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13. ABSTRACT <i>(Maximum 200 words)</i> Purpose: This study examined the impact of 10-days of exercise-heat acclimation on sweat mineral concentrations. Methods: Eight male subjects walked on a treadmill at 3.5 mph, 4% grade for 100 continuous minutes or until rectal temperature reached 39.5°C on 10 consecutive days in an environmental chamber set at 45°C, 20% relative humidity. Arm sweat samples were collected during the first 30 minutes of exercise-heat stress on days 1 and 10 using a polyethylene arm glove. Results: Final core temperature and HR values were significantly lower (P< 0.05) on day 10 versus day 1. Whole body sweating rates increased by ~6% (P= 0.12). Sweat sodium concentration on day 10 (36.22 ± 7.22 mmoloL-1) was significantly lower than day 1 (54.49 ± 16.18 mmoloL-1) (P< 0.05). Sweat mineral concentrations of calcium (~29%), copper (~50%), and magnesium (~43%) were also significantly lower on day 10 versus day 1 of heat acclimation (P< 0.05). A trend for lower sweat iron (~75%; P= 0.07) and zinc (~23%; P= 0.10) concentrations were observed from day 1 to day 10. The estimated hourly sweat mineral losses (arm concentration x whole-body sweat rate) were reduced for calcium (~27%), copper (~46%), magnesium (~42%) (P< 0.05), but not iron (75%) and zinc (~16%) (P> 0.05) from day 1 to day 10. Conclusion: Exercise-heat acclimation conserves arm sweat mineral concentrations and possibly whole-body sweat losses of calcium, copper, and magnesium; and may reduce sweat iron and zinc concentrations.			
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Effect of Heat Acclimation on Sweat Minerals

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ABSTRACT

CHINEVERE, T. D., R. W. KENEFICK, S. N. CHEUVRONT, H. C. LUKASKI, and M. N. SAWKA. Effect of Heat Acclimation on Sweat Minerals. *Med. Sci. Sports Exerc.*, Vol. 40, No. 5, pp. 886–891, 2008. **Purpose:** This study examined the impact of 10 d of exercise-heat acclimation on sweat mineral concentrations. **Methods:** Eight male subjects walked on a treadmill at 3.5 mph, 4% grade for 100 continuous minutes or until rectal temperature reached 39.5°C on 10 consecutive days in an environmental chamber set at 45°C, 20% relative humidity. Arm sweat samples were collected during the first 30 min of exercise-heat stress on days 1 and 10 using a polyethylene arm glove. **Results:** Final core temperature and HR values were significantly lower ($P < 0.05$) on day 10 versus day 1. Whole-body sweating rates increased by approximately 6% ($P = 0.12$). Sweat sodium concentration on day 10 (36.22 ± 7.22 mM) was significantly lower than day 1 (54.49 ± 16.18 mM) ($P < 0.05$). Sweat mineral concentrations of calcium (~29%), copper (~50%), and magnesium (~43%) were also significantly lower on day 10 versus day 1 of heat acclimation ($P < 0.05$). A trend for lower sweat iron (~75%; $P = 0.07$) and zinc (~23%; $P = 0.10$) concentrations were observed from day 1 to day 10. The estimated hourly sweat mineral losses (arm concentration \times whole-body sweat rate) were reduced for calcium (~27%), copper (~46%), and magnesium (~42%) ($P < 0.05$), but not iron (75%) or zinc (~16%) ($P > 0.05$), from day 1 to day 10. **Conclusion:** Exercise-heat acclimation conserves arm sweat mineral concentrations and possibly whole-body sweat losses of calcium, copper, and magnesium, and may reduce sweat iron and zinc concentrations. **Key Words:** CALCIUM, COPPER, IRON, MAGNESIUM, ZINC

Heat acclimation from repeated prolonged exposure to exercise-heat stress will induce biological adaptations that reduce physiological strain, improve thermal comfort, and increase exercise tolerance time in the heat (24,37). It is well established that heat acclimation reduces sweat sodium concentration (1,4,14,23), but since sweating rate increases (33), there can be significant whole-body sodium deficits that need to be replaced (20). It is known that sweat contains minerals; however, the effects of heat acclimation on sweat mineral concentrations, or whole-body sweat mineral losses, have not been well studied (10–12).

