The Effect of Heat Acclimation on Sweat Microminerals: Artifact of Surface Contamination

Matthew R. Ely, Robert W. Kenefick, Samuel N. Cheuvront, Troy Chinevere, Craig P. Lacher, Henry C. Lukaski, and Scott J. Montain

Heat acclimation (HA) reportedly conveys conservation in sweat micromineral concentrations when sampled from arm sweat, but time course is unknown. The observation that comprehensive cleaning of the skin surface negates sweat micromineral reductions during prolonged sweating raises the question of whether the reported HA effect is real or artifact of surface contamination. **Purpose:** To measure sweat mineral concentrations serially during HA and determine if surface contamination plays a role in the reported mineral reductions. **Methods:** Calcium (Ca), copper (Cu), magnesium (Mg), and zinc (Zn) were measured in sweat obtained from 17 male volunteers using an arm bag on Day 1, 5, and 10 of a HA protocol. To study the role of contamination, sweat was simultaneously (n = 10 subjects) sampled twice daily from a cleaned site (WASH) and unclean site (NO WASH) on the scapular surface. **Results:** Sweat Ca, Cu, and Mg from Arm Bag trended progressively downward from Day 1 to Day 10 of HA (p = .10–0.25). Micromineral concentrations from the WASH site did not change between Day 1, 5, or 10 (Ca = 0.30 ± 0.12 mmol/L, Cu 0.41 ± 0.53 μmol/L; Zn 1.11 ± 0.80 μmol/L). Surface contamination can confound sweat mineral estimates, as sweat Ca and Cu from NO WASH site were initially higher than WASH (p < .05) but became similar to WASH when sampled serially. **Conclusion:** Heat acclimation does not confer reductions in sweat Ca, Cu, Mg, or Zn. When the skin surface is not cleaned, mineral residue inflates initial sweat mineral concentrations. Earlier reports of micromineral reductions during HA may have been confounded by interday cleaning variability.

**Keywords:** calcium, copper, iron, magnesium, zinc

If sweating is a significant contributor to mineral losses from the body, dietary mineral requirements might be appreciable, particularly for people living and/or training in hot environments. The Institute of Medicine has identified five minerals—calcium, copper, magnesium, iron, and zinc—for which sweat losses could be an important determinant of dietary requirements (Committee on the Scientific Evaluation of Dietary Reference Intakes, 2001; Committee on Military Nutrition Research, 2006a). However, historical estimations of these mineral losses in sweat have been highly variable (as summarized in Chinevere et al., 2007, and Committee on Military Nutrition Research, 2006a). Surface contamination and state of heat acclimation have been suggested as potential contributors to between study variability.

Skin surface mineral residue (surface contamination) may be a major confounder to accurate estimation of sweat mineral loss (Adams et al., 1950; Talbert et al., 1933). Multiple studies have observed serial reductions in sweat micromineral concentrations during prolonged sweating (DeRuisseau et al., 2002; Montain et al., 2007; Tipton et al., 1993; Weller & Haymes, 1996) with the decrement occurring soon after the onset of sweating (Paulev et al., 1983). A recent study (Ely et al., 2011) was able to eliminate the decrements in sweat mineral concentrations by applying a rigorous cleaning procedure to the flat skin surface. The same cleaning procedure applied to an irregular skin surface was not as effective, as mineral decrements similar to previous reports persisted (Ely et al., 2011). The decreasing mineral concentrations on the irregular surface were suspected to be a consequence of surface contamination from an area that could not be thoroughly cleaned (Ely et al., 2011). These findings strongly question the biological origins of sweat minerals in samples collected at the onset of sweating.

Heat acclimation reduces sweat sodium concentration (Allan & Wilson, 1971; Buono et al., 2007; Dill et al., 1938; Kirby & Convertino, 1986). Other minerals lost in sweat during acclimatization are less well studied, and are limited to seasonal (Hoshi et al., 2002) or geographic (Omokhodion & Howard, 1994) effects. In the only comprehensive study examining mineral loss during a laboratory heat acclimation protocol, Chinevere et al. (Chinevere et al., 2008) noted significant reductions in

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calcium, copper, and magnesium from Day 1 to Day 10 of acclimation. Although a cleaning procedure was used, the arm bag methodology employed may have still confounded the results, since large irregular sweat collection surfaces are difficult to clean (Ely et al., 2011). Moreover, multiple days of repeated vigorous sweating may have flushed mineral residue lying on the skin surface, contributing to a progressive decline in sweat mineral concentrations over repeated days of acclimation testing.

