et al, 1995; Walsh et al, 1994).

McConell et al (1999) determined the effect of fluid ingestion on heart rate, rectal temperature and performance during exercise in temperate conditions (21°C, 41% RH). Eight males cycled for 45 minutes at 80% VO<sub>2</sub>max whilst consuming either a volume of water that replaced 100% of sweat loss, 50% of sweat loss or no fluid. Immediately following the 45 minute bout of exercise, subjects completed a 15 minute time trial. As a result of exercise and fluid ingestion, subjects lost 1.9% BM (no fluid), 1.0% BM and 0% BM. No differences in heart rate, rectal temperature or performance were observed. Below et al (1995) determined the effects of fluid ingestion during 1h cycle exercise (50 minutes at 80% VO<sub>2</sub>max + 10min performance ride) in the heat (31°C, 54% RH). Eight males ingested either a large (~1330mL) or small (~200mL) volume of water which resulted in dehydration of 0.5% BM and 2.0% BM, respectively. Performance was improved by approximately 6% when the large volume of water was consumed and this was accompanied by a lower rectal temperature and heart rate.

## **1.11** Fluid and electrolyte intake before exercise

As dehydration can be detrimental to performance and athletes typically consume fluids at a rate below sweat rate during exercise, strategies that promote hyperhydration prior to exercise have been investigated. One hyperhydration strategy involves the ingestion of a solution with a high sodium content (Harrison et al, 1976; Coles & Luetkemeier, 2005; Sims et al, 2007; Nielsen et al, 1971; Greenleaf & Brock, 1980).

Hyperhydration with solutions containing sodium before exercise result in an expanded plasma volume compared to drinks with a low-sodium content (Coles & Luetkemeier, 2005; Sims et al, 2007; Greenleaf & Brock, 1980). Some (Sims et al, 2007) but not all (Coles & Luetkemeier, 2005) investigators report this to attenuate the cardiovascular drift during exercise. The effects on the thermoregulatory system are also unclear. Nielsen et al (1971) determined the effects of hyperhydration with water and a sodium chloride solution (342mmol Na<sup>+</sup>/L) on thermoregulatory responses during 60 minutes of exercise at ~50% VO<sub>2</sub>max in temperate conditions (20°C). Rectal temperature was reported to plateau in all trials after approximately 30 minutes of exercise, but the plateau in rectal temperature was  $0.4^{\circ}$ C higher than the control trial when hyperhydrated with the sodium chloride solution

and 0.5°C below the control trial when hyperhydrated with water. As a strong positive relationship was observed between plasma osmolality and rectal temperature, the higher rectal temperature with sodium chloride ingestion was attributed to the elevated plasma osmolality. It has since been shown that an increase in plasma osmolality will increase the sweating threshold and the threshold for peripheral vasodilation (Fortney et al 1984). Consequently, this has raised concerns about hyperhydration with salt solutions (Coyle & Montain, 1993).

Coles & Luetkemeier (2005) investigated whether sodium loading would increase plasma volume and improve cycling time trial performance. Fourteen males (with a VO<sub>2</sub>max of ~50ml/kg/min) ingested 10mL/kg (~800mL) of a high sodium (164mmol Na<sup>+</sup>/L) or placebo (no sodium) solution over a 30 minute period followed by a 15 minute equilibration period before exercise. Exercise involved 45 minutes of cycling at 70% VO<sub>2</sub>max in 22°C, followed immediately by a 15 minute time trial. Plasma volume was 3.1% higher than baseline at the start of exercise when the sodium solution was consumed but had declined by 4.7% on the placebo trial. Interestingly, at the onset of the time trial, no differences in plasma volume were observed, yet a significant improvement in performance was seen on the sodium trial. Despite a higher serum osmolality with sodium loading, no differences in sweat loss or rectal temperature were observed between trials. Sims et al (2007) investigated whether the ingestion of a sodium solution would induce hypervolaemia and enhance exercise capacity in the heat. Eight males (with VO<sub>2</sub>max of 57ml/kg/min) ingested 10mL/kg (~757mL) of either a high-sodium (164mmol Na<sup>+</sup>/L) or low-sodium (10mmol Na<sup>+</sup>/L) solution, 45 minutes before running to exhaustion at 70% VO<sub>2</sub>max in the heat (32°C, 50% RH). They reported a significant improvement in exercise capacity, with 7 out of 8 subjects running for longer on the high-sodium trial. There were no differences in serum osmolality between trials, but neither were there differences in plasma volume between trials at the start of exercise. Not all studies report benefits from plasma volume expansion prior to exercise on performance (Warburton et al, 1999, 2000; Watt et al, 2000). As endurance training results in an expansion in plasma volume, investigators have reported that endurance athletes will not benefit as much, if at all, from plasma volume expansion as those individuals with a low aerobic fitness as endurancetrained athletes already possess a high blood volume (Coles & Luetkemeier, 2005; Watt et al, 2000; Warburton et al, 1999).

## 1.12 Fluid and electrolyte intake during exercise

It is currently recommended that fluid intake during exercise should be sufficient to limit dehydration to no more than 2% BM (ACSM, 2007). Recommendations for electrolyte replacement have proven more difficult to formulate due to the aforementioned wide variation in sweat composition and losses between individuals. Current guidelines suggest that there is no conclusive evidence to ingest magnesium, calcium or potassium during exercise (Coyle, 2004). In contrast, there is a general consensus that the inclusion of sodium in drinks consumed during exercise will confer some benefit and is considered especially important if exercise is prolonged (>2h) or when high sodium losses (>3-4g) are expected (Coyle, 2004; ACSM, 2009). The inclusion of sodium is justified because sodium is the predominant electrolyte in sweat (Table 1.2), improves drink palatability (Passe et al, 2006; Wemple et al, 1997), maintains the drive to drink (Wemple et al, 1997; Dill et al, 1973), maintains extracellular volume (Criswell et al, 1992, Sanders et al, 2001) and may attenuate the decline in serum sodium concentration that is seen when plain water is consumed at rates equal to or greater than sweat loss (Vrijens & Rehrer, 1999; Twerenbold et al, 2003).

Although the presence of sodium in the small intestine is important for water absorption, there is evidence that the inclusion of sodium in a drink consumed at rest (Gisolfi et al, 1995; Jeukendrup et al, 2009) or during exercise (Gisolfi et al, 2001) is not necessary as the intestinal secretion of sodium into the intestinal lumen is sufficient to promote water and solute absorption. Gisolfi et al (1995) reported that the addition of sodium (0, 25 or 50mmol/L) to a 6% carbohydrate solution did not affect water absorption in the jejunum at rest. Similarly, Gisolfi et al (2001) reported that the ingestion of a 6% carbohydrate solution containing either 0, 20 or 50mmol/L of sodium did not alter water absorption in the duodenum or jejunum during cycling exercise at 65% VO<sub>2</sub>peak in temperate conditions (22°C and 40% relative humidity). However, the inclusion of sodium in a drink may reduce the rate at which endogenous sodium is secreted into the intestinal lumen and hence may also attenuate the efflux of water entering the lumen (Gisolfi et al, 2001; Leiper, 1998). As a result of this inconclusive evidence, it is hard to justify the inclusion of sodium in a drink to be consumed during exercise based solely upon on water absorption.

## 1.12.1 Sodium intake and performance

Pitts et al (1944) investigated whether the addition of sodium chloride to water, consumed at a rate equal to sweat loss during exercise would be advantageous to performance. Six heat-acclimatised men who were receiving adequate amounts of dietary salt (although the amount of which was not specified) marched in the heat (37.8°C, 35% RH) for anywhere between 1-6h. They reported that because the benefits of consuming water (only) on heart rate, rectal temperature and performance were so striking compared to when no fluid was consumed, that the addition of sodium chloride was no more or less beneficial than water alone. More recently, Hew-Butler et al (2006) determined whether sodium supplementation could improve ironman performance. Four hundred and thirteen triathletes were split into a placebo, sodium or control (no supplementation) group. Subjects receiving sodium supplements were asked to consume 1-4 salt capsules per hour which resulted in an average sodium intake of  $156 \pm 88$  mmol during the race. This is equivalent to  $6.8 \pm 3.8$ g of sodium. They reported no difference in performance between placebo (762min), sodium supplementation (758min) and control (741min) groups. However, athletes were allowed ad libitum access to food and fluid during the race which was not measured and therefore the true differences in sodium intake cannot be ascertained. Similarly, Speedy et al (2002) reported no effect of sodium supplementation (6.34g, range 4.09 - 9.13g) on ironman performance, but food and fluid intake were also not measured. Twerenbold et al (2003) investigated the effect of different drink sodium concentrations on endurance performance. Thirteen endurance runners completed three, 4h runs on an outdoor track. During each run they ingested either water, a high-sodium (30mmol/L) or low-sodium (18mmol/L) drink at a rate of 1 L/h. Although no significant differences were reported between the high-sodium (39.91km), low-sodium (42.03km) and water trials (40.55km), subjects ran slower on their first trial than trials 2 and 3, indicating a learning effect. Additionally, the environmental temperature varied between trials (5.3 -19°C) which may have also influenced performance (Galloway & Maughan, 1997).

#### 1.12.2 Hyponatraemia

Hyponatraemia is defined as a serum sodium concentration below 135mmol/L (Hew-Butler et al, 2008). Mild hyponatraemia (131-134mmol/L) is generally asymptomatic, but moderate hyponatraemia (126 – 130mmol/L) can result in bloating, malaise, headache, nausea, vomiting and fatigue. Severe hyponatraemia (<126mmol/L) can result in a coma, seizures and even death (Sallis, 2008). Hyponatraemia has been reported in endurance

events, such as the marathon (Kipps et al, in press; Almond et al, 2005) and ironman triathlon (Wharam et al, 2006; Speedy et al, 1999). The primary cause of symptomatic hyponatraemia is the excess consumption of fluids resulting in a positive fluid balance, but other factors purported to be involved in the aetiology of hyponatraemia include the loss of sodium in sweat (Hew-Butler et al, 2008; Montain et al, 2006).

The loss of sodium may either act to stimulate vasopressin release due to the hypovolaemia that accompanies the loss of sodium and water in sweat or directly via the loss of sodium itself reducing the pool of exchangeable sodium (Montain et al, 2006). In the situation of fluid overload, the presence of vasopressin is considered inappropriate as even small amounts can markedly influence kidney function and it is therefore termed Syndrome of Inappropriate Anti-Diuretic Hormone (SIADH). Montain et al (2006) used a mathematical model to predict the effects of various hydration regimens, sweat rates, running speeds and sweat sodium concentrations in the pathogenesis of hyponatraemia. They reported that individuals who lost large amounts of sodium in sweat could finish ultraendurance exercise hyponatraemic even in the absence of body weight gain. There has also been interest as to whether solutions containing sodium attenuate the decline in serum sodium concentrations or even prevent hyponatraemia (Sanders et al, 1999, 2001; Barr et al, 1991; Stofan et al, 2006; Twerenbold et al, 2003; Speedy et al, 2002; Hew-Butler et al, 2006; Baker et al, 2008).

In the study of Barr et al (1991), 8 cyclists rode intermittently (13 minutes cycling and 2 minutes rest) for 6h at 55% VO<sub>2</sub>max in the heat (30°C and 50% RH). Subjects ingested either water or a solution containing 25mmol/L of sodium at a rate equal to sweat loss. No differences between drinks were reported for performance, RPE, heart rate, rectal temperature or plasma sodium concentration, but plasma volume tended to be better maintained when sodium was ingested. They suggested the reason why no difference in serum sodium concentration was observed between trials, despite approximately 170mmol of more sodium being consumed when the sodium solution was ingested, was due to the significant increase in urine sodium excretion and the possible concealment of a change by the changes in plasma volume. Nevertheless, the presence of sodium in a drink (25mmol/L) was not sufficient to prevent a decline in serum sodium concentration when fluid was ingested at a rate close to sweat rate. However, other investigators report

contradictory findings (Vrijens & Rehrer, 1999; Stofan et al, 2006; Twerenbold et al, 2003; Baker et al, 2008; Montain et al, 2006).

In the study of Vrijens & Rehrer (1999), 10 cyclists completed 3h cycling at 55% VO<sub>2</sub>max in the heat (34°C, 65% RH) whilst ingesting either a sports drink (18mmol Na<sup>+</sup>/L) or water at a rate equivalent to sweat loss. The decline in serum sodium concentration was significantly less when the sports drink (-0.86mmol/L/h) rather than when water (-2.48mmol/L/h) was consumed. Only one subject was considered hyponatraemic and this occurred on the water trial. Stofan et al (2006) reported that the addition of electrolytes (including 36mmol/L of sodium) to a 6% carbohydrate drink consumed during 3h cycle exercise at 55% VO<sub>2</sub>peak in the heat (28.3°C WBGT) at a rate equal to sweat loss, attenuated the decline in serum sodium concentration. In the study of Baker et al (2008), 8 subjects with no prior history of exercise-associated hyponatraemia performed 2h of intermittent running exercise at 70% VO<sub>2</sub>max followed by a run to exhaustion at 85% VO<sub>2</sub>max, both of which took place in the heat (30°C, 40% RH). Subjects consumed a 6% carbohydrate solution during exercise which contained 3 different sodium concentrations (0, 18 and 30mmol/L). The volume ingested varied in such a way that subjects either gained body mass (+1.8% BM), maintained BM (-0.1% BM) or lost BM (-2.1% BM and -3.4% BM). They reported that the addition of sodium to drinks consumed at a rate equal to or greater than sweat loss attenuated the decline in serum sodium concentration. Although this attenuation did not reach significance, the decline in serum sodium concentration was related to the sodium content of the drinks consumed, with the greatest attenuation observed when the highest sodium concentration was consumed. The general consensus is that the addition of sodium to drinks ingested during exercise at a rate equal to or greater than sweat loss will attenuate the decline in serum sodium concentration, but will not prevent hyponatraemia (Montain et al, 2006; Hew-Butler et al, 2008).

## 1.12.3 Cramp

The association between heat cramps and sweat sodium losses has been largely observational due to the inherent problem of inducing muscle cramps within the laboratory. Nevertheless several investigators have reported cramp-prone individuals to lose large amounts of sodium in sweat (Talbott & Michelsen, 1933; Ladell, 1949; Bergeron, 1996; Stofan et al, 2001; Stofan et al, 2005). This association is further strengthened by findings that the ingestion of salt can alleviate signs and symptoms of

cramping (Brockbank, 1929; Talbott & Michelsen, 1933; Bergeron, 1996; Bergeron, 2007). Early investigations report the occurrence of heat cramps in miners (Brockbank, 1929) and construction workers (Talbott & Michelsen, 1933). Talbott & Michelsen (1933) described 5 construction workers of the Hoover Dam who suffered from heat cramps. They reported that heat cramps occurred when the loss of salt in sweat was not replaced and that symptoms were rapidly ameliorated after IV administration of saline. Indeed in all 5 cases, subjects were symptom free within 6h after initiating this treatment. The amelioration of symptoms is also reported when salt is ingested orally. Bergeron (1996) described a male tennis player who had a history of muscle cramps. These muscle cramps persisted despite the ingestion of calcium, magnesium or potassium supplements which are suggested as possible remedies for muscle cramping (O'Toole et al, 1993; Bergeron, 2003). During a tennis match which took place during a training session in the heat (31.6°C, 62% RH), sweat sodium losses of 162mmol were reported. These losses were similar to, or higher than, his typical daily sodium intake (87 - 174 mmol/day). It was recommended that he increase his sodium intake to 261-348 mmol (6 - 8g/d). During the following 9 months, no heat cramps were reported during competition or training. Stofan et al (2005) investigated whether cramp-prone American Football players lost more fluid and sodium in sweat than teammates who did not cramp. Sweat composition was assessed using a forearm sweat patch during two, ~2.5h training sessions. In the first training session of the day, cramp-prone players tended (P=0.063) to have a higher sweat sodium concentration (56mmol/L) than non-crampers (22mmol/L) and this was also found in the second training session (54mmol/L and 29mmol/L, respectively; P=0.063). Although sweat loss was not different between groups, total sweat sodium losses tended to be higher in the group of cramp-prone players. Over the entire day, sweat sodium losses were 10.4g for crampers and 4.9g of sodium for non-crampers.

It is suggested that the excessive loss of sodium and water in sweat results in a decreased extracellular volume and the mechanical deformation of nerve endings. These motor-nerve endings become hyperexcitable and spontaneously discharge leading to heat cramps (Bergeron, 1996). The ingestion of salt-containing solutions may therefore attenuate the perturbations in extracellular volume and composition and therefore the prevalence of heat cramps. Current recommendations are to consume 3g of salt in 500mL of a sports drink (Bergeron, 2007) within a 5-10 minute time period. This is approximately equivalent to 50% of the current upper limit (UL) for sodium (Institute of Medicine, 2004). Cramp-

prone individuals are also advised to increase their dietary salt intake to help prevent heat cramps from re-occurring (Bergeron, 1996; Eichner, 2007).

#### 1.12.4 Drink palatability

The indiscriminate use of large amounts of sodium during exercise is not always encouraged as this can lead to a reduced drink palatability (Passe et al, 2006; Wemple et al, 1997) and consequently fluid intake. Passe et al (2006) determined the palatability of a 6% carbohydrate solution which contained 5 different concentrations of sodium (0, 18, 30, 40, 60mmol/L) at rest and during exercise. They reported that ad libitum fluid intake tended (P=0.058) to be greater when a solution contained 30mmol/L of sodium than a sodium-free solution. As athletes typically ingest an amount of fluid equivalent to  $\sim 50\%$  of sweat losses, an increased palatability and therefore fluid intake would be important to delay the onset of dehydration. Therefore commercially available sports drinks are carefully designed to meet a balance between efficacy and palatability. Current recommendations suggest sports drinks should contain 22-30mmol/L (0.5-0.7g/L) of sodium (ACSM, 2009). Given that these sodium concentrations lie at the bottom of the range of sweat sodium concentrations (20-80mmol/L) (Table 1.1) and that fluids are consumed at rates below sweat rate, sweat sodium losses are not entirely replaced during exercise (Maughan et al, 2004; 2005; Shirreffs et al, 2005; Palmer & Spriet, 2008). For example, Shirreffs et al (2005) reported football players to replace 23% (range 0-62%) of the sodium lost in sweat during a 90 minute training session. The importance of sodium replacement after exercise has therefore been studied extensively.

#### **1.13** Fluid and electrolyte intake after exercise

## 1.13.1 Effect of drink composition

The ingestion of plain water following exercise results in a fall in plasma osmolality and sodium concentration. This leads to a decline in plasma vasopressin concentration and subsequently stimulates urine production and reduces the drive to drink (Nose et al, 1988a), both of which are detrimental to the rehydration process. In contrast, the addition of sodium chloride to plain water increases intake and reduces urine output (Wemple et al, 1997). Wemple et al (1997) had 6 individuals dehydrate by 3.0% BM by exercising in the heat. During the following 3h rehydration period, subjects had ad libitum access to either water, a 6% CHO-E solution (containing 25mmol  $Na^+/L$ ) or a 6% CHO-E solution

(containing 50mmol Na<sup>+</sup>/L). Fluid intake was significantly greater when the CHO-E solution containing 25mmol/L of sodium was ingested than water alone.

The importance of sodium in rehydration drinks has been reported by others (Shirreffs et al, 1996; Mitchell et al, 2000) and has been systematically investigated in several studies (Maughan and Leiper, 1995; Shirreffs and Maughan, 1998; Merson et al, 2008). Maughan and Leiper (1995) reported that when a volume equal to 150% of BM loss was ingested following exercise-induced dehydration, the amount of fluid retained was inversely related to the drink's sodium concentration. Shirreffs & Maughan (1998) had 6 individuals dehydrate by 1.9% BM by intermittent cycle exercise in the heat (34°C, 60-70% RH). During the 1h rehydration period, subjects ingested 4 drinks containing 0, 25, 50 or 100mmol/L of sodium, in a volume equal to 150% of body mass loss. Urine volume was also found to be inversely related to the amount of sodium ingested. However, the positive relationship between urine volume and sodium intake is not always reported (Mitchell et al, 2000). Mitchell et al (2000) reported that the ingestion of a 50mmol/L sodium solution was no more beneficial than a 25mmol/L solution in restoring fluid balance after exerciseinduced dehydration. The reason for this discrepancy is likely due to the shorter monitoring period in the study of Mitchell et al (2000) (3h) compared to the other investigations (6-7h) (Maughan and Leiper, 1995; Shirreffs and Maughan, 1998). This is likely too short for any changes in urine volume between drinks to have been observed.

In some situations sodium may not be the only electrolyte that can improve fluid retention. The ingestion of a drink containing primarily potassium may favour intracellular rehydration compared to solutions with either a low electrolyte content or that contain primarily sodium (Maughan et al, 1994; Nielsen et al, 1986; Shirreffs et al, 2007a) and this may also be beneficial for rehydration. Maughan et al (1994) reported that the addition of either potassium (25mmol/L) or sodium (60mmol/L) to a rehydration drink proved equally effective in retaining fluid although their effects were not additive when ingested in a volume equal to BM loss. However, it would seem that drinks which contain primarily potassium may not promote the recovery of fluid balance as favourably as sodium when fluid is given in amounts equal to 150% of BM loss (Shirreffs et al, 2007a). Shirreffs et al (2007a) determined the effectiveness of 4 commonly used beverages in restoring fluid balance after exercise-induced dehydration of 1.9% BM. These included a carbonated water/apple juice mixture (8 mmol Na<sup>+</sup>/L and 30mmol K<sup>+</sup>/L), a sports drink (23mmol

 $Na^{+}/L$  and 6mmol K<sup>+</sup>/L) and 2 brands of mineral water (0-1mmol Na<sup>+</sup>/L and 0mmol K<sup>+</sup>/L). At the end of the 4h recovery period, subjects were considered euhydrated when a sports drink was consumed, but hypohydrated when water and the carbonated water/apple juice mixture were consumed.

#### 1.13.2 Effect of drink volume

For complete fluid restoration following dehydration, a volume greater than sweat loss must be consumed due to the on-going obligatory urine, faecal and respiratory losses that persist despite individuals being in a body water deficit (Shirreffs et al, 1996; Mitchell et al, 2000). Shirreffs et al (1996) reported that 150% of BM loss needs to be consumed if fluid balance is to be fully restored, and maintained, during the ensuing hours after rehydration has ceased. In some circumstances, the consumption of fluid equivalent to 120% of BM loss may be sufficient if the rehydration period is extended from 1 to 3h (Kovacs et al, 2002) as the perturbations to plasma volume and sodium concentration are reduced.

# 1.13.3 Effect of food ingestion

The addition of sodium to fluids ingested after exercise is not the only delivery method that has been investigated. The efficacy of food ingestion during the rehydration period has also been reported (Ray et al, 1998; Maughan et al, 1996). Ray et al (1998) administered 350mL of either water, CHO-E (containing 16mmol Na<sup>+</sup>/L), chicken broth (110mmol  $Na^{+}/L$ ) or soup (334mmol Na<sup>+</sup>/L) followed by water ingestion at a rate equivalent to sweat loss to subjects who had dehydrated by 2.5% BM. Despite the small volume of test drinks given, subjects excreted significantly less urine on the chicken broth and soup trials than water. This was attributed to the higher sodium intake during the chicken broth and soup trials. Maughan et al (1996) had 8 subjects cycle intermittently in the heat until they were dehydrated by 2.1% BM. Subjects were then fed either a carbohydrate-electrolyte drink (containing 21mmol/L of sodium) or a standardised meal plus fluid, so that water intake corresponded to 150% of BM loss in both trials. Cumulative urine volume was lower when the meal was consumed and as a result individuals were considered euhydrated at the end of the 6h recovery period but in negative fluid balance when only the sports drink was consumed. Again these findings were attributed to the higher electrolyte content of the test meal (63mmol of sodium and 21mmol of potassium) than was provided by the carbohydrate-electrolyte drink (42mmol of sodium and 7mmol of potassium). The consumption of food also stimulates fluid intake (Szlyk et al, 1990) and there appears a

general consensus that athletes seem capable of replacing sweat losses with normal food and fluid intake when the interval between exercise bouts is more than 24 h (Casa et al, 2000). When repeated exercise sessions are scheduled on the same day or the fluid deficit is large, the importance of re-establishing fluid and electrolyte balance is accentuated and more aggressive rehydration strategies may need to be undertaken (Kovacs et al, 2002).

Takamata et al (1994) showed that despite ad libitum access to water, individuals who refrained from consuming sodium during the same 23h post-exercise time period failed to return to euhydration. In a more recent study Godek et al (2005) determined whether American Football players exposed to multiple exercise sessions each day could replace the fluid losses incurred. They found that players failed to replace sweat losses incurred each day as indicated by changes in BM and the specific gravity of urine samples, despite the provision of fluids. Although dietary sodium intake and 24h urine sodium excretion were not measured and therefore sodium balance could not be calculated, they found that despite educating the players on the importance of salting their food, urine sodium concentrations were depressed throughout the 8-day study period. This occurred despite an estimated salt intake of 8-11g per day. For some individuals urine sodium was undetectable in a number of samples which they suggested to indicate a negative sodium balance.

## **1.14** Aims of this thesis

The aims of this thesis are to:

- 1. Determine current sodium, potassium and chloride intakes of healthy recreationally active individuals (Chapters 3, 4, 5 and 7).
- 2. Determine whether exercise affects sodium intake (Chapters 5 and 7).
- 3. Determine whether exercise affects urine sodium excretion (Chapters 5 and 7).
- 4. Determine whether exercise affects sodium balance (Chapters 5 and 7).
- 5. Determine whether exercise affects dietary potassium intake, urine potassium excretion and potassium balance (Chapters 5 and 7).
- 6. Determine whether prior exercise alters sweat electrolyte composition during a second exercise session later that same day (Chapters 6 and 7).
- 7. Determine whether the high electrolyte content of milk is effective in restoring fluid balance post-exercise (Chapter 8).
- 8. Identify whether there are specific situations or individuals that may require an increased sodium intake (Chapters 5, 6, 7 and 8).

# Chapter 2

# General methods

## 2.1 Introduction

This chapter will describe the experimental, analytical and statistical procedures that were implemented in the following chapters of this thesis. Any deviation from the methods described here will be detailed in that specific chapter.

## 2.2 Ethical approval

All studies reported in this thesis were approved by the Loughborough University Ethical Advisory Committee and carried out in the physiology laboratories at Loughborough University. All volunteers were informed of the purpose and procedures involved in each study, including the potential risks and nature of the exercise tasks (in chapters 4, 6, 7 and 8). Each subject gave their full written and verbal consent and completed a health screen questionnaire prior to the start of each experiment. By doing so they agreed to participate in the study on the understanding that they had the right to withdraw at any time.

## 2.3 Subjects

Volunteers were recruited for all studies by word of mouth, email, posters or in person from the staff and student population at Loughborough University. All were considered healthy and recreationally active and aged between 18 and 35 years of age. Subjects were not acclimatised to the heat.

# 2.4 Preliminary trials

## 2.4.1 Familiarisation

No familiarisation trial was completed in chapters 4, 6 and 7 as blood samples were not required in the aforementioned chapters, nor was the exercise task used as an indicator of performance.

## 2.4.2 Anthropometry

In chapters 3, 4 and 8, body mass (BM) was measured to the nearest 0.01kg (CFW-150K, Adam Equipment Co Ltd, Milton Keynes, UK). In chapters 5, 6 and 7, BM was measured to the nearest 0.02kg (AFW-120K, Adam Equipment Co Ltd, Milton Keynes, UK). Height was measured to the nearest 0.5cm using a stadiometer. In chapter 3, body mass index (BMI) was calculated using the formula (BM in kg) / (Height in m)<sup>2</sup>. Skinfold measurements were taken on the right hand side of the body at 4 sites (biceps, triceps,

subscapular, suprailiac) in triplicate using Harpenden Skinfold Callipers. Body fat was then estimated according to the method of Durnin & Rahaman (1974).

## 2.5 Pre-trial standardisation

No pre-trial standardisation was carried out in chapters 3, 4, 5 and 7, because the aim of these experiments was to study the volunteers in normal, everyday circumstances and therefore any restrictions on activity or diet were carefully avoided. In chapters 6 and 8, subjects were asked to keep a diary of their dietary and exercise patterns for the 48h period preceding the first experimental trial and were asked to replicate this behaviour prior to the second trial. Subjects were asked to refrain from strenuous exercise and alcohol intake during the 24h period before each trial. In a further attempt to standardise the state of hydration and electrolyte balance prior to each trial, subjects arrived in the laboratory after an overnight fast, only having consumed 500mL of water 1.5h beforehand.

## 2.6 Urine collection and analysis

## 2.6.1 24 h urine collection

Twenty-four hour urine collections were made in chapters 3, 4, 5 and 7. On the first day of collection the subject's first pass of urine was not collected but instead they made a note of the time this was done. They now had an empty bladder which started the collection period. From then on, all urine was collected into a plastic container; the volume measured using a 500mL measuring cylinder, a 5mL sample retained for analysis and the time recorded. The remaining urine was flushed down the toilet and the cylinder rinsed with water. This was repeated for all subsequent voids. The 24h urine collection was completed by going to the toilet at the same time the next morning. Participants were encouraged to make this as close to 24h as possible. This process was continued for all 5 days. The multiple samples that made up each 24h collection were returned to the laboratory daily.

#### 2.6.2 Laboratory-based urine collection

In chapters 6 and 8, subjects urinated at specific time points during the trial, details of which are given in each chapter. On each occasion they were asked to empty their bladder as completely as possible with the entire volume collected and a 5mL sample retained for subsequent analysis.

## 2.6.3 Urine analysis

The total volume of each urine sample was measured using a 500mL measuring cylinder and a 5mL sample retained in a small container for analysis. Upon the return of each 24h urine collection to the laboratory in chapters 5 and 7, samples were centrifuged at 1500g for 15 minutes, before 1.5mL was dispensed into a small container and immediately frozen at -20°C for creatinine analysis. The remaining 3.5mL of each sample was stored at 4°C and later analysed for sodium and potassium by flame photometry (Clinical flame photometer 410C, Corning, Halstead, UK), chloride by coulometric titration (Jenway Ltd, Dunmow, UK) and osmolality by freezing point depression (Osmomat 030, Gonotec GmbH, Gonotec, Berlin, Germany). Creatinine was analysed using the kinetic colorimetric method (Fixed rate) Jaffe reaction without de-proteinisation (Jaffé, 1886). In chapters 3, 4, 6 and 8, urine samples were not centrifuged as creatine was not measured, but urine electrolytes were analysed using the techniques described above. Samples were analysed in duplicate and the equipment was calibrated before, during and after sample analysis within the physiology laboratory at Loughborough University to enhance the accuracy of each technique.

## 2.7 Sweat collection and analysis

#### 2.7.1 Regional collection

Regional sweat collection took place in chapters 4, 6 and 8. Sweat patches consisted of a transparent dressing (5cm x 7cm) which enclosed an absorbent pad with dimensions, 2.5cm x 4cm (Tegaderm, 3M, Loughborough, UK). To determine the extent of any electrolyte contamination of sweat patches, de-ionised water was added to 5 sweat patches. The values obtained for sodium and potassium were  $1 \pm 1 \text{ mmol/L}$  and  $0.1 \pm 0.1 \text{ mmol/L}$ , respectively. When a sodium chloride solution of known concentration (50 mmol/L) was added to 5 sweat patches, values obtained for sodium were  $52 \pm 1 \text{ mmol/L}$ . Five sterile syringes (that are used to extract samples of sweat from the absorbent pads) were found to be free from contamination of sodium ( $0 \pm 0 \text{ mmol/L}$ ) and potassium ( $0 \pm 0 \text{ mmol/L}$ ).

In chapters 4 and 6, sweat patches were placed at 4 sites on the right-hand side of the body before exercise. These skin sites were the scapula (over the spine of the scapula and ~7cm lateral from the vertebral column), chest (superior to the nipple), forearm (mid-dorsal) and thigh (mid-ventral) (Patterson et al, 2000). Before patch application the skin was cleaned with distilled, de-ionised water to remove any residual sweat or cosmetic products that may

have been present. Upon the cessation of the third exercise bout, patches were removed using tweezers and placed into a 10mL sterile syringe. A sample was immediately extracted into a small container and stored for later analysis. A similar procedure was followed in chapter 8 except with two absorbent patches. Both patches were placed on the subject's scapula at the onset of exercise. One was removed after approximately 1% BM had been lost and the other upon the cessation of the final exercise bout. Electrolyte analysis was carried out as described for urine except for sweat patches obtained in chapter 6, which were analysed by ion chromatography (DX-80 Ion Analyser, Dionex). Estimates of whole body sweat electrolyte composition were made by taking the average electrolyte concentration at each regional sweat collection site.

## 2.7.2 Whole body sweat collection

In chapters 6 and 7 a whole body washdown technique (Shirreffs & Maughan, 1997) was implemented. Subjects were asked to shower with water and soap before rinsing with 4 litres of distilled water which had been divided into 4 sports drink bottles. The latter took place in a stainless steel tray. Subjects then dried themselves with a pre-washed towel and put on a pair of overshoes and a pre-washed gown before entering the environmental chamber which was maintained at approximately 35°C and 60% relative humidity (RH). Nude BM (with the exception of overshoes) was measured before subjects entered a polyethylene bag which contained the cycle ergometer (Monark) and pre-washed shorts. The polyethylene bag was stretched over a plastic frame so that it did not touch the subject's skin. Subjects commenced the dehydration procedure which involved intermittent exercise on a cycle ergometer at an intensity which corresponded to  $\sim 2 \text{ W/kg}$ BM. Exercise periods of 10 minutes were separated by 5 minutes of rest, during which subjects remained inside the bag. This pattern continued until 40 minutes of exercise was completed. Upon cessation of exercise, subjects washed themselves with 4 litres of 20 mmol/L ammonium sulphate solution. A further 1 litre of ammonium sulphate solution (20mmol/L) was used to wash down the bike and bag before the bag contents were mixed and duplicate samples obtained for analysis. After a shower, final nude BM was obtained.

To determine the extent of any electrolyte contamination during the washdown procedure, the bike, plastic frame, polyethylene bag and shorts were all assembled and then washed with 5 litres of de-ionised water without any subject present. This protocol was carried out on two occasions. The values indicated the procedure to be free from contamination of

sodium (0  $\pm$  0mmol/L) and potassium (0  $\pm$  0mmol/L). To assess the recovery of electrolytes, the assembly procedure was repeated as previously described. One litre of a solution containing 50mmol of sodium chloride and 5mmol of potassium chloride was added, followed by an additional 5 litres of an ammonium sulphate solution (20mmol/L). This protocol was carried out on two occasions. The values obtained for sodium and potassium were 49.4  $\pm$  1.9mmol/L and 4.2  $\pm$  0.1mmol/L, respectively. This process was again repeated but in the presence of a subject who had previously undergone the shower and washdown procedure. The total duration of time between the start of the washdown procedure to obtaining a sample was approximately 11-12 minutes. During this time the polyethylene bag contents were mixed and duplicate samples were taken from the bottom of the bag for analysis. This protocol was carried out on four occasions. The values obtained for sodium and potassium were 52  $\pm$  3mmol/L and 4.5  $\pm$  0.3mmol/L, respectively. Whole body sweat samples were analysed by ion chromatography (DX-80 Ion Analyser, Dionex).

### 2.8 Dietary analysis

In chapters 6 and 8, subjects standardised their diet for the 48h period preceding each trial using household measures as descriptors of the food consumed, but no dietary analysis was undertaken on this data. In chapters 4, 5 and 7, subjects were asked to weigh (to the nearest 1g) and record all food and drink consumed during 5 consecutive days using food scales (Ohaus LS2000, New Jersey, USA). In chapters 4 and 7, subjects consumed their normal diet under free-living conditions which varied from day to day. In chapter 4, subjects were asked to consume the same amount and type of food each day. In all cases their diet was analysed for energy, protein, carbohydrate, fat, fibre, sodium, potassium and chloride using specialist dietary software (Compeat Pro 5.8.0). The validity of this software was checked by comparing 30 named products to current food labels. The dietary analysis software was found to overestimate sodium values by  $41 \pm 60$  mg/serving (2  $\pm$ 3mmol/serving), but this was not statistically significant (P=0.673). The amount of herbs, spices and discretionary salt used in chapter 5 was determined by the use of household measures (for example 1 tsp,  $\frac{1}{2}$  tsp). In chapters 4 and 7, discretionary salt use was assessed by using a saltshaker which was provided on day 1 and measured to the nearest 1mg (Mettler Toledo AG245, UK) in the laboratory before and after its use.

## 2.9 Blood collection, sampling and analysis

Blood samples were taken only in chapter 8. The details of blood collection and analysis can be found in that specific chapter.

# 2.10 Coefficients of variation

The coefficient of variation [(SD/Mean)\*100] for the analytical procedures implemented in this thesis was calculated from 30 random samples of each assay (Table 2.1).

**Table 2.1**Mean, standard deviation and coefficient of variation of duplicate samples<br/>using the analytical procedures administered during experimental chapters<br/>in this thesis.

| Assay                                | Method                                 | Mean (SD)                    | CV (%) |
|--------------------------------------|--|------------------------------|--------|
|                                      |  |                              |        |
| Urine Na <sup>+</sup>                | Flame Photometry                       | $104 \pm 48 \text{ mmol/L}$  | 0.7    |
| Urine K <sup>+</sup>                 | Flame Photometry                       | $57 \pm 30 \text{ mmol/L}$   | 1.1    |
| Urine Cl <sup>-</sup>                | Coulometric Titration                  | $84\pm56\ mmol/L$            | 1.4    |
| Urine Osmolality                     | Freezing Point Depression              | $480\pm285\ mosmol/kg$       | 0.4    |
| Urine Creatinine                     | Jaffe Method                           | $94 \pm 59 \text{ mg/dL}$    | 1.4    |
|                                      |  |                              |        |
| Sweat Na <sup>+</sup>                | Flame Photometry                       | $60 \pm 19 \text{ mmol/L}$   | 1.1    |
| Washdown Solution (Na <sup>+</sup> ) | Ion Chromatography                     | $51 \pm 35 \text{ mmol/L}$   | 3.0    |
| Sweat K <sup>+</sup>                 | Flame Photometry                       | $4.8\pm0.7~mmol/L$           | 1.8    |
| Washdown Solution (K <sup>+</sup> )  | Ion Chromatography                     | $5.1 \pm 1.3 \text{ mmol/L}$ | 2.6    |
| Sweat Cl                             | Coulometric Titration                  | $54\pm20\ mmol/L$            | 1.8    |
| Washdown Solution (Cl <sup>-</sup> ) | Ion Chromatography                     | $49 \pm 34 \text{ mmol/L}$   | 2.3    |
|                                      |  |                              |        |
| Serum Na <sup>+</sup>                | Flame Photometry                       | $140\pm2\ mmol/L$            | 0.9    |
| Serum K <sup>+</sup>                 | Flame Photometry                       | $6.4\pm0.8\ mmol/L$          | 1.9    |
| Serum Cl <sup>-</sup>                | Coulometric Titration                  | $104 \pm 3 \text{ mmol/L}$   | 0.8    |
| Serum Osmolality                     | molality Freezing Point Depression 281 |                              | 0.3    |
| Blood Glucose                        | d Glucose GOD-PAP (Randox)             |                              | 1.2    |
| Haemoglobin                          | Cynamethemoglobin                      | $14.9 \pm 0.8 \ g/100 mL$    | 0.7    |
| Haematocrit                          | Microcentrifugation                    | $44 \pm 2$ %                 | 0.4    |

## 2.11 Statistical analysis

The statistical procedures employed in this thesis are detailed in the methods section of each experimental chapter. In summary, data was initially tested for normality. Data were then analysed by repeated measures ANOVA followed by paired t-tests with Holm-Bonferroni adjustment for multiple comparisons or Freidman's Test followed by Wilcoxon tests when found not to be normally distributed. Correlations were assessed using Pearson's correlation or Spearman's Rank when found to be not normally distributed. Data are expressed as mean  $\pm$  SD or median (range) when found not to be normally distributed. In some circumstances a range has been reported regardless of the distribution of data as it was deemed to provide further useful information. Statistical significance was set at P<0.05. Statistical analysis was performed using SPSS 16.0.

# Chapter 3

# Daily variation in electrolyte excretion

## 3.1 Introduction

Humans can survive on extremely low-sodium diets (Oliver et al, 1975) as obligatory urine, skin and faecal sodium losses are very small (1.7 to 8.0mmol of sodium per day; Dahl, 1958; Dahl et al, 1955; Dole et al, 1950). Nevertheless, dietary surveys report a wide range of sodium intakes well in excess of these values (Gregory et al, 1990; Henderson et al, 2003). In a recent UK-based survey (Henderson et al, 2003), the average dietary sodium and potassium intakes according to a 7-day weighed food diary were 144  $\pm$ 44mmol and  $86 \pm 17$ mmol for males and  $100 \pm 30$ mmol and  $68 \pm 19$ mmol for females. As the amount of sodium above basal requirements is excreted primarily in the urine, urinary sodium excretion can provide a good measure of intake in non-sweating individuals (Holbrook et al, 1984; Taseveska et al, 2006). Although potassium losses in faeces are greater than sodium, amounting to between 5–15mmol/day, urine potassium excretion can also provide a good measure of potassium intake (Holbrook et al, 1984; Taseveska et al, 2006). In the same UK-based survey as discussed previously (Henderson et al, 2003), the sodium and potassium intakes according to the collection of a single 24h urine sample were  $187 \pm 86$  mmol and  $81 \pm 33$  mmol for males and  $138 \pm 66$  mmol and  $67 \pm 30$  mmol for females (Henderson et al, 2003). The discrepancy between methods for sodium intake was attributed to the ability of urine collections to account for discretionary salt use. However, whilst urine sodium and potassium collections can provide an objective measure of dietary sodium and potassium intake, one-off 24h urine collections have been criticised by some (Sowers & Stumbo, 1986; Liu & Stamler, 1984; Caggiula et al, 1985; Dyer et al, 1997) although not all (Kesteloot & Joossens, 1990), as a measure of an individual's habitual intake due to the large day to day variation in urine electrolyte excretion.

In 2004, the Food Standards Agency in the UK launched a campaign aimed at reducing the average salt intake of the British population to 6g/day (2.4g/d of sodium). Since the survey of Henderson et al (2003) is based on data collected between 2000/01, the first aim of this study was to determine the daily excretion of sodium and potassium in a population of healthy subjects and to compare this to data collected previously (Henderson et al, 2003). The second aim was to determine the variation in urinary electrolyte excretion between multiple 24h urine collections.

# 3.2 Method

# 3.2.1 Subjects

Eighteen healthy volunteers (10 female, 8 male) participated in this study, which had received prior approval from the Loughborough University Ethical Advisory Committee (R05-P39). All subjects were informed about the experimental procedures and associated risks before their written consent was obtained. Their physical characteristics are shown in Table 3.1.

**Table 3.1**Physical characteristics of individuals. Values are mean  $\pm$  SD. \*denotes<br/>significant difference between males and females (P<0.05).</th>

|                          | Males        | Females         |
|--------------------------|--------------|-----------------|
| Body Mass (kg)           | 80.3 ± 15.5* | $62.9 \pm 10.6$ |
| Height (cm)              | $176 \pm 7*$ | $164 \pm 7$     |
| BMI (kg/m <sup>2</sup> ) | $26 \pm 4$   | $23 \pm 3$      |
| Age (y)                  | $23 \pm 1$   | $25 \pm 4$      |

# 3.2.2 Experimental protocol

The data presented here were collected as part of a study looking at the contamination of dietary supplements (Judkins et al, 2006). As a consequence, subjects reported to the laboratory each morning where they ingested 5g of creatine dissolved in 500mL of water, daily for 5 consecutive days. After supplement ingestion individuals were free to leave the laboratory and carry on with their normal daily activities. During one 5-day period, the creatine supplement was spiked with 10µg of 19-norandrostenedione (SPIKED), but not during a separate 5-day period (CONTROL). Figure 3.1 shows a schematic representation of the study protocol. A 5-day period of creatine supplementation was followed by a minimum 48h washout period and then creatine supplementation proceeded during a further 5-day period in the same manner. The order of supplement ingestion was randomised and administered in a crossover design. Body mass was measured at the beginning and end of the study period (CFW-150K, Adam Equipment Co Ltd, Milton Keynes, UK).