The most thorough examination of sweat mineral changes associated with heat acclimation is from a series of studies (10–12) that observed a decline in the losses of sweat calcium, copper, zinc, and concentration of iron following heat acclimation. However, these observations were made on only three men and were not examined for statistical differences. In addition, when reviewing all studies reporting the impact of heat acclimation on sweat mineral con-

centrations, the findings are contradictory. Hoshi et al. (19) reported significantly lower sweat concentrations of magnesium and copper in the summer versus winter season. In contrast, Omokhodion and Howard (31) reported no differences in sweat calcium, copper, and zinc concentrations in individuals living in the tropics compared with those living in temperate regions.

The Institute of Medicine has raised concern that current mineral dietary reference intakes (DRI), which are established for the general population, may be inadequate for certain occupational segments (e.g., athletes, soldiers, etc.) who work hard for prolonged periods in hot climates (21). Chronic, profuse sweating may raise the potential for clinical mineral deficiencies when long-term intake is not sufficient to balance substantial mineral losses in sweat (6,21). Indeed, some studies have shown lower-than-normal blood mineral levels in athletes (5,15,17,30), which could be the result of excessive mineral losses in sweat. Thus, sweat mineral losses may need to be accounted for in future DRI (7,26), much as they have been recently modified for sodium (21). If conservation of sweat minerals (relative to increased sweating rate) occurs as a result of heat acclimation, then this mineral conservation may minimize or obviate the need for increased dietary mineral intake in individuals frequently exposed to hot climates. Therefore, this study examined the impact of exercise-heat acclimation status on sweat mineral concentrations. As heat acclimation has previously been shown to reduce sweat sodium concentration (1,4,14), we hypothesized that 10 d of exercise-heat

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acclimation would have a similar impact on other sweat minerals.

METHODS

Subjects. Eight healthy men (mean \pm SD, age 20.2 ± 2 yr, height 1.7 ± 0.1 m, body mass 74.6 ± 6.5 kg, BSA 1.94 m²) volunteered to participate in this study. The subjects were not accustomed to exercise-heat stress and, thus, were not acclimatized to hot weather. This study was approved by the U.S. Army Research Institute of Environmental Medicine's scientific and human use review committees. Written informed consent was obtained from each volunteer after being informed of the purpose, experimental procedures, and known risks of the study. Investigators adhered to U.S. Army Regulation 70-25 and U.S. Army Medical Research and Materiel Command Regulation 70-25 on the Use of Volunteers in Research.

Design. Experiments were conducted in the northeastern United States ($> 42^\circ$ latitude) during the late winter/early spring, where the high daytime air temperatures never rose above 14°C . Volunteers performed exercise-heat acclimation (24) by walking on a treadmill (1.56 m·s⁻¹, 4% grade) in a 45°C , 20% rh (31.3°C WBGT) environment for 10 d while wearing a T-shirt, shorts, and athletic shoes. Volunteers discontinued walking on reaching the first of three criteria: 1) 100 continuous minutes of exercise, 2) core temperature of 39.5°C , or 3) voluntary cessation.

Procedures. Volunteers consumed a small, standardized meal 2 h before and drank 250 mL of water 1 h prior to beginning each heat acclimation session. Nude body weights were recorded after self-placement of a flexible rectal thermistor (Yellow Springs Instruments, Yellow Springs, OH) inserted 10 cm beyond the anal sphincter for core temperature (T_{re}) measurements. After dressing, a heart rate monitor (Polar a₃, Polar Electro, Inc, Woodbury, NY) was secured around the chest. Core temperature and heart rate were monitored continuously throughout all heat stress exposures. Upon commencement of each session, volunteers undressed, were weighed (with rectal thermistor), and then drank sufficient liquid to return within 1% of their first morning weight to ensure that they did not undergo a progressive dehydration that would negatively affect core temperature and heart rate responses (36).