The purpose of this study was to reevaluate the effect of heat acclimation on sweat micromineral concentrations and examine the time course of the adaptive response. In addition, subexperiments were included to study the potential role of skin surface contamination on sweat mineral composition. To accomplish these tasks sweat samples were collected from a cleaned (WASH) and a noncleaned (NO WASH) upper-back site on Day 1, Day 5, and Day 10 of heat acclimation. Skin surface mineral residue was studied by performing an arm rinse before exercise on Day 1, 5, and 10 of heat acclimation, and by collecting serial sweat samples from the WASH and NO WASH sites. It was hypothesized that 1) sweat calcium, copper, iron, magnesium, and zinc concentrations would decrease from Day 1–5 and 10 of heat acclimation from arm but not from the WASH site; 2) the arm skin surface would possess residual amounts of minerals; 3) the initial samples of sweat collected from the NO WASH site would be substantially greater than those measured at the WASH site but differences would be substantially less when a second serial sweat sample was collected at each site; and 4) the concentration of minerals in sweat from initial sweat samples collected from the NO WASH site would remain stable over the 10 days of heat acclimation.

### Methods

Seventeen healthy male soldiers (age: 22 ± 5 yrs; weight: 77.5 ± 14.6 kg; height: 1.77 ± 0.06 m; body surface area: 1.93 ± 0.19 m²) volunteered for the study. They were acclimated to the heat by performing up to 100 min of continuous walking, core temperature of 39.5 °C, or voluntary cessation. The heat acclimation protocol is identical to Chinevere et al. (2008) and follows the classic acclimation outline described by Lind and Bass (1963). Total body sweat rate was calculated from changes in body mass (± 0.05 kg, Metler Toledo WSI-600 Toledo, OH) divided by the total exercise time. No fluid was provided to the volunteers during exercise so comparisons could be made with previous acclimation studies (Chinevere et al., 2008). The volunteers ate ad libitum over the 10-day heat acclimation period with the exception of receiving the same standardized meal the morning of HA Day 1, 5, and 10. To study the time course of sweat micromineral conservation, sweat was obtained from an arm bag Day 1, 5, and 10 of the heat acclimation protocol. To examine if any changes seen could be confounded by residual skin surface contamination at the arm site, additional sweat samples were collected on a subset of the volunteers (n = 10) from sweat pouches located on the upper back.

#### Blood

It is generally believed that blood is the precursor fluid for sweat formation. Therefore, to monitor changes in blood mineral concentrations that may occur with heat acclimation, a 5 ml blood sample was obtained from the antecubital space using trace element free tubes (Becton Dickinson, Franklin Lakes, NJ) approximately 1 hour before Day 1, 5, and 10 of heat acclimation. Blood samples were centrifuged and plasma was extracted and placed into sterile Cryule Vials (Wheaton NJ), which were then frozen at –80 °C until analysis.

#### Surface Mineral Residue

To assess the amount of mineral residue on the skin surface, an uncleaned arm was exposed to a distilled water rinse. On 10 of the subjects, the arm and hand opposite the side from which blood was drawn were rinsed with 10 ml of 18 MΩ deionized water within an arm bag (Continental Plastic Corp) before cleaning on Day 1, 5, and 10 of heat acclimation. A 10 ml volume rinse was chosen as it approximated the volume of sweat obtained using the arm bag methodology (Chinevere et al., 2008; Ely et al., 2011), and pilot testing indicated a 10 ml volume of deionized water could coat the length of the arm and a significant portion recovered for analysis. The rinse water was in contact with the arm and hand skin surface for less than 1 min and was slowly worked down the arm bag from the volunteer’s deltoid insertion to fingertips. For estimation of rinse recovery, a measure of the empty arm bag was subtracted from the final weight of the arm bag plus rinse to the nearest 0.1 g (Ohaus Scale Corp., Florham Park NJ). Two ml of the rinse was withdrawn using a blunt stainless steel syringe, placed into sterile Cryule Vials (Wheaton NJ), and frozen at –80 °C until analysis. The 18 MΩ deionized water did contain minerals of interest (calcium: 0.09 mmol/L, copper: 0.05 μmol/L, potassium: 20 μmol/L).
and NO WASH sides approximately 20 min after starting exercise (Sample 1) and a second sample (Sample 2) was obtained approximately 20 min later. Sweat samples were transferred from the Luer Lock syringes into Cryule Vials that were weighed and frozen. The regional sweat rate of the back area was calculated using the surface area of the sweat pouches and dividing by the volume of sweat collected over the time of the collection period.

