The purpose of this study was to examine the effects of a 2-hour exercise bout on sweat iron and zinc concentrations and losses in males and females. Nine male and 9 female recreational cyclists exercised at ~50% VO\textsubscript{peak} in a temperate environment (Ta = 23 °C, RH = 51%). Sweat samples were collected for 15 min during each of four 30-min exercise bouts. No significant differences were observed between males’ and females’ sweat iron or zinc concentrations or losses. Sweat iron concentrations decreased significantly between 60 and 90 min of exercise. Sweating rates increased significantly from 30 to 60 min and remained constant during the second hour. Sweat iron losses were significantly lower during the second hour (0.042 mg/m\textsuperscript{2}/h) than the first hour of exercise (0.060 mg/m\textsuperscript{2}/h). Sweat zinc concentrations also decreased significantly over the 2-hour exercise bout. Dietary intakes of iron and zinc were not significantly correlated to sweat iron and zinc concentrations. Sweat iron and zinc losses during 2 hours of exercise represented 3% and 1% of the RDA for iron and 9% and 8% of the RDA for zinc for men and women, respectively. These results suggest a possible iron conservation that prevents excessive iron loss during prolonged exercise.

**Key Words:** gender, endurance exercise, sweat rate, mineral losses, iron intake, zinc intake

**Introduction**

There is limited research examining the loss of trace minerals like iron and zinc during exercise of long duration. Most studies have measured sweat iron and/or zinc concentrations during exercise lasting less than one hour (1, 6, 10, 12). Large losses of iron and zinc in sweat during prolonged exercise could lead to negative iron and zinc balance, especially in individuals with low dietary intakes of these minerals.

Previous research in our laboratory found that both sweat iron and zinc concentrations decreased significantly from the first 30 min to the end of 60 min of exercise (14, 16). Sweat iron also decreases with repeated resting exposures to a hot environment (3), and lower sweat iron concentrations were found after subjects...
exercised 2 h/d for several days in a hot environment (17). Brune et al. (3) suggested the sweat iron concentration was elevated in the initial sweat secretions due to the presence of cellular iron debris and environmental iron. It is also possible that iron may be conserved during periods of prolonged sweating thus limiting the amount of iron lost in prolonged exercise. Sweat zinc concentrations decrease during periods of low dietary zinc intake (11). On the other hand, sweat zinc concentration during maximal exercise increased in athletes following 2 months of competition (6). Cordova and Navas (6) suggested that zinc excretion increases during stressful exercise due to a redistribution of zinc in the body or muscle damage.

The purpose of the present study was to examine the effects of a 2-hour exercise bout on sweat iron and zinc concentrations and losses in men and women. It was hypothesized that sweat iron and zinc concentrations would decrease during the second hour of exercise compared to the first hour. A secondary purpose of this study was to examine the relationship between dietary intakes of iron and zinc and their respective sweat concentrations.

Materials and Methods

Subjects

Nine male and 9 female recreational cyclists from the Florida State University and the surrounding area volunteered to serve as subjects for this study. All subjects completed a health history form and gave written informed consent prior to participating in the study. The study was approved by the Florida State University Institutional Review Board.

Procedures

During the subjects’ initial visit to the laboratory, peak oxygen uptake was measured on a Monark 868 cycle ergometer. Using a continuous protocol, subjects pedaled at 80 rpm with a resistance of 1.0 kp, and the resistance was increased 0.5 kp every 2 min until the subject could no longer maintain cycling cadence. Heart rate (Polar CIC, Inc., Port Washington, NY) and expired air were monitored continuously with a Parvo Medics Metabolic System (Consentius Technologies, Sandy, UT). Oxygen uptake values were averaged over 30-s intervals.

During the second visit, subjects completed four 30-min exercise bouts at 50% VO2peak in a temperate environment (Ta = 22.9 ± 1.3 °C; RH = 51 ± 5%). Oxygen uptake was measured for a 5-min duration at 10 min into the second exercise bout to substantiate exercise intensity. Prior to exercise, subjects thoroughly washed both arms with liquid soap and water and dried with towels that had been rinsed in distilled water. Body mass was measured to the nearest 28 g immediately prior to exercise and at the end of each 30-min exercise bout on a Homs beam balance scale. Subjects consumed 225 ml of water prior to each 30-min exercise bout. Time intervals between exercise bouts were less than 5 min. After rinsing both arms with distilled water and drying with a towel, a pre-weighed polyethylene arm bag was placed on one arm and secured with an elastic band at the deltoid tuberosity for the last 15 min of each exercise bout. Sweat was collected from alternating arms for each bout. Arm bag sweat volume was measured in grams on an Ohaus digital scale (Ohaus Scale Corp., Florham Park, NJ). Total body sweat rate was calculated from
change in body mass measured to the nearest ounce and corrected for fluid intake and urine volume.

