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Effect of Stratum Corneum Hydration on the Composition of Sweat Collected by a Local Sweat Patch Method

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EFFECT OF STRATUM CORNEUM HYDRATION ON THE COMPOSITION OF SWEAT COLLECTED BY A LOCAL SWEAT PATCH METHOD

by

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GRADUATE COMMITTEE APPROVAL

of a thesis submitted by

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This thesis has been read by each member of the following graduate committee and by majority vote has been found to be satisfactory.

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BRIGHAM YOUNG UNIVERSITY

As chair of the candidate's graduate committee, I have read the thesis of Penny Renee Taylor in its final form and have found that (1) its format, citations, and bibliographical style are consistent and acceptable and fulfill university and department style requirements; (2) its illustrative materials including figures, tables, and charts are in place; and (3) the final manuscript is satisfactory to the graduate committee and is ready for submission to the university library.

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ABSTRACT

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The purpose of this study was to determine the effect of stratum corneum (SC) hydration by distilled water on SC ion content and sweat ion concentrations as measured by occlusive sweat patch. 10 men and 10 women completed approximately 40 minutes of moderate exercise in the heat. Select skin sites were hydrated before sweating by adhering cylinders of distilled water to forearm skin. SC samples were taken before and after exercise using the tape stripping (TS) method and sweat samples were taken with homemade filter paper sweat patches with a tegaderm backing. An increase in SC hydration was verified by a reduction in SC potassium concentration ($p<0.05$). SC hydration caused a significant decrease in sweat potassium (K^+), calcium (Ca^{++}), and lactate (Lac^-) concentration: K^+ =8.14 ± 0.46 to 6.56 ± 0.46, Ca^{++} = 0.86 ± 0.17 to 0.67 ± 0.18, Lac^- = 11.64 ± 1.36 to 8.82 ± 1.11, euhydrated to hyperhydrated respectively ($p<0.05$). SC sodium (Na^+) and K^+ concentration increased after sweating without a

sweat patch ($p<0.05$). Our data do not dispute the idea that electrolytes can be leached from the SC by distilled water or sweat trapped within an occlusive dressing. However, our data indicate that during normal sweating the SC "dehydrates" resulting in an increase in the electrolyte concentration. As such, we propose that the occlusive dressing does trap sweat on the skin but the important end result is that it prevents water movement out of the SC and thereby producing a more concentrated sweat.

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Effect of stratum corneum hydration on the composition of sweat collected by a local sweat patch method

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Abstract

The purpose of this study was to determine the effect of stratum corneum (SC) hydration by distilled water on SC ion content and sweat ion concentrations as measured by occlusive sweat patch. 10 men and 10 women completed approximately 40 minutes of moderate exercise in the heat. Select skin sites were hydrated before sweating by adhering cylinders of distilled water to forearm skin. SC samples were taken before and after exercise using the tape stripping (TS) method and sweat samples were taken with filter paper sweat patches made in our laboratory with a tegaderm backing. An increase in SC hydration was verified by a reduction in SC potassium concentration ($p < 0.05$). SC hydration caused a significant decrease in sweat potassium (K^+), calcium (Ca^{++}), and lactate (Lac^-) concentration: $K^+ = 8.14 \pm 0.46$ to 6.56 ± 0.46 mmol/l, $Ca^{++} = 0.86 \pm 0.17$ to 0.67 ± 0.18 mmol/l, $Lac^- = 11.64 \pm 1.36$ to 8.82 ± 1.11 mmol/l, euhydrated to hyperhydrated respectively ($p < 0.05$). SC sodium (Na^+) and K^+ concentration increased after sweating without a sweat patch ($p < 0.05$). Our data do not dispute the idea that electrolytes can be leached from the SC by distilled water or sweat trapped within an occlusive dressing. However, our data does indicate that during normal sweating the SC "dehydrates" resulting in an increase in the electrolyte concentration. Consequently, we propose that the occlusive dressing does trap sweat on the skin but the important end result is that it prevents water movement out of the SC and thereby producing a more concentrated sweat.

Introduction

Sweat patches are a simple and easily repeatable way to measure sweat output from various body sites. However, their extrapolation to whole body estimates of sweat output has often been in question, in particular because of the large variations in regional sweat output. Recently, Weschler[1] hypothesized that any aqueous solution remaining on the skin can leach electrolytes from the stratum corneum (SC), the skin's outermost layer. This hypothesis claims that sweat from skin patches has erroneously high concentrations of electrolytes due to this leaching and therefore cannot be considered true sweat. Much of the basis for Weschler's hypothesis rests on comparative data from whole body washdown (WBW) and sweat patch studies.[1] This research indicates that regional sweat output, as collected by a local sweat patch, bag or pouch does not correlate well to whole body sweat data.[2,3,4] The implications of this hypothesis, if true, are many and include the idea that sweat from patches always results in an overestimation of electrolyte loss in sweat. This would make data from skin patch studies unsuitable for electrolyte replacement recommendations or electrolyte mass balance studies examining hyponatremia.[1]

Nakagawa et al[5] showed that a 5 min application of water to the skin lowers SC conductance and presumably the composition of the SC, specifically the concentration of various natural moisturizing factors (NMF) like lactate. Egawa and Tagami[6] showed that a brief period of hydration (1.5 minutes of a wet cotton ball placed on the skin) did not alter SC hydration but that 15 min of the same treatment did. These data indicate that when a fluid (water) is placed on the skin more water moves into the skin than

electrolytes move out. Thus, the high concentration of electrolytes in samples of sweat from patches may be due to a concentrating effect of water moving into the skin rather than the fluid leaching electrolytes from the SC.[6] Alternately, it is known that the electrochemical properties of the SC are modified as a function of its water content.[7,8] If an aqueous solution remains in contact with skin, the changing chemical and electrical gradients across the SC may allow ions to be leached out.

Because sweat patches are commonly used in research, a direct test of the Weschler hypothesis is necessary to ensure accurate data collection in subsequent studies. In the current study we tested the hypothesis that changes in SC hydration level, via applying water to the skin, will alter SC and sweat composition. Based upon the Weschler hypothesis a reduction in electrolyte concentration in the SC with hydration should also reduce the electrolyte composition of the sweat collected by a sweat patch.

Methods

Subjects

Ten men and 10 women between the ages of 18 and 35 volunteered for this study. All subjects were in good health and participated in structured exercise at least 3 times per week for a half hour. They had a mean (± SD) age, height and body mass of 27.8 ± 4.9 years, 1.77 ± 0.09 m, and 73.99 ± 12.98 kg, respectively and a mean body surface area of 1.91 ± 0.2 m^2.[9] The study was approved by Brigham Young University's Institutional Review Board and all subjects were required to provide written informed consent before engaging in the study. Subjects were also asked to complete a medical health history questionnaire. Volunteers were excluded if they had any chronic illness

that would affect their sweat output, and if they were not at least moderately active. Moderate activity was defined as at least 30 minutes of exercise 3 times per week. Subjects were not required to follow a specific diet before exercise but were asked to ingest at least 5 ml/kg body weight of water the night previous to their session to ensure adequate hydration.

