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Section: Original Investigation

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Variability of measurements of sweat sodium using the regional absorbent patch method

Original investigation

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ABSTRACT

There is interest in including recommendations for the replacement of the sodium lost in sweat in individualized hydration plans for athletes. **Purpose:** Although the regional absorbent patch method provides a practical approach to measuring sweat sodium losses in field conditions, there is a need to understand the variability of estimates associated with this technique. **Methods:** Sweat samples were collected from the forearms, chest, scapula and thigh of 12 cyclists during two standardized cycling time trials in the heat, and two in temperate conditions. Single measure analysis of sodium concentration was conducted immediately by ion-selective electrodes (ISE). A subset of 30 samples was frozen for re-analysis of sodium concentration using ISE, flame photometry (FP) and conductivity (SC). **Results:** Sweat samples collected in hot conditions produced higher sweat sodium concentrations than those from the temperate environment ($p=0.0032$). A significant difference ($p=0.0048$) in estimates of sweat sodium concentration was evident when calculated from the forearm average (mean \pm 95% CI; 64 ± 12 mmol \cdot L $^{-1}$) compared to using a four-site equation (70 ± 12 mmol \cdot L $^{-1}$). There was a high correlation between the values produced using different analytical techniques ($r^2=0.95$), however mean values were different between treatments (frozen-FP, frozen-SC>ISE>frozen-ISE; $p<0.0001$). **Conclusion:** Whole-body sweat sodium concentration estimates differed depending on the number of sites included in the calculation. Environmental testing conditions should be considered in the interpretation of results. The impact of sample freezing and subsequent analytical technique was small but statistically significant. Nevertheless, when undertaken using a standardized protocol, the regional absorbent patch method appears to be a relatively robust field test.

Keywords: Sweat testing, salt, fluid balance, hydration, electrolytes

INTRODUCTION

The replacement of sweat sodium losses plays an important role in the restoration of fluid balance following exercise-induced hypohydration.¹ In the absence of sodium replacement, the intake of fluid is associated with a decrease in thirst² and an increased diuresis,³ despite the continued presence of a significant fluid deficit. It has been recommended that a targeted intake of sodium be incorporated into personalized hydration plans that are now promoted as best practice for athletes.^{4,5}

While the more precise technique for assessment of sweat electrolyte content and loss is the whole-body washdown protocol,⁶ this is largely a laboratory technique. The regional absorbent patch method, in which sweat is collected from various sites of the body, is a reasonable proxy for the whole-body washdown protocol^{7,8} that is more practical for field testing.

The reported normal range of sodium concentration in sweat is 20-80 mmol·L⁻¹.¹ While a large degree of inter-individual variability is expected, a certain level of variation can exist within the same individual. The variability in measurements of sweat sodium concentration may be derived from biological sources such as fitness level,⁹ acclimation status,¹⁰ environmental conditions¹¹ or dietary sodium intake,¹² but may also arise from technical errors associated with the regional absorbent patch method.

The variability introduced by the lack of standardized protocols and potential errors in carrying out sweat testing in the field are yet to be quantified. The following variants have been identified as being of frequent occurrence in the presently available literature: number of sites and site selection for patch application, duration of patch application/sweat collection, the interval between sweat collection and analysis and the method of sweat sodium concentration analysis.

Given that testing of sweat sodium concentration is becoming commonplace in athlete servicing activities, it is important that results can be interpreted to provide athletes with meaningful feedback. Accordingly, the aim of the current investigation was to identify and quantify the sources of variability in measures of sodium concentration associated with the collection of sweat via the regional absorbent patch method by mimicking conditions or variations to the protocol that might occur with field testing. Additionally, this paper will consider whether any variability in sweat sodium concentrations arising from protocol differences contribute to meaningful changes in the practical outcomes or interpretation of results based on some of the ways in which sweat sodium data are currently used.

METHODS

Subjects. Twelve well-trained male cyclists (mean±SD; age 31.9±7.0 y, body mass 74.4±5.1 kg, maximal aerobic power 477±34 W, peak oxygen consumption 72.4±6.4 ml·kg⁻¹·min⁻¹) were recruited for this randomized controlled trial. Prior to the study, each subject was informed of the nature and risks of the study before completing a medical questionnaire and giving their written informed consent. The study was approved by the Australian Sports Commission Human Ethics Committee.