# 3.2.3 Urine collection

Twenty four hour urine collections were made on days 1 and 5 of each 5-day period. This corresponded to a Monday (day 1) and Friday (day 5) of each week. The 24h urine collection procedures are described fully in chapter 2.



**Figure 3.1** Schematic of the study period. 24h urine collection (U), first void of urine (AM) and creatine ingestion (Cr). Each 24h urine collection started the morning of one day and was terminated the morning on the following day, to account for the lag in electrolyte excretion.

## 3.2.4 Dietary intake

Apart from the ingestion of creatine (5g), water (500mL) and during the SPIKED trial 10µg of 19-norandrostenedione on the morning of days 1-5, volunteers consumed their normal diets, but did not weigh or record their food intake.

## 3.2.5 Physical activity

During the collection period, volunteers were free to continue their normal exercise patterns.

# 3.2.6 Sample analysis

Urine samples were analysed for sodium and potassium. Completeness of each 24h urinary collection was self-reported by individuals. All analytical procedures are described fully in Chapter 2.

# 3.2.7 Statistical analysis

All data were tested for normality of distribution. Data were then analysed by repeated measures ANOVA followed by paired t-tests with Holm-Bonferroni adjustment for multiple comparisons or Freidman's Test followed by Wilcoxon tests when found not to be normally distributed. Gender differences were analysed by independent T-tests where appropriate. Pearson's correlation was used to assess relationships between variables. Data are expressed as mean  $\pm$  SD. Statistical significance was set at P<0.05.

## 3.3 Results

## 3.3.1 Subject characteristics and body mass changes

Males were heavier (P=0.01) and taller (P=0.03) than females and tended to be younger (P=0.073) and have a higher BMI (P=0.083) (Table 3.1). There was an increase (P<0.05) in body mass over the entire study period for both males (0.46kg [range -0.05 to 1.78kg]) and females (0.48kg [range 0.05 to 0.77kg]).

# 3.3.2 Duration of urine collection

Subjects reported 64 out of 72 collections to be complete. Only collections reported to be complete were included in data analysis. There were no differences (P=0.863) in the duration of each day's urine collection which on average was 23.9h (range 20.0 - 27.3h) (Table 3.2). All urine data for each collection period were adjusted to 24h, with this value being used in all subsequent analysis. The within-individual CV for the duration of urine collection was 4% (range 1 - 10%).

| Table 3.2 | The duration | of each day' | s urine co | ollection. | Values are | median | (range) |
|-----------|--------------|--------------|------------|------------|------------|--------|---------|
|-----------|--------------|--------------|------------|------------|------------|--------|---------|

|                | SPIKED        |               | CONTROL       |               |  |
|----------------|---------------|---------------|---------------|---------------|--|
|                | Day 1         | Day 5         | Day 1         | Day 5         |  |
| Duration of    | 23.7          | 24.0          | 24.3          | 23.9          |  |
| Collection (h) | (20.0 - 24.7) | (21.5 - 25.5) | (22.0 - 25.8) | (22.0 - 27.3) |  |

# 3.3.3 Urine volume & electrolyte excretion

Urine volume, and sodium and potassium excretion during each 24h period are shown in Figure 3.2. There was no significant difference in the volume of urine excreted between each 24h collection period (P=0.131). Urine volume was not significantly different between males and females in any 24h collection period (P=0.164), but when expressed as the average daily urine volume over the 4 collection periods, urine volume tended to be greater for females ( $2443 \pm 702$ mL) than males ( $1921 \pm 513$ mL; P=0.098).

There was no significant difference in the amount of sodium excreted in the urine between each 24h collection period (P=0.107). Urine sodium excretion was significantly higher for males than females on day  $1_{spiked}$  (P=0.009), but not during any other 24h collection period. When expressed as the average daily urine sodium excretion over the 4 collection periods, urine sodium excretion was significantly higher in males (200 ± 48mmol) than females (157 ± 33mmol; P=0.035).

There was no significant difference in the amount of potassium excreted in the urine between each 24h collection period (P=0.767). There were no gender differences in urine potassium excretion during any 24h collection period (P=0.668), neither was there a difference in the average urine potassium excretion over the 4 collection periods between males ( $114 \pm 32$ mmol) and females ( $103 \pm 20$ mmol; P=0.379).

The average urine volume, sodium and potassium excretion from all 24h urine collections for males and females combined was  $2220 \pm 854$ ml,  $176 \pm 68$ mmol and  $106 \pm 27$ , respectively.

The coefficients of variation (CV) for urine volume  $(18 \pm 8\% \text{ vs } 23 \pm 11\%; \text{ P=0.271})$ , urine sodium  $(31 \pm 14\% \text{ vs } 30 \pm 13\%; \text{ P=0.817})$  and urine potassium  $(15 \pm 7\% \text{ vs } 17 \pm 9\%; \text{ P=0.742})$  excretion were not different between males and females, respectively. Table 3.3 shows the CV for urine volume and electrolyte excretion when the values for males and females were pooled into one data set. All subsequent analysis was carried out on pooled data from males and females.

**Table 3.3**The within-individual CV (%) in urine excretion for day 1 (from trial<br/>SPIKED and trial CONTROL), day 5 (from trial SPIKED and trial<br/>CONTROL), trial SPIKED (day 1 and 5), trial CONTROL (day 1 and 5)<br/>and over all days.

|                 | Day 1       | Day 5       | SPIKED      | CONTROL     | All Days    |
|-----------------|-------------|-------------|-------------|-------------|-------------|
| Urine Volume    | $17 \pm 12$ | $18 \pm 16$ | $22 \pm 14$ | $21 \pm 16$ | $21 \pm 10$ |
| Urine Sodium    | $23 \pm 19$ | $28\pm20$   | $16 \pm 13$ | $38 \pm 24$ | $30 \pm 13$ |
| Urine Potassium | $12 \pm 9$  | $16 \pm 15$ | $13 \pm 9$  | $13 \pm 11$ | $16 \pm 8$  |



**Figure 3.2** Urine Volume (A), sodium (C) and potassium (E) excreted during each 24h collection period. Gender comparisons are shown for urine volume (B), sodium (D) and potassium (F) during each 24h collection period. \*denotes significant difference between males and females (P<0.05).
#### 3.3.4 Relationship in the excretion of urine parameters between each 24h collection

The relationship of urine volume excretion between each 24h period was moderate to strong (Table 3.4), but was not strengthened if 24h urine collections were collected in the same week (r = 0.40 and r = 0.68) or on the same day of different weeks (r = 0.63 and r = 0.71). The relationship of urine sodium excretion between each 24h period was weak to moderate (Table 3.4), but was not strengthened if 24h collections were collected in the same week (r = 0.00 and r = 0.42) or on the same day of different weeks (r = 0.11 and r = 0.43). The relationship of urine potassium excretion between each 24h period was moderate (Table 3.4), but was not strengthened if 24h collections were collected in the same week (r = 0.45 and r = 0.70) or on the same day of different weeks (r = 0.50 and r = 0.55).

**Table 3.4**The relationship of urine volume or urine electrolyte excretion between<br/>each 24h period. Values are correlation coefficients. \*denotes significant<br/>relationship (P<0.05).</th>

|   | Urine Vol                  | Urine Vol                  | Urine Vol                   | Urine Vol                   |
|---|----------------------------|----------------------------|-----------------------------|-----------------------------|
|   | (Day 1 <sub>spiked</sub> ) | (Day 5 <sub>spiked</sub> ) | (Day 1 <sub>control</sub> ) | (Day 5 <sub>control</sub> ) |
| Urine Vol (Day 1 <sub>spiked</sub> )              |                            | 0.40                       | 0.71*                       | 0.78*                       |
| Urine Vol (Day 5 <sub>spiked</sub> )              |                            |                            | 0.80*                       | 0.63*                       |
| Urine Vol (Day 1 <sub>control</sub> )             |                            |                            |                             | 0.68*                       |
| Urine Vol (Day 5 <sub>control</sub> )             |                            |                            |                             |                             |
|   | Urine Na <sup>+</sup>      | Urine Na <sup>+</sup>      | Urine Na <sup>+</sup>       | Urine Na <sup>+</sup>       |
|   | (Day 1 <sub>spiked</sub> ) | (Day 5 <sub>spiked</sub> ) | (Day 1 <sub>control</sub> ) | (Day 5 <sub>control</sub> ) |
| Urine Na <sup>+</sup> (Day 1 <sub>spiked</sub> )  |                            | 0.42                       | 0.43                        | 0.54*                       |
| Urine Na <sup>+</sup> (Day 5 <sub>spiked</sub> )  |                            |                            | 0.35                        | 0.11                        |
| Urine Na <sup>+</sup> (Day 1 <sub>control</sub> ) |                            |                            |                             | 0.00                        |
| Urine Na <sup>+</sup> (Day 5 <sub>control</sub> ) |                            |                            |                             |                             |
|   | Urine K <sup>+</sup>       | Urine K <sup>+</sup>       | Urine K <sup>+</sup>        | Urine K <sup>+</sup>        |
|   | (Day 1 <sub>spiked</sub> ) | (Day 5 <sub>spiked</sub> ) | (Day 1 <sub>control</sub> ) | (Day 5 <sub>control</sub> ) |
| Urine K <sup>+</sup> (Day 1 <sub>spiked</sub> )   |                            | 0.70*                      | 0.55*                       | 0.37                        |
| Urine $K^+$ (Day $5_{spiked}$ )                   |                            |                            | 0.75*                       | 0.50                        |
| Urine K <sup>+</sup> (Day 1 <sub>control</sub> )  |                            |                            |                             | 0.45                        |
| Urine K <sup>+</sup> (Day 5 <sub>control</sub> )  |                            |                            |                             |                             |

#### 3.3.5 Relationship between urine volume and urine electrolyte excretion

Urine sodium excretion was unrelated to urine volume during each 24h collection period or when each was expressed as the average excretion over the study period (Table 3.5). The relationship between urine potassium excretion and urine volume showed great variation between collection periods, but this relationship was moderate and significant when expressed as the average excretion over the study period (Table 3.5).

| (P<0.05).             |       |       |       |       |         |
|-----------------------|-------|-------|-------|-------|---------|
|                       | SPI   | KED   | CON   | FROL  | All Day |
|                       | Day 1 | Day 5 | Day 1 | Day 5 | Average |
| Urine Volume & Sodium | 0.09  | 0.07  | -0.22 | 0.24  | -0.08   |

0.74\*

0.60\*

0.21

0.54\*

0.45

**Table 3.5**The relationship between urine volume and urine electrolyte excretion.<br/>Values are correlation coefficients. \*denotes significant relationship<br/>(P<0.05).

#### 3.3.6 Relationship between urine sodium and potassium excretion.

Urine Volume & Potassium

On day  $5_{spiked}$ , the relationship between sodium and potassium excretion was moderate and significant, but during all other 24h collection periods and when data was expressed as the average over all 4 days, the relationship between sodium and potassium excretion was weak (Table 3.6).

Table 3.6The relationship between urine sodium and urine potassium excretion.<br/>Values are correlation coefficients. \*denotes significant relationship<br/>(P<0.05).</th>

|                          | SPI   | KED   | CONTROL |       | All Day |
|--------------------------|-------|-------|---------|-------|---------|
|                          | Day 1 | Day 5 | Day 1   | Day 5 | Average |
| Urine Sodium & Potassium | 0.21  | 0.51* | 0.28    | 0.27  | 0.37    |

#### 3.4 Discussion

This study found that 6 males and 9 females excreted more sodium in the urine than the average male and female in the UK (Henderson et al, 2003). Although there was no significant difference in urine sodium excretion between days, there was no relationship between the amount of sodium excreted in each 24h urine collection which suggests that a single 24h urine collection is not representative of an individual's habitual sodium intake.

#### 3.4.1 Body mass

There was a small, but significant increase in body mass during the study period. Body mass measurements were separated by a minimum of 2 weeks and therefore it is possible that the gain in body weight was the result of an imbalance between energy intake and energy expenditure. Nevertheless, given that subjects were not asked to eat/refrain from specific food types or to weigh and record food intake, disruption to their normal eating patterns would be expected to be minimal and therefore the data presented here are assumed to be representative of their normal dietary intake. Several studies have reported that a rapid creatine loading protocol (20g creatine/day for 5-6 days) results in a body mass

increase of 0.5 to 1.0kg (Hultman et al, 1996). This is due to the retention of fluid, as urine volume declines during this corresponding period (Hultman et al, 1996). Although the gain in body mass was not as great in the current study, subjects ingested only 5g of creatine per day which increases muscle creatine levels more gradually (Hultman et al, 1996) and would be expected to result in less fluid retention. No differences in urine volume were observed in this study between the first and fifth day of each 5-day period which is similar to the findings of Hultman et al (1996). They reported urine volume to return to normal after 3 days of creatine supplementation, despite the higher creatine intake (20g/day) than in the current study (5g/day).

#### 3.4.2 Sodium

On average subjects excreted 176mmol of sodium per day, with males  $(200 \pm 48 \text{mmol})$  excreting significantly more sodium than females  $(157 \pm 33 \text{mmol})$ . In total 6 males and 9 females excreted more sodium than the average reported urine sodium excretion for males (187mmol/day) and females (138mmol/day) in the UK (Henderson et al, 2003). Sodium is found in a vast array of foods, but is found in large quantities in processed foods (Henderson et al, 2003). Despite targets set by the government directed at food manufacturers to reduce the amount of salt added during food processing, there is a trend for an increased sodium intake (Gregory et al, 1990; Henderson et al, 2003) in the British population. This trend could still be persisting, but it must be considered that subjects were not asked to record their dietary intake and therefore because of the positive relationship between sodium intake and energy intake that has been reported (Bingham et al, 1994; Holbrook et al, 1984; Pietinen, 1982), the higher sodium intake of subjects may solely be due to a higher energy intake, rather than a preference for salty foods.

Although no significant difference was found in the amount of sodium excreted in the urine between each 24h period, the relationship of sodium excretion between separate 24h collections was weak and there appeared to be no improvement in the relationship when collections were made in the same week (eg Day  $1_{control}$  and Day  $5_{control}$ ) or on the same days of the week (eg Day  $1_{spiked}$  and Day  $1_{control}$ ). The wide daily variation in sodium excretion within individuals has led to a criticism by some (Sowers & Stumbo, 1986; Liu & Stamler, 1984; Caggiula et al, 1985; Dyer et al, 1997) although not all (Kesteloot & Joosens, 1990) of the ability of a single 24h urine collection to accurately estimate an individual's habitual sodium intake. The within-individual variation in sodium excretion

was 30% in the current study, which is at the lower end of the range reported in the literature (30 - 43%; Day et al, 2001; Caggiula et al, 1985; Bingham et al, 1988; Sowers & Stumbo, 1986; Knuiman et al, 1988). Whilst the variation in urine sodium excretion may reflect a variation in sodium intake, urine sodium excretion can also be influenced by several other factors including potassium intake (Van Buren et al, 1992), fibre intake (Cummings et al, 1976) and losses of sodium through sweating (Holbrook et al, 1984; Consolazio et al, 1963). In the current study there was only a weak relationship between potassium excretion and sodium excretion. Previously, Van Buren et al (1992) have reported the oral administration of potassium salts to cause an immediate increase in potassium and sodium excretion. Despite this acute effect, sodium excretion returned to normal levels quickly and resulted in no significant increase in cumulative sodium excretion over the 8h monitoring period, which is considerably shorter than the 24h monitoring period in the current study.

Although a large proportion of dietary sodium is excreted in the urine, some investigators have reported a seasonal variation in the percentage of dietary sodium being excreted in the urine. A lower percentage of dietary sodium is excreted in the urine during the summer months and this is attributed to the loss of sodium in sweat (Holbrook et al, 1984; James et al, 1987). Sweat sodium losses can be large and influence the amount of sodium present in the urine (Consolazio et al, 1963; Robinson et al, 1955; Godek et al, 2005; Lichton 1957). Lichton (1957) exposed one heat-acclimated subject to the heat on 48 separate occasions. Each 1h exposure to the heat involved cycling exercise. It was concluded that the sweat glands have priority over the kidneys in the demand for salt and therefore urine sodium excretion is reduced due to the loss of sodium in sweat. Subjects in the current study were not restricted in their daily activities, nor were these recorded, so it cannot be ruled out that sweat losses contributed to the day to day variation in urine sodium excretion.

Whilst the completeness of 24h urine collections or alterations in the duration of each collection are potential factors responsible for a variation in urine sodium excretion, incomplete urine collections were omitted from analysis and all urine collections were corrected to 24h. Although creatinine has been used as a marker of completeness, it is not without its limitations (Bingham & Cummings, 1985; Flynn et al, 1990). Flynn et al (1990) found the self-reporting method to be more accurate than creatinine in assessing the completeness of urine collections and taken in combination with the similar duration of

each collection seen in the current study, subject self-report was deemed to be an adequate method in the determination of completeness.

#### 3.4.3 Potassium

Subjects excreted on average 106 mmol of potassium per day. All 8 males and 10 females excreted more potassium than the average reported urine potassium excretion for males (81 mmol/d) and females (67 mmol/d) in the UK (Henderson et al, 2003). This supports the notion for a growing trend for higher potassium intakes (Henderson et al, 2003). However, because a relationship between potassium and energy intake has been reported (Bingham et al, 1994; Holbrook et al, 1984; Pietinen 1982), the higher potassium intakes found in this study may be due to an increased energy intake, although this is purely speculative as energy intake was not measured.

The amount of potassium excreted in the urine was similar between each 24h collection period and the relationship of potassium excretion between each 24h collection was Nevertheless, their appeared to be no improvement in the relationship if moderate. collections were made in the same week (eg Day 1<sub>control</sub> and Day 5<sub>control</sub>) or on the same days of the week (eg Day  $1_{spiked}$  and Day  $1_{control}$ ). The strength of these relationships was stronger than those seen for urine sodium, possibly due to the lower day to day variation in potassium excretion (16%). This is also lower than those previously reported by other investigators (19%; Taseveska et al, 2006; Day et al, 2001; Willett 1990). Urine potassium excretion is suggested to provide an accurate measure of potassium intake (Taseveska et al, 2006), but the use of 24h urine potassium excretion as a marker of potassium intake has been questioned because faecal potassium excretion is greater and more variable than sodium (Cummings et al, 1976). Although faecal potassium excretion was not measured in the present study, it usually amounts to 5 to 15mmol per day (Cummings et al, 1976; Arn & Reimer, 1950; Mickelson et al, 1977), but is influenced by the fibre content of the diet (Cummings et al, 1976; Taseveska et al, 2006). However, fibre intake and therefore variation in fibre intake was not measured in this study, so the contribution to the variation in urine potassium excretion is unknown.

Sweat potassium losses have been suggested to result in a reduced percentage of dietary potassium being excreted in the urine during summer months (Holbrook et al, 1984), and as a consequence the loss of potassium in sweat may have also been responsible for some

of the variation in potassium excretion. However, in contrast to sodium, potassium is lost in relatively small amounts in sweat (Maughan & Nadel, 2000), especially when considered relative to urine losses, and it is therefore likely only to have a small effect.

#### 3.4.4 Conclusion

The average amount of sodium and potassium excreted in the urine was greater in this study than those reported in a recent survey of the British population (Henderson et al, 2003). Although no significant differences were found in the amount of sodium excreted between each 24h collection period, a lack of correlation between 24h collections supports the suggestion that a one-off 24h urine collection is not representative of an individual's habitual sodium intake. The variation in potassium excretion was smaller than sodium and resulted in moderate correlations in urine potassium between 24h collection periods.

## Chapter 4

## Variation in electrolyte excretion whilst consuming the same diet

#### 4.1 Introduction

The wide daily variation in sodium intake both between and within individuals has led to a criticism by some (Sowers & Stumbo, 1986; Liu & Stamler, 1984; Caggiula et al, 1985; Dyer et al, 1997) although not all (Kesteloot & Joosens, 1990) of the accuracy of one-off dietary collections in attempting to determine an individual's habitual sodium intake.

Sodium is found in a wide variety of everyday food items but is especially prevalent in manufactured foods (Henderson et al, 2003; James et al, 1987). The main reasons why salt is added to processed foods are for flavour, texture and preservation (SACN, 2003). Several methods have previously been used to assess the sodium, potassium and chloride intakes of individuals. These include weighed food records (Day et al, 2001; Gregory et al, 1990; Henderson et al, 2003), 24h recalls (Leiba et al, 2005; Espeland et al, 2001), food frequency questionnaires (Day et al, 2001; McKeown et al, 2001), duplicate portion analysis (Schacter et al, 1980; Clark & Mossholder, 1986) and urine collections (Holbrook et al, 1984; Clark & Mossholder, 1986; Henderson et al, 2003). Each method is associated with problems (Bingham, 1987), but most notable is the inability to accurately assess the discretionary salt use of an individual (Caggiula et al, 1985; Clark & Mossholder, 1986). Urine sodium and potassium excretion can provide an objective estimate of an individual's intake of these electrolytes from food and drink and can account for discretionary salt use, thus overcoming some of the problems with the other dietary methods. However, in addition to being directly affected by dietary sodium intake, urine sodium excretion is also affected by several other factors including sweat sodium loss (Lichton, 1957), potassium intake (Mickelson et al, 1977; van Buren et al, 1992) and hydration status (Ladd, 1951). The loss of sodium in sweat will lower the amount of dietary sodium excreted in the urine (Lichton, 1957), whereas an increase in potassium intake will increase urine sodium excretion (van Buren et al, 1992). As a result even when sodium intake is kept constant, the day to day variation in sodium excretion may still persist (Baldwin, 1960). Baldwin et al (1960) reported the magnitude of the day to day variation in sodium excretion to be related to dietary sodium intake, with those individuals who consume larger but constant amounts of sodium to have a greater variation.

The aim of this study was to determine the variation in electrolyte excretion whilst individuals were consuming constant self-selected diets.

#### 4.2 Method

#### 4.2.1 Subjects

Eight healthy male volunteers participated in this study, which had received prior approval from the Loughborough University Ethical Advisory Committee (R07-P20). All subjects were informed about the experimental procedures and associated risks before their written consent was obtained. Their physical characteristics (mean  $\pm$  SD) were: age 21  $\pm$  2y, height 1.76  $\pm$  0.07m, body mass 77.5  $\pm$  13.9kg and body fat 16  $\pm$  3%.

#### 4.2.2 Experimental protocol

Subjects reported to the laboratory at least one day prior to the commencement of the study period when they were given the equipment for dietary and urinary collections and a detailed briefing of the collection procedures. In addition, the logbooks provided contained written instructions about the dietary and urine collection process. Figure 4.1 shows a schematic representation of the study protocol. On the morning of day 1, subjects arrived in the laboratory and nude body mass (BM) was measured (AFW-120K, Adam Equipment Co Ltd, Milton Keynes, UK). Subjects returned to the laboratory each day only to return urine samples and on the morning of day 6, when a final BM was obtained.



**Figure 4.1** Schematic of the study period. Body mass (BM), dietary (D) and urine (U) collections. Each 24h urine collection started on the morning of one day and was terminated on the morning of the following day.

#### 4.2.3 Dietary monitoring

On day 1 of the collection period subjects were asked to consume a diet considered to be consistent with their normal dietary behaviour and to weigh all food and drink ingested. The food and corresponding amounts consumed on day 1 were then to be ingested on each of the remaining 4 days. The amount of discretionary salt used on day 1 was given to the subject in a small container on each of the remaining days. This was to be consumed with

the same meal each day. Water was allowed ad libitum during the 5-day period. The dietary collection procedures are described fully in Chapter 2.

#### 4.2.4 Urine collection

During the same 5-day period and on the morning of day 6, subjects were asked to collect all urine passed. On the first day of collection, the first pass of urine was collected, but was not included in any calculations apart from the assessment of hydration status. The 24h urine collection procedures are described fully in chapter 2.

#### 4.2.5 Physical activity

During the study, subjects were asked to refrain from any strenuous exercise that would incur sweat loss.

#### 4.2.6 Sample analysis

Urine samples were analysed for sodium, potassium, chloride and osmolality. Completeness of each 24h urinary collection was reported by subjects each day and assessed by creatinine analysis based upon the Jaffe method (Jaffé, 1886). Weighed food intakes were analysed using Compeat Pro 5.8.0 Software. All analytical procedures are described fully in Chapter 2.

#### 4.2.7 Statistical analysis

All data were tested for normality of distribution. As urine electrolyte excretion can provide an accurate estimate of electrolyte intake in non-sweating individuals (Holbrook et al, 1984), electrolyte intake assessed by weighed food diaries and 24h urine excretion were subject to a two-factor repeated measures ANOVA, followed by paired t-tests with Holm-Bonferroni adjustment for multiple comparisons. Other data were analysed by one-factor repeated measures ANOVA followed by paired t-tests with Holm-Bonferroni adjustment for multiple comparisons or Freidman's Test followed by Wilcoxon tests when found not to be normally distributed. Correlations were assessed using Pearson's correlation or Spearman's Rank when found not to be normally distributed. Parametric data are expressed as mean  $\pm$  SD and non-parametric data as median (range). In some circumstances a range has been reported regardless of the distribution of data as it was deemed to provide further useful information. Statistical significance was set at P<0.05.

#### 4.3 Results

#### 4.3.1 Body mass

Subjects lost 0.64kg (-3.18 to +0.03kg) in BM between the morning of day 1 and day 6 (Table 4.1). The coefficient of variation (CV) in BM between days 1 and 6 was  $1.2 \pm 0.8\%$  (range 0.0 to 2.1%).

**Table 4.1**BM on the first (day 1) and last day (day 6) of the study period. Values are<br/>median (range). \* denotes significantly different from day 1 (P<0.05).</th>

|                | Day 1            | Day 6            |
|----------------|------------------|------------------|
| Pody Mass (kg) | 73.24            | 72.60*           |
| Douy Mass (kg) | (64.39 - 109.64) | (64.09 - 106.46) |

#### 4.3.2 Duration of urine collection

There were no significant differences in the duration of each day's urine collection (Table 4.2; P>0.05), but all urine data for each collection period were adjusted to 24h, with this value being used in all subsequent analysis. The median duration of each urine collection over the 5-day period was 24.00h (23.00 – 25.00h). The within-individual CV for the duration of each urine collection was  $1 \pm 1\%$ .

**Table 4.2**The duration of each day's urine collection. Values are median (range).

|                | Day 1         | Day 2         | Day 3         | Day 4         | Day 5         |
|----------------|---------------|---------------|---------------|---------------|---------------|
| Duration of    | 24.00         | 24.00         | 24.00         | 24.00         | 24.00         |
| Collection (h) | (23.92-24.25) | (24.00-24.08) | (23.00-24.67) | (23.75-25.00) | (23.83-24.50) |

#### 4.3.3 Completeness of urine collection

Two subjects reported a failure to collect one complete 24h collection. There were no significant differences (P>0.05) in the amount of creatinine excreted during each 24h collection period regardless of whether the missed collections were included or excluded (Table 4.3) in data analysis. The CV for urinary creatinine excretion for the two subjects with incomplete collections was 9% and 20%. The incomplete collections have been omitted from the data reported here. The average amount of creatinine excreted in the urine each day over the 5-day period was 1900  $\pm$  283mg. The within-individual CV for urinary creatinine excretion was 9  $\pm$  5%.

**Table 4.3**Urine creatinine excretion during each 24h period. Values are mean  $\pm$  SD.

|                       | Day 1        | Day 2        | Day 3        | Day 4        | Day 5        |
|-----------------------|--------------|--------------|--------------|--------------|--------------|
| Urine Creatinine (mg) | $1838\pm201$ | $1862\pm306$ | $2018\pm261$ | $1867\pm355$ | $1912\pm302$ |

#### 4.3.4 Dietary intake and urine excretion

Urine volume, nutrient intake and urine electrolyte excretion during each 24h period are shown in Table 4.4, Table 4.5 and Figure 4.2, respectively. There were no significant differences (P>0.05) in energy, carbohydrate, protein, fat, fibre or fluid intake between days. The CV's for nutrient intakes are shown in Table 4.6.

Urine sodium excretion and dietary sodium intake did not change significantly over time (P=0.209; Figure 4.2). There was a tendency (P=0.082) for urine sodium excretion to exceed dietary sodium intake on day 1, but urine sodium was similar (P>0.05) to dietary sodium intake on days 2, 3, 4 and 5. The mean sodium intake and urine sodium excretion over the last 4 days of the study were  $170 \pm 23$ mmol/d and  $184 \pm 41$ mmol/d, respectively. One subject was a discretionary salt user. The amount of sodium added at the table (11.7mmol/d) represented 7% of their total sodium intake.

Dietary potassium intake and urinary potassium excretion did not change significantly over time (P=0.218; Figure 4.2). Urine potassium excretion was similar to dietary potassium intake in all 24h collection periods (P=0.423). The mean potassium intake and urine potassium excretion over the last 4 days of the study were  $108 \pm 39$ mmol/d and  $93 \pm 26$ mmol/d, respectively.

Dietary chloride intake and urinary chloride excretion did not change significantly over time (P=0.235; Figure 4.2). Urine chloride excretion was similar to dietary chloride intake in all collection periods (P=0.271). The mean chloride intake and urine chloride excretion over the last 4 days of the study were  $160 \pm 21$  mmol/d and  $151 \pm 46$  mmol/d, respectively.

The CV for urine sodium excretion was 23% over all days but tended to decline over the study period (P=0.062) (Table 4.6). Similarly, the CV for urine chloride excretion tended to decline as the study progressed (P=0.060), but the CV for urine potassium excretion was little affected (P=0.795).

|  | Day 1                                | Day 2            | Day 3             | Day 4              | Day 5                        | 5 Day<br>Average  | 4 Day<br>Average |
|--|--------------------------------------|------------------|-------------------|--------------------|------------------------------|-------------------|------------------|
| Urine Volume (mL)                                    | $2279 \pm 1324$                      | $2202\pm940$     | $2094\pm901$      | $1836\pm815$       | $1594\pm546$                 | $2004\pm917$      | $1942\pm816$     |
|  |                                      |                  |                   |                    |                              |                   |                  |
| Table 4.5 Dietary nutrien   Average). Values are mea | t intake during eacl<br>$m \pm SD$ . | h 24h period and | l the average ove | er all days (5 Day | <sup>7</sup> Average) and da | ays 2, 3, 4 and 5 | (4 Day           |
|  | Day 1                                | Day 2            | Day 3             | Day 4              | Day 5                        | 5 Day<br>Average  | 4 Day<br>Average |
| Water (g)  | $3238 \pm 1758$                      | $3104 \pm 1233$  | $2926\pm1092$     | $2858\pm1180$      | $2984 \pm 1237$              | $3022\pm1249$     | $2968 \pm 1123$  |
| Energy (kcal)  | $2598\pm550$                         | $2591\pm498$     | $2579\pm501$      | $2568\pm507$       | $2566 \pm 497$               | $2580\pm484$      | $2576\pm476$     |
| Carbohydrate (g)                                     | $368 \pm 114$                        | $365\pm104$      | $366\pm105$       | $360 \pm 107$      | $361 \pm 105$                | $364 \pm 102$     | $363 \pm 100$    |
| Protein (g)  | $127 \pm 30$                         | $125 \pm 31$     | $124 \pm 33$      | $124 \pm 31$       | $123 \pm 33$                 | $125 \pm 30$      | $124 \pm 31$     |
| Fat (g)  | $80 \pm 15$                          | $81 \pm 15$      | 80 ± 13           | $81 \pm 14$        | $80 \pm 14$                  | $80 \pm 13$       | 80 ± 13          |
| Fibre (mmol)   | $18.2\pm9.1$                         | $18.3\pm9.0$     | $18.3\pm9.0$      | $18.3 \pm 9.0$     | $18.7\pm9.0$                 | $18.4\pm8.5$      | $18.4\pm8.5$     |
|  |                                      |                  |                   |                    |                              |                   |                  |

**Table 4.4** Urine volume during each 24h collection period and the average over all days (5 Day Average) and days 2, 3, 4 and 5 (4 Day Average). Values are mean ± SD.

**Table 4.6**The within-individual CV (%) in nutrient intake and urine electrolyte<br/>excretion over all experimental days (5 Day), days 2, 3, 4 & 5 (4 Day), days<br/>3, 4 & 5 (3 Day) and days 4 & 5 (2 Day)

|              | <b>Dietary Intake</b> |             | Urine Ex   | cretion    |            |
|--------------|-----------------------|-------------|------------|------------|------------|
|              | 5 Day                 | 5 Day       | 4 Day      | 3 Day      | 2 Day      |
|              | Average               | Average     | Average    | Average    | Average    |
| Water        | $7\pm7$               |             |            |            |            |
| Energy       | $1 \pm 1$             |             |            |            |            |
| Carbohydrate | $2\pm 2$              |             |            |            |            |
| Protein      | $3 \pm 4$             |             |            |            |            |
| Fat          | $2 \pm 1$             |             |            |            |            |
| Fibre        | $2\pm 2$              |             |            |            |            |
| Sodium       | $2 \pm 2$             | $23 \pm 13$ | $13\pm5$   | $11 \pm 6$ | $9\pm7$    |
| Potassium    | $2 \pm 1$             | $13 \pm 6$  | $12 \pm 5$ | $13 \pm 6$ | $12 \pm 6$ |
| Chloride     | $1 \pm 2$             | $26 \pm 14$ | $16\pm7$   | $13\pm8$   | $10\pm7$   |

A



Figure 4.2 Dietary intake and urine excretion of sodium (A), potassium (B) and chloride (C) during each 24h period. All Values are mean  $\pm$  SD.

#### 4.3.5 Relationship between energy intake and electrolyte intake

The relationship between sodium intake and energy intake was weak and non-significant when each was expressed as the 5-day average (r = 0.20; P=0.630). The relationship between potassium intake and energy intake was strong and tended to be significant when expressed over all 5 days (r = 0.71; P=0.051). The relationship between chloride intake and energy intake was weak and non-significant when expressed over all 5 days (r = 0.30; P=0.466). The subject who ingested the highest amount of sodium and chloride consumed the second least amount of energy. When this subject was removed, the relationship between sodium intake and energy intake over the 5 days improved but remained non-significant (r = 0.64; P=0.124). Similarly, the relationship between chloride intake and energy intake over the 5 days improved (r = 0.54; P=0.213), but the relationship between energy intake and potassium intake was little affected (r = 0.64; P=0.119). The sodium and potassium density of diets were 1.6 ± 0.4g (68mmol)/1000kcal and 1.6 ± 0.5g (41mmol)/1000kcal, respectively.

#### 5.3.6 Relationship between electrolyte intake and electrolyte excretion

Figure 4.3 shows the relationships between the mean electrolyte intake and electrolyte excretion over the 5-day period for sodium, potassium and chloride. The relationship between dietary sodium intake and urine sodium excretion showed great variation between each 24h period, but when each was expressed as an average over the 5-day period, a moderate, positive relationship was observed (r = 0.66; P=0.075) (Figure 4.3). The relationship between chloride intake and urine chloride excretion was weak and non-significant during all 24h collection periods and when expressed as an average over the 5-day period (r = 0.30; P=0.472) (Figure 4.3). There was a strong positive relationship between potassium intake and urine potassium excretion during each 24h period which reached significance on days 2, 3 and 5 and when expressed as an average over the 5-day study period (r = 0.82; P=0.014) (Figure 4.3).



**Figure 4.3** The relationship between (A) dietary sodium intake and urine sodium excretion, (B) dietary potassium intake and urine potassium excretion, (C) dietary chloride intake and urine chloride excretion. Values represent the average electrolyte intake and excretion over all 5 experimental days.

#### 5.3.7 Electrolyte balance

Electrolyte balance was estimated from dietary electrolyte intake and urinary electrolyte excretion. Dermal and faecal electrolyte losses were assumed to be similar between days but were not included in the balance calculations reported here. There were no significant differences in sodium balance (P=0.217), potassium balance (P=0.296) or chloride balance (P=0.210) over the 5 days (Figure 4.4). The mean electrolyte balance over the last 4 days of the study period was  $-12 \pm 29$ mmol for sodium,  $16 \pm 25$ mmol for potassium and  $11 \pm 41$ mmol for chloride. Individual's daily electrolyte balance (calculated in relation to the mean electrolyte balance from the last 4 days of the study) is shown in figures 4.5, 4.6 and 4.7. Fluctuations in balance for most individuals appeared sinusoidal in nature, although the frequency and amplitude varied between subjects and the electrolyte studied.



**Figure 4.4** Sodium, potassium and chloride balance during the 5-day period. Values are calculated from dietary and urinary analysis.



**Figure 4.5** Variation in sodium balance from the mean sodium balance of individuals calculated from days 2, 3, 4 and 5. (A) Subject 1, (B) Subject 2, (C) Subject 3, (D) Subject 4, (E) Subject 5, (F) Subject 6, (G) Subject 7, (H) Subject 8.



**Figure 4.6** Variation in potassium balance from the mean potassium balance of individuals calculated from days 2, 3, 4 and 5. (A) Subject 1, (B) Subject 2, (C) Subject 3, (D) Subject 4, (E) Subject 5, (F) Subject 6, (G) Subject 7, (H) Subject 8.



**Figure 4.7** Variation in chloride balance from the mean chloride balance of individuals calculated from days 2, 3, 4 and 5. (A) Subject 1, (B) Subject 2, (C) Subject 3, (D) Subject 4, (E) Subject 5, (F) Subject 6, (G) Subject 7, (H) Subject 8.

#### 4.3.8 Relationship between oscillations in electrolyte excretion and electrolyte intake

The standard deviation in individual's daily urine electrolyte excretion between days 2, 3, 4 and 5 was determined to indicate the magnitude of the oscillations in electrolyte excretion. The relationship between the oscillations in urine sodium excretion and dietary sodium intake was weak (r = 0.38; P=0.353) (Figure 4.8). The oscillations in urine potassium excretion were moderately related to dietary potassium intake (r = 0.55; P = 0.162) (Figure 4.8). The relationship between the oscillations in urine chloride excretion and dietary chloride intake was moderate (r = 0.59; P=0.125) (Figure 4.8).



**Figure 4.8** The relationship between (A) oscillations in sodium excretion and sodium intake; (B) oscillations in potassium balance and potassium intake; (C) oscillations in chloride balance and chloride intake. Electrolyte intake is based on the 5-Day average and oscillations in electrolyte excretion on the 4-Day average.

# 4.3.9 Relationship between oscillations in sodium and chloride excretion with potassium intake and the relationship between oscillations in potassium excretion with sodium intake

The within-individual oscillations in urine sodium (r = -0.25; P=0.551) and chloride (r = -0.15; P=0.722) excretion were not significantly related to dietary potassium intake (Figure 4.9). Oscillations in potassium excretion were not related to sodium intake (r = 0.34; P=0.407).



**Figure 4.9** The relationship between potassium intake and (A) oscillations in sodium excretion, (B) oscillations in chloride excretion and (C) between sodium intake and oscillations in potassium excretion. Electrolyte intake is based on the 5-Day average and oscillations in electrolyte balance are based on the 4-Day average.

#### 4.3.10 Hydration status

There was no difference (P=0.618) in the osmolality of the first pass of urine between days (Table 4.7). The average urine osmolality of the first void was  $691 \pm 227$ mosmol/kg.

Table 4.7Urine Osmolality (mosmol/kg) of the first void from each day. Values are<br/>mean  $\pm$  SD.

|                                  | Day 1       | Day 2         | Day 3       | Day 4         | Day 5       | Day 6       |
|----------------------------------|-------------|---------------|-------------|---------------|-------------|-------------|
| Urine Osmolality<br>(First Void) | $704\pm287$ | $627 \pm 176$ | $651\pm275$ | $705 \pm 189$ | $746\pm214$ | $714\pm255$ |

#### 4.3.11 Relationships between sodium, potassium and chloride excretion.

The relationship between urine sodium and urine potassium excretion was significant on day 1 (P<0.05) but not significant on any of the remaining days or when expressed as the average excretion over the 5-day study period (P=0.413). The relationship between urine chloride and urine potassium excretion was significant on day 1 (P<0.05) but did not reach significance over the remaining days or when expressed as the average excretion over the 5-day study period (P=0.888). The positive relationship between urine sodium and urine chloride excretion was strong and significant in every 24h period and when expressed as the average excretion over the 5-day study period (P<0.001) (Table 4.8).

**Table 4.8**Correlation coefficients between electrolytes excreted in the urine. \*denotes<br/>significant relationship (P<0.05).</th>

|   | Day 1 | Day 2 | Day 3 | Day 4 | Day 5 | All   | 4 Day   |
|---|-------|-------|-------|-------|-------|-------|---------|
|   |       |       |       |       |       | Days  | Average |
| Urine Na <sup>+</sup> & K <sup>+</sup>  | 0.90* | 0.57  | 0.15  | 0.05  | -0.14 | 0.34  | 0.16    |
| Urine Na <sup>+</sup> & Cl <sup>-</sup> | 0.96* | 0.86* | 0.99* | 0.97* | 0.95* | 0.95* | 0.95*   |
| Urine K <sup>+</sup> & Cl <sup>-</sup>  | 0.78* | 0.28  | 0.13  | -0.15 | -0.33 | 0.06  | -0.06   |

#### 4.3.12 Relationship between urine volume and urine electrolyte excretion

The relationship between urine volume and urine chloride excretion and between urine volume and urine sodium excretion was not significant (P>0.05). The relationship between urine volume and urine potassium was moderate but not significant (Table 4.9).

|                                   | Day 1 | Day 2 | Day 3 | Day 4 | Day 5 | All   | 4 Day   |
|-----------------------------------|-------|-------|-------|-------|-------|-------|---------|
|                                   |       |       |       |       |       | Days  | Average |
| Urine Vol & Urine Na <sup>+</sup> | 0.61  | 0.02  | -0.63 | -0.08 | -0.06 | 0.01  | -0.32   |
| Urine Vol & Urine K <sup>+</sup>  | 0.75  | 0.51  | 0.25  | 0.32  | 0.64  | 0.42  | 0.36    |
| Urine Vol & Urine Cl <sup>-</sup> | 0.51  | -0.02 | -0.58 | -0.18 | -0.23 | -0.17 | -0.42   |

**Table 4.9**Correlation coefficients between urine volume and urine electrolytes during<br/>each 24h period and the average over all days and 4 days (days 2, 3, 4, 5).

#### 4.4 Discussion

This study investigated the variation in electrolyte excretion whilst consuming the same diet for 5 consecutive days. Despite the ingestion of similar amounts of sodium, potassium and chloride each day, the within-individual variation in urine sodium, potassium and chloride excretion was 23%, 13% and 26%, respectively. Although one specific factor could not primarily account for this variation, at least part of the variation may be due to a lag in electrolyte excretion following a change in diet.