**Trace Mineral Analysis**

Sweat samples were stored at 4 °C in iron-free glass tubes until analysis. Samples were centrifuged at 3000 rpm for 15 min, and the supernatant was filtered through a Whatman 542 filter (Whatman International Ltd., Maidstone, England) to remove cellular debris prior to analysis. Sweat iron concentration was measured in duplicate using the ferrozine method (procedure #565-C, Sigma, St. Louis, MO) on a Beckman spectrophotometer. The standard curve was prepared from an iron standard containing 50 μg/dl. Sweat zinc concentration was measured using a Perkin-Elmer model 3110 atomic absorption spectrophotometer (Perkin-Elmer, Norwalk, CT). Samples were aspirated into an air-acetylene flame and optical density recorded at a wavelength of 214 nm, with a slit width setting of 0.7 nm. Samples were measured in duplicate. Optical density was plotted against a zinc standard curve, which was prepared from an original stock solution of 1.0 g/L (Sigma, St. Louis, MO). Estimated whole body sweat iron and zinc losses were calculated by multiplying the sweat iron or zinc concentration times the sweat rate (L/m²/h).

**Dietary Intake**

Each subject kept a food diary for the 3 days immediately prior to the prolonged exercise test. Instructions for recording food intake were given by a registered dietitian prior to completing the food diaries. Dietary analysis was carried out using Nutritionist III software (N-Squared Computing, Silverton, OR).

**Statistical Analyses**

A 2 × 4 (gender × exercise bout) analysis of variance with repeated measures was used to determine differences between genders, exercise bouts, and the interaction between gender and exercise bouts. The Tukey procedure was used for follow-up comparisons when significant $F$ ratios were found for exercise bouts. Pearson Correlations were calculated between iron and zinc sweat concentrations and dietary intakes and sweat rates. A probability of .05 was accepted as significant. Unpaired $t$ tests were used to test for significant differences in subject characteristics.

**Results**

Physical characteristics of the subjects are presented in Table 1. Male cyclists were significantly taller, heavier, had larger body surface areas, and higher peak $\dot{V}O_2 \text{peak}$ than female cyclists. During the 2-hour exercise bout, there was no significant difference in relative exercise intensity between the females and males. Dietary iron intake was not significantly different between females and males, while dietary zinc intake was significantly different ($p = .05$) and 50% lower for the females than males.

There was a main effect for sweat iron concentration between exercise bouts. Tukey post hoc comparisons indicated sweat iron concentrations were significantly higher at 30 and 60 min than at 90 and 120 min (Figure 1). There was no main effect
for gender or interaction between gender and exercise bouts for sweat iron concentration. Arm bag sweat volumes were not significantly different between exercise bouts or gender. Mean arm bag sweat was 14.3 ± 6.0 g and 8.9 ± 6.4 g per 15-min collection period for men and women, respectively (p = .07). Main effects for exercise bouts and gender were significant for sweat rate. Sweat rate (g/m²/h) was significantly lower during the first 30 min of exercise than in subsequent exercise bouts (Figure 2), and males had significantly higher sweat rates than females (p < .05). There was also a significant main effect for exercise bouts for sweat iron loss. Estimated total sweat iron loss (mg/m²/h) was significantly greater during the first hour of exercise than the second hour (Figure 3). There was no significant main effect for gender in sweat iron loss or interaction between gender and exercise bouts.

Sweat iron concentrations were not significantly correlated with sweat rates or dietary iron intake. A significant correlation was found between sweat iron concentrations during the first and second hours of exercise (r = 0.615).

