The Effects of Acute Exercise Bouts on Hepcidin in Women

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Purpose: To investigate the effects of acute exercise on serum hepcidin and iron (sFe) in active women. Changes in interleukin-6 (IL-6), hepcidin, ferritin, and sFe in response to 2 different exercise durations were compared.

Methods: Twelve women age 19–32 yr performed 2 treadmill runs (60 and 120 min) at 65% of VO2max. Blood samples were obtained before, immediately after, and 3, 6, 9, and 24 hr after exercise. Two-way repeated-measures ANOVA was conducted to examine changes in measured variables. Significance was accepted at \( p < .05 \).

Results: Significant effects for trial were observed for hepcidin (60 min: 1.15 ± 0.48 nmol/L; 120 min: 2.28 ± 1.44 nmol/L) and for time, with hepcidin significantly increased 3 hr postexercise in both trials (60 min: 3 hr – 1.99 ± 2.00 nmol/L; 120 min: 3 hr – 4.60 ± 4.61 nmol/L). Significant main effects for time occurred for sFe, ferritin, and IL-6. sFe was significantly decreased 9 hr postexercise compared with 3 and 24 hr postexercise. IL-6 was significantly increased immediately postexercise. Conclusions: Both runs resulted in significant increases in hepcidin 3 hr after exercise. Increases in hepcidin were preceded by significant increases in IL-6 immediately postexercise and followed by significant decreases in sFe 9 hr postexercise. It was concluded that endurance exercise increases the production of hepcidin, which affects sFe. The 2-hr exercise bout stimulated greater changes in serum hepcidin than the 1-hr bout.

Keywords: iron, interleukin-6, endurance exercise

Iron is an essential element with many important roles in the body, most notably as a carrier of oxygen and as a component necessary for the production of energy. Despite iron’s importance, iron deficiency is the most common nutritional deficiency worldwide, and a significant prevalence of iron deficiency has been reported in the United States (“Iron Deficiency,” 2002).

Iron deficiency, with and without anemia, has frequently been demonstrated in female athletes, particularly those participating in endurance-type exercise (Gropper, Blessing, Dunham, & Barksdale, 2006; Sinclair & Hinton, 2005). The increased incidence of iron deficiency in female endurance athletes is thought to be the result of low dietary iron intake in this population, losses of iron in menstrual blood, sweat iron loss, and gastrointestinal blood loss (Gropper et al., 2006; Waller & Haymes, 1996). Correcting inadequacies in iron status is important to athletes participating in endurance-types of activities, because iron deficiency (with and without anemia) has been shown to compromise aerobic performance and hinder adaptations to training (Brownlie, Utermohlen, Hinton, Giordano, & Haas, 2002; Brownlie, Utermohlen, Hinton, & Haas, 2004).

Hepcidin regulation occurs via several different stimuli including inflammatory cytokines, hypoxia, and plasma iron status (Nicolas, et al., 2002). Its association with inflammation provides a means by which hepcidin may be influenced by acute exercise. Hepcidin expression increases in response to inflammation in both animals and humans (Nemeth et al., 2003; Pigeon et al., 2001) and is mediated by interleukin-6 (IL-6). IL-6 production increases during the acute-phase inflammatory response, which up-regulates hepcidin (Nemeth, Rivera et al., 2004). Kemna, Pickkers, Nemeth, van der Hoeven, and Swinkels (2005) observed a corresponding decrease in serum iron after the increases in IL-6 and hepcidin, thus providing a link between inflammation, hepcidin, and decreased iron status.
Strenuous exercise has been shown to induce increases in inflammatory cytokines and acute-phase proteins (Fallon, 2001; Hoffman-Goetz & Pedersen, 1994) and is frequently used as a model to investigate the acute-phase response. Only a few studies (Peeling et al., 2009a, 2009c; Roecker, Meier-Buttermilch, Brechtel, Nemeth, & Ganz, 2005) have observed the effect that strenuous exercise has on urinary hepcidin. Peeling et al. (2009a) found significant increases in urinary hepcidin 3 hr after high-intensity interval-type running bouts and a moderate-intensity long-distance run. Similar results were found when they examined the effects of the same exercise protocol performed on different training surfaces (i.e., a road surface and a grass surface; Peeling et al., 2009c). Urinary hepcidin levels were significantly higher 3 hr after all the exercise bouts than preexercise levels, regardless of the training surface. Peeling et al. (2009a, 2009c) suggested that the increases in hepcidin resulted from exercise-induced inflammation and hemolysis, as evidenced by significant increases in IL-6, increased serum iron levels, and significant decreases in serum haptoglobin. Roecker et al. (2005) observed significant increases in urinary hepcidin levels in female athletes after the completion of a marathon compared with preexercise levels. Those authors proposed that the increases in hepcidin were induced by increases in IL-6; however, they did not measure this variable.