#### 4.4.1 Body mass

Subjects were asked to consume their normal diet on day 1 and to repeat this on each of the following 4 days. As a consequence they lost on average 0.62 kg over the 5-day period. The variation in BM between day 1 and day 6 was  $1.2 \pm 0.8\%$  (range 0 to 2.1%). Previous investigators have reported that an individuals' euhydrated BM may fluctuate daily by ~1% (Casa et al, 2000; Cheuvront et al, 2004). Therefore at least part of the variation for some individuals in the current study was due to factors other than body water, especially considering the similar osmolality of morning urine samples that were obtained on each day. It has been reported that the within-subject variation in daily energy intake can vary greatly (14 - 43%), with mean values of 23% (Acheson et al, 1980; Bingham, 1987; Nelson et al, 1989). Therefore asking an individual to self-select a diet that will meet their energy needs for an extended period may lead to an inappropriate amount of energy being consumed. Consequently, it could be argued that individuals did not consume their normal diet during the study period, the consequences of which are discussed later.

#### 4.4.2 Sodium & chloride

On average subjects consumed 171 mmol of sodium per day, with only 2 individuals consuming more than the average reported sodium intake (187 mmol) for males in the UK (Henderson et al, 2003). Although the sodium density of diets in the current study (1.6g/1000kcal) were lower than those estimated from data presented by Henderson et al

(2003) (1.9g/1000kcal), they were higher than those seen in chapter 5 of this thesis (1.2g/1000kcal). The relationship between energy intake and sodium intake was weak in this study and although strengthened when one outlier was removed, the relationship did not reach significance. Given that sodium is found in a vast array of foods and in large quantities particularly in processed foods (Henderson et al, 2003), individual eating habits could predispose some to choose high-sodium products that are not accompanied by a high-energy content, as witnessed here. It is also possible that although subjects were encouraged to follow their normal dietary habits, the requirement to eat the same foods each day, may have inadvertently caused an alteration in behaviour, detracting from their normal eating habits, in favour of foods more convenient for this purpose. Examination of the food diaries revealed that processed foods were consumed in by all subjects. However, one would speculate that individuals would choose their favourite foods and hence still be representative of the diet they would normally consume.

There was a tendency for urine sodium excretion to exceed dietary sodium intake on day 1, but not during any other 24h period. It is possible that the elevated urine sodium excretion on day 1 was either due to a carry-over effect from the previous day's intake or a lag in excretion following a change in diet. The 5-day study period in the current study started on a Monday and finished on the Friday of the same week. Given that the intake of nutrients varies between weekdays and weekends (Acheson et al, 1980), an altered eating pattern and daily routine on the days preceding the trial may have inadvertently affected urine variables on day 1. Previously, Watson et al (1970) have reported that a sodium load even when ingested late in the day is largely excreted in the first morning void the next day. Others have also suggested that a 24h urine collection be obtained between the morning of one day and the morning of the following day in order to account for the lag between sodium ingestion and excretion (Schacter et al, 1980). Nevertheless, some investigators have reported similar conflicting results between dietary sodium intake and urine sodium excretion on the first day of collection, leading them to either ignore the first day of collection (Sowers & Stumbo, 1986) or to start dietary collection the day before urinary collection (Pietinen, 1982). Other studies have reported that when subjects abruptly reduce their sodium intake, a lag in sodium excretion of between 3 to 5 days is observed before urine sodium excretion is reduced and sodium balance is achieved (Simpson, 1988; Hollenberg et al, 1972). Likewise, although sodium excretion is prompt following an increase in sodium intake (Hollenberg et al, 1972; Simpson, 1988), it may still take some days before balance is achieved. It is most likely that a change in eating behaviour in the current study may have led to a change in sodium intake, but the severity of this change is likely to have been smaller in magnitude than previous reports (Simpson, 1988; Hollenberg et al, 1972; Leaf & Couter, 1949) because subjects were asked to follow their normal dietary behaviour, rather than to substantially decrease or increase the amount of sodium in their diet. Consequently, this would potentially minimise any great deviation in sodium intake from their "norm", resulting in a shorter lag period.

The correlation between dietary sodium intake and urinary sodium excretion showed great variation between each 24h collection period but when the 5-day average was taken, the relationship was moderate and tended to be significant (r = 0.66; P=0.075). Other studies have shown weaker (r = 0.26-0.42; Gregory et al, 1990; Bingham et al, 1995; Day et al, 2001; Clark & Mossholder, 1986), similar (r = 0.61; Caggiula et al, 1985) and stronger correlations (r = 0.76; Holbrook et al, 1984) which may be explained by the method of dietary assessment, the number of collection days or the individual variation in both measures (Bingham et al, 1988; Caggiula et al, 1985). The wide daily variation in sodium and chloride intakes both between and within individuals has led to a criticism by some (Sowers & Stumbo, 1986; Liu & Stamler, 1984; Caggiula et al, 1985; Dyer et al, 1997) although not all (Kesteloot & Joosens, 1990) of the accuracy of one-off urine collections to determine an individual's habitual sodium intake. Despite ingesting the same food each day, urine sodium excretion did fluctuate in the current study. The CV for urine sodium excretion over all 5 days was 23%, but was reduced to 13% when expressed over the last 4 days of the study period, which is considerably lower than the reported within-individual variations for urine sodium excretion in the literature for individuals consuming ad libitum diets (35 - 43%; Knuiman et al, 1988; Day et al, 2001; Caggiula et al, 1985). Therefore, whilst placing individuals on a constant sodium intake and restricting their physical activity levels reduces the daily variation in sodium excretion, it cannot completely remove it. Others have also reported a variation in sodium excretion whilst on constant sodium intakes (Baldwin et al, 1960). Baldwin et al (1960) reported the amplitude of the observed daily oscillations in sodium excretion to be related to sodium intake (r = 0.82), which orientated itself around a balance point. In the current study, the relationship between oscillations in sodium excretion and sodium intake was only weak (r = 0.38), but this may partly be due to the homogenous nature of sodium intakes. Sodium intakes ranged between 138 to 208 mmol in this study compared to 87 to 299 mmol reported by Baldwin

et al (1960). Several other factors have been suggested to influence these daily oscillations including the amount of potassium ingested (Mickelson et al, 1977; Van Buren et al, 1992), level of hydration (Ladd, 1951) and the delay in equilibration following a change in intake (Leaf & Couter, 1949; Hollenberg et al, 1972). Nevertheless, only weak correlations were observed between oscillations in urine sodium excretion and potassium intake (r = -0.25) and urine volume was not related to urine sodium excretion (r = -0.32). Van Buren et al (1992) have reported the oral administration of potassium salts to cause an immediate increase in potassium and sodium excretion. Despite this acute effect, sodium excretion returned to normal levels quickly and resulted in no significant increase in cumulative sodium excretion over the 8h monitoring period, which is considerably shorter than the 24h monitoring period in the current study.

Although other avenues of sodium loss were not measured (skin, faeces) in the current study, faecal sodium excretion is considered negligible (Allsopp et al, 1998; Baldwin et al, 1960), and is not responsible for the fluctuations in sodium excretion (Baldwin et al, 1960). Insensible losses of sodium via the skin are also small (Dahl, 1958) and considering the current study was undertaken during the months of November and December in the UK, insensible sodium losses were assumed not to be a contributing factor to the variation in urine sodium excretion. Similarly, subjects were asked to refrain from strenuous physical activity in an attempt to minimise any losses of sodium in sweat. A potential source of variation is due to incomplete urine collections and/or alterations in the duration of each collection. In this study no differences were reported in the duration of each day's urine collection, with a CV of  $1 \pm 1\%$  between collections. Nevertheless, all urine variables were corrected to a 24h period. The completeness of collection was determined by both subject self-report and urine creatinine excretion. Two individuals reported one incomplete 24h urine collection and these collections were omitted from further analysis. The CV for creatinine excretion was  $9 \pm 5\%$  when these two incomplete collections were removed. Many investigators have reported a large within-individual variation for urine creatinine (Garde et al, 2004; Greenblatt et al, 1976; Vestergaard & Leverett, 1958; Ricos et al, 1994; Curtis & Fogel, 1970; Webster & Garrow, 1985) as a large number of factors can influence urine creatinine excretion including exercise (Calles-Escandon et al, 1984) and the meat content of the diet (Lykken et al, 1980). Both these factors were controlled for in the current study and as a result all individuals demonstrated a within-individual variation well within, or below, the normal range reported for collections known to be complete (9-24%; Garde et al, 2004). Although it is unclear why the daily variation in urine sodium excretion persists, Baldwin et al (1960) have reported these oscillations to diminish after a more prolonged period of monitoring, a finding replicated in the current study.

#### 4.4.3 Potassium

On average subjects consumed 108mmol of potassium per day, with 6 individuals consuming more than the average reported potassium intake (81mmol) for males in the UK (Henderson et al, 2003). The potassium density of diets in the current study (1.6g/1000kcal) was slightly higher than the 1.5g/1000kcal estimated from Henderson et al (2003), both of which were higher than previous values reported for the British population (1.3g/1000kcal; Gregory et al, 1990).

A large number of factors can influence the amount of potassium excreted in the urine, including the absolute level of potassium intake (Voors et al, 1983), seasonal variation (Holbrook et al, 1984) and fibre intake (Cummings et al, 1976). Consequently, the use of urine potassium excretion as a marker of dietary potassium intake has been questioned (Cummings et al, 1976). In the current study there were no significant differences between dietary potassium intake and urine potassium excretion during any 24h period and the relationship between urine potassium excretion and dietary potassium intake was strong (r = 0.82). Others have reported weaker (0.23 - 0.64; Clark & Mossholder, 1986; McKeown et al, 2001; Day et al, 2001; Caggiula et al, 1985; Pietinen, 1982) or similar correlations (r = 0.73 - 0.82; Bingham et al, 1995; Bingham et al, 1997; Holbrook et al, 1984). Although the fibre content of the diet can influence faecal potassium losses (Cummings et al, 1976; Taseveska et al, 2006), the day to day variation in dietary fibre was small (2%) in the current study, and as a result may partly explain the stronger relationship between dietary potassium intake and urinary potassium excretion. This, however, is purely speculative as faecal potassium excretion was not measured.

Similar to sodium, oscillations in potassium excretion were observed for individuals. The magnitude of these oscillations was moderately related to dietary potassium intake, with individuals who were consuming larger amounts of potassium exhibiting larger oscillations in excretion. However, the within-individual variation in potassium excretion was 12%, which is lower than values previously reported in the literature (19%; Taseveska et al,

2006, Day et al, 2001; Willett, 1990). Interestingly the variation in potassium excretion (12%) was similar to that of sodium excretion (13%). Considering that sodium is lost in greater quantities than potassium in sweat, this suggests that the day to day variation is due to factors other than sweat loss.

#### 4.4.4 Conclusion

In summary, despite consuming the same diet for 5 consecutive days, the within individual variation in sodium and potassium excretion was 23% and 13%, respectively. This was reduced to 13% for sodium when measured over the last 4 days but remained similar for potassium (12%). This is most likely due to the lag in sodium excretion following a change in diet, but in both cases sodium and potassium orientated itself around a balance point for each individual.

### Chapter 5

# The effect of exercise on water and electrolyte balance

#### 5.1 Introduction

Sodium is needed for various processes in the body and as the principal cation in the extracellular fluid it is intimately linked with body water balance. Potassium is the principal cation in the intracellular space and is important for nerve transmission and muscle function (Institute of Medicine, 2004).

Obligatory urine, skin and faecal sodium losses are small (Dole et al, 1950; Dahl et al, 1955; Dahl, 1958) amounting to between 1.7 - 8.0 mmol of sodium per day (Dahl, 1958). Although humans can survive on extremely low sodium diets (as evidenced by a urine sodium excretion of  $1.0 \pm 1.5$  mmol/24h; Oliver et al, 1975), dietary surveys report a wide range of sodium intakes usually well in excess of these values (Gregory et al, 1990; Henderson et al, 2003). In a recent UK-based survey (Henderson et al, 2003), males were reported to have an average dietary sodium intake of 3.3g/d (144mmol/d) according to a 7day weighed food record, but this increased to 4.3g/d (187mmol/d) according to a single 24h urine collection. The amount of sodium above basal requirements is excreted primarily in the urine and therefore urinary sodium excretion can provide a good estimate of intake (Holbrook et al, 1984; Taseveska et al, 2006). The difference between weighed food records and 24h urine collections in the above survey (Henderson et al, 2003) was attributed to the use of discretionary salt (salt added during cooking or at the table), which was not measured by the weighed food records. However, the use of 24h urine collections as a measure of sodium intake is not suitable for individuals who are subjected to either manual labour, hot environments (Consolazio et al 1963) or exercise training (Shirreffs et al, 2006) as they may lose large amounts of sodium in sweat. This is because the sweat glands have precedence over the kidneys for sodium and therefore urinary sodium excretion is reduced as a result of sweat sodium loss (Lichton, 1957).

The extent of sweat electrolyte losses is dictated by the sweat electrolyte concentration and the volume of sweat lost. Sodium losses in sweat can be high and can account for a large proportion of dietary intake. Sweat sodium losses as high as 11.2g (486mmol) have been reported during 7.5h of heat exposure (Consolazio et al, 1963). Recent studies on football (Maughan et al, 2004; Maughan et al, 2005; Shirreffs et al, 2005) and American football players (Stofan et al, 2002b) indicate that substantial electrolyte losses can occur during a single training session, particularly for sodium. Sodium losses are typically around 2.0g

(85mmol), but can be as high as 9.9g (430mmol) during a 90-120 minute training session which corresponds to a salt loss of 5.0g and 25.1g, respectively.

The purpose of this study was to determine the effect of intermittent moderate intensity exercise in the heat on fluid and electrolyte balance in subjects consuming their normal diet.

#### 5.2 Method

#### 5.2.1 Subjects

Nine healthy male volunteers participated in this study, which had received prior approval from the Loughborough University Ethical Advisory Committee (R05/P137). All subjects were informed about the experimental procedures and associated risks before their written consent was obtained. Their physical characteristics (mean  $\pm$  SD) were: age 24  $\pm$  4y, height 1.79  $\pm$  0.08m, body mass 80.3  $\pm$  12.4kg and body fat 13  $\pm$  3%.

#### 5.2.2 Experimental protocol

Subjects reported to the laboratory at least one day prior to the commencement of the study period when they were given the equipment for dietary and urinary collections and a detailed briefing of the collection procedures. In addition, the logbooks provided contained written instructions about the dietary and urine collection process. Figure 5.1 shows a schematic representation of the study protocol.



**Figure 5.1** Schematic of the study period. Body mass (BM), dietary collection period (D), 24h urine collection (U) and exercise (Ex).

On the morning of day 1, subjects arrived in the laboratory and nude body mass (BM) was measured. Subjects returned to the laboratory each day to return urine samples, on the morning of day 4 for the exercise trial and on day 6, when a final BM was obtained.

#### 5.2.3 Dietary monitoring

Subjects were asked to follow their normal dietary behaviour, but to weigh and record all food and drink consumed throughout the 5-day collection period. The dietary collection procedures are described fully in Chapter 2.

#### 5.2.4 Urine collection

During this 5-day period and on the morning of day 6, subjects were asked to collect all urine passed except for the first void on day 1. The 24h urine collection procedures are described fully in chapter 2.

#### 5.2.5 Physical activity

During the collection period, subjects were asked to refrain from strenuous exercise that would incur sweat losses (apart from the exercise task on day 4). On arrival at the laboratory on the morning of day 4, subjects were asked to empty their bladder as completely as possible. Subjects then entered a room maintained at approximately 35°C and 60-70% relative humidity (RH). Nude BM was measured to the nearest 10g (CFW-150K, Adam Equipment Co Ltd, Milton Keynes, UK) and then the skin was cleaned with distilled, de-ionised water before four absorbent patches (Tegaderm, 3M, Loughborough, UK) for sweat collection were placed on 4 regional skin sites (Chapter 2). Dehydration was induced by intermittent exercise on a cycle ergometer (Monark) at an intensity of 161  $\pm$  48W. Exercise periods of 10 minutes were separated by 5 minutes of rest during which subjects towel dried and nude BM loss was obtained. This pattern continued until subjects were dehydrated by almost 2% of BM, with the remaining BM loss achieved via the ongoing perspiration that followed exercise. Upon completion of the third exercise bout, sweat patches were removed and placed into a 10mL syringe with a sample being immediately extracted into a small container for subsequent analysis. After a shower, final nude BM was obtained before subjects dressed and were free to leave the laboratory and continue with their normal daily activities.

#### 5.2.6 Sample analysis

Urine and sweat samples were analysed for sodium, potassium, chloride and osmolality as described in Chapter 2. Completeness of each 24h urinary collection was reported by subject self-report each day. Weighed food intakes were analysed using Compeat Pro 5.8.0 Software. The regional sweat collection technique overestimates sweat sodium concentrations by 30-40% (Shirreffs et al 2006), therefore a 35% correction factor has been applied to sweat sodium and chloride concentrations. Sweat potassium concentration is not consistently affected by the sweat collection method and no correction factor has been applied.

#### 5.2.7 Statistical analysis

All data were tested for normality of distribution. As urine electrolyte excretion can provide an accurate estimate of electrolyte intake in non-sweating individuals (Holbrook et al, 1984), electrolyte intake assessed by weighed food diaries and 24h urine excretion were subject to a two-factor repeated measures ANOVA, followed by paired t-tests with Holm-Bonferroni adjustment for multiple comparisons. Other data were analysed by one-factor repeated measures ANOVA followed by paired t-tests with Holm-Bonferroni adjustment for multiple comparisons or Freidman's Test followed by Wilcoxon tests when found not to be normally distributed. Correlations were assessed using Pearson's correlation or Spearman's Rank when found not to be normally distributed. Parametric data are expressed as mean  $\pm$  SD and non-parametric data as median (range). In some circumstances a range has been reported regardless of the distribution of data as it was deemed to provide further useful information. Statistical significance was set at P<0.05.

#### 5.3 Results

#### 5.3.1 Exercise

The mean BM loss during the dehydration procedure was  $1.51 \pm 0.19$ kg. This corresponded to a  $1.9 \pm 0.2\%$  reduction of the pre-exercise BM. The mean exercise time to achieve this was  $49.4 \pm 6.8$  minutes. Due to the intermittent nature of the exercise session, subjects remained in the heat chamber for  $73.8 \pm 13.5$  minutes. Estimated whole body sweat rates were  $1.24 \pm 0.13$  Litres/hour (L/h).

#### 5.3.2 Estimated whole body sweat composition and loss

A total of 5 sweat patches out of 36 were dislodged during the trials. These 5 sweat patches were not included in data analysis. Estimated whole-body sweat composition and total electrolyte losses are shown in Table 5.1.

|                                   | Arithmetic    | Calculation  |
|-----------------------------------|---------------|--------------|
|                                   | $Mean \pm SD$ | Range        |
| Sweat [Sodium] (mmol/L)           | $44 \pm 11$   | (28 - 62)    |
| Sweat [Potassium] (mmol/L)        | $6.0 \pm 1.2$ | (4.1 - 7.6)  |
| Sweat [Chloride] (mmol/L)         | $41 \pm 15$   | (26 - 67)    |
| Total Sweat Sodium Loss (mmol)    | $66 \pm 16$   | (32 - 86)    |
| Total Sweat Potassium Loss (mmol) | $8.9 \pm 1.8$ | (6.8 - 12.1) |
| Total Sweat Chloride Loss (mmol)  | $62 \pm 21$   | (29 - 97)    |

**Table 5.1**Estimated whole-body sweat electrolyte concentrations ([electrolyte]) and<br/>total electrolyte losses during exercise.

#### 5.3.3 Sweat composition at regional collection sites

Sweat sodium and chloride concentrations were significantly lower on the thigh than all other collection sites (P<0.05) (Table 5.2). Sweat potassium concentration was significantly lower on the back than the forearm (P=0.012) and tended to be lower on the back than the thigh (P=0.050).

**Table 5.2**Sweat electrolyte concentrations ([electrolyte]) at each regional collection<br/>site. <sup>a</sup> denotes significantly different to the forearm, <sup>b</sup> denotes significantly<br/>different from the thigh.

|         | [Sodium] (mmol/L) |           | [Potassium] (mmol/L) |             | [Chloride] (mmol/L) |           |
|---------|-------------------|-----------|----------------------|-------------|---------------------|-----------|
|         | Mean $\pm$ SD     | Range     | $Mean \pm SD$        | Range       | $Mean \pm SD$       | Range     |
| Back    | $46 \pm 12^{b}$   | (28 - 68) | $4.8\pm0.9^{a}$      | (3.7 - 6.0) | $45 \pm 16^{b}$     | (27 - 77) |
| Chest   | $48 \pm 12^{b}$   | (27 - 64) | $6.2 \pm 1.7$        | (3.5 - 9.9) | $45 \pm 16^{b}$     | (27 - 70) |
| Forearm | $42 \pm 11^{b}$   | (29 - 63) | $7.4 \pm 1.8$        | (5.1 - 9.0) | $37 \pm 15^{b}$     | (24 - 66) |
| Thigh   | $37 \pm 9$        | (27 – 53) | $6.0 \pm 1.4$        | (4.0 - 7.6) | $32 \pm 12$         | (20 - 57) |

#### 4.3.4 Relationship between sweat rate & sweat composition

Sweat sodium, potassium and chloride concentrations were not related (P>0.05) to whole body sweat rate at any collection site (Table 5.3). Similarly, estimated whole body sweat sodium, potassium and chloride concentrations were not related to whole body sweat rate (Table 4.3).
|                                 | Back | Chest | Forearm | Thigh | WB   |
|---------------------------------|------|-------|---------|-------|------|
| Sweat Rate & [Na <sup>+</sup> ] | 0.01 | 0.13  | 0.27    | 0.07  | 0.03 |
| Sweat Rate & [K <sup>+</sup> ]  | 0.18 | -0.02 | 0.43    | 0.34  | 0.31 |
| Sweat Rate & [Cl <sup>-</sup> ] | 0.13 | 0.22  | 0.25    | 0.22  | 0.13 |

**Table 5.3**The relationship between whole body sweat rate and regional sweat<br/>composition at each collection site and estimated whole body (WB) sweat<br/>composition. Values are correlation coefficients.

#### 5.3.5 Hydration status

There was no difference (P=0.325) in the osmolality of the first pass of urine between days (Table 5.4). Individuals have been categorised as hypohydrated (>900mosmol/kg) and euhydrated (<700mosmol/kg) (Cheuvront & Sawka 2005; Shirreffs & Maughan 1998a). The average osmolality of the first void of urine each day was  $692 \pm 199$ mosmol/kg.

**Table 5.4**Osmolality of the first void of each day. Values are mean  $\pm$  SD and (range).

|                  | Day 2         | Day 3         | Day 4         | Day 5         | Day 6         |
|------------------|---------------|---------------|---------------|---------------|---------------|
| Urine Osmolality | $725 \pm 229$ | $586 \pm 157$ | $639 \pm 160$ | $696 \pm 249$ | $752 \pm 189$ |
| (First Void)     | (399 – 1082)  | (335 – 797)   | (408 - 917)   | (327 – 1025)  | (404 - 1004)  |
| >900mosomol/kg   | 2             | 0             | 1             | 3             | 2             |
| 700-900mosmol/kg | 3             | 3             | 2             | 1             | 4             |
| <700mosmol/kg    | 4             | 6             | 6             | 5             | 3             |

# 5.3.6 Body mass

Subjects BM remained stable (P>0.05) between the morning of day 1 ( $80.28 \pm 12.43$ kg) and day 6 ( $80.29 \pm 12.52$ kg). The coefficient of variation (CV) in BM between days 1, 4 and 6 was  $0.6 \pm 0.3\%$  (range 0.2 to 1.1%).

#### 5.3.7 Duration of urine collection

Subjects reported 42 out of 45 24h urine collections to be complete. The 3 incomplete collections were omitted from further analysis. There were no significant differences (P=0.110) in the duration of each day's urine collection (Table 5.5) but all urine data for each collection period were adjusted to 24h, with this value being used in all subsequent analysis. The average duration of each urine collection over the 5-day period was 24.00h (22.50 - 26.33). The CV for the duration of urine collection was 2 % (0 - 4%).

**Table 5.5**Duration of each day's urine collection. Values are median (range).

|                | Day 1           | Day 2           | Day 3           | Day 4           | Day 5           |
|----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Duration of    | 24.00           | 23.98           | 24.00           | 24.08           | 24.00           |
| Collection (h) | (22.58 - 24.58) | (23.42 - 24.50) | (22.50 - 24.42) | (23.72 - 25.52) | (23.92 - 26.33) |

# 5.3.8 Dietary intake and urine excretion

Nutrient intake and electrolyte excretion during each 24h period are shown in Table 5.6, Table 5.7 and Figure 5.2. There were no significant differences (P>0.05) in energy, carbohydrate, protein, fat or fibre intake between days. A significantly (P<0.05) greater amount of fluid was consumed on day 4 than on day 3 and day 5 and the amount of fluid consumed on day 4 tended to be greater than on day 2 (P=0.072). The CV's for nutrient intakes and urine excretion are shown in Table 5.8.

Dietary sodium intake and urinary sodium excretion did not change significantly over time (P=0.530; Figure 5.2). There was a tendency (P=0.069) for urine sodium excretion to exceed dietary sodium intake on day 1, but urine sodium excretion was similar (P>0.05) to dietary sodium intake on days 2, 3 and 5. On day 4 (the day of exercise) the amount of sodium excreted in the urine ( $122 \pm 30$ mmol) was significantly less than dietary sodium intake ( $181 \pm 89$ mmol; P=0.021).

Dietary potassium intake and urinary potassium excretion did not change significantly over time (P=0.698; Figure 5.2). Urine potassium excretion was similar to dietary potassium intake in all 24h collection periods (P=0.805) and was little affected on the day of exercise. On day 4, the amount of potassium consumed in the diet and excreted in the urine was 139  $\pm$  61mmol and 112  $\pm$  20mmol, respectively.

Dietary chloride intake and urinary chloride excretion did not change significantly over time (P=0.575; Figure 5.2). Urine chloride excretion was similar to dietary chloride intake in all collection periods except for day 4, when the amount of chloride excreted in the urine (96  $\pm$  36mmol) was significantly less than chloride intake (186  $\pm$  103mmol; P=0.010).

The mean dietary sodium, potassium and chloride intake over the 5-day period was  $172 \pm 78$  mmol,  $131 \pm 52$  mmol and  $162 \pm 81$  mmol, respectively.

|                  | Day 1        | Day 2         | Day 3            | Day 4           | Day 5            | 5 Day<br>Average | 3 Day<br>Average |
|------------------|--------------|---------------|------------------|-----------------|------------------|------------------|------------------|
| Water (g)        | $3336\pm903$ | $3319\pm723$  | $3508 \pm 1169*$ | $4711 \pm 1738$ | $3341 \pm 1512*$ | $3643 \pm 1324$  | $3388\pm917$     |
| Energy (kcal)    | $3196\pm658$ | $2921\pm460$  | $3446 \pm 762$   | $3495 \pm 1519$ | $3368 \pm 1337$  | $3285\pm1006$    | $3188\pm652$     |
| Carbohydrate (g) | $490\pm125$  | $441 \pm 100$ | $498 \pm 135$    | $542\pm268$     | $476 \pm 231$    | $489 \pm 178$    | $476\pm119$      |
| Protein (g)      | $125 \pm 55$ | $121 \pm 42$  | $141 \pm 46$     | $132 \pm 41$    | $133 \pm 51$     | $130 \pm 45$     | $129\pm47$       |
| Fat (g)          | $106 \pm 30$ | $92 \pm 29$   | $119 \pm 43$     | $113 \pm 65$    | $116 \pm 61$     | $109 \pm 47$     | $106 \pm 35$     |
| Fibre (mmol)     |              | $23 \pm 10$   | 26 + 10          | $28 \pm 14$     | $20 \pm 11$      | $25 \pm 12$      | $25\pm12$        |

| Day 1Day 2Day 3Day 4Day 55 Day3 DayAverageAverageAverageAverage | $2177\pm542$     | $2200\pm806$     | $2281 \pm 1113$ | $2092\pm995$ | $2145\pm531$ | $2212\pm588$ | 2274 ± 804 | Urine Volume (mL) |
|---|------------------|------------------|-----------------|--------------|--------------|--------------|------------|-------------------|
|   | 3 Day<br>Average | 5 Day<br>Average | Day 5           | Day 4        | Day 3        | Day 2        | Day 1      |                   |

|              | Dietary     | Intake      | Urine Ex    | cretion     |
|--------------|-------------|-------------|-------------|-------------|
|              | All Days    | 3 Days      | All Days    | 3 Days      |
| Water        | $23 \pm 7$  | $10 \pm 5$  |             |             |
| Energy       | $19\pm 8$   | $12 \pm 5$  |             |             |
| Carbohydrate | $22 \pm 11$ | $15 \pm 7$  |             |             |
| Protein      | $26 \pm 12$ | $26 \pm 14$ |             |             |
| Fat          | $29 \pm 10$ | $19 \pm 10$ |             |             |
| Fibre        | $25 \pm 12$ | $23 \pm 15$ |             |             |
| Sodium       | $34 \pm 16$ | $21\pm 8$   | $34 \pm 13$ | $22\pm9$    |
| Potassium    | $24 \pm 9$  | $18 \pm 11$ | $21 \pm 11$ | $17 \pm 15$ |
| Chloride     | $38 \pm 14$ | $20 \pm 7$  | $37 \pm 14$ | $21 \pm 14$ |

**Table 5.8**The CV (%) in nutrient intake and urine excretion over all experimental<br/>days (All Days) and days 1, 2 and 3 (3 Days).



**Figure 5.2** Dietary intake and urine excretion of sodium (A), potassium (B) and chloride (C) during each 24h period. All Values are mean  $\pm$  SD. \* denotes significant difference between dietary intake and urine excretion.

### 5.3.9 Electrolyte balance

Electrolyte balance was estimated from dietary electrolyte intake, urinary electrolyte excretion and sweat electrolyte loss (during the exercise trial). There were no significant differences in net sodium (P=0.370), potassium (P=0.176) or chloride (P=0.158) balance between any 24h period (Figure 5.3). The mean sodium, potassium and chloride balance over the last 4 days of the study period was  $-4 \pm 68$ mmol,  $5 \pm 54$ mmol and  $18 \pm 67$ mmol, respectively.



Figure 5.3 Sodium (A), potassium (B) and chloride (C) balance over each 24h period. Values are calculated from urinary electrolyte excretion, dietary electrolyte intake and sweat electrolyte loss. Values are mean  $\pm$  SD.

# 4.3.10 Relationship between electrolyte intake and electrolyte excretion

Fluid intake was positively related (P<0.05) to urine volume on days 1, 2, 4 and 5 (Table 5.9). Dietary sodium intake was positively related (P<0.05) to urine sodium excretion on days 4 and 5, and when expressed over the 5-day period, the relationship was moderate and tended to be significant (P=0.067). Dietary chloride intake was positively related to urine chloride excretion on days 4 and 5 (P<0.05) and when expressed over the 5-day period (P=0.011). Potassium intake was unrelated (P>0.05) to urine potassium excretion during all 24h periods. When expressed as the average intake and excretion over 5 days, the relationship was moderate but not significant (P=0.123)

**Table 5.9** The relationship between dietary water intake and urine volume, dietary sodium intake and urine sodium, dietary potassium intake and urine potassium and dietary chloride intake and urine chloride excretion in each 24h period, the 3 Day Average (days 1, 2, 3) and the 5 day Average (all experimental days). Values are correlation coefficients. \*denotes significant relationship (P<0.05).

|  |       |       |       |       |       | 5 Day   | 3 Day   |
|--|-------|-------|-------|-------|-------|---------|---------|
|  | Day 1 | Day 2 | Day 3 | Day 4 | Day 5 | Average | Average |
| Water intake & urine volume                    | 0.84* | 0.87* | 0.40  | 0.98* | 0.87* | 0.94*   | 0.84*   |
| Na <sup>+</sup> intake & urine Na <sup>+</sup> | 0.25  | 0.32  | 0.45  | 0.75* | 0.86* | 0.63    | 0.36    |
| K <sup>+</sup> intake & urine K <sup>+</sup>   | 0.34  | 0.37  | -0.06 | 0.58  | 0.30  | 0.55    | 0.41    |
| Cl <sup>-</sup> intake & urine Cl <sup>-</sup> | 0.32  | 0.63  | 0.60  | 0.71* | 0.81* | 0.79*   | 0.53    |

# 5.3.11 Relationship between energy intake and electrolyte intake

Dietary sodium intake was positively related (P<0.05) to energy intake on days 3, 4 and 5 and this relationship persisted when calculated over all 5 days (P=0.016) (Table 5.10). No significant relationship between potassium intake and energy intake was observed, although the relationship tended to reach significance on days 4 (P=0.071) and 5 (P=0.088) (Table 5.10). When expressed over the 5-day study period, the relationship between potassium intake and energy intake was moderate (P=0.180). Chloride intake was positively (P<0.05) related to energy intake on days 3, 4 and 5 and this relationship persisted when calculated over all 5 experimental days (P=0.036) (Table 5.10). The average sodium and potassium densities of the diets were 1.2g (52mmol)/1000kcal and 1.6g (41mmol)/1000kcal, respectively.

**Table 5.10** The relationship between dietary energy intake (kcal) and dietary electrolyte intake (mmol) during each 24h period, the 3 Day Average (days 1, 2, 3) and 5 Day Average (days 1, 2, 3, 4, 5). Values are correlation coefficients. \*denotes a significant relationship (P<0.05).

|                                 | Day 1 | Day 2 | Day 3 | Day 4 | Day 5 | 5 Day<br>Average | 3 Day<br>Average |
|---------------------------------|-------|-------|-------|-------|-------|------------------|------------------|
| Energy & Na <sup>+</sup> Intake | 0.22  | 0.47  | 0.78* | 0.90* | 0.92* | 0.77*            | 0.62             |
| Energy & K <sup>+</sup> Intake  | 0.09  | -0.12 | 0.28  | 0.63  | 0.60  | 0.49             | 0.12             |
| Energy & Cl Intake              | 0.19  | 0.41  | 0.79* | 0.83* | 0.67* | 0.70*            | 0.56             |

# 5.3.12 Relationship between electrolyte intake and sweat electrolyte loss

The total amount of sodium, potassium and chloride lost in sweat was unrelated to the dietary intake of these electrolytes on the days preceding exercise (Table 5.11). Similarly, no relationship was observed between dietary electrolyte intake and sweat electrolyte loss for sodium, potassium or chloride on day 4. The relationship between the total volume of sweat lost during exercise and the volume of fluid consumed during the same day was moderate and approached significance (P=0.071). The sweat electrolyte losses incurred during the exercise bout on day 4 accounted for  $43 \pm 21\%$  (range 17 - 89%),  $8 \pm 4\%$  (range 3-16%) and  $40 \pm 18\%$  (range 14-68%) of the dietary sodium, potassium and chloride intake for that day, respectively.

**Table 5.11**The relationship between dietary electrolyte intake (mmol) and estimated<br/>whole body sweat electrolyte loss (mmol). Dietary values are the means of<br/>days 1, 2 and 3 (3 Day Average), day 3 and day 4. Values are correlation<br/>coefficients.

|   | 3 Day   | Day 3 | Day 4 |
|---|---------|-------|-------|
|   | Average |       |       |
| Dietary Na <sup>+</sup> Intake & Sweat Na <sup>+</sup> Loss | 0.34    | -0.14 | 0.01  |
| Dietary K <sup>+</sup> Intake & Sweat K <sup>+</sup> Loss   | -0.31   | -0.01 | -0.18 |
| Dietary Cl <sup>-</sup> Intake & Sweat Cl <sup>-</sup> Loss | 0.25    | 0.18  | 0.10  |
| Dietary Fluid Intake & Sweat Loss                           |         |       | 0.63  |

# 5.4 Discussion

This study investigated the effect of a single session of moderate intensity, intermittent exercise in the heat on electrolyte balance in individuals who were consuming their normal self-selected diet for 5 consecutive days. Despite the loss of sodium and chloride in sweat during exercise, no significant differences in sodium or chloride balance were seen due to the conservation of urine sodium and chloride by the kidney on the day of exercise. Sweat potassium losses were of much smaller magnitude than sodium and chloride and as a result urine potassium excretion and potassium balance were unaffected by exercise.

# 5.4.1 Sodium and chloride

The magnitude of sweat sodium losses can vary greatly between individuals due to the large variation in sweat composition and sweat rate. In this study individuals lost between 32 and 86mmol (0.7 - 2.0g) of sodium in sweat during exercise, representing between 17 and 89% of dietary sodium intake for that day. There is a consensus that regional sweat collection techniques overestimate sweat sodium concentrations obtained using whole body collection methods (Patterson et al, 2000; Shirreffs & Maughan, 1997; Palacios et al, 2003; Van Heyningen & Weiner, 1952; Stofan et al, 2002a) and therefore sweat sodium concentrations were adjusted to account for this 30-40% overestimation (Shirreffs et al, 2006). The overestimation may be due to the suggested regional differences in sweat rate and composition (Costa et al, 1969; Patterson et al, 2000; Lemon et al, 1986; Havenith et al, 2008), the formation of an artificial environment beneath the sweat patch (Van Heyningen & Weiner, 1952; Shirreffs et al, 2006) or the leaching of electrolytes from the stratum corneum (Weschler, 2008). In the current study, regional differences in sweat composition were found, as sweat sodium and chloride concentrations were lower on the thigh compared to all other collection sites. Nevertheless, estimated whole body sweat sodium concentrations obtained from regional collection techniques correlate well with whole body sweat sodium concentrations obtained by whole-body washdown methods (Ladell, 1948; Patterson et al, 2000).

An important question that has been posed (Stofan et al, 2005) is whether high sodium intakes drive sweat sodium concentration or whether sweat sodium losses drive sodium intake. Those that report a relationship between dietary sodium intake and sweat sodium concentration have typically imposed a salt deficiency on subjects (Robinson et al 1950b; Armstrong et al 1985; McCance 1938), but other investigators have reported a similar effect in the absence of a salt deficiency (Costa et al 1969; Allsopp et al 1998). In the current study no relationship was found between dietary sodium intake and sweat sodium loss, but it is possible that the dietary collection period was too short to gain an accurate representation of an individual's sodium intake, considering that greater than 8 days collection is needed to do so (Liu & Stamler 1984; Sowers & Stumbo 1986).

On average subjects consumed 171mmol of sodium per day during the first 3 days of the trial and 172mmol over all 5 days. Although these values are lower than those in a recent survey of the UK population (187mmol/day; Henderson et al, 2003), 4 individuals in the

current study consumed more sodium than the UK average. As the energy intake of individuals in the current study (3188kcal/day) was higher than the UK average (2323kcal/day), the sodium densities of diets in the current study (1.2g Na<sup>+</sup>/1000kcal) were lower than those of the UK population (1.9g Na<sup>+</sup>/1000kcal) (Henderson et al, 2003).

A sodium appetite has previously been reported by others following exercise (Takamata et al, 1994; Leshem et al, 1999), but the sodium intake on the day of exercise in the current study was not different to that on any other day. Furthermore there was no relationship between the amount of sodium lost in sweat and dietary sodium intake on day 4, suggesting individuals did not show a preference for salty foods following exercise. Instead there was evidence of significant sodium conservation by the kidneys in response to the sweat sodium losses imposed by the exercise task on day 4. Lichton (1957) has previously described a competition between the sweat glands and kidneys for salt during exercise. It was concluded that the sweat gland has precedence over the kidney for sodium, with any remaining sodium being available for excretion in the urine. This finding is further supported by the current study. The kidneys can respond quickly in response to sweat sodium and chloride losses incurred, with some reports showing this to occur within 1 or 2h of exercise (Robinson et al, 1955). On day 4 urine sodium excretion was significantly lower than dietary sodium intake on average by 60mmol which could account for the majority of the sweat sodium losses and the maintenance of sodium balance. Although faecal and insensible sodium losses were not accounted for in this study, they were assumed to be negligible and consistent between days (Allsopp et al, 1998; Dahl et al 1955).

In non-sweating individuals, it is suggested that urine sodium excretion can provide an objective marker of dietary sodium intake (Holbrook et al, 1984) as urine excretion represents the main avenue of sodium loss from the body. However, the correlation between dietary sodium intake and urinary sodium excretion was weak (r = 0.36) and non-significant when calculated over the first 3 days. Surprisingly the relationship was stronger when calculated over all 5 experimental days despite the sweat losses incurred on day 4. Other studies have reported similar (r = 0.26-0.42; Gregory et al, 1990; Clark & Mossholder, 1986; Bingham et al, 1995; Day et al, 2001), weaker (r = 0.05; Sowers & Stumbo, 1986) and stronger (r = 0.48-0.76; Clark & Mossholder, 1986; Holbrook et al, 1984; McKeown et al, 2001; Caggiula et al, 1985; Pietinen, 1982) correlations between

sodium intake and urine sodium excretion in free-living individuals. The weak correlations may be partly explained by the high day to day variation in both sodium intake and excretion (Sowers & Stumbo, 1986; Caggiula et al, 1985; Knuiman et al, 1988, Liu et al, 1979), and the contradictory findings in the literature may be explained by differences in the number of days of collection, dietary assessment method or a lack of detailed calculation of discretionary salt use. Stronger correlations are generally reported when duplicate portion analysis is implemented (Clark & Mossholder, 1986; Holbrook et al, 1984), when an accurate assessment of discretionary salt use was made (Clark & Mossholder, 1986; Caggiula et al, 1985) or when an increased number of collection days were included (Clark & Mossholder, 1986; McKeown et al, 2001). Nevertheless, despite the increased subject burden of keeping a weighed dietary record, this method is frequently reported to be better than a food frequency questionnaire, food diary and 24h recall methods (Bingham et al, 1995; Porrini et al, 1995; McKeown et al, 2001). A potential source of variability is due to incomplete urine collections or alterations in the duration of each collection. In this study, the completeness of collection was verified by subject selfreport, with three subjects reporting one incomplete collection. Although creatinine has been used as a marker of completeness, it is not without its limitations (Bingham & Cummings, 1985; Flynn et al, 1990) and is reported to vary as a result of diet (Jacobsen et al, 1979) and exercise (Calles-Escandon et al, 1984). Flynn et al (1990) found the selfreporting method to be more accurate than creatinine in assessing the completeness of collection and taken in combination with the similar duration of each collection seen in this study, was deemed to be an adequate method in the determination of completeness.

Although sweat sodium and chloride losses did not exceed dietary sodium and chloride intake in the current study, if a longer, more intense and/or second exercise bout was scheduled for later that same day, sweat losses may be of sufficient magnitude to exceed dietary intake in some individuals. Similarly, if dietary salt intake was reduced, as has been recently recommended to 6g per day (SACN, 2003) (equivalent to 2.3g or 103 mmol of sodium), sweat sodium losses would account for a greater proportion of dietary intake, if not exceed it. This may place some individuals at an increased risk of muscle cramps (Dill et al, 1938; Stofan et al, 2005) and possibly hyponatraemia (Montain et al, 2006).

# 5.4.2 Potassium

In contrast to sodium, potassium is lost in relatively small amounts in sweat, especially when considered relative to urine potassium losses. Sweat potassium concentrations seem largely unaffected by differences in sweat rate (Schwartz & Thaysen, 1956; Verde et al, 1982) and the findings of the current study support this. Regional differences in sweat composition were observed as sweat potassium concentration was lower on the back than the arm and the thigh, a finding that has been reported previously (Patterson et al, 2000; Maughan et al, 2004) and may be attributable to regional differences in sweat gland function and density (Sato & Dobson, 1970). Subjects in this study lost between 7 and 12mmol of potassium in sweat, equivalent to between 3 and 16% of their dietary potassium intake on day 4 and were too small to cause any significant alteration in potassium balance on the day of exercise. Unlike sodium there is no consensus as to whether regional collection techniques provide accurate estimates of whole body electrolyte composition, with some studies reporting regional techniques to underestimate (Shirreffs & Maughan, 1997) or overestimate (Patterson et al, 2000) potassium concentrations. However, these differences ( $\pm 2$ mmol/L) would be small relative to the potassium intake of individuals in the current study.

