

Sweat Mineral-Element Responses During 7 h of Exercise-Heat Stress

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Context: Uncertainty exists regarding the effect of sustained sweating on sweat mineral-element composition. **Purpose:** To determine the effect of multiple hours of exercise-heat stress on sweat mineral concentrations. **Methods:** Seven heat-acclimated subjects (6 males, 1 female) completed 5 × 60 min of treadmill exercise (1.56 m/s, 2% grade) with 20 min rest between exercise periods in 2 weather conditions (27 °C, 40% relative humidity, 1 m/s and 35 °C, 30%, 1 m/s). Sweat was collected from a sweat-collection pouch attached to the upper back during exercise bouts 1, 3, and 5. Mineral elements were determined by using inductively coupled plasma-emission spectrography. **Results:** At 27 °C, sweat sodium (863 [563] µg/mL; mean [SD]), potassium (222 [48] µg/mL), calcium (16 [7]) µg/mL), magnesium (1265 [566] ng/mL), and copper (80 [56] ng/mL) remained similar to baseline over 7 h of exercise-heat stress, whereas sweat zinc declined 42–45% after the initial hour of exercise-heat stress (Ex1 = 655 [362], Ex3 = 382 [168], Ex5 = 355 [288] µg/mL, $P < 0.05$). Similar outcomes were observed for sweat zinc at 35 °C when sweat rates were higher. Sweat rate had no effect on sweat trace-element composition. **Conclusions:** Sweat sodium, potassium, and calcium losses during multiple hours of sustained sweating can be predicted from initial sweat composition. Estimates of sweat zinc losses, however, will be overestimated if sweat zinc conservation is not accounted for in sweat zinc-loss estimates.

Key Words: sodium, potassium, zinc, copper, magnesium, calcium

Dietary requirements for mineral elements are a function of intake, absorption, and losses. Although a considerable amount of research has been conducted to examine regulation and secretion of sweat sodium and potassium, fewer studies have examined how exercise affects sweat trace-mineral concentrations, particularly when exercise is prolonged and results in sustained sweating. Other studies have limited practical application because they report daily dermal losses without important reference to sweat volume or energy expenditure—factors necessary for inference to exercise–nutrient-loss interaction (8, 15, 19).

Studies of sweat sodium, potassium, and calcium composition demonstrate that there is considerable between-subjects variability in sweat sodium, and within-subject concentrations depend on the rate of sweating (1, 21, 23). Based on the

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available literature, sweat sodium is expected to be 460–1609 $\mu\text{g}/\text{mL}$ (20–70 mEq/L) with an average value of ~ 800 $\mu\text{g}/\text{mL}$ (~ 35 mEq/L). Sweat potassium and calcium do not appear to be affected by sweat rate, and typical values are 156–274 $\mu\text{g}/\text{mL}$ (4–7 mEq/L) and 28–80 $\mu\text{g}/\text{mL}$ (0.7–2 mEq/L), respectively. Whether these values can be used to predict daily sweat losses, however, remains uncertain because no study has collected repeated sweat samples during multiple hours of work to assess whether conservation develops with sustained sweating. If conservation occurs, it could represent one explanation of why local collection samples that are often collected during short periods of time (and often early in exercise) are typically higher than whole-body wash-down methods, the latter providing a time-averaged sweat-composition assessment (20, 21).

Less is understood regarding sweat trace-mineral-element composition. Sweat contains measurable quantities of copper, magnesium, and zinc. Actual composition, however, remains uncertain because differences between sampling techniques, sample sites, sample timing, and experimental conditions might be contributing to between-studies differences in experimental values (12). That said, heat acclimation produces sweat zinc and calcium conservation (7, 8), there are data suggesting that concentrations might decline as sweat rate increases (13, 22, 24), and there is evidence that sweat zinc levels decline during prolonged sweating (9, 22). Specifically, Tipton et al. (22) reported a 54% reduction in sweat zinc concentration over the first hour of exercise in a hot environment. Subsequently, DeRuisseau et al. (9) reported a 38% reduction in sweat zinc during the second hour of exercise relative to composition during the first hour of exercise. Whether further conservation occurs over time has not been investigated.