The purpose of this study was to investigate whether acute exercise affects serum hepcidin levels and iron status in active women. The effects of exercise duration on changes in IL-6, serum hepcidin, ferritin, and serum iron were investigated by comparing 60- and 120-min exercise bouts at the same exercise intensity.

Methods and Materials

Subjects

Twelve female runners were recruited for this study. An effect-size and power-calculation analysis based on a previous study that observed the responses of hepcidin to pharmaceutically induced inflammation was conducted to ensure that this number of subjects would be adequate to obtain significant results (Kemna et al., 2005). With alpha set at .05, it was predicted that 7 subjects would be needed for a power of 80% (Marks, 1982). The study used for the calculation of effect size included 10 subjects, so this number was sought for the current study. Twelve women who expressed an interest in participating were included as subjects to make a final \( N = 12 \). All women were active runners who were free of any existing health problems and were not currently taking any anti-inflammatory medications. This study was approved by the Florida State University Human Subjects Research Committee before data collection was begun.

Procedures and Techniques

All testing was conducted in the exercise physiology laboratory at Florida State University. Subjects completed three different test sessions, consisting of one aerobic-capacity test (\( \text{VO}_{2\text{max}} \) test) and two submaximal exercise bouts. All exercise testing took place in the morning to control for diurnal variations in serum iron (Dale, Burritt, & Zinsmeister, 2002). Before all visits, subjects were asked to refrain from exercise or the consumption of caffeine, nicotine, nonsteroidal anti-inflammatory drugs, or antihistamines 24 hr before coming to the laboratory and to refrain from eating 2 hr before coming to the laboratory. During the first visit, subjects were asked to sign the informed consent and to complete medical- and training-history questionnaires. Subjects’ weight and height were measured in running clothes without shoes, using a balance-beam scale and stadiometer, respectively. Body fat was estimated from using the skinfold-thickness method, according to the American College of Sports Medicine (2006).

Aerobic-Capacity Testing

Subjects completed an incremental running protocol to determine their \( \text{VO}_{2\text{max}} \) on a treadmill using a modified Astrand progressive run-to-exhaustion protocol (Miller, Dougherty, Green, & Crouse, 2007). Gas-exchange and ventilatory parameters were measured by indirect calorimetry using a ParvoMedics TrueMax 2400 metabolic cart. Heart rate was monitored using a Polar heart-rate monitor. The test protocol consisted of 2-min stages during which the subjects ran at their individual predetermined pace (5–8 mile/hr, 8–13 km/hr), with the treadmill grade increasing 2% every stage. Before beginning the test, subjects performed a 5-min warm-up, after which the speed was increased to each individual’s predetermined pace and grade was increased to 2%. Tests were terminated when subjects reached volitional fatigue. The criterion for achievement of \( \text{VO}_{2\text{max}} \) was to have reached at least three of the following criteria: a plateau in oxygen consumption for an increase in exercise intensity \( \geq 2.0 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1} \) increase), respiratory-exchange ratio \( \geq 1.05 \), heart rate 85% of age-predicted maximum \( (220 – \text{age}) \), and rating of perceived exertion \( >18 \) (Howley, Bassett, & Welch, 1995). If the subjects did not meet three of the four criteria, the test was repeated. Each subject’s pace for the submaximal exercise bouts was based on the result of \( \text{VO}_{2\text{max}} \) test and the pace that elicited 65% of the value achieved by the subject. After the \( \text{VO}_{2\text{max}} \) test, subjects were instructed to follow the same pretest instructions for each submaximal exercise bout. They were given a dietary record and instructed to record their diet (using household measures) for 48 hr before and 24 hr after their first submaximal bout. This record was then used by the subjects to repeat their diet before and after their second submaximal bout to control for any dietary effects.