Although one study has reported large sweat potassium losses during exercise of up to 44% of dietary potassium intake (Consolazio et al, 1963) others report values similar to those in the current study (Costill et al, 1982). The discrepancy between these two studies can be primarily explained by the different durations of exercise or heat exposures (7.5h and 2h, respectively). As sweat potassium concentration is unrelated to dietary potassium intake (Malhotra et al, 1981; Costill et al, 1982), does not fall as exercise duration increases (Montain et al, 2007) or change as a result of heat acclimation (Chinevere et al, 2008); if the duration of exercise was increased or a second exercise bout was scheduled for later that same day, the percentage of dietary potassium lost in sweat would increase, provided there is not a compensatory increase in potassium intake.

On average subjects consumed 133mmol of potassium per day during the first 3 days and 131mmol per day over the entire 5 day study period. Eight individuals consumed more than the average reported potassium intake (81mmol) for males in the UK (Henderson et al, 2003). The higher values in the current study may be partly explained by a positive relationship between potassium intake and energy intake. Although the relationship did

not reach significance in this study, a positive relationship has been reported previously (Holbrook et al, 1984; Bingham et al, 1994; Pietinen, 1982). The potassium density of diets in the current study (1.6g/1000kcal) is slightly higher than the 1.5g/1000kcal estimated from Henderson et al (2003), both being higher than other values previously reported in the literature (1.3 - 1.4g/1000kcal; Holbrook et al, 1984; Gregory et al, 1990). This supports the notion of a growing trend for higher potassium intakes as previously reported by Henderson et al (2003).

Although urine potassium excretion is suggested to provide an accurate estimate of potassium intake (Taseveska et al, 2006), only a weak and non-significant relationship was observed between dietary potassium intake and urine potassium excretion (r = 0.41) in the current study. Others have reported similar (r = 0.36-0.40; Gregory et al, 1990; Day et al, 2001; Leiba et al, 2005), lower (r = 0.23; Clark & Mossholder, 1986) and stronger correlations (r = 0.46-0.82; Pietinen, 1982; Clark & Mossholder ,1986; Bingham et al, 1995; Bingham et al, 1997; Holbrook et al, 1984; Caggiula et al, 1985; McKeown et al, 2001). Stronger correlations are generally reported when duplicate portion analysis is implemented (Clark & Mossholder, 1986; Holbrook et al, 1984), or when an increased number of collection days were included (Caggiula et al, 1985; Bingham et al, 1997; Bingham et al, 1995; McKeown et al, 2001; Clark & Mossholder, 1986). There are several factors that can influence the amount of potassium excreted, including the absolute level of potassium intake (Voors et al, 1983) and dietary fibre intake (Cummings et al, 1976; Taseveska et al 2006). Faecal potassium excretion is greater and more variable than sodium (Cummings et al, 1976) and although not measured in the present study, it usually amounts to between 5 to 15mmol/d (Dempsey et al, 1958; Cummings et al, 1976; Arn & Reimer, 1950; Mickelson et al, 1977). Considering the day to day variation in dietary fibre intake was 25% in the current study, it may partly explain the weak relationship between dietary and urinary potassium.

# 5.4.3 Hydration status

Cheuvront et al (2004) have previously shown that an individuals' euhydrated BM can fluctuate daily by up to 1.1%. The variation in BM between days 1, 4 and 6 of the current study was 0.6% (range 0.2 to 1.1%) indicating that individuals were in a similar state of hydration at these times. This was supported by the finding that there were no differences in the osmolality of urine samples obtained from the first void of each morning. Other

investigators have reported urine indices of hydration status indicate individuals fail to fully replace sweat losses during daily two-a-day exercise sessions in American football players (Stover et al, 2006; Godek et al, 2005). It is likely that because one, not two, exercise sessions took place and that this single exercise session took place on the morning of day 4 in the current study, individuals had sufficient time and access to foods and fluids to replace the sweat losses incurred (Casa et al, 2005).

#### 5.4.4 Conclusion

A single bout of exercise in the heat resulted in the loss of 66mmol (1.5g sodium) of sodium in sweat, but this was not sufficient to exceed dietary sodium intake for any individual. The maintenance of sodium balance was largely attributable to a decline in urine sodium excretion compared to dietary sodium intake on the day of exercise. Sweat potassium losses were 8.9mmol (0.3g potassium) and represented only 8% of dietary potassium intake. Consequently dietary potassium intake, urine potassium excretion and potassium balance were unaltered by exercise.

# Chapter 6

# The effect of prior exercise on sweat composition and loss

#### 6.1 Introduction

Several field studies report large amounts of sodium can be lost in sweat during a single 60-120 minute training session (Palmer & Spriet, 2008; Stofan et al, 2002, 2005; Shirreffs et al, 2006). If two exercise sessions were scheduled for the same day, sweat sodium losses could be very high. Given that large sweat sodium losses have been linked to muscle cramps (Stofan et al, 2005) and hyponatraemia (Montain et al, 2006), further study appears warranted.

There is only limited data on the impact of two-a-day exercise sessions on sweat composition. Stofan et al (2005) reported similar sweat sodium concentrations between the first and second training sessions of the day in a group of cramp-prone American Football players (57mmol/L and 52mmol/L, respectively). Similar sweat sodium concentrations were also observed in both training sessions in a separate group of American Football players that were not cramp-prone (22mmol/L and 30mmol/L, respectively). However, no statistical analysis was performed on the data to confirm this finding. Shirreffs et al (2005) reported similar sweat sodium concentrations in one group of football players completing their second training session of the day as those obtained from players from other clubs who only participated in one training session (Maughan et al 2004, 2005). However, Palmer & Spriet (2008) reported that sweat sodium concentrations obtained from a group of ice hockey players in the second of two training sessions were lower than a separate group of players who were tested during the first session of the day. But, these inter-individual comparisons cannot be used to determine whether or not sweat composition was altered by the first exercise session because of the large variation in sweat sodium concentrations between individuals. In the above investigations training sessions were separated by 5.5h (Stofan et al, 2005), 8.5h (Shirreffs et al, 2005) or was not stated (Palmer & Spriet, 2008).

Previously, investigators have reported a decline in sweat sodium concentration in subjects exposed to the heat approximately 5-12h after an aldosterone injection (Sato & Dobson, 1970b; Collins, 1966). Plasma aldosterone concentration increases in response to exercise in the heat (Francesconi et al, 1983; Kirby & Convertino, 1986; Morgan et al, 2004), and this increase is of greater magnitude when an individual is dehydrated (Francesconi et al, 1983; Morgan et al, 2004). Robinson et al (1955) reported that individuals who were

exposed to prolonged work (3-4h) in the heat (45-46°C) showed evidence of a decline in sweat sodium concentrations approximately 4-5h after previous exercise-heat exposure.

In the aforementioned studies (Stofan et al, 2005; Shirreffs et al, 2005, 2006; Palmer & Spriet, 2008; Sato & Dobson, 1970b; Collins, 1966; Robinson et al, 1955) regional sweat collection techniques were implemented. Whilst sweat composition ascertained from regional sweat collection techniques correlate well with the more time-consuming whole body washdown method (Patterson et al, 2000), they consistently overestimate total sodium and chloride losses (Dill et al, 1967; Van Heynigen & Weiner, 1952; Consolazio et al, 1966; Shirreffs & Maughan, 1997; Patterson et al, 2000). As there are regional differences in sweat gland function (Sato & Dobson, 1970a) and sweat composition (Patterson et al, 2000; Costa et al, 1969), assessment of whole body sweat composition may allow a more accurate assessment of the effects of prior exercise to be made.

The aim of this study was to investigate the impact of prior exercise on sweat composition and loss during a second bout of exercise completed later that same day.

# 6.2 Method

# 6.2.1 Subjects

Eight physically active male volunteers participated in this study, which had received prior approval from the Loughborough University Ethical Advisory Committee (R07-P1). All subjects were informed of the experimental procedures and associated risks before their written consent was obtained. Their physical characteristics (mean  $\pm$  SD) were: age 25  $\pm$  4y, height 1.80  $\pm$  0.08m and body mass 77.4  $\pm$  10.2kg.

# 6.2.2 Pre-trial standardisation

In an attempt to standardise the state of hydration prior to each trial, subjects kept a diary of their dietary and exercise regimens in the 48h period preceding the first experimental trial and were asked to replicate their behaviour prior to the second trial. Subjects were asked to refrain from strenuous exercise and alcohol intake during the 24h period immediately preceding each trial. All experimental trials began in the morning following an overnight fast and the consumption of 500mL of water 1.5h before arrival in the laboratory.



**Figure 6.1** Schematic of schedule for both (A) SINGLE and (B) MULTIPLE exercise sessions. Body mass (BM), urine collection (U), breakfast (B), lunch (L) and whole body washdown procedure (WBW).

# 6.2.3 Experimental protocol

Figure 6.1 shows a schematic representation of the study protocol. Subjects completed either one (SINGLE-PM) or two (MULTI-AM and MULTI-PM) exercise sessions on one day in a randomised, crossover design. Trials were separated by 7 days. On arrival at the laboratory, subjects were asked to empty their bladder as completely as possible with the entire volume collected and a sample retained for subsequent analysis. Nude body mass (BM) was measured to the nearest 20g (AFW-120K, Adam Equipment Co Ltd, Milton Keynes, UK), before a standardised breakfast was consumed. Individuals weighed their desired breakfast portion on trial one and this amount was then given on trial two. Following 1.5h of rest on the two-a-day trial, subjects provided another urine sample and then commenced the whole body washdown procedure which is described fully in Chapter 2. Four absorbent patches (Tegaderm, 3M, Loughborough, UK) for sweat collection were also positioned on the subject's scapula, chest, forearm and thigh. Dehydration was induced by intermittent exercise on a cycle ergometer at an intensity which corresponded to  $153 \pm 15W$ . Exercise periods of 10 minutes were separated by 5 minutes of rest, during which subjects remained inside the bag. This pattern continued until 40 minutes of exercise was completed. After 30 minutes of exercise, sweat patches were carefully removed with tweezers and a sample extracted using a 10mL syringe into a small container for subsequent analysis. Upon the completion of exercise subjects showered and a final nude BM was obtained before subjects dressed and returned to a comfortable environment

within 15 minutes of the completion of exercise. Over the following 30 minutes, subjects consumed a pre-prepared, standardised lunch consisting of sandwiches, cereal bars and fruit squash (Table 6.5). Subjects were then free to leave the laboratory. Approximately 4.5h following the cessation of exercise, subjects returned to the laboratory. They were asked to provide a urine sample, before completing the same washdown procedures as those carried out in the morning. Similarly, the exercise task and sweat collection methods were repeated as per the morning session with the onset of exercise occurring 5h after the cessation of the morning session. Upon the completion of exercise and the washdown procedure, subjects were free to leave the laboratory and continue with their normal daily activities.

When only one exercise session was to be completed, subjects followed the same schedule as has been previously described except that after the breakfast meal was consumed, subjects were free to leave the laboratory. Subjects returned to the laboratory to consume lunch and then for the afternoon exercise session (SINGLE-PM). On one, and two-a-day trials, subjects were allowed to consume water ad libitum during the resting period but the amount was recorded by weighing a sports drink bottle before and after its use.

# 6.2.4 Sample analysis

The total volume of each urine sample was measured using 500mL measuring cylinder and each sample was analysed for sodium, potassium and chloride. Whole body and regional sweat samples were analysed by ion chromatography (DX-80 Ion Analyser, Dionex). Dietary food intake were analysed using Compeat Pro 5.8.0 Software. All analytical procedures are described fully in Chapter 2.

# 6.2.5 Statistical analysis

All data were tested for normality of distribution. Data were analysed by repeated measures ANOVA followed by paired t-tests with Holm-Bonferroni adjustment for multiple comparisons or Freidman's Test followed by Wilcoxon tests when found not to be normally distributed. Correlations were assessed using Pearson's correlation or Spearman's Rank when found not to be normally distributed. Parametric data are expressed as mean  $\pm$  SD and non-parametric data as median (range). In some circumstances a range has been reported regardless of the distribution of data as it was deemed to provide further useful information. Statistical significance was set at P<0.05.

# 6.3 Results

# 6.3.1 Exercise

Despite subjects completing each trial at the same (P>0.05) workload, temperature, humidity and duration (Table 6.1) there was a tendency (P=0.082) for sweat loss during exercise to be lower on trial SINGLE-PM ( $1.26 \pm 0.26L$ ) than MULTI-AM ( $1.36 \pm 0.29L$ ) and MULTI-PM ( $1.36 \pm 0.28L$ ). The percentage dehydration was similar between trials (Table 6.2).

|                            |              | Trial        |              |
|----------------------------|--------------|--------------|--------------|
|                            | MULTI-AM     | MULTI-PM     | SINGLE-PM    |
| Room Temperature (°C)      | $34.9\pm0.4$ | $34.9\pm0.4$ | $34.9\pm0.4$ |
| Room Relative Humidity (%) | $59\pm2$     | $58\pm2$     | $59\pm2$     |
| Bag Temperature (°C)       | $34.8\pm0.4$ | $34.8\pm0.4$ | $34.8\pm0.4$ |
| Bag Relative Humidity (%)  | $65 \pm 4$   | $65 \pm 4$   | $65 \pm 4$   |
| Workload (watts)           | $152 \pm 16$ | $152 \pm 16$ | $154 \pm 14$ |

**Table 6.1**Ambient temperature and humidity inside (Bag) and outside (Room) of the<br/>large polyethylene bag and the workload during each exercise trial

# **Table 6.2**Sweat loss and % dehydration during exercise.

|                   |                 | Trial         |               |
|-------------------|-----------------|---------------|---------------|
|                   | MULTI-AM        | MULTI-PM      | SINGLE-PM     |
| Sweat Loss (L)    | $1.36 \pm 0.29$ | $1.36\pm0.28$ | $1.26\pm0.26$ |
|                   | (0.99–1.89)     | (0.94–1.82)   | (0.89 - 1.78) |
| Dehydration (%BM) | 1.8             | 1.8           | 1.7           |
|                   | (1.3–2.1)       | (1.3–2.0)     | (1.3 - 1.8)   |

# 6.3.2 Whole body sweat collection

Whole body sweat sodium concentration was significantly higher on trial MULTI-AM (P=0.046) and trial MULTI-PM (P=0.048) than trial SINGLE-PM, but there was no difference in whole body sweat sodium concentration between trials MULTI-AM and MULTI-PM (P=0.914) (Figure 6.2). Whole body sweat sodium loss tended to be higher on trial MULTI-AM (59mmol [20-268]; P=0.078) and trial MULTI-PM (58mmol [24-262]; P=0.069) than trial SINGLE-PM (42mmol [range 13–183]), but there was no difference in whole body sweat sodium loss between trials MULTI-AM and MULTI-PM (P=0.625) (Figure 6.2).

There were no differences in whole body sweat potassium concentration (P=0.304) or whole body sweat potassium losses (P=0.285) between any trial (Figure 6.2). Whole body sweat potassium losses on trials MULTI-AM, MULTI-PM and SINGLE-PM were 7.3mmol [3.3-12.5], 8.0mmol [3.0-12.9] and 4.9mmol [3.3-11.6], respectively (Figure 6.2).

Whole body sweat chloride concentration tended to be higher on trial MULTI-AM (P=0.069) and trial MULTI-PM (P=0.062) than trial SINGLE-PM, but there was no difference in whole body sweat chloride concentration between trial MULTI-AM and MULTI-PM (P=0.922) (Figure 6.2). Whole body sweat chloride losses tended to be higher on trial MULTI-AM (55mmol [20-265]; P=0.078) and trial MULTI-PM (56mmol [22-257]; P=0.078) than trial SINGLE-PM (41mmol [15-176]), but there was no difference in whole body sweat chloride losses between trials MULTI-AM and MULTI-PM (P=0.430) (Figure 6.2).



**Figure 6.2** Sweat electrolyte composition (A) and total sweat electrolyte losses (B) obtained from whole body washdown method. <sup>a</sup> denotes significantly different from trial MULTI-AM, <sup>b</sup> denotes significantly different from MULTI-PM.

# 6.3.3 Regional sweat collection

Regional sweat sodium concentration was significantly higher on trial MULTI-AM than trial SINGLE-PM (P=0.038) and trial MULTI-PM (P=0.045) (Figure 6.3). There was a tendency for regional sweat sodium concentration to be higher on trial MULTI-PM than SINGLE-PM (P=0.106). Sweat sodium losses on trial MULTI-AM (88  $\pm$  56mmol) were significantly higher than SINGLE-PM (68  $\pm$  44mmol; P=0.026) and tended to be higher than MULTI-PM (78  $\pm$  48mmol; P=0.094). Total sweat sodium losses tended to be higher on MULTI-PM than SINGLE-PM (P=0.078) (Figure 6.3).

There were no significant differences in regional sweat potassium concentration (P=0.512) or total sweat potassium losses (P=0.295) between trials (Figure 6.3). Total sweat potassium losses on trials MULTI-AM, MULTI-PM and SINGLE-PM were 7.0  $\pm$  1.6mmol, 6.6  $\pm$  1.4mmol and 6.3  $\pm$  1.2mmol, respectively.

Regional sweat chloride concentration on trial MULTI-AM was significantly higher than trial MULTI-PM (P=0.045) and trial SINGLE-PM (P=0.045). There was also a tendency for regional sweat chloride concentration to be higher on trial MULTI-PM than SINGLE-PM (P=0.086) (Figure 6.3). Sweat chloride losses were significantly higher on MULTI-AM (81  $\pm$  56mmol) than SINGLE-PM (62  $\pm$  44mmol; P=0.027), and tended to be higher on MULTI-PM than SINGLE-PM (71  $\pm$  48mmol; P=0.076). Sweat chloride losses also tended to be higher on MULTI-PM than SINGLE-PM (P=0.064) (Figure 6.3).



**Figure 6.3** Sweat electrolyte composition (A) and total sweat electrolyte losses (B) obtained from regional collection methods. <sup>a</sup> denotes significantly different from trial MULTI-AM.

# 6.3.4 Sweat composition at regional collection sites

A repeated measures ANOVA revealed a significant regional site effect (P=0.002), a trial effect (P=0.015) but no interaction effect (P=0.278) for sweat electrolyte composition. Therefore the data from each trial was pooled (n=24) to assess regional differences in sweat composition. Sweat sodium and chloride concentrations were significantly lower on the thigh than all other collection sites (P<0.05) and were significantly lower on the back and forearm than the chest (P<0.05; Figure 6.4). Sweat potassium concentration was significantly lower on the back than all other collection sites (P<0.05).



**Figure 6.4** Regional (back, chest, forearm and thigh) sweat electrolyte concentrations. <sup>a</sup> denotes significantly different from the back, <sup>b</sup> denotes significantly different from the chest, <sup>c</sup> denotes significantly different from the arm, <sup>d</sup> denotes significantly different from the thigh (P<0.05).

#### 6.3.5 Relationship between sweat rate & sweat composition

Regional sweat sodium (r = 0.13; P=0.555) and chloride (r = 0.15; P=0.493) concentrations were unrelated to sweat rate, but sweat potassium concentration tended to be negatively related to sweat rate (r = -0.37; P=0.079) (Figure 6.5). Sweat electrolyte concentrations obtained from the whole body washdown technique showed sweat sodium (r = 0.36; P=0.081) and chloride (r = 0.40; P=0.053) concentration tended to be positively related to sweat rate. The relationship between sweat potassium concentration and sweat rate was significant (r = 0.90; P<0.001) (Figure 6.5).



**Figure 6.5** The relationship between whole body sweat rate and sweat electrolyte concentrations obtained from the regional (A, C, E) and whole body (B, D, F) sweat collection techniques.

# 6.3.6 Relationship between regional and whole body washdown techniques

The regional sweat collection technique produced significantly higher values for sweat sodium concentration (P=0.010) and tended to produce higher values for sweat chloride concentration (P=0.079) than the whole body washdown technique (Table 6.3). There were no differences in sweat potassium concentrations between the collection techniques (P=0.403; Table 6.3). There was a strong correlation between the sweat sodium concentration measured by each technique (r = 0.90; P<0.001). Similarly there was a strong correlation between the sweat sodium (r = 0.88; P<0.001). The correlation between sweat potassium concentration measured by each technique (r = 0.20; P=0.341)

| Table 6.3 | Sweat electrolyte composition from the regional sweat collection technique |
|-----------|--|
|           | and whole body washdown technique. Values are mean $\pm$ SD and median     |
|           | (range). $*$ denotes significant difference between techniques.            |

|                               | Sweat Electrolyte Concentration |             |               |
|-------------------------------|---------------------------------|-------------|---------------|
|                               | Sodium                          | Potassium   | Chloride      |
| Whole Body Washdown           | 43 (11 – 144)                   | $4.9\pm1.3$ | 40 (12 - 141) |
| <b>Regional Sweat Patches</b> | $58 \pm 29*$                    | $5.0\pm0.9$ | $53\pm30$     |

# 6.3.7 Dietary intake

Table 6.4 reports the nutrient composition of the test meals provided at breakfast and lunch during each trial. The sweat electrolyte losses incurred during SINGLE-PM according to the whole body washdown technique accounted for 39% (range 13-155%),  $20 \pm 7\%$  and 38% (14-149%) of the dietary sodium, potassium and chloride intake consumed in the laboratory, respectively. When two exercise sessions took place (MULTI-AM and MULTI-PM), the percentage of dietary sodium, potassium and chloride lost in sweat were 112% (range 42-450%),  $48 \pm 19\%$  and 107% (42-441%), respectively.

Fluid intake was calculated based on ad libitum water intake and the water content of foods consumed at each meal. Subjects consumed significantly more fluid during the morning of the SINGLE session compared to when MULTIPLE exercise sessions took place. Subjects consumed a significantly greater amount of fluid in the rest period between lunch and the afternoon exercise session on the MULTIPLE trial ( $2216 \pm 634$ mL) than the SINGLE trial ( $1256 \pm 321$ mL) (Figure 6.6). As a result there was a tendency (P=0.078) for more fluid to be consumed on the MULTIPLE trial ( $2822 \pm 670$ mL) than the SINGLE trial ( $2297 \pm 1000$  more fluid to be consumed on the MULTIPLE trial ( $2822 \pm 670$ mL) than the SINGLE trial ( $2297 \pm 1000$  more fluid to be consumed on the MULTIPLE trial ( $2822 \pm 670$ mL) than the SINGLE trial ( $2297 \pm 1000$  more fluid to be consumed on the MULTIPLE trial ( $2822 \pm 670$ mL) than the SINGLE trial ( $2297 \pm 1000$  more fluid to fluid the fluid to flui

465mL) over the entire study period. The amount of fluid consumed between MULTI-AM and MULTI-PM represented  $167 \pm 50\%$  of BM lost during MULTI-AM.

**Table 6.4**Energy, carbohydrate, protein, fat and electrolyte (sodium, potassium,<br/>chloride) content of breakfast, lunch and the total consumed during the<br/>study period.

| Nutrient         | Breakfast      | Lunch | Total          |
|------------------|----------------|-------|----------------|
| Energy (kcal)    | $432\pm87$     | 1166  | $1598\pm87$    |
| Carbohydrate (g) | $86\pm20$      | 157   | $243\pm20$     |
| Protein (g)      | $15 \pm 3$     | 37    | $53 \pm 3$     |
| Fat (g)          | $6 \pm 1$      | 48    | $53 \pm 1$     |
| Sodium (mg)      | $742 \pm 118$  | 1786  | $2528 \pm 118$ |
| Potassium (mg)   | $716\pm161$    | 444   | $1160\pm161$   |
| Chloride (mg)    | $1191 \pm 182$ | 2729  | $3920\pm182$   |
| Sodium (mmol)    | $32 \pm 5$     | 78    | $110 \pm 5$    |
| Potassium (mmol) | $18 \pm 4$     | 11    | $30 \pm 4$     |
| Chloride (mmol)  | $34\pm5$       | 77    | $111 \pm 5$    |



**Figure 6.6** Fluid intakes during the morning, afternoon and entire study period. \* denotes significant difference between trials (P<0.05).

# 6.3.8 Hydration status and urine variables

There was no difference in the osmolality of pre-exercise urine samples (P=0.454) or preexercise BM (P=0.526) between trials (Table 6.5). There were no differences in the sodium (P=0.986), chloride (P=0.920) or potassium (P=0.980) concentrations of preexercise urine samples between trials (Table 6.5).

Table 6.5Pre-exercise urine osmolality and body mass. Values are mean  $\pm$  SD or<br/>median (range).

|   | MULTI-AM          | MULTI-PM       | SINGLE-PM         |
|---|-------------------|----------------|-------------------|
| Pre-exercise urine Osmolality (mosmol/kg) | 313 (146 - 624)   | 230 (56 - 762) | 259 (102-682)     |
| Pre-exercise body mass (kg)               | $77.51 \pm 10.16$ | $77.27\pm9.78$ | $77.69 \pm 10.18$ |
| Pre-exercise Urine Sodium (mmol/L)        | $70 \pm 42$       | $66 \pm 56$    | $69 \pm 40$       |
| Pre-exercise Urine Potassium (mmol/L)     | $38 \pm 15$       | $41 \pm 34$    | $41 \pm 29$       |
| Pre-exercise Urine Chloride (mmol/L)      | $56 \pm 35$       | $54 \pm 55$    | $48\pm41$         |
|   |                   |                |                   |

# 6.4 Discussion

It has been established that some individuals can lose large amounts of sodium in sweat during a typical training session (Stofan et al, 2002, 2005; Palmer & Spriet, 2008; Shirreffs et al, 2006) and this may account for a large proportion of dietary sodium intake (Chapter 5). If a second exercise session is scheduled for later the same day, the extent of sweat electrolyte losses will be increased, but it is currently unclear as to whether the sweat losses incurred during the second exercise bout are affected by prior exercise. The main finding of the current study was that sweat sodium and potassium concentrations obtained by the whole body washdown technique remained unchanged in the second of two exercise bouts undertaken on the same day.

Previously, Stofan et al (2005) reported similar sweat sodium concentrations during both morning and afternoon practices in two groups of American Football players, but this was not the focus of the study and as a consequence no statistics were reported on the data. Other investigators have reported that aldosterone, administered intravenously, did not reduce sweat sodium concentration until a period of at least 5-12h had elapsed (Sato & Dobson, 1970b; Collins, 1966). In one of a series of experiments, Robinson et al (1955) exposed subjects to 3-4h of exercise-heat exposure (45-46°C). After a 4-5h rest period in cool conditions, subjects returned to the heat for a further exercise session. They reported sweat sodium concentration to be lower in the second exercise in the heat increases plasma aldosterone concentration (Francesconi et al, 1983; Kirby & Convertino, 1986;

Morgan et al 2004) and this increase is of greater magnitude in individuals who are dehydrated (Francesconi et al, 1983; Morgan et al, 2004), it is possible that the 5h time period that elapsed between exercise sessions in the current study was too short for aldosterone to exert a significant effect on sweat sodium concentrations. Alternatively, several other factors may be responsible for the absence of changes in sweat sodium concentration including the method of sweat collection, the foods and fluids provided, sweat rate and circadian rhythm.

In the current study, regional and whole body sweat collection techniques were contradictory in determining the effect of prior exercise on sweat sodium concentration. Using the regional sweat patch technique, sweat sodium concentration on trial MULTI-PM was significantly lower than on trial MULTI-AM, whereas sweat sodium concentrations were similar on trial MULTI-AM and MULTI-PM using the whole body washdown technique. This may indicate that the effect of exercise on sweat sodium concentration was different depending on the region of the body that sweat was collected.

Inoue et al (1998) investigated the relationship between sweat rate and sweat sodium concentration in 4 different regions of the body (chest, back, forearm and thigh) during exercise. A significant relationship was reported between sweat rate and sweat sodium concentration at each collection site, but the relationship was steeper on the limbs than the torso, suggesting regional differences in the ability of sweat glands to reabsorb sodium. One factor that was suggested to be responsible for this finding was that the sensitivity of the sweat glands to aldosterone was different in different regions of the body. Kirby & Convertino (1986) have previously reported an increased sensitivity of sweat glands to aldosterone following heat exposure and because of the regional differences in sweat gland function (Inoue et al, 1998; Sato & Dobson, 1970a), the sensitivity of sweat glands in different regions of the body may be affected differently by prior exercise. Support for this comes from the study of Bates & Miller (2008) who collected sweat from the upper arm and thigh during exercise on two consecutive days. They reported that sweat sodium concentrations obtained from the upper arm were significantly lower on day 2 than day 1 by 9mmol/L. The decline was smaller in magnitude and did not reach significance in sweat obtained from the thigh (4mmol/L). In the current study sweat was collected from 4 skin sites (back, chest, forearm and thigh). The average sweat sodium concentration of these 4 sites was taken as an indirect estimate of whole body sweat sodium concentration.

Statistical analysis of the data revealed that although regional differences in sweat composition were found, there was no interaction between exercise session and the collection site, indicating that all sites responded in a similar manner to exercise regardless of whether that session was the first or second of the day. On close inspection the difference in sweat sodium concentration between MULTI-AM and MULTI-PM using the whole body washdown technique was 4mmol/L compared to 6mmol/L using the regional sweat patch technique. Therefore the difference between techniques, although significant, was small. Furthermore, no differences in total sweat sodium loss were observed between MULTI-AM and MULTI-PM, regardless of the sweat collection technique used.

In the studies of Robinson et al (1955), subjects refrained from consuming salt during the recovery period and this was suggested to potentiate the decline in sweat sodium concentrations that were seen in the exercise sessions that took place between 4h and 22h later. Indeed, other investigators have reported a period of salt deficiency is important for changes in sweat sodium concentration to be seen (McCance, 1938; Robinson et al, 1956). In the current study, food was provided at breakfast and lunch. For 6 out of the 8 subjects, the lunch provided a sufficient amount of sodium to replace all the sodium lost in sweat during MULTI-AM. Consequently, the replacement of sweat sodium losses may have masked any effect of prior exercise on sweat sodium composition.

Whilst both lunch and breakfast were standardised for each individual, the amount of sodium ingested at lunch (78mmol) was greater than that ingested at breakfast (32mmol). However, this is unlikely to have altered sweat composition during the following bout of exercise as such acute changes in diet have not been reported to have an effect on sweat composition (Chapter 5; Stofan et al, 2005; Barnett & Maughan, 1996). Stofan et al (2005) reported that sodium-rich foods consumed in the morning, did not affect sweat composition during an exercise session completed in the evening later that same day. Barnett & Maughan (1996) investigated the effect of fluid intake before and during exercise on sweat composition. Subjects consumed either a low-sodium (4mmol/L) or high-sodium (40mmol/L) solution in an amount equal to sweat loss. There was no effect of drink sodium concentration was unrelated to dietary sodium intake on the 3 days preceding exercise. Furthermore, if this acute intake was to affect sweat sodium concentration, sweat

sodium concentration would have been higher on SINGLE-PM than MULTI-AM, but this was not observed.

During the study period, food intake was standardised as was the time of day that exercise took place, but fluid intake was allowed ad libitum. The hydration status of an individual has the potential to affect both sweat rate and composition (Cage et al, 1970; Morgan et al, 2004; Montain et al, 1995; Robinson et al, 1956). However, pre-exercise urine samples did not indicate individuals were hypohydrated prior to MULTI-PM. Although urine indices may not accurately reflect hydration status during acute periods when individuals are consuming large amounts of fluid (Popowski et al, 2001; Cheuvront & Sawka, 2005), pre-exercise body mass was similar to those obtained before MULTI-AM. Furthermore, analysis of fluid intake patterns showed that individuals consumed approximately 167% of body mass loss incurred during MULTI-AM, which in combination with the sodium content of lunch, would be expected to assist in the ability of the body to retain the ingested fluid volume and restore fluid balance (Shirreffs et al, 1996; Ray et al, 1998; Maughan et al, 1996).

Whilst no difference in sweat rate or sweat sodium concentration were found between MULTI-AM and MULTI-PM, both sweat rate and sweat sodium concentration were lower on trial SINGLE-PM than trial MULTI-AM and trial MULTI-PM. The sweating threshold is elevated in the afternoon compared to early morning (Stephenson et al, 1984; Wenger et al, 1976) and this may explain the differences, albeit small, in sweat rate between trial SINGLE-PM and MULTI-AM. The lower sweat rate on trial SINGLE-PM may also explain the lower sweat sodium concentration between trials, given the positive relationship between sweat rate and sweat sodium concentration (Allan & Wilson, 1971), although this relationship did not reach significance in the current study. But, Collins (1966) reported a diurnal variation in sweat sodium composition, but not sweat potassium concentration that was independent of changes in sweat rate. Sweat sodium concentration was 6-8mmol/L lower in the afternoon than the morning, and this could also explain the lower values seen on trial SINGLE-PM. Interestingly, the lower sweat rate was not apparent on trial MULTI-PM and as mentioned previously, neither was a change in sweat composition. Therefore whilst it was postulated that sweat sodium concentrations may be lower in the second of two exercise bouts due to sodium conservation, this does not seem to be the case, indicating that prior exercise session may alter this response in some way.

Kenefick et al (2009) have recently determined the effect of prior heat stress on exercise performance during a subsequent exercise bout in the heat. There was no effect of prior heat stress on rectal temperature, heart rate or performance, but sweat rate was not measured. In the current study, although subjects did not replace sweat loss during the first exercise bout, fluid intake, urine osmolality and body mass data indicate that fluid losses had been replaced during the 5h recovery period. At present the mechanism that is responsible for preventing a circadian rhythm in sweating response is unclear. Alternatively, the differences in sweat composition and rate between SINGLE-PM and MULTI-AM and MULTI-PM may be attributed to the day to day variation in both these factors.

There is little data on the day to day variation in sweat sodium concentrations. Shirreffs & Maughan (1997) collected sweat using the whole body washdown technique from 5 subjects who cycled in the heat until approximately 2% of BM was lost. This was repeated on 4 occasions each separated by 7 days. Subjects standardised their diet for the 2 days prior to exercise. The intra-individual range in sweat sodium concentrations obtained was  $22 \pm 5$ mmol/L and the coefficient of variation was between 10-23%. In the current study the intra-individual range in sweat sodium concentration obtained between all 3 exercise sessions was  $17 \pm 11$ mmol/L. This value was inflated by one individual, but upon their removal, was reduced to  $13 \pm 6$ mmol/L. This corresponded to a CV of 18% (range 5-29%) for all individuals. It is therefore possible that this day to day variation contributed to the changes observed between the two-a-day trial and the one-a-day trial, but may also mask any differences between MULTI-AM and MULTI-PM.

The amount of sodium lost in sweat when one or two exercise sessions were completed on the same day was 42mmol (0.97g) and 117mmol (2.69g), respectively. The loss of sodium in sweat during SINGLE-PM represented 39% of sodium intake for that day, but this increased to 112% of sodium intake when two exercise sessions (MULTI-AM and MULTI-PM) took place. It should be emphasised that this refers to the intake of sodium from the foods and fluids consumed within the laboratory and does not take into account any foods ingested after the afternoon exercise session. Although sodium balance was not measured in the current study, the loss of sodium in sweat has the potential to cause perturbations in sodium balance. In an attempt to compensate for sweat sodium losses, there is a decrease in urine sodium excretion (Lichton, 1957; Robinson et al, 1955; Chapter 5). In the current study, electrolyte balance throughout the day was not measured but urine samples obtained before MULTI-PM failed to indicate significant sodium conservation by the kidneys, despite the sweat sodium losses that were incurred during MULTI-AM. Godek et al (2005) have previously reported a decline in urine sodium concentration in American Football players undertaking two exercise sessions per day for 8 consecutive days, indicating significant sodium conservation by the kidneys. However the decline in urine sodium excretion was not observed until the day following the first two-a-day session. In the current study, a single urine collection was made prior to the second exercise bout and in the study of Godek et al (2005) the average sodium conservation by the kidneys. These "spot check" urine collections have their limitations as they cannot assess sodium balance and are prone to inaccuracies especially during acute periods of sweat loss and drink ingestion (Popowski et al, 2001; Cheuvront & Sawka, 2005). Further insight into the effects of 2-a-day exercise sessions on sodium balance would be gained if a more accurate assessment of intake and excretion were to be made.

Sweat sodium concentrations were 32% greater with the regional collection method than the whole body washdown technique. In contrast no differences were observed for sweat potassium concentration. The overestimation of sweat sodium concentration by regional collection techniques is in accordance with other studies (Dill et al, 1967; Van Heynigen & Weiner, 1952; Consolazio et al, 1966; Shirreffs & Maughan, 1997; Patterson et al, 2000) and has been attributed to the formation of an artificial environment beneath the sweat patch (Van Heynigen & Weiner, 1952; Shirreffs et al, 2006), leaching of electrolytes from the stratum corneum (Weschler 2008) and regional differences in sweat rate and composition (Costa et al, 1969; Patterson et al, 2000; Lemon et al, 1986; Havenith et al, 2008). Nevertheless, despite the aforementioned problems associated with regional sweat collection methods, indirect estimates of whole body sweat sodium concentration obtained from regional sweat sodium concentrations are reported to correlate well with the whole body washdown technique (Patterson et al, 2000) and this was supported by the strong correlations found in the current study.

In conclusion, sweat sodium concentration remains unaltered in the second of two exercise sessions scheduled on the same day when exercise bouts were separated by 5h. Further evidence is provided to suggest that regional sweat collection techniques overestimate

sweat sodium concentrations obtained from the whole body washdown technique, but there is a strong correlation between the two collection methods.

# Chapter 7

# The effect of two bouts of exercise on water and electrolyte balance

# 7.1 Introduction

Individual sweat electrolyte losses during exercise can vary greatly. Recent studies have reported average sweat sodium losses of between 1.5 - 2.3g (67 - 99mmol) during a 90 minute training session, but for some individuals sodium losses were as high as 3.1g (133mmol) (Maughan et al, 2004, 2005; Shirreffs et al, 2005). Stofan et al (2002) have also reported large sweat sodium losses in American Football players during training. For one individual, 9.9g of sodium was lost in a 2h training session. Whilst the values reported above most likely overestimate sweat sodium losses by approximately 30-40% due to the regional sweat collection method that was employed (Shirreffs et al, 2006), even when this is corrected for, individuals would still lose substantial amounts of sodium.

In some sports, athletes participate in training sessions twice a day and there may be situations in other sports where this occurs on an infrequent basis during the season. It has been previously reported that sweat electrolyte losses during a second exercise bout were similar to the first exercise bout undertaken earlier that same day (Chapter 6). Provided there is not a compensatory increase in sodium intake, sweat sodium losses would account for a greater proportion of dietary sodium intake, if not exceed it. Those individuals prone to large sweat sodium losses may be predisposed to an increased risk of muscle cramps (Dill et al, 1938; Stofan et al, 2005) and possibly hyponatraemia (Montain et al, 2006).

The sweat glands have priority over the kidneys for sodium and therefore as a result of the sweat sodium losses incurred during exercise, urine sodium excretion is reduced (Lichton, 1957). In Chapter 6, sodium balance was not measured, but Godek et al (2005) reported urine sodium concentrations during two-a-day practices in 10 college American Football players to be significantly depressed compared to baseline values during the 8 day study period. In some individuals urine sodium concentrations were undetectable on a number of days, indicating sodium retention by the kidneys. Whilst the authors suggested this indicated a negative sodium balance, urine sodium concentrations were averaged from 4 urine collections per day and sodium intake and sweat sodium losses were not measured.

In Chapter 5 of this thesis, sweat sodium losses during a single exercise session did not exceed sodium intake and were sufficiently offset by the conservation of sodium by the kidney. Additionally, there were no differences in the urine osmolality of the first void on each day, indicating that individuals successfully replaced the fluid and electrolyte losses

incurred during the exercise bout. The aim of this study was to determine if two exercise sessions on the same day, would 1) result in negative sodium balance or 2) render an individual hypohydrated the following day.

#### 7.2 Method

#### 7.2.1 Subjects

Nine healthy male volunteers participated in this study which had received prior approval from the Loughborough University Ethical Advisory Committee (R08-P55). All subjects were informed about the experimental procedures and associated risks before their written consent was obtained. Their physical characteristics (mean  $\pm$  SD) were: age 24  $\pm$  4y, height 1.81  $\pm$  0.08m, body mass 77.2  $\pm$  7.1kg and body fat 12  $\pm$  3%.

#### 7.2.2 Experimental protocol

Subjects reported to the laboratory at least one day prior to the commencement of the study period when they were given the equipment for dietary and urinary collections and a detailed briefing of the collection procedures. In addition, the logbooks provided contained written instructions about the dietary and urine collection process. Figure 7.1 shows a schematic representation of the study protocol.



**Figure 7.1** Schematic of the study period. Body mass (BM), dietary collection period (D), 24h urine collection (U) and whole body washdown procedure (WBW). Each 24h urine collection started the morning of one day and was terminated on the morning the following day.
On the morning of day 1, subjects arrived in the laboratory and nude body mass (BM) was measured (AFW-120K, Adam Equipment Co Ltd, Milton Keynes, UK). Body fat was then estimated using Harpenden Skinfold Callipers according to the method of Durnin & Rahaman (1967). Subjects were then free to leave the laboratory and continue with their normal daily activities but were asked to follow the instructions for the dietary and urine collection procedures (described below).

Subjects reported to the laboratory on the morning and afternoon of day 4 for the first (AM) and second (PM) exercise sessions which were separated by a 5h recovery period. Subjects were allowed to consume their normal breakfast or lunch before arrival at the laboratory if they wished. On day 6 a final nude BM was obtained.

#### 7.2.3 Dietary monitoring

Subjects were asked to follow their normal dietary behaviour, but to weigh and record all food and drink consumed for 5 consecutive days using electronic scales. The dietary collection procedures are described fully in Chapter 2.

#### 7.2.4 Urine collection

During the same 5-day period and on the morning of day 6, subjects were asked to collect all urine passed. On the first day of collection, the first pass of urine was collected, but was not included in any calculations apart from the assessment of hydration status. The 24h urine collection procedures are described fully in chapter 2.

## 7.2.5 Physical activity

During the collection period, subjects were asked to refrain from strenuous exercise that would provoke sweat losses (apart from the exercise sessions on day 4). On arrival at the laboratory on the morning of day 4, subjects were asked to empty their bladder as completely as possible. This urine sample was included in 24h collections but was also used as an indicator of pre-exercise hydration status. Subjects then commenced the whole body washdown procedure (Shirreffs & Maughan 1997) as described in Chapter 2.

Dehydration was induced by intermittent exercise on a cycle ergometer at an intensity which corresponded to  $153 \pm 12W$ . Exercise periods of 10 minutes were separated by 10 minutes of rest, during which subjects remained inside the bag. This pattern continued

until 40 minutes of exercise was completed. Heart rate (Polar Favor, Kempele, Finland), ambient temperature and relative humidity were recorded every 5 minutes throughout exercise. Upon the completion of exercise subjects showered and a final nude BM was obtained before subjects dressed and returned to a comfortable environment. Subjects were then free to leave the laboratory and continue with their normal daily activities. Approximately 4.5h following the completion of the first exercise bout, subjects returned to the laboratory and provided a urine sample before completing the same washdown procedure as that carried out in the morning. The exercise task and sweat collection method were repeated in exactly the same manner as the morning session with the onset of exercise occurring 5h after the cessation of the morning exercise session. After the completion of the afternoon session, subjects were free to leave the laboratory and continue with their normal daily activities.