Design. On separate days, subjects undertook a total of four 45.6 km standardized self-paced cycling time trials. Two trials were undertaken in a hot [31.9±0.7 °C, 35±7% relative humidity (r.h.)] environment with the other two being completed in temperate (21.2±0.6 °C, 54±11% r.h.) conditions. Two testing protocols were used to collect sweat samples (A and B, described below) and subjects were allocated into the four different treatment groups (based on environmental conditions and sweat collection protocol) in a randomized counterbalanced order. Trials were separated by 3-7 d with a consistent recovery time between trials for each subject. This study took place toward the end of the Australian

summer, such that all subjects were naturally acclimatized to, and experienced at cycling in hot conditions. In each experimental trial, estimations of fluid balance including sweat loss and sweat sodium concentration were made. Sweat sodium measurements generated from each method of analysis were categorized against a set of diagnostic criteria¹³ and counted to explore the practical application and interpretation of results in the absence of sports-specific criteria.

Experimental time trials. A ‘first waking’ urine sample was analyzed (Digital hand-held ‘pocket’ urine S.G. refractometer PAL-10s, Atago Company Ltd., Tokyo, Japan) for specific gravity to assess each subject’s hydration status. Subjects performed each 46.5 km cycling time trial on a magnetically braked ergometer (Velotron, Racermate Inc., Seattle, WA, USA). All experimental trials were undertaken in the afternoon. Subjects completed a standardized 20 min warm-up on the Velotron ergometer.

Fluid balance analysis. Immediately prior to, and immediately following each trial (after removing any surface sweat from their bodies), subjects were required to void their bladder and have a nude body mass measured (A&D Precision Personal Scales UC-321, Tokyo, Japan), accurate to ± 50 g. Subjects were provided with a 6% carbohydrate-containing fluid that included 51 mg sodium per 100 ml (Gatorade, Pepsico Australia, Chatswood, Australia) and were permitted to drink ad libitum during the first time trial. Drink bottles were weighed (A&D Compact Scales HL-4000, Tokyo, Japan), accurate to ± 1 g, immediately before and after each trial to calculate ingested fluid volume. The volume that was consumed during the first trial was measured and repeated for subsequent trials. Whole-body sweat loss was determined by the change in body mass during the time trial after correction for the mass of fluid ingested and volume of urine passed. Changes in mass due to substrate exchange and respiratory water loss were uncorrected.¹⁴ The calculations of fluid and sodium balance over each experimental trial were made according to methods detailed previously.¹⁵

Sweat sample collection and storage. The regional absorbent patch method was used to collect sweat samples during the experimental trials. Two sweat collection protocols were employed to allow variables of interest to be investigated in each of the environmental conditions (Table 1). Sweat patches (Tegaderm+Pad, 3M Health Care, Minnesota, USA) were applied 5 min prior to the start of each time trial (i.e. after completion of the standardized warm-up). Each site was cleaned with distilled water and dried with sterile gauze before application. All samples were removed using metal forceps (washed with distilled water and dried before use) and instantly placed into clean filtered centrifuge tubes (Salivette, Sarstedt AG & Co, Germany). After removal, sweat patches were immediately centrifuged at 10 °C for 5 min at 4500 revolutions per min (relative centrifugal force = 3940 × g; Heraeus Labofuge 400R, GmbH, Hanau, Germany).

Protocol A. Protocol A investigated the effect of sample storage on sweat sodium concentrations. Two sweat patches were applied on the left forearm and two on the right forearm. Patches were placed on the dorsal surface with the first patch immediately distal to the elbow and the second distal to the first patch by ~1 cm. All sweat patches were removed 30 min into the experimental trial. One sample was analyzed immediately, with the other samples stored in sealed centrifuge tubes for seven days in one of three conditions: in a refrigerated cool room (7 °C), in a temperature-controlled, air-conditioned laboratory (21 °C) or in an incubator (Memmert U30, GmbH, Hanau, Germany; maintained at 32 °C), before being analyzed by indirect ion-selective electrodes (ISE).