**Analysis**

All mineral analysis was conducted at the USDA-ARS Grand Forks Human Nutrition Research Center, Grand Forks, ND. To minimize sample contamination, all Cryule Vials and stainless-steel syringes were pretreated in a 10% nitric acid solution for 8 hr and air-dried before use and did not contain any mineral of interest. Samples (plasma, sweat, and rinse) were vortexed and an aliquot of the supernatant was placed in a plastic tube to which 0.1 ml of ultrapure 6M nitric acid was added to ensure liberation of all cations from any associations (binding) with proteins, amino acids, and contaminants. Capped tubes containing the samples were then stored at room temperature for 12 hr. Diluted plasma, sweat and rinse aliquots (typically 3–4 times) were then analyzed for mineral concentrations by inductively coupled argon plasma emission spectrometry using a Perkin Elmer 3300 DV ICP instrument (Perkin Elmer Corp., Wellesley, MA) equipped with automated sample injection. To prevent carry-over contamination between samples the instrument sampler and internal sampling chambers were flushed after each sample. Quality control procedures demonstrated that the mineral analysis procedures produced outcomes within 10% of known standards within the physiological range expected for sweat and plasma mineral concentrations. Minimal detection limits for calcium, copper, iron, magnesium, and zinc were 4, 0.2, 0.3, 8, and 0.1 μmol/L, respectively.

**Statistics**

Sample size was estimated (Potvin & Schutz, 2000) based on the desire to detect a minimum 30% reduction in sweat calcium mineral concentration (−0.46 mmol/L) previously reported with heat acclimation (Chinever et al., 2008). When conventional alpha (.05) values were applied to include an autocorrelation parameter for repeated measures equal to 0.80, 10 subjects were estimated to provide sufficient power (beta = 0.20) to detect an effect size of 0.6 (main effects) to > 1.0 (interaction). The denominator for the effect size estimate (i.e., $SD$) was set to 0.70 mmol/L (the between-subjects $SD$ (Chinever et al., 2008) and assumes that the within subjects $SD$ would be smaller (Fraser, 2001; Hayden et al., 2004).

To examine the effectiveness of the heat acclimation procedure, the exercise time, core temperature, heart rate, and sweating rates measured on HA Day 1 and Day 10 were compared using a paired $t$ test. The effect of heat acclimation on mineral levels measured in plasma, arm bag, WASH, and NO WASH sites as well as

**Arm Bag**

Following the rinse, the same arm was washed using liberal amounts of 18 MΩ deionized water and scrubbed with unscented liquid antibacterial Dial soap using a surgical scrub brush (Becton-Dickinson), the same washing procedure used in a previous study (Ely et al., 2011). The soap did contain minerals of interest (Calcium 0.6 mmol/L, Copper 3.38 μmol/L, iron 0.9 μmol/L, magnesium 0.05 mmol/L, sodium 151.1 mmol/L, zinc 2.2 μmol/L), but no correction was made to the sweat samples as the area was doused multiple times with deionized water after the soap scrub. The arm was left to air dry and the volunteers were careful not to touch any object before a preweighed disposable polyethylene shoulder-length arm bag (Continental Plastic Corp., Delavan, WI) was secured at the deltoid insertion by Velcro strap. The band was not tight enough to restrict blood flow, and suppression of sweating over time was minimized by removing arm bags 20–25 min into exercise. Arm bag sweat volume was measured by subtracting a measure of an empty arm bag from the final weight of the arm bag containing sweat to the nearest 0.1 g (Ohaus Scale Corp., Florham Park NJ). Sweat samples were withdrawn using a blunt stainless-steel needle attached to a Luer lock syringe and transferred to Cryule Vials (Wheaton, NJ) that were frozen at −80 °C until analysis. The stainless-steel needle was rinsed in deionized water and a new Luer lock was secured at the deltoid insertion by Velcro strap. The same arm was used to clean the right side (WASH) of the scapula regions. The cleaning procedure described above for the arm was used to clean the right side (WASH) of the scapula regions. The cleaning procedure described above for the arm was used to clean the right side (WASH) of the scapula regions.

