Exercise-Induced Trace Mineral Element Concentration in Regional Versus Whole-Body Wash-Down Sweat

Lindsay B. Baker, John R. Stofan, Henry C. Lukaski, and Craig A. Horswill

Simultaneous whole-body wash-down (WBW) and regional skin surface sweat collections were completed to compare regional patch and WBW sweat calcium (Ca), magnesium (Mg), copper (Cu), manganese (Mn), iron (Fe), and zinc (Zn) concentrations. Athletes (4 men, 4 women) cycled in a plastic open-air chamber for 90 min in the heat. Before exercise, the subjects and cycle ergometer (covered in plastic) were washed with deionized water. After the onset of sweating, sterile patches were attached to the forearm, back, chest, forehead, and thigh and removed on saturation. After exercise, the subjects and cycle ergometer were washed with 5 L of 15-mM ammonium sulfate solution to collect all sweat minerals and determine the volume of unevaporated sweat. Control trials were performed to measure mineral contamination in regional and WBW methods. Because background contamination in the collection system was high for WBW Mn, Fe, and Zn, method comparisons were not made for these minerals. After correction for minimal background contamination, WBW sweat [Ca], [Mg], and [Cu] were 44.6 ± 20.0, 9.8 ± 4.8, and 0.125 ± 0.069 mg/L, respectively, and 5-site regional (weighted for local sweat rate and body surface area) sweat [Ca], [Mg], and [Cu] were 59.0 ± 15.9, 14.5 ± 4.8, and 0.166 ± 0.031 mg/L, respectively. Five-site regional [Ca], [Mg], and [Cu] overestimated WBW by 32%, 48%, and 33%, respectively. No individual regional patch site or 5-site regional was significantly correlated with WBW sweat [Ca] (r = –.21, p = .65), [Mg] (r = .49, p = .33), or [Cu] (r = .17, p = .74). In conclusion, regional sweat [Ca], [Mg], and [Cu] are not accurate surrogates for or significantly correlated with WBW sweat composition.

Keywords: sweat composition, sweat calcium concentration, sweat copper concentration, sweat magnesium concentration

Mineral elements such as calcium (Ca), magnesium (Mg), copper (Cu), manganese (Mn), iron (Fe), and zinc (Zn) are important in the regulation of many physiological processes, including those affecting physical performance. There is evidence that some athletes have inadequate mineral intakes, as well as lower than normal mineral status (Clarkson & Haymes, 1995; Haralambie, 1981; Lukaski, 1995, 2004; Sinclair & Hinton, 2005). Mg deficiency has been shown to impair performance, and Mg supplementation may improve muscle strength and power and aerobic performance in Mg-deficient individuals (Brilla & Gunther, 1995; Brilla & Haley, 1992; Institute of Medicine [IOM], 2006). Ca balance plays an important role in determining bone-mineral density, and poor Ca status may be related to increased risk for stress fractures (American Dietetic Association [ADA] et al., 2009; Clarkson & Haymes, 1995). As a functional component of superoxide dismutase, Cu acts to control damage from reactive oxygen species in cells. Mn and Zn are critical in many aspects of energy metabolism, and Fe is essential for oxygen transport to active muscles (Clarkson & Haymes, 1995; Lukaski, 1995).

Daily dietary mineral requirements are determined in part by the output of minerals via urinary, fecal, and dermal losses. Because of increased dermal (specifically, cell-free sweat) mineral losses during exercise and evidence that some athletes have lower than normal mineral status, there has been much interest and discussion regarding the potentially higher mineral requirements for athletes than for nonathletes (ADA et al., 2009; Clarkson & Haymes, 1994; IOM, 2006; Lukaski, 1995). In fact, the IOM (2006) has stressed the need for more research to understand the effects of physical activity on mineral losses and to determine how much of each mineral is required to replenish losses incurred during exercise.