Procedure

Subjects reported once to the Human Performance Research Laboratory at Brigham Young University and were prepared by putting on heart rate monitors and recording pre-exercise body weight. Subjects then had their forearm skin cleaned with isopropyl alcohol and gently cleansed with deionized water, using ion free towels. After cleansing, SC hydration was performed by placing glass cylinders containing distilled water on selected skin sites for 15 minutes. After SC hydration pre-exercise tape stripping on selected sites was completed and the subject was moved to the environmental chamber. The chamber was preheated to 38 ± 1.2°C with a relative humidity between 15-16%. Subjects exercised on a cycle ergometer at a workload that produced a heart rate between 140 and 160 beats per minute. After the onset of sweat, which typically occurred between minute 6 and 11, the subjects exercised an additional 30 minutes and then exited the chamber for removal of sweat patches and postexercise skin stripping.

Design

Eight skin sites were chosen on the dorsal aspect of the forearm, 4 sites per arm, for six separate treatments. Treatment locations were randomized for each subject and were defined as:

Site 1: normal SC hydration, sweat patch, tape strip post sweating

Site 2: hydrated SC, no sweat patch, tape strip post sweating

Site 3: hydrated SC, sweat patch, tape strip post sweating

Site 4: normal SC hydration, no sweat patch, tape strip pre sweating

Site 5: normal SC hydration, no sweat patch, tape strip post sweating

Site 6: hydrated SC, no sweat patch, tape strip pre sweating

Sites 1 and 3 were performed in duplicate to allow collection of sufficient sweat for analysis.

SC hydration

Open-ended cylinders with a diameter of approximately 2.2 cm were filled with 8-9 ml of distilled water. These cylinders were attached to predetermined sites on nonglabrous forearm skin for 15 minutes.

Tape Stripping

DSquame Sampling Discs (CuDerme Corporation, Dallas, Texas) were applied to the skin and held in place with a constant pressure applicator (236 g/in^2) for 10 seconds. The tape strip was then removed with tweezers over the course of 5 seconds. Five tape strips per site were taken and placed in tightly screw capped bottles for later analysis. A 6^{th} strip was taken and used for SC protein analysis.

Extraction of Stratum Corneum Electrolytes

The 5 strips for each site were cut into 3 pieces and placed into a 15 ml falcon tube. Eight milliliters of deionized water was then added and each tube was vortexed for 30 seconds. Each tube was then sonicated at 4 Hz for 2 minutes and all samples were allowed to incubate for 24 hours. The fluid in each tube was then transferred equally to 5 ml test tubes and placed in a drying centrifuge (Savant Vacuum Centrifuge, Savant Instruments Inc, Holbrook, NY) at 70°C under vacuum pressure for 5-6 hours or until all of the water had evaporated. Each test tube was then reconstituted with 250 ml of a standard dilutant. The dilutant was composed of 203 mM Na+, 5 mM K+, 1.9 mM Ca++, and 1.9 mM Mg++. The dilutant was formulated to provide each sample with sufficient NaCL to allow measurement in several instruments that required minimal Na levels in the sample for proper operation (NOVA 8, Chloride Titrator, and YSI Lactate Analyzer).

Sweat Patches

Individual filter paper patches were constructed from thick absorbent filter paper (Biorad Laboratories, Hercules, Ca) using a 22 mm diameter punch. Each patch was then soaked for 24-48 hr in a large quantity of distilled water (to leak out any residual electrolytes), and dried at 37°C for 24 hr. Each sweat patch was applied directly to the treatment sites and was covered with a Tegaderm backing (3M Medical Technologies, Minneapolis, MN).

Sample analysis

Magnesium (Mg^{++}), Na^{+}, K^{+}, and Ca^{++} concentrations in each sample were measured using ion selective electrodes (NOVA 8, Nova Biomedical, Waltham, MA) while chloride (Cl^{-}) was determined by coulometric titration (Digital Chloridometer, Labconco, Kansas City, MO). Sweat and SC Lac^{-} concentration was determined by an YSI 2300 lactate analyzer (YSI, Yellow Springs, OH).

Protein Analysis

A single DSquame Sampling Disc was placed in a 1.5 ml eppendorf tube with 1.0 ml of 1 N NaOH and incubated with mixing for 2 hr at 37°C. After incubation 500 µl of the incubation solution was transferred to a 1,5 ml eppendorf tube and was neutralized by the addition of 500 µl of 1 N HCL. This neutralized solution was used to determined total protein using a Biorad BCG reaction. Standard curves were constructed using blank DSquame sampling discs combined with known amounts of protein added to the 1 N NaOH incubation tube.

Statistical Analysis

Changes in SC composition and sweat composition of saturated and nonsaturated sweat patches were analyzed using repeated measures analysis of variance, with site and/or time as repeated measures. Specific areas of significance were determined using a student's paired *t* test with a Bonferroni correction. Because of insufficient sweat collection in two of the subjects sweat composition analysis was limited to 18 subjects. Due to technical errors some stratum corneum samples were lost resulting in a reduction in sample size for Na^{+} to n = 17 and Mg^{++} and Ca^{++} to n = 19. Significance was set to the

$p < 0.05$ level. Statistics were performed using SAS Statistical Software (SAS Institute Inc., Cary, NC)

Results

Sweat rate and composition.

Whole body sweat rate during the exercise bout averaged 7.57 ± 1.81 $g \bullet min^{-1} \bullet m^{-2}$. Local sweat rate at the hyperhydrated skin site on the forearm averaged 2.03 $mg \bullet min^{-1} \bullet cm^{-2}$ which was similar to local sweat rate measured at the euhydrated skin site, 2.24 $mg \bullet min^{-1} \bullet cm^{-2}$. Mean electrolyte concentrations of sweat collected at the euhydrated skin site (Site 1) and the hyperhydrated skin site (Site 3) are compared in Figure 1. An increase in skin hydration caused a decrease in sweat K^+, Ca^{++}, and Lac^- concentration ($p < 0.05$, Table 1). K^+ decreased 19.4% (8.14 ± 0.46 to 6.56 ± 0.41 mmol/L), Ca^{++} decreased 22.1% (0.86 ± 0.17 to 0.67 ± 0.18 mmol/L) and Lac^- decreased 24.2% (11.64 ± 1.36 to 8.82 ± 1.11 mmol/L).

Stratum corneum composition.

The SC composition was measured at skin sites before and after exercised-induced sweating with and without a sweat patch present on the skin. In the absence of a sweat patch the SC composition of euhydrated (Site 4) and hyperhydrated (Site 6) skin sites were similar prior to sweating (Table 1) and after sweating (euhydrated -Site 5 versus hyperhydrated -Site 2)(Table 2). Tables 1 and 2 demonstrate that hyperhydrating the skin with water for 15 min did not significantly alter the composition of the SC before sweating or following 30 min of exercise-induced sweating.

Sweating itself did alter SC composition. Figure 2 illustrates the impact of sweating for 30 min on SC composition at both euhydrated and hyperhydrated skin sites. In euhydrated skin sweating caused the SC Na^+ and K^+ concentrations to increase regardless of hydration status (Site 4 versus Site 5 or Site 6 versus Site 2). The increase in SC Na^+ was 2.70 ± 0.44 mmol/L representing an increase of 75% over the presweat SC composition.

With the application of a sweat patch the SC Na^+ concentrations at the euhydrated skin site (Table 5, Site 1, 3.63 ± 0.79 mmol/L) and hyperhydrated site (Table 3, Site 3, 3.73 ± 0.71 mmol/L) were lower than at the skin site without a sweat patch (Table 3, Site 5, 6.29 ± 0.82 mmol/L, $p<0.05$). SC K^+ concentrations at both sweat patch sites (Site 1 and Site 3) were also lower than at the skin site without a sweat patch (Site 5). In general, Table 5 illustrates that in the presence of a sweat patch that SC composition is relatively unaltered by a 30 min bout of exercise-induced sweating.