The purpose of this investigation was to examine the effect of exercise duration on sweat mineral composition. It was hypothesized that sweat sodium and potassium concentrations would be stable over time but that sweat mineral conservation would produce reductions in sweat trace-mineral-element concentrations over multiple hours of work, and this effect would be independent of sweat rate.

Methods

Subjects

Seven heat-acclimated volunteers (6 men and 1 woman, body mass 72.7 [9.7] kg, height 1.76 [0.04] m, age 24 [3] y) participated in the study. They were briefed orally regarding the experiment and inherent risks and provided written consent before participation. The experiment was approved by institutional scientific review and human use committees before implementation, and we adhered to AR72-25 and CFR 46 throughout the experiment.

Experimental Design

Before experimental testing the volunteers were heat acclimated by performing 100 min of treadmill walking (1.56 m/s, 4% grade) in a hot room (49 °C, 20% relative humidity, 1-m/s wind) for 10 consecutive days (16). For the experimental tests, subjects completed 5 \times 60 min of treadmill exercise (1.56 m/s, 2% grade) separated by 20 min rest in 2 environments (27 °C, 40% relative humidity,

1-m/s wind, and 35 °C, 30% relative humidity, 1-m/s wind). Sweat samples were obtained using the closed-pouch sweat-collection method described by Brisson et al. (3), which has an 8–11% within-subject coefficient of variation for sodium and potassium (11). Briefly, a 62.5-cm² piece of parafilm was adhered to sterile dressing (Tegaderm, 3M), and this, in turn, was attached to the upper back after the site had been cleaned with alcohol, rinsed with distilled water, and allowed to air dry before adhesion. Samples were collected for the entire 60-min exercise period, during exercise bouts 1, 3, and 5 (10–70 min, 170–230 min, and 330–390 min of heat exposure). Sweat samples were withdrawn using a blunt stainless-steel syringe and transferred to a screw-top cryovial that was weighed and frozen until analysis. To avoid hydromeiosis, sweat-collection pouches were only attached to the skin during the time of sample collection and the sampling site was varied over time. Sweat sample 1 was obtained from the upper left back, sample 2 was collected from a sweat-collection pouch attached to the upper right back just before exercise bout 3, and sample 3 was collected from a sweat pouch applied to same location as sample 1 just before exercise bout 5. Clothing worn was army battle dress uniform (BDU), warm weather (insulation = 1.08 clo, vapor permeability = 0.49 im/clo at wind speed 1 m/s), which consists of undershorts, pants, T-shirt, long-sleeved blouse, and hat. To collect sweat samples from the pouch, the subjects sat on a bench, unbuttoned their blouse, and with investigative-team assistance, pulled T-shirt and blouse up to expose the pouch. Water was available ad libitum. Each subject was also provided 1 L of sports drink (7% carbohydrate, 17 mEq/L sodium, and 5 mEq/L potassium) after exercise bout 2, and this was available to drink during remaining breaks. Lunch was provided between exercise bouts 3 and 4. Metabolic rate was determined via indirect calorimetry from 90 s of expired gas collected into Mylar bags ~25 min into each exercise period.

No attempt was made to standardize food intake between trials or between subjects during the 24 h before the experiments.

Experimental Methods

Sweat samples were centrifuged to remove cellular debris and stored in a refrigerator. Before analysis, sweat samples were vortexed, and a 0.25-mL 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 stored at room temperature for 12 h. Sweat electrolyte and mineral composition were measured using inductively coupled plasma-emission spectrography (PerkinElmer model 3300, Norwalk, CT). Whole-body sweat losses were calculated from nude body weights obtained before and after the total exercise-heat exposure and were adjusted for fluid and food intake, urine and fecal mass losses, respiratory water loss, and metabolic weight loss. Clothed body masses were obtained before and after each exercise to calculate exercise-only sweat rates. Indirect calorimetry was performed using a dry-gas meter and metabolic cart (TrueMax, ParvoMedics, Sandy, UT).