The concentrations of Ca, Mg, Cu, Mn, Fe, and Zn in sweat collected from exercising humans vary widely among studies. The reported ranges include 14–74, 1–17, 0.03–2.51, 0.02–0.07, 0.11–0.92, and 0.29–2.19 mg/L for Ca, Mg, Cu, Mn, Fe, and Zn, respectively (Aruoma, Reilly, MacLaren, & Halliwell, 1988; Bullen, O’Toole, & Johnson, 1999; Chinevere, Kenefick, Cheuvront, Lukaski, & Sawka, 2008; Cohn & Emmett, 1978; Costa, Calloway, & Margen, 1969; DeRuisseau, Cheuvront, Haymes, & Sharp, 2002; Kilding et al., 2009; Lamanca, Haymes, Daly, Moffatt, & Waller, 1988; Montain, Cheuvront, & Lukaski, 2007; Paulev, Jordal, & Pedersen, 1983; Shirreffs & Maughan, 1997; Verde, Shephard, Corey, & Moore, 1982; Waller & Haymes, 1996). Although it is likely that physiologic variability (acquired or inherent differences at the level of the sweat gland) accounts for some of the intersubject and interstudy variability.

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in sweat [Ca], [Mg], [Cu], [Mn], [Fe], and [Zn], the inconsistent results may also be the result of a lack of standardized techniques and collection sites among studies. Previous data may also be confounded by technical and analytical issues. These may include failure to control for mineral contamination of sweat samples and lack of quality control to ensure accurate and precise analytical values (Lukaski, 2008). The use of contemporary analytical instrumentation such as inductively coupled plasma emission spectrometry would enable measurement of sweat mineral concentrations with greater sensitivity and precision than in past studies.

Most studies have assessed sweat mineral losses via regional skin-surface collections. The validity of using regional sweat mineral concentration as a surrogate for whole-body sweat is questionable because sweat rate and composition vary across different regions of the body (Costa et al., 1969; Palacios, Wigeritz, & Weaver, 2003), and covering the skin surface with a patch suppresses sweat evaporation in that specific region (Collins & Weiner, 1962). Instead, sweat collection via whole-body wash-down (WBW) is the criterion method to determine whole-body sweat mineral loss, because all sweat runoff is collected without interference with normal evaporative sweating processes (Lemon, Yarasheski, & Dolny, 1986; Shirreffs & Maughan, 1997).

Cohn and Emmett (1978) measured regional and WBW Zn, Cu, Fe, Mn, nickel, cadmium, lead, sodium (Na), and chloride (Cl) concentrations in 6 subjects (3 men, 3 women) during 90 min of intermittent cycling. They found that regional sweat mineral concentrations were higher and more variable than values obtained with the WBW procedure (Cohn & Emmett, 1978). In their study, regional sweat was collected with an occlusive arm bag. The arm bag has practical limitations for sweat collection in the field and only collects sweat from one site. Conversely, patch collections are more practical for field studies because they do not interfere with the subject’s hand and arm function during sports and exercise. The patch sweat-collection technique has been widely used to measure sweat mineral concentration; however, this method has not been validated against the criterion WBW method for Ca, Mg, Cu, Mn, Fe, and Zn.

Establishing an accurate and practical method for measuring exercise-induced sweat mineral loss is important for determining whether athletes have higher requirements than the dietary reference intakes (IOM, 1997, 2001). Therefore, research to validate the regional sweat patch versus the criterion sweat-collection method is warranted. No study to date has compared regional sweat patches with WBW Ca, Mg, Cu, Mn, Fe, and Zn concentrations during exercise. Therefore, the aim of the current investigation was to compare simultaneous WBW and regional sweat collections to determine the relation between regional and WBW sweat [Ca], [Mg], [Cu], [Mn], [Fe], and [Zn] in exercising men and women. We used inductively coupled plasma emission spectrometry to measure sweat mineral concentrations with greater sensitivity and precision than previous studies comparing regional and whole-body methods.

Materials and Methods

Subjects

Four male and four female well-trained athletes (36 ± 8 years, 72 ± 28 kg, 171 ± 7 cm, 1.84 ± 0.16 m²; runners, cyclists, and triathletes) participated in this study. The experimental protocol was approved by the Human Subjects Review Committee of the Gatorade Sports Science Institute, and all subjects gave written informed consent before participation. The data in this article were collected during the experiment described by Baker, Stofan, Hamilton, and Horswill (2009).