Lactate composition of the SC varied significantly ($F = 2.37$, $p < 0.045$) across the skin sites. However, post-hoc analysis using the Tukey's minimum significant difference test did not reveal any specific pairs of data that were different. The largest differences in SC Lac^- were observed between skin sites with (Sites 1 or 3) and without a sweat patch (Sites 2 or 5) after 30 min of sweating.

SC protein analysis, taken from the 6^{th} tape strip at each site found no significant differences between any site or treatment. Table 4 shows the mean for each testing site.

Discussion

The most significant new finding of this study is that pre-treating the skin with distilled water alters the composition of sweat collected by a sweat patch over this same area. Specifically, hydrating the skin for 15 min resulted in a reduction in the concentration of K^+, Mg^{++}, Ca^{++}, and Lac^- in the collected sweat sample (Figure 1). The ability of the distilled water to hydrate the SC was demonstrated by a modest reduction in SC K^+ concentration estimated from tape stripping prior to sweating. Our data indicate that changing the concentration profile of the SC will impact the composition of sweat collected using an occlusive sweat patch.

The concentration profile of the SC is affected by many factors including age, time of year, and artificially forced hydration.[5,6] In the present study we noted that sweating also resulted in a change in the SC composition. Specifically, we noted an increase in SC Na^+ and K^+ concentration (Figure 2) at euhydrated skin sites in the absence of an occlusive sweat patch. Following forced hydration we also noted an increase in Na^+, K^+, Ca^{++} ($p < 0.05$), and Lac^- ($p = 0.08$) concentrations following 30 min of exercise-induced sweating in the absence of an occlusive sweat patch. Thus, during normal exercise-induced sweating, if the sweat is allowed to evaporate from the skin the SC electrolyte concentration rises. As such, it appears as if water is leaving the SC during normal, non occluded sweating.

When a sweat patch with an occlusive covering is placed on the skin the concentrating effect of sweating on the SC electrolyte composition is markedly attenuated. At euhydrated skin sites we noted only a small increase in SC calcium

($p < 0.05$) and possibly K^+ ($p = 0.07$) (Table 3) while at the hydrated skin sites we noted a small increase in SC K^+ (p=0.048). We suspect that the occlusive covering placed over the skin prevented water from moving out of the SC thus limiting the concentrating effects of normal sweating.

The impact of our SC hydrating protocol on the SC composition was limited, with only a small decrease in potassium concentration. However, K^+ is considered a major component of natural mosturizing factor[10] (NMF) and as such represents a significant change in SC composition. Skin NMF, chiefly amino acids and metabolites of amino acids, act to retain water in the outermost layers of the SC.[10] Lactate and potassium levels in the SC correlated well with SC hydration.[5] Nakagawa et al[5] showed that components of NMF, specifically K^+ and Lac^-, could be extracted from the skin using distilled water blanks with a 10 min application. Egawa and Tagami[6] demonstrated a similar effect using cotton balls soaked in distilled water and placed on the skin for 15 min. However, Egawa and Tagami showed a significant hydration of the SC with the application of distilled water. So while distilled water can leach NMF from the SC it also increases the water content. Our data are in agreement with these studies in that SC K^+ was reduced following application of the distilled water blanks. In addition, SC Lac^- was lowest on the site where skin was treated with the distilled water.

Our data for sweat electrolyte concentration using occlusive sweat patches are similar to some but not other studies.[11-13] These differences are clearly the result of differences in environmental temperature and eventually local sweat rate. When we compare sweat sodium concentration from sites with similar sweat rates our data are

consistent with the literature.[11] Whole body data tends to put Na^+ concentration at or around 50 mmol/L.[13] Our data showed similar concentrations in the hyperhydrated skin site at a concentration of approximately 54 mmol/L. The standard sweat patch did however show a higher concentration at approximately 62 mmol/L. Nevertheless, the differences in Na^+ concentrations between the two treatments in this study were not significant.

It has been known for some time that the various methods used to determine regional sweat composition overestimates the values obtained from the WBW technique.[1,3,4] Because many of the methods used to collect regional sweat involve an occlusive covering, it has been hypothesized that some interaction is taking place while sweat remains in contact with the skin.[1] By hydrating the skin with distilled water we increased the water content[6] and reduced the concentration of NMF. The result was sweat collected over the hydrated skin showed a lower concentration of electrolytes. It is possible that any differences in concentration may be due to movement of water and/or electrolytes between sweat and the SC. Weschler[1] postulates that electrolytes are leached from the SC into the sweat that is left on the skin and trapped by the occlusive dressing. Our data do not dispute the idea that electrolytes can be leached from the SC. However, our data indicate that during normal sweating the SC "dehydrates" resulting in an increase in the electrolyte concentration. As such, we propose that the occlusive dressing does trap sweat on the skin as Weschler[1] posulates but the important end result is that it prevents water movement out of the SC and thereby producing a more concentrated sweat.

It is possible and logical to concede that human skin loses water over time as our data indicate. What parameters are required for as significant a water loss as our data shows is uncertain. It does however, bring to light the need for a certain distinction about the definition of "true sweat." For purposes of this discussion, "true sweat" will be defined as only the water and electrolytes that physically exit the sweat gland. Therefore, WBW is not measuring only true sweat because the calculations made for concentration cannot take into account insensible water loss from the skin, which our data indicates is in addition to water lost through sweating. However, it does not follow that this data is false, or erroneous in any way. Whole body washdown is certainly appropriate for recommendations to athletes exercising in hot dry environments.

That being said, if our postulations are correct, then measuring sweat by means of WBW may not be accurate or applicable to all exercising, work or life related situations. For instance, if exercising in a humid environment leaves sweat clinging to skin instead of evaporating, the insensible water loss from the skin would likely not occur. The same would be true if clothing or accessories kept the skin moist in any way. These scenarios would prevent the dilution of sweat that occurs because of insensible water loss from the skin and would increase the concentration of electrolytes in sweat.

Revisiting again the definition of "true sweat;" it would seem that those primarily concerned with only what is exiting the sweat gland would do better to develop a method that only collects "true sweat," that is easily repeatable. This would aid in the development of a greater understanding of sweat gland function, sweat genesis and concentration gradients within sweat genesis.

This study, of course, does not provide irrefutable evidence against the "faux sweat" theory proposed by Weschler.[1] It does however, cast significant doubt about whether that term can be applied to sweat collected by sweat patch. It seems more likely that this term may be applied based on the avenue of research being done and the application thereof, and is therefore unfit for any generalized use within the scientific community. More research is needed to determine what conditions make insensible skin water loss significant and, conversely, what conditions keep it from occurring. It is possible that, due to athletic clothing, during exercise different body parts are experiencing a different type of sweating. How we take such scenarios into account in research remains to be seen.

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Table 1. Effects of skin hydration on SC composition prior to sweating.

Site	Na^+ (mmol/L)	K^+ (mmol/L)	Mg^{++} (mmol/L)	Ca^{++} (mmol/L)	Cl^- (meq/L)	Lac^- (mmol/L)
4	3.59 ± 0.58	0.28 ± 0.04	0.07 ± 0.02	0.10 ± 0.02	7.43 ± 1.34	0.13 ± 0.08
6	3.42 ± 0.44	0.13 ± 0.02*	0.10 ± 0.04	0.16 ± 0.09	6.45 ± 0.94	0.05 ± 0.03

Values are presented as means ± 1 SD. Site 4 : euhydrated SC, pre exercise. Site 6, hyperhydrated SC, pre exercise. * $p < 0.05$ different from Site 4.