**Scapular Sweat Pouches**

Upper-back sweat was collected from the left and right scapula regions. The cleaning procedure described above for the arm was used to clean the right side (WASH) of the back while the left side was not cleaned (NO WASH). The cleaned area was allowed to air dry and two closed pouch (60 cm²) sweat collectors described by Brisson (Brisson et al., 1991) were affixed to the left and right side. The tubing portion of a catheter was inserted into each pouch with the Luer Lock end protruding out of a hole near the top of the pouch to allow repeated sampling of sweat using a Luer Lock syringe (Monoject, Sherwood Medical, Deland, FL). Sweat was collected from the WASH and NO WASH sides approximately 20 min after starting

0.01 mmol/L, sodium: 0.02 mmol/L, zinc: 0.05 μmol/L); therefore, a correction was made to the rinse samples mineral concentration values. An estimation of absolute surface mineral residue per square meter of skin surface area was made by multiplying the concentration of the rinse by the volume of recovered rinse and dividing by the surface area of the arm using the equations of Du Bois et al. (1916) and Lund and Browder (1944).
surface mineral residue estimates were analyzed using a one-way repeated measure ANOVA. The impact of skin mineral residue on sample concentrations was examined by evaluating the interaction between sweat samples collected serially from the NO WASH and WASH sites on HA Day 5 using a two-way repeated-measures ANOVA (Site × Time). Differences between the WASH and NO WASH sites on HA Day 1 and Day 10 were not included because of missing samples. Tukey’s post hoc procedures were used to identify differences among means following significant main and/or interaction effects. All analyses were conducted using SPSS 19 (SPSS Inc). Data are presented as mean ± SD.

**Results**

All 17 volunteers completed the 10-day heat acclimation protocol. Indications of heat acclimation included statistically significant increases in exercise time (81.6 ± 21.3 to 97.1 ± 12.1 min; p = .03), decreased core temperature at 40 min (38.4 ± 0.5 °C to 37.9 ± 0.3 °C; p < .01), and decreased heart rate at 40 min (151 ± 21 to 136 ± 15 bpm; p < .01). The 40-min time point was used for comparison across all subjects, as it was the amount of time that treadmill walking was completed by all subjects during the heat acclimation. Mean whole-body sweat rate increased from Day 1 (1.08 ± 0.20 L/h) to Day 10 (1.18 ± 0.20 L/h; p = .01). Sweat sodium concentrations at Arm Bag site remained similar across heat acclimation (Day 1 = 42 ± 21, Day 10 = 43 ± 15 mmol/L; p = .99); the concentrations trended downward at the W ASH site but the difference did not achieve statistical significance (Day 1 = 72 ± 24, Day 5 = 64 ± 21, Day 10 = 56 ± 18 mmol/L; p = .11). Sweat potassium concentrations were likewise similar over time (Arm Bag Day 1 = 10.8 ± 2.6, Day 10 = 11.3 ± 3.1 mmol/L; WASH day 1 = 5.1 ± 0.6, Day 10 = 6.0 ± 0.9 mmol/L).

**Effect of HA on Sweat Microminerals**

Tables 1 and 2 present the effects of heat acclimation on the sweat microminerals from the Arm Bag and the WASH site, respectively. At the Arm Bag site, the heat acclimation protocol produced downward trends in sweat calcium, copper and magnesium, but the magnitude of the change over time did not reach statistical significance. Sweat zinc levels were stable over time. Sweat iron concentrations were often below detectable limits, and, as a consequence, no analysis was attempted. In the subset of volunteers where sweat microminerals were measured at the upper-back WASH site, where cleaning can effectively remove surface contaminants, heat acclimation had no appreciable effect on sweat calcium, copper, and zinc values. Unfortunately, the iron and magnesium in the sweat were often below detectable limits, so the effect of acclimation was not considered. Consistent with an earlier paper (Ely et al., 2011), the sweat micromineral levels measured from the upper-back WASH site were considerably lower than values from Arm Bag collection site.

With regards to sweat collection, the arm bags were worn for 22 ± 2, 23 ± 8, and 22 ± 3 min of exercise-heat stress on Day 1, 5, and 10, respectively. The bags collected 11.3 ± 4.7, 13.4 ± 6.3, and 15.0 ± 6.6 ml of sweat (p = .08). The local sweat rate at the Arm site averaged 0.36 ± 0.15, 0.43 ± 0.21, and 0.48 ± 0.24 L/m²/hr on Day 1, 5, and 10, respectively (p = .12). The local sweating rate at the upper-back WASH site averaged 0.5 ± 0.2, 0.7 ± 0.4 and 0.4 ± 0.2 L/m²/hr on Day 1, 5, and 10 of heat acclimation, respectively.