Protocol B. Protocol B investigated the effects of patch application site and the duration of sample collection. Seven sweat patches were applied during protocol B. These included the collection sites from protocol A with the addition of chest (7 cm superior to the nipple, immediately below the clavicle), scapula (immediately superior to the scapula spine, 15 cm lateral of the vertebral column) and thigh (equidistant between the knee and hip on the

anterior surface) sites. One left and one right forearm sweat patch were removed at 30 min into the trial. The remaining forearm sweat patches, as well as the chest, scapula and thigh patches were removed on completion of the trial. All samples were analysed by ISE immediately after extraction.

Analytical technique. A subset of 30 samples were randomly selected from forearm sites removed at 30 min (from either protocol) to be frozen and re-analyzed using three different techniques (described below). After an initial 0.15 ml aliquot of each sample was treated as stated above in either protocol A or B, the remaining sample was divided into a further three 0.15 ml aliquots into clean, standard conical microtubes with a flip top lid (1.7ml; Scientific Specialities Inc., California) and frozen at -80 °C.

Analysis of sweat sodium. Sweat sodium, chloride and potassium concentration were measured by indirect ISE (Hitachi, Model 911; CV 0.24%). At the time of re-analysis, frozen samples were thawed on the lab bench and re-analyzed by ISE, as well as by flame photometry (Corning, Model 410C; CV<1.5%) and a semi-portable conductivity analyzer (Sweat Check 3120, Wescor, Utah, USA). ISE and flame photometry analysis were performed by experienced technicians using standard laboratory protocols for assessment of electrolytes in biological fluids appropriate for each technique. Both analyzers were calibrated according to manufacturers’ guidelines. To carry out conductivity analysis, the final aliquot of each sample was injected into a capillary tube that was connected to the measuring cell of the ‘desktop’ device. Calibration of the Sweat Check was verified with a standard solution of sodium chloride (80 mmol·L⁻¹).

Ranges for ‘high’, ‘moderate’ and ‘low’ sweat sodium concentrations used as clinical diagnostic criteria were provided within the guidelines for the use of the Sweat Check machine. To test the effect of analytical techniques on practical interpretation of sweat

sodium measurements, the subset of 30 samples were analyzed according to the four different techniques (detailed above) and categorized against these criteria.

Statistical analysis. Linear mixed models were fitted using JMP Pro 10 (SAS). In each case, random effects for subject and trial (nested within subject) were fitted to account for possible correlation within subjects and within trials within subjects. To confirm a difference in total sweat loss between the temperate and hot environmental conditions, a linear mixed model was fitted to sweat loss (L) with environmental condition as a fixed effect.

Results were excluded if samples produced a sweat potassium concentration of greater than or equal to $11 \text{ mmol}\cdot\text{L}^{-1}$. This exclusion value was set in accordance with previous literature^{5,16} reporting the typical concentration of sweat potassium to be 2–8 $\text{mmol}\cdot\text{L}^{-1}$, and seldom exceeding $10 \text{ mmol}\cdot\text{L}^{-1}$. Unusually high potassium concentration measures indicate a high likelihood of sweat sample contamination.⁵

Statistical significance of the F statistics for fixed effects was set at an alpha level of $p\leq 0.05$. When an F statistic was significant, post hoc analysis was conducted using Tukey’s Honestly Significant Difference Test.

RESULTS

Results for total sweat loss, sweat rate and fluid intake for each trial (self-paced, approximately 70 min) are presented in Table 2. Values for pre-exercise USG did not differ between trials ($F_{3,33}=1.16$, $p=0.34$), verifying that subjects began each trial with a similar hydration status. No significant difference in forearm sweat sodium concentration ($[\text{Na}^+]$) was observed across trial days ($F_{3,46.05}=1.33$, $p=0.28$), confirming that subjects were accustomed to exercising in the heat prior to the commencement of the study, and that the

measurement of sweat sodium concentration from forearm samples using the regional absorption patch method has reasonable test-retest repeatability.

Protocol A. Environmental conditions influenced sweat sodium concentration, with samples collected in hot conditions (mean \pm 95% CL; 73 \pm 9 mmol \cdot L⁻¹) producing higher estimates than those collected in a temperate environment (61 \pm 10 mmol \cdot L⁻¹; $F_{1,11.14}=13.56$, $p=0.0035$). When comparing the results of samples that were stored for seven days, only those stored at 7 °C were significantly different ($F_{3,62.46}=7.31$, $p=0.0003$) to sweat samples that were analyzed immediately (Table 3).