Table 2. Effects of skin hydration on SC composition after sweating in the absence of a sweat patch.

Site	Na^+ (mmol/L)	K^+ (mmol/L)	Mg^{++} (mmol/L)	Ca^{++} (mmol/L)	Cl^- (meq/L)	Lac^- (mmol/L)
5	6.29 ± 0.82	0.48 ± 0.05	0.08 ± 0.04	0.10 ± 0.02	7.31 ± 0.96	0.21 ± 0.09
2	4.99 ± 0.82*	0.53 ± 0.10	0.04 ± 0.01	0.06 ± 0.01*	7.90 ± 1.42	0.32± 0.15

Values are presented as means ± 1 SD. Site 5 : euhydrated SC, post sweating. Site 2, hyperhydrated SC, post sweating. * $p < 0.05$ different from Site 5.

Table 3. Effects of sweating on SC composition at euhydrated and hyperhydrated skin sites in the presence of a sweat patch.

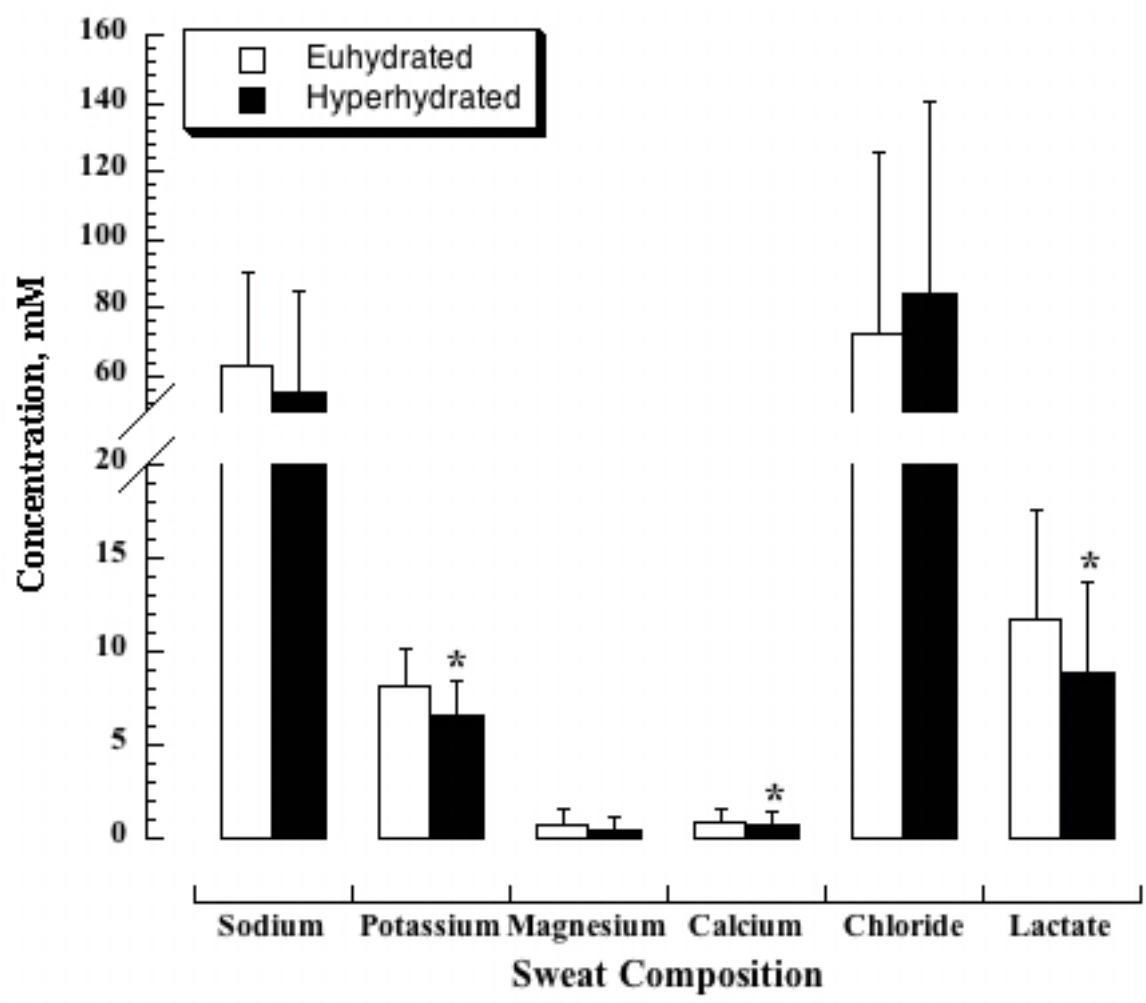
Site	Sweating	Na+ (mmol/L)	K+ (mmol/L)	Mg++ (mmol/L)	Ca++ (mmol/L)	Cl- (meq/L)	Lac- (mmol/L)
4	Pre	3.59 ± 0.58	0.28 ± 0.04	0.07 ± 0.02	0.10 ± 0.02	7.43 ± 1.34	0.13 ± 0.08
1	Post	3.63 ± 0.79	0.20 ± 0.02	0.08 ± 0.02	0.07 ± 0.02	7.52 ± 1.15	0.05 ± 0.03
6	Pre	3.42 ± 0.44	0.13 ± 0.02	0.10 ± 0.04	0.16 ± 0.09	6.45 ± 0.94	0.05 ± 0.03
3	Post	3.73 ± 0.71	0.18 ± 0.02	0.07 ± 0.02	0.06 ± 0.01	6.93 ± 1.34	0.05 ± 0.03

Values are presented as means ± 1 SD. Site 4 : euhydrated SC, pre sweating. Site 1: euhydrated SC, post sweating with patch. Site 6, hyperhydrated SC, pre sweating. Site 3, hyperhydrated SC, post sweating with patch. SC stratum corneum. *$p < 0.05$ different from Pre.