#### 7.2.6 Sample analysis

Urine samples were analysed for sodium, potassium, chloride, creatinine and osmolality as described in Chapter 2. Sweat samples were analysed for sodium, potassium and chloride by ion chromatography (DX-80 Ion Analyser, Dionex). Completeness of each 24h urinary collection was reported by subject self-report each day and via creatinine analysis (Jaffe 1886). Weighed food intakes were analysed using Compeat Pro 5.8.0 Software.

# 7.2.7 Statistical analysis

All data were tested for normality of distribution. As urine electrolyte excretion can provide an accurate estimate of electrolyte intake in non-sweating individuals (Holbrook et al, 1984) electrolyte intake assessed by weighed food diaries and 24h urine excretion were subject to a two-factor repeated measures ANOVA, followed by paired t-tests with Holm-Bonferroni adjustment for multiple comparisons. Other data were analysed by one-factor repeated measures ANOVA followed by paired t-tests with Holm-Bonferroni adjustment for multiple comparisons or Freidman's Test followed by Wilcoxon tests when found not to be normally distributed. Correlations were assessed using Pearson's correlation or Spearman's Rank when found not to be normally distributed. Parametric data are expressed as mean  $\pm$  SD and non-parametric data as median (range). In some circumstances a range has been reported regardless of the distribution of data as it was deemed to provide further useful information. Statistical significance was set at P<0.05.

## 7.3 Results

# 7.3.1 Exercise

The mean BM loss during exercise was similar (P=0.555) on AM and PM (Table 7.1). Estimated whole body sweat rates were 1.15 Litres/hour (L/h) (0.74 - 1.54 L/h) and 1.11 L/h (0.79 - 1.49 L/h) on AM and PM, respectively. There was no difference in whole body sweat sodium (P=0.969), potassium (P=0.484) or chloride (P=0.715) concentration between AM and PM (Table 7.1). Similarly, there was no difference in total sweat losses of sodium (P=0.934), potassium (P=1.000) or chloride (P=0.539) between AM and PM (Table 7.1).

**Table 7.1**Whole body sweat loss, sweat electrolyte concentrations ([electrolyte]) and<br/>total electrolyte losses during AM and PM and cumulative losses for both<br/>exercise sessions (Total).

|                                   | Exercise Session              |                               |   |  |
|-----------------------------------|-------------------------------|-------------------------------|---|--|
|                                   | AM                            | PM                            | Total   |  |
| Sweat Loss (L)                    | 1.34<br>(0.86-1.80)           | 1.30<br>(0.92-1.74)           | 2.64<br>(1.80-3.48)   |  |
| Dehydration (% BM)                | $1.7\pm0.4$                   | $1.7 \pm 0.4$                 |   |  |
| Sweat [Na <sup>+</sup> ] (mmol/L) | $48\pm31$                     | $47 \pm 25$                   |   |  |
| Sweat [K <sup>+</sup> ] (mmol/L)  | $5.2 \pm 1.5$                 | $5.3 \pm 1.4$                 |   |  |
| Sweat [Cl <sup>-</sup> ] (mmol/L) | $46 \pm 31$                   | $46 \pm 26$                   |   |  |
| Sweat Na <sup>+</sup> Loss (mmol) | $71 \pm 59$<br>(14 - 163)     | $67 \pm 47$<br>(18 - 129)     | $138 \pm 106$<br>(32 - 287)                                 |  |
| Sweat K <sup>+</sup> Loss (mmol)  | $7.5 \pm 3.9$<br>(3.0 - 11.8) | $7.4 \pm 3.4$<br>(3.3 - 11.4) | $\begin{array}{c} 14.9 \pm 7.3 \\ (6.7 - 23.1) \end{array}$ |  |
| Sweat Cl <sup>-</sup> Loss (mmol) | $70 \pm 59$<br>(14 - 163)     | $66 \pm 48$<br>(18 - 129)     | $135 \pm 106$<br>(32 - 287)                                 |  |

#### 7.3.2 Relationship between sweat rate & sweat composition

There were no differences in sweat or electrolyte losses between exercise bouts, therefore data from both the AM and PM exercise bouts were pooled (n=18) to assess the relationship between sweat rate and composition. Sweat sodium (r = 0.56; P=0.017), potassium (r = 0.69; P=0.001) and chloride (r = 0.55; P=0.017) concentrations were all positively related to sweat rate.

## 7.3.3 Hydration status

There was no difference (P=0.803) in the osmolality of the first pass of urine between days (Table 7.2). The average osmolality of the first void was  $480 \pm 156$  mosmol/kg. There was

no difference (P=0.766) in the osmolality of urine samples obtained immediately before AM ( $335 \pm 266$ mosmol/kg) and PM ( $378 \pm 235$ mosmol/kg).

|                  | Day 1       | Day 2         | Day 3         | Day 4         | Day 5         |
|------------------|-------------|---------------|---------------|---------------|---------------|
| Urine Osmolality | $500\pm189$ | $486 \pm 153$ | $462 \pm 122$ | $463 \pm 138$ | $490 \pm 197$ |
| (mosmol/kg)      |             |               |               |               |               |
| >900mosomol/kg   | 0           | 0             | 0             | 0             | 0             |
| 700-900mosmol/kg | 1           | 1             | 0             | 0             | 2             |
| <700mosmol/kg    | 8           | 8             | 9             | 9             | 7             |

**Table 7.2**Osmolality of the first void of each day. Values are mean  $\pm$  SD and (range).

## 7.3.4 Body mass

Subjects' BM remained stable (P=0.496) between the morning of day 1 (77.2  $\pm$  7.1kg) and day 6 (77.1  $\pm$  7.5kg). The coefficient of variation (CV) in BM over the study period was 0.6  $\pm$  0.2% (range 0.4 to 1.0%). There was no difference in pre-exercise body mass between trial AM (77.0  $\pm$  7.4kg) and PM (77.1  $\pm$  7.4kg; P = 0.662).

## 7.3.5 Duration of urine collection

There were no differences (P=0.850) in the duration of each day's urine collection (Table 7.3) but all urine data for each collection period were adjusted to 24h, with this value being used in all subsequent analysis. The average duration of each urine collection over the 5-day period was 24.00h (23.53 – 24.33h). The CV for the duration of urine collection was 0.1 % (0.0 – 1.0%).

**Table 7.3**Duration of each day's urine collection. Values are median (range).

|                | Day 1           | Day 2           | Day 3           | Day 4           | Day 5           |
|----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Duration of    | 24.00           | 24.00           | 24.00           | 24.00           | 24.00           |
| Collection (h) | (23.91 - 24.17) | (24.00 - 24.15) | (23.75 - 24.10) | (23.92 - 24.33) | (23.53 - 24.17) |

#### 7.3.6 Completeness of urine collections

Two subjects reported a failure to collect one complete 24h collection. There were no significant differences (P>0.05) in the amount of creatinine excreted during each 24h collection period when the missed collections were included (data not shown) or excluded (Table 7.4) in data analysis. The CV for urinary creatinine excretion for the two subjects with incomplete collections was 8% and 22%. The incomplete collections have been omitted from all further data reported here. The average amount of creatinine excreted in the urine each day over the 5-day period was  $2024 \pm 339$  mg. The within-individual CV for urinary creatinine excretion was  $9 \pm 5\%$ .

|                     | Day 1        | Day 2        | Day 3        | Day 4        | Day 5        |
|---------------------|--------------|--------------|--------------|--------------|--------------|
| Urine<br>Creatinine | $2040\pm350$ | $2012\pm346$ | $2085\pm435$ | $2072\pm357$ | $1918\pm236$ |

**Table 7.4** Urine creatinine excretion (mg) during each 24h period. Values are mean  $\pm$  SD.

#### 7.3.7 Dietary intake and urine excretion

Urine volume and dietary nutrient intake during each 24h period are shown in Tables 7.5 and 7.6. There were no significant differences (P>0.05) in energy, carbohydrate, protein, fat or fibre intake between days. A significantly greater amount of fluid was consumed on day 4 ( $5492 \pm 1015$ mL) than on day 5 ( $3733 \pm 978$ mL; P=0.040). The CV's for nutrient intakes and urine excretion are shown in Table 7.7.

Figure 7.2 shows the dietary electrolyte intake and urine electrolyte excretion during each 24h period. Dietary sodium intake did not change significantly over time, but urine sodium excretion was significantly lower on day 4 than day 1 (P=0.036) and day 3 (P=0.020). Dietary sodium intake was similar to urine sodium excretion on days 2 and 3 (P>0.05) but on day 1 urine sodium excretion tended to be greater than dietary sodium intake (P=0.070). On the day of exercise (day 4) urine sodium excretion ( $80 \pm 35$ mmol) was significantly lower than dietary sodium intake ( $151 \pm 52$ mmol; P=0.017) and on day 5 urine sodium excretion ( $109 \pm 42$ mmol) remained significantly lower than sodium intake ( $138 \pm 34$ mmol; P=0.031).

Dietary potassium intake and urinary potassium excretion did not change significantly over the 5-day study period (P=0.354) (Figure 7.2). There were no differences (P>0.05) between urine potassium excretion and dietary potassium intake on day 3 and day 4, but urine potassium excretion was lower than dietary potassium intake on day 1 (P=0.016), day 2 (P=0.049) and day 5 (P<0.001).

Dietary chloride intake did not change significantly over time, but urine chloride excretion was significantly lower on day 4 than day 1 (P=0.020) and day 3 (P=0.020) (Figure 7.2). Dietary chloride intake was similar to urine chloride excretion on days 1, 2 and 3 (P>0.05) but on the day of exercise (day 4) urine chloride excretion ( $63 \pm 26$ mmol) was significantly lower than dietary chloride intake ( $148 \pm 54$ mmol; P=0.008). On day 5 urine chloride excretion ( $82 \pm 34$ mmol) remained lower than chloride intake ( $134 \pm 30$ mmol; P=0.002).

|  | Day 1                                      | Day 2                        | Day 3              | Day 4             | Day 5                | 5 Day<br>Average | 3 Day<br>Average |
|--|--|------------------------------|--------------------|-------------------|----------------------|------------------|------------------|
| Urine Volume (mL)  | $3595\pm1868$                              | $3422\pm1592$                | $3162\pm1265$      | $2386\pm824$      | $2546 \pm 1067$      | $3024\pm1388$    | $3385\pm1527$    |
|  |  |                              |                    |                   |                      |                  |                  |
| Table 7.6 Dietary nutrient int   are mean ± SD. * denotes sign | ake during each 24<br>nificantly different | h period and the from day 4. | average over all o | days (5 Day Avera | tge) and days $1, 2$ | and 3 (3 Day Ave | rage). Values    |
|  | Day 1                                      | Day 2                        | Day 3              | Day 4             | Day 5                | 5 Day<br>Average | 3 Day<br>Average |
| Water (g)  | $4212\pm2087$                              | $4198 \pm 1085$              | $3859 \pm 1488$    | $5492 \pm 1015$   | $3733\pm978$         | $4299 \pm 1473$  | $4090 \pm 1553$  |
| Energy (kcal)  | $3082\pm574$                               | $3050\pm584$                 | $2861\pm574$       | $3249\pm888$      | $2643\pm597$         | $2977\pm659$     | $2998\pm563$     |
| Carbohydrate (g)   | $358\pm154$                                | $410\pm80$                   | $376\pm96$         | $492\pm149$       | $373\pm116$          | $402\pm126$      | 381 ± 112        |
| Protein (g)  | $116 \pm 24$                               | $117 \pm 35$                 | $122 \pm 38$       | $109 \pm 36$      | $116 \pm 29$         | $116 \pm 32$     | $119 \pm 32$     |
| Fat (g)  | $110 \pm 38$                               | $108 \pm 42$                 | $103\pm28$         | $105 \pm 42$      | $87\pm40$            | $103 \pm 38$     | $107 \pm 35$     |
| Fibre (g)  | $22 \pm 11$                                | 23 ± 7                       | 21 ± 11            | $23 \pm 13$       | $24 \pm 11$          | 23 ± 11          | $22 \pm 10$      |
|  |  |                              |                    |                   |                      |                  |                  |

| verage). Values | are mean $\pm$ SD. | Table 7.5 Urine volume during each 24h collection period and the average over all days (5 Day Average) and days 1, 2 and 3 (3 Day A |
|-----------------|--------------------|---|
|                 |                    | and 3 (3 Day Average). Values   |

|              | Dietary     | Intake      | <b>Urine Excretion</b> |             |
|--------------|-------------|-------------|------------------------|-------------|
|              | All Days    | 3 Days      | All Days               | 3 Days      |
| Water        | $26 \pm 9$  | $17 \pm 6$  |                        |             |
| Energy       | $19 \pm 4$  | $17\pm8$    |                        |             |
| Carbohydrate | $26 \pm 11$ | $25 \pm 20$ |                        |             |
| Protein      | $22\pm 6$   | $21 \pm 10$ |                        |             |
| Fat          | $27 \pm 11$ | $21 \pm 13$ |                        |             |
| Fibre        | $29\pm8$    | $31 \pm 11$ |                        |             |
| Sodium       | $25\pm9$    | $21 \pm 12$ | $39 \pm 19$            | $25 \pm 11$ |
| Potassium    | $22 \pm 12$ | $21 \pm 16$ | $22 \pm 7$             | $19\pm9$    |
| Chloride     | $25 \pm 10$ | $23 \pm 12$ | $41 \pm 18$            | $21 \pm 10$ |

**Table 7.7**The CV (%) in nutrient intake and urine excretion over all experimental<br/>days (All Days) and days 1, 2 and 3 (3 Days).





**Figure 7.2** Dietary intake and urine excretion of sodium (A), potassium (B) and chloride (C) during each 24h period. All Values are mean ± SD. \* denotes significant difference between dietary intake and urine excretion. <sup>a</sup> denotes urine electrolyte excretion significantly different from day 1, <sup>b</sup> denotes urine electrolyte excretion significantly different from day 3.

#### 7.3.8 Electrolyte balance

Electrolyte balance was estimated from dietary electrolyte intake, urinary electrolyte excretion and sweat electrolyte loss. There were no significant differences in net potassium (P=0.583) or chloride (P=0.058) balance between any 24h period (Figure 7.3). Sodium balance was more positive on day 5 ( $29 \pm 33$ mmol) than day 1 (-40 ± 30mmol; P=0.030). The mean electrolyte balance over the last 4 days of the study period were -4 ± 24mmol, 19 ± 19mmol and 9 ± 24mmol for sodium, potassium and chloride, respectively.





# 7.3.9 Relationship between electrolyte intake and electrolyte excretion

Fluid intake was positively related (P<0.05) to urine volume on days 1, 2 and 3 and tended to be related on day 5 (P=0.052) (Table 7.8). Dietary sodium intake was positively related (P<0.05) to urine sodium excretion on days 2 and 3 and when expressed over the 5-day period (r = 0.67; P=0.050). The relationship between dietary potassium intake and urine potassium excretion was positive (P<0.05) on days 1 and 5 and when expressed over the 5 day study period (r = 0.71; P=0.033). The relationship between dietary chloride intake and urine chloride excretion showed great variability each day, but when expressed over the 5 day period the relationship was positive and significant (r = 0.67; P=0.049).

**Table 7.8** The relationship between dietary water intake and urine volume and dietary electrolyte intake and urine electrolyte excretion in each 24h period, the 3 day average (days 1, 2, 3) and the 5 day average (all 5 days). Values are correlation coefficients. \*denotes significant relationship (P<0.05).

|  | Doy 1 | Day 2 | Day 3 | Day A | Dov 5 | 5 Day   | 3 Day   |
|--|-------|-------|-------|-------|-------|---------|---------|
|  | Day 1 | Day 2 | Day 5 | Day 4 | Day 5 | Average | Average |
| Water intake & urine volume                    | 0.97* | 0.90* | 0.86* | 0.22  | 0.66  | 0.92*   | 0.96*   |
| Na <sup>+</sup> intake & urine Na <sup>+</sup> | 0.39  | 0.70* | 0.68* | 0.06  | 0.65  | 0.67    | 0.84*   |
| K <sup>+</sup> intake & urine K <sup>+</sup>   | 0.71* | 0.27  | 0.61  | 0.20  | 0.95* | 0.71*   | 0.62*   |
| Cl <sup>-</sup> intake & urine Cl <sup>-</sup> | 0.40  | 0.50  | 0.18  | 0.07  | 0.44  | 0.67*   | 0.82*   |

# 7.3.10 Relationship between energy intake and electrolyte intake.

Sodium intake was positively related to energy intake on day 1, but when expressed over all 5 days the relationship was weak (r = 0.22; P=0.564). The relationship between potassium intake and energy intake was significant only on day 4. When expressed over all 5 days the relationship was moderate but not significant (r = 0.51; P=0.163). Chloride intake was positively related to energy intake on day 1, but when expressed over all 5 days the relationship was weak and non-significant (r = 0.35; P=0.350). The average sodium and potassium densities of the diets were 1.2g (53mmol)/1000kcal and 1.5g (38mmol)/1000kcal, respectively.

**Table 7.9**The correlation coefficients between dietary energy intake (kcal) and<br/>dietary electrolyte intake (mmol) during each 24h period, the 3 Day<br/>Average (days 1, 2, 3) and 5 Day Average (days 1, 2, 3, 4, 5). \*denotes<br/>significant relationship (P<0.05).</th>

|                                 | Day 1 | Day 2 | Day 3 | Day 4 | Day 5 | 5 Day<br>Average | 3 Day<br>Average |
|---------------------------------|-------|-------|-------|-------|-------|------------------|------------------|
| Energy & Na <sup>+</sup> Intake | 0.74* | 0.34  | 0.45  | 0.56  | 0.33  | 0.22             | 0.27             |
| Energy & K <sup>+</sup> Intake  | 0.48  | 0.30  | 0.64  | 0.89* | 0.61  | 0.51             | 0.02             |
| Energy & Cl <sup>-</sup> Intake | 0.86* | 0.48  | 0.46  | 0.58  | 0.17  | 0.35             | 0.35             |

# 7.3.11 Relationship between electrolyte intake & sweat electrolyte loss

The total amount of sodium, potassium and chloride lost in sweat was unrelated to the dietary intake of these electrolytes on the days preceding exercise. (Table 7.10). The sweat electrolyte losses incurred during the exercise bout on day 4 accounted for  $94 \pm 72\%$  (range 27 - 226%),  $14 \pm 6\%$  (range 5-23%) and  $94 \pm 71\%$  (range 26-221%) of the dietary sodium, potassium and chloride intake for that day, respectively. The relationship between the total volume of sweat lost during exercise and the volume of fluid consumed during the same day was moderate and approached significance (r = 0.67; P=0.050). However, no relationship was seen on day 4 between dietary electrolyte intake and sweat electrolyte loss for sodium, potassium or chloride (Table 7.10).

**Table 7.10**The relationship between dietary electrolyte intake (mmol) and whole body<br/>sweat electrolyte loss (mmol). Dietary values are the means of days 1, 2<br/>and 3 (3 Day Average), day 3 and day 4. Values are correlation<br/>coefficients.

|   | 3 Day<br>Average | Day 3 | Day 4 |
|---|------------------|-------|-------|
| Dietary Na <sup>+</sup> Intake & Sweat Na <sup>+</sup> Loss | 0.52             | -0.13 | 0.33  |
| Dietary K <sup>+</sup> Intake & Sweat K <sup>+</sup> Loss   | 0.18             | -0.35 | 0.33  |
| Dietary Cl <sup>-</sup> Intake & Sweat Cl <sup>-</sup> Loss | 0.37             | -0.30 | 0.18  |
| Dietary Fluid Intake & Sweat Loss                           |                  |       | 0.67  |

# 7.4 Discussion

This study investigated the effect of two exercise sessions in the heat on electrolyte balance in individuals who were consuming their normal self-selected diets. The loss of sodium in sweat during exercise accounted for 94% (range 27-226%) of dietary sodium intake for that day. Whilst fluid losses were adequately replaced on day 4, there was evidence of significant urinary sodium conservation on the day of exercise and on the following day but no evidence of an increased sodium intake.

Each exercise session involved 40 minutes of exercise, totalling 70 minutes of heat exposure (including rest periods) and resulted in approximately 1.3L of sweat being lost per exercise session. This is not dissimilar to typical sweat losses incurred during a training session or competition (Burke & Hawley, 1997; Broad et al, 1996). Therefore whilst the protocol was considered representative of the normal sweat losses that are encountered by athletes during exercise, it is acknowledged that the extent of sweat

electrolyte losses can vary greatly between individuals due to the large variation in sweat composition and rate.

#### 7.4.1 Sodium

It was reported previously that sweat sodium losses during a single exercise session accounted for ~43% of dietary sodium intake (Chapter 5). In Chapter 6, it was also shown that whole body sweat composition remained un-altered despite the loss of electrolytes in sweat during a previous exercise bout undertaken earlier that same day. Indeed the finding that prior exercise had no significant effect on sweat composition was replicated in the present study when exercise bouts were separated by 5h. Whilst a reduction in sweat sodium concentration has been reported to occur approximately 4h after a previous exercise bout, it may take ~22h before a significant reduction in sweat sodium concentration is observed (Robinson et al, 1955). As a result of the two exercise sessions in the current study, cumulative sweat sodium losses amounted to 138 mmol (range 32 - 287mmol) on day 4. This is equivalent to 3.1g of sodium (range 0.7 - 6.6g).

Sweat sodium losses could potentially be off-set by an increase in sodium intake and indeed other investigators have previously reported an increased sodium intake following exercise (Takamata et al, 1994; Leshem et al, 1999). Individuals consumed on average 154mmol of sodium per day during the current study, but their sodium intake on day 4 (151mmol) was not significantly greater than that on any other day. Furthermore there was no relationship between the amount of sodium lost in sweat and dietary sodium intake on day 4 (r = 0.33). Taken together this would suggest individuals did not show a preference for salty foods following exercise which is in contrast to the studies mentioned previously (Takamata et al, 1994; Leshem et al, 1999). There are several factors which may explain this finding. Subjects in the current study were all university students and were encouraged to follow their normal daily routine. This involved lectures and other commitments and therefore there may have been only a limited period of time to consume foods during the 5h break between exercise bouts or following the second exercise bout. Alternatively, just by the nature of completing two exercise bouts and the whole-body washdown procedure, approximately 4h of their day was spent in the laboratory where food and fluid intake was restricted. These potential time demands are most likely experienced by other populations to a greater or lesser degree including athletes who may have team meetings or other responsibilities during the intervening period between training sessions. Lastly, exercise may reduce appetite especially when undertaken in the heat (Herman, 1993). These factors alone or in combination may have inadvertently led to a reduced opportunity and/or a desire to ingest food and therefore sodium on the day of exercise which was sufficient to override any desire for sodium. As a result sweat sodium losses represented 94% (range 27 to 226%) of dietary sodium intake for that day.

Instead of a change in sodium intake, there was evidence of significant sodium conservation by the kidneys on both day 4 and day 5 as a consequence of the sweat sodium losses incurred. Lichton (1957) previously described a competition between the kidneys and sweat glands for sodium. It was concluded that the sweat gland has priority over the kidney for sodium and as a result urine sodium excretion is reduced. The kidneys can respond quickly in response to sweat sodium losses, with some reports showing this to occur within 1 or 2h of exercise (Robinson et al, 1955). On day 4 urine sodium excretion was significantly lower than dietary sodium intake and remained significantly lower than dietary sodium intake on the day following exercise (day 5). This carry-over effect was not seen when one exercise session was completed (Chapter 5). Whilst this is most likely explained by the extra sweat sodium losses incurred during the second exercise bout; the scheduling of this second bout so that it was completed in the afternoon also limits the time available for sodium repletion post-exercise. Godek et al (2005) have previously reported urine sodium excretion to be significantly depressed the day after the first of several 2-aday practices in American Football players which was suggested to indicate a negative sodium balance. For some individuals urine sodium concentration was undetectable. However, urine sodium concentration was averaged from 4 collections and dietary intake was not recorded. In the current study, despite the loss of sodium in sweat, sodium balance was not significantly different on day 4 than any other day. The reduction in urine sodium excretion potentially minimised the perturbations in sodium balance for some individuals. But, for 3 individuals sweat sodium losses exceeded dietary sodium intake on day 4 and therefore regardless of the effectiveness of urinary sodium conservation would remain in negative sodium balance. The positive relationship between sweat rate and sweat sodium concentration found in this study and by others (Allan & Wilson, 1971), meant that the two individuals with the highest sweat rates, had the highest sweat sodium concentrations. This combination produced the largest total sweat sodium losses. As a consequence, these two individuals produced urine samples with an average sodium concentration of 22mmol/L and 9mmol/L on day 4, and 12mmol/L and 9mmol/L on day 5, thus indicating

considerable sodium conservation by the kidneys. Therefore whilst there was no significant change in sodium balance as a group, individual responses did suggest significant sodium losses and negative sodium balance for some individuals. Whilst sweat loss can be easily monitored in athletes by changes in body mass, the assessment of sweat composition requires specialist equipment (Burke, 2005) and is therefore not as easily accessible to athletes. Without knowing sweat composition, it is hard to accurately identify individuals "at risk" of large sodium losses.

It is unclear as to why cumulative sweat sodium losses (138mmol) could not be completely accounted for by the decrease in urinary sodium excretion on day 4 and day 5 (100mmol) in the current study. Dietary intake, urine excretion and whole body sweat composition were measured, but dermal losses outside of the laboratory and faecal electrolyte losses were not as they were considered negligible and consistent between days (Allsopp et al, 1998). Allsopp et al (1998) have reported faecal sodium excretion to be 4-5mmol/day and others have reported cutaneous losses of sodium to be small (0.09 - 2.59 mmol/d; Dahl et al)1955) so there is little scope for sodium conservation by these avenues. Alternatively, sweat sodium losses may have been overestimated. Many factors can influence sweat composition including the method of collection. However, it is unlikely that the whole body washdown technique used in this study overestimated losses as it has been consistently shown to be more accurate than regional techniques which are prone to overestimation (Patterson et al, 2000; Shirreffs & Maughan, 1997; Van Heyningen & Weiner, 1952). Indeed, the whole body washdown technique has been shown to be valid and reproducible (Shirreffs & Maughan, 1997; Chapter 2). At least part of this discrepancy may be explained by individual variation, with those individuals who lost the greatest amount of sodium in sweat, demonstrating renal sodium conservation on day 4 and day 5, which may have continued into day 6 although data was not obtained on this day.

#### 7.4.2 Potassium

In contrast to sodium, potassium is lost in relatively small amounts in sweat. Although Consolazio et al (1963) have previously reported sweat potassium losses during exercise to account for 44% of dietary potassium intake, this was largely due to the prolonged duration (7.5h) of exercise-heat exposure. Other investigators have suggested exercise can result in a potassium deficiency (Knochtel et al, 1972) but this was due to the determination of a potassium deficiency indirectly via muscle potassium concentrations, which has since been

suggested as an unsuitable method (Costill et al, 1982). Indeed given the physiological range of sweat potassium concentrations (4-8mmol/L) an individual in the current study would have needed to lose between 14-28 litres of sweat before 100% of their dietary potassium intake was lost.

Subjects in the current study lost between 7 and 23 mmol of potassium in sweat, equivalent to between 5 and 23% of their dietary potassium intake on day 4. The discrepancy between the current study and that of Consolazio et al (1963) could also be due to the different potassium intakes of individuals. In the current study, individuals consumed 113mmol of potassium per day during the 5-day study period. Eight individuals consumed more than the average reported potassium intake (81mmol) for males in the UK (Henderson et al, 2003) and all individuals consumed more than the subjects in the study of Consolazio et al (1963) (64mmol/day). In Chapter 5 of this thesis, potassium intake was also higher than the UK average and in both instances the higher potassium intake was not related to energy intake, but instead due to an increased potassium density of diets (1.5 -1.6g/1000 kcal). This supports the notion of a growing trend for higher potassium intakes as previously reported by Henderson et al (2003). Indeed even on very low potassium diets (25mmol/day), a potassium deficiency proved difficult to induce due to a concomitant reduction in urinary potassium excretion (Costill et al, 1982). Consequently it would appear potassium losses in sweat are far less likely than sodium to result in a negative balance.

#### 7.4.3 Hydration status

There were no significant differences in the osmolality of urine samples obtained from the first void of each morning, all of which indicated individuals were not hypohydrated according to the cut-off criteria reported in the literature (Cheuvront & Sawka, 2005; Shirreffs & Maughan, 1998). This is in contrast to other investigators who have reported urine parameters of hydration status to indicate American Football players fail to replace sweat losses during daily two-a-day exercise sessions (Godek et al, 2005). This discrepancy may be due to the fact that players lost greater amounts of sweat (although they had free access to fluids to help off-set sweat losses) and/or the longer training session duration (2h 15min v 1h 10min) compared to the current study. Nevertheless, other studies (Fudge et al, 2008) have reported runners can replace sweat losses incurred during two-a-day exercise session.

# 7.4.4 Conclusion

Two exercise bouts scheduled on the same day resulted in the loss of large amounts of sodium, but not potassium, in sweat. Sweat sodium losses corresponded to 94% of dietary intake. In an attempt to maintain sodium balance there was evidence of significant sodium conservation by the kidneys on the day of exercise and on the following day. In contrast no change in sodium intake was observed.

# Chapter 8

# The effects of two commercially available drinks on restoring fluid balance and subsequent exercise performance in the heat

#### 8.1 Introduction

The relationship between carbohydrate availability and fatigue during prolonged exercise is widely acknowledged (Coggan & Coyle, 1987), but when exercising in the heat the problems of both hyperthermia and dehydration become relatively more important (Febbraio et al, 1996; Coyle, 2004; Nielsen et al, 1993; Bilzon et al, 2002). Current recommendations encourage athletes to drink at a rate that will prevent excessive dehydration (>2% BM) during exercise (ACSM, 2007) and although individual drinking practices vary, most will finish a bout of exercise in a hypohydrated state (Sawka & Pandolf, 1990). Therefore, the restoration of fluid and electrolyte losses should form an integral part of the recovery process. Athletes seem capable of replacing these losses with normal food and fluid intake when the interval between exercise bouts is more than 24h (Casa et al, 2000), but when repeated exercise sessions are scheduled on the same day or the fluid deficit is large, some athletes may fail to re-establish fluid and electrolyte balance (Godek et al, 2005).

Although current guidelines suggest individuals should reduce their sodium intake (SACN 2003; Institute of Medicine 2004), there are certain situations in a sporting environment when sodium ingestion is recommended. The previous experimental chapters of this thesis have focused on the effects of exercise on sodium intake and excretion over a 24h period, but this chapter will focus on fluid and electrolyte intake in an acute period post-exercise.

The ingestion of plain water following exercise results in a fall in plasma osmolality and sodium concentration which stimulates urine production and reduces the drive to drink (Nose et al, 1988a), both of which will delay the rehydration process. In contrast, the addition of sodium chloride to plain water increases intake and reduces urine output (Wemple et al, 1997). The importance of sodium in rehydration drinks has been reported by others (Shirreffs et al, 1996; Mitchell et al, 2000) and has been systematically evaluated in several studies (Maughan and Leiper, 1995; Shirreffs and Maughan, 1998b; Merson et al, 2008). Maughan and Leiper (1995) reported that when a volume equal to 150% of body mass (BM) loss was ingested following exercise-induced dehydration, the amount of fluid retained was inversely related to the drinks sodium concentration. In some situations sodium may not be the only electrolyte that can improve fluid retention. Maughan et al (1994) reported that the addition of either potassium (25mmol/L) or sodium (60mmol/L) to

a rehydration beverage proved equally effective in retaining fluid although their effects were not additive when ingested in a volume of fluid equal to BM loss.

For complete fluid restoration following dehydration, a volume greater than sweat loss must be consumed due to the ongoing obligatory water losses that persist despite an individual being in body water deficit (Shirreffs et al, 1996; Mitchell et al, 2000). In day to day situations both psychological and physiological factors govern intake and therefore the palatability of ingested fluids can prove a determining factor in the rehydration process. Whilst the addition of sodium chloride to fluids can increase volitional intake (Wemple et al, 1997), a high-sodium content may make a drink unpalatable (Wemple et al, 1997; Nadel et al, 1990). Commercially available sports drinks are therefore designed to meet a balance between efficacy and palatability and typically contain around 25mmol/L of sodium (Shirreffs, 2003). Investigators have reported a sports drink to have a slight advantage over plain water in the restoration of fluid balance (Costill & Sparks, 1973; Gonzalez-Alonso et al, 1992; Shirreffs et al, 2004), may confer a distinct advantage in terms of rehydration.

Despite the relatively high electrolyte content of milk (Shirreffs, 2003; Table 8.1), it seems well tolerated and is widely consumed in Europe, North America, Australia and New Zealand (Amanatidis, 2002). Investigations administering milk following exercise have looked at muscle protein synthesis (Elliott et al, 2006; Wilkinson et al, 2007), muscle function (Wojcik et al, 2001), glycogen synthesis (Wojcik et al, 2001; Karp et al, 2006) and endurance capacity (Thomas et al, 2009; Karp et al, 2006), but until recently there was little information on the efficacy of milk in the restoration of fluid balance following exercise-induced dehydration (Shirreffs et al, 2007b). Shirreffs et al (2007b) reported that following exercise-induced dehydration of 1.8% BM, the consumption of skimmed milk in an amount equal to 150% of BM loss, resulted in a reduced urine output over the following 4h recovery period compared to a sports drink. Consequently subjects remained euhydrated during the milk trial but were in negative fluid balance having consumed the sports drink. Whilst the effects of dehydration on performance are well documented, if individuals commence exercise in a hypohydrated state, further decrements in performance may be experienced. Even low levels of dehydration (~1.8% of BM) can impair

performance (Walsh et al, 1994; Armstrong et al, 1985), with detrimental effects occurring in a graded manner as dehydration progresses (Montain & Coyle, 1992).

The purpose of this study was to determine the effectiveness of a sports drink or skimmed milk in restoring fluid and electrolyte balance following intermittent exercise and on subsequent cycling capacity in the heat.

#### 8.2 Method

#### 8.2.1 Subjects

Seven physically active male volunteers participated in this study which had received prior approval from the Loughborough University Ethical Advisory Committee (R06-P15). Their physical characteristics (mean  $\pm$  SD) were: age 23  $\pm$  3y, height 1.80  $\pm$  0.10m, body mass 75.6  $\pm$  11.1kg, body fat 10  $\pm$  2%, peak oxygen consumption (VO<sub>2peak</sub>) 58.7  $\pm$  4.6 ml/kg/min.

#### 8.2.2 Preliminary trials

Subjects completed two preliminary trials. The first was to determine peak oxygen uptake and the second acted as a familiarisation trial. Peak oxygen uptake (VO<sub>2</sub>peak) was determined by a discontinuous, incremental exercise protocol on a cycle ergometer (Gould Corival 300, Groningen, Holland). The test commenced at a workload of 100 watts (W) for 5 minutes. All subsequent stages were 3 minutes in duration and subject to workload increments of 25 or 50W which were determined after consultation with the subject and depended on their performance during the previous stage. This process continued until volitional exhaustion. Heart rate (HR) (Polar Favor, Kempele, Finland) and ratings of perceived exertion (RPE) (Borg, 1973) were assessed during the last 30 seconds of each stage. Gas samples were obtained in the last 2 minutes of the first stage and in the last minute of all subsequent stages. Expired gas was collected using Douglas bags and analysed for oxygen and carbon dioxide (Servomex 1440, Crowborough, UK), volume (Harvard Apparatus Ltd, Edenbridge, UK) and temperature (Edale Instruments, Cambridge UK). Oxygen uptake, carbon dioxide production and respiratory exchange ratio (RER) were subsequently calculated and used to determine the workload that would be undertaken during experimental trials. VO<sub>2peak</sub> was deemed to be achieved if two or more of the following criteria were met: 1) A plateau in oxygen consumption, 2) an RER  $\geq 1.15$ , 3) HR  $\pm$  10 bpm of age-predicted maximum, and 4) RPE of 19 or 20 (British Association of Sports & Exercise Sciences, 1997).

A preliminary trial was completed approximately one week prior to the first experimental trial during which subjects underwent all testing procedures in an attempt to familiarise them with the laboratory setting and the sensation of exercising to exhaustion. The point of exhaustion was determined when the subject failed to maintain a cadence above 60 rpm despite verbal encouragement. Blood samples were not taken from subjects already familiarised to this procedure.

#### 8.2.3 Pre-trial standardisation

In an attempt to standardise the state of hydration prior to each trial, subjects kept a diary of their dietary and exercise regimens in the 48h period preceding the first experimental trial and were asked to replicate their behaviour prior to the second trial. Subjects were asked to refrain from strenuous exercise and alcohol intake during the 24h period immediately preceding each trial. All experimental trials began in the morning following an overnight fast and the consumption of 500mL of water 1.5h before arrival in the laboratory.

#### 8.2.4 Experimental protocol

Figure 8.1 shows a schematic representation of the study protocol. Upon arrival in the laboratory, subjects were seated in a comfortable environment (approximately 25°C) for 15 minutes [to control for the postural effects on blood volume (Shirreffs & Maughan, 1994)], before a 5mL blood sample was obtained from a superficial antecubital vein of the arm. Subjects were then asked to provide a urine sample and to complete a subjective feelings questionnaire which required them to rate their feelings of thirst, stomach fullness, bloatedness, hunger, mouth feel, tiredness, alertness, ability to concentrate and head soreness (Appendix A). Upon entering the heat room which was maintained at approximately 35°C and 65% relative humidity (RH), nude body mass (BM) was measured to the nearest 10g (Adam CFW150 digital scale, Milton Keynes, UK). Two sweat patches (Tegaderm, 3M, Loughborough, UK) were placed on the subject's back, with the skin being initially cleaned with distilled, de-ionised water. Dehydration was induced by intermittent exercise on a cycle ergometer at an intensity which elicited 58  $\pm$  4% VO<sub>2peak</sub>.

towel dried and nude BM was obtained. This pattern continued until subjects were dehydrated by almost 2% BM, with the remaining BM loss achieved via the ongoing perspiration that followed exercise. The first sweat patch was removed when the subject had lost approximately 1% BM and the other upon the completion of the last exercise bout, with a sample being immediately extracted for subsequent analysis in both instances. After a shower, final BM was obtained before subjects dressed and returned to a comfortable environment within 15 minutes of the completion of exercise. A 21-gauge butterfly cannula was then introduced into a superficial forearm vein and was kept in place for the remainder of the trial. Following 15 minutes of seated rest a blood sample was taken, a urine sample obtained and a questionnaire completed.



**Figure 8.1** Schematic of the study period. Drink consumption (D), blood (B), urine (U), questionnaire (Q) and expired air (GAS) collection and body mass (BM), ratings of perceived exertion (RPE), thermal comfort (TC), heart rate (HR), skin temperature ( $T_{sk}$ ) and rectal temperature ( $T_{re}$ ) measurements.

Over the following 60 minutes subjects ingested either a sports drink (Powerade, The Coca Cola Company) (CHO-E) or skimmed milk (Tesco PLC, Cheshunt, UK) (M) in a volume equal to 150% of the BM loss (Table 8.1). This was divided into 4 equal volumes with one given every 15 minutes. Drinks were served between 10-12°C. The order of administration of test drinks was randomised using a crossover design, and separated by a minimum of 7 days to avoid any training or acclimation effect (Barnett & Maughan, 1993). Further blood and urine samples were obtained following rehydration (0h) and at 1, 2 and 3h. At these time points, questionnaires were administered that did not differ in content to those administered before exercise, with the exception of those given at the end of the rehydration period which had additional questions regarding subjective perceptions of the test drink (How sweet, salty, bitter and pleasant did your drink taste? How refreshed do

you feel?). In each case a 100mm visual analogue scale was used, anchored at each end by appropriate verbal cues (Appendix B).

Approximately 2.5h following the rehydration period, subjects were reweighed and asked to position a rectal probe 10cm beyond the anal sphincter to allow rectal temperature to be measured. Skin probes (YSI UK Ltd, Hampshire, UK) were then attached at four body sites (chest, arm, thigh, calf) and finally a heart rate monitor (Polar Favor, Kempele, Finland) was positioned.

CHO-E Μ Energy (kcal/L) \* 340 240 Carbohydrate (g/L) \* 50 60 Fat (g/L) \* 0 1 Protein (g/L) \* 0 33 Sodium (mmol/L)  $23 \pm 1$  $32 \pm 1$ Potassium (mmol/L)  $1.6 \pm 0.1$  $42 \pm 0$ Chloride (mmol/L)  $36 \pm 2$  $1 \pm 0$ Osmolality (mosmol/kg)  $280\pm2$  $278 \pm 4$ 

Table 8.1Composition of test drinks. Electrolyte concentrations and osmolality are<br/>expressed as mean  $\pm$  SD. \* denotes values obtained from drink labels

Subjects re-entered the heat chamber at the end of the 3h recovery period which was maintained at  $35.3 \pm 0.5^{\circ}$ C and  $63 \pm 2\%$  relative humidity during exercise. Subjects mounted an electronically braked cycle ergometer (Gould Corival 300, Groningen, Holland) and began cycling at an intensity eliciting  $62 \pm 2\%$  VO<sub>2peak</sub>. Rectal temperature (T<sub>re</sub>), skin temperatures and heart rate (HR) were recorded every 5 minutes throughout exercise. Weighted mean skin temperature (T<sub>sk</sub>) was subsequently calculated according to the method of Ramanathan (1964). Ratings of perceived exertion (Borg 1973; Appendix C) and thermal sensation (a 21-point scale ranging from unbearably cold (-10) to unbearably hot (+10)) (Appendix D) were assessed by questionnaire at this time and every 10 minutes thereafter during exercise. Expired gas samples were collected every 15 minutes and analysed for oxygen and carbon dioxide to allow substrate oxidation to be calculated. The point of exhaustion was determined when the subject failed to maintain a cadence above 60 rpm despite verbal encouragement. Immediately upon the cessation of exercise a blood sample was obtained. BM was measured following exercise and sweat loss during exercise was determined.

#### 8.2.5 Sample analysis

Urine and sweat samples were analysed for sodium, potassium, chloride and osmolality as described in chapter 2. A portion (2.5mL) of each blood sample was mixed with EDTA and duplicate 100µl aliquots were deproteinised immediately in 1000µl of 0.3 N perchloric acid. Following centrifugation, the supernatant was analysed for blood glucose (God-PAP, Randox, Co. Antrim, UK, Maughan, 1982). The remaining EDTA treated blood sample was used for measurements of haematocrit (in triplicate by centrifugation; Hawksley, Sussex, UK) and haemoglobin by the cynamethaemoglobin method. The changes in plasma, blood and red cell volume were then estimated (Dill & Costill, 1974). The remaining, untreated, 2.5mL of each blood sample was dispensed into a container (holding no anti-coagulant) and stored on ice before being centrifuged at 1500g for 15 minutes at 4°C. Serum samples were then separated, refrigerated and later analysed for sodium, potassium, chloride and osmolality. as described for urine analysis (chapter 2).

#### 8.2.6 Statistical analysis

Data were analysed by a two-way repeated measures ANOVA followed by paired t-tests with Holm-Bonferroni adjustment for multiple comparisons when found to be normally distributed, and are expressed as mean  $\pm$  SD. Data not normally distributed are expressed as median (range) and were analysed by Wilcoxon tests where appropriate. Statistical significance was set at P<0.05.

#### 8.3 Results

The mean BM loss during the dehydration procedure was  $1.52 \pm 0.17$ kg during trial CHO-E and  $1.51 \pm 0.16$ kg during trial M (P=0.735). This corresponded to a  $2.0 \pm 0.1\%$  reduction of the pre-exercise BM in both trials. The mean exercise time to achieve this was similar (P=0.296) on trial CHO-E (36.2 ± 4.4 minutes) and trial M (37.2 ± 3.2 minutes). The volume of fluid ingested during the rehydration period was equivalent to 150% of BM loss, equating to  $2.28 \pm 0.25$ L and  $2.26 \pm 0.24$ L in trial CHO-E and M (P=0.735), respectively.