Protocol B. A significant effect of application site ($F_{4,84.25}=56.00$, $p<0.0001$) was evident, with the estimated mean sodium concentration of samples taken from the chest found to be higher than the back, left and right forearm, and thigh (Figure 1). In the case of the averaged forearm values, no significant effect of application time (mean \pm 95% CL; 30 min: 62 \pm 13 mmol \cdot L⁻¹ vs. 70 min: 64 \pm 13 mmol \cdot L⁻¹; $F_{1,18.25}=1.52$, $p=0.23$) or environmental testing condition (temperate: 60 \pm 13 mmol \cdot L⁻¹ vs. hot: 65 \pm 14 mmol \cdot L⁻¹; $F_{1,10.05}=0.73$, $p=0.26$) on sweat sodium concentration was observed. There was no interaction between application time and environmental testing condition.

To confirm the effect of environmental conditions observed in protocol A, data collected with consistent methodology across both protocols were combined. Sweat samples collected in hot environmental conditions (69 \pm 9 mmol \cdot L⁻¹) produced significantly higher sweat sodium concentration values than those from the temperate environment (60 \pm 9 mmol \cdot L⁻¹; $F_{1,40.44}=9.83$, $p=0.0032$).

A significant difference ($F_{1,18.99}=10.21$, $p=0.0048$) in estimates of sweat sodium concentration was evident when calculated from the forearm average (64 \pm 12 mmol \cdot L⁻¹) compared to calculations from a four-site equation (70 \pm 12 mmol \cdot L⁻¹).

Analytical technique. Immediate analysis of a subset of 30 samples by ISE resulted in an estimated mean sweat sodium concentration of $58 \pm 20 \text{ mmol} \cdot \text{L}^{-1}$ (range: 17-97 $\text{mmol} \cdot \text{L}^{-1}$). Re-analysis of stored samples by ISE (frozen-ISE) produced a mean of $54 \pm 20 \text{ mmol} \cdot \text{L}^{-1}$ (22-89 $\text{mmol} \cdot \text{L}^{-1}$). A mean of $65 \pm 20 \text{ mmol} \cdot \text{L}^{-1}$ (23-109 $\text{mmol} \cdot \text{L}^{-1}$) and $62 \pm 20 \text{ mmol} \cdot \text{L}^{-1}$ (25-97 $\text{mmol} \cdot \text{L}^{-1}$) were found using flame photometry and sweat conductivity, respectively. There was a high correlation ($r^2=0.95$) between the values produced under each condition, and although there was no statistical difference in sweat sodium results produced by flame photometry and sweat conductivity, estimated values were statistically different between the frozen-thawed flame photometry and ISE samples and those analyzed immediately by ISE (frozen-FP , frozen-SC > ISE > frozen-ISE; $F_{3,87}=24.61$, $p<0.0001$). The number of samples falling into the ranges defined as ‘normal’, ‘borderline’ and ‘high’ for sweat sodium concentration, using each technique is compared in Table 4.

DISCUSSION

This is the first study to investigate the variability in estimates of sweat sodium concentrations using the regional absorbent patch method due to differences in protocols commonly used to test athletes in field conditions.

Environmental testing conditions. Sweat samples collected during exercise in hot environmental conditions had a higher sodium concentration than those collected in a temperate environment. Since sweat loss was high in hot conditions, our results are consistent with previous research which has shown that the sodium concentration of sweat increases with sweat rate.¹⁷⁻¹⁹ This confirms that sweat testing protocols must be interpreted with consideration of the environmental testing conditions, with the additional note that this is still relevant irrespective of athletes’ acclimation status.

Site selection and estimates of whole-body sweat sodium concentration. In accordance with previous literature,^{7,8} the current investigation found significant differences in the sodium concentration of sweat collected from different body sites. Sweat samples collected from sites on the trunk have been shown to be higher in sodium concentrations than limb sites.⁸ Our results showed highest sodium concentrations in sweat collected at the chest followed by the back, forearms and thigh. Earlier investigations attempted to determine the best site(s) to use as a proxy for sweat sodium concentrations measured by the whole-body washdown techniques. A surface area-weighted calculation using four regional sites was found to be strongly associated ($r=0.88-0.97$) with the whole-body washdown technique^{7,8} and has been employed in studies undertaken in various sports settings.^{20,21} However, practical challenges in collecting sweat samples in the field have promoted the forearm as the site that is most accessible across a variety of sports settings. Coupled with its high correlation ($r=0.88$) with whole-body washdown results,⁷ the forearm has become the most frequently used site for measuring sweat sodium concentration in various athlete groups.^{19,22,23}