Experimental Procedure

Subjects were required to refrain from exercise, alcohol, and caffeine for 24 hr before testing and to fast for 1 hr before each experiment. During the experimental trial, subjects cycled on a friction-braked ergometer (Monark Model 828 E) at 75% maximum heart rate in a plastic isolation chamber (consisting of a silage bag inside a plastic frame) for 90 min in a heated (30 °C, 43% relative humidity) laboratory. Heart rate was monitored by telemetry (Polar). A 90-min protocol was used to ensure that a sufficient volume of sweat was generated for collection and analyses. During exercise, subjects were allowed to drink a carbohydrate-electrolyte solution (18 mEq/L Na, 3 mEq/L potassium, and 0 mEq/L of Ca, Mg, Cu, Mn, Fe, and Mn) ad libitum. Total whole-body sweat loss was calculated from the change in pre- to postexercise nude body mass, corrected for fluid intake, urine loss, respiratory water loss (calculated from American College of Sports Medicine, 2000; Mitchell, Nadel, & Stolwijk, 1972), and weight loss resulting from substrate oxidation (calculated from Brooks & Mercier, 1994; Mitchell et al., 1972; assuming R = 1.0).

WBW Sweat Collection

The WBW method described by Shirreffs and Maughan (1997) was used to determine sweat [Ca], [Mg], [Cu], [Mn], [Fe], and [Zn] from the entire body. The day before testing, subjects’ clothes (shorts, sports bra) were cleaned with deionized water to remove extraneous minerals and then air dried. Before entering the plastic isolation chamber on test day, subjects were washed with 4 L of deionized water and dried with sterile towels. They then donned previously demineralized shorts or shorts and sport bra and a Polar heart-rate monitor (subjects did not wear socks or shoes). To ensure collection of all WBW mineral loss and to determine the volume of unevaporated sweat collected in the plastic isolation chamber, each subject (nude) and cycle ergometer were showered thoroughly with 5 L of deionized water containing 15-mM ammonium sulfate (98% pure, manufactured by J.T. Baker) immediately after completing the 90-min exercise protocol. After showering, the subject dried off with sterile towels and stepped out of the isolation chamber. The heart-rate monitor and subject’s clothing, as well as all towels,
gauze, and gloves that touched the subject during the experiment, were put in the bottom of the silage bag (with the sweat and wash solution). After thoroughly mixing the contents collected at the bottom of the silage bag, a sample (postwash) was collected for later analysis. In addition, a 200-ml sample of the 5 L 15-mM ammonium sulfate solution was retained for electrolyte analysis before washing the subject and cycle ergometer (prewash).

**Regional Patch Sweat Collection**

Twenty minutes into exercise, one sterile patch (3M Medical Sciences) was attached to the anterior midthigh. Then, 30 min into exercise, one patch each was placed on the upper chest, back (superior scapula), forehead, and posterior midforearm (in that order). The patches were composed of a 15.4-cm² absorbent pad surrounded by an occlusive dressing (Tegaderm). The total patch size was 42 cm². The thigh, back, chest, and forearm patches were occlusive dressing (Tegaderm). The total patch size was 42 cm². The thigh, back, chest, and forearm patches were placed on the right side of each subject’s body. The subjects’ skin was cleaned with deionized water and sterile gauze before patch application. Patches were removed on saturation and placed in a sterile Petri dish. At the end of the experiment, the patches were transferred into air-tight plastic tubes (Sarstedt Salivette) and centrifuged at 3,500 rpm and 4 °C for 15 min. Finally, the sweat samples were extracted from the tubes, aliquotted into cryovials, and refrigerated until analysis. The five-site regional sweat mineral concentrations were calculated based on the following equation, which is weighted for local sweat rate and body surface area (Takamata, Mack, Gillen, & Nadel, 1994):

\[
[M] = (0.07[M]_FH\cdot SR_{FH} + 0.36[M]_TR\cdot SR_{TR} + 0.13[M]_FA\cdot SR_{FA} + 0.32[M]_T\cdot SR_T)/(0.07SR_{FH} + 0.36SR_{TR} + 0.13SR_{FA} + 0.32SR_T)
\]

where [M] is the mineral concentration (Ca, Mg, Cu, Mn, Fe, or Zn) in the sweat (mg/L), SR is the local sweat rate (mg · min⁻¹ · cm⁻²), FH is the forehead, FA is the forearm, T is the thigh, TR is the trunk (average of back and chest), and the constants represent the percentage distribution of body surface area. SR was calculated from the pre- to postexperiment change in patch + Petri-dish weight (mg), the amount of time that the patch was on the skin (min), and the patch surface area (42 cm²).

