Effects of Exercise on Hepcidin Response and Iron Metabolism During Recovery

Peter Peeling, Brian Dawson, Carmel Goodman, Grant Landers, Erwin T. Wiegerinck, Dorine W. Swinkels, and Debbie Trinder

Urinary hepcidin, inflammation, and iron metabolism were examined during the 24 hr after exercise. Eight moderately trained athletes (6 men, 2 women) completed a 60-min running trial (15-min warm-up at 75–80% HR_{peak} + 45 min at 85–90% HR_{peak}) and a 60-min trial of seated rest in a randomized, crossover design. Venous blood and urine samples were collected pretrial, immediately posttrial, and at 3, 6, and 24 hr posttrial. Samples were analyzed for interleukin-6 (IL-6), C-reactive protein (CRP), serum iron, serum ferritin, and urinary hepcidin. The immediate postrun levels of IL-6 and 24-hr postrun levels of CRP were significantly increased from baseline (6.9 and 2.6 times greater, respectively) and when compared with the rest trial ($p \leq .05$). Hepcidin levels in the run trial after 3, 6, and 24 hr of recovery were significantly greater (1.7–3.1 times) than the pre- and immediate postrun levels ($p \leq .05$). This outcome was consistent in all participants, despite marked variation in the magnitude of rise. In addition, the 3-hr postrun levels of hepcidin were significantly greater than at 3 hr in the rest trial (3.0 times greater, $p \leq .05$). Hepcidin levels continued to increase at 6 hr postrun but failed to significantly differ from the rest trial ($p = .071$), possibly because of diurnal influence. Finally, serum iron levels were significantly increased immediately postrun (1.3 times, $p \leq .05$). The authors concluded that high-intensity exercise was responsible for a significant increase in hepcidin levels subsequent to a significant increase in IL-6 and serum iron.

**Keywords:** inflammation, cytokines, high-intensity running

Despite the important roles played by iron in oxygen delivery and energy production, it is the world’s most common diet-scarce nutrient (Umbreit, 2005), and iron deficiency is a frequent diagnosis among athletes, particularly those involved in endurance sports (Beard & Tobin, 2000; Zoller & Vogel, 2004). Low body-iron
stores have been linked to reduced hemoglobin concentration, decreased red cell volume, and a reduction in myoglobin levels (Beard & Tobin). Combined, these limitations to the oxygen transport and delivery system are potentially detrimental to work capacity and athletic performance.

It is proposed that during exercise, iron losses may result from several mechanisms including hemolysis, hematuria, sweating, and gastrointestinal bleeding (Babic et al., 2001; DeRuisseau, Cheuvront, Haymes, & Sharp, 2002; McInnis, Newhouse, von Duvillard, & Thayer, 1998; Zoller & Vogel, 2004). Over time, the cumulative effect of such mechanisms may increase the incidence of iron deficiency should the ingestion and absorption of dietary iron not meet the levels needed to replenish depleted stores.

In addition to such avenues of iron loss, up-regulation of the hormone hepcidin might be a potential mechanism leading to iron deficiency in athletes. Hepcidin is a peptide produced in the liver that is the key regulator of iron metabolism (Nicolas, Viatte, et al., 2002). Hepcidin acts to internalize and degrade the ferroportin iron-transport channels in the intestine and on the surfaces of macrophages, ultimately reducing the absorption of iron from the duodenum and the release of iron recycled from senescent red blood cells (Nemeth, Rivera, et al., 2004; Nemeth, Tuttle, et al., 2004). The expression of hepcidin is tightly controlled by a number of factors, including body-iron levels, erythropoiesis, hypoxia, and, of particular relevance to exercise, inflammatory responses (Nicolas, Chauvet, et al., 2002).

To date, only one investigation has explored the influence of exercise on hepcidin in humans. Roecker, Meier-Buttermilch, Brechtel, Nemeth, and Ganz (2005) collected urine samples from 14 female runners before and immediately after a competitive marathon run and at 24 and 72 hr of recovery. Urinary hepcidin levels were significantly increased at 24 hr after the 42.2-km run compared with preexercise levels and had returned to baseline by 72 hr of recovery. It was proposed that the increased hepcidin levels were driven by an increase in circulating inflammatory cytokines. However, no blood analysis of serum inflammatory cytokines or iron levels was conducted during the investigation. In addition, Roecker et al. reported that only 8 of the 14 participants responded with increased urinary hepcidin levels at 24 hr postexercise. The other 6 participants were classified as nonresponders. Caution should be applied when interpreting these outcomes, because results were not compared with hepcidin levels in a true rested control condition, nor were urinary hepcidin levels measured in samples collected in the period between the completion of exercise and 24 hr later. Kemna, Pickkers, Nemeth, van der Hoeven, and Swinkels (2005) showed that urinary hepcidin levels peaked after 6 hr in healthy participants intravenously injected with a bolus of lipopolysaccharide (an endotoxin that induces a robust immune and inflammatory response), after which the urinary hormone levels began to fall. Therefore, it is possible that the urinary hepcidin concentrations of the nonresponders indicated by Roecker et al. might have peaked and recovered before the 24-hr sample was taken.

Therefore, it is possible that exercise-induced inflammatory increases in hepcidin levels may have an impact on the iron levels of athletes. However, the response of hepcidin levels during the initial 24-hr period postexercise has yet to be conclusively investigated. Therefore, it was the aim of this study to examine the time course of inflammation and hepcidin levels and any effect on serum iron during the 24 hr after high-intensity endurance exercise.
Methods

Participants

Eleven moderately trained endurance runners (6 men and 5 women) running four or more sessions per week were recruited for participation in this study (Table 1). All participants were briefed on the purpose, requirements, and risks involved and were required to provide written informed consent before commencement of the study. Ethical approval for this study was granted by the human ethics committee of The University of Western Australia.

Experimental Overview

Participants were required to attend three laboratory testing sessions during the experimental period, consecutive sessions being separated by a minimum of 28 days. During each visit, the female participants were in Days 1–7 (follicular phase) of the menstrual cycle and were taking no birth control medication. Furthermore, no participants were taking iron supplements. In the 24 hr before each testing day, participants were instructed to refrain from exercise and heavy manual labor.

The first visit to the laboratory involved an initial familiarization with the treadmill and metabolic cart and was concluded with a graded exercise test (GXT), used to determine each individual’s peak oxygen uptake ($\text{VO}_{2\text{peak}}$) and peak heart rate ($\text{HR}_{\text{peak}}$; Table 1). The subsequent two testing sessions were applied in a counterbalanced, randomized order and included a 60-min running trial and a 60-min trial of seated rest. Both the run and rest trials were followed by 6 hr of monitored rest and a 24-hr follow-up appointment.

On the run and rest days, participants reported to the laboratory at 6:30 a.m., after a minimum overnight fast of 10 hr. During the initial 30 min, baseline urine and blood samples were collected. At 7 a.m., the participants began the 60 min of either running or seated rest. The run included a 15-min warm-up at 75–80% $\text{HR}_{\text{peak}}$ (9.6 ± 0.2 km/hr), followed by 45 min at 85–90% $\text{HR}_{\text{peak}}$ (12.0 ± 0.3 km/hr). Immediately on completion of the 60-min run or rest period, venous blood and urine samples were collected. Over the next 6 hr, all participants remained in a rested state at the laboratory. During this time, venous blood and urine were collected at the 3- and 6-hr time points, and serial feedings of a standardized meal were

<table>
<thead>
<tr>
<th>Group</th>
<th>Age (years)</th>
<th>Height (m)</th>
<th>Body mass (kg)</th>
<th>$\text{VO}_{2\text{peak}}$ (ml · kg$^{-1}$ · min$^{-1}$)</th>
<th>$\text{HR}_{\text{peak}}$ (beats/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole group (n = 6 men, 5 women)</td>
<td>25.3 ± 1.0</td>
<td>1.77 ± 0.03</td>
<td>66.9 ± 3.3</td>
<td>51.3 ± 1.9</td>
<td>188 ± 2</td>
</tr>
<tr>
<td>Serum ferritin &gt;35 μg/L (n = 6 men, 2 women)</td>
<td>24.1 ± 0.6</td>
<td>1.80 ± 0.03</td>
<td>71.9 ± 3.1</td>
<td>53.3 ± 2.1</td>
<td>189 ± 1</td>
</tr>
<tr>
<td>Iron deficient (n = 3 women)</td>
<td>29.0 ± 1.4</td>
<td>1.73 ± 0.01</td>
<td>57.9 ± 1.7</td>
<td>48.4 ± 1.1</td>
<td>184 ± 2</td>
</tr>
</tbody>
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Note. $\text{VO}_{2\text{peak}}$ = peak oxygen consumption; $\text{HR}_{\text{peak}}$ = peak heart rate.
provided 1.5 and 4 hr postexercise. Subsequently, all participants were permitted to leave the laboratory and returned at 8 a.m. the following day to provide a final 24-hr postintervention venous blood and urine sample. The participants performed no exercise during the 24-hr postintervention.

**Experimental Procedures**

**GXT.** The GXT was conducted on a motorized treadmill with 4-min work and 1-min rest periods. The initial workload was 8 km/hr, with subsequent 1-km/hr increments over each work period until volitional exhaustion. Over the duration of the GXT the treadmill was set to a gradient of 1% to simulate conditions commonly encountered outside the laboratory (Jones & Doust, 1996). During the GXT, expired air was analyzed for concentrations of $O_2$ and $CO_2$ (Ametek gas analyzers, Applied Electrochemistry, SOV S-3A/1 and COV CD-3A, Pittsburgh, PA). The gas analyzers were calibrated pretest and verified posttest with certified gravimetric gas mixtures (BOC Gases, Chatswood, Australia). Ventilation was recorded at 15-s intervals via a turbine ventilometer (Morgan, 225 A, Kent, England), which was calibrated before and verified after exercise using a 1-L syringe in accordance with the manufacturer’s specifications. $VO_2^{\text{peak}}$ was determined by summing the four highest consecutive 15-s $VO_2$ values.

**Urine.** Urine samples were collected into a 75-ml, sterilized container and were subsequently centrifuged at 10 °C and 3,000 rpm for 10 min. The supernatant was divided into aliquots and stored at –80 °C until analysis. Urinary hepcidin-25 was measured at the Department of Clinical Chemistry, Radboud University Nijmegen Medical Centre, The Netherlands, as described previously (Kemna, Tjalsma, Podust, & Swinkels, 2007; Swinkels et al., 2008). In brief, 5 µl (0.3 µM) of the solution of the lyophilized internal standard (synthetic hepcidin-24, Peptide Int., Louisville KY) in distilled water was added to 495 µL of urine before hepcidin was extracted from the sample with the use of Macro-Prep CM support beads (Biorad Laboratories) as previously described (Park, Valore, Waring, & Ganz, 2001). Next, 1 µl of the extract of hepcidin was applied to a normal-phase chip (NP20, Bio-Rad Laboratories, Hercules, CA) followed by the addition of energy-absorbing matrix (Kemna et al., 2007), all in a nitrogen atmosphere. Hepcidin quantification via surface-enhanced laser desorption/ionization time-of-flight mass spectrometry has been described previously (Kemna, Tjalsma, et al., 2005; Kemna et al., 2007). The standard curve of hepcidin-25 that was constructed by exploiting hepcidin-24 as the internal standard and by serially diluting hepcidin-25 in tubes with blank urine from a patient with juvenile hemochromatosis (van Dijk et al., 2007) was linear ($R^2 > .99$). In addition, spiking of both hepcidin isoforms in different concentration combinations to blank urine revealed no influence of the internal standard hepcidin-24 on the peak height and position of the human hepcidin-25. Hepcidin results are expressed here relative to urinary creatinine levels. The lower limit of detection with this method is 0.05 nM, with an intrarun variation of 3.0% at 3.3 nM and 9.9 nM and an interrun variation of 12.6% at 1.5 nM and 10.2% at 9.1 nM.

**Blood.** Venous blood was collected via venipuncture of an antecubital vein in the forearm. An 8.5-ml SST II gel Vacutainer was filled and allowed to clot for
60 min at room temperature. Subsequently, the sample was centrifuged at 10 °C and 3,000 rpm for 10 min. Serum supernatant was then divided into 1-ml aliquots and stored at −80 °C until further analysis. Serum interleukin-6 (IL-6), C-reactive protein (CRP), iron, and ferritin were measured at the Sir Charles Gairdner Hospital’s pathology laboratory (Perth, Western Australia).

Serum IL-6 was measured using a commercially available ELISA (Quantikine HS, R&D Systems, Minneapolis, MN) with an assay range of 0.38–10 ng/L. The analytical coefficients of variation (CVs) for IL-6 determination at 0.49 and 2.78 ng/L were 9.6% and 7.2%, respectively. The CRP was measured using a Roche CRPLX particle-enhanced immunoturbidimetric assay kit with an analytical CV of 1.8%. Iron, transferrin, and ferritin were measured on the Roche modular system (Roche Diagnostics, Switzerland). Iron levels were determined using FerroZine reagent, and absorbance was measured at 570 nM. The analytical CVs for iron determination at 13 and 43.5 mmol/L were 1.4% and 0.9%, respectively. Ferritin levels were determined using a sandwich IRMA assay (Roche Diagnostics). The analytical CVs for ferritin determination at 30.7 and 232 µg/L were 2.9% and 3.2%, respectively.

Data Analysis

The following data are presented as $M \pm SEM$. Blood analysis showed that 3 of the participants had serum ferritin levels characteristic of Stage 1 iron depletion (serum ferritin <35 µg/L) according to the criteria for stages of iron deficiency outlined by Peeling et al. (2007). As such, these 3 participants were excluded from the main analysis, and the results are relevant only to athletes with serum ferritin levels >35 µg/L ($n = 8$). A repeated-measures ANOVA for time, trial, and Time × Trial effects was conducted between the run and rest conditions. Post hoc, paired-samples $t$ tests were performed in the event of a main effect. The alpha level was accepted at $p \leq .05$. The results of the iron-deficient group (Table 2) were not excluded from presentation in this article, because observations from their data provided interesting insights to suggest a possible effect of iron status on hepcidin production. However, because the iron-deficient group is small, no statistical analysis was carried out on these data.

**Results**

**Run**

The HR recorded during the 15-min warm-up was $151 \pm 4$ beats/min ($80\% \pm 2\% HR_{peak}$). During the 45 min at 85–90% $HR_{peak}$, the HR was $166 \pm 2$ beats/min ($88\% \pm 1\% HR_{peak}$). The running velocity during the 15-min warm-up was $9.6 \pm 0.2$ km/hr. This velocity was subsequently increased for the remaining 45 min to $12.0 \pm 0.3$ km/hr.

**IL-6**

There were significant time ($p = .0001$), trial ($p = .005$), and Time × Trial ($p = .0001$) effects for IL-6 differences between the run and rest conditions (Figure 1).
Table 2  Levels of Interleukin-6 (IL-6), C-Reactive Protein (CRP), Urinary Hepcidin, Serum Iron, and Serum Ferritin for the Iron-Deficient Participants (n = 3) During the Run and Rest Trials, M ± SEM

<table>
<thead>
<tr>
<th>Variable</th>
<th>Rest</th>
<th>Run</th>
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<tbody>
<tr>
<td></td>
<td>Pre</td>
<td>Post</td>
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<tr>
<td>IL-6 (pg/ml)</td>
<td>0.40 ± 0.06</td>
<td>0.32 ± 0.08</td>
</tr>
<tr>
<td>CRP (µg/L)</td>
<td>0.27 ± 0.18</td>
<td>0.23 ± 0.03</td>
</tr>
<tr>
<td>Hepcidin (nM/mmol creatine)</td>
<td>0.04 ± 0.01</td>
<td>0.03 ± 0.01</td>
</tr>
<tr>
<td>Serum iron (µmol/L)</td>
<td>10.10 ± 4.08</td>
<td>9.87 ± 3.41</td>
</tr>
<tr>
<td>Serum ferritin (µg/L)</td>
<td>17.35 ± 4.38</td>
<td>16.96 ± 3.59</td>
</tr>
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</table>
The IL-6 recorded immediately postrun ($p = .002$) and 3 hr postrun ($p = .010$) were significantly greater than at the same time points in the rest trial. The time effect showed that the immediate postrun levels were significantly greater than prerun ($p = .001$) but had returned to baseline levels by 3 hr of recovery.

**CRP**

Significant time ($p = .015$) and Time × Trial ($p = .015$) effects for the CRP levels between the run and rest conditions were found (Figure 2). Here, the 24-hr postrun levels were significantly greater ($p = .049$) than those recorded at 24 hr in the rest condition. The time effect showed that the 24-hr postrun levels were significantly greater than at 6 hr postrun and at prerun ($p = .041$ and $p = .022$, respectively).

**Hepcidin**

There were significant time ($p = .011$) and Time × Trial ($p = .049$) effects for the hepcidin levels between the run and rest conditions (Figure 3). Specifically, the 3-hr postrun hepcidin levels were significantly greater than in the rest condition ($p = .048$), in addition to a strong effect size ($ES = .80$) for greater hepcidin levels in the run condition at the 6-hr time point ($p = .072$). The time effect showed that the 3-hr postrun hepcidin levels were significantly greater than those recorded immediately postrun ($p = .012$) and the 6- and 24-hr postrun levels were significantly greater than the prerun values ($p = .028$ and $p = .030$, respectively). The time effect also showed that the 6-hr hepcidin value in the rest trial was significantly greater than
Figure 2 — C-reactive protein levels during the run and rest trials, \( M \pm SEM \).

Figure 3 — Urinary hepcidin levels during the run and rest trials, \( M \pm SEM \).
at 3 hr \((p = .046)\). Figure 4 represents the individual hepcidin responses of the 8 participants in the run trial. The ranges of hepcidin values were 0.05–1.00 nM/mmol of creatine at prerun, 0.12–1.51 nM/mmol of creatine at 3 hr postrun, and 0.36–4.50 nM/mmol of creatine at 6 hr postrun.

**Serum Iron**

There were significant time \((p = .003)\), trial \((p = .036)\), and Time \(\times\) Trial \((p = .013)\) effects for differences between the run and rest conditions (Figure 5). The trial effect showed that the 6-hr and the 24-hr postrun serum iron levels were significantly lower than in the rest trial \((p = .001\) and \(p = .009\), respectively\). The time effect showed the postrun serum iron to be significantly increased from baseline \((p = .0001)\), which fell back to baseline by 3 hr postrun.

**Serum Ferritin**

The range of serum ferritin was 131 \(\pm\) 19 \(\mu\)g/L to 134 \(\pm\) 18 \(\mu\)g/L in the rest condition and 133 \(\pm\) 25 \(\mu\)g/L to 144 \(\pm\) 26 \(\mu\)g/L in the run trial. There were no significant time \((p = .536)\), trial \((p = .860)\), or Time \(\times\) Trial \((p = .861)\) effects for measured levels of serum ferritin.

**Iron-Deficient Participants**

Table 2 presents the data collected from the 3 female participants removed from the analysis on the basis of Stage 1 iron depletion (serum ferritin <35 \(\mu\)g/L). No
statistical analysis was carried out on these data because of the small sample size. However, observation of the data would suggest that the run trial was still responsible for invoking an acute inflammatory response in these participants (as shown by an increase in IL-6). However, the resultant influence on urinary hepcidin output appears attenuated.

**Discussion**

The findings of this study showed that a 60-min period of high-intensity running in moderately endurance-trained participants with serum ferritin levels >35 µg/L increased the circulating levels of IL-6 (6.9 times) and iron (1.3 times) immediately postexercise and increased the levels of CRP (2.6 times) 24 hr later. Subsequent to this response, urinary hepcidin levels were significantly increased over time when compared with prerun levels (1.7–3.1 times greater) and when compared with a resting control condition at 3 hr postexercise (3.0 times greater). It was noted that the hepcidin response appeared attenuated in participants with a compromised iron status (serum ferritin levels <35 µg/L, n = 3), despite still incurring an inflammatory response.

**Inflammatory and Hepcidin Responses**

The exercise protocol employed during this investigation produced a large acute-phase inflammatory response. In fact, the postrun levels of IL-6 were 6.9 times...
greater than at baseline, and the CRP levels were 2.6 times greater 24 hr later. These results are similar to those of previous investigations that have shown exercise-induced IL-6 increases of 3–25 times and CRP increases of 2–15 times over baseline levels (Brenner et al., 1999; Fallon, 2001; Ostrowski et al., 1998). Circulating levels of IL-6 have been implicated as the primary mediator of hepcidin production (Nemeth, Tuttle, et al., 2004). