Surface contamination artificially elevates initial sweat mineral concentrations

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Ely MR, Kenefick RW, Cheuvront SN, Chinevere TD, Lacher CP, Lukaski HC, Montain SJ. Surface contamination artificially elevates initial sweat mineral concentrations. J Appl Physiol 110: 1534–1540, 2011. First published April 21, 2011; doi:10.1152/japplphysiol.01437.2010.—Several sweat mineral element concentrations decline with serial sampling. Possible causes include reduced dermal mineral concentrations or flushing of surface contamination. The purpose of this study was to simultaneously sample mineral concentrations in transdermal fluid (TDF), sweat, and serum during extended exercise to determine if these compartments show the same serial changes during repeat sampling. Sixteen heat-acclimated individuals walked on a treadmill (1.56 m/s, 3.0% grade) in a 35°C, 20% relative humidity (RH), 1 m/s wind environment 50 min each hour for 3 h. Mineral concentrations of Ca, Cu, Fe, K, Mg, Na, and Zn were measured each hour from serum, sweat from upper back (sweat pouch) and arm (bag), and TDF from the upper back. Sites were meticulously cleaned to minimize surface contamination. Mineral concentrations were determined by spectrometry. TDF remained stable over time, with exception of a modest increase in TDF [Fe] (15%) and decrease in TDF [Zn] (−18%). Likewise, serum and pouch sweat mineral concentrations were stable over time. In contrast, the initial arm bag sweat mineral concentrations were greater than those in the sweat pouch, and [Ca], [Cu], [Mg], and [Zn] declined 26–76% from initial to the subsequent samples, becoming similar to sweat pouch. Nominal TDF mineral shifts do not affect sweat mineral concentrations. Arm bag sweat mineral concentrations are initially elevated due to skin surface contaminants that are not removed despite meticulous cleaning (e.g., under fingernails, on arm hair), then decrease with extended sweating and approach those measured from the scapular region.

zinc; iron; copper; calcium; electrolytes

ACCUARTE ESTIMATION of dermal mineral loss due to extended sweating is important for determination of dietary reference intakes (8). When serial sweat samples are collected during exercise, the sweat captured initially often has higher mineral element concentrations than subsequent samples. For example, sweat iron concentrations reportedly declined 30% (14) during 120 min of sustained exercise-induced sweating. Similarly, sweat zinc concentrations obtained after 60 and 120 min of exercise were ~40% lower than samples obtained at the initiation of sweating with analogous trends also observed for sweat copper and magnesium (27). Paulev et al, (33) also noted that sweat iron concentration fell 36% between two successive 10-min sweat samples with no further reductions thereafter. The fall in exercise sweat mineral concentrations has been observed in a range of environments (23–35°C) (14, 27, 33). They also appear to be independent of exercise, as the same pattern of sweat composition changes have been reported during passive heat exposure (10, 37).

It remains unclear whether the concentrated sweat at onset occurs as a result of a biological mechanism related to changing mineral concentration in dermal fluid, or is simply an artifact of contamination by minerals lodged in the sweat pore or on the skin surface. The epidermis is abundant in minerals (5, 6), with greatest concentrations in the superficial layers (40, 41). The distribution profile of the minerals does vary, as calcium appears isolated to the stratum corneum, whereas zinc and iron are highly concentrated at the epidermal-dermal interface (40). No known study has directly compared dermal fluid and sweat mineral concentrations during prolonged exercise to determine if changes in the dermal fluid may be contributing to the observed reduction of sweat mineral concentrations over time.

Differences in sweat composition are reported in studies that have compared regional sites (1, 2, 31), but the observation is not universal (30). Arm bag sweat collection, a method in which the hand and forearm are inserted inside a plastic bag, has been the predominant method used to determine mineral concentrations in sweat (11, 14, 38). This anatomical region encompasses a large surface area that produces a robust quantity of sweat, but is probably susceptible to confounding effects of skin surface contamination due the extent of the area and the challenges inherent in cleaning between fingers and under fingernails. A popular alternate site of sweat collection is the scapular surface on the back (3, 27), which is relatively flat and less challenging to clean. Collection of serial samples from these two sweat collection sites should provide insight into the contribution of surface contamination to sweat captured for mineral analysis.