**Measurement of Contamination in the Collection Systems**

The regional and WBW methods were checked for background contamination of Ca, Mg, Cu, Mn, Fe, and Zn in the collection systems. To determine any possible contamination in the regional methodology, 10 patches (each placed inside a separate Petri dish) were saturated with 1.0 ml of deionized water. Next, they were spun in the air-tight plastic tubes and then extracted and stored in vials until later analysis. To determine any possible contamination in the WBW collection system, a mock trial was performed in which the plastic isolation chamber and its contents (including cycle ergometer, towels, shorts, and heart-rate-monitor strap, but no subject) were washed with 5 L of 15-mM ammonium sulfate and 2 L of artificial sweat (with known mineral concentrations; prepared at the Gatorade Sports Science Institute). After the contents collected at the bottom of the plastic bag were thoroughly mixed, a sample was collected for later analysis.

**Sweat Analysis**

An ion-specific electrode (Symphony, VWR Scientific) and standard curve (average \( r = .99 \)) were used to quantify the concentration of ammonium ion in the prewash and postwash solutions for dilution calculations (i.e., determining volume of unevaporated sweat in the silage bag at the end of the experiment). The average within-sample coefficient of variation for triplicate measures of ammonium ion concentration in pre- and postwash samples was 0.5%.

The WBW and regional sweat samples were analyzed at the USDA, ARS Grand Forks Human Nutrition Research Center. Before analysis, sweat samples were vortexed and a 0.5-ml sample of the supernatant, or, in some cases the smallest volume that could be accurately measured, was placed in an acid-washed plastic tube to which 0.1 ml of ultrapure 6-M nitric acid was added to ensure liberation of all cations from bound proteins, amino acids, and contaminants. Sample tubes were capped and stored at room temperature for 12 hr. The next day the samples were brought up to a volume of 2.5 ml with high-quality, deionized water (18 MΩ). WBW and regional sweat mineral concentrations were measured in duplicate using inductively coupled plasma emission spectrometry (PerkinElmer Model 3300; Norwalk, CT). The minimum detection limits were <0.006 μg/ml for Cu, Fe, Mn, and Zn and <0.1 μg/ml for Ca and Mg. The concentration of these elements in the deionized water used for preparation of collection sites was below detection limits. Quality control was ensured by running a traceable standard after every 10 samples to check for instrument drift. A standard reference material (SRM 1643e, National Institute of Standards and Technology) was analyzed four times throughout the course of the analysis and produced results ±4.5% of the certified values for each element.

**Statistical Analysis**

Mean differences between regional and whole-body sweat mineral concentrations with 95% confidence intervals and effect sizes (Cohen, 1988) were calculated. In addition, linear regression was used to plot regional versus WBW sweat mineral concentrations. Pearson’s product–moment correlations were calculated to assess the relationship between regional and WBW sweat mineral concentrations. The significance level for all correlations was set at \( \alpha = .05 \). Data are presented as \( M \pm SD \) unless otherwise indicated.
Results

A total of 8 subjects participated in this study; however, for some of the analyses \( N \) is less than 8 because of insufficient sweat volume obtained at some of the sites on some of the subjects. The \( N \) is provided in the footnotes of Tables 1, 2, and 3 for sweat \([Ca]\), \([Mg]\), and \([Cu]\), respectively.

The subjects’ total sweat loss, calculated respiratory water loss, and calculated weight loss resulting from

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Regional (REG) Versus Whole-Body Wash-Down (WBW) Sweat Calcium [Ca]</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Sweat [Ca], mg/L</td>
</tr>
<tr>
<td>WBW</td>
<td>44.6 ± 20.0</td>
</tr>
<tr>
<td>5-site REG(^a)</td>
<td>59.0 ± 15.9</td>
</tr>
<tr>
<td>Forearm</td>
<td>59.6 ± 12.1</td>
</tr>
<tr>
<td>Back</td>
<td>55.0 ± 21.0</td>
</tr>
<tr>
<td>Chest</td>
<td>88.8 ± 34.9</td>
</tr>
<tr>
<td>Forehead(^a)</td>
<td>36.3 ± 15.0</td>
</tr>
<tr>
<td>Thigh</td>
<td>49.2 ± 9.2</td>
</tr>
</tbody>
</table>

Note. Values are \( M \pm SD \), mean differences (95% CI), percent differences, effect sizes of differences (Cohen’s \( d \) pooled; Cohen, 1988), and correlations (95% CI) for 8 subjects (4 men, 4 women). WBW and REG sweat [Ca] have been corrected for contamination in the WBW and REG methods. \(^a\)\( N = 7 \) (3 men, 4 women).