In the current study, although highly correlated ($r=0.91$), whole-body sweat sodium estimates using results from sweat collected from the chest, back, right forearm and thigh in the four-site equation⁷ were significantly higher than estimates produced from the arithmetic mean of forearm measures. Although the needs and strategies for replacement of total sodium loss from sweat are currently uncertain, it is worth considering the magnitude of change in salt replacement targets as a result of variations in sweat sodium estimates arising from different calculation methods. As a working example of this, and using 1.5 L as an arbitrary sweat volume, results from the present study of total sodium loss from whole-body sweat sodium concentrations using the forearm average and four-site equation would equate to 2.2 g and 2.4 g, respectively. Taking into consideration the 95% CL of the difference of the means

of sweat sodium concentration ($6\pm 4 \text{ mmol}\cdot\text{L}^{-1}$) the difference in total sodium loss generated by each method of calculation may be an under- or over-estimation by 0.12 g (approximately 0.5 g salt). This error is unlikely to translate into substantial differences in individual nutrition plans in the present situation, but might be compounded in scenarios involving higher sweat losses over many hours of exercise such as lengthy two-a-day training practices or ultraendurance events such as an Ironman triathlon.

Handling and storage. Since the analysis of sodium from a sweat sample is not readily available to most practitioners, there may be occasions when storage of samples is required before subsequent laboratory analysis. This raises a question of the stability of sweat samples when they are not able to be analyzed immediately following collection. Storage of sweat samples for seven days in a temperature-controlled laboratory and incubator appeared to have no significant effect on sweat sodium concentration results. Unexpectedly, the samples stored in the refrigerator produced measures of sweat sodium that were significantly higher than the others. While it has been reported that certain analytes in urine samples are unstable after 24 h refrigeration,²⁴ the same justification cannot be found in the literature for sodium in sweat samples. A limitation of the current study was that the placement of patches on the forearms was not randomised against the storage condition, therefore any methodological issues with patches at that particular forearm site (e.g. greater local sweat rate leading to potential issues with patch adhesion or contamination of sample) may have influenced subsequent results and led to a type I error.

Analytical technique. The freezing and thawing of samples caused an approximate 7% decrease in sweat sodium estimates using the ISE method. There was a difference in sweat sodium concentrations across analytical techniques with the flame photometry method producing estimates that were ~20% higher than the ISE technique. The sodium concentration estimates produced by sweat conductivity using the semi-portable desktop

device (Sweat Check) fell between the estimates from the two laboratory-based methods. These results support previous work that showed a very high correlation ($r=0.98$) between sweat sodium results produced by the Sweat Check and those using flame photometry.²⁵ The ISE method is not a direct measure of concentration but rather a measure of ion activity, and despite well-reported correlation with direct measures of sodium concentration using flame photometry,^{26,27} part of the discrepancy in the findings of this study may be attributed to inherent limitations of the ISE technique, namely the effects of the ionic strength of the sample reducing the measured activity. While there is no literature on which to draw, the procedure of freezing and thawing sweat samples may potentiate this effect.

Currently there is no published literature on the classification of ‘high’, ‘moderate’ and ‘low’ sweat sodium concentrations in relation to issues of exercise or athletic performance. However, reference ranges for sweat sodium concentration have been suggested for clinical use.¹³ When values from the present study are compared to such a reference range, the choice of analytical technique significantly affects the interpretation of results. Based on these results, half of the samples could potentially be misclassified. Differences in the handling of sample (freezing and thawing) led to 17% of samples being categorized into a different classification. If sports-specific criteria existed and were to be employed to categorize athletes sweat characteristics, small variations in results, as demonstrated here, may influence subsequent recommendations regarding sodium replacement during or after exercise. It is therefore essential to have an understanding of the cumulative variability introduced at each point of sweat testing to allow an appreciation of the margins of error to be built into the interpretation of the results.