### Table 1  Effect of Heat Acclimation on Arm Bag Sweat Mineral Concentration (n = 17)

<table>
<thead>
<tr>
<th></th>
<th>Day 1</th>
<th>Day 5</th>
<th>Day 10</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium (mmol/L)</td>
<td>1.01 ± 0.63</td>
<td>0.95 ± 0.68</td>
<td>0.75 ± 0.33</td>
<td>.10</td>
</tr>
<tr>
<td>Copper (μmol/L)</td>
<td>4.21 ± 2.52</td>
<td>3.69 ± 1.86</td>
<td>3.07 ± 1.74</td>
<td>.25</td>
</tr>
<tr>
<td>Magnesium (mmol/L)</td>
<td>0.21 ± 0.16</td>
<td>0.18 ± 0.15</td>
<td>0.15 ± 0.07</td>
<td>.10</td>
</tr>
<tr>
<td>Zinc (μmol/L)</td>
<td>7.54 ± 6.47</td>
<td>6.04 ± 3.62</td>
<td>7.73 ± 3.96</td>
<td>.56</td>
</tr>
</tbody>
</table>

*Note. Data are M ± SD.*

### Table 2  Effect of Heat Acclimation on Sweat Mineral Concentrations From Sweat Patch on WASH Upper-Back Site (n = 8)

<table>
<thead>
<tr>
<th></th>
<th>Day 1</th>
<th>Day 5</th>
<th>Day 10</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium (mmol/L)</td>
<td>0.26 ± 0.11</td>
<td>0.25 ± 0.03</td>
<td>0.38 ± 0.13</td>
<td>.05</td>
</tr>
<tr>
<td>Copper (μmol/L)</td>
<td>0.30 ± 0.30</td>
<td>0.26 ± 0.11</td>
<td>0.68 ± 0.84</td>
<td>.24</td>
</tr>
<tr>
<td>Zinc (μmol/L)</td>
<td>0.80 ± 0.73</td>
<td>0.97 ± 0.60</td>
<td>1.56 ± 0.93</td>
<td>.15</td>
</tr>
</tbody>
</table>

*Note. Data are M ± SD.*
Effect of Skin Surface Contamination

As illustrated in Table 3, the arm/hand surface area was abundant in surface mineral residue. The volume of the recovered rinse did not differ between Day 1, 5, and 10 and averaged 6.4 ± 0.7 ml of the original 10 ml. The mineral concentrations in the recovered rinse also did not differ from Day 1, 5, or 10 and averaged 1.2 ± 0.49 mmol/L, 10.08 ± 6.72 μmol/L, 1.02 ± 0.14 mmol/L, and 29.75 ± 20.86 μmol/L for calcium, copper, magnesium, and zinc, respectively. These rinse mineral concentrations equate to surface mineral residues of 3.55 ± 1.53, 0.05 ± 1.53, 0.46 ± 0.32, and 0.13± 0.11 mg per square meter (mg/m²) of skin surface area for calcium, copper, magnesium, and zinc, respectively.

The impact of cleaning (or the lack thereof) is illustrated in Figure 1. Initial sweat micromineral concentrations collected from the NO WASH site were higher than those collected from the WASH site. For calcium, copper, and zinc, there was a main effect of time but the effect was dependent on sampling site (p < .01). Post hoc analysis revealed that the initial NO WASH calcium and copper values were higher than those from the WASH site, and both minerals at the WASH site were stable over time. A similar pattern was present for zinc, but differences between NO WASH and WASH at either Sample 1 or 2 did not reach statistical significance. Cleaning or lack thereof had minimal effect on sweat sodium values as initial sodium concentration at the NO WASH and WASH sites were 55 ± 23 and 59± 22 mmol/L, respectively, and there was no time interaction (p = .19). In contrast, sweat potassium was higher on the NO WASH site when compared with WASH site (p = .03), and there was a trend for interaction (p = .06) consequent to initial potassium of 7.8 ± 2.1 vs. 6.22 ± 0.8 mmol/L at NO WASH and WASH sites respectively, and subsequent values of 6.4 ± 0.8 and 6.2 ± 1.3 mmol/L.

The difference in initial mineral concentration between NO WASH and WASH cannot be explained by the amount of sweat collected at each site (1.3 ± 0.5 vs. 1.4 ± 0.7 ml) nor by the collection time of Sample 1 (26 ± 3 vs. 23 ± 6 min) as they were similar. The mean local sweat rates were 0.50 ± 0.22, 0.73 ± 0.49, 0.62 ± 0.25 and 0.69 ± 0.30 L/m²/h for NO WASH Collection 1 and 2 and WASH Collection 1 and 2, respectively.

The profuse and sustained sweating associated with the daily exercise heat exposures had no appreciable effect on the composition of initial sweat collected from the NO WASH site (Table 4).