Quality control procedures demonstrated that our electrolyte- and mineral-analysis procedures produced outcomes within 7% of known standards for sodium, potassium, calcium, copper, magnesium, and zinc. Comparison of triplicates of

unknown sample produced a coefficient of variation of 1% for calcium, potassium, and sodium; 2% for magnesium and zinc; and 4% for copper. The distilled-water rinse did contain small amounts of zinc (15 ng/mL) and magnesium (~100 ng/mL) and could be a small source of contamination. Because sample-site preparation was standardized, no correction was made to sweat samples collected.

Statistical Analysis

A 1-way repeated-measures ANOVA was used to examine the effect of exercise duration on sweat composition. A probability of $P < 0.05$ was used for establishing statistical difference. Tukey's honestly significant difference post hoc procedure was used to identify differences between mean values. It was estimated that 5–8 subjects would provide adequate statistical power ($\beta = 0.2$) to detect reductions in sweat mineral concentrations (9, 22) in excess of the anticipated within-subject coefficient of variation (11) (i.e., effect size > 1.0). Data are presented as mean (standard deviation).

Results

The exercise intensity (421 [63] W) and rest periods produced average sweat rates of 0.33 (0.07) and 0.58 (0.15) L/h, respectively, in the 27 °C and 35 °C experiments. Ad libitum fluid intake was sufficient to prevent dehydration in excess of 2% of initial body mass regardless of experimental condition. For 27 °C trials, trace-mineral results were obtained for all 7 subjects. Because of insufficient sample 2 volume for 1 subject, however, complete sodium, potassium, and calcium data sets were only available for 6 subjects. For 35 °C trials, missing samples resulted in the loss of paired data for 2 subjects and the necessity to collapse data for samples obtained during exercise bouts 3 and 5 for the remaining 5 subjects. The reasons for lost data for these 5 included discontinuation of exercise ($n = 2$) and insufficient sample volume ($n = 3$). To accommodate the loss of data and test the experimental hypotheses, the 27 °C experiment was used to examine the effect of exercise duration on sweat composition, whereas the 35 °C paired data were examined separately to see whether the same pattern over time occurred despite the higher sweat rate. Hydromieosis did not appear to be a confounding factor because there was no redness or induration at sampling sites. Exercise-only sweat rates were stable over time, averaging 0.41 (0.10), 0.42 (0.10), and 0.44 (0.10) L/h for 27 °C exercise bouts 1, 2, and 3, respectively. At 35 °C, exercise-only sweat rates averaged 0.52 (0.10) and 0.59 (0.11) L/h during initial and subsequent sweat-sample collections.

As shown in Table 1, during the 27 °C experiment exercise duration appeared to have minimal effect on sweat sodium, potassium, or calcium concentrations because sweat samples collected during the third and fifth exercise periods (170–230 and 330–390 min of heat exposure, respectively) were similar to initial values. The average value of the 3 time points was 863 (563) $\mu\text{g/mL}$ (38 [24] mEq/L), 222 (48) $\mu\text{g/mL}$ (5.7 [1.2] mEq/L), and 16(7) $\mu\text{g/mL}$ (0.4 [0.2] mEq/L) for sodium, potassium, and calcium, respectively.

The trace minerals showed a somewhat different pattern of response—sweat zinc concentration fell 42% ($P = 0.03$) from initial values (655 [362] ng/mL) during the third exercise bout with no further apparent zinc conservation thereafter (Table 1,

Table 1 Effect of Exercise Duration on Sweat Sodium, Potassium, Calcium, Zinc, and Magnesium Composition (27 °C)

	10-70 min	170-230 min	330-390 min
Na, $\mu\text{g/mL}^a$	874 (485) 175-1512	888 (568) 199-1658	828 (471) 268-1625
K, $\mu\text{g/mL}^a$	196 (23) 167-236	223 (71) 151-358	246 (47) 186-313
Ca, $\mu\text{g/mL}^a$	18 (9) 11-36	15 (6) 8-22	16 (7) 6-30
Zn, ng/mL^b	655 (362) 292-1234	382 (168)* 169-641	355 (288)* 110-910
Cu, ng/mL^b	106 (71) 40-220	59 (32) 31-112	79 (63) 27-200
Mg, ng/mL^b	1434 (532) 838-2358	1203 (630) 441-2015	1164 (531) 359-1854

Data are mean (standard deviation) and range for ^a $n = 6$ subjects and ^b $n = 7$ subjects.