In the experiment described herein, transdermal fluid (TDF) and sweat were serially sampled during 3 h of exercise-heat stress to determine the contribution of changes in dermal fluid and surface contamination to sweat mineral element composition. A secondary interest was to compare regional differences in sweat mineral concentrations between the arm and scapular regions. It was hypothesized that the there would be reductions in sweat iron, zinc, copper, and magnesium after the first-hour sweat sample and this would be associated with changes in iron, zinc, magnesium, and copper TDF concentrations. It was also hypothesized that arm sweat mineral concentrations would be greater than sweat minerals measured on the scapular region.

METHODS

Sixteen healthy, heat-acclimated individuals (14 male, 2 female; age 23 ± 6 yr, weight 80.0 ± 16.4 kg, height 1.77 ± 0.09 m, BSA 1.96 ± 0.21 m²) volunteered for the study. Volunteers were provided informational briefings and gave voluntary, informed written consent.
to participate. Investigators adhered to the U.S. Army Medical Research and Materiel Command Regulation 72–25 and CRF 46 on the use of volunteers in research, and the appropriate Institutional Review Boards approved this study.

Volunteers were initially acclimated to the heat by performing up to 100 min of continuous walking (1.56 m/s, 4% grade) on a motor-driven treadmill in a hot environment [45°C, 20% relative humidity (RH), 1 m/s wind] for 10 consecutive days (24). The purpose of the heat acclimatization was to reduce thermoregulatory strain accompanying prolonged exercise in a warm environment. Three to four days after the acclimation protocol subjects were exposed to a 35°C, 20% RH, 1 m/s wind environment for 180 min while walking on a treadmill (1.56 m/s, 3.0% grade), alternating 50 min of exercise followed by 10 min of a seated sample collection. This work/rest protocol was selected to provide three exercise bouts of sufficient duration to collect three discrete TDF samples with sufficient volume for mineral analysis. During heat acclimation and the subsequent testing sessions, heart rate (HR) was measured every 10 min using a Polar heart rate monitor (Polar a3, Polar Electro, Woodbury, NY). Rectal temperatures were obtained from a telemetric temperature sensor (VitalSense Jonah Ingestible Capsule, Minimitter, Bend, OR) inserted 8–10 cm (length of gloved index finger) beyond the anal sphincter. This approach was used to clean the back. The area was allowed to air dry, and two closed pouch (62.5 cm²) sweat collectors described by Brisson (4) were affixed to the left and right side. The tubing portion of a blood catheter was inserted into the pouch with the luer-lock end protruding out of the top to allow repeated sampling of sweat using a luer-lock syringe (Monoject, Sherwood Medical). Sweat samples were transferred from the luer-lock syringes into Cryule vials that were weighed and frozen at −80°C. Left and right pouch samples were combined. 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. To obtain TDF, a laser poration device (Spectrex) was used to create 80-µm-deep pores, which allowed TDF to be extracted via vacuum pump from below the stratum corneum. In total, six sampling sites, three around each sweat pouch on the precleaned skin on the upper back, allowed collection of 60 µl of TDF each hour. The TDF was sampled during the 10 min of seated rest and was placed into Cryule vials, and immediately stored at −80°C. TDF sampling from the forearm site was not performed as preliminary experiments revealed a low laser poration success rate (~10%) on this skin surface.