Table 4. Mean (± SD) protein obtained from one tape strip at each site

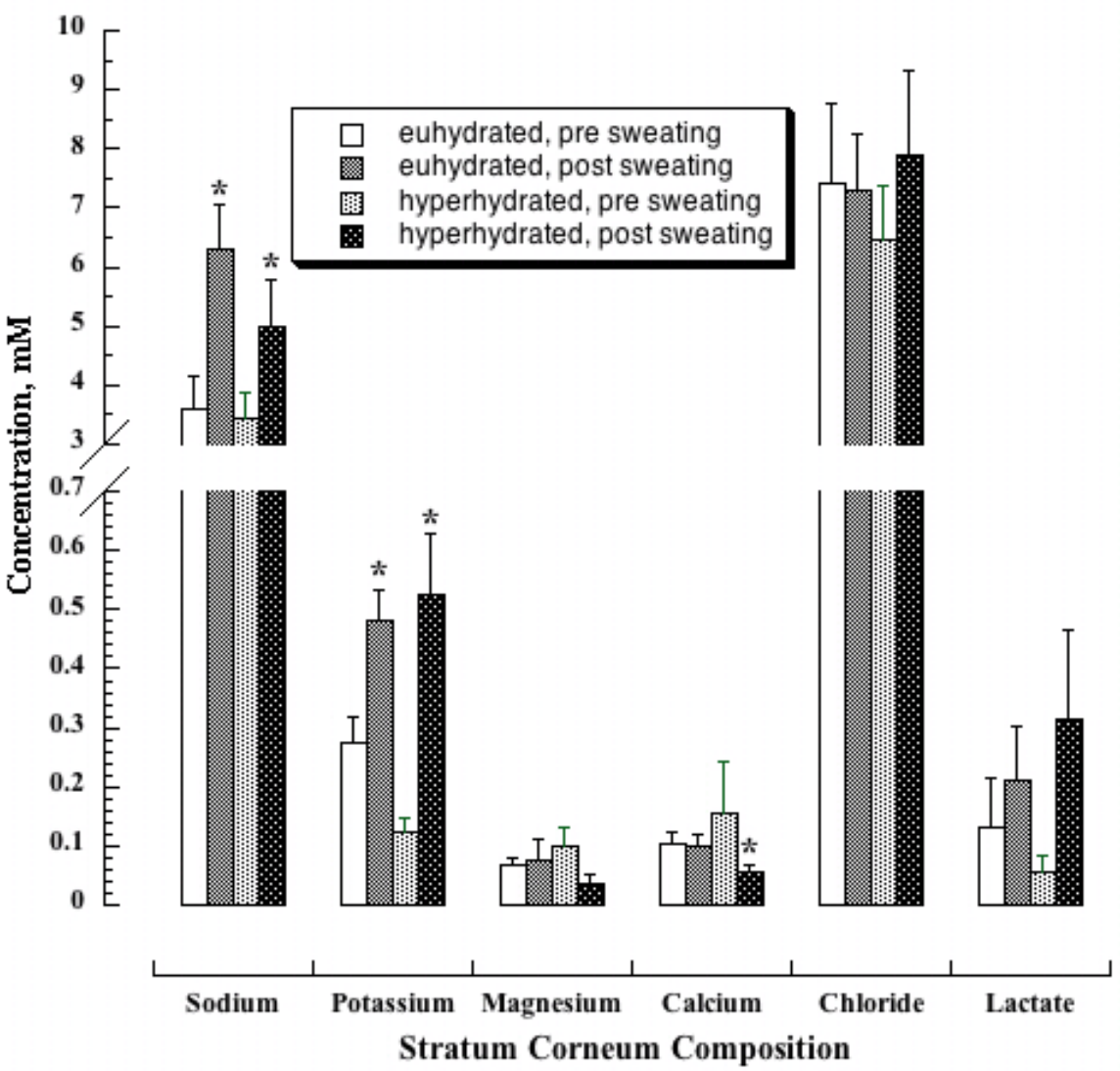
Site	1	2	3	4	5	6
Protein Amount (μg)	21.58 ± 1.82	22.36 ± 2.01	24.21 ± 2.28	18.45 ± 1.11	20.17 ± 2.45	19.77 ± 2.78

Figure 1. Sweat composition at euhydrated and hyperhydrated skin sites.



Values are presented as means ± 1 SD. * $p < 0.05$ different from euhydrated site.

Figure 2. Effects of sweating on SC composition at euhydrated and hyperhydrated skin sites in the absence of a sweat patch.



Values are presented as means ± 1 SD. *$p < 0.05$ different from pre sweating.

Appendix A

Prospectus

Chapter 1

Introduction

Thermoregulation is one of the human body's most important capabilities, essential for maintaining homeostasis. The most obvious mechanism of thermoregulation for the body is sweating; the release of fluid onto the skin, which then evaporates, taking heat away from the body.[1] In fact, sweating is the main avenue of heat removal during exercise, regardless of the temperature. The stimulation of sweat production comes not only from heat but also some hormones.[2]

Sweat glands in the skin release a hypotonic solution that contains several ions such as chloride, sodium and potassium as well as waste products such as urea.[1] Under normal conditions, humans produce approximately 500 ml of sweat a day.[3] However, this amount can be increased by ambient temperature and a higher activity level. Sweating rates are affected by the training level of the individual as well as acclimatization to heat, both of which increase sweat rate.[1,4] Additionally, sweat rate varies on different parts and regions of the body.[5,6] The sweating rate is important for retention of sodium, as sweat sodium concentration decreases as sweat rate increases. Sweat rate can also affect the concentration of other sweat solutes.[3]

Over the years several different methods for measuring sweat output and concentration have been used in research.[4-8] Each method has its own methodological problems and limitations, which are often addressed by refining procedures. Whole body washdown (WBW) is considered the "gold standard" for sweat collection because regional sweat rate differences are not a concern.[9] There are several variations on the

whole body washdown technique, most of which involve determining sweat loss by measuring the change in body mass. As described by Shirriffs and Maughan,[7] sweat is allowed to evaporate and, after exercise, body and clothes are washed down with distilled water which is collected and analyzed for its electrolyte content.

Another commonly used sweat collection method is the sweat patch, of which there are many variations.[2,5-6,8,10-12] Brisson et al[10] described a procedure for one type of sweat patch involving attaching parafilm to the skin with a wound dressing. After the experimental procedure the patches are aspirated using a sterile syringe which removes the collected sweat.[10] Filter paper patches are also used in research,[2,11] as well as sterile gauze patches.[6] Both methods involve an impermeable backing allowing the patch to absorb sweat which is then analyzed for content. Many other sweat collection procedures have been described such as arm bags[5] and the capsule method.[8,12]

Sweat patches are a simple and easily repeatable way to measure sweat output from various bodily sites. However, their extrapolation to whole body estimates of sweat output has always been in question, in particular because of the variations in regional sweat output. Recently another attack has been initiated on the validity of using sweat patches for even regional sweat output. In an article in press, Weschler[9] has hypothesized that any aqueous solution remaining on the skin can leach electrolytes from the stratum corneum (SC), the skin's outermost layer. This hypothesis claims that sweat from skin patches has erroneously high concentrations of electrolytes due to this leaching and therefore cannot be considered true sweat. The implications of this hypothesis, if true, are many and include the idea that sweat from patches always results in an overestimation

of electrolyte loss in sweat. This would make data from skin patch studies unsuitable for electrolyte replacement recommendations as well as mass balance studies and studies concerning hyponatremia. Additionally, there is the idea that sweat patches would be more useful if they utilize a wicking material that would keep moisture away from the skin and hence prevent any leaching.[9]

Because sweat patches are widely used in research, a validation of this hypothesis is necessary to ensure accurate data collection from subsequent studies. If confirmed, this hypothesis would necessitate the reinvention of the sweat patch for accuracy. To this end, the current study will evaluate the effect of SC hydration on sweat composition and the effect of sweating on the composition of the SC.

Problem Statement

The purpose of this study will be to determine if ion leaching from the SC onto forearm skin during sweating is occurring with the use of a sweat collection patch.

Research Questions

1. Does the presence of a sweat collection patch cause the leaching of ions from the stratum corneum of the skin onto the surface?

2. If a sweat patch is not saturated with sweat, will electrolyte leaching from the stratum corneum occur?

3. Will changing the hydration of the stratum corneum change sweat composition?

Assumptions

1. Subjects will be free from preexisting medical conditions that affect sweat output.

2. Subjects will prehydrate the night before exercise by drinking 10 milliliter (ml) of water per kilogram (kg) of body weight.

3. Subjects will not otherwise change their daily routines.

4. Stratum corneum composition is uniform on both sides of the body.

Delimitations

Objectives for this study

1. Determine if there are changes in sodium, potassium, chloride and lactate in the stratum corneum (SC) after sweating, with and without an occlusive covering.

2. Determine if there is a difference between saturated sweat patch ion content and non-saturated patch ion content.

3. Determine if changing the hydration status of the SC prior to sweating will alter sweat composition.

Participants will be men and women aged 18 to 35 years that live in Utah County. Each participant will have the same number of sweat patches applied and the room conditions will be identical for each participant. Data collection will take place during winter semester 2009.