PRACTICAL APPLICATIONS

These results highlight the need for researchers and practitioners to report details of their testing procedures to enhance the interpretation of sweat sodium results. Furthermore, they encourage the development of standardized protocols for the collection, storage and analysis of sweat sodium samples, especially if longitudinal or comparative monitoring is planned. It is now important to determine what is seen as the smallest worthwhile or physiologically meaningful change in sweat sodium concentration. Such information will inform our understanding of the sensitivity of the regional absorbent patch method and whether standardized protocols that improve the reliability of measurement are of practical importance.

CONCLUSIONS

The regional absorbent patch method appears to be a relatively robust field test when undertaken by an experienced tester, and withstands protocol variations in relation to the duration of sweat collection time. Environmental conditions and the intensity of exercise should be noted and considered in the interpretation and comparison of results. Indeed, it would be prudent to undertake sweat testing in conditions that are likely to reflect the scenario in which specific individualized hydration plans will be put to use. Care should be taken when deciding on which sites to include in testing, as the sampling protocol will determine how the results can be used to estimate whole-body sweat sodium losses. The difference in estimates of sweat sodium concentrations attributed to specific protocols for storing samples for later analysis or from different analytical techniques is small but statistically significant, and of potential impact on the interpretation of results.

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REFERENCES

1. Maughan RJ. Fluid and electrolyte loss and replacement in exercise. *Journal of sports sciences* 1991;9 Spec No:117-142.
2. Nose H, Mack GW, Shi XR, Nadel ER. Role of osmolality and plasma volume during rehydration in humans. *Journal of applied physiology* 1988;65:325-331.
3. Maughan RJ, Leiper JB, Shirreffs SM. Factors influencing the restoration of fluid and electrolyte balance after exercise in the heat. *British journal of sports medicine* 1997;31:175-182.
4. Sawka MN, Burke LM, Eichner ER, Maughan RJ, Montain SJ, Stachenfeld NS. American College of Sports Medicine position stand. Exercise and fluid replacement. *Medicine and science in sports and exercise* 2007;39:377-390.
5. Maughan RJ, Shirreffs SM. Development of individual hydration strategies for athletes. *International journal of sport nutrition and exercise metabolism* 2008;18:457-472.
6. Shirreffs SM, Maughan RJ. Whole body sweat collection in humans: an improved method with preliminary data on electrolyte content. *Journal of applied physiology* 1997;82:336-341.
7. Patterson MJ, Galloway SD, Nimmo MA. Variations in regional sweat composition in normal human males. *Experimental physiology* 2000;85:869-875.
8. Baker LB, Stofan JR, Hamilton AA, Horswill CA. Comparison of regional patch collection vs. whole body washdown for measuring sweat sodium and potassium loss during exercise. *Journal of applied physiology* 2009;107:887-895.
9. Wilmore JHC, D.L. *Environmental influences on performances*. Champaign: Human Kinetics; 2004.
10. Chenevere TD, Kenefick RW, Chevront SN, Lukaski HC, Sawka MN. Effect of heat acclimation on sweat minerals. *Medicine and science in sports and exercise* 2008;40:886-891.
11. Buono MJ, Claros R, Deboer T, Wong J. Na⁺ secretion rate increases proportionally more than the Na⁺ reabsorption rate with increases in sweat rate. *Journal of applied physiology* 2008;105:1044-1048.
12. Allsopp AJ, Sutherland R, Wood P, Wootton SA. The effect of sodium balance on sweat sodium secretion and plasma aldosterone concentration. *European journal of applied physiology and occupational physiology* 1998;78:516-521.
13. Wescor Incorporated. *Sweat Check Sweat Conductivity Analyzer Model 3120: Instruction/Service Manual*. United States of America: Wescor Incorporated; 2005.
14. Maughan RJ, Watson P, Evans GH, Broad N, Shirreffs SM. Water balance and salt losses in competitive football. *International journal of sport nutrition and exercise metabolism* 2007;17:583-594.

15. Palmer MS, Spriet LL. 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 = Physiologie appliquee, nutrition et metabolisme* 2008;33:263-271.
16. Kenney WL. Dietary water and sodium requirements for active adults. *Sports Science Exchange*. <http://www.gssiweb.org/Article/sse-92-dietary-water-and-sodium-requirements-for-active-adults>: Gatorade Sports Science Institute, 2004.
17. Allan JR, Wilson CG. Influence of acclimatization on sweat sodium concentration. *Journal of applied physiology* 1971;30:708-712.
18. Buono MJ, Ball KD, Kolkhorst FW. Sodium ion concentration vs. sweat rate relationship in humans. *Journal of applied physiology* 2007;103:990-994.
19. Horswill CA, Stofan JR, Lacambra M, Toriscelli TA, Eichner ER, Murray R. Sodium balance during U. S. football training in the heat: cramp-prone vs. reference players. *International journal of sports medicine* 2009;30:789-794.
20. Kilding AE, Tunstall H, Wraith E, Good M, Gammon C, Smith C. Sweat rate and sweat electrolyte composition in international female soccer players during game specific training. *International journal of sports medicine* 2009;30:443-447.
21. Lott MJ, Galloway SD. Fluid balance and sodium losses during indoor tennis match play. *International journal of sport nutrition and exercise metabolism* 2011;21:492-500.
22. Hamouti NDC, J.; Estevez, E.; Mora-Rodriguez, R. Dehydration and sodium deficit during indoor practice in elite European male team players. *European Journal of Sport Science* 2010;10:329-336.
23. Stofan JR, Zachwieja JJ, Horswill CA, Murray R, Anderson SA, Eichner ER. Sweat and sodium losses in NCAA football players: a precursor to heat cramps? *International journal of sport nutrition and exercise metabolism* 2005;15:641-652.
24. Froom P, Bieganiec B, Ehrenrich Z, Barak M. Stability of common analytes in urine refrigerated for 24 h before automated analysis by test strips. *Clinical chemistry* 2000;46:1384-1386.
25. Boisvert P, Candas V. Validity of the Wescor's sweat conductivity analyzer for the assessment of sweat electrolyte concentrations. *European journal of applied physiology and occupational physiology* 1994;69:176-178.
26. Preusse CJ, Fuchs C. [Comparison of ion-selective electrodes and flame photometry for the determination of serum Na⁺ and K⁺ for clinical purposes (author's transl)]. *Journal of clinical chemistry and clinical biochemistry* 1979;17:639-645.
27. Kulpmann WR, Lagemann J, Sander R, Maibaum P. A comparison of reference method values for sodium, potassium and chloride with method-dependent assigned values. *Journal of clinical chemistry and clinical biochemistry* 1985;23:865-874.