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Regional (REG) Versus Whole-Body Wash-Down (WBW) Sweat Magnesium [Mg]</th>
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<tbody>
<tr>
<td></td>
<td>Sweat [Mg], mg/L</td>
</tr>
<tr>
<td>WBW</td>
<td>9.8 ± 4.8</td>
</tr>
<tr>
<td>5-site REG(^a)</td>
<td>14.5 ± 4.8</td>
</tr>
<tr>
<td>Forearm</td>
<td>15.6 ± 4.4</td>
</tr>
<tr>
<td>Back</td>
<td>13.9 ± 8.9</td>
</tr>
<tr>
<td>Chest</td>
<td>24.1 ± 10.6</td>
</tr>
<tr>
<td>Forehead(^a)</td>
<td>7.4 ± 5.1</td>
</tr>
<tr>
<td>Thigh</td>
<td>11.8 ± 2.3</td>
</tr>
</tbody>
</table>

Note. Values are \( M \pm SD \), mean differences (95% CI), percent differences, effect sizes of differences (Cohen’s \( d \) pooled; Cohen, 1988), and correlations (95% CI) for 7 subjects (3 men, 4 women). WBW and REG sweat [Mg] have been corrected for contamination in the WBW and REG methods. \(^a\)\( N = 6 \) (2 men, 4 women).

<table>
<thead>
<tr>
<th>Table 3</th>
<th>Regional (REG) Versus Whole-Body Wash-Down (WBW) Sweat Copper [Cu]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sweat [Cu], mg/L</td>
</tr>
<tr>
<td>WBW</td>
<td>0.125 ± 0.069</td>
</tr>
<tr>
<td>5-site REG(^a)</td>
<td>0.166 ± 0.031</td>
</tr>
<tr>
<td>Forearm</td>
<td>0.159 ± 0.031</td>
</tr>
<tr>
<td>Back</td>
<td>0.191 ± 0.105</td>
</tr>
<tr>
<td>Chest</td>
<td>0.239 ± 0.071</td>
</tr>
<tr>
<td>Forehead(^a)</td>
<td>0.089 ± 0.036</td>
</tr>
<tr>
<td>Thigh</td>
<td>0.126 ± 0.028</td>
</tr>
</tbody>
</table>

Note. Values are \( M \pm SD \), mean differences (95% CI), percent differences, effect sizes of differences (Cohen’s \( d \) pooled; Cohen, 1988), and correlations (95% CI) for 7 subjects (4 men, 3 women). WBW and REG sweat [Cu] have been corrected for contamination in the WBW and REG methods. \(^a\)\( N = 6 \) (3 men, 3 women).
substrate oxidation, unevaporated sweat, and fluid intake were 1,283 ± 372, 86 ± 19, 76 ± 15, 214 ± 140, and 1,252 ± 576 g, respectively. The average change in body mass was –0.3% ± 0.5%.

Regional and WBW Contamination Results

The average amounts of background contamination found in the regional patch collection method were 6.34, 2.21, 0.009, 0.003, 0.021, and 0.016 mg/L for [Ca], [Mg], [Cu], [Mn], [Fe], and [Zn], respectively. These concentrations represented approximately 5–15% of measured regional sweat mineral concentrations. The amounts of background contamination found in the WBW collection system were 4.19, 0.008, 0.028, 0.138, and 0.979 mg/L for [Ca], [Cu], [Mn], [Fe], and [Zn], respectively. No background Mg was detected in the WBW system. These concentrations represented approximately 9%, 0%, 6%, 25%, 53%, and 68% of measured WBW sweat [Ca], [Mg], [Cu], [Mn], [Fe], and [Zn], respectively. Because the levels of Mn, Fe, and Zn contamination in the WBW collection were rather high, the regional versus WBW comparisons that follow are limited to the minerals Ca, Mg, and Cu. All reported regional and WBW mineral concentrations have been corrected for background contamination in the respective collection systems.