Medical history will be taken by having all subjects complete a medical history questionnaire previous to being accepted into the study. The medical history questionnaire has been used at BYU by other graduate students.

Limitations

1. Subjects may not comply with hydration instructions.

2. Subjects may unknowingly suffer from an illness that affects their sweating rate or composition.

Operational Definitions

Faux sweat - Term proposed by Weschler[9] to describe sweat collected in occlusive coverings, meant to denote that the sweat is not, compositionally, what originally left the sweat gland.

Ion leaching - In sweating, the movement of ions out of the SC into fluid that is left on the skin.

Stratum corneum (SC) - The outermost layer of skin in humans.

Sweat patch - An absorbent device used for sweat collection. It is usually composed of an absorbent material such as filter paper or gauze covered with a nonabsorbent, occlusive covering that can be adhered to skin.

Thermoregulation - The regulation of body temperature.

Whole body washdown (WBW) - Technique for measuring sweat rate and composition; allowing sweat to dry on skin and then washing the body with a known amount of distilled water, and measuring weight change over time.

Chapter 2

Review of Literature

In an article still in press Louise Weschler[9] reviews previous work on the composition of sweat and proposes a hypothesis about how sweat measurement affects sweat content. The hypothesis suggests that whenever fluid of any kind remains on the skin during sweat collection with an occlusive covering electrolytes from the stratum corneum are leached into the sweat, causing an anomalously high concentration. According to Weschler[9], this leaching creates "faux sweat" or sweat that is not what originally came out of the sweat gland. She cites three main lines of evidence based on published studies to support her hypothesis and also cites articles criticizing other forms of nonevaporating sweat collection.[9] This review will attempt to examine how previous studies may or may not support the faux sweat hypothesis to show how testing this hypothesis can bring factual accuracy to future research on the composition of sweat.

One of the most important parts of sweat research is sweat collection. In the 1930s, DB Dill (cited inWeschler[9]) developed a method of analyzing sweat electrolyte composition called whole body washdown (WBW). Although the exact parameters have varied and changed throughout the years, the basic concept remains the same. Total body sweat loss is calculated by weighing the subject before and after exercise, taking into account expiratory and urinal water loss when applicable.[7] Rather than analyzing wet sweat, the body is allowed to dry, allowing water to evaporate off of the skin leaving electrolytes dried onto the skin surface. Then the subject is washed-down with a known amount of distilled water and the resulting fluid is analyzed for content and

concentration.[7] The data resulting from studies using WBW is considered the most accurate, and all other collection techniques are compared to it. However, WBW is impractical for measuring regional sweat output and is also time consuming. Therefore, there have been many attempts to develop ways to more simply collect sweat for analysis. Many of these methods involve regional sweat collection and a device with an impermeable barrier that prevents evaporation. It is these devices that Weschler[9] claims alters sweat composition.

This aspect of sweat research is measuring the concentration of various ions that are excreted in sweat. Costill[1] points out that ionic concentration in sweat varies between individuals. Using data from WBW we learn that sodium and chloride in sweat increase in concentration when sweat rate increases at given states of heat acclimatization. However, concentrations of other ions do not follow the same pattern; potassium and magnesium concentrations do not change with sweat rate and calcium concentration decreases.[1] Similar findings for chloride were reported by Dill et al.[4] In their study, the relationship of sweat chloride concentrations to sweat rate and skin temperature in different age groups is analyzed. Researchers conclude that with increasing sweat rate you have an increase in chloride concentration without relation to skin or rectal temperature.[4] Also observed was that individuals with the same sweating rate had different chloride concentrations, suggesting that hydration state and/or genetics play a role. Some of the subjects are father and son, and chloride concentrations are similar in these family groupings.[4]

Several times in the literature we see studies that attempt to control some of the intraindividual differences in sweat electrolyte concentration by having control over the subject's diet and fluid intake. An example of this approach is seen in the Costa et al[6] study in which the researchers examine regional and total body sweat composition using two groups of males. One group received a "space" diet provided by National Aeronautics and Space Administration (NASA) and the other group eating a purified formula used often in the Costa et al lab. These diets were consumed for six weeks and testing was purposed to determine the differences in responses to these nutritionally adequate diets. Sweat was collected during cycle ergometer exercise on four different occasions. Gauze sweat patches placed on the mid back, mid front chest and mid upper arm as well as an arm bag were used to collect sweat samples.[6] They found that sweat rate was indeed individual and was not related to measured energy expenditure, which is different than the results of Costill[1] and Dill.[4] Comparing the sweat patches they discovered that the lowest sweat rate was consistently found on the upper arm and that these regional rates did not correlate well with total body sweat loss.[6] This is the first argument toward accepting the faux sweat hypothesis: regional sweat output, as collected by sweat patch, bag or pouch does not correlate well to whole body sweat data.[1,5,13]

This argument is also seen in a study by Dill et al[14] measuring sweat electrolytes in desert walks. The sweating rate and concentrations of chloride, sodium, and potassium were observed during 80 or 100 minute walks in the desert. Whole body sweat concentrations are measured by WBW and sweat collected in a glove is measured to compare it to whole body sweat composition. When comparing glove to WBW data we

find that hand sweat concentration was almost always higher than whole body measurements for chloride, sodium, and potassium. It is suggested that sweat concentrations taken from a gloved hand may be inaccurate.[14]

There are numerous reports of high concentrations of sodium and potassium in studies using regional sweat collectors. For instance, Kleeman et al[5] publishes a study evaluating the effect that an impermeable barrier has on the electrolyte concentration in arm bag sweat. The report points out that when collecting local site sweat one assumes that the local site is representative of whole body sweat and that the technique does not alter electrolyte concentrations. These assumptions, however, are not true. This study indicates that different body sites yield different electrolyte concentrations under identical conditions. Kleeman and his associates[5] conclude that using arm bag sweat can be used to predict total body losses of electrolytes, but that the accuracy of prediction is not very high. Van Heyningen and Weiner[15] make the same comparison in their study and find that electrolyte concentrations are higher in arm bag collection than in whole body analysis.

Additionally, Collins[16] uses the capsule method in his study on palmer and forearm sweat. He endeavors to determine local sweat rate and composition from the two sites using indirect heating as well as methacholine injection. In this study, similar values for chloride and sodium are present at both the forearm and palmer sites. The two sites differ, however, in potassium and lactate content with the palmer site having higher concentrations of both. The concentration of potassium is the most interesting to note; palmer sweat values at their highest show a mean of 12 meq/L which is greater by a

factor of 3 than plasma levels. Contrary to Weschler's theory,[9] Collins[16] theorizes that at high rates of sweating under an occlusive covering the skin may reabsorb some of the water, leading to high levels of electrolytes. Still, it is possible to conclude, from these studies, that occlusive coverings increase the secretion of certain electrolytes in the sweat or that, like Weschler[9] hypothesizes, electrolytes are being leached from the skin.