To minimize sample contamination, all Cryule vials and stainless steel syringes were pretreated in a 10% nitric acid solution for 8 h and air-dried before use. The cleaned Cryule vials and the arm gloves were analyzed by the Grand Forks Human Nutrition Research Center (U.S. Department of Agriculture, Grand Forks, ND) and did not contain any mineral of interest. Additionally, samples (serum, TDF, and sweat) were analyzed by the Grand Forks Human Nutrition Research Center. Samples were vortexed and an aliquot of the supernatant was placed in a plastic tube to which 0.1 ml of ultrapure 6 M 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 h. Mineral concentrations were analyzed by inductively coupled argon plasma emission spectrometry using a Perkin Elmer 3100 XL ICP instrument (Perkin Elmer, Waltham, MA) equipped with automated sample introduction. To prevent carryover 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 for all minerals. Serum sodium concentrations were additionally analyzed using a PolyChem analyzer (Polymedco, Cortlandt Manor, NY).

Statistics. A blood sample during the second hour was not obtained from one volunteer; therefore, the subject’s serum mineral concentrations during the first and third hours were averaged to complete the data set. The upper back sweat pouches provided sufficient samples for 15/16 subjects for hour 1, 16/16 subjects for hour 2, and 12/16 subjects for hour 3. The group mean for each sweat mineral was used for the missing sample in hour 1, and the analysis was completed using the 12 volunteers with samples at each of the three time points.

Following tests for normality of distribution (Kolmogorov-Smirnov) and equality of variances (Levene Median), data were analyzed using a two-way repeated-measures analysis of variance (sample site X time). Tukey’s post hoc procedure was used to identify differences among means following significant main and/or interaction effects. Sample size was estimated based on sweat zinc concentrations of DeRuisseaux et al. (14). When conventional α = 0.05 and β = 0.20 values were applied, 12 subjects were estimated to provide sufficient power to detect a 30% change in sweat zinc concentrations (14) when coupled to a within-subjects coefficient of variation (CV_within) <55% (effect size ~0.50). The latter value was estimated from the measured between-subjects CV (CV_between) of 55% for sweat zinc, since CV_within is less than CV_between for nearly all biological measures in...
humans (17). However, due to potential subject attrition and lost data, 16 volunteers were tested. All analyses were conducted using SigmaStat 3.5 (Systat Software). Data are presented as means ± SD.

RESULTS

All subjects completed the 3 h of exercise-heat stress. Data for men (n = 14) and women (n = 2) were combined as sweat mineral concentrations do not appear sex dependent (14, 38). Subjects consumed 1.0 ± 0.3 liters of water during the 3 h of exercise-heat stress and the body mass loss was 2.0 ± 0.4 kg, or −2.4 ± 0.3% of initial body mass. Whole body sweat rate averaged 0.33 ± 0.05 l·m⁻²·h⁻¹ over the 3-h period. Oxygen consumption was stable over the 3 h and averaged 19.2 ± 1.9 ml·kg⁻¹·min⁻¹. Exercise heart rates and core temperatures averaged 124 ± 17 beats/min and 37.93 ± 0.39°C during the last 10 min of each 50-min exercise bout, respectively. The serum mineral concentrations were stable and were generally within the typical population reference intervals (13), and similar to the findings of others during exercise (1, 28, 32, 39). The mean 3-h serum mineral concentrations were Ca, 2.3 ± 0.2 mmol/l; Cu, 14.4 ± 3.0 μmol/l; Fe, 24.2 ± 7.7 μmol/l; K, 4.3 ± 1.2 mmol/l; Mg, 0.7 ± 0.1 mmol/l; Na, 132 ± 3 mmol/l; and Zn, 13.2 ± 3.1 μmol/l (Fig. 1).