On the first and last days of heat acclimation, volunteers' right arms were washed by a technician wearing nitrile gloves. Arms were first rinsed thoroughly with tap water, washed with soap, and rinsed again with tap water. A final rinse was made with liberal amounts (5–6 L) of mineral-free (verified by previous analysis) deionized water (18.2 Mohm) and allowed to air dry. A polyethylene arm glove was placed on the arm and secured with a Velcro strap just below the axilla. Subjects then entered the environmental chamber to begin treadmill walking. After 30 min of walking, the arm gloves were removed to avoid hidromeio-

sis (8) and possible increased mineral reabsorption due to increased skin temperature of the encapsulated arm (38). Sweat samples were withdrawn from the arm gloves using a 10-mL syringe and stainless-steel needle. Samples were then stored (3°C) in screwtop Cryule vials prior to analysis. All vials and needles were prewashed overnight in a 5% nitric acid solution, followed with a deionized water rinse, and allowed to air dry. Prior to analysis, sweat samples were centrifuged to remove cellular debris. 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 6 M nitric acid was added to ensure liberation of all cations from all bounded proteins, amino acids, and contaminants. Sample tubes were capped and stored at room temperature for 12 h. Sweat electrolyte and mineral composition was measured using inductively coupled plasma emission spectrography (PerkinElmer model 3300; Norwalk, CT). Mineral analyses of the deionized water used during all washing procedures yielded < 0.003 mg·L⁻¹ for all minerals except calcium (< 0.15 mg·L⁻¹). All sweat mineral concentrations are reported as millimoles per liter. Sweat mineral losses were estimated as the product of arm sweat mineral concentration (mg·L⁻¹) \times the whole-body sweating rate (L·h⁻¹) (13,39).

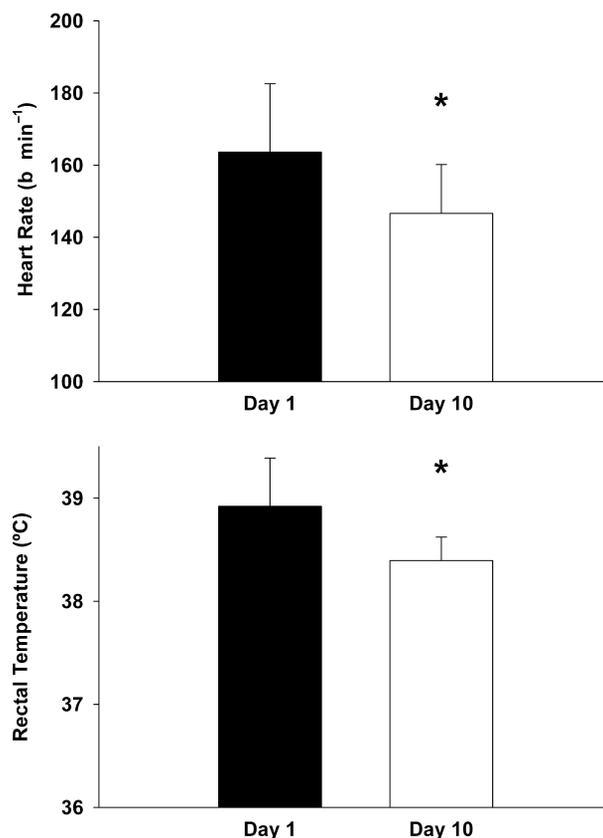


FIGURE 1—Final heart rate and rectal temperature comparisons on day 1 and day 10 of heat acclimation. * Significantly lower than day 1 ($P < 0.05$). Values are means \pm SD.

TABLE 1. Comparison of mean and ranges of sweat mineral concentrations and estimated whole-body losses after 10 d of exercise-heat acclimation in a 45°C, 20% rh environment.