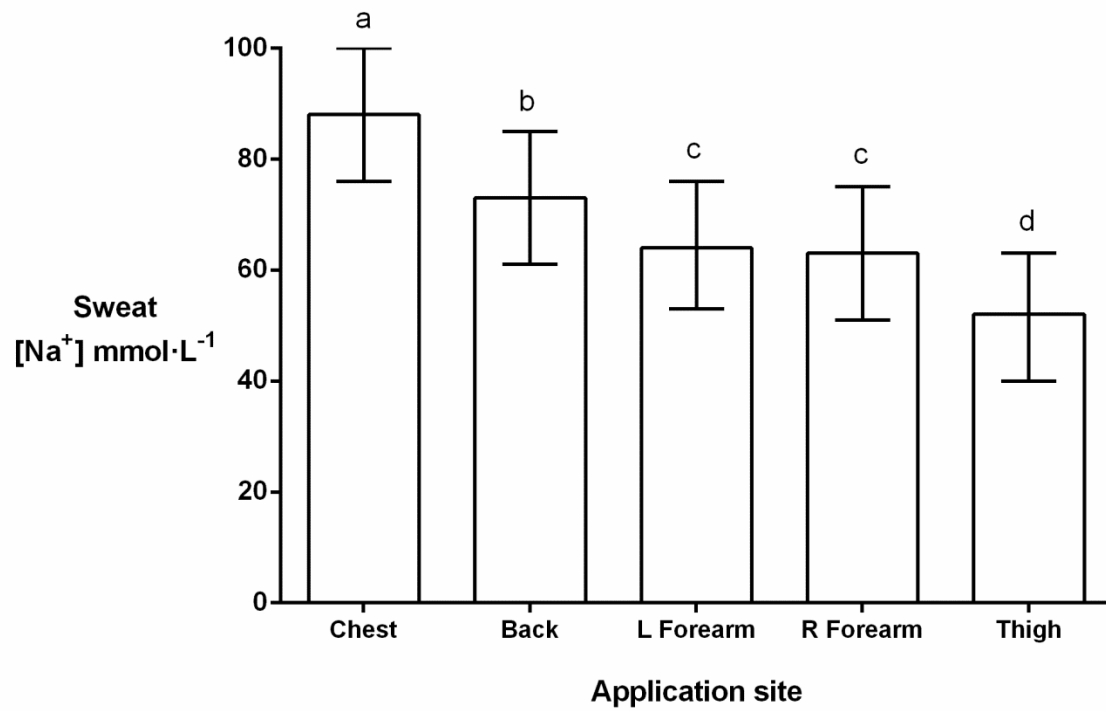


Figure 1. Estimated mean sodium concentrations of sweat sampled from five body sites.

L – left, R – right. $a > b > c > d$ ($p < 0.008$). Error bars represent 95% CI.

Table 1. Summary of sweat collection, handling and analysis protocols.

	Protocol A				Protocol B						
Site application	Forearm				Forearm				Chest	Scapula	Thigh
	Left		Right		Left		Right				
Duration of sample collection (min)	30	30	30	30	30	70	30	70	70	70	70
Storage	Immediate analysis	7 day storage			Immediate analysis						
		7 °C	21 °C	32 °C							
Analysis	ISE	ISE			ISE						
Re-analysis (frozen-thawed samples only, n = 30)	ISE	-			ISE	-	ISE	-			
	FP				FP		FP				
	SC				SC		SC				

Each protocol was undertaken in both temperate (21.2 ± 0.6 °C, $54 \pm 11\%$ r.h.) and hot (31.9 ± 0.7 °C, $35 \pm 7\%$ r.h.) environments. r.h. – relative humidity, ISE – ion selective electrode, FP – flame photometry, SC – sweat conductivity.

Table 2. Sweat rate, sweat loss and fluid intake for each time trial (self-paced, approximately 70 min) in both environmental conditions; mean \pm SD.

Variable	Hot environment (31.9 ± 0.7 °C, $35 \pm 7\%$ r.h.)		Temperate environment (21.2 ± 0.6 °C, $54 \pm 11\%$ r.h.)	
	Protocol A	Protocol B	Protocol A	Protocol B
	Sweat loss (L)*	1.53 ± 0.46	1.58 ± 0.42	0.91 ± 0.41
Sweat rate ($L \cdot h^{-1}$)*	1.30 ± 0.41	1.34 ± 0.37	0.79 ± 0.37	0.84 ± 0.31
Fluid intake (L)	0.50 ± 0.13	0.50 ± 0.12	0.50 ± 0.12	0.49 ± 0.11

*represents significant effect of different environmental testing conditions on sweat characteristics ($p < 0.0001$). Typical error (TE) for sweat loss: Hot – 0.22 L, Temperate – 0.21 L. r.h. – relative humidity.

Table 3. Estimated mean \pm 95% CL forearm sweat sodium concentration measurements analysed (ion-selective electrode method) immediately or after storage for seven days in 7 °C, 21 °C or 32 °C.

	Immediately analysed (n = 24)	Stored for 7 days		
		7 °C (n = 22)	21 °C (n = 23)	32 °C (n = 23)
Estimated mean sweat [Na ⁺] (mmol·L ⁻¹)	64 \pm 9	73 \pm 9*	65 \pm 9	65 \pm 9

*represents significant effect of storage of samples at 7 °C compared to those analysed immediately and stored at 21 °C and 32 °C (p < 0.004).

Table 4. The number of individual samples (n = 30) classified into the reportable ranges from clinical diagnostic criteria¹³ using different analytical techniques to measure forearm sweat sodium concentration.

Classification	ISE	S _{ISE}	S _{FP}	S _{SC}
0 - 59 mmol·L ⁻¹	18	19	11	12
60 - 79 mmol·L ⁻¹	8	9	10	11
\geq 80 mmol·L ⁻¹	4	2	9	7

ISE – sample analysed immediately by the ion-selective electrode method; S_{ISE} – frozen/thawed sample analysed by the ion-selective electrode method; S_{FP} – frozen/thawed sample analysed by flame photometry; S_{SC} – frozen/thawed sample analysed by conductivity.