The TDF mineral concentrations of Ca, Cu, K, Mg, and Na exhibited small intrasubject variation through the exercise heat exposure at 2.3 ± 0.2 mmol/l, 6.4 ± 2.2 μmol/l, 4.3 ± 1.3 mmol/l, 0.5 ± 0.1 μmol/l, and 124.9 ± 16.7 μmol/l, respectively (Fig. 1). The TDF concentrations of Fe increased from hour 2 to hour 3 (15.1 ± 5.7 to 17.7 ± 6.9 μmol/l, P < 0.05) (Fig. 1), and Zn concentrations in TDF decreased from exercise

![Fig. 1. Comparison of transdermal fluid (TDF), serum, and sweat pouch mineral concentrations during 180 min of exercise heat stress. Gray banded areas are typical population reference intervals for serum (13). All minerals are in Système International (SI) units. †Hour 3 TDF is greater than hour 2 and hour 1 (P < 0.05). #Hour 1 TDF is greater than hour 2 and hour 3 (P < 0.05); n = 12.](http://jap.physiology.org/doi/10.1152/jappl.00662.2010)
hour 1 to hour 2 (16.2 ± 5.5 to 13.2 ± 3.6 μmol/l, P < 0.05) and remained stable into hour 3 (13.8 ± 3.2 μmol/l) (Fig. 1). TDF mineral concentrations were significantly lower than serum for Ca, Cu, and Mg, whereas TDF Zn, Fe, Na, and K mineral concentrations were similar to serum (Fig. 1).

The back sweat pouches collected 2.2 ± 1.0 ml of sweat per hour. The local sweat rate did not significantly change from hour 1 to hour 2 but decreased from hour 2 to hour 3 (0.51 ± 0.15, 0.43 ± 0.19, and 0.30 ± 0.17 l-m⁻²-h⁻¹, P < 0.05). Pouch mineral concentrations were stable through the exercise heat exposure for Ca (0.36 ± 0.16 mmol/l), Cu (0.72 ± 0.39 μmol/l), Fe (0.28 ± 0.29 μmol/l), K (5.25 ± 0.97 mmol/l), Mg (0.05 ± 0.02 mmol/l), and Zn (3.64 ± 2.64 μmol/l). Na concentrations significantly increased (P < 0.05) from hour 1 (54.7 ± 11.4 mmol/l) to hour 3 (69.4 ± 22.8 mmol/l). Sweat pouch mineral concentrations were in all cases significantly lower than serum and TDF levels except for K where they were similar (Fig. 1).

The arm bags collected sweat for the first 32.0 ± 7.0, 24.0 ± 8.0, and 20.5 ± 10.0 min of each hour of exercise and the estimated regional sweat rate increased significantly (P < 0.05) from the hour 1 to hour 2, then was similar to hour 3, 0.17 ± 0.11, 0.29 ± 0.15, and 0.29 ± 0.14 l-m⁻²-h⁻¹, respectively. The initial sweat sample had significantly higher Ca, Cu, Mg, and Zn than subsequent samples, whereas K and Na increased, and Fe did not change over time (Fig. 2). During hour 1 the Ca, Cu, and Zn concentrations were similar to the initial TDF concentrations but fell to levels approximating those measured from upper back sweat pouch at hour 2 and hour 3 samples. Hour 1 arm bag concentrations of Fe, K, and Na were significantly higher than sweat pouch concentrations during 180 min of exercise heat stress. All minerals are in SI units. *Arm bag significantly different from sweat pouch (P < 0.05). †Significantly different from hour 1 (P < 0.05); n = 12.
The novelty of the present investigation is the concurrent, serial determinations of TDF, blood serum, and two-site (arm and scapular back regions) sweat mineral concentrations. The subjects performed three 50-min exercise bouts in an environment that produced near-continuous sweating. Sweat samples were obtained using widely employed techniques (4, 9, 19), and mineral concentrations were measured using the most precise analytical technique available. Additionally, precautions to avoid external contamination of minerals were taken, and any possible contaminator of samples was analyzed for mineral content. Furthermore, all subjects participated in a 10-day heat acclimation protocol beforehand to maximize heat tolerance, reduce task attrition, and for consistency for comparisons to avoid external contamination of minerals were taken, and any possible contaminator of samples was analyzed for mineral content. Furthermore, all subjects participated in a 10-day heat acclimation protocol beforehand to maximize heat tolerance, reduce task attrition, and for consistency for comparison to like studies (27).