### Plasma Mineral Concentrations.

The plasma mineral concentrations of calcium, copper, iron, magnesium, and zinc were within the typical population reference intervals (Daniels, 2003). Plasma calcium and copper were similar Day 1, Day 5, and Day 10 of heat acclimation, averaging 2.4 ± 0.1 mmol/L and 13.7 ± 2.7 μmol/L, respectively. In contrast, plasma magnesium concentration was lower on day 1 when compared with Day 5 and Day 10 (0.72 ± 0.09–0.75 ± 0.07 and 0.75 ± 0.07 mmol/L; p = .01) and plasma zinc concentrations were higher on Day 10 when compared with Day 5 or Day 1 (Day 1: 13.7 ± 1.7, Day 5: 13.6 ± 1.4, Day 10: 15.6 ± 2.1 μmol/L; p < .01). Although the magnesium and zinc concentrations modestly increased, both were still within the typical population reference intervals (Daniels, 2003).

### Table 3 Arm Rinse Mineral Concentrations and Surface Mineral Residue Estimates (n = 10)

<table>
<thead>
<tr>
<th></th>
<th>Concentration</th>
<th>Absolute Content (mg/m²)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 1</td>
<td>Day 5</td>
</tr>
<tr>
<td>Calcium (mmol/L)</td>
<td>1.2 ± 0.6</td>
<td>1.3 ± 0.4</td>
</tr>
<tr>
<td>Copper (μmol/L)</td>
<td>8.4 ± 3.7</td>
<td>12.0 ± 7.4</td>
</tr>
<tr>
<td>Magnesium (mmol/L)</td>
<td>0.3 ± 0.1</td>
<td>0.3 ± 0.2</td>
</tr>
<tr>
<td>Zinc (μmol/L)</td>
<td>34.1 ± 21.3</td>
<td>34.0 ± 23.4</td>
</tr>
</tbody>
</table>

*Note. Data are corrected M ± SD.*

### Table 4 Effect of Heat Acclimation on Sweat Mineral Concentrations From Sweat Patch on NO WASH Upper-Back Site (n = 8)

<table>
<thead>
<tr>
<th></th>
<th>Day 1</th>
<th>Day 5</th>
<th>Day 10</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium (mmol/L)</td>
<td>0.60 ± 0.20</td>
<td>0.63 ± 0.35</td>
<td>0.64 ± 0.26</td>
<td>.93</td>
</tr>
<tr>
<td>Copper (μmol/L)</td>
<td>2.02 ± 1.86</td>
<td>1.92 ± 1.21</td>
<td>3.43 ± 4.12</td>
<td>.43</td>
</tr>
<tr>
<td>Zinc (μmol/L)</td>
<td>3.70 ± 1.81</td>
<td>3.24 ± 1.92</td>
<td>4.46 ± 2.71</td>
<td>.44</td>
</tr>
</tbody>
</table>

*Note. Data are M ± SD.*
Discussion

The novelty of the present investigation was the reevaluation of the effect of heat acclimation on sweat micromineral concentrations using methods that allowed examination of the impact of skin contamination on the observed mineral concentrations. The data provide supportive evidence that the sweat is quite dilute with respect to microminerals and is unlikely to be a major contributor to daily mineral requirements. To study the impact of surface contamination on minerals in sweat, this study used concurrent, serial determinations of sweat mineral concentrations from a thoroughly cleaned (WASH) and uncleaned (NO WASH) skin site in addition to estimating surface mineral residue by use of a skin rinse at the beginning, midpoint, and end of a heat acclimation protocol. To study the impact of heat acclimation on sweat microminerals, the volunteers performed up to 100 min of exercise in a hot environment for 10 consecutive days. The volunteers exhibited classical signs of heat acclimation of increased exercise time, decreased core temperature, decreased heart rate, and increased sweating rates. Sweat samples were obtained using widely employed techniques (Brune et al., 1986; Conn, 1949; Brisson et al., 1991) and mineral concentrations were measured using the most precise analytical technique available.

Heat Acclimation

The primary observation was that sweat micromineral concentrations did not decrease with acclimation to the heat. Sweat micromineral concentrations from the arm
bag and WASH sites remained relatively stable between Day 1, 5, and 10 (Tables 1 & 2). Moreover, the mean sweat mineral concentrations measured at both sites were similar to the mineral concentrations obtained in an earlier study employing the same techniques (Ely et al., 2011). This observation is in contrast to the main hypothesis and prior laboratory heat acclimation findings reported by Chinevere et al. (Chinevere et al., 2008) where arm sweat calcium, copper, and magnesium significantly decreased from Day 1 to Day 10. However, it is in agreement with the observation that heat acclimatized and unacclimatized individuals have similar trace elements concentrations in their sweat (Omokhodion & Howard, 1994). Moreover, in the study of Chinevere et al. (2008), it appears as though the group mineral reductions were primarily driven by large mineral reductions in a few individuals, while others had stable mineral concentrations between Day 1 and 10 of heat acclimation.