Table 1  Physical Characteristics (Mean ± SD) of Male and Female Cyclists

<table>
<thead>
<tr>
<th>Variable</th>
<th>Males (n = 9)</th>
<th>Females (n = 9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>27.7 ± 5.0</td>
<td>24.0 ± 4.3</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>179.1 ± 4.8*</td>
<td>162.2 ± 4.3</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>74.1 ± 4.6*</td>
<td>58.6 ± 6.8</td>
</tr>
<tr>
<td>Body surface area (m²)</td>
<td>1.92 ± 0.08*</td>
<td>1.63 ± 0.13</td>
</tr>
<tr>
<td>$\dot{V}O_2^{peak}$ (ml/kg/min)</td>
<td>56.0 ± 4.7*</td>
<td>45.1 ± 6.7</td>
</tr>
<tr>
<td>$%\dot{V}O_2^{peak}$ (%)</td>
<td>49.8 ± 3.4</td>
<td>51.9 ± 5.3</td>
</tr>
<tr>
<td>$\dot{V}O_2$ (ml/kg/min)</td>
<td>27.8 ± 7.8</td>
<td>23.4 ± 11.2</td>
</tr>
<tr>
<td>Iron intake (mg/d)</td>
<td>23.6 ± 7.8</td>
<td>22.4 ± 11.2</td>
</tr>
<tr>
<td>Zinc intake (mg/d)</td>
<td>22.5 ± 14.0†</td>
<td>11.2 ± 7.4</td>
</tr>
</tbody>
</table>

Note. *Significant differences between males and females, p < .01. †Significant difference between males and females, p = .05.
Figure 1 — Male and female sweat iron concentrations (mean ± SD) during exercise. *Significant differences between 30 and 60 min vs. 90 and 120 min ($p < .01$).

Figure 2 — Male and female sweat rates (mean ± SD) per hour during exercise. *Significant differences between males and females ($p < .05$). ‡Significant differences between 30 min vs. 60, 90, and 120 min ($p < .001$).
Figure 3 — Male and female sweat iron loss relative to body surface area (mean ± SD) during exercise. *Significant differences between 30 and 60 min vs. 90 and 120 min ($p < .01$).

Figure 4 — Male ($n = 9$) and female ($n = 6$) sweat zinc concentrations (mean ± SD) during the first and second hours of exercise. *Significant difference between 60 min vs. 120 min ($p < .05$).
The major findings of this study were that cell-free sweat iron concentrations and losses decrease significantly after the first hour of a prolonged exercise bout. Although sweat rates remained relatively constant during the final 90 min of exercise, the sweat iron concentration was significantly lower during the second hour. Sweat iron concentrations were not significantly correlated with whole body sweat rates or the volume of sweat in the arm bag. These results suggest that the decline in iron concentration is not due to a simple dilution from an increased sweat volume. Decreases in sweat iron concentration have been previously observed with repeated resting heat exposure (3) and following 3 days of exercise in a hot, humid environment (17). Brune and colleagues (3) suggested the initial sweat contains iron present in the cellular debris. Wheeler et al. (17) attributed the decrease in sweat iron concentration to an increase in sweat rate because the total sweat iron loss remained constant. Another possible explanation is an iron conservation mechanism that may prevent excessive iron loss during prolonged exercise or exposure to hot environments. It is well known that serum iron concentration decreases during acute phase responses to inflammation, and it has been suggested that a similar response occurs during prolonged exercise such as triathlons (13). A sequestering of iron by the tissues could reduce the amount of iron present in the extracellular fluid and available to the sweat gland.

Sweat zinc concentration also decreased significantly between the first and second hours of exercise. This finding extends the results of Tipton et al. (14), who found sweat zinc concentrations decreased at 60 min of exercise compared to 30 min. The decline in sweat zinc concentration in the present study was more gradual between the first and second hours (0.90 to 0.56 mg/L) than the sharp drop (0.97 to
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0.41 mg/L) between 30 and 60 min reported by Tipton. Our sweat zinc concentration during the second hour is slightly higher than the sweat zinc concentration at 60 min in the Tipton study and in arm sweat (0.44 mg/L) after 30–40 min of heavy exercise (1). One possible explanation for the differences in sweat zinc concentration decrease between the present study and Tipton et al. (14) could be the greater increase in sweat rate between 30 and 60 min in the Tipton study. Sweat rate increased ~50% in the Tipton study, while the zinc concentration decreased 58%. In the present study, sweat rate increased 25% and zinc concentration decreased 38% between the first and second hours. However, sweat zinc concentrations and sweat rates during the first and second hours of the present study were not significantly correlated. Subjects in the Tipton study were acclimatized to a warm, humid environment, which results in higher sweat rates after 60 min (2). Subjects in the present study were tested in the cooler months of the year and unlikely to have been heat acclimated. It is possible that a zinc conservation mechanism is present in heat acclimatized individuals. Consolazio et al. (5) found a large decrease in sweat zinc concentration after 4 days of exercise in a hot (37.8 °C) environment.