	Concentrations (mM)		P	Estimated Losses (mg·h ⁻¹)		P
	Day 1	Day 10		Day 1	Day 10	
Ca	1.61 ± 0.70 [0.62–2.47]	1.15 ± 0.53 [0.54–2.06]	0.01	75 ± 35 [30–132]	55 ± 23 [28–95]	0.03
Cu	0.01 ± 0.00 [0.00–0.01]	0.00 ± 0.00 [0.00–0.01]	0.01	0.41 ± 0.29 [0.13–1.01]	0.22 ± 0.17 [0.10–0.67]	0.01
Fe	0.01 ± 0.01 [0.00–0.02]	0.00 ± 0.00 [0.00–0.00]	0.07	0.32 ± 0.39 [0.00–1.06]	0.08 ± 0.09 [0.00–0.27]	0.08
Mg	0.30 ± 0.17 [0.09–0.57]	0.17 ± 0.15 [0.00–0.41]	0.00	8.36 ± 4.39 [2.28–13.99]	4.85 ± 3.91 [0.00–10.81]	0.01
Zn	0.01 ± 0.01 [0.00–0.03]	0.01 ± 0.01 [0.00–0.02]	0.10	0.88 ± 0.58 [0.29–1.93]	0.74 ± 0.55 [0.26–2.09]	0.15

Data are means ± SD [range]. Significance set at $P < 0.05$.

Statistical Analyses

Day 1 and day 10 final HR and T_{re} values, sweating rate, sweat mineral concentrations, and losses were analyzed with paired t -tests. We estimated that eight subjects would provide sufficient statistical power ($\beta = 0.20$) to detect a difference in T_{re} between days 1 and 10 larger than the typical standard deviation ($\sim 0.20^\circ\text{C}$) (9). In addition, eight subjects would provide sufficient power to detect a change in sweat sodium concentration equal to the typical day-to-day variability within subjects ($\sim 10\%$) (18). Statistical significance was accepted at $P < 0.05$.

RESULTS

Heat acclimation. Pretrial body weights for day 1 (74.8 ± 6.9 kg) and day 10 (74.6 ± 6.8 kg) were not different ($P > 0.05$). Exercise tolerance time increased an average of 8 min on day 10 (97 ± 8 min) compared with day 1 (89 ± 16 min), but this change was not significant ($P > 0.05$). Final T_{re} and HR values were significantly lower on day 10 compared with day 1 ($P < 0.05$) (Fig. 1). The

mean whole-body sweating rate was 1.16 ± 0.19 L·h⁻¹ on day 1 and 1.23 ± 0.17 L·h⁻¹ on day 10, which was not significantly different ($P = 0.12$).

Sweat electrolytes and minerals. Sweat sodium and potassium concentrations have been extensively studied and were used as markers to verify that heat acclimation induced the expected responses of these electrolytes. Sweat sodium concentration declined from 54.49 ± 16.18 mM on day 1 to 36.22 ± 7.22 mM on day 10 ($P < 0.05$). Estimated sweat sodium losses were significantly lower on day 10 (1008 ± 220 mg·h⁻¹) versus day 1 (1413 ± 339 mg·h⁻¹) ($P < 0.05$). No difference was observed between day 1 (11.72 ± 4.44 mM) and day 10 (11.26 ± 2.13 mM) for sweat potassium concentrations ($P > 0.05$). Similar to previous findings, the sodium/potassium ratio declined from 2.74 to 1.89 in response to heat acclimation (25).

Table 1 provides mean values for sweat mineral concentrations and estimated sweat mineral losses during exercise-heat stress on day 1 and day 10 of heat acclimation. Sweat concentrations and estimated losses of calcium, copper, and magnesium were all significantly lower on day 10 versus day 1 ($P < 0.05$) (Table 1). Mean sweat concentrations of iron and zinc tended to decline (75% and 23%, respectively) from day 1 to day 10, but these changes were not significantly different ($P > 0.05$), nor were estimated losses (Table 1). It is likely, given the consistency and magnitude of the decline in sweat iron and zinc, that statistical significance was not reached because of the limited sample size and also because of the higher individual variability of these minerals. Figure 2 presents the individual data for sweat concentrations and demonstrates the consistency of the lower mineral concentrations after heat acclimation. Sweat concentration of calcium, copper, and magnesium declined in seven of eight subjects, five of eight for iron, and six of eight for zinc.