Submaximal Exercise Bouts

To control for changes in iron status that may have occurred as a result of the menstrual cycle, both submaximal exercise bouts were performed 7–10 days after the onset of the menses. Subjects performed the first of
these bouts and then returned after their next menstrual period for the second bout. The order of the two trials was randomized and counterbalanced. During the two trials, subjects were required to run on a treadmill at 65% of their VO2max for 60 min during one trial and 120 min during the second trial. Before each trial subjects were weighed in running clothes without shoes, and blood samples were obtained for analysis of both inflammatory and iron markers. During both trials ventilatory rates and gases were measured for 5 min beginning at Minute 15 and Minute 45. During the 120-min trial the same measurements were conducted at Minute 75 and Minute 105 for 5 min. The last 2 min of these measurements were averaged for each trial to determine the average intensity of the exercise bout (i.e., the average percentage of VO2max). Immediately after each trial a second blood sample was obtained. Additional blood samples were obtained 3, 6, 9, and 24 hr postexercise. All pre- and posttest measures were repeated for the second submaximal trial.

Blood Collection and Analysis

Blood samples were collected into Vacutainer tubes using standard procedures via venipuncture. Serum was used for the measurement of ferritin, serum iron, hepcidin, IL-6, and high-sensitivity C-reactive protein (CRP). Three-hundred-microliter aliquots of serum were separated and then stored at −80 °C for later analyses. Whole blood collected in Vacutainer tubes containing 10.8 mg of spray-dried K2EDTA was used for the measurement of hematocrit and hemoglobin.

Commercially available ELISA kits were used for the measurement of ferritin (Bio-Quant, Inc., San Diego, CA) and IL-6 (Quantikine HS, R&D Systems, Minneapolis, MN), and a microplate reader was used to determine the absorbency of the samples and controls. Serum hepcidin measurements were performed in July 2009 (testing laboratory: Hepcidinanalysis.com, Nijmegen, The Netherlands) by a combination of weak cation-exchange chromatography and time-of-flight mass spectrometry (TOF MS). An internal standard (synthetic hepcidin-24; Peptide International Inc.) was used for quantification (Swinkels et al., 2008). Peptide spectra were generated on a Microflex LT matrix-enhanced laser desorption/ionization TOF MS platform (Bruker Daltonics). Serum hepcidin-25 concentrations were expressed as nM/L. The lower limit of detection of this method was 0.5 nM; average coefficients of variation were 2.7% (intrarun) and 6.5% (interrun). The median reference level of serum hepcidin-25 is 4.2 nM, range 0.5–13.9 nM; for women it is slightly lower (Kroot et al., 2009). Serum iron and CRP were measured using the Sirrus clinical chemistry analyzer and commercially available reagents (serum iron: Stanbio Laboratory, Boerne, TX; CRP: Kamiya Biomedical Co., Seattle, WA). Hematocrit was determined from microcentrifugation of whole blood and measured using a microhematocrit reader. Hemoglobin was measured using the HemoCue Hb 201+ analyzer (HemoCue, Inc., Lake Forest, CA). Changes in plasma volume after exercise were calculated using the equation developed by Dill and Costill (1974). All immediately postexercise values were corrected for changes in plasma volume.

Statistical Analysis

An intention-to-treat analysis was used to evaluate data, and missing data points were filled in using the mean for the preceding and following time points for that individual. For 3 of the subjects in this study, data were missing for one time point on one of the submaximal trials (different time points or trials for each subject) because of the inability to obtain an adequate amount of blood from the venipuncture. Descriptive statistics including means, standard deviations, and ranges were calculated for all measures. A two-way ANOVA (2 × 6, Trial × Time) was used to analyze the dependent measures of hepcidin, IL-6, CRP, serum iron, ferritin, hemoglobin, and hematocrit, with repeated measures on both factors. Tukey’s post hoc analysis was used to determine significant findings over time, with significance accepted at p ≤ .05. A one-way ANOVA with repeated measures was performed for all variables in which significant main effects of time were observed. Pearson’s product–moment correlations were calculated to determine whether there were relationships between dependent variables. All statistical analyses were performed using PASW (formerly SPSS) software.

Results

Subjects

Twelve women 19–32 years of age participated in this study. They were highly active women who performed endurance exercise on a regular basis and used running as their primary form of aerobic activity. Their descriptive and anthropometric data are presented in Table 1. All participants achieved a VO2max using the criteria cited previously with an $M \pm SD$ of 52.1 ± 3.9 ml · kg⁻¹ · min⁻¹ (range 47.5–60.3 ml · kg⁻¹ · min⁻¹). Corresponding criteria supporting the achievement of maximum aerobic capacity included a mean maximum heart rate of 188 ± 7 beats/min, maximum respiratory-exchange ratio of 1.11 ± 0.04, and maximum rating of perceived exertion of 18 ± 1. The resulting VO2max for each subject was used to determine the intensity that would be used (i.e., 65% of this value) for each submaximal exercise bout.