* $P < 0.05$ vs. baseline.

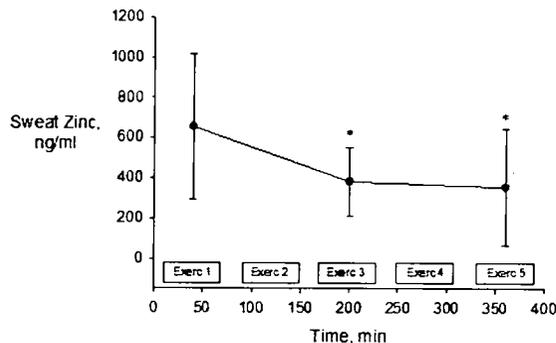


Figure 1 — Effect of sweat duration on sweat zinc composition. Sweat was collected for entire 60-min exercise period during exercise bouts 1, 3, and 5. Data are mean \pm standard deviation.

Figure 1). This pattern of reduction was observed in 5 of 7 subjects and could not be attributed to changes in sweat rate because the exercise-only whole-body sweat rates were stable over time. Sweat copper and magnesium had a pattern of response similar to that of zinc (copper 5 of 7 subjects, magnesium 4 of 7 subjects), but differences from baseline were smaller and did not reach statistical significance (Table 1; $P = 0.38$ and $P = 0.26$, respectively). Across the entire trial, sweat magnesium and copper averaged 1265 (566) and 80 (56) ng/mL , respectively.

Table 2 Effect of Exercise Duration on Sweat Mineral Concentrations During 35 °C Trials

Variable	10-70 min	170-390 min
Na, $\mu\text{g/mL}^a$	986 (294) 720-1431	1106 (395) 686-1738
K, $\mu\text{g/mL}^a$	232 (27) 201-262	238 (44) 200-313
Ca, $\mu\text{g/mL}^a$	15 (7) 8-25	14 (5) 6-21
Zn, ng/mL^b	811 (953) 227-2501	286 (189)* 89-584
Cu, ng/mL^b	108 (136) 27-349	30 (13) 13-46
Mg, ng/mL^b	1196 (526) 623-1723	1059 (178) 801-1262

Data are mean (standard deviation) and range for $n = 5$ subjects. The 170- to 390-min values are single sweat samples collected after either 170-230 or 330-390 min of heat exposure.

* $P < 0.05$ vs. baseline.

The results of the 35 °C trial are presented in Table 2. Sweat sodium, potassium, and calcium concentrations remained similar to initial values during subsequent sampling, averaging 1046 (333), 235 (35), and 14 (6) $\mu\text{g/mL}$, respectively. Similar to the 27 °C trial, sweat zinc, copper, and magnesium concentrations appeared to be higher during the initial sweat sample and to become dilute over time, because 5 of 5, 4 of 5, and 3 of 5 subjects displayed reductions over time, respectively. Changes in mineral concentrations at 35 °C did not reach statistical significance, however ($P > 0.05$).

Discussion

To test the experimental hypothesis that sustained sweating would affect sweat composition, the subjects performed multiple hours of work at an exercise intensity and in environmental conditions that produced near-continuous sweating. The sweat samples were collected using a validated technique (3, 11) and analyzed using the most sensitive and precise analytical techniques available. Furthermore, all subjects participated in a 10-successive-day heat-acclimation protocol to avoid changes in sweat composition associated with repeated exercise-heat stress.

The sodium and potassium values obtained using the sweat-pouch technique are in agreement with those of other investigations that collected local sweat samples from exercising heat-acclimated subjects (11, 20) and are similar to values obtained with whole-body wash-down techniques (21). Our sweat calcium values are similar to some reported in the literature (7, 14, 17) but lower than others (5, 18, 23). Analytical error is unlikely to explain our low calcium values because our methods were validated against certified standard references and calcium demonstrated a coefficient of variation of 4%. Differences in sample-collection methods are a possibility. Heat acclimation has also been shown to lower sweat calcium

concentrations (7). The between-subjects variabilities (as reflected by standard deviation, as well as range) for sweat sodium, potassium, and calcium are very similar to those observed in earlier studies.

Our 27 °C data extend the outcomes of previous studies by repeatedly sampling sweat composition during multiple hours of work to test the hypothesis that prolonged sweating might produce sweat conservation. The experimental results support the null hypothesis, that is, that no change in sweat sodium or potassium conservation occurs over multiple hours of work, because sweat sodium and potassium values remained similar to initial exercise values during the third and fifth hours of exercise (170–230 and 330–390 min of heat exposure, respectively). In addition, it was observed that sweat calcium levels remained stable over the duration of the experimental period. Thus, our data suggest that sweat losses of these elements can be modeled adequately over multiple hours of work if initial values for these sweat components are previously known. Our calcium outcomes are in contrast to those of Consolazio et al. (7), who reported that calcium values fell 60% during the initial 3 h of a 15-h heat exposure. In contrast to the current study, their subjects were sedentary and sweat rates were consequently lower.

Compared with other studies in the literature, which have primarily relied on the arm-bag technique to obtain sweat samples, our initial sweat zinc concentrations are consistent with expected values, whereas magnesium and copper concentrations were on the lower end of the range reported in the literature. Limited data are available regarding sweat trace-mineral levels from the torso region, but it is unlikely that sampling from the back versus the arm is responsible for differences, because Aruoma et al. (2) reported similar values from arm and back locations. A more likely explanation is that the aggressive heat-acclimatization protocol produced modifications in sweat composition (6, 7, 8).

The current experiment extends our understanding of sweat zinc, magnesium, and copper by characterizing changes in these minerals during prolonged periods of sweating. In agreement with earlier studies, there was a trend for several of the sweat minerals to decline after the initial minutes of sweating. Consistent with earlier reports (9, 22), we observed substantial zinc conservation after 2 h of sweating. Specifically, sweat zinc concentrations fell 42% from the first to the third hour of exercise. The novel contribution provided by our data is that there appears to be no further conservation with additional hours of exercise.

Why zinc conservation occurs remains uncertain. The reduction in zinc concentration cannot be attributed to increasing sweat rate over time, because whole-body exercise-only sweat rates were stable over the sampling duration. This conclusion is also supported by the observation of others that the effect of sweat duration persists when zinc is normalized to sweat rate (22). An alternative explanation is that initial values are contaminated by mineral residues either on skin *per se* or from skin-cell exfoliation consequent to sweating. The fall in sweat zinc (and possibly other minerals), however, could also be caused by changes in the ionic milieu surrounding the sweat duct or reabsorption by the sweat gland. Although minerals from sources extraneous to those secreted from the sweat gland could be responsible, the observation that heat acclimation produces reductions in zinc and other minerals argues that physiological adaptations independent of skin contamination might play a role. To our knowledge, no investigation has measured the interstitial milieu during sweat-gland secretion. It has been well described, however, that plasma zinc

levels remain stable or increase during prolonged exercise (4, 10). Therefore, if plasma is representative of the interstitial milieu, reabsorption by the sweat gland per se might play a role.

In summary, multiple hours of work and sweating have limited effect on sodium, potassium, or calcium in sweat. Thus, mass-balance models attempting to predict sodium and potassium losses in sweat are likely to accurately reflect sweat losses from a single sweat sample. In contrast, sweat zinc conservation occurs after the initial hour of sweating, and similar patterns of response might also affect the accuracy of predicting sweat losses of magnesium and copper. Therefore, changes in sweat trace-mineral levels can affect predictions of sweat losses and subsequent dietary requirements if sweat conservation is not considered in the estimate of sweat losses.

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