## 8.3.1 Pre-exercise hydration status

Four pre-exercise urine samples had an osmolality of more than 700mosmol/kg (712, 729, 789mosmol/kg), but only one sample was deemed to indicate a hypohydrated state (930 mosmol/kg). The remaining 10 pre-exercise urine samples were lower than

700mosmol/kg. This, in combination with the constancy of pre-exercise BM and serum osmolality on trials CHO-E and M, suggest that the subjects' hydration status was similar prior to each trial and could be considered euhydrated (Cheuvront et al, 2004; Cheuvront & Sawka, 2005; Shirreffs & Maughan, 1998a) (Table 8.2).

|                  | CHO-E             | М                | P-value |
|------------------|-------------------|------------------|---------|
| Body Mass        | $75.39 \pm 10.17$ | $75.13 \pm 9.93$ | 0.534   |
| Urine Osmolality | $460 \pm 326$     | $376 \pm 242$    | 0.558   |
| Serum Osmolality | $279 \pm 4$       | $279 \pm 3$      | 0.916   |

Table 8.2Pre-exercise body mass (kg), urine and serum osmolality (mosmol/kg).<br/>Values are mean  $\pm$  SD.

#### 8.3.2 Urine volume

The volume of urine excreted following rehydration varied over time (P=0.002) and tended to be greater on trial CHO-E than trial M (P=0.056) (Figure 8.2). Peak urine volume occurred 1h following the rehydration period on trial CHO-E but was delayed until the 2h time-point on trial M. Cumulative urine volume was lower on trial M than trial CHO-E at time-points 0, 1 and 2h after the rehydration period and tended to be lower at 3h (P=0.054) (Table 8.3).



**Figure 8.2** Urine volume (mL) over the duration of the experiment. The pre-exercise sample has been omitted. Points are mean  $\pm$  SD. <sup>b</sup> denotes trial CHO-E significantly different (P<0.05) from post-exercise. <sup>c</sup> denotes trial M significantly different (P<0.05) from post-exercise.

**Table 8.3**Cumulative urine volume (mL) produced during the recovery period. Pre<br/>and post-exercise time points have been omitted from calculations. Values<br/>are mean  $\pm$  SD. <sup>a</sup> denotes trial CHO-E significantly different from trial M<br/>(P<0.05).</th>

| Time<br>post-rehydration (h) | CHO -E            | М             |
|------------------------------|-------------------|---------------|
| 0                            | $32 \pm 19^{a}$   | 45 ± 18       |
| 1                            | $393 \pm 215^{a}$ | $206 \pm 105$ |
| 2                            | $679 \pm 297^{a}$ | $400 \pm 84$  |
| 3                            | $861\pm396$       | $525 \pm 118$ |

# 8.3.3 Percentage of drink retained

The percentage of test drink retained was calculated from the cumulative urine volume excreted and drink volume ingested. The percentage of test drink retained was significantly greater at all time points during recovery on trial M than trial CHO-E (P<0.05) (Table 8.4).

**Table 8.4**Percentage of drink retained (%). Values are mean  $\pm$  SD. <sup>a</sup> denotes trial<br/>CHO-E significantly different from trial M (P<0.05).</th>

| Time after rehydration (h) | CHO-E                        | М            |
|----------------------------|------------------------------|--------------|
| 0                          | $98.6 \pm 0.8^{a}$           | $98.0\pm0.8$ |
| 1                          | $83.0 \pm 8.3^{a}$           | $91.0\pm4.1$ |
| 2                          | $70.2 \pm 12.5$ <sup>a</sup> | $82.3\pm3.3$ |
| 3                          | $62.2 \pm 17.1$ <sup>a</sup> | $76.6\pm5.8$ |

#### 8.3.4 Urine osmolality

Urine osmolality was higher post-exercise and immediately after rehydration (0h) than preexercise on trial M (P<0.05). On trial CHO-E, urine osmolality tended to be higher immediately after rehydration (P=0.100) and tended to be lower 1h after rehydration (P=0.090) than pre-exercise. Urine osmolality was significantly (P<0.05) higher on trial M than trial CHO-E at 1, 2 and 3h after the end of the rehydration period (Figure 8.3).



**Figure 8.3** Urine osmolality over the duration of the experiment. <sup>a</sup> denotes CHO-E significantly different from M (P<0.05). <sup>c</sup> denotes trial M significantly different (P <0.05) from pre-exercise.

#### 8.3.5 Net fluid balance

Net fluid balance was calculated relative to the pre-exercise time point taking into account the volume of sweat lost, drink ingested and urine excreted (Figure 8.4). Subjects on both trials were in negative fluid balance following exercise and in positive fluid balance as a result of drink ingestion. Subjects remained in positive fluid balance on trial M for the remainder of the recovery period but returned to pre-exercise values 2h after the rehydration period on trial CHO-E. There was a significant difference in fluid balance between trials 2h after rehydration. By the end of the 3h recovery period there was a tendency for fluid balance to continue to differ between trial M (191  $\pm$  162mL) and trial CHO-E (-135  $\pm$  392mL; P=0.051). This represents a difference in net fluid balance of 326  $\pm$  354mL or 0.4% BM.



**Figure 8.4** Whole body net fluid balance over the duration of the experiment. <sup>a</sup> denotes CHO-E significantly different from M (P<0.05). <sup>b</sup> denotes trial CHO-E significantly different (P <0.05) from pre-exercise. <sup>c</sup> denotes trial M significantly different (P <0.05) from pre-exercise.

#### 8.3.6 Sweat composition

There were no differences in sweat electrolyte composition between sweat patches removed at approximately 1%  $(1.01 \pm 0.18\%)$  or 2%  $(1.72 \pm 0.09\%)$  BM loss. Therefore the sweat composition of patches removed at 1% BM loss was used in all subsequent calculations. The mean sweat sodium, potassium and chloride concentrations over all trials were 56 ± 17mmol/L, 4.8 ± 0.5mmol/L and 47 ± 16 mmol/L, respectively. Mean total sweat sodium (P=0.964), potassium (P=0.469) and chloride (P=0.688) losses did not differ between trials (Table 8.5).

**Table 8.5**Total sweat electrolyte losses (mmol) and electrolyte intake (mmol) from<br/>test drinks. Values are mean  $\pm$  SD. <sup>a</sup> denotes trial M significantly different<br/>from trial CHO-E (P<0.05).</th>

|                            | CHO-E         | М                     |
|----------------------------|---------------|-----------------------|
| Sweat Na <sup>+</sup> Loss | $85\pm29$     | $85 \pm 30$           |
| Sweat K <sup>+</sup> Loss  | $7.1 \pm 1.3$ | $7.4\pm0.9$           |
| Sweat Cl <sup>-</sup> Loss | $70\pm26$     | $74 \pm 29$           |
| Na <sup>+</sup> intake     | $53 \pm 6$    | $72\pm8$ <sup>a</sup> |
| K <sup>+</sup> intake      | $4 \pm 1$     | $95\pm10^{a}$         |
| Cl <sup>-</sup> intake     | $2\pm0$       | $81\pm10~^{a}$        |

## 8.3.7 Electrolyte balance

Net sodium, potassium and chloride balance were calculated as for net fluid balance. Subjects were in negative sodium balance immediately following exercise in both trials, but remained in negative sodium balance for the entire recovery period on trial CHO-E as insufficient sodium was consumed to replace all sodium losses incurred with dehydration (Table 8.5; Figure 8.5A). Although a significantly greater amount of sodium was consumed on trial M than trial CHO-E, subjects tended to remain in negative sodium balance (P=0.092) on trial M immediately following the end of the rehydration period. Due to the ongoing urinary sodium losses, subjects returned to a negative sodium balance 1h following rehydration on trial M which persisted until the end of recovery. The increased urinary sodium excretion observed on trial M (Table 8.6), meant this deficit amounted to  $68 \pm 25$ mmol and  $68 \pm 26$ mmol by the end of the recovery period on trials CHO-E and M, respectively.

On trial CHO-E, insufficient potassium was consumed to replace that lost in sweat (Table 8.5) and as a result, subjects were in net negative potassium balance for the entire experiment. In contrast the amount of potassium ingested on trial M was far in excess of sweat potassium losses (Table 8.5) and despite a greater quantity of potassium being excreted in the urine (Table 8.6), subjects remained in positive potassium balance throughout recovery (Figure 8.5B).

On trial CHO-E, insufficient chloride was ingested to replace that lost in sweat (Table 8.5), consequently subjects were in net negative chloride balance for the entire experiment. Although sweat chloride losses were replaced on trial M, ongoing urinary chloride losses resulted in a net chloride deficit of  $46 \pm 24$  mmol and  $85 \pm 24$ mmol by the end of the recovery period on trial M and CHO-E, respectively (Figure 8.5C).

|             | Time after Rehydration (h) |                       |                        |                        |  |
|-------------|----------------------------|-----------------------|------------------------|------------------------|--|
|             | 0                          | 1                     | 2                      | 3                      |  |
| Sodium      |                            |                       |                        |                        |  |
| Trial CHO-E | $3 \pm 2$                  | $12 \pm 5$            | $22 \pm 7$             | $33 \pm 10$            |  |
| Trial M     | $5\pm2^{a}$                | $19\pm10^{a}$         | $39 \pm 12^{a}$        | $52\pm17$ <sup>a</sup> |  |
| Potassium   |                            |                       |                        |                        |  |
| Trial CHO-E | $3 \pm 2$                  | $8 \pm 4$             | $16 \pm 6$             | $25\pm 8$              |  |
| Trial M     | $4\pm2^{a}$                | $18\pm8$ <sup>a</sup> | $39 \pm 12^{a}$        | $54\pm15$ <sup>a</sup> |  |
| Chloride    |                            |                       |                        |                        |  |
| Trial CHO-E | $2 \pm 1$                  | $6 \pm 4$             | $10 \pm 6$             | $15 \pm 7$             |  |
| Trial M     | $3\pm 2$                   | $17 \pm 10^{a}$       | $39\pm13$ <sup>a</sup> | $51\pm17$ $^{\rm a}$   |  |

**Table 8.6**Cumulative urinary electrolyte excretion (mmol). Pre and post-exercise time<br/>points have been omitted from calculations. Values are mean  $\pm$  SD. <sup>a</sup><br/>denotes trial CHO-E significantly different from trial M (P<0.05).</th>



**Figure 8.5** Whole body net sodium (A), potassium (B) and chloride (C) balance over the duration of the experiment. <sup>a</sup> denotes trial M significantly different from trial CHO-E (P <0.05), <sup>b</sup> denotes trial CHO-E significantly different from pre-exercise (P <0.05), <sup>c</sup> denotes trial M significantly different from pre-exercise (P <0.05).

# 8.3.8 Serum electrolyte concentration

Serum sodium concentration was elevated immediately after exercise on trial M and tended to remain elevated at 0h (P=0.068) and 2h (P=0.096) after rehydration compared to preexercise values. On trial CHO-E, serum sodium concentration tended to increase as a result of exercise (P=0.068) and was elevated significantly 1h after rehydration compared to pre-exercise values. But, no differences were observed between trials (P=0.804) (Table 8.7). A significant difference in serum chloride concentration was observed over time but there were no significant differences between trials (P=0.895). Serum potassium concentration was significantly higher throughout the recovery period on trial M than trial CHO-E (P=0.030).

**Table 8.7**Serum electrolyte concentrations (mmol/L) over the duration of the<br/>experiment. Values are mean  $\pm$  SD. <sup>a</sup> denotes trial CHO-E significantly<br/>different from trial M (P<0.05), <sup>b</sup> denotes trial CHO-E significantly<br/>different from pre-exercise value (P <0.05), <sup>c</sup> denotes trial M significantly<br/>different from pre-exercise value (P <0.05).</th>

| Time after<br>Rehydration | Pre                        | Post                            | 0                      | 1                      | 2                      | 3                       | Exh                         |
|---------------------------|----------------------------|---------------------------------|------------------------|------------------------|------------------------|-------------------------|-----------------------------|
| Sodium                    |                            |                                 |                        |                        |                        |                         |                             |
| CHO-E                     | 141 + 3                    | 144 + 2                         | 142 + 1                | $143 + 2^{b}$          | 142 + 1                | 142 + 2                 | $145 + 1^{b}$               |
| M                         | $141 \pm 3$<br>$141 \pm 2$ | $144 \pm 2^{\circ}$<br>144 ± 2° | $142 \pm 1$<br>144 ± 2 | $143 \pm 2$<br>141 ± 1 | $142 \pm 1$<br>142 ± 1 | $142 \pm 2$ $142 \pm 2$ | $145 \pm 1$ $146 \pm 2^{c}$ |
| Potassium                 |                            |                                 |                        |                        |                        |                         |                             |
| CHO-E                     | $6.5 \pm 0.7$              | $5.9 \pm 0.6$                   | $5.2 \pm 0.5$          | $5.1 \pm 0.5$          | $4.9 \pm 0.5$          | $4.6 \pm 0.5$           | $5.4 \pm 0.7$               |
| Μ                         | $6.8\pm0.9$                | $6.2\pm0.7$                     | $6.6\pm0.8^{a}$        | $6.4\pm0.7^{\:a}$      | $5.9\pm0.7^{a}$        | $5.2\pm0.6^{a}$         | $6.1\pm0.7$                 |
| Chloride                  |                            |                                 |                        |                        |                        |                         |                             |
| CHO-E                     | $104 \pm 2$                | $106 \pm 3$                     | $104 \pm 3$            | $105 \pm 3$            | $104 \pm 2$            | $103 \pm 2$             | $104 \pm 3$                 |
| М                         | $104 \pm 4$                | $104 \pm 2$                     | $105 \pm 3$            | $105 \pm 4$            | $105 \pm 3$            | $104 \pm 3$             | $106 \pm 3$                 |

#### 8.3.9 Serum osmolality

Serum osmolality increased in all trials as a result of exercise. Serum osmolality remained elevated for the remainder of the study on trial M (Figure 8.6) but returned to baseline values 1h after the end of the rehydration period on trial CHO-E. Serum osmolality was significantly lower on trial CHO-E than trial M at 0, 1 and 2h after rehydration and at exhaustion.



**Figure 8.6** Serum osmolality (mosmol/kg) over the duration of the experiment. Values are mean  $\pm$  SD. <sup>a</sup> denotes trial CHO-E significantly different from trial M (P<0.05), <sup>b</sup> denotes trial CHO-E significantly different from pre-exercise value (P <0.05), <sup>c</sup> denotes trial M significantly different from pre-exercise value (P <0.05).

#### 8.3.10 Plasma volume

Plasma volume decreased significantly on trial M (P=0.006) but not on trial CHO-E (P=0.273) in response to exercise (Figure 8.7). Immediately after rehydration plasma volume increased on trial M and remained elevated throughout recovery. On trial CHO-E there was a tendency for plasma volume to increase immediately after the rehydration period (P=0.070) but the increase in plasma volume reached significance 1h later and remained elevated throughout recovery. There were no differences in plasma volume between trials at any time-point.



**Figure 8.7** Changes in plasma volume over the duration of the experiment relative to the post-exercise time point. Values are mean  $\pm$  SD. <sup>b</sup> denotes trial CHO-E significantly different from post-exercise value (P <0.05), <sup>c</sup> denotes trial M significantly different from post-exercise value (P <0.05).

#### 8.3.11 Blood glucose

Blood glucose concentrations were similar between trials at rest (P=0.342) but were higher immediately following rehydration on trial CHO-E ( $7.9 \pm 1.3$ mmol/L) than trial M ( $5.0 \pm 0.5$ mmol/L; P=0.001) (Figure 8.8). Blood glucose concentration was slightly elevated compared to pre-exercise values throughout recovery on trial M (P<0.05), but tended to decline during recovery on trial CHO-E reaching significance 2h after rehydration (P=0.044). Blood glucose concentrations at exhaustion were similar on trial CHO-E ( $5.28 \pm 0.36$ mmol/L) and trial M ( $5.75 \pm 0.50$ mmol/L; P=0.109).



**Figure 8.8** Blood glucose (mmol/L) over the duration of the experiment. Values are mean  $\pm$  SD. <sup>a</sup> denotes trial CHO-E significantly different from trial M (P<0.05), <sup>b</sup> denotes trial CHO-E significantly different from pre-exercise (P <0.05), <sup>c</sup> denotes trial M significantly different from pre-exercise (P <0.05).

# 8.3.12 Subjective feelings and drink palatability

Subjects reported drink CHO-E (71  $\pm$  25) to be sweeter than drink M (38  $\pm$  23; P=0.007), but no differences in drink saltiness (P=0.398), bitterness (P=0.823), pleasantness (P=0.345) or how refreshing (P=0.498) were found between trials (Figure 8.9). Similarly, no significant differences between drinks were reported for subjective feelings of thirst (P=0.441), bloatedness (P=0.069), mouth feel (P=0.609), tiredness (P=0.396), alertness (P=0.077), ability to concentrate (P=0.087) or head feel (P=0.441). However, stomach fullness was higher on trial M (P=0.033), whilst subjects reported feeling more hungry on trial CHO-E (P=0.010) (Figure 8.9).



**Figure 8.9** Perceived drink characteristics (A). Values are mean  $\pm$  SD or median where appropriate. <sup>a</sup> denotes CHO-E significantly different from M (P<0.05). Subjective feelings of thirst (B), fullness (C), bloatedness (D), hunger (E), mouth feel (F), tiredness (G), alertness (H), ability to concentrate (I) and headache (J). Values are mean  $\pm$  SD. <sup>a</sup> denotes CHO-E significantly different from M (P<0.05).
#### 8.3.13 Exercise capacity test

One subject on trial M was stopped due to the obtainment of a high  $T_{re}$  (40.23°C). However, data from this subject were included in all calculations. The mean ambient temperature (P=0.509) and relative humidity (P=0.188) on trial CHO-E (35.2 ± 0.4°C, 64 ± 2% RH) and M (35.2 ± 0.5°C, 63 ± 2% RH) were the same. The mean cycling time to exhaustion was similar on trial CHO-E (39.6 ± 7.3 minutes) and trial M (39.7 ± 8.1 minutes; P=0.952) (Figure 8.10). No trial order effect was observed (P=0.879) with subjects riding for 39.5 ± 7.7 minutes on trial one and 39.7 ± 7.6 minutes on trial two.



Figure 8.10 Exercise time to exhaustion for each individual on trial CHO-E and trial M.

Rectal temperature was higher on trial M than trial CHO-E at rest and after 5 minutes of exercise (Table 8.8). There was a tendency for this to persist at the 10 minute time point (P=0.059) and at fatigue (P=0.055). When expressed as an overall increase in  $T_{re}$ , no differences were observed between trial CHO-E ( $2.48 \pm 0.47^{\circ}$ C) and M ( $2.41\pm 0.48^{\circ}$ C; P=0.755). Skin temperature increased during exercise but no differences were located between trials (P=0.242). Heart rate tended to be elevated at rest on trial M (P=0.073) and was significantly elevated throughout exercise on trial M compared to trial CHO-E (P<0.05) (Table 8.8). There were no significant differences between trials for perceived thermal stress (P=0.094) or ratings of perceived exertion (P=0.744). Sweat rate was similar on trial M (2.72 ± 0.46 L/h) and trial CHO-E (2.56 ± 0.48 L/h; P=0.108). No differences were observed between trials in VO<sub>2</sub> obtained from gas samples after 15 minutes of exercise, nor were there differences in RER, or estimated rates of carbohydrate and fat oxidation (P>0.05).

| (P<0.00).  | 0                             | 5                             | 10                     | 15              | 20              | 25              |
|------------|-------------------------------|-------------------------------|------------------------|-----------------|-----------------|-----------------|
|            | 0                             | 5                             | 10                     | 15              | 20              | 25              |
| Heart Rate |                               |                               |                        |                 |                 |                 |
| CHO-E      | $59 \pm 9$                    | $151 \pm 6$                   | $162 \pm 6$            | $168 \pm 6$     | $174 \pm 4$     | $182 \pm 3$     |
| Μ          | $66 \pm 9$                    | $156 \pm 4$ <sup>a</sup>      | $168\pm5^{\mathrm{a}}$ | $172 \pm 4^{a}$ | $180 \pm 4^{a}$ | $186 \pm 4^{a}$ |
| Tsk        |                               |                               |                        |                 |                 |                 |
| CHO-E      | $33.6\pm0.5$                  | $35.4 \pm 0.4$                | $35.9\pm0.4$           | $36.1 \pm 0.5$  | $36.2\pm0.5$    | $36.3\pm0.5$    |
| Μ          | $33.4\pm0.8$                  | $35.3\pm0.4$                  | $35.9\pm0.3$           | $36.2\pm0.3$    | $36.3 \pm 0.3$  | $36.5\pm0.3$    |
| Tre        |                               |                               |                        |                 |                 |                 |
| CHO-E      | $36.87\pm0.22$                | $37.04 \pm 0.23$              | $37.35\pm0.27$         | $37.70\pm0.28$  | $38.02\pm0.30$  | $38.36\pm0.32$  |
| Μ          | $37.20 \pm 0.22$ <sup>a</sup> | $37.31 \pm 0.22$ <sup>a</sup> | $37.59 \pm 0.19$       | $37.92\pm0.21$  | $38.25\pm0.18$  | $38.57\pm0.23$  |
| TS         |                               |                               |                        |                 |                 |                 |
| CHO-E      | $2 \pm 1$                     | I                             | 4 ± 1                  | ı               | 5 ± 2           | ı               |
| Μ          | $2 \pm 1$                     | ı                             | 4 ± 1                  | ı               | $6\pm 2$        | ı               |
| RPE        |                               |                               |                        |                 |                 |                 |
| CHO-E      | $6\pm0$                       | I                             | $13 \pm 2$             | I               | $16 \pm 2$      | ı               |
| Μ          | $6\pm0$                       | I                             | $14 \pm 1$             | I               | $16 \pm 2$      | I               |
|            |                               |                               |                        |                 |                 |                 |

#### 8.4 Discussion

This study investigated the effectiveness of two commercially available drinks in restoring fluid and electrolyte balance following exercise-induced dehydration and on subsequent endurance capacity in the heat. Despite subjects being in positive fluid balance on trial M, but not so on trial CHO-E at the end of the 3h recovery period, no difference in exercise time to exhaustion was observed.

The ingestion of a carbohydrate-electrolyte solution has been reported to result in a more effective rehydration than plain water (Costill & Sparks, 1973; Gonzalez-Alonso et al, 1992) and diet cola (Gonzalez-Alonso et al, 1992), but in both of these studies a volume equivalent to 100% of BM loss was ingested and as a result of ongoing urine excretion, subjects were in negative fluid balance throughout the recovery period regardless of the drink that was consumed. In the current study a volume equal to 150% of BM loss was ingested, and subjects were essentially euhydrated on trial CHO-E at the end of the 3h recovery period. This is in agreement with some (Shirreffs et al, 2007a), but not all (Maughan et al, 1996; Shirreffs et al, 2007b) investigations. Shirreffs et al (2007a) reported that only when a CHO-E solution (23mmol  $Na^+/L$ ) was consumed during the 1h rehydration period following exercise-induced dehydration, did subjects remain euhydrated throughout the 4h recovery period compared to either mineral water (0-1mmol Na<sup>+</sup>/L) or apfelschorle (8mmol Na<sup>+</sup>/L). However, Maughan et al (1996) reported that when a sports drink (containing 21mmol Na<sup>+</sup>/L) was consumed during a 1h rehydration period postexercise, subjects were essentially euhydrated after 2h but were in negative fluid balance at 4h and 6h after rehydration. It is currently unclear as to why these studies produced contradictory results, as the exercise task, rehydration regimen and drink composition were similar. It could be that the sodium content of the CHO-E solutions consumed was on the borderline of what is needed to provide significant effects on fluid balance (Maughan & Leiper, 1995). Maughan & Leiper (1995) reported that following exercise-induced dehydration, there was no significant difference in cumulative urine volume between two solutions containing either 2mmol/L or 26mmol/L of sodium when they were ingested in an amount equal to 150% of BM loss. However, when the sodium concentration was increased to 52 or 100mmol/L, a significant reduction in cumulative urine excretion was seen. Nevertheless, there is a general consensus that urine volume is inversely related to the sodium concentration of the drink that is ingested (Maughan & Leiper, 1995; Shirreffs & Maughan, 1998b; Merson et al, 2009).

Milk has a relatively high electrolyte content compared to fruit juices, soft drinks and other commercially available fluids (Shirreffs, 2003) which may lend itself to the rehydration process. Previously, Shirreffs et al (2007b) investigated the effects of a CHO-E solution (containing 23mmol Na<sup>+</sup>/L), skimmed milk (39mmol Na<sup>+</sup>/L) and skimmed milk with added sodium (58mmol Na<sup>+</sup>/L) on restoring fluid balance post-exercise. Both milk drinks significantly increased fluid retention and prevented subjects returning to negative fluid balance by the end of the 4h recovery period. In the current study there was a tendency for a reduced urine excretion when subjects ingested drink M compared to CHO-E. As a result subjects were in positive fluid balance at the end of the 3h recovery period on trial M, but not on trial CHO-E.

There is a need to replace the electrolytes lost in sweat following exercise and because sodium is the primary cation in sweat, its replacement is a priority. In this study sweat composition was measured which allowed electrolyte balance to be calculated. Sweat sodium concentrations have been estimated to be between 20-80mmol/L (Verde et al, 1982) and the values obtained in this study fall in the middle of this range. Shirreffs and Maughan (1998b) suggested that for subjects to remain in positive fluid balance, the amount of sodium consumed needs to be greater than sweat sodium loss. In neither trial was sufficient sodium consumed to replace all sweat sodium losses and in combination with urinary sodium excretion, subjects were in a sodium deficit throughout, yet were essentially euhydrated on trial CHO-E and in positive fluid balance on trial M at the end of the recovery period. It is likely that the regional sweat sodium concentrations obtained in this study overestimated losses compared to whole body sweat collection techniques (Patterson et al, 2000; Shirreffs & Maughan, 1997; Chapter 6), although previously, Mitchell et al (2000) reported that provided a sufficient volume was ingested, rehydration was achieved after consuming a beverage with a sodium concentration of 25mmol/L, despite subjects remaining in negative sodium balance. Potassium losses in sweat were small in comparison to sodium, but only on trial M was a sufficient amount of potassium ingested to replace these losses. On trial M, potassium intake was ~1200% greater than that lost in sweat and despite an increased potassium excretion compared to trial CHO-E, subjects remained in positive potassium balance at the end of the study period.

The addition of either potassium (25mmol/L) or sodium (60mmol/L) to a rehydration drink has been reported to confer a similar benefit in terms of fluid retention when given in a volume equal to BM loss (Maughan et al, 1994), but their effects were not additive. However, it is likely that no further reductions in urine output were possible as subjects were hypohydrated throughout the study (Maughan et al, 1994). In a subsequent investigation, Shirreffs et al (2007a) suggested that potassium was not as effective as sodium in promoting fluid retention when drinks were ingested in an amount equal to 150% of BM loss. However, direct comparison between this study and that cannot be made due to commercially-available products being administered that differed in several respects.

Although there was a tendency for a greater volume of urine to be produced on trial CHO-E, serum sodium concentrations were similar between trials despite a greater amount of sodium being ingested on trial M. But it is possible that the rapid increase in urine sodium excretion following drink ingestion on trial M, concealed any potential differences in serum sodium concentrations at the sampling timepoints. Serum potassium concentrations were unexpectedly elevated in both trials before and after exercise. Although postural changes are known to influence serum potassium concentration (Shirreffs & Maughan, 1994), it seems unlikely to be responsible due to the careful control of posture during this study. During the first 2h of recovery, serum osmolality was higher on trial M than CHO-E. The importance in maintaining serum osmolality during rehydration has previously been reported (Nose et al, 1988a) and this may affect the efficacy of a drink by influencing the amount of fluid consumed and the amount of fluid retained.

Costill and Sparks (1973) reported that the ingestion of a glucose-electrolyte beverage resulted in a greater recovery of plasma volume than plain water, and other investigators (Nose et al, 1988a; Shirreffs & Maughan, 1998b; Wemple et al, 1997) have reported a preferential restoration of plasma volume following the ingestion of sodium-containing beverages. In contrast, the ingestion of a drink primarily containing potassium results in a slower rate of plasma volume recovery compared to beverages with either a low electrolyte content or that contain primarily sodium (Maughan et al, 1994; Nielsen et al, 1986; Shirreffs et al, 2007a). This initial delay in plasma volume recovery is suggested to be due to a preferential restoration of the intracellular fluid compartment (Nadel et al, 1990). Despite potassium being the predominant electrolyte in drink M, a delay in plasma volume

restoration was not observed which may be due to the sodium content of milk opposing such an effect.

Whilst the electrolyte content of a drink is a major determinant of its efficacy in terms of fluid retention (Shirreffs et al, 1996), a role of gastric emptying in this case cannot be discounted. Recently, Evans et al (2009) determined the affect of a solutions carbohydrate content on the restoration of fluid balance following exercise-induced dehydration. All drinks contained 25mmol/L of sodium, but the 10% glucose solution extended the duration of time that subjects remained euhydrated by 1h compared to a 2% glucose solution and by 2h compared to a 0% glucose solution. This may be due to a slower gastric emptying of the 10% glucose solution. A myriad of factors influence gastric emptying including the volume, energy density and osmolality of the fluid ingested (Calbet & MacLean, 1997; Vist & Maughan, 1995). A delay in fluid delivery could minimise the perturbations in blood chemistry which have a strong influence in the secretion of fluid regulatory hormones (Nose et al, 1988a). Although the volume and osmolality of test drinks in the current study were similar, drink M had a higher energy content and this could have resulted in a delayed gastric emptying (Calbet & MacLean, 1997; McHugh & Moran, 1979). Whilst it appears that the energy content of a drink is more important than the macronutrient composition, some (Mahe et al, 1992) although not all investigators (Calbet & MacLean, 1997; McHugh & Moran, 1979; Calbet & Holst, 2004) suggest that the casein in milk may clot in the stomach and contribute to a slowing of gastric emptying. In the current study subjects reported increased sensations of fullness on trial M, which supports, albeit indirectly, the suggestion that gastric emptying may have been slowed on this trial.

The addition of protein to a rehydration drink may also affect fluid retention (Seifert et al, 2006; Gisolfi et al, 1990). Seifert et al (2006) compared the efficacy of a carbohydrate solution (containing 20mmol Na<sup>+</sup>/L), a carbohydrate plus protein solution (23mmol Na<sup>+</sup>/L) and water in the restoration of fluid balance following exercise-induced dehydration of 2.5% BM. The amount of fluid retained at the end of the 3h recovery period was significantly greater when the carbohydrate plus protein solution was consumed compared to when the carbohydrate-electrolyte solution or water was ingested. As the difference in sodium concentration between the carbohydrate-electrolyte solution and carbohydrate plus protein solution was small (3mmol/L), the authors attributed the increased efficacy of the carbohydrate plus protein solution to the protein content of the drink. They suggested that

the presence of protein improved intestinal water absorption (Gisolfi et al, 1990), rather than slowing gastric emptying presumably because drinks were isocaloric and the protein was whey, not casein.

In the current study, subjects returned to the heat and cycled to exhaustion at 60% VO<sub>2</sub>max at the end of the recovery period. Despite a difference in fluid balance of 326mL or 0.4% BM between trials, there was no difference in exercise capacity between trial M and trial CHO-E. Merson et al (2008) have recently shown that following exercise-induced dehydration, the ingestion of a solution containing 50mmol/L of sodium resulted in significantly less urine production than when a sodium-free solution was consumed. Nevertheless, the difference in fluid balance (549mL or 0.7% BM) did not confer a performance benefit in the subsequent exercise task, which involved cycling at 95% VO<sub>2</sub>peak to exhaustion in temperate conditions (<25°C).

Current recommendations encourage athletes to drink at a rate that will prevent excessive dehydration (>2% BM) during exercise (ACSM, 2007), as even low levels of dehydration may be detrimental to performance (Armstrong et al, 1985; Walsh et al, 1994), especially in the heat (Coyle, 2004). But despite the difference of 0.4% BM between trials, in neither trial were subjects considered hypohydrated immediately prior to the exercise capacity test, as indicated by net fluid balance. Furthermore, the decrement in performance reported by others when initiating a bout of exercise in a hypohydrated state has been attributed in some part to a reduction in plasma volume (Armstrong et al, 1985). Although the water lost in sweat is most likely to originate from the extracellular space when exercising in the heat (Kozlowski & Saltin, 1964; Nose et al, 1988b), diuretic induced dehydration, as used by Armstrong et al (1985), results in greater reduction in plasma volume than exerciseinduced dehydration (Caldwell et al, 1984). It is likely that a redistribution of fluid losses between body compartments (Nose et al, 1988b) in conjunction with differences in dehydration procedure and expansion of plasma volume via the rehydration regimen may have further negated the impact of the difference in fluid balance on performance in the current study.

The effect of a chocolate milk drink ingested after glycogen depleting exercise is reported to increase subsequent exercise capacity (Karp et al, 2006; Thomas et al, 2009). But, this occurred when the exercise capacity test was completed in temperate conditions (Thomas

et al, 2009), or the environmental conditions were not stated (Karp et al, 2006). Other investigators have also reported benefits of ingesting CHO-E drinks during a 4h recovery period on subsequent exercise capacity (Fallowfield et al, 1995; Wong et al, 2000). Both these studies also took place in temperate conditions (~20°C) and the improvement in performance with a CHO-E solution was attributed to the replenishment of glycogen stores and consequently a higher rate of carbohydrate oxidation during exercise. In the current study blood glucose concentrations at exhaustion did not indicate that carbohydrate availability was the cause of the cessation of exercise, which is consistent with the suggestion that exhaustion in the heat is due to dehydration and hyperthermia (Gonzalez-Alonso et al, 1999; Nielsen et al, 1993; Bilzon et al, 2002), despite an increased reliance on muscle glycogen during exercise (Jentjens et al, 2002).

Time to exhaustion in hot conditions has been reported to be related to initial body temperature (Gonzalez-Alonso et al, 1999) and in this study rectal temperature was significantly lower at rest on trial CHO-E than trial M. Although the difference in rectal temperature tended to persist at exhaustion, no difference in exercise time was observed. Trials were completed at the same time of day to control for circadian variation in rectal temperature (Waterhouse et al, 2004) and subjects performed the initial bout of exercise for the same duration and intensity. The volume and temperature of the drinks ingested and the environmental conditions during recovery were also similar. Whilst hyperosmolality is beneficial in terms of the rehydration process, it may have negative effects on thermoregulatory responses at rest and during exercise (Harrison et al, 1978; Fortney et al, 1984). Harrison et al (1978) reported that ingestion of a saline solution (342mmol/L) increased serum osmolality by ~14mosmol/kg compared to when plain water was consumed. As a result, rectal temperature was  $\sim 0.3^{\circ}$ C higher on the saline trial at rest and during subsequent exercise in the heat (45°C, 30% RH). However, the difference in serum osmolality between trials was far smaller in the current study (2-4mosmol/kg). It is possible that the mechanism responsible for the observed differences in rectal temperature is linked to the different macronutrient and energy content of test drinks and the effect this has on the thermic effect of food (Belko et al, 1986).

Although the addition of sodium to plain water may increase volitional intake (Wemple et al, 1997), a high-sodium content may make a drink unpalatable (Nadel et al 1990; Wemple et al, 1997). Commercially available sports drinks are therefore designed to meet a balance

between efficacy and palatability and typically contain around 25mmol/L of sodium (Shirreffs, 2003). Although a fixed volume of fluid equivalent of 150% of BM loss was consumed during this study, no differences in drink pleasantness or saltiness were found, despite their differing electrolyte content. However, the sweetness of drink CHO-E may enhance fluid intake after exercise in some but not all individuals (Passe, 2001). Considering that both the volume and composition of fluids ingested can affect the restoration of fluid balance (Maughan & Leiper, 1993; Shirreffs et al, 1996), the efficacy of drink M allows further scope in the variety of drinks recommended to be consumed after exercise. This becomes especially important when the recovery time is short, the fluid deficit large or when access to food is not possible or practical (Casa et al, 2000).

In summary, when a volume equal to 150% of BM loss was consumed following exerciseinduced dehydration of  $2.0 \pm 0.1\%$  BM, subjects were essentially euhydrated on trial CHO-E and in positive fluid balance on trial M at the end of the 3h recovery period. This was achieved with only partial replacement of sweat sodium losses by both drink CHO-E and M and complete recovery of sweat potassium losses by drink M. Despite the difference between trials in net fluid balance corresponding to approximately 0.4% BM, no difference in endurance capacity were observed.

# Chapter 9

# General discussion

#### 9.1 Overview

Dietary sodium intake has been, and still is, the topic of much debate, mainly surrounding its association with blood pressure and other health-related concerns (Institute of Medicine, 2004; SACN, 2003). In the sporting arena, exercise can result in sweat loss which gives rise to the loss of both water and other constituents such as sodium, chloride and potassium from the body. Whilst water losses are governed by the amount of sweat lost, electrolyte losses are influenced by both the amount and the composition of sweat. Both these factors exhibit wide variation and therefore can result in substantially different electrolyte losses between individuals despite completing the same exercise session. Sodium is the primary cation in sweat and is therefore lost in greater quantities than potassium. The loss of sodium in sweat may have detrimental effects on health and performance as it has been linked with hyponatraemia (Montain et al, 2006), muscle cramps (Dill et al, 1938; Stofan et al, 2005) and perturbations in fluid balance (Taylor et al, 1943). Recent guidelines (ACSM, 2009) stipulate that the Upper Limit (2.3g (100mmol)/day) (Institute of Medicine 2004) for sodium is too low for many athletes, due to the importance and increased requirement of sodium in this athletic population. In fact the consumption of sodium is encouraged for some individuals (Bergeron, 1996; ACSM, 2009) and in some situations such as during (ACSM, 2009) and after exercise (Shirreffs and Maughan, 1998b). This is in direct contrast to current dietary guidelines aimed at the general population. The aim of this thesis was to address the following questions:

- 1. What are the current sodium, potassium and chloride intakes of healthy recreationally active individuals ? (Chapters 3, 4, 5 and 7)
- 2. Does exercise affect sodium intake? (Chapters 5 and 7)
- 3. Does exercise affect urine sodium excretion? (Chapters 5 and 7)
- 4. Does exercise affect sodium balance? (Chapters 5 and 7)
- Does exercise affect dietary potassium intake, urine potassium excretion and potassium balance? (Chapters 5 and 7)
- Does prior exercise alter sweat electrolyte composition in a second exercise session later that same day? (Chapters 6 and 7)
- 7. Does the electrolyte content of milk enhance the recovery of fluid balance after exercise? (Chapter 8)
- 8. Are there individuals or specific situations which may require an increased sodium intake? (Chapters 5, 6, 7 and 8).

#### 9.2 Dietary sodium intake

According to a recent dietary survey of the British population, the average male and female are consuming 11.0g and 8.1g of salt per day, respectively (Henderson et al, 2003). This is equivalent to 4.3g (187mmol) and 3.2g (138mmol) of sodium per day, respectively. Humans can survive on extremely low sodium diets (Oliver et al, 1975) as obligatory urine, skin and faecal sodium losses are very small (1.7 to 8.0 mmol of sodium per day; Dahl, 1958; Dahl et al, 1955; Dole et al, 1950). Therefore current salt intakes are far greater than requirement for most individuals. In 2004, the Food Standards Agency in the UK launched a campaign aimed at reducing the average salt intake of the British population to 6g/day (2.4g/d of sodium). Whilst these values are based on a significant improvement to the health of the population (SACN, 2003), further decreases would enhance health still further (He & MacGregor, 2003). In the meta-analysis of He & MacGregor (2003) a dose response relationship was observed between blood pressure and a reduction in salt intake. A reduction in salt intake of 3g/d (from an initial salt intake of 12g/d), would decrease (systolic/diastolic) blood pressure by 2.5/1.4mmHg, reduce stroke death by 12-14% and ischemic heart disease deaths by 9-10%. This would result in a 7,300 - 8,300 reduction in stroke deaths and 10,600 - 12,400 reduction in ischemic heart disease deaths per year. If salt intake was reduced by 6g/d, blood pressure would decrease by 5/2.8mmHg and the deaths via strokes and ischemic heart disease would be reduced by 23-25% and 16-19%, respectively.

The assessment of dietary sodium intake has been made by food frequency questionnaire (Day et al, 2001; McKeown et al, 2001), food recall (Leiba et al, 2005; Espeland et al, 2001), food diary (Day et al, 2001; Gregory et al, 1990; Henderson et al, 2003), duplicate portion analysis (Schacter et al, 1980; Clark & Mossholder, 1986) and urine collection (Holbrook et al, 1984; Clark & Mossholder, 1986). All of these techniques have their advantages and disadvantages, but despite the increased subject burden of keeping a weighed dietary record, this method is reported to be more accurate than a food frequency questionnaire, food diary and 24h recall methods (Bingham et al, 1995; Porrini et al, 1995; McKeown et al, 2001). The main limitation of weighed food records is their inability to account for discretionary salt use (Caggiula et al, 1985; Clark & Mossholder, 1986; Melse-Boonstra, 1999; James et al, 1987). Therefore 24h urine collections have been suggested to provide a more accurate estimate of sodium intake. Sodium in excess of requirement is

excreted primarily in the urine and therefore 24h urine sodium collections can provide a good estimate of dietary sodium intake (Holbrook et al, 1984; Taseveska et al, 2006).

The aim of chapter 3 was to determine the current dietary sodium intake and the day to day variation in sodium intake of free-living individuals by the collection of 24h urine samples. The main finding was that subjects consumed more sodium per day than the average sodium intake of the British population. However, in chapters 4, 5 and 7 the average sodium intake was lower than the British average. Nevertheless it is difficult to make accurate comparisons between the studies in these thesis and that of Henderson et al (2003), as only a single 24h urine collection was made in the UK-based survey, which may not be representative of an individuals habitual sodium intake.

The sodium densities of diets in Chapter 5 and Chapter 7 (1.2g/1000kcal) were also lower than a previous survey of the British population (1.9g/1000kcal) (Henderson et al, 2003). Interestingly, in Chapter 4, subjects were asked to consume the same foods each day for a 5-day period. As a result the sodium density of diets was higher (1.6g/1000kcal). This was due to an increased reliance on convenience foods during this research design. Despite the lower sodium densities reported in the chapters of this thesis, energy intakes were greater than those reported by Henderson et al (2003) and considering the positive relationship between sodium intake and energy intake found in Chapter 5 and by others (Pietinen, 1982; Holbrook et al, 1984) this may partly explain why sodium intake was only slightly lower than those reported by Henderson et al (2003). However, in Chapters 5 and 7 the relationship between energy intake and sodium intake was weak. Consequently, although athletes would typically consume diets that provide more energy than the general public due to their higher energy requirement, it appears that an individual's sodium intake can vary greatly due to the selection or avoidance of salty foods. The avoidance of saltcontaining foods is not without consequence, as this can impose great restriction on the consumption of certain food groups. Two food groups that provide substantial amounts of salt in the current diet are cereal products (35% of total salt intake) and meat products (26% of total salt intake) (Figure 9.1; Henderson et al, 2003). The ramifications of avoiding these foods could be a reduction in energy, carbohydrate, fat, vitamin A, vitamin D, folate, iron, magnesium, calcium, zinc and copper as the aforementioned food groups also serve as major providers of these nutrients (Henderson et al, 2003; Morris, 1997). As athletes are encouraged to follow a high carbohydrate diet (Burke et al, 2001) any

restrictions placed upon the major provider of carbohydrate may potentially be detrimental to training and competition (Achten et al, 2004; Costill et al, 1988).