<table>
<thead>
<tr>
<th>Subject Characteristics (N = 12)</th>
<th>M ± SD</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>23.8 ± 3.8</td>
<td>19–32</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>161.4 ± 2.9</td>
<td>156.2–167.6</td>
</tr>
<tr>
<td>Weight (lb)</td>
<td>123.5 ± 8.7</td>
<td>114–141</td>
</tr>
<tr>
<td>Body fat (%)</td>
<td>18.4 ± 4.1</td>
<td>11.6–23.4</td>
</tr>
<tr>
<td>VO2max (ml · kg⁻¹ · min⁻¹)</td>
<td>52.1 ± 3.9</td>
<td>47.5–60.3</td>
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</tbody>
</table>
**Hemoglobin and Hematocrit**

The combined means and standard deviations for the variables for both trials are reported in Table 2. Means and standard deviations for hemoglobin and hematocrit levels for each of the trials are reported in Tables 3 and 4. Significant effects for time were observed for hemoglobin ($p < .001$) and hematocrit ($p < .001$); however, no significant effect for trial and no Trial × Time interaction occurred for either variable, suggesting that although both of these variables changed as a result of exercise, the change was not significantly different between the trials. Decreases in plasma volume immediately after exercise in both trials were substantial; therefore, all immediately postexercise serum concentrations were corrected for changes in plasma volume. When the combined trial means for time were compared using Tukey’s honestly significant difference method, it was observed that the immediately postexercise hemoglobin concentrations were significantly higher than at all other time points for this variable.

**Hepcidin**

Significant effects for trial ($p = .024$) and for time ($p = .018$) were observed in hepcidin; however, the Trial × Time interaction was not statistically significant ($p = .063$). The effect for trial showed that hepcidin concentrations were significantly higher for the 120-min run. One-way ANOVA with time as the independent factor was conducted for each of the trials to determine between which time points the significant mean differences occurred. One-way ANOVA analyses indicated that hepcidin levels were significantly higher 3 hr postexercise than the preexercise ($p = .011$), immediately postexercise

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**Table 2  Combined Hemoglobin, Hematocrit, Hepcidin, Interleukin-6, C-Reactive Protein, Serum Iron, and Ferritin Levels for the 60-min and 120-min Runs, $M \pm SD$**

<table>
<thead>
<tr>
<th></th>
<th>Pre</th>
<th>Post</th>
<th>3 hr</th>
<th>6 hr</th>
<th>9 hr</th>
<th>24 hr</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemoglobin, g/dl</td>
<td>12.4 ± 1.1</td>
<td>13.4 ± 1.2</td>
<td>12.4 ± 0.9</td>
<td>12.3 ± 0.9</td>
<td>12.3 ± 0.9</td>
<td>12.5 ± 1.0</td>
</tr>
<tr>
<td>Hematocrit, %</td>
<td>38.1 ± 3.3</td>
<td>40.3 ± 3.0</td>
<td>38.3 ± 3.1</td>
<td>37.9 ± 2.9</td>
<td>37.7 ± 2.8</td>
<td>38.2 ± 2.72</td>
</tr>
<tr>
<td>Hepcidin, nmol/L</td>
<td>0.9 ± 0.8</td>
<td>1.1 ± 0.9</td>
<td>3.3 ± 3.7</td>
<td>2.4 ± 3.1</td>
<td>1.5 ± 1.4</td>
<td>1.0 ± 1.2</td>
</tr>
<tr>
<td>Interleukin-6, pg/ml</td>
<td>1.5 ± 1.9</td>
<td>3.5 ± 2.4*¥≠@‡</td>
<td>1.9 ± 2.0</td>
<td>1.6 ± 2.0</td>
<td>1.8 ± 2.0</td>
<td>1.4 ± 2.0</td>
</tr>
<tr>
<td>C-reactive protein, mg/L</td>
<td>1.3 ± 1.7</td>
<td>1.2 ± 1.7</td>
<td>1.4 ± 1.8</td>
<td>1.6 ± 1.9</td>
<td>2.0 ± 2.1</td>
<td>2.1 ± 2.4</td>
</tr>
<tr>
<td>Serum iron, μg/L</td>
<td>68.9 ± 32.0</td>
<td>70.2 ± 30.7</td>
<td>75.3 ± 30.1</td>
<td>62.3 ± 21.7</td>
<td>55.6 ± 20.9¥</td>
<td>79.9 ± 41.1</td>
</tr>
<tr>
<td>Ferritin, ng/ml</td>
<td>42.7 ± 67.9</td>
<td>50.0 ± 69.4</td>
<td>48.9 ± 70.9</td>
<td>50.1 ± 73.0</td>
<td>45.2 ± 69.5</td>
<td>40.3 ± 54.6</td>
</tr>
</tbody>
</table>