The ability to collect sweat from various regions of the body and extrapolate the concentration to whole body sweat composition is the subject of many studies. Concerned with finding a simple technique for regional sweat analysis, Verde et al[17] used a homemade gauze sweat patch to collect sweat samples from forearm skin. These patches also had an impermeable backing. Additionally, researchers were concerned about ensuring that the sweat patch did not become saturated, which one could assume meant that they left little to no fluid on the skin surface during collection. According to their data there was no inhibition of sweat rate with the sweat patch and they resulted in sodium and chloride concentrations that are similar to those obtained by WBW[17]. In her article Weschler[9] claims that Verde[17] found sodium concentrations that were higher than plasma concentrations. However this researcher found that Verde et al[17] reported the opposite, sodium concentrations for the three conditions tested were found to be slightly lower than plasma sodium concentrations. Potassium concentrations were however higher than plasma potassium concentrations, an average of 8.8 meq/L, whereas plasma levels are about 4 meq/L and WBW values tend to be around plasma levels.[1,13-14, 16, 18-22]

Patterson et al's[13] efforts to quantify sweat composition's regional distribution in an attempt to determine if regional samples can be used to predict whole body

concentrations. Researchers collected samples from 11 sites using the parafilm patch method as well as WBW. From regional site data whole body mean estimation was found using a weighted equation using both 4 and 8 sites. They found large variations in sweat rate and concentrations of various ions between regional sites. Also, derived whole body data from regional sites overestimated whole body concentrations with the estimations sometimes being almost double that which was found using WBW. The data also showed strong correlations between sodium and chloride regional concentrations and whole body values, with some regions showing a stronger correlation than others. The opposite is true however, for the lactate and potassium concentrations, where there was no relationship.[13]

The next argument for faux sweat involves observations of urocanic acid and urea in sweat.[9] Urocanic acid is a breakdown product of histidine and is found in the SC as part of a group of water soluble compounds known collectively as Natural Moisturizing Factor (NMF).[23,24] However, research done by Brusilow and Ikai[24] using an anaerobic sweat collection technique show that urocanic acid is not present in sweat exiting the sweat gland at as high a concentration as is found when collecting sweat with filter paper patches under the same time and conditions.[24]

Likewise, urea concentrations in sweat have been shown to be higher than those in plasma.[12,25] Schwartz and Thaysen[12] postulated that a passive reabsorption of water must occur in the sweat duct to produce the higher concentration. But, by using ^{14}C labeling Brusilow[25] was able to show that hypothesis as invalid. Brusilow's[25] hypothesis was that excess urea found in sweat must be from a non-plasma source and that either the

sweat gland must create the urea or that there is a pool of urea somewhere in the skin that sweat comes in contact with.[25] Gordon et al[26] examined these two possibilities and concluded that the urea pool theory was the correct one and that this pool is not in equilibrium with the plasma or any other body water. These researchers discovered that urea builds up in the epidermis over time and that the source is blood plasma. They also postulated that the high amount of urea in sweat was due to sweat passing over a urea pool and dissolving more urea into the sweat.[26]

Other incidental observations of ion diffusion out of the skin are reported by Nakagawa et al.[27] Using distilled water in open ended cylinders they showed that components of NMF can be extracted in as little as 5 minutes, enough to lower the hydration state of the SC as measured by conductance.[27] Egawa and Tagami,[28] however, showed no difference in hydration state of the SC after 1.5 minutes of a wet cotton ball being placed on the arm, but significant hydration increases after 15 minutes. The differences in these two studies may suggest that over time more water moves into the skin than ions move out, suggesting that high concentrations in sweat patches may be from concentration and loss of water from excreted sweat.[28] However, it is difficult to make any sure hypothesis due to the fact that distilled water and not sweat was the substance on the skin. The presence of original sweat ions may make the results different.

What is also interesting to note from the Nakagawa[27] study is the differences between sweat ion concentration and SC content. They showed that flowing sweat contains far more sodium and chloride than does SC, but that potassium and lactate are in

higher concentration in the SC. These results may be a little skewed as SC samples were taken from the arm and sweat samples taken from the face,[27] and it has been shown that NMF distribution differs between those two areas with differences in age by Egawa and Tagami.[28]

The last line of evidence for faux sweat is the "changing nature of the SC with increasing water content."[9] The NMF in the SC is a mixture of amino acids, derivatives of amino acids and salts. In fact, 10% of the dry weight of SC is the components of NMF. These components are highly water soluble and highly hygroscopic and will absorb atmospheric water vapor as low as 50% humidity. When water content is low, the water is bound and immobile and the mobility increases after reaching approximately 33%.[29-30] As the water content increases NMF dissolves into its own water, hence maintaining SC hydration[24,31] and increasing ionic mobility.[32] Kalia et al[32] measured ion mobility in human SC and found that sodium has almost twice the mobility of chloride. Additionally it was found that ion mobility decreases as a function of position within the SC, meaning that ions deeper have lower mobilities. Researchers also point out that water movement is driven by a powerful concentration gradient, approximately 55 M below the SC and near 0 at the skin's surface.[32] Alternately, the driving force for ions is an electrical gradient and the skin holds a net charge at physiological pH. However, at high water content the ions exist in an aqueous environment and would have a mobility closely resembling water, regardless of the depth. When aqueous substances are present on the skin surface much of the electrical impedance is taken away and ion mobility through the skin surface would also be similar to that of water. Therefore, changing the

hydration status of the SC could conceivably increase the concentration of ions seen in sweat collection.[32]

Still, with the removal of the electrical gradient, a concentration gradient would become the driving force for ion movement through the SC and then into or out of sweat. As mentioned previously, Nakagawa et al[27] showed figures that sweat concentrations of sodium and chloride were far greater than concentrations in the stratum corneum. Again, different bodily surfaces were used for sweat and SC measurements and must be taken into consideration. Additionally, Tanaka et al[33] found significant quantities of sodium, potassium and chloride in distilled water blanks applied before sweating or exercise.

It is apparent from research that WBW and regional sweat collection techniques show data with different concentrations for sweat electrolytes. If regional sweat collection techniques are truly artificially high, data from such studies would always extrapolate to higher estimations of body sweat electrolyte loss. Other implications pointed out by Weschler[9] include the possibility that particles not present in interstitial fluid but reported in sweat may need to be reevaluated. Additionally, potassium concentrations higher than plasma levels may indicate leaching in regional and WBW sweat collection.[9] Weschler[9] also points out that using a wicking material during collection, to keep the skin dry may make it possible to determine local sweat electrolyte concentrations.

It is important for researchers to be able to collect data that are accurate and reliable. Research suggests that a model like the faux sweat hypothesis could explain differences between WBW and regional sweat collection techniques. Current theories on

sweat production may need to be reworked if the faux sweat hypothesis produces significant differences in sweat ion concentration data. Alternately, the answer may simply be to follow example of Verde et al[17] and ensure that, during data collection, the sweat patch does not become saturated.

Chapter 3

Methods

Subjects

Participants will be 15-18 adult males and females between the ages of 18 and 35 who volunteer for the study. Participants will be recruited from the Brigham Young University campus in Provo, Utah and the surrounding Utah County area. Participants will fill out a health screening questionnaire to ensure that no subject has any chronic illness and that they are at least moderately active. Moderate activity will be defined as engaging in exercise for at least 30 minutes, 3 times per week. All subjects will sign an informed consent form and the study will be approved by the Brigham Young University Institutional Review Board prior to data collection.

Design

The proposed study is a repeated measures design with each subject serving as their own control and all measurements are made on similar forearm skin. Local sweat induced by exercise in the heat will be collected from 3 skin sites on the dorsal aspect of the forearm using sweat patches. Each local site will represent a different skin treatment condition as outlined in

Table 1. Three additional skin sites will be used to measure the composition of the SC using a tape stripping technique. The dependent variable will therefore be sweat and SC composition of sodium, potassium, chloride, and lactate. The independent conditions will be defined by the skin treatment. For sweat composition this will consist of 1) saturated versus nonsaturated sweat patches and 2) euhydrated versus hyperhydrated SC.