### Surface Mineral Residue

Surface mineral residue has long been suspected as contributing to elevated sweat mineral concentrations (Brune et al., 1986; Hohnadel et al., 1973), but it is unknown if any previous study attempted to quantify the impact of surface mineral residue. This study used a 10-ml deionized water rinse over the arm/hand, an area suspected to contain high amounts of surface mineral residue (Hohnadel et al., 1973; Ely et al., 2011). It was found that minerals reside on the skin surface in relatively high amounts (Table 3), and the concentration of mineral residue does not appear to decrease with multiple days of repeated vigorous sweating, as rinse mineral concentrations were similar on Day 1, 5, and 10 (Table 3). The Day 1 arm sweat mineral concentrations reported by Chinevere et al. (2008) are similar to the current study’s Day 1 rinse concentrations (Table 3), while Chinevere’s Day 10 sweat mineral concentrations are closer to the current study’s Day 10 arm bag sweat values (Table 1). Differences in sweat rate did not contribute to the differences in mineral concentrations, as arm bag sweating rates were similar in the two studies. Therefore, the Chinevere et al. (2008) observation of mineral conservation with heat acclimation may have been due to a more thorough cleaning on Day 10 compared with Day 1 rather than heat acclimation per se.

### Scapular Sweat Pouches

Further evidence that surface contamination inflates sweat mineral concentrations is apparent when sweat mineral concentrations from the NO WASH and WASH scapular skin sites are compared. In agreement with the stated hypotheses, sweat obtained from the NO WASH site contained greater initial mineral concentrations than those obtained from the cleaned WASH (Figure 1). In addition, the NO WASH mineral concentrations then declined 48–65% from initial to subsequent within-day (Sample 1 and 2) samples becoming similar to the mineral concentrations at the WASH site. The 48–65% drop in sweat mineral concentrations is very similar to mineral decrements reported by others during prolonged sweating (DeRuisseau et al., 2002; Ely et al., 2011; Montain, et al., 2007; Paulev et al., 1983; Tipton et al., 1993). For example, Tipton et al. (1993) reported zinc mineral concentrations were 58% lower at a 60-min sample compared with a 30-min sample and DeRuisseau et al. (2002) reported sweat zinc and iron concentrations decreased 38% and 40% respectively, from 60 to 120 min of exercise. These studies collected sweat from sites that had apparently not been cleaned (Paulev et al., 1983), underwent a less extensive cleaning (DeRuisseau et al., 2002; Montain et al., 2007; Tipton et al., 1993), and/or were collected from the arm, an area where a thorough cleaning procedure probably could not remove all mineral residue (DeRuisseau et al., 2002; Ely et al., 2011; Tipton et al., 1993).

### Arm Bag

It was previously suggested that thoroughly cleaning the arm/hand does not allow removal of surface contamination as the arm hair and fingers are possible sources of mineral residue (Ely et al., 2011). Although arm bag and sweat pouch comparisons were not the aim of the current study, the mineral concentrations from the arm bag were similar to the initial values obtained from the upper-back NO WASH site (Figure 1; Table 1). For example, exercise sweat calcium concentrations from the arm bag were 0.68 ± 0.17 mmol/L and 0.57 ± 0.34 mmol/L for the initial upper-back NO WASH site on Day 5. This appears to corroborate previous assertions that surface contamination is a confounder to accurate sweat mineral loss (Ely et al., 2011; Hohnadel et al., 1973; Paulev et al., 1983; Omokhodion & Howard, 1994).

### Blood

Plasma minerals were assessed to provide a reference for sweat values. It was anticipated that plasma mineral concentrations would remain stable over the 10-day heat acclimation. Other than sodium, potassium, and chloride (Allan & Wilson, 1971; Buono et al., 2007; Dill et al., 1938; Kirby & Convertino, 1986), it is unknown if plasma mineral concentrations have been previously examined over the course of heat acclimation. Regardless, the plasma mineral concentrations of Ca and Cu did not change over the course of the study and were within the typical reference intervals. Minor increases in plasma zinc and magnesium did occur but stayed within the typical population reference intervals. While the changes observed in blood did not appear to be related to changes in sweat when measured at arm bag site, the increase in plasma zinc observed on Day 10 of HA was coincident with an increase in sweat zinc concentration at the WASH site. The latter observation suggests the possibility that the observed sweat change was consequent to greater amounts of zinc in the sweat gland source fluid.
Limitations

The possibility remains that heat acclimation produces subtle but real reductions in sweat micromineral concentrations. While our heat acclimation protocol provided sufficient stimulus to produce several of the classic signs of heat adaptation, e.g., lowered core temperatures and heart rate, it did not produce statistically lower sweat sodium values. Therefore, the heat acclimation effect produced by the HA protocol might have been too subtle to detect sweat micromineral changes. That said, the experiments were conducted during the cold winter months of the northeast United States, and volunteers would not have been exposed to such heat stress for more than 4 months. There is also the possibility that subtle changes in sweat composition are not detectable when the experimental situation produces sweating rates in excess of 1 L/h.