There were no significant differences in sweat iron or zinc concentrations or losses between the male and female cyclists. Although the male and female cyclists in this study had similar dietary iron intakes, sweat iron concentration was not significantly related to a subject’s dietary iron intake during the 3 days preceding sweat iron collection. Only 5 subjects, all women, had dietary iron intakes that fell below the RDA of 18 mg/d for females (15). Dietary zinc was significantly lower in the women than the men. Dietary zinc intakes for 4 women and 1 man were below the RDA for zinc of 8 and 11 mg/d for women and men, respectively (15). There was no significant correlation between dietary zinc intake and sweat zinc concentration in the present study.

Estimations of whole body sweat iron and zinc losses during exercise are difficult because of the potential for environmental contamination. Although fully clothed subjects have been used to determine whole body daily iron and zinc losses (9, 11), this is not practical in prolonged exercise bouts. Sweat iron and zinc concentrations vary at different body sites, with the arm sweat iron and zinc concentrations falling slightly below the average of other sites (abdomen, chest, and back) measured during exercise (1). Sweat iron concentrations were highly correlated between the right and left arm in the present study, while the sweat zinc concentration was more variable, in agreement with the results of Jacob et al. (9). Suppression of sweating has been observed when arm bags are left in place for more than an hour (4). Lower sweat rates in the arm bag could lead to higher iron and zinc concentrations and overestimations of sweat losses. However, arm bags were only worn for 15 min on one arm each hour to reduce the risk of sweat suppression. The amount of arm bag sweat did not vary between the two arms or over time.

Sweat iron loss per m² was lower during the second hour of exercise (0.042 mg/m²/h) than observed at 60 min (0.060 mg/m²/h) in both this study and by Waller and Haymes (16). Estimated whole body iron loss during the first 60 min of exercise (0.105 ± 0.082 mg/h) was significantly higher than during the second 60 min (0.078 ± 0.077 mg/h). Our results suggest that use of sweat iron concentrations during the first hour of exercise to estimate iron loss during exercise lasting 2 hours will lead to a 15% overestimation of total iron loss. The daily loss of iron from the skin is estimated to be 0.24–0.33 mg/d (7, 9). The combined 2-hour sweat iron loss (0.183 mg) during exercise would represent 55–76% of the estimated daily iron loss from
the skin. Because the men had higher sweat rates, their 2-hour sweat iron loss (0.23 mg) represented 23% of estimated total daily iron loss (1 mg/d), while the women’s 2-hour sweat iron loss (0.14 mg) represented 10% of estimated total daily iron loss (1.3 mg/d; 18). Sweat iron losses over 2 hours represented 3% and 1% of the RDA for iron for the men and women, respectively.

The estimated whole body sweat zinc loss did not change significantly during 2 hours of exercise. This may be due to the small sample size leading to a type II error. Calculated power for zinc loss with 15 subjects was only 0.307. In contrast, the calculated power for iron loss with 18 subjects was 0.76. Because of the large variability in sweat zinc within subjects, a larger number of subjects would be required when examining sweat zinc loss.

Daily whole body surface loss of zinc for men and women ranges from 0.40–0.76 mg/d (8, 9, 11). Average sweat zinc loss for the men in this study was 0.50 mg/h and for women was 0.33 mg/h. Milne et al. (11) found that daily sweat zinc of men was 6% of the daily zinc intake on a normal diet. The sweat zinc lost during 2 hours of exercise was 4.5% of the daily zinc intake (9.3% of the RDA for zinc) for the men in this study and 6.6% of the daily zinc intake (8.2% of the RDA) for the women. Prolonged periods of low dietary zinc intake result in a significant reduction in sweat zinc loss, suggesting zinc is conserved during periods of zinc depletion (11).

It is concluded that sweat iron concentration and loss are reduced during the second hour of exercise while sweat rate remains constant. Sweat zinc concentrations are lower also during the second hour of exercise. The decreases in sweat iron and zinc concentrations are not due to a simple dilution by an increasing sweat volume.

References


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