For SC composition this will consist of 1) euhydrated versus hyperhydrated SC and 2) pre and postsweating (nonocclusive dressing).

Procedure

Each subject will read and sign informed consent which will contain all procedures and the risk involved with such a study. A medical history will be taken to ensure the safety of the subject for the conditions of the experiment. Subjects who do not meet the criteria for exercise testing as defined by the American College of Sports Medicine will not be allowed to participate.

Subjects will report the Human Performance Research Laboratory at Brigham Young University in Provo, Utah for one appointment. Subjects will be asked to ingest at least 10 ml/kg body weight of water the night before coming to the lab. To ensure uniform sweat composition over the course of the session, participants will not be allowed to ingest water or food during the exercise. Subjects will be allowed to wear their own exercise clothing, providing that it is not restrictive, has short sleeves and it is appropriate for stationary cycling. Before beginning the experiment, each subject will have the 6 chosen skin sites shaved and washed with distilled water and allowed to dry.

Sweat Collection

Local forearm sweat will be collected using an absorbent pad with a Tegaderm covering that can be adhered to skin. The sweat collection patches and skin stripping sites will be on nonglabrous skin of either the left or right forearm (assuming no significant differences in sweat production or composition between the two forearms). For local sweat collection each skin site is defined by the following parameters: a) SC

hydration using a distilled water blank placed on the skin site for 5 min prior to sweat collection; and b) saturation of the sweat patch or not. For determination of SC composition each skin site is defined by the following parameters: a) skin stripping prior to stimulating sweating; b) skin stripping following thermal stress and local sweat production, and c) skin stripping following SC hydration with a distilled water blank.

Table 1. Delineation of sites for sweat analysis

	Sweat Composition			SC Composition		
Treatment Site	1	2	3	4	5	6
Distilled Water Blank	N	N	Y	N	N	Y
Strip Skin Before Patch	N	N	N	Y	N	Y
Patch Application	Y	Y	Y	N	N	N
Strip Skin After Patch	Y	Y	Y	N	Y	N
Patch Saturated or NonSaturated	NonSaturated	Saturated	NonSaturated	N/A	N/A	N/A

Skin sites will be thoroughly washed with distilled water and dried before the application of the sweat patches or the distilled water blank. Treatment 1 is to measure sweat and SC composition following sweating with an occlusive nonsaturated sweat patch. Treatment 2 will analyze sweat and SC composition following sweating with an occlusive saturated

patch. Sweat and SC composition following hydration with distilled water of the SC is performed in Treatment 3. Baseline SC composition (without occlusive dressing) will be determined with Treatment 4 and SC composition following sweating (without occlusive dressing) is determined in Treatment 5. Treatment 6 determines SC composition following hydration with distilled water. Measurements from each skin treatment site will be collected during a single sweating bout induced by mild exercise in a hot environment. Each forearm will receive 3 (of the total 6) skin treatments (3 treatments per forearm). Each treatment site will be assigned in a random fashion.

Stratum corneum hydration will be performed by placing a distilled water "blank" made from a test tube containing 2 milliliters distilled water, placed on the skin site and held there for 15 minutes and then allowed to air dry. This method has been shown by Tanaka et al[33] to produce the appearance of sodium, potassium and chloride in the distilled water blank after 1 minute. Nakagawa et al[27] showed that NMF could be extracted with a distilled water blank and lower the hydration status of the SC. Egawa and Tagami[28] have shown that at 15 minutes after applying a moistened cotton ball the hydration status of the SC is significantly increased.

The sweat patches will be applied after the onset of sweating and timed after application. The nonsaturated patch will be removed approximately 15-20 minutes after patch application and the saturated patches will be removed after approximately 30-40 minutes. After each patch is removed it will be placed into an airtight container until analysis can be completed.

Sweat Induction

In order to induce sweating participants will exercise on a semi-recumbent cycle ergometer for 40 minutes at 34°C. Each participant will exercise at a steady state of 60% of their age-predicted max heart rate.

Sweat Analysis

Sealed absorbent patches (Pacific Biometrics, Seattle, Washington) will be placed on the forearm for 15 to 40 minute periods. The sweat patch consists of 4.7 x 3.1 cm filter paper, sealed and affixed to the skin with tegaderm (3M Medical Technologies, Minneapolis, MN.) The skin site will be cleansed with deionized water prior to placement and wiped with a clean dry towel. Local sweat rate is determined by patch weight increase (to 0.0001 g) from the dry weight per minute on the skin. After weighing, the patches are transferred to plastic screw-capped bottles. The fluid in the patches will be collected by centrifugation with nylon Microfuge centrifuge filter tubes and analyzed for sodium (Na^+), potassium (K^+), chloride (Cl^-) and lactate (Lac^-) concentrations. Na^+ and K^+ will be measured using ion selective electrodes (NOVA 8, Nova Biomedical, Waltham, MA.) Cl^- will be determined by coulometric titration (Digital Chloridometer, Labconco, Kansas City, MO) and Lac^- by an YSI 2300 lactate analyzer (YSI, Yellow Springs, OH.)

Stratum Corneum Analysis

The change in stratum corneum content before and after sweating with and without a patch will be analyzed to determine if sweating has changed the content, which would infer leaching into the sweat patch. In order to determine if the ion content of the

SC has been altered by the leaching of ions into sweat, we will sample the outer layers of the SC by using the tape-stripping method outlined by Nakagawa et al.[27] Ion levels in the stratum corneum will be determined by stripping off portions of forearm SC with adhesive tape and then soaking the tape in distilled water for 24 hours to extract the ions (sodium, potassium, chloride and lactate.)[27] Individual ion levels will be determined by the methods described for sweat samples.

Data Analysis

Changes in stratum corneum composition and sweat composition of saturated and nonsaturated sweat patches will be analyzed using repeated measures analysis of variance, with subject and/or time as repeated measures. Differences in sweat content under the various conditions will be analyzed using ANOVA. Significance will be set at the 0.05 level.

Sample Size/Power Calculation

The average difference between WBW and patch sweat sodium concentrations on the forearm skin are 18.1 mEq/L according to data from Patterson et al.[13] We do not expect to see this large of a difference between hyper- and euhydrated skin. We do expect a difference of between 5 and 10 mEq/L. The standard deviation of sweat patch sweat sodium measurements range from 4 to 8 mEq/L (within subject, consecutive sweat patch estimate.) The sample size required to detect a true difference between means is calculation using the following equation:

$$n \geq 2\left(\frac{\sigma}{\delta}\right)^2 \left\{t_{\alpha\{v\}} + t_{2(1-P)\{v\}}\right\}^2$$

where

- σ = the standard deviation of the measurement
- δ = the smallest true difference we desire to detect:
- v = degrees of freedom of the sample standard deviation with a groups (a = 1) and n (13) replicates per group
- α = significance level
- P = power of the test
- $t_{a\{v\}}$ and $t_{2(1-P)\{v\}}$ = values from a two tailed t table

If we start with a prediction of n≈13, $\sigma = 6$, and $\delta = 7.5$, we solve the equation:

$$n \geq 2\left(\frac{6}{7.5}\right)^2 \{2.179 + .868\}^2$$

$$n \geq 11.88$$

To be conservative we round up to n = 12 and we predict that we can detect a 7.5 mEq/L difference between euhydrated and hyperhydrated skin sweat samples using 12 subjects. In this study we plan to use 15 subjects because the difference between two sweat sample compositions may be smaller than anticipated.

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