A primary observation was that TDF mineral concentrations were stable over several hours of persistent sweating. There were modest changes in TDF Fe and Zn concentration. The TDF concentrations of Fe increased (15%) from hour 2 to hour 3 (15.1 ± 5.7 to 17.7 ± 6.9 μmol/l, P < 0.05) (Fig. 1) and Zn concentrations in TDF decreased (18%) from exercise hour 1 to hour 2 (16.2 ± 5.5 to 13.2 ± 3.6 μmol/l, P < 0.05) and remained stable into hour 3 (13.8 ± 3.2 μmol/l) (Fig. 1). These small shifts were not associated with alterations in the scapular sweat iron or zinc or with circulating serum concentrations (Fig. 1). Therefore, the physiological importance of the small but statistically significant changes in TDF Fe and Zn concentrations is uncertain.

It was anticipated that there would be substantial reductions in sweat iron, zinc, copper, and magnesium after the initial sweat sample. This expectation was based on prior findings that sweat iron and zinc collected during the second hour of exercise was ~40% lower than sweat collected during the initial hour, with similar patterns over time for copper and magnesium (14, 27). Contrary to the expectation, upper back sweat iron, zinc, copper, and magnesium as well as calcium and potassium concentrations were stable between samples (Fig. 1). The present study utilized a similar sweat collection site, method, and environmental conditions as Montain et al. (27); however, the current study incorporated more rigorous skin preparation procedures to minimize contamination of sweat from minerals on the skin surface and presumably trapped in the sweat duct. Therefore, the thorough cleaning procedures used in the present study to remove contaminants are thought to account for the lack of decreasing sweat mineral concentrations previously reported (14, 27, 33, 38). In contrast to the initial sweat samples in the sweat pouch, the arm bag contained sweat with higher mineral concentrations during the first hour of exercise (Fig. 2). Arm bag sweat Ca, Cu, Mg, and Zn then declined 26%, 76%, 49% and 41% from hour 1 to hour 2, reductions very similar in magnitude to earlier studies (10, 14, 27, 38).

After mineral concentration declined from hour 1 to hour 2, the sweat composition from samples collected at the arm and upper back were quite similar (Fig. 2). If TDF in the upper back is representative of the TDF in the forearm and hand, then the decreased mineral concentration in the arm sweat is independent of changes in the TDF. It is more likely that the initial burst of concentrated sweat is primarily the consequence of sweat mixing with minerals trapped in the sweat pore or residing on the skin surface than transpiring from alterations in TDF or other unknown mechanisms. Skin desquamation and mineral residues have long been recognized in contributing to the sweat mineral concentrations. Brune et al. (7) documented that soap, dirt, or metabolic by-products of skin flora increase the mineral content of the initial sweat loss. Moreover, Hohnadel et al. (20) reported that small amounts of minerals remain on the skin surface after a standardized washing procedure. Although the same rigor was used to clean the arm and hand as the scapular region of the back, the larger surface area and physical characteristics of the hand make cleaning of this anatomic region more difficult. It is likely that minerals remained on the arm and hand and contributed to the elevated initial concentrations in the arm bag compared with the back.