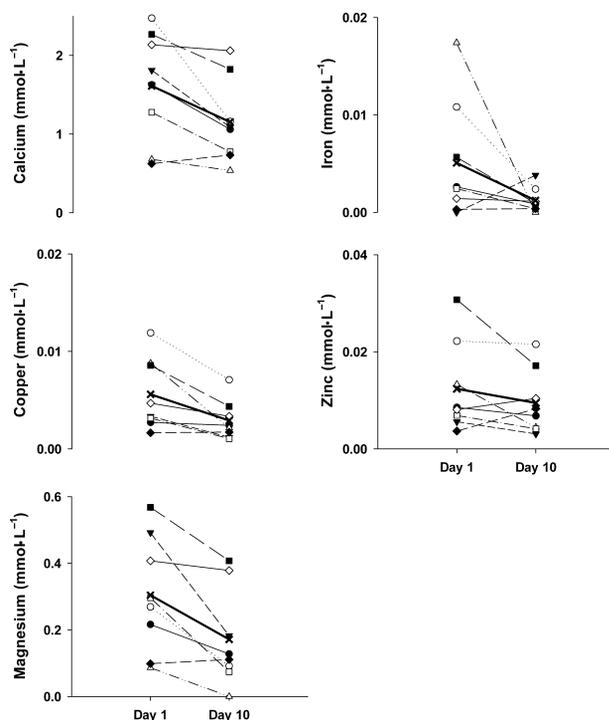


FIGURE 2—Individual and mean sweat mineral concentrations on day 1 and day 10 of heat acclimation. Solid, bold line indicates mean.

DISCUSSION

This was the first study to provide extensive data on sweat mineral concentrations and losses following heat acclimation. Sweat mineral changes following heat acclimation have not been thoroughly studied, and conclusions on sweat mineral changes have been made only through inference of seasonal variations. In agreement with our hypothesis, the primary finding was that heat acclimation induced significantly lower sweat concentrations and estimated whole-body sweat losses for calcium, copper, and magnesium. Although sweat concentrations for iron and zinc tended to be lower on day 10, the

results were not significantly different. However, when observing individual data (Fig. 2), lower sweat mineral concentrations were observed as a whole after heat acclimation.

Since sweat sodium concentrations are influenced by hydration status (26), the present study controlled for subject hydration levels by ensuring that fluid intake was sufficient to maintain baseline body weight levels. In agreement with previous studies (1,23), the sweat sodium concentrations were reduced following heat acclimation. Heat acclimation sweat sodium conservation appears to be due to increased sodium ion conservation within the reabsorptive duct of the sweat gland possibly mediated by aldosterone (34,35). No data are available on the mechanisms for conservation on other sweat minerals, but might also be related to increased sweat gland reabsorption.

Our observation of reduced sweat copper concentrations after heat acclimation are consistent with findings from previous studies (12,19); however, our observation of lower calcium and magnesium concentrations after heat acclimation has mixed support (10,11,19). Consolazio et al. (11) found that whole-body sweat calcium losses (did not report sweat concentrations) declined by about 50% after 16 d of heat acclimation. In another study, Consolazio et al. (10) found that that sweat magnesium concentration declined by about 8% after 16 d of heat acclimation. The sweat mineral data from both Consolazio studies (10,11) should be interpreted cautiously because of their small sample size ($N = 3$) and lack of statistical comparisons. Hoshi et al. (19) reported no difference (10 sedentary graduate students) in sweat calcium concentrations, but lower (~66%) sweat magnesium concentrations in summer compared with winter in 10 sedentary subjects. However, it was only assumed that heat acclimation was “absent” in winter and “present” in summer. In addition, Hoshi et al. (19) employed a nonstandardized exercise intensity between the seasonal trials with no ambient heat stress to induce sweating.

The studies of Consolazio et al. (10,11) employed sweat collection site preparation procedures that may have confounded their results. They rinsed without washing the collection site with distilled water which may not sufficiently remove exfoliated skin cells that could contain significant amounts of some minerals (31). In addition, the mineral concentration of their distilled water was not reported, leaving open the possibility that mineral contamination was introduced. In the present study, the skin was thoroughly washed and rinsed with liberal amounts of deionized water that contained negligible amounts of minerals.