*Significantly different from preexercise. ¥Significantly different from 3 hr postexercise. ≠Significantly different from 6 hr postexercise. @Significantly different from 9 hr postexercise. ‡Significantly different from 24 hr postexercise.

**Table 3  Hemoglobin, Hematocrit, C-Reactive Protein, and Ferritin Levels for the 60-min Run, $M \pm SD$**

<table>
<thead>
<tr>
<th></th>
<th>Pre</th>
<th>Post</th>
<th>3 hr</th>
<th>6 hr</th>
<th>9 hr</th>
<th>24 hr</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemoglobin, g/dl</td>
<td>12.4 ± 1.8</td>
<td>13.3 ± 1.3</td>
<td>12.3 ± 1.0</td>
<td>12.3 ± 1.0</td>
<td>12.3 ± 1.0</td>
<td>12.5 ± 1.0</td>
</tr>
<tr>
<td>Hematocrit, %</td>
<td>38.0 ± 4.0</td>
<td>40.4 ± 3.3</td>
<td>38.4 ± 2.8</td>
<td>38.1 ± 3.1</td>
<td>37.9 ± 3.1</td>
<td>38.4 ± 3.3</td>
</tr>
<tr>
<td>C-reactive protein, mg/L</td>
<td>1.9 ± 2.2</td>
<td>1.7 ± 2.2</td>
<td>1.9 ± 2.5</td>
<td>2.1 ± 2.6</td>
<td>2.3 ± 2.8</td>
<td>2.3 ± 2.7</td>
</tr>
<tr>
<td>Ferritin, ng/ml</td>
<td>53.7 ± 89.4</td>
<td>56.5 ± 88.8</td>
<td>56.8 ± 91.7</td>
<td>59.7 ± 95.7</td>
<td>52.6 ± 91.6</td>
<td>45.5 ± 69.4</td>
</tr>
</tbody>
</table>

**Table 4  Hemoglobin, Hematocrit, C-Reactive Protein, and Ferritin Levels for the 120-min Run, $M \pm SD$**

<table>
<thead>
<tr>
<th></th>
<th>Pre</th>
<th>Post</th>
<th>3 hr</th>
<th>6 hr</th>
<th>9 hr</th>
<th>24 hr</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemoglobin, g/dl</td>
<td>12.5 ± 1.0</td>
<td>13.5 ± 1.2</td>
<td>12.6 ± 0.9</td>
<td>12.3 ± 0.9</td>
<td>12.2 ± 0.9</td>
<td>12.4 ± 0.9</td>
</tr>
<tr>
<td>Hematocrit, %</td>
<td>38.3 ± 2.6</td>
<td>40.2 ± 2.9</td>
<td>38.2 ± 3.5</td>
<td>37.8 ± 2.8</td>
<td>37.5 ± 2.7</td>
<td>38.0 ± 2.2</td>
</tr>
<tr>
<td>C-reactive protein, mg/L</td>
<td>0.8 ± 0.5</td>
<td>0.6 ± 0.5</td>
<td>0.8 ± 0.7</td>
<td>1.1 ± 0.8</td>
<td>1.7 ± 1.0</td>
<td>1.9 ± 2.1</td>
</tr>
<tr>
<td>Ferritin, ng/ml</td>
<td>31.8 ± 37.4</td>
<td>43.6 ± 45.5</td>
<td>41.1 ± 44.3</td>
<td>40.5 ± 42.3</td>
<td>37.9 ± 39.8</td>
<td>35.0 ± 36.8</td>
</tr>
</tbody>
</table>
Acute Exercise Bouts and Hepcidin in Women

Hepcidin began to decline by 6 and 9 hr postexercise and was only slightly higher than preexercise values 24 hr postexercise. This pattern was observed in both the 60- and 120-min runs. This pattern is illustrated in Figure 1.