The initial mineral concentrations found in the arm bag compare favorably to arm bag values reported in the literature over the last 20 years (14, 29, 34, 35, 38) although these values have wide intersubject variability (Fig. 2). The concentration of minerals in arm bags is often much greater than values reported for sweat pouches (23, 27, 30), which assess mineral concentrations at localized sites. During extended sweating, the arm bag mineral concentrations approached those of the pouch (Fig. 2). It is at these extended sweating time points that mineral concentrations from the present study are similar to whole body concentrations reported by others (7, 21, 36). For example, Hoshi et al. (21) reported sweat calcium, magnesium, copper, iron, and zinc concentration obtained via whole body wash down of 0.1 ± 0.1 mmol/l, 0.2 ± 0.1 mmol/l, 0.6 ± 0.4 mmol/l, 0.6 ± 0.3 mmol/l, and 5.2 ± 4.7 μmol/l, respectively, whereas Shirreffs and Maughan (36) reported sweat calcium and magnesium concentrations of 1.3 ± 0.9 mmol/l and 0.5 ± 0.5 mmol/l, respectively. In the only known study in which serial measures of whole body sweat have been obtained, Brune et al. (7) reported that Fe concentration fell 56% between an initial 15-min collection and a second subsequent sample (0.91 ± 0.13 to 0.40 ± 0.00 mmol/l). These results imply that mineral concentrations from sweat samples obtained during the initial minutes of sweating should not be used to extrapolate whole body sweat mineral losses associated with prolonged exercise as most mineral needs will likely be grossly overestimated.

Serum minerals were assessed to provide a reference for TDF and sweat values. More specifically, it was anticipated that serum mineral concentrations would remain stable over time and that sweat concentrations would fall after the initial sample. In agreement with preceding literature (1, 28, 32, 39), serum minerals were stable over time and the values were generally within the typical population reference interval (13). One exception was serum sodium which measured consistently in the hyponatremic range (Fig. 1). There is no explanation for the low serum concentrations, as subjects were not drinking excessive quantities of water before or during exercise and they were entirely asymptomatic. The relatively low values were also not due to instrument error as the same outcomes were produced when the samples were analyzed on a separate analytical instrument. Importantly, it is unlikely that the concentration of sweat minerals, the primary interest in this study, would be affected by low serum sodium values.
A limitation of the present study was the reliance on TDF collection to represent the fluid surrounding the sweat gland. The TDF sampling technique created pores a~80-μm deep into the skin. Therefore, the TDF technique sampled from the epidermis rather than directly from the dermal and hypodermal fluid spaces where the sweat coil resides. Minerals are distributed unevenly across the epidermis and dermal layers and there may be differences in concentrations consequent to the sampling technique. Ideally, TDF samples would have been obtained from the forearm for comparison to arm bag sweat samples. Pilot testing of TDF sampling from this body location had an ~10% success rate in creating an acceptable poration to sample TDF. The low success rate is presumably due to a thicker epidermal layer of the forearm compared with the upper back. A separate limitation is that TDF sample analysis measured total mineral element concentration rather than restricting the analysis to only those forms (e.g., free or unbound) that could pass through the sweat gland. As 97–99% of the body Zn and Fe is bound to relatively large proteins (as referenced in 12), measurement of total mineral concentration in the TDF may have lacked the sensitivity to capture shifts in the free or unbound forms capable of entering the sweat gland and being secreted onto the skin surface.

The practical implications of this work relate to the importance of accurately estimating sweat mineral losses for determination of dietary reference intakes (8). The present findings indicate that surface contamination may result in overestimations of mineral losses during prolonged heat and exercise induced sweating. Additionally, an extensive cleaning protocol may not sufficiently remove all surface contaminants from an area widely used for sweat collection. Therefore, when accurate estimations of sweat minerals are of interest it is recommended that initial sweat be removed and subsequent samples be used for analysis.

Conclusion. During 3 h of exercise-heat stress, TDF concentrations of minerals were stable. Sweat minerals sampled from a very thoroughly cleaned scapular surface also produced stable mineral sweat concentrations. Sweat collection using the arm bag technique produced a significantly elevated mineral concentration compared with the sweat pouch at the initial sample, but further samples decreased in mineral concentrations to levels similar to the samples obtained from scapular sweat pouches. The elevated initial arm bag mineral concentrations are most likely related to surface contamination. Therefore, sweat mineral concentrations obtained from samples collected during the initial minutes of sweating should not be used to extrapolate to whole body sweat mineral losses.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

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