Regional Versus WBW Sweat Ca, Mg, and Cu

The regression results for five-site regional patch versus WBW sweat [Ca], [Mg], and [Cu] are presented in Figure 1. The correlation results and difference scores for regional versus WBW sweat [Ca], [Mg], and [Cu] are presented in Tables 1, 2, and 3, respectively.

Discussion

The main findings from this study were that there were medium to large mean differences between most regional sites and WBW sweat [Ca], [Mg], and [Cu]; no regional sites were significantly correlated with WBW sweat [Ca], [Mg], or [Cu]; and a considerable amount of Mn, Fe, and Zn contamination in the WBW system precluded a valid comparison between methods.

Simultaneous WBW and regional sweat collections have been compared in previous studies, most of which concluded that regional methods overestimate and are not accurate surrogates of whole-body sweat constituent loss (Cohn & Emmett, 1978; Jacob, Sandstead, Munoz, Klevay, & Milne, 1981; Lemon et al., 1986; Palacios et al., 2003; Patterson, Galloway, & Nimmo, 2000; Shirreffs & Maughan, 1997; van Heyningen & Weiner, 1952; Vellar, 1968). However, most of those studies either did not involve exercise or only measured Na, Cl, potassium, lactate, and/or urea concentrations in exercising subjects. One study (Cohn & Emmett, 1978) compared regional (arm bag) and WBW sweat mineral (Zn, Cu, Fe, nickel, cadmium, lead, Mn, Na, and Cl) concentration during exercise but did not measure Ca and Mg. The results of the current study support the findings of previous work and extend the notion that regional methods do not provide accurate estimates of whole-body sweat constituent loss of the minerals Ca and Mg.

Cohn and Emmett (1978) measured sweat mineral concentration in six exercising subjects using regional arm-bag and WBW procedures. They did not report on the correlation between methods, but in agreement with the
Limitations and Future Directions

The WBW procedure used in the current study resulted in a considerable amount of Mn, Fe, and Zn contamination. The background [Mn], [Fe], and [Zn] detected in the WBW collection system were 0.028, 0.138, and 0.979 mg/L, respectively, whereas only 0.003, 0.021, and 0.016 mg/L were found in the regional patch collection system. The cycle ergometer (and its metal components) was most likely the source of most of the mineral contamination. Thus, perhaps an improved WBW method would involve a different mode of exercise, such as the stepping protocol used in the WBW study by van Heyningen and Weiner (1952). Using a plastic stepstool inside the isolation chamber would eliminate the contamination presumably introduced by the metal components of a cycle ergometer. Future research is required to develop and validate a WBW method to more precisely measure whole-body sweat Mn, Fe, and Zn loss in exercising subjects. Accurate measurement of whole-body sweat Fe and Zn loss is especially important for determining whether female athletes have increased dietary requirements, because this population appears to be most susceptible to insufficient Fe and Zn intake and status (Clarkson & Haymes, 1994; Haralambie, 1981; Sinclair & Hinton, 2005).

Summary

In conclusion, background contamination of Mn, Fe, and Zn in the WBW method and large mean differences and lack of significant correlations between regional and WBW sweat [Ca], [Mg], and [Cu] make it difficult to estimate whole-body sweat mineral concentrations from regional collections. Therefore, WBW values for sweat [Ca], [Mg], and [Cu] should be used to determine the impact of exercise-induced sweat loss on athletes’ mineral requirements. Further work is needed to develop and validate regional methods if a more practical means of estimating whole-body sweat [Ca], [Mg], and [Cu] loss is desired. In addition, future research is needed to develop and validate a contamination-free WBW method for collecting sweat Mn, Fe, and Zn from exercising men and women.

Acknowledgments

We thank the subjects who gave their time and effort to make this study possible, Dennis Passe for statistical consultation, and the staff of the Mineral Analysis Laboratory at the USDA, ARS Grand Forks Human Nutrition Research Center for their technical contributions.

This study was funded by the Gatorade Sports Science Institute and the USDA, ARS Grand Forks Human Nutrition Research Center. Authors Lindsay B. Baker and John R. Stofan are employees of the Gatorade Sports Science Institute.

References