IL-6

Serum IL-6 concentrations are illustrated in Figure 2. A significant effect for time (p = .000) was observed for IL-6, but no significant effect for trial and no Trial × Time interaction were observed. When the combined trial means for time were compared, IL-6 levels were significantly elevated immediately postexercise. These levels began to decline and, while still slightly elevated, were no longer significantly different from preexercise values by 3 hr postexercise. IL-6 means and standard deviations for the combined data from both trials are reported in Table 2.

CRP

Means and standard deviations for CRP levels for each of the trials are reported in Tables 3 and 4. The combined means and standard deviations for both trials are reported in Table 2. A significant main effect for time was observed for CRP (p = .027). However, no significant main effect for trial (p = .404) and no Trial × Time interaction (p = .242), were observed for this variable. When the combined trial means for time were compared using Tukey’s honestly significant difference method, no significant differences were found between time intervals.

Iron-Status Markers

Serum Iron. Serum iron concentrations for each trial are illustrated in Figure 3 and reported in Tables 3 and 4 (M ± SD). Combined means and standard deviations for both trials are reported in Table 2. A significant effect for time (p = .011), but no significant effect for trial (p = .404) and no Trial × Time interaction (p = .242), was observed for serum iron. Serum iron concentrations reached their lowest levels 9 hr postexercise in both trials. Post hoc testing indicated that the 9-hr-postexercise serum iron was significantly different than the 3-hr- and 24-hr-postexercise values.

Ferritin. Means and standard deviations for ferritin concentrations for each of the trials are reported in Tables 3 and 4. Combined means and standard deviations for both trials are reported in Table 2. A significant effect for time was observed for ferritin (p = .040), but no significant effect for trial (p = .343) and no Trial × Time interaction (p = .440) were found. Ferritin concentrations were elevated immediately after exercise. Post hoc tests indicated that the immediately postexercise ferritin concentration was significantly higher than the 24-hr-postexercise mean. Changes in ferritin are illustrated in Figure 4.

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**Figure 1** — Hepcidin concentrations (M ± SD) before and after the 60- and 120-min runs. Significance accepted at p < .05. *Significantly different from before the 60-min run. †Significantly different from after the 60-min run. ‡Significantly different from 24 hr after the 60-min run. §Significantly different from before the 120-min run. †‡Significantly different from after the 120-min run. †§Significantly different from 24 hr after the 120-min run.
Figure 2 — Serum interleukin-6 (IL-6) concentrations ($M \pm SD$) before and after the 60- and 120-min runs. Significance accepted at $p < .05$. *Significantly different from preexercise. ¥Significantly different from 3 hr postexercise. ≠Significantly different from 6 hr postexercise. @Significantly different from 9 hr postexercise. ‡Significantly different from 24 hr postexercise.

Figure 3 — Serum iron concentrations ($M \pm SD$) before and after the 60- and 120-min runs. Significance accepted at $p < .05$. ¥Significantly different from 3 hr postexercise. ‡Significantly different from 24 hr postexercise.
Individual Data

Investigation of the individual data indicated that 5 of the subjects had large hepcidin responses at the 3-hr-postexercise time point, while 7 of the subjects had moderate to very small increases in hepcidin. Subjects were separated into responders (peak hepcidin concentrations >5 nmol/L) and nonresponders (peak hepcidin concentrations <2.4 nmol/L). The responder and nonresponder groups were then analyzed separately, using a three-way ANOVA (Trial × Time × Group, 2 × 6 × 2), for hepcidin, IL-6, and serum iron. Significant effects for each factor; significant Trial × Group, Time × Group, and Trial × Time × Group interactions for hepcidin; and a significant effect for time and significant Trial × Time interaction for IL-6 were found. No significant effects or interactions were observed for serum iron; however, it was observed that responders had a higher mean serum iron concentration than nonresponders, 78.8 ± 37.0 μg/L and 61.5 ± 23.3 μg/L, respectively.

Discussion

This study investigated the effects of two acute bouts of running on hepcidin, markers of inflammation, and iron status in women athletes, including the time course for changes in IL-6, serum hepcidin, and serum iron during and after exercise. Results of this study indicate that significant increases in serum hepcidin occurred approximately 3 hr after acute bouts of exercise. This increase in hepcidin was preceded by a significant increase in IL-6 and was followed by a significant decrease in serum iron. Changes in serum hepcidin were significantly greater after the 120-min run than with the 60-min run.