**Figure 9.1** The percentage contribution of food types to average daily sodium intake (Henderson et al, 2003).

Like most nutrients, sodium and potassium intakes demonstrate considerable day to day variation both between and within individuals which has led to a criticism by some (Sowers & Stumbo, 1986; Liu & Stamler, 1984; Caggiula et al, 1985; Dyer et al, 1997) although not all (Kesteloot & Joosens, 1990) of the accuracy of one-off urine collections to determine an individual's habitual sodium intake. In Chapter 3, the day to day variation in urine sodium excretion was evident and it appears that the variation was not consistently reduced in samples collected on the same day of different weeks or on different days in the same week. Nevertheless, the within-individual variation in sodium excretion reported in Chapter 3 was at the lower end of the range reported in the literature. This finding was also observed in Chapter 5 and Chapter 7 and is most likely attributable to the study period falling largely on weekdays given that the intake of nutrients varies between weekdays and weekends (Acheson et al, 1980). In chapter 4, sodium intake was held constant, yet the daily fluctuations in sodium excretion persisted. These daily oscillations in sodium excretion have been reported previously (Baldwin et al, 1960), the magnitude of which was positively related to the amount of sodium being consumed (Baldwin et al, 1960). However, in Chapter 4 the relationship between oscillations in urine sodium excretion and sodium intake was only moderate and did not reach significance, a finding most likely due to the homogenous nature of sodium intakes.

Whilst the variation in urine sodium excretion is influenced by a true variation in sodium intake, it may also be attributed to several other factors including potassium intake (Mickelson et al, 1977; Van Buren et al, 1992) and the loss of sodium in sweat (Holbrook et al, 1984; Consolazio et al, 1963). Van Buren et al (1992) reported that the oral administration of potassium salts caused an immediate increase in potassium and sodium excretion. Despite this acute effect, sodium excretion returned to normal levels quickly and resulted in no significant increase in cumulative sodium excretion over an 8h monitoring period, which is considerably shorter than the 24h monitoring period used in the chapters 3, 4, 5 and 7 of this thesis. Although Mickelson et al (1977) reported that the replacement of sodium with a 1:1 sodium-potassium salt in a saltshaker, caused a significant increase in 24h urine sodium excretion, it is unclear as to whether this was due to the addition of potassium or the lag in excretion following the change in sodium intake. There is a seasonal variation in the amount of dietary sodium excreted in the urine, with a decline of 7% seen in the summer compared to winter which was attributed to cutaneous losses of sodium (Holbrook et al, 1984). In an exercise setting, large amounts of sodium can be lost in sweat (Maughan et al, 2004; Maughan et al, 2005; Shirreffs et al, 2005; Stofan et al, 2002; Stofan et al, 2005) and this could potentially cause large perturbations in sodium balance.

### 9.3 Effect of Exercise on Urine Sodium Excretion

Lichton (1957) described a competition between the sweat glands and kidneys for salt in an individual exposed to exercise at varying temperatures. It was concluded that the sweat gland has precedence over the kidney for sodium, with any remaining sodium being available for excretion in the urine. Consequently, urine sodium would be expected to decline as a result of the loss of sodium in sweat. The kidneys can respond quickly in response to sweat sodium losses, with some reports showing this to occur within 1 or 2h of exercise (Robinson et al, 1955). In Chapter 5, the response to a single bout of exercise which resulted in a sweat sodium loss of 66mmol (1.5g sodium), was a significant conservation of sodium by the kidneys on that same day. In Chapter 7, two exercise sessions were scheduled for the same day which resulted in a cumulative sweat sodium loss of 138mmol (3.2g sodium). Urine sodium was significantly depressed on both the day of exercise (day 4) and the day following exercise (day 5), again indicating significant sodium conservation by the kidneys. For the two individuals who lost the largest amount of sodium in sweat, average urine sodium concentrations were 22mmol/L and 9mmol/L on

day 4 and 12mmol/L and 9mmol/L on day 5. The carry-over effect when two exercise sessions were scheduled was most likely due to the greater magnitude of sweat sodium losses compared to when one session took place, but may also be partly explained by the second exercise session being completed in the afternoon. This potentially reduces the time available for dietary consumption of sodium. Godek et al (2005) have also reported urine sodium excretion to be significantly depressed the day after the first two-a-day practice in American Football players. The reason why urine sodium conservation was seen on the same day of exercise in the current study, in addition to evidence of sodium conservation the following day, is most likely due to the collection of all urine samples, instead of the collection of only 4 urine samples in the study of Godek and colleagues.

#### 9.4 Effect of exercise on dietary sodium intake

It is currently recommended that fluid intake during exercise should be sufficient to limit dehydration to no more than 2% BM (ACSM 2007). In addition sports drinks containing sodium (0.5 - 0.7g/L or 22-30mmol/L) are also recommended to athletes (ACSM, 2009). Given that these drink sodium concentrations lie at the bottom of the range of sweat sodium concentrations (20-80mmol/L) (Maughan & Nadel, 2000) and that fluids are typically consumed at rates below sweat rate (Sawka & Pandolf, 1990), sweat sodium losses are not entirely replaced during exercise. Indeed, field studies report sodium ingestion during exercise to replace between 0-23% of the sodium lost in sweat (Maughan et al, 2004; 2005; Shirreffs et al, 2005; Palmer & Spriet, 2008).

The addition of sodium chloride to fluids can increase volitional intake (Wemple et al, 1997), but a high-sodium content may make a drink unpalatable (Passe et al, 2006; Wemple et al, 1997; Nadel et al, 1990). Therefore commercially available sports drinks are carefully designed to meet a balance between efficacy and palatability. Recently, it has been shown that exercise can extend the range of sodium concentrations which appear palatable to the consumer (Passe et al, 2006). A similar phenomenon has been reported to occur after exercise and has been termed sodium appetite (Takamata et al, 1994). Takamata et al (1994) investigated the effects of prolonged exercise and heat exposure on the palatability of several rehydration solutions that differed in their salt concentration. They reported evidence of a salt appetite which appeared 3h after exercise and strengthened over the next 20h. Currently it is unclear as to what drives sodium appetite. Many mechanisms have been postulated including the actions of aldosterone (Geerling &

Loewy, 2008). In chapter 5 of this thesis, subjects completed one exercise session involving approximately 74 minutes of heat exposure with no access to fluids during this period. Exercise has been shown to stimulate the release of aldosterone and this increase is of greater magnitude when an individual is dehydrated (Morgan et al, 2004; Francesconi et al, 1983). Nevertheless, there was no alteration in sodium intake on the day of exercise. In chapter 7 of this thesis, subjects completed two exercise sessions involving a total of 120 minutes heat exposure. This resulted in a total sweat sodium loss of 138mmol, but again sodium intake did not increase on the day of exercise.

Stofan et al (2005) posed the question, does sweat sodium loss drive sodium intake or does sodium intake drive sweat sodium loss? Wald & Leshem (2003) reported the preference for sodium to increase after exercise particularly in individuals that lost the greatest amounts of sweat. This is in contrast to both Chapter 5 and Chapter 7 of this thesis where no relationship was observed between the amount of sodium lost and the amount of sodium ingested on that same day. Some have suggested a delay before sodium intake is seen, typically around 24h later (Takamata et al, 1994). However, there was no significant increase in sodium intake on day 5 in Chapter 5 or 7. Given the already elevated sodium intakes of individuals in the current study, it is most likely that the majority of individuals did not experience a sodium deficit that was severe, or of sufficient duration, to stimulate an increased sodium intake. Alternatively, for those individuals who lost the largest amount of sodium, it may be that access to fluids and foods was the main limitation on sodium intake, but this is purely speculation.

#### 9.5 Effect of exercise on sodium balance

In this thesis, sodium balance was calculated from sodium intake, urine sodium excretion and sweat sodium loss. In chapter 4, sodium balance appeared to fluctuate around a central point from day to day, rather like the sinusoidal nature of water balance (Greenleaf, 1992). Exercise poses a risk to the maintenance of sodium balance especially for some individuals who lose large amounts of sodium in sweat. Such disturbances appear manageable by most individuals when one exercise session is completed, as urinary sodium conservation provides the main mechanism by which sodium balance is maintained (Chapter 5). When two exercise sessions are scheduled for the same day, sweat composition in the second bout of exercise is unaffected, despite the loss of sodium during exercise earlier that same day (Chapter 6; Chapter 7). Although plasma aldosterone concentrations were not measured in this thesis, exercise has been shown to increase plasma aldosterone concentration (Francesconi et al, 1983; Kirby & Convertino, 1986; Morgan et al, 2004) and this would theoretically result in a conservation of sodium by the sweat gland. However, studies have reported a change in aldosterone concentration is not necessary to alter sweat composition (Allsopp et al, 1998; Morgan et al, 2004) although it may play a potentiating role (Allsopp et al, 1998). Robinson et al (1955) reported that sweat sodium concentrations did not start to decline until 4-5h after the first exercise bout but this decline in sweat sodium concentration did not reach significance until the following day ( $\sim$ 22h), which is considerably longer than the 5h time period between exercise sessions in chapter 6 and 7 of this thesis. Despite the large cumulative loss of sodium in sweat during two-aday exercise, sodium balance was not significantly affected in Chapter 7. Nevertheless, some individuals were in negative sodium balance. The consequences of these large unreplaced sodium losses could be muscle cramps (Stofan et al, 2005), hyponatraemia (Montain et al, 2006) or a contracted extracellular volume (Sanders et al, 2001). Although no subject in this thesis suffered from muscle cramps during the exercise tasks performed in Chapters 4, 6, 7 or 8, it would seem wise to offset these losses due to the potentially serious consequences of hyponatraemia and the detrimental effects of a reduction in plasma volume (Armstrong et al, 1985a). Body mass changes during an exercise bout are relatively simple to measure (Maughan et al, 2007) and allow estimates of sweat loss to be made. The assessment of sweat composition is also relatively simple to measure, but requires specialist laboratory equipment for analysis (Burke, 2005). The benefits of acquiring information on sweat composition and sweat loss will allow a nutrition strategy to be tailored to an individual's requirement and allow individuals at risk of large sweat sodium losses to be identified.

## 9.6 Effect of exercise on potassium balance

The concentration of potassium (4-8mmol/L) in sweat is lower than sodium (20-80mmol/L) (Maughan & Nadel, 2000), consequently sweat potassium losses are smaller in magnitude than sweat sodium losses. Despite this there has been some concern as to whether large sweat potassium losses could place an individual in negative potassium balance (Consolazio et al, 1963; Knochtel et al, 1972). Consolazio et al (1963) reported large losses of potassium in sweat during exercise, in some cases equivalent to 44% of dietary potassium intake. However, this was largely due to the prolonged duration of the exercise-heat exposure (7.5h). Other investigators have suggested that exercise resulted in

a potassium deficiency (Knochtel et al, 1972) but this was due to the indirect determination of potassium deficiency via muscle potassium concentrations which has since been suggested as an un-suitable method (Costill et al, 1982). In Chapter 5, subjects lost between 7 and 12mmol of potassium in sweat during exercise, equivalent to between 3 and 16% of dietary potassium intake for that day. These losses were too small to cause any significant alteration in potassium excretion, intake, or balance on the day of exercise. In Chapter 7, two exercise sessions were scheduled on the same day which resulted in subjects losing between 7 and 23mmol of potassium in sweat, equivalent to between 5 and 23% of dietary potassium intake. There was also no effect of exercise on potassium excretion or intake. At least part of the discrepancy between the findings of this thesis and that of Consolazio et al (1963) may be attributed to the different lengths of heat exposures, but also to differences in dietary potassium intake. A consistent finding of this thesis was the higher than average potassium intakes of individuals. In Chapter 5, individuals consumed 131mmol per day and in chapter 7, 113mmol per day. In total 17 (out of 18) individuals in these chapters consumed more than the average reported intake (86mmol) for males in the UK, all of whom consumed more than those in the study of Consolazio and colleagues (64mmol/day). In both Chapter 4 and 7, potassium intake was un-related to energy intake, but instead was due to the increased potassium density of the diets. This supports the notion of a growing trend for higher potassium intakes as previously reported by Henderson et al (2003) and it may be that this pattern is continuing. Even on very low potassium diets (25mmol/day), a potassium deficiency proved difficult to induce due to a concomitant reduction in the amount of dietary potassium excreted in the urine (Costill et al, 1982). Consequently it would appear potassium losses in sweat are far less likely than sodium to result in a negative balance.

In Chapter 8, the efficacy of milk as a rehydration drink was investigated. Milk has a relatively high-sodium (32mmol/L) and potassium (42mmol/L) content compared to fruit juices, soft drinks and most other commercially available fluids (Shirreffs, 2003) which may lend itself to the rehydration process. It has previously been reported that the addition of either potassium (25mmol/L) or sodium (60mmol/L) to a rehydration drink confers a similar benefit in terms of fluid retention when given in a volume equal to BM loss (Maughan et al, 1994), but their effects were not additive. However, it is likely that no further reductions in urine output were possible as subjects were hypohydrated throughout the study (Maughan et al, 1994). In a recent study, skimmed milk was shown to be more

effective than both water and a sports drink in replacing the fluid losses incurred during exercise-induced dehydration (Shirreffs et al, 2007b). In Chapter 8 there was also a tendency for a reduced urine excretion when subjects ingested skimmed milk compared to CHO-E drink. Whilst it is not possible to discern if potassium was primarily responsible for the beneficial effect on fluid restoration, Shirreffs et al (2007a) suggest that potassium is not as effective as sodium in aiding fluid retention when drinks are ingested in an amount equal to 150% of BM loss. However, direct comparison between Shirreffs et al (2007a) and Chapter 8 cannot be made due to commercially-available products being administered that differed in several respects. The ingestion of a drink primarily containing potassium results in a slower rate of plasma volume recovery compared to beverages with either a low electrolyte content or that contain primarily sodium (Maughan et al, 1994; Nielsen et al, 1986; Shirreffs et al, 2007a). This initial delay in plasma volume recovery has been suggested to be due to a preferential restoration of the intracellular fluid compartment. Despite potassium being the predominant electrolyte in milk, a delay in plasma volume restoration was not observed which may be due to the sodium content of milk opposing such an effect. Considering sodium is the primary cation lost in sweat, its inclusion in drinks helps replenish sweat losses, prevents the decline in serum sodium concentration and maintains the drive to drink (Nose et al, 1988a). It therefore appears that sodium should remain the primary cation present in a rehydration drink, although the addition of potassium does not appear to impede the rehydration process.

## 9.7 Effect of exercise on hydration status

Current guidelines encourage athletes to drink at a rate that will prevent excessive dehydration (>2% BM) during exercise (ACSM, 2007). Although individual drinking practices vary, most will finish a bout of exercise in a hypohydrated state (Sawka & Pandolf, 1990). Therefore, restoration of fluid and electrolyte losses should form an integral part of the recovery process. Casa et al (2000) report athletes to be capable of replacing these losses with normal food and fluid intake when the interval between exercise bouts is more than 24 h, but when repeated exercise sessions are scheduled on the same day or the fluid deficit is large, athletes may fail to fully replace fluid losses (Godek et al, 2005).

In Chapter 5, one exercise session was undertaken on the morning of day 4 which resulted in a sweat loss equivalent to 1.51 litres, equivalent to 1.9% BM. The first void the following day (day 5) indicated that all subjects had consumed enough fluid to replace these losses as no individual was considered dehydrated according to the cut-off criteria reported in the literature (Cheuvront & Sawka, 2005; Shirreffs & Maughan, 1998a). In Chapter 7, two exercise sessions were completed on day 4 which resulted in a cumulative sweat loss of 2.64 litres. There were no significant differences in the osmolality of urine samples obtained from the first void each morning, all of which indicated individuals were not hypohydrated. This is in contrast to other investigators who have reported urine parameters of hydration status to indicate American Football players fail to replace sweat losses during daily two-a-day exercise sessions (Godek et al, 2005). This discrepancy may be due to the fact that players lost greater amounts of sweat (although they had free access to fluids to help off-set sweat losses) and/or the longer training session duration (2h 15min v 1h 10min) compared to the current study. In contrast, other investigators (Fudge et al 2008) have reported runners to successfully replace the sweat losses incurred during two-aday exercise sessions. Interestingly, Godek et al (2005) also suggest that urine indicators of hydration status may not be suitable for determining hydration status in American Football players due to their large body size and findings of a persistently elevated urine specific gravity in this population.

In Chapter 6, two exercise sessions were also scheduled on the same day, separated by a 5h recovery period. Food was provided during the recovery period and fluid was allowed ad libitum. Urine samples obtained before the second exercise bout indicated individuals were not hypohydrated. In some situations, such as periods of acute rehydration, urine indices may not be an appropriate indicator of hydration status (Popowski et al, 2001; Cheuvront & Sawka, 2005). Therefore in conjunction with urine indices, fluid intake was monitored and indicated that 167% of sweat loss had been ingested during the intervening 5h recovery period between exercise sessions. Considering the volume consumed and the favourable effects of this prolonged type of drinking regimen, as opposed to a shorter rehydration period, it may lend itself to a more complete restoration of fluid balance Although only water was permitted, this was ingested in (Kovacs et al, 2002). combination with a standardised lunch. The ingestion of sodium, via the foods provided, may have also proven beneficial for fluid retention and maintaining the drive to drink (Ray et al, 1998; Maughan et al, 1996; Nose et al, 1988a).

A considerable amount of research has looked at post-exercise rehydration and the importance of sodium in rehydration drinks has been reported by many investigators (Shirreffs et al, 1996; Wemple et al, 1997; Mitchell et al, 2000) and has been systematically evaluated in several studies (Maughan and Leiper, 1995; Shirreffs and Maughan, 1998b; Merson et al, 2008). Maughan and Leiper (1995) reported that when a volume equal to 150% of body mass (BM) loss was ingested following exercise-induced dehydration, the amount of fluid retained was inversely related to the drinks sodium concentration. Milk has a relatively high sodium content compared to other commercially available fluids (Shirreffs, 2003). In a recent study, skimmed milk was shown to be more effective than both water and a sports drink in replacing fluid losses incurred during exercise-induced dehydration (Shirreffs et al, 2007b). In Chapter 8, the effectiveness of a sports drink and skimmed milk in restoring fluid and electrolyte balance and endurance capacity was investigated. There was a tendency for a reduced urine excretion with milk compared to a sports drink, during the 4h recovery period. This resulted in a difference in fluid balance between trials of 326mL or 0.4% BM at the onset of the endurance capacity test. Despite this, no benefits in endurance capacity were observed, possibly as a result of the lack of sensitivity in the endurance capacity test employed (Jeukendrup et al, 1996) or the similar alterations in plasma volume observed between drinks. Nevertheless, skimmed milk was well tolerated and therefore it seems appropriate to recommend that milk is added to the list of products that are suitable for ingestion during the recovery period after exercise.

#### 9.9 Conclusions

- 1. The healthy individuals that participated in the studies within this thesis ingested less sodium than the average sodium intake reported for the UK.
- 2. The lower sodium intake occurred despite a higher energy intake of individuals.
- 3. The healthy individuals that participated in the studies within this thesis consumed more potassium than the average potassium intake reported for the UK.
- 4. The increased potassium intake may reflect the increased energy intake of individuals but may also reflect the higher potassium density of diets.
- 5. Sweat potassium losses during single and multiple exercise sessions did not exceed dietary intake for any individual.
- 6. Urine potassium excretion, dietary potassium intake and net potassium balance were not significantly affected by either single and multiple exercise sessions

- 7. A single exercise session results in a significant decline in urine sodium excretion on the day of exercise which helps to maintain sodium balance.
- 8. When two exercise sessions were scheduled, a reduced urine sodium excretion was again the main mechanism by which perturbations in sodium balance were minimised and sodium balance was maintained. Unlike when the single exercise session was completed, urine sodium conservation persisted during the day following exercise most likely due to the extent of sodium losses and/or the timing of the second session later in the day.
- 9. Sodium intake was unaffected by the exercise-induced sodium losses incurred when one or two-a-day exercise sessions were completed. This may be due to the already high sodium intakes of individuals meaning that sodium deficits were not as severe or prolonged as other studies. Alternatively it may in part be due to a reduced availability/accessibility to foods during the exercise task and intervening recovery period.
- 10. Prior exercise and the associated loss of sodium in sweat did not alter sweat composition during a second exercise bout undertaken later that same day. The repercussions of this mean that sweat sodium losses can be large when repeated exercise sessions are scheduled.
- 11. Sweat sodium concentrations and losses demonstrate wide variation between individuals.
- 12. Sweat sodium concentration was not related to dietary sodium intake
- 13. The ingestion of skimmed milk tended to improve rehydration compared to a sports drink during the acute recovery period post-exercise when no food was consumed, but did not affect subsequent endurance capacity in the heat. However it is not possible to discern if the beneficial effects upon fluid retention were attributable to its relatively high sodium or potassium content or to an effect on gastric emptying.
- 14. Due to the high sodium intake of most individuals, there is no need to advise all individuals participating in exercise to consume large amounts of sodium. Indeed this would be unwise considering the health implications of such recommendations. However, the provision of sodium after exercise has been shown to help replenish fluid balance and there may be some individuals who lose large amounts of sodium in sweat, especially when multiple exercise sessions are completed.
- 15. Without knowledge of both sweat loss and sweat composition it is not possible to accurately identify individuals at risk of large sodium losses. However, high sweat

rates may be used as a crude indicator for identifying individuals at risk of high sweat sodium losses.

# References

Acheson KJ, Campbell IT, Edholm OG, Miller DS, Stock MJ (1980) The measurement of food and energy intake in man-an evaluation of some techniques. The American Journal of Clinical Nutrition, 33: 1147-54.

Achten J, Halson SL, Moseley L, Rayson MP, Casey A, Jeukendrup AE (2004) Higher dietary carbohydrate content during intensified running training results in better maintenance of performance. Journal of Applied Physiology, 96: 1331-1340.

Allan JR, Wilson CG (1971) Influence of acclimatization on sweat sodium concentration. Journal of Applied Physiology, 30:708-12.

Allsopp AJ, Sutherland R, Wood P, Wootton SA (1998) The effect of sodium balance on sweat sodium secretion and plasma aldosterone concentration. European Journal of Applied Physiology, 78: 516-521.

Almond CSD, Shin AY, Fortescue EB, Mannix RC, Wypij D, Binstadt BA, Duncan CN, Olson DP, Salerno AE, Newburger JW, Greenes DS (2005) Hyponatremia among runners in the Boston marathon. The New England Journal of Medicine, 352: 1550-1556.

Amantidis S (2002). Milk and milk products. In: *Essentials of human nutrition*. Mann, J. and Truswell A.S. (eds). Second Edition, Oxford University press, 394-397.

American College of Sports Medicine, American Dietetic Association, Dietitians of Canada (2009) Joint Position Statement: Nutrition and Athletic Performance. Medicine and Science in Sports and Exercise, 41: 709-731

American College of Sports Medicine Position Stand: Exercise and Fluid replacement (2007) Medicine and Science in Sports and Exercise, 39: 377-390.

Anderson RK, Kenney WL (1987) Effect of age on heat activated sweat gland density and flow during exercise in dry heat. Journal of Applied Physiology, 63: 1089-1094.

Armstrong LE, Costill DL, Fink WJ (1985a). Influence of diuretic-induced dehydration on competitive running performance. Medicine and Science in Sports and Exercise, 17: 456-61.

Armstrong LE, Costill DL, Fink WJ, Bassett D, Hargreaves M, Nishibata I, King DS (1985b) Effects of dietary sodium on body and muscle potassium content during heat acclimation. European Journal of Applied Physiology, 54: 391-397.

Armstrong CG, Kenney WL (1993) Effects of age and acclimation on responses to passive heat exposure. Journal of Applied Physiology, 75: 2162-2167.

Armstrong LE, Hubbard RW, Askew EW, Francesconi RP (1993) Responses of soldiers to 4g and 8g Salt diets during 10 days of heat acclimation. In: Nutritional needs in hot environments, Applications for military personnel in field operations, edited by Marriott BM, National Academy Press, Washington DC, pg 247-258.

Armstrong LE, Costill DL, Fink WJ (1987) Changes in body water and electrolytes during heat acclimation: effects of dietary sodium. Aviation, Space and Environmental Medicine 58: 143-148.

Arn KD, Reimer A (1950) Minimal sodium losses through the skin. Journal of Clinical Investigation, 29: 1342-1346.

Avellini BA, Kamon E, Krajewski JT (1980a) Physiological responses of physically fit men and women to acclimation to humid heat. Journal of Applied Physiology, 49: 254-261

Avellini BA, Shapiro Y, Pandolf KB, Pimental NA, Goldman RF (1980b) Physiological responses of men and women to prolonged dry heat exposure. Aviation Space and Environmental Medicine, 51: 1081-5.

Baker LB, Lang JA, Kenney WL (2008) Quantitative analysis of serum sodium concentration after prolonged running in the heat. Journal of Applied Physiology, 105: 91-99.

Baldwin D, Alexander R, Warner EJ. (1960). Chronic sodium chloride challenge studies in man. *J Lab Clin Med* **55**, 362-75.

Barnett A and Maughan RJ (1993). Response of unacclimatized males to repeated weekly bouts of exercise in the heat. British Journal of Sports Medicine, 27: 39-44.

Barnett A, Maughan RJ (1996) Effect of fluid replacement on the ionic concentration of local back sweat during exercise. In: Communications to the international conference on dehydration, rehydration and exercise in the heat. Journal of Sports Sciences, 14: 347-370.

Barr SI, Costill DL, Fink WJ (1991) Fluid replacement during prolonged exercise: effects of water, saline or no fluid. Medicine and Science in Sports and Exercise, 23: 811-817.

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

Bates GP, Miller VS (2008) Sweat rate and sodium loss during work in the heat. Journal of Occupational Medicine and Toxicology, 3: 4-

Baylis PH (1987) Osmoregulation and control of vasopressin secretion in healthy humans. American Journal of Physiology, 253: R671-R678.

Belko AZ, Barbieri TF, Wong EC (1986). Effect of energy and protein intake and exercise intensity on the thermic effect of food. The American Journal of Clinical Nutrition, 43(6): 863-9.

Below PR, Mora-Rodriguez R, Gonzalez-Alonso J, Coyle EF (1995) Fluid and carbohydrate ingestion independently improve performance during 1h of intense exercise. Medicine and Science in Sports and Exercise, 27: 200-210.

Bergeron MF (1996) Heat cramps during tennis: A case report. International Journal of Sport Nutrition, 6:62-8.

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

Bergeron MF (2007) Exertional heat cramps: Recovery and return to play. Journal of Sport Rehabilitation, 16: 190-196.

Bilzon JL, Murphy JL, Allsopp AJ, Wootton SA, Williams C (2002) Influence of glucose ingestion by humans during recovery from exercise on substrate utilisation during subsequent exercise in a warm environment. European Journal of Applied Physiology, 87: 318-326.

Bingham S (1987) The dietary assessment of individuals: methods, accuracy, new techniques and recommendations. Nutrition Abstracts and Reviews, 57: 705-742.

Bingham SA, Cummings JH (1985) The use of creatinine output as a check on the completeness of 24 hour urine collections. Human Nutrition, Clinical Nutrition, 39: 343-353.

Bingham SA, Cassidy A, Cole TJ, Welch A, Runswick SA, Black AE, Thurnham D, Bates C, Khaw KT, Key TJA, Day NE (1995). Validation of weighed records and other methods of dietary assessment using the 24 h urine nitrogen technique and other biological markers. British Journal of Nutrition, 73: 531-50.

Bingham SA, Gill C, Welch A, Cassidy A, Runswick SA, Oakes S, Lubin R, Thurnham DI, Key TJA, Roe L, Khaw KT, Day NE (1997). Validation of dietary assessment methods in the UK arm of EPIC using weighed records, and 24-hour urinary nitrogen and potassium and serum vitamin C and carotenoids as biomarkers. International Journal of Epidemiology, 26: S137-S151

Bingham SA, Gill C, Welch A, Day K, Cassidy A, Khaw KT, Sneyd MU, Key TJA, Roe L, Day NE (1994) Comparison of dietary assessment methods in nutritional epidemiology: weighed records v 24h recalls, food frequency questionnaires and estimated diet records. British Journal of Nutrition, 72: 619-643.

Bingham S, Williams R, Cole T, Price C, Cummings J. (1988). Reference values for analytes of 24-h urine collections known to be complete. Annals of Clinical Biochemistry, 25: 610-9.

Bishop D (2003) Warm Up I: Potential mechanisms and the effects of passive warm up on exercise performance. Sports Medicine, 33: 439 – 454.

Borg GA (1973). Perceived exertion: a note on "history" and methods. *Med.Sci.Sports* 5: 90-3.

Boysen TC, Yanagawa S, Sato F, Sato K (1984) A modified anaerobic method of sweat collection. Journal of Applied Physiology, 56: 1302-1307.

Brisson GR, Boisvert P, Peronnet F, Perrault H, Boisvert D, Lafond JS (1991) A simple and disposable sweat collector. European Journal of Applied Physiology, 63: 269-272.

British Association of Sports & Exercise Sciences (1997) Guidelines for the physiological testing of athletes. Eds Bird S, Davison R. British Association of Sport & Exercise Sciences, Leeds, UK.

Broad EM, Burke LM, Cox GR, Heeley P, Riley M (1996) Body weight changes and voluntary fluid intakes during training and competition sessions in team sports. International Journal of Sport Nutrition, 6: 307-320.

Brockbank EM (1929) Miners Cramp. British Medical Journal, 1: 65-66.

Brown G, Dobson RL (1967) Sweat sodium excretion in normal women. Journal of Applied Physiology, 23: 97-99.

Buono MJ, Sjoholm NT (1988) Effect of physical training on peripheral sweat production. Journal of Applied Physiology, 65: 811-814.

Buono MJ, Ball KD, Kolkhorst FW (2007) Sodium ion concentration vs. sweat rate relationship in humans. Journal of Applied Physiology, 103: 990-994.

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

Burke LM, Cox GR, Cummings NK, Desbrow B (2001) Guidelines for daily carbohydrate intake. Do athletes achieve them? Sports Medicine, 31: 267-299.

Burke LM, Hawley JA (1997). Fluid balance in team sports: Guidelines for optimal practices. Sports Medicine, 24: 38 – 54.

Caggiula AW, Wing RR, Nowalk MP, Milas NC, Lee S, Langford H (1985) The measurement of sodium and potassium intake. The American Journal of Clinical Nutrition, 42: 391-8.

Calbet JA, Holst JJ (2004) Gastric emptying, gastric secretion and enterogastrone response after administration of milk proteins or their peptide hydrolysates in humans. European Journal of Nutrition, 43: 127-139.

Calbet JA, MacLean DA (1997). Role of caloric content on gastric emptying in humans. J of Physiology, 498: 553-559.

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

Calles-Escandon J, Cunningham JJ, Snyder P, Jacob R, Huszar G, Loke J, Felig P (1984) Influence of exercise on urea, creatinine and methylhistidine excretion in normal human subjects. American Journal of Physiology, 246: E334-E338.

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

Cage GW, Wolfe SM, Thompson RH, Gordon RS (1970) Effects of water intake on composition of thermal sweat in normal human volunteers. Journal of Applied Physiology, 29:687-90.

Caggiula AW, Wing RR, Nowalk MP, Milas NC, Lee S, Langford H. (1985). The measurement of sodium and potassium intake. The American Journal of Clinical Nutrition, 42: 391-8.

Casa DJ, Armstrong LE, Hillman S, Montain SJ, Reiff RV, Rich BSE, Roberts WO, Stone JA (2000). National athletic trainers association position statement: Fluid replacement for athletes. Journal of Athletic Training, 35: 212-224.

Casa DJ, Clarkson PM, Roberts WO (2005) American College of Sports Medicine Roundtable on Hydration and Physical Activity: Consensus Statement. Current Sports Medicine Reports, 4: 115-127.

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

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

Cheuvront SN, Sawka MN (2005) Hydration Assessment of Athletes. *Gatorade Sports* Science Exchange, 18:2.

Chinevere TD, Kenefick RW, Cheuvront SN, Lukaski HC, Sawka MN (2008) Effect of heat acclimation on sweat minerals. Medicine and Science in Sports and Exercise, 40: 886-891.

Clark AJ, Mossholder S (1986) Sodium and potassium intake measurements: Dietary methodology problems. The American Journal of Clinical Nutrition, 43: 470-6.

Coles MG, Luetkemeier MJ (2005) Sodium facilitated hypervolemia, endurance performance and thermoregulation. International Journal of Sports Medicine, 26: 182-187.

Collins KJ, Crockford GW, Weiner JS (1966) The local training effect of secretory activity on the response of eccrine sweat glands. Journal of Physiology, 184: 203-214.

Collins (1966) The action of exogenous aldosterone on the secretion and composition of drug induced sweat. Clinical Science, 30: 207-221.

Collins KJ, Weiner JS (1962) Observations on arm-bag suppression of sweating and its relationship to thermal sweat gland fatigue. Journal of Physiology, 161: 538-556.

Consolazio C, Matoush L, Nelson R, Harding R, Canham J. (1963). Excretion of sodium, potassium, magnesium and iron in human sweat and the relation of each to balance and requirements. Journal of Nutrition, 79: 407-15.

Consolazio CF, Matoush LO, Nelson RA, Isaac GJ, Canham JE. (1966). Comparisons of nitrogen, calcium and iodine excretion in arm and total body sweat. The American Journal of Clinical Nutrition, 18: 443-8.

Comar & Bronner (1960) Mineral Metabolism: An Advanced Treatise, Volume 1 (part A), pg 342. New York, Academic Press.

Coggan AR and Coyle EF (1987). Reversal of fatigue during prolonged exercise by carbohydrate infusion or ingestion. Journal of Applied Physiology, 63: 2388-2395.

Costa F, Calloway DH, Margen S (1969) Regional and total body sweat composition of men fed controlled diets. The American Journal of Clinical Nutrition, 22: 52-58.

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

Costill DL, Cote R, Fink WJ (1982) Dietary potassium and heavy exercise: effects on muscle water and electrolytes. The American Journal of Clinical Nutrition, 36: 266-275.

Costill DL, Flynn MG, Kirwan JP, Houmard JA, Mitchell JB, Thomas R, Park SH (1988) Effects of repeated days of intensified training on muscle glycogen and swimming performance. Medicine and Science in Sports and Exercise, 20: 249-254. Costill DL, Sparks KE (1973). Rapid fluid replacement following thermal dehydration. Journal of Applied Physiology, 34: 299-303.

Cox GR, Broad EM, Riley MD, Burke LM (2002) Body Mass changes and voluntary fluid intakes of elite level water polo players and swimmers. Journal of Science and Medicine in Sport, 5: 183-193.

Coyle EF (2004) Fluid and fuel intake during exercise. Journal of Sports Sciences, 22: 39-55.

Criswell D, Renshler K, Powers SK, Tulley R, Cicale M, Wheeler K (1992) Fluid replacement beverages and maintenance of plasma volume during exercise: role of aldosterone and vasopressin. European Journal of Applied Physiology, 65: 445-451.

Cummings JH, Hill MJ, Jenkins DJ, Pearson JR, Wiggins HS (1976) Changes in fecal composition and colonic function due to cereal fiber. The American Journal of Clinical Nutrition, 29: 1468-73.

Curtis G, Fogel M (1970) Creatinine Excretion: Diurnal Variation and Variability of Whole and Part Day Measures. A methodologic issue in psychoendocrine research. Journal of the American Psychosomatic Society, 32: 337- 350.

Dahl LK. (1958). Salt intake and salt need. The New England Journal of Medicine 258, 1152-7.

Dahl LK, Stall BG, Cotzias GC. (1955). Metabolic effects of marked sodium restriction in hypertensive patients: Skin electrolyte losses. Journal of Clinical Investigation, 34: 462-70.

Davies JA, Harrison MH, Cochrane LA, Edwards RJ, Gibson TM (1981) Effect of saline loading during heat acclimation on adrenocortical hormone levels. Journal of Applied Physiology, 50:605-12.

Davies CTM (1979) Thermoregulation during exercise in relation to sex and age. European Journal of Applied Physiology, 42: 71-79.

Day N, McKeown N, Wong M, Welch A, Bingham S. (2001). Epidemiological assessment of diet: A comparison of a 7-day diary with a food frequency questionnaire using urinary markers of nitrogen, potassium and sodium. International Journal of Epidemiology, 30: 309-17.

Dempsey EF, Carroll EL, Albright F, Henneman PH (1958) A study of factors determining fecal electrolyte excretion. Metabolism, 7:108-118.

Dill DB, Costill DL (1974) Calculation of percentage changes in volumes of blood, plasma and red cells in dehydration. Journal of Applied Physiology, 37: 247-248.

Dill DB, Yousef MK, Nelson JD (1973) Responses of men and women to two-hour walks in desert heat. Journal of Applied Physiology, 35: 231-235.

Dill DB, Horvath SM, Van Beaumont W, Gehlsen G, Burrus K (1967) Sweat electrolytes in desert walks. Journal of Applied Physiology, 23: 746-751.

Dill DB, Hall FG, Edwards HT (1938) Changes in composition of sweat during acclimatization to heat. The American Journal of Physiology, 123: 412-419.

Dole VP, Dahl LK, Cotzias GC, Eder HA, Krebs ME (1950) Dietary treatment of hypertension; clinical and metabolic studies of patients on the rice-fruit diet. Journal of Clinical Investigation, 29, 1189-206.

Drinkwater BL, Kupprat IC, Denton JE, Crist JL, Horvath SM (1977) Response of prepubertal girls and college women to work in the heat. Journal of Applied Physiology, 43: 1046-1053.

Durnin JV, Rahaman MM. (1967). The assessment of the amount of fat in the human body from measurements of skinfold thickness. British Journal of Nutrition, 21: 681-9.

Dyer A, Elliott P, Chee D, Stamler J (1997) Urinary biochemical markers of dietary intake in the INTERSALT study. The American Journal of Clinical Nutrition, 65: 1246S-1253S.

Eichner ER (2007) The role of sodium in heat cramping. Sports Medicine, 37: 368-370.

Edelman IS, James AH, Baden H, Moore FD (1954) Electrolyte composition of bone and the penetration of radiosodium and deuterium oxide in dog and human bone. The Journal of Clinical Investigation, 33: 122-131.

Elliot TA, Cree MG, Sanford AP, Wolfe RR, Tipton KD (2006). Milk ingestion stimulates net muscle protein synthesis following resistance exercise. Medicine and Science in Sports and Exercise, 38: 667-74.

Ellis FP, Ferres HM, Lind AR (1954) The effect of water intake on sweat production in hot environments. The Journal of Physiology, 125:61-62P.

Ellis KJ, Vaswani A, Zanzi I, Cohn SH (1976) Total body sodium and chlorine in normal adults. Metabolism, 25: 645-654.

Espeland MA, Kumanyika S, Wilson AC, Reboussin DM, Easter L, Self M, Robertson J, Brown WM, McFarlane M (2001) Statistical issues in analysing 24-hour dietary recall and 24-hour urine collection data for sodium and potassium intakes. American Journal of Epidemiology, 153: 996-1006

Evans GH, Shirreffs SM, Maughan RJ (2009) Postexercise rehydration in man: The effects of osmolality and carbohydrate content of ingested drinks. Nutrition, 25: 905-913.

Falk B, Bar-Or O, MacDougall JD (1991) Aldosterone and prolactin response to exercise in the heat in circumpubertal boys. Journal of Applied Physiology, 71: 1741-1745.

Fallowfield JL, Williams C, Singh R (1995). The influence of ingesting a carbohydrate electrolyte beverage during 4 hours of recovery on subsequent endurance capacity. International Journal of Sport Nutrition, 5: 285-299.

Febbraio MA, Murton P, Selig SE, Clark SA, Lambert DL, Angus DJ, Carey MF (1996). Effect of CHO ingestion on exercise metabolism and performance in different ambient temperatures. Medicine and Science in Sports and Exercise, 28: 1380-7.
Flynn A, Shortt C, Morrissey PA (1990) Sodium and potassium intakes in Ireland. Proceedings of the Nutrition Society, 49: 323-332.

Forbes GB, Lewis AM (1956) Total sodium, potassium and chloride in adult man. The Journal of Clinical Investigation, 35: 596 – 600.

Forbes GB, Perley A (1951) Estimation of total body sodium by isotopic dilution. I. Studies on young adults. The Journal of Clinical Investigation, 30: 558 -

Fortney SM, Wenger CB, Bove JR, Nadel ER (1984) Effect of hyperosmolality on control of blood flow and sweating. Journal of Applied Physiology, 57: 1688-1695.

Fortney SM, Nadel ER, Wenger CB, Bove JR (1981) Effect of blood volume on sweating rate and body fluids in exercising humans. Journal of Applied Physiology, 51: 1594-1600.

Fox RH, Lofstedt BE, Woodward PM, Eriksson E, Werkstrom B (1969) Comparison of thermoregulatory function in men and women. Journal of Applied Physiology, 26: 444-453.

Francesconi RP, Sawka MN, Pandolf KB (1983) Hypohydration and heat acclimation: plasma renin and aldosterone during exercise. Journal of Applied Physiology, 55: 1790-1794.

Frye AJ, Kamon E (1981) Responses to dry heat of men and women with similar aerobic capacities. Journal of Applied Physiology, 50: 65-70.

Frye AJ, Kamon E (1983) Sweating efficiency in acclimated men and women exercising in humid and dry heat. Journal of Applied Physiology, 54: 972-977.

Fudge BW, Easton C, Kingsmore D, Kiplamai FK, Onywera VO, Westerterp KR, Kayser B, Noakes TD, Pitsiladis YP (2008) Elite Kenyan endurance runners are hydrated day-today with ad libitum fluid intake. Medicine and Science in Sports and Exercise, 40:1171-1179. Galloway SDR, Maughan RJ (1997) Effects of ambient temperature on the capacity to perform prolonged cycle exercise in man. Medicine and Science in Sports and Exercise, 29: 1240-1249.

Garcia AMC, Lacerda MG, Fonseca IAT, Reis FM, Rodrigues LOC, Silami-Garcia E (2006) Luteal phase of the menstrual cycle increases sweating rate during exercise. Brazilian Journal of Medical and Biological research, 39:1255-1261.

Garde AH, Hansen AM, Kristiansen J, Knudsen LE (2004) Comparison of uncertainties related to standardisation of urine samples with volume and creatinine concentration. The Annals of Occupational Hygiene, 48: 171-179.

Geerling JC, Lowey AD (2008) Central regulation of sodium appetite. Experimental Physiology, 93: 177-209.

Gisolfi C, Robinson S (1969) Relations between physical training, acclimatization, and heat tolerance. Journal of Applied Physiology, 26: 530-534.

Gisolfi CV, Summers R, Schedl H (1990). Intestinal absorption of fluids during rest and exercise. In: Perspectives in Exercise Science and Sports Medicine, Vol. 3, Fluid Homeostasis During Exercise. Gisolfi C.V. and D.R. Lamb (eds.), Carmel, Benchmark Press, 1990, pp. 129-180.

Gisolfi CV, Summers RD, Schedl HR, Bleiler TL (1995) Effect of sodium concentration in a carbohydrate-electrolyte solution on intestinal absorption. Medicine and Science in Sports and Exercise, 27: 1414-1420.

Gisolfi CV, Lambert GP, Summers RW (2001) Intestinal fluid absorption during exercise: role of sport drink osmolality and [Na+]. Medicine and Science in Sports and Exercise, 33: 907-915.