Sweat zinc and iron might be conserved in response to heat acclimation, even though our findings for sweat iron and zinc concentrations were not statistically different. The average sweat zinc and iron concentration declined by 23 and 75%, respectively, and both minerals declined in six of eight of subjects on day 10 (Fig. 2). In addition, previous studies support the possibility of sweat zinc and iron being conserved with heat acclimation. Consolazio et al. (12) observed whole-body sweat zinc losses to decline by about

84% during 16 d of heat acclimation. In another study, those investigators (10) observed the arm sweat iron concentrations to decline by about 30% during 16 d of heat acclimation. Omokhodian and Howard (31) reported sweat zinc concentrations of about $0.5 \text{ mg}\cdot\text{L}^{-1}$ for heat-acclimatized persons living in tropical climates, which are comparable with our postacclimation concentrations.

The mechanisms of sweat mineral conservation are unclear, although previous data suggest sweat sodium conservation following heat acclimation involves increased sodium ion reuptake within the reabsorptive duct of the sweat gland (34,35). If, like sodium, the decline in sweat mineral concentrations can be attributed to mineral reabsorption versus excess dermal loss, then it remains to be shown if eccrine sweat glands are capable of reabsorbing other minerals. Studies have shown that sweat zinc (13,27) and iron (13,39) concentrations decline over time during a single bout of prolonged ($\geq 1 \text{ h}$) exercise, leading to speculation that initial sweating might include mineral-containing cellular debris and external mineral contamination. In support of this supposition, steady-state sweat iron losses were not achieved until after several repeated 30-min (separated by 15-min breaks in temperate conditions) sauna bath exposures (2). Brune et al. (2) suggested that prewashing the collection site may not completely remove iron trapped within the sweat ducts (2). By extension of this reasoning, perhaps repeated, daily sweating continuously flushes minerals trapped in sweat pores and does not allow sufficient time for dermal mineral replenishment. In contrast to this argument, it has been shown that plasma minerals either do not change or increase during repeated days of prolonged exercise in a warm environment (16). Assuming that plasma and interstitial fluid mineral concentrations are similar raises the possibility that sweat mineral conservation might be related to sweat gland reabsorption. Therefore, the measurement of interstitial fluid mineral concentrations pre and post heat acclimation would be one indirect approach to answer the question about glandular mineral reabsorption *in vivo*. However, current capabilities only allow for microvolume collection over extended periods. Advances in analytical technology allowing for precise measurement of these minerals from microvolumes should assist in answering these questions.

Applying the results of this study to male workers performing 8 h of work at similar workloads in hot conditions would lead us to conclude that the mineral losses in sweat could contribute up to about 40% of the DRI for calcium, about 200% for copper, about 80% for iron, about 10% for magnesium, and about 50% for zinc. However, the validity of calculating whole-body sweat mineral losses from local sweat mineral concentrations and whole-body sweating rates should be viewed cautiously. First, regional sweat mineral concentrations vary widely (2) and have been reported to be higher than whole-body sweat mineral concentrations (7,22,32). Secondly, arm sweating rates differ from other body regions being much lower than torso sweating rates (29). Finally,

sweat mineral concentrations may vary as a function of sweating rate, as does sweat sodium where concentrations increase with sweating rate (1). In addition, despite that calculation of whole-body mineral losses from regional sweat mineral concentrations may overestimate actual whole-body losses, dismissal regarding changes to future mineral DRI is premature. Consider, for example, that heat acclimated individuals exposed to hot climates for many hours would still accrue appreciable absolute sweat mineral losses. Therefore, future studies calculating whole-body sweat mineral losses, and possibly balance studies, may be required for future DRI recommendations.

CONCLUSIONS

This study demonstrates that 10 d of exercise-heat acclimation significantly reduces sweat mineral concentra-

tions and possibly whole-body losses of calcium, copper, and magnesium. Although exercise-heat acclimation did not significantly reduce mean sweat concentrations of iron and zinc, the individual data suggest a trend for reduced concentrations in most subjects. Future studies should further investigate whole-body sweat mineral losses and determine possible mechanism(s) responsible for changes in sweat minerals following heat acclimation.

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The results of the present study do not constitute endorsement by the American College of Sports Medicine. The view, opinions, and/or findings contained in this report are those of the authors and should not be construed as an official Department of the Army position, or decision, unless so designated by other official documentation. Approved for public release; distribution unlimited.

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