Serum Hepcidin

The primary goal of this study was to investigate changes that occurred in the iron-regulatory peptide hepcidin as a result of two running bouts of different durations. It was hypothesized that significant increases in serum hepcidin would be greater after the 120-min run than after the 60-min run. Results of our study support this hypothesis as significant effects for trial and for time were observed for hepcidin. The Trial × Time interaction approached but did not reach statistical significance. The large variation in individual responses to hepcidin may explain the lack of significance in the Trial × Time interaction, and the small sample size would have contributed to the low power for this analysis. Large interindividual variability in the response of urinary hepcidin to exercise was observed in a study by Roecker et al. (2005), with 6 of the 14 female subjects in their study being designated as nonresponders and 8 designated as responders.

There was a significant effect for time in the hepcidin response in the current study, with peak serum hepcidin occurring 3 hr postexercise in both trials. These results suggest that women frequently participating in endurance exercise lasting 60 min or longer could potentially
experience increases in serum hepcidin concentration. The peak in serum hepcidin concentration 3 hr postexercise is similar to that found in studies by Peeling et al. (2009a, 2009c), who observed significantly higher urinary hepcidin levels in male subjects at baseline than 3 hr after continuous and interval running sessions, both with durations of around 40 min. In both studies, the increase in urinary hepcidin followed a peak in IL-6 that was observed immediately postexercise. The same pattern with an increase in IL-6 occurred before the peak in serum hepcidin in our study. In another study including both male and female subjects, Peeling et al. (2009b) observed significant increases in IL-6 immediately after a 60-min run at 75–90% HRpeak, which was followed by a significant increase in urinary hepcidin. Urinary hepcidin levels at 3, 6, and 24 hr postexercise were significantly greater than preexercise levels. Increased urinary hepcidin was observed in all participants, although there was variation between participants with regard to the magnitude of the rise. Only the 3-hr-postrun levels of hepcidin were significantly greater than the same time point compared with a 60-min trial of seated rest.

Increases in the inflammatory cytokine IL-6 are frequently observed after prolonged or intense bouts of acute exercise (Fallon, 2001; Hoffman-Goetz & Pedersen, 1994). The current study hypothesized that both the 60- and 120-min runs would induce increases in IL-6, thus reflecting an acute-phase inflammatory response. Increases were observed in IL-6 in both time trials, with the significant changes in IL-6 concentrations observed immediately after exercise. These results are in agreement with those of Gusba, Wilson, Robinson, and Graham (2008), who observed the highest peak in IL-6 levels immediately after exhaustive cycling at 65% of VO2max. When the 60- and 120-min trials were compared in the current study, no significant differences were observed for IL-6. The lack of significant difference between the trials may be a result of the large amount of variability observed within subjects with respect to IL-6 response, as well as the characteristics associated with the relationship between exercise duration and the response of IL-6 (Fischer, 2006).

Kemna et al. (2005) observed a significant increase in hepcidin after a peak in IL-6 resulting from an injection of lipopolysaccharide. However, both the increase in IL-6 and the increase in hepcidin occurred slightly later after the lipopolysaccharide stimulus than with the exercise stimulus. This relationship between increases in IL-6 activity and corresponding increases in hepcidin has been reported in other studies (Huang, Constante, Layoun, & Santos, 2009; Nemeth, Rivera, et al., 2004; Wrighting & Andrews, 2006) and provides the most likely explanation for the increase in hepcidin observed in the current study.

Peeling et al. (2009b) observed that urinary hepcidin levels obtained 12 hr postexercise were no longer significantly different than baseline levels, although still slightly elevated. In the current study serum hepcidin concentrations remained elevated at 6 hr and 9 hr postexercise, although they were not significantly different from baseline. This is in contrast to the study by Roecker et al. (2005), who observed significant increases in urinary hepcidin 1 day after the completion of a marathon. In a recent study, Peeling et al. (2009b) also observed significantly elevated urinary hepcidin concentration 24 hr postexercise compared with preexercise, but it was not significant compared with 24 hr in a resting trial. The persistent increases in urinary hepcidin found in the Roecker and Peeling studies may be a result of greater stress on the body during longer duration (marathon) and higher intensity continuous running than with moderate-intensity exercise. Our study examined changes in serum hepcidin that began to decrease by 6 hr after moderate-intensity exercise.

Iron Status

The study of the relationship between exercise, hepcidin release, and iron status in women bears practical significance, because women tend to be at greater risk for iron deficiency (“Iron Deficiency,” 2002), particularly those participating in endurance training (Gropper et al., 2006; Magazanik et al., 1988; Sinclair & Hinton, 2005). In the current study, hemoglobin, hematocrit, serum iron, and ferritin were used to reflect the iron status of individual subjects. For each of these variables there was a significant effect for time. However, no significant Trial × Time interactions were found, nor were there any significant effects for trial for any of these variables.

Serum Iron. Serum iron concentrations decreased after both exercise bouts in the current study and were significantly lower 9 hr postexercise than the 3- and 24-hr-postexercise means. This decrease at 9 hr postexercise occurred after the peak level of hepcidin (at 3 hr postexercise). Hepcidin inhibits the release of iron from duodenal enterocytes and macrophages, via its action on the iron exporter ferroportin (Andrews, 2000; Nemeth, Tuttle, et al., 2004), which decreases the release and recycling of iron. There was a 10.1% decrease in serum iron after the 60-min run and a 26.7% decrease in iron after the 120-min run at 9 hr postexercise in the current study. These decreases in iron were preceded by increases in hepcidin 3 hr after both 60- and 120-min runs, with larger increases in hepcidin observed after the 120-min run. Serum iron decreased to a low at 9 hr postexercise and returned to baseline by 24 hr in both exercise trials. This effect of exercise on serum iron could have practical implications for women athletes who are participating in vigorous training schedules, particularly those that include multiple exercise bouts on the same day. Repeated increases in hepcidin activity may produce a situation in which serum iron levels are below the normal range, thus contributing to a state of iron deficiency.

Ferritin. An increase in ferritin was observed immediately after exercise, but this increase was only significantly different compared with the 24-hr-postexercise ferritin concentration. Ferritin remained slightly elevated at 6- and 9-hr-postexercise time points but was slightly below
baseline 24 hr postexercise. The changes in ferritin were similar to the results of Lampe, Slavin, and Apple (1986), who observed significant increases in serum ferritin in 9 female runners after completion of a marathon. Their study, as well as data from the current study, suggests that an increase in ferritin occurs after acute exercise, presumably as a result of the acute-phase inflammatory response experienced with endurance exercise (Fallon, 2001; Hoffman-Goetz & Pedersen, 1994). The magnitude of this increase, however, is quite variable, which is probably the result of the combination of factors relating to the exercise bout (intensity, duration), variation in the response of inflammatory cytokines between individuals, and individual iron status.

Responders Versus Nonresponders

Data from individual subjects indicated that 5 had high hepcidin responses at the 3-hr-postexercise time point (responders) and 7 of the subjects had moderate to very small increases (nonresponders). Our results are similar to those in the study by Roecker et al. (2005) after a marathon run. Mean serum iron concentration, although not significantly different, was lower in the nonresponders than in the responders. This suggests that low serum iron concentrations could attenuate the rise in hepcidin associated with endurance exercise in the nonresponder group. This agrees with the results of Peeling et al. (2009b), who observed a greater increase in urinary hepcidin after exercise than both prerun and the rest trial when 3 participants considered iron-deficient were excluded from their analysis. Those authors suggested that the decreased iron levels may have attenuated the hepcidin response to exercise.

In the current study, a slightly different pattern in the response of IL-6 to the 120-min trial was observed in the responders group than with the nonresponders. Both groups had a similar peak IL-6 concentration immediately after the 120-min trial (responders: 3.9 ± 3.6 pg/ml; nonresponders: 4.4 ± 2.6 pg/ml). However, this increased IL-6 concentration was maintained in the responders group at 3 and 6 hr postexercise, compared with the nonresponders, in whom concentrations of IL-6 quickly decreased back to baseline by 3 hr postexercise. These differences were not statistically significant because of the small sample size, but the results suggest that the maintenance of an elevated concentration of IL-6 may also contribute to the higher hepcidin concentrations in the responders group.

Conclusions

Based on the results of this study it was concluded that acute bouts of running lasting 60 min and longer increase the release of hepcidin. It was also concluded that longer exercise durations result in greater increases in hepcidin. Peak increases in hepcidin were preceded by increases in IL-6, reflecting the acute-phase response elicited by the running. Peaks in hepcidin were followed by significant decreases in serum iron 9 hr postexercise, presumably because of hepcidin’s effects on the iron exporter ferroportin.

Both exercise durations used in this study resulted in increases in hepcidin. The 120-min trial produced higher mean hepcidin concentrations than the 60-min trial, as evidenced by the statistically significant main effect for trial. The results for each trial support the conclusion that moderate-intensity running for as little as 60 min will result in increased hepcidin.

References