Other limitations include the use of a water rinse volume that was less than the quantity of sweat collected. The present results indicate that the rinse mineral concentrations are far greater than sweat mineral concentrations collected in the arm bag (Table 1 and 3). It is possible that use of a greater volume of deionized water rinse (e.g., 20 ml) could have removed an even greater amount of surface minerals or possibly could have diluted the sample. Therefore, comparisons of rinse mineral concentrations to arm bag sweat mineral concentrations were not made. Regardless, the high mineral concentrations of the rinse certainly indicate that the skin surface is abundant in mineral residue. Equally important, daily profuse sweating does not appear to reduce the confounding effects of skin surface residue as neither the arm rinse nor the artificially high mineral levels in initial sweat from NO WASH site fell over the course of 10 days of heat acclimation. We were unable to study these sweating responses to HA in women, as no females volunteered to participate in the study. However, it is unlikely that a woman’s sweat would respond differently to heat acclimation than observed for men, as there is a lack of evidence to suggest sweat micromineral concentrations, or reported mineral reductions during prolonged sweating, differ between sexes (Baker et al., 2011; DeRuisseau et al., 2002; Ely et al., 2011; Montain et al., 2007; Tipton et al., 1993, Waller & Haymes, 1996).

Implications

The practical implications of this work relate to the importance of accurately estimating sweat mineral losses for determination of dietary requirements. Micromineral loss estimates from the past ~80 years (first known documentation in Talbert et al., 1933) encompass very large ranges. The upper end of these ranges could signify the need for increased mineral intakes as outlined by the Food and Nutritional Boards of the Institute of Medicine (Committee on Military Nutrition Research, 2006b). Besides individual variation, many factors were assumed to contribute to the large range of micromineral concentrations—including state of heat acclimation, conservation with prolonged sweating, sweat collection method, collection site, hidromeiosis, and mineral leaching (Baker et al., 2011; Chinevere et al., 2008; Collins & Weiner, 1962; DeRuisseau et al., 2002; Patterson et al., 2000, Paulev et al., 1983; Shamsuddin et al., 2005). It now appears that much of the variation has been due to the amount of surface mineral residue in the sweat samples. The present study (n = 17) along with that of Ely et al. (2011; n = 16) and Omokhodion and Howard (1994; n = 15) demonstrate that exercise-induced sweat contains relatively low concentrations of microminerals when surface contamination is removed. In addition, it confirms that sweat collected at onset of sweating likely contains mineral contamination, but subsequent sweat samples will produce sweat with low micromineral concentrations (Tipton et al., 1993, n = 18; Waller & Haymes, 1996, n = 18; DeRuisseau et al., 2002, n = 18; and Montain et al. 2007, n = 7). Taken together (N = 107), these studies provide compelling evidence that relatively little calcium, copper, iron, magnesium, and zinc are lost in sweat, and adjustments to dietary mineral requirements to mitigate sweat losses are not warranted. The low amounts of microminerals found in sweat from a cleaned surface or one where the initial sweat is removed (Sample 2, NO WASH) are similar to the low whole-body wash down values reported by others (i.e., Hoshi et al., 2001; Baker et al., 2011; Cohn & Emmett, 1978). Methodologically, sweat should be obtained from a cleaned flat skin surface and/or initial sweat should be discarded and subsequent samples used for analysis when accurate measures of sweat minerals are of interest.

Conclusion

The findings of this study demonstrate that heat acclimation does not confer a reduction in sweat calcium, copper, magnesium, or zinc concentrations, whether the sweat is obtained from a clean or an unclean surface. Surface contamination can elevate sweat micromineral concentrations, as sweat sampled initially from an unclean surface contained significantly greater micromineral concentrations than sweat sampled from a cleaned surface, and the initially elevated micromineral concentrations at the unclean site become similar to concentrations from clean sites with subsequent sampling. In addition, surface mineral residue does not considerably change over the heat acclimation period. Therefore, when accurate estimation of sweat micromineral loss is of interest, sweat should be sampled from a relatively flat skin surface that can be thoroughly cleaned, or the initial sweat should be discarded and subsequent samples obtained for analysis.

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