Godek SF, Godek J, McCrossin J, Bartolozzi A (2006) Sweat and sodium losses in professional ice hockey players during a pre-season practice and a game. Medicine and Science in Sports and Exercise, 38: S218-S219.

Godek S, Godek JJ, Bartolozzi AR (2005) Hydration status in college football players during consecutive days of twice a day preseason practices. American Journal of Sports Medicine, 33: 843-851.

Gonzalez-Alonso J, Heaps CL ,Coyle EF (1992). Rehydration after exercise with common beverages and water. International Journal of Sports Medicine, 13: 399-406.

Gonzalez-Alonso J, Teller C, Andersen SL, Jensen FB, Hyldig T, Nielsen B (1999). Influence of body temperature on the development of fatigue during prolonged exercise in the heat. Journal of Applied Physiology, 86: 1032-9.

Greenblatt DJ, Ransil BJ, Harmatz JS, Smith TW, Duhme DW, Koch-Weser J (1976) Variability of 24hr urinary creatinine excretion by normal subjects. The Journal of Clinical Pharmacology, 16: 321-328.

Greenhaff PL, Clough PJ (1989) Predictors of sweat loss in man during prolonged exercise. European Journal of Applied Physiology, 58: 348-352

Greenleaf JE (1992) Problem: thirst, drinking behaviour and involuntary dehydration. Medicine and Science in Sports and Exercise, 24: 645-656.

Greenleaf JE, Brock PJ (1980) Na+ and Ca2+ ingestion: plasma volume-electrolyte distribution at rest and exercise. Journal of Applied Physiology, 48: 838-847.

Gregory J, Foster K, Tyler H, Wiseman M (1990) The Dietary and Nutritional Survey of British Adults. London, HMSO.

Harrison MH, Edwards RJ, Fennessy PA (1978) Intravascular volume and tonicity as factors in the regulation of body temperature. Journal of Applied Physiology, 44: 69-75.

Havenith G, Fogarty A, Bartlett R, Smith CJ, Ventenat V (2008) Male and female upper body sweat distribution during running measured with technical absorbents. European Journal of Applied Physiology, 104: 245-255. Hayden G, Milne HC, Patterson MJ, Nimmo MA (2004) The reproducibility of a closedpouch sweat collection and thermoregulatory responses to exercise-heat stress. European Journal of Applied Physiology, 91: 748-751.

Hazlehurst LT, Claassen N (2006) Gender differences in the sweat responses during spinning exercise. Journal of Strength and Conditioning Research, 20: 723-724.

He FJ, MacGregor GA (2003) How far should salt intake be reduced? Hypertension, 42: 1093-1099.

Henane R, Flandrois R, Charbonnier JP (1977) Increase in sweating sensitivity by endurance conditioning in man. Journal of Applied Physiology, 43: 822-828.

Henderson L, Irving K, Gregory J, Bates CJ, Prentice A, Perks J, Swan G, Farron M (2003) National Diet and Nutrition Survey: adults aged 19-64years. Volume 3: Vitamin and mineral intake and urinary analytes. London, TSO.

Herman CP (1993) Effects of Heat on Appetite. In: Nutritional Needs in Hot Environments. National Academy Press, Chapter 10, pages 187-213.

Hew-Butler TD, Sharwood K, Collins M, Speedy D, Noakes T (2006) Sodium supplementation is not required to maintain serum sodium concentrations during an ironman triathlon. British Journal of Sports Medicine, 40: 255 – 259.

Hew-Butler T, Ayus JC, Kipps C, Maughan RJ, Mettler S, Meeuwisse WH, Page AJ, Reid SA, Rehrer NJ, Roberts WO, Rogers IR, Rosner MH, Siegel AJ, Speedy DB, Stuempfle KJ, Verbalis JG, Weschler LB, Wharam P (2008) Statement of the Second International Exercise-Associated Hyponatremia Consensus Development Conference, New Zealand, 2007. Clinical Journal of Sports Medicine, 18: 111-121.

Holbrook JT, Patterson KY, Bodner JE, Douglas LW, Veillon C, Kelsay JL, Mertz W, Smith JC (1984) Sodium and potassium intake and balance in adults consuming self-selected diets. The American Journal of Clinical Nutrition, 40:786-93.

Hollenberg NK, Adams DF, Solomon HS, Abrams HL, Merrill JP (1972) What mediates the renal vascular response to a salt load in normal man? Journal of Applied Physiology 33: 491-495.

Horswill CA, Stofan JR, Lacambra M, Toriscelli TA, Eichner ER, Murray R (2009) Sodium balance during US football training in the heat: Cramp-prone vs Reference players. International Journal of Sports Medicine, 30: 789-794.

Hubbard RW, Szlyk C, Armstrong LE (1990). Influence of thirst and fluid palatability of fluid ingestion during exercise. In: *Perspectives in Exercise Science and Sports Medicine*, *Vol. 3, Fluid Homeostasis During Exercise*. Gisolfi C.V. and D.R. Lamb (eds.), Carmel, Benchmark Press, 1990, pp. 39-96.

Hultman E, Soderlund K, Timmons JA, Cederblad G, Greenhaff PL (1996) Muscle creatine loading in men. Journal of Applied Physiology, 81: 232-237

Inoue Y, Nakao M, Ishizashi H, Tsujita J, Araki T (1998) Regional differences in the Na+ reabsorption of sweat glands. Applied Human Science, 17: 219-221.

Inoue Y, Nakao M, Araki T, Murakami H (1991) Regional differences in the sweating responses of older and younger men. Journal of Applied Physiology, 71: 2453-2459.

Inoue Y, Tanaka Y, Omori K, Kuwahara T, Ogura Y, Ueda H (2005) Sex and menstrual cycle related differences in sweating and cutaneous blood flow in response to passive heat exposure. European Journal of Applied Physiology, 94: 323-332.

Institute of Medicine (2004). Dietary reference intakes for water, potassium, sodium, chloride and sulfate. Washington D.C. The National Academies Press.

Jacobsen FK, Christensen CK, Mogensen CE, Andreasen F and Heilskov NS (1979) Pronounced increase in serum creatinine concentration after eating cooked meat. British Medical Journal, 21: 1049-1050. Jaffe M (1886) Measurement of creatinine using picric acid. Physiological Chemistry, 10: 391-400

James W, Ralph A, Sanchez-Castillo C. (1987). The dominance of salt in manufactured food in the sodium intake of affluent societies. Lancet, 21, 426-9.

Jentjens RLPG, Wagenmakers JM, Jeukendrup AE (2002) Heat stress increases muscle glycogen use but reduces the oxidation of ingested carbohydrates during exercise. Journal of Applied Physiology, 92: 1562-1572.

Jeukendrup A, Saris WH, Brouns F, Kester AD (1996) A new validated endurance performance test. Medicine and Science in Sports and Exercise, 28: 266-270.

Jeukendrup AE, Currell K, Clarke J, Cole J, Blannin AK (2009) Effect of beverage glucose and sodium content on fluid delivery. Nutrition and Metabolism, 6: 9-15.

Judkins C, Watson P, Russell C (2006) A crossover study of 19-nor-androstenedione contamination in sports supplements: preliminary findings. Proceedings of the 5th International congress on Medical Polymers. Cologne, Germany 2006.

Karp JR, Johnston JD, Tecklenburg S, Mickleborough TD, Fly AD, Stager JM (2006). Chocolate milk as a post-exercise recovery aid. International Journal of Sport Nutrition and Exercise Metabolism, 16: 78-91.

Kenefick RW, Ely BR, Cheuvront SN, Palombo LJ, Goodman DA, Sawka MN (2009) Prior heat stress: Effect on subsequent 15min time trial performance in the heat. Medicine and Science in Sports and Exercise, 41: 1311-1316.

Kenney LA, Fowler SR (1988) Methycholine-activated eccrine sweat gland density and output as a function of age. Journal of Applied Physiology, 65: 1082-1086.

Kesteloot H, Joossens J (1990) The relationship between dietary intake and urinary excretion of sodium, potassium, calcium and magnesium: Belgian interuniversity research on nutrition and health. Journal of Human Hypertension, 4: 527-33.

Kipps C, Sharma S, Pedoe DT (in press) The incidence of exercise-associated hyponatraemia in the London marathon. British Journal of Sports Medicine.

Kirby CR, Convertino VA (1986) Plasma aldosterone and sweat sodium concentrations after exercise and heat acclimation. Journal of Applied Physiology, 61: 967-970.

Kilding AE, Tunstall H, Wraith E, Good M, Gammon C, Smith C (2009) Sweat Rate and sweat electrolyte composition in international female soccer players during game specific training. International Journal of Sports Medicine, 30: 443-447.

Knochtel JP, Dotin LN, Hamburger RJ (1972) Pathophysiology of intense physical conditioning in a hot climate I: Mechanisms of potassium depletion. The Journal of Clinical Investigation, 51: 242-255.

Knuiman JT, van Poppel G, Burema J, van der Heijden L, Hautvast JG (1988) Multiple overnight urine collections may be used for estimating the excretion of electrolytes and creatinine. Clinical Chemistry, 34: 135-138.

Kovacs EMR, Schmahl RM, Senden JMG, Brouns F (2002) Effect of high and low rates of fluid intake on post-exercise rehydration. International Journal of Sport Nutrition and Exercise Metabolism, 12: 14-23

Kozlowski S, Saltin B (1964) Effect of sweat loss on body fluids. Journal of Applied Physiology, 19: 1119-1124.

Ladd M. (1951). Effect of prehydration upon renal excretion of sodium in man. Journal of Applied Physiology, 3: 603-9.

Ladell WSS (1949) Heat cramps. Lancet, 2: 836-839.

Ladell WSS (1948) The measurement of chloride losses in the sweat. The Journal of Physiology, 107: 465-471.

Leaf A, Couter WT (1949) Evidence that renal sodium excretion by normal human subjects is regulated by adrenal cortical activity. The Journal of Clinical Investigation, 28: 1067–1081.

Leiba A, Vald A, Peleg E, Shamiss A, Grossman E (2005) Does dietary recall adequately assess sodium, potassium and calcium intake in hypertensive patients? Nutrition, 21: 462-466.

Leiper JB (1998) Intestinal water absorption – Implications for the formulation of rehydration solutions. International Journal of Sports Medicine, 19: S129-S132.

Lemon PW, Yarasheski KE, Dolny DG. (1986). Validity/reliability of sweat analysis by whole-body washdown vs. regional collections. Journal of Applied Physiology, 61: 1967-71.

Leshem M, Abutbul A, Eilon R. (1999). Exercise increases the preference for salt in humans. Appetite, 32: 251-60.

Lichton (1957) Competition between sweat glands and kidneys for salt and water in man. Journal of Applied Physiology, 11: 223-226.

Lieberman J (1966) Cyclic fluctuation of sweat electrolytes in women. Journal of the American Medical Association, 195: 629-635.

Liu K, Cooper R, McKeever J, McKeever P, Byington R, Soltero I, Stamler R, Gosch F, Stevens E, Stamler J (1979) Assessment of the association between habitual salt intake and high blood pressure: methodological problems. American Journal of Epidemiology 110: 219-226.

Liu K, Stamler J (1984). Assessment of sodium intake in epidemiological studies on blood pressure. Annals of Clinical Research, 16: 49-54.

Lobeck CC, Huebner D (1962) Effect of age, sex and cystic fibrosis on sodium and potassium content of human sweat. Pediatrics, 30: 172-179.

Lote CJ (1994) Principles of Renal Physiology, third edition, Chapman & Hall, London.

Lykken GI, Jacob RA, Munoz JM, Sandstead HH (1980) A mathematical model of creatine metabolism in normal males - comparison between theory and experiment. The American Journal of Clinical Nutrition, 33: 2674-2685.

Mahe S, Huneau JF, Marteau P, Thuillier F, Tome D (1992) Gastroileal nitrogen and electrolyte movements after bovine milk ingestion in humans. The American Journal of Clinical Nutrition, 56: 410-416.

Malhotra MS, Sridharan K, Venkataswamy Y, Rai RM, Pichan G, Radhakrishnan U, Grover SK (1981) Effect of restricted potassium intake on its excretion and on physiological responses during heat stress. European Journal of Applied Physiology, 47: 169-179.

Mao IF, Chen ML, Ko YC (2001) Electrolyte loss in sweat and iodine deficiency in a hot environment. Archives of Environmental Health, 56: 271-277

Maughan RJ (1982) A simple, rapid method for the determination of glucose, lactate, pyruvate, alanine, 3-hydroxybutarate and acetoacetate on a single 20-ul blood sample. Clinica Chimica Acta, 122: 231-240.

Maughan RJ, Nadel ER (2000) Temperature regulation and fluid and electrolyte balance. In: Nutrition in Sport, Maughan RJ (ed), Blackwell Science, pg 203 – 215.

Maughan RJ, Merson SJ, Broad NP, Shirreffs SM (2004) Fluid and electrolyte intake and loss in elite soccer players during training. International Journal of Sport Nutrition and Exercise Metabolism, 14: 327-340.

Maughan RJ, Shirreffs SM, Merson SJ, Horswill CA (2005) Fluid and electrolyte balance in elite male football (soccer) players training in a cool environment. Journal of Sports Sciences, 23: 73-79. Maughan RJ, Watson P, Evans GH, Broad N, Shirreffs SM (2007) Water balance and salt losses in competitive football. International Journal of Sport Nutrition and Exercise Metabolism, 17: 583-594.

Maughan RJ and Leiper JB (1993). Post-exercise rehydration in man: Effects of voluntary intake of four different beverages. Medicine and Science in Sports and Exercise, 25:S2.

Maughan RJ and Leiper JB (1995). Sodium intake and post-exercise rehydration in man. European Journal of Applied Physiology and Occupational Physiology, 71: 311-9.

Maughan RJ, Owen JH, Shirreffs SM, Leiper JB (1994). Post-exercise rehydration in man: effects of electrolyte addition to ingested fluids. European Journal of Applied Physiology and Occupational Physiology, 69: 209-15.

Maughan RJ, Leiper JB, Shirreffs SM (1996) Restoration of fluid balance after exerciseinduced dehydration: effects of food and fluid intake. European Journal of Applied Physiology and Occupational Physiology, 73: 317-325.

Maughan RJ, Shirreffs SM, JB Leiper (2007) Errors in the estimation of hydration status from changes in body mass. Journal of Sports Sciences 25: 797-804.

McCance RA (1936) Medical problems in mineral metabolism. III - Experimental Salt Deficiency. Lancet, 1: 823-830

McCance RA (1938) The effect of salt deficiency in man on the volume of the extracellular fluids and on the composition of sweat, saliva, gastric juice and cerebrospinal fluid. Journal of Physiology, 92: 208-218.

McHugh PR and Moran TH (1979) Calories and gastric emptying: a regulatory capacity with implications for feeding. American Journal of Physiology, 139: R254-R260.

McConell GK, Stephens TJ, Benedict J (1999) Fluid ingestion does not influence intense 1-h exercise performance in a mild environment. Medicine and Science in Sports and Exercise, 31: 386-392. McKeown NM, Day NE, Welch AA, Runswick SA, Luben RN, Mulligan AA, McTaggart A, Bingham SA (2001) Use of biological markers to validate self reported dietary intake in a random sample of the European Prospective Investigation into Cancer United Kingdom Norfolk cohort. American Journal of Clinical Nutrition, 74: 188-196.

Melin B, Koulmann N, Jimenez C, Savourey G, Launay JC, Cottet-Emard JM, Peguignot J M, Alllevard AM, Gharib C (2001) Comparison of passive heat or exercise induced dehydration on renal water and electrolyte excretion: the hormonal involvement. European Journal of Applied Physiology, 85: 250-258.

Melse-Boonstra A, Rozendaal, Rexwinkel H, Gerichhausen MJW, van den Briel T, Bulux J, West CE (1998) Determination of discretionary salt intake in rural Guatemala and Benin to determine the iodine fortification of salt required to control iodine fortification of salt required to control iodine deficiency disorders: studies using lithium-labelled salt. American Journal of Clinical Nutrition, 68: 636-641.

Merson SJ, Maughan RJ, Shirreffs SM (2008) Rehydration with drinks differing in sodium concentration and recovery from moderate exercise induced hypohydration in man. European Journal of Applied Physiology, 103: 585-594.

Meyer F, Bar-Or O, MacDougall D, Heigenhauser GJ (1992) Sweat electrolyte loss during exercise in the heat: effects of gender and maturation. Medicine and Science in Sports and Exercise, 24:776-781

Mickelsen O, Makdani D, Gill JL, Frank RL. (1977) Sodium and potassium intakes and excretions of normal men consuming sodium chloride or a 1:1 mixture of sodium and potassium chlorides. The American Journal of Clinical Nutrition, 30: 2033-2040.

Mitchell JB, Phillips MD, Mercer SP, Baylies HL, Pizza FX (2000). Postexercise rehydration: effect of Na(+) and volume on restoration of fluid spaces and cardiovascular function. Journal of Applied Physiology, 89: 1302-9.

Mitchell D, Senay LC, Wyndham CH, van Rensburg AJ, Rogers GG, Strydom NB (1976) Acclimatization in a hot humid environment: energy exchange, body temperature and sweating. Journal of Applied Physiology, 40: 768-778.

Montain SJ, Coyle EF (1992) Influence of graded dehydration on hyperthermia and cardiovascular drift during exercise. Journal of Applied Physiology, 73: 1340-1350.

Montain SJ, Latzka WA, Sawka MN (1995) Control of thermoregulatory sweating is altered by hydration level and exercise intensity. Journal of Applied Physiology, 79:1434-1439.

Montain SJ, Cheuvront SN, Lukaski HC (2007) Sweat mineral-element responses during 7h of exercise heat stress. International Journal of Sport Nutrition and Exercise Metabolism, 17: 574-582.

Montain SJ, Cheuvront SN, Sawka MN (2006) Exercise associated hyponatraemia: quantitative analysis to understand the aetiology. British Journal of Sports Medicine, 40: 98-106.

Morgan RM, Patterson MJ, Nimmo MA (2004) Acute effects of dehydration on sweat composition in men during prolonged exercise in the heat. Acta Physiologica Scandinavica, 182:37-43

Morimoto T, Slabochova Z, Naman RK, Sargent F (1967) Sex differences in physiological reactions to thermal stress. Journal of Applied Physiology, 22: 526-532.

Morris CD (1997) Effect of dietary sodium restriction on overall nutrient intake. The American Journal of Clinical Nutrition, 65: 687S-691S.

Nadel E R, Bullard R W, Stolwijk J A J (1971) Importance of skin temperature in the regulation of sweating. Journal of Applied Physiology, 31: 80-87.

Nadel ER, Mack GW, Nose H (1990). Influence of fluid replacement beverages on body fluid homeostasis during exercise and recovery. In: *Perspectives in Exercise Science and* 

Sports Medicine, Vol. 3, Fluid Homeostasis During Exercise. Gisolfi C.V. and D.R. Lamb (eds.), Carmel, Benchmark Press, 1990, pp. 181-205.

Nadel ER, Pandolf KB, Roberts MF, Stolwijk JAJ (1974) Mechanisms of thermal acclimation to exercise and heat. Journal of Applied Physiology, 37: 515-520.

Nelson M, Black AE, Morris JA, Cole TJ (1989) Between and within subject variation in nutrient intake from infancy to old age: estimating the number of days required to rank dietary intakes with desired precision. The American Journal of Clinical Nutrition, 50: 155-167.

Neville N, Gant N, Folland JP (in press) Thermoregulatory demands of elite professional America's Cup yacht racing. Scandinavian Journal of Medicine and Science in Sport.

Nielsen B, Sjogaard G, Ugelvig J, Knudsen B, Dohlmann B (1986). Fluid balance in exercise dehydration and rehydration with different glucose-electrolyte drinks. European Journal of Applied Physiology and Occupational Physiology, 55: 318-25.

Nielsen B, Hales JR, Strange S, Christensen NJ, Warberg J, Saltin B (1993). Human circulatory and thermoregulatory adaptations with heat acclimation and exercise in a hot, dry environment. Journal of Physiology, 460: 467-85.

Nielsen B, Hansen G, Jorgensen SO, Nielsen E (1971) Thermoregulation in exercising man during dehydration and hyperhydration with water and saline. International Journal of Biometeorology, 15: 195-200.

Nose H, Mack GW, Shi XR, Nadel ER (1988a). Role of osmolality and plasma volume during rehydration in humans. Journal of Applied Physiology, 65: 325-31.

Nose H, Mack G W, Shi X, Nadel E R (1988b) Shift in body fluid compartments after dehydration in humans. Journal of Applied Physiology, 65: 318-324.

Oliver W, Cohen E, Neel J. (1975). Blood pressure, sodium intake, and sodium related hormones in the yanomamo indians, a "no-salt" culture. Circulation 52: 146-51.

O'Toole ML, Douglas PS, Lebrun CM, Laird RH, Miller TK, Miller GC, Miller WDB. Magnesium in the treatment of exertional muscle cramps. Medicine and Science in Sports and Exercise, 25: S19

Palacios C, Wigertz K, Martin B, Weaver CM (2003a) Sweat mineral loss from whole body, patch and arm bag in white and black girls. Nutrition Research, 23: 401-411.

Palacios C, Wigertz K, Weaver CM (2003b) Comparison of 24 Hour whole body versus patch tests for estimating body surface electrolyte losses. International Journal of Sport Nutrition and Exercise Metabolism, 13: 479-488.

Palmer MS, Spriet LL (2008) Sweat rate, salt loss, and fluid intake during an intense on-ice practice in elite Canadian male junior hockey players. Applied Physiology, Nutrition and Metabolism, 33: 263-271.

Passe DH, Horn M, Stofan J, Murray R (2004). Palatability and voluntary intake of sports beverages, diluted orange juice, and water during exercise. International Journal of Sport Nutrition and Exercise Metabolism, 14: 272-84.

Passe DH, Stofan J, Rowe C, Horn MK, Horswill C, Murray R (2006) Exercise affects hedonic responses to sodium in a CHO-E beverage. Medicine and Science in Sports and Exercise, 38: S192.

Passe DH (2001). Physiological and psychological determinants of fluid intake. In Sports Drinks: Basic Science and Practical Aspects. Maughan R.J. and R. Murray (eds.), Boca Raton, CRC Press, pp. 45-87, 2001.

Patterson M J, Galloway S D R, Nimmo M A (2000) Variations in regional sweat composition in normal human males. Experimental Physiology, 85: 869-875

Pearcy M, Robinson S, Miller DI, Thomas JT, DeBrota J (1956) Effects of dehydration, salt depletion and pitressin on sweat rate and urine flow. Journal of Applied Physiology, 8: 621-626.

Pietinen P (1982) Estimating sodium intake from food consumption data. Annals of Nutrition and Metabolism, 26: 90-9.

Pitts GC, Johnson RE, Consolazio FC (1944) Work in the heat is affected by intake of water, salt and glucose. American Journal of Physiology, 142: 253-259.

Pivarnik JM, Marichal CJ, Spillman T, Morrow JR (1992) Menstrual cycle phase affects temperature regulation during endurance exercise. Journal of Applied Physiology, 72: 543-548.

Piwonka RW, Robinson S, Gay VL, Manalis RS (1965) Preacclimatization of men to heat by training. Journal of Applied Physiology, 20: 379-384.

Popowski LA, Oppliger RA, Lambert GP, Johnson RF, Johnson AK, Gisolfi CV (2001) Blood and urinary measures of hydration status during progressive acute dehydration. Medicine and Science in Sports and Exercise, 33: 747-753.

Porrini M, Gentile M, Fidanza F. (1995). Biochemical validation of a self-administered semi-quantitative food-frequency questionnaire. British Journal of Nutrition, 74: 323-33.

Ramanathan NL (1964). A new weighting system for mean surface temperature of the human body. Journal of Applied Physiology, 19: 531-3.

Ray ML, Bryan MW, Ruden TM, Baier SM, Sharp RL, King DS (1998) Effect of sodium in a rehydration beverage when consumed as a fluid or meal. Journal of Applied Physiology, 85: 1329-1336.

Ricos C, Jimenez CV, Hernandez A, Simon M, Perich C, Alvarez V, Minchinela J, Macia M (1994) Biological Variation in Urine Samples Used for Analyte Measurements. Clinical Chemistry, 40: 472-477.

Roberts MF, Wenger CB, Stolwijk JAJ, Nadel ER (1977) Skin blood flow and sweating changes following exercise training and heat acclimation. Journal of Applied Physiology, 43: 133-137.

Robinson S, Maletich RT, Robinson WS, Rohrer BB, Kunz AL (1956) Output of NaCl by sweat glands and kidney in relation to dehydration and to salt depletion. Journal of Applied Physiology, 8: 615-620.

Robinson S, Gerking SD, Turrell ES, Kincaid RK (1950a) Effect of skin temperature on salt concentration of sweat. Journal of Applied Physiology, 2: 654-662.

Robinson S, Kincaid RK, Rhamy RK (1950b) Effect of salt deficiency on the salt concentration in sweat. Journal of Applied Physiology, 3: 55-62.

Robinson S, Nicholas JR, Smith JH, Daly WJ, Pearcy M (1955) Time relation of renal and sweat gland adjustments to salt deficiency in men. Journal of Applied Physiology, 8: 159-165.

Sallis RE (2008) Fluid balance and dysnatremias in athletes. Current Sports Medicine Reports, 7: S14-S19.

Saltin B, Hermansen L (1966) Esophageal, rectal and muscle temperature during exercise. Journal of Applied Physiology, 21: 1757-1762.

Sanders B, Noakes TD, Dennis SC (1999) Water and electrolyte shifts with partial fluid replacement during exercise. European Journal of Applied Physiology, 80: 318-323.

Sanders B, Noakes TD, Dennis SC (2001) Sodium replacement and fluid shifts during prolonged exercise in humans. European Journal of Applied Physiology, 84: 419-425.

Sato S, Dobson RL (1970a) Regional and individual variations in the function of the human eccrine sweat gland. The Journal of Investigative Dermatology, 54: 443-449.

Sato K, Dobson RL (1970b) The effect of intracutaneous d-aldosterone and hydrocortisone on human eccrine sweat gland function. The Journal of Investigative Dermatology, 54: 450-462 Sato K, Sato F (1983) Individual variations in structure and function of human eccrine sweat gland. American Journal of Physiology, 245: R203-R208.

Sato K, Nishiyama A, Kobayashi M (1979) Mechanical properties and functions of the myoepithelium in the eccrine sweat gland. American Journal of Physiology, 237: C177-C184

Sato K, Leidal R, Sato F (1987) Morphology and development of an apoeccrine sweat gland in human axillae. American Journal of Physiology, 252: R166-R180.

Sato K, Sato F (1987b) Sweat secretion by human axillary apoeccrine sweat gland in vitro. The American Journal of Physiology, 252: R181-R187.

Sawka MN, Gonzalez RR, Young AJ, Dennis RC, Valeri CR, Pandolf KB (1989) Control of thermoregulatory sweating during exercise in the heat. American Journal of Physiology 257: R311-R316.

Sawka MN, Cheuvront SN, Carter R (2005) Human water needs. Nutrition Reviews, 63: S30-S39.

Sawka MN and Pandolf KB (1990). Effects of body water loss on physiological function and exercise performance. In: *Perspectives in Exercise Science and Sports Medicine, Vol. 3, Fluid Homeostasis During Exercise*. Gisolfi C.V. and D.R. Lamb (eds.), Carmel, Benchmark Press, 1990, pp. 1-38.

Schachter J, Harper P, Radin M, Caggiula A, McDonald R, Diven W. (1980). Comparison of sodium and potassium intake with excretion. Hypertension 2: 695-9.

Schwartz IL, Thaysen JH (1956) Excretion of sodium and potassium in human sweat. The Journal of Clinical Investigation, 35: 114-21

Scientific Advisory Committee on Nutrition (SACN) (2003) Salt and Health. London, HMSO.

Seifert J, Harmon J, Declercq P (2006). Protein added to a sports drink improves fluid retention. International Journal of Sport Nutrition and Exercise Metabolism, 16: 420-429.

Shamsuddin AKM, Yanagimoto S, Kuwahara T, Zhang Y, Nomura C, Kondo N (2005) Changes in the index of sweat ion concentration with increasing sweat during passive heat stress in humans. European Journal of Applied Physiology, 94: 292-297.

Shapiro Y, Pandolf KB, Avellini BA, Pimental NA, Goldman RF (1980) Physiological responses of men and women to humid and dry heat. Journal of Applied Physiology, 49: 1-8.

Shirreffs SM (2003). The optimal sports drink. Schweizerische Zeitschrift für, 51: 25–29.

Shirreffs SM, Aragon-Vargas LF, Kiel M, Love TD, Phillips S (2007a) Rehydration following exercise in the heat: a comparison of four commonly used drinks. International Journal of Sport Nutrition and Exercise Metabolism, 17: 244-258.

Shirreffs SM, Watson P, Maughan RJ (2007b) Milk as an effective post-exercise rehydration drink. British Journal of Nutrition, 98: 173-180.

Shirreffs SM, Aragon-Vargas LF, Chamorro M, Maughan RJ, Serratosa L, Zachwieja JJ (2005) The sweating response of elite professional soccer players to training in the heat. International Journal of Sports Medicine, 26: 90-95.

Shirreffs SM, Taylor AJ, Leiper JB, Maughan RJ (1996). Post-exercise rehydration in man: effects of volume consumed and drink sodium content. Medicine and Science in Sports and Exercise, 28: 1260-71.

Shirreffs SM, Maughan RJ (1998b). Volume repletion after exercise-induced volume depletion in humans: replacement of water and sodium losses. American Journal of Physiology, 274: F868-F875

Shirreffs SM, Maughan RJ (1998a) Urine osmolality and conductivity as indices of hydration status. Medicine and Science in Sports and Exercise, 30: 1598-1602.

Shirreffs SM, Maughan RJ (2008) Water and salt balance in young male football players in training during the holy month of Ramadan. Journal of Sports Sciences, 26: S47-S54.

Shirreffs SM, Sawka MN, Stone M (2006) Water and electrolyte needs for football training and match play. Journal of Sports Sciences, 24: 699-707.

Shirreffs SM, Maughan RJ (1994) The effect of posture change on blood volume, serum potassium and whole body electrical impedance. European Journal of Applied Physiology, 69: 461-463.

Shirreffs SM, Maughan RJ (1997) Whole body sweat collection in humans: An improved method with preliminary data on electrolyte content. Journal of Applied Physiology, 82: 336-41.

Shvartz E, Shapiro Y, Magazanik A, Meroz A, Birnfeld H, Mechtinger A, Shibolet S (1977) Heat acclimation, physical fitness and responses to exercise in temperate and hot environments. Journal of Applied Physiology, 43: 678-683.

Sigal CB, Dobson RL (1968) The effect of salt intake on sweat gland function. The Journal of Investigative Dermatology, 50: 451-455.

Simpson FO (1988) Sodium intake, body sodium and sodium excretion. The Lancet, 332: 25-29.

Sims ST, van Vliet L, Cotter JD, Rehrer NJ (2007) Sodium loading aids fluid balance and reduces physiological strain of trained men exercising in the heat. Medicine and Science in Sports and Exercise, 39: 123-130.

Smiles KA, Robinson S (1971) Sodium ion conservation during acclimatisation of men to work in the heat. Journal of Applied Physiology, 31: 63-69

Soo K, Naughton G (2007) The hydration profile of female cricket players during competition. International Journal of Sport Nutrition and Exercise Metabolism, 17:14-26.

Sowers M, Stumbo P (1986) A method to assess sodium intake in populations. Journal of the American Dietetic Association, 86: 1196-1202.

Speedy DB, Thompson JMD, Rodgers I, Collins M, Sharwood K (2002) Oral salt supplementation during ultraendurance exercise. Clinical Journal of Sports Medicine, 12: 279-284.

Speedy DB, Noakes TD, Rogers IR, Thompson JMD, Campbell RGD, Kuttner JA, Boswell DR, Wright S, Hamlin M (1999) Hyponatremia in ultradistance triathletes. Medicine and Science in Sports and Exercise, 31: 809-815.

Stephenson LA, Wenger LB, O'Donovan BH, Nadel ER (1984) Circadian rhythm in sweating and cutaneous blood flow. American Journal of Physiology, 246: R321-R324.

Stofan J, Niksich D, Horswill CA, Martin R, Bream T, Murray R (2001) Sweat and sodium losses in cramp-prone professional football players. Medicine and Science in Sports and Exercise, 33: S256

Stofan JR, Stover EA, Lovett SC, Horswill CA, Murray R (2006) A carbohydrateelectrolyte drink attenuates decline in blood sodium during prolonged exercise in the heat. Medicine and Science in Sports and Exercise, 38: S217

Stofan JR, Zachwieja JJ, Horswill CA, Murray R, Anderson SA, Eichner ER (2005) Sweat and Sodium Losses in NCAA Football Players: A precursor to Heat Cramps? International Journal of Sport and Exercise Nutrition and Exercise Metabolism, 15:641-652

Stofan JR, Zachwieja JJ, Horswill CA, Horn MK, Osterberg K, Murray R (2002a) Assessment of sweat electrolyte losses in humans during exercise: Whole body versus regional measurements. FASEB, 16: A1141.

Stofan JR, Zachwieja JJ, Horswill CA, Murray R (2002b) Sweat and sodium losses during practice in professional football players. Medicine and Science in Sports and Exercise, 34:S113

Stover EA, Zachwieja J, Stofan J, Murray, R, Horswill CA (2006) Consistently high urine specific gravity in adolescent American football players and the impact of an acute drinking strategy. International Journal of Sports Medicine, 27: 330-335.

Szlyk PC, Sils IV, Francesconi RP, Hubbard RW (1990) Patterns of human drinking: effects of exercise, water temperature, and food consumption. Aviation, Space and Environmental Medicine, 61: 43-48.

Takamata A, Mack GW, Gillen CM, Nadel ER. (1994). Sodium appetite, thirst, and body fluid regulation in humans during rehydration without sodium replacement. American Journal of Physiology, 266: R1493-R1502

Talbott JH, Michelsen J (1933) Heat cramps. A clinical and chemical study. The Journal of Clinical Investigation, 12: 533-549.

Tankersley CG, Smolander J, Kenney WL, Fortney SM (1991) Sweating and skin blood flow during exercise: effects of age and maximal oxygen uptake. Journal of Applied Physiology, 71: 236-242.

Tasevska N, Runswick SA, Bingham SA (2006) Urinary potassium is as reliable as urinary nitrogen for use as a recovery biomarker in dietary studies of free living individuals. The Journal of Nutrition, 136: 1334-40.

Taylor HL, Henschel A, Mickelsen O, Keys A (1943) The effect of the sodium chloride intake on the work performance of man during exposure to dry heat and experimental heat exhaustion. American Journal of Physiology, 140: 439-451.

Thomas K, Morris P, Stevenson E (2009) Improved endurance capacity following chocolate milk consumption compared with 2 commercially available sports drinks. Applied Physiology, Nutrition and Metabolism 34: 78-82.

Twerenbold R, Knechtle B, Kakebeeke TH, Eser P, Muller G, von Arx P, Knecht H (2003) Effects of different sodium concentrations in replacement fluids during prolonged exercise in women. British Journal of Sports Medicine, 37: 300-303. Van Buren M, Rabelink TJ, Van Rijn HJM, Koomans HA. (1992) Effects of acute NaCl, KCl and KHCO<sub>3</sub> loads on renal electrolyte excretion in humans. Clinical Science, 83:567–574

Van Heyningen R, Weiner JS (1952). A comparison of arm-bag sweat and body sweat. Journal of Physiology, 116: 395-403.

Vellar OD (1968) Studies on sweat losses of nutrients. I. Iron content of whole body sweat and its association with other sweat constituents, serum iron levels, haematological indices, body surface area and sweat rate. Scand J Clin Lab Invest, 21: 157-167.

Verde T, Shephard RJ, Corey P, Moore R (1982) Sweat composition in exercise and heat. Journal of Applied Physiology, 53: 1540-1545.

Vestergaard P, Leverett R (1958) Constancy of urinary creatinine excretion. The Journal of Laboratory and Clinical Medicine, 51: 211-218.

Vist GE and Maughan RJ (1995) The effect of osmolality and carbohydrate content on the rate of gastric emptying of liquids in man. Journal of Physiology, 486: 523-531.

Voors AW, Dalferes ER, Frank GC, Aristimuno GG, Berenson GS (1983) Relation between ingested potassium and sodium balance in young blacks and whites. The American Journal of Clinical Nutrition, 37: 583-594.

Vrijens DMJ, Rehrer NJ (1999) Sodium free fluid ingestion decreases plasma sodium during exercise in the heat. Journal of Applied Physiology, 86: 1847-1851.

Wald N, Leshem M (2003) Salt conditions a flavour preference or aversion after exercise depending on NaCl dose and sweat loss. Appetite, 40: 277-284.

Walsh RM, Noakes TD, Hawley JA, Dennis SC (1994). Impaired high-intensity cycling performance time at low levels of dehydration. International Journal of Sports Medicine, 15: 392-8.

Wagner JA, Robinson S, Tzankoff SP, Marino RP (1972) Heat tolerance and acclimatisation to work in the heat in relation to age. Journal of Applied Physiology, 33: 616-622.

Warburton DE, Gledhill N, Quinney HA (2000) Blood volume, aerobic power and endurance performance: potential ergogenic effect of volume loading. Clinical Journal of Sport Medicine, 10: 59-66

Warburton DE, Gledhill N, Jamnik VK, Krip B, Card N (1999) Induced hypervolemia, cardiac function and performance of elite cyclists. Medicine and Science in Sports and Exercise, 31: 800-808.

Waterhouse J, Edwards B, Bedford P, Hughes A, Robinson K, Nevill A, Weinert D, Reilly T (2004). Thermoregulation during mild exercise at different circadian times. Chronobiology International 21: 253-75.

Watson RL, Langford HG (1970) Usefulness of overnight urines in population groups. Pilot studies of sodium, potassium and calcium excretion. The American Journal of Clinical Nutrition, 23: 290-304.

Watt MJ, Garnham AP, Febbraio MA, Hargreaves M (2000) Effect of acute plasma volume expansion on thermoregulation and exercise performance in the heat. Medicine and Science in Sports and Exercise, 32: 958-962.

Webster J, Garrow JS (1985) Creatinine excretion over 24hours as a measure of body composition or of completeness of urine collection. Human Nutrition, Clinical Nutrition, 39: 101-106.

Weiner J S, van Heyningen R E (1952) Relation of skin temperature to salt concentration of general body sweat. Journal of Applied Physiology, 4: 725-733.

Wemple RD, Morocco TS, Mack GW (1997) Influence of sodium replacement on fluid ingestion following exercise-induced dehydration. International Journal of Sports Nutrition, 7: 104-16.

Wenger CB, Roberts MF, Stolwijk JAJ, Nadel ER (1976) Nocturnal lowering of thresholds for sweating and vasodilation. Journal of Applied Physiology, 41: 15-19.

Weschler LB (2008) Sweat electrolyte concentrations obtained from within occlusive coverings are falsely high because sweat itself leaches skin electrolytes. Journal of Applied Physiology, 105: 1376-1377

Wharam PC, Speedy DB, Noakes TD, Thompson JMD, Reid SA, Holtzhausen LM (2006) NSAID use increases the risk of developing hyponatremia during an ironman triathlon. Medicine and Science in Sports and Exercise, 38: 618-622.

Wilkinson SB, Tarnopolsky MA, MacDonald JR, Armstrong D, Phillips SM (2007) Consumption of fluid skim milk promotes greater muscle accretion after resistance exercise than does consumption of isonitrogenous and isoenergetic soy protein beverage. The American Journal of Clinical Nutrition, 85: 1031-1040.

Willett, W (1990) Nutritional Epidemiology. Oxford University press.

Wojcik JR, Walberg-Rankin J, Smith LL, Gwazdauskas FC (2001). Comparison of carbohydrate and milk-based beverages on muscle damage and glycogen following exercise. International Journal of Sport Nutrition and Exercise Metabolism, 11: 406-19.

Wong SH, Williams C, Adams N (2000) Effects of ingesting a large volume of carbohydrate-electrolyte solution on rehydration during recovery and subsequent exercise capacity. International Journal of Sport Nutrition and Exercise Metabolism, 10: 375-393.

Wyndham CH, Morrison JF, Williams CG (1965) Heat reactions of male and female Caucasians. Journal of Applied Physiology, 20: 357-364.

Yanagawa S, Yokozeki H, Sato K (1986) Origin of periodic acid Schiff reactive glycoprotein in human eccrine sweat. Journal of Applied Physiology, 60: 1615-1622.

# Appendix A

| Subject Number :    | Trial Number :                      | Date:         |
|---------------------|-------------------------------------|---------------|
|                     | How thirsty do you feel now?        |               |
| not at all thirsty  |                                     | very thirsty  |
|                     | How full does your stomach feel no  | w?            |
| not at all full     |                                     | very full     |
|                     | How bloated do you feel now?        |               |
| not at all bloated  |                                     | very bloated  |
|                     | How hungry do you feel now?         |               |
| not at all hungry   |                                     | very hungry   |
|                     | How does your mouth feel now?       |               |
| not at all pleasant |                                     | very pleasant |
|                     | How tired do you feel now?          |               |
| not at all tired    |                                     | very tired    |
|                     | How alert do you feel now?          |               |
| not at all alert    |                                     | very alert    |
|                     | How well can you concentrate just n | ow?           |
| not at all well     |                                     | very well     |
|                     | How does your head feel now?        |               |
| not at all sore     |                                     | very sore     |

#### Subjective Feelings Questionnaire

# Appendix B

| Subjective reenings Questionnaire |                                    |                |
|-----------------------------------|------------------------------------|----------------|
| Subject Number :                  | Trial Number : Da                  | te:            |
|                                   |                                    |                |
|                                   | How sweet did your drink taste?    |                |
| not at all sweet                  |                                    | very sweet     |
|                                   | How salty did your drink taste?    |                |
| not at all salty                  |                                    | very salty     |
|                                   | How bitter did your drink taste?   |                |
| not at all bitter                 |                                    | very bitter    |
|                                   | How pleasant did your drink taste? |                |
| not at all pleasant               |                                    | very pleasant  |
|                                   | How refreshed do you feel now?     |                |
| not at all refreshed              |                                    | very refreshed |

#### **Subjective Feelings Questionnaire**

# Appendix C

#### **RATINGS OF PERCEIVED EXERTION**

| 6  |                  |
|----|------------------|
| 7  | Very, very light |
| 8  |                  |
| 9  | Very Light       |
| 10 |                  |
| 11 | Fairly Light     |
| 12 |                  |
| 13 | Somewhat hard    |
| 14 |                  |
| 15 | Hard             |
| 16 |                  |
| 17 | Very Hard        |
| 18 |                  |
| 19 | Very, very hard  |
| 20 |                  |

# Appendix D

#### THERMAL SENSATION SCALE

| -10 | COLD, IMPOSSIBLE TO BEAR     |
|-----|------------------------------|
| -9  |                              |
| -8  | VERY COLD, SHIVERING HARD    |
| -7  |                              |
| -6  | COLD, LIGHT SHIVERING        |
| -5  |                              |
| -4  | MOST AREAS OF BODY FEEL COLD |
| -3  |                              |
| -2  | SOME AREAS OF BODY FEEL COLD |
| -1  |                              |
| 0   | NEUTRAL                      |
| 1   |                              |
| 2   | SOME AREAS OF BODY FEEL WARM |
| 3   |                              |
| 4   | SOME AREAS OF BODY FEEL HOT  |
| 5   |                              |
| 6   | MOST AREAS OF BODY FEEL HOT  |
| 7   |                              |
| 8   | VERY HOT, UNCOMFORTABLE      |
| 9   |                              |
| 10  | HEAT IMPOSSIBLE TO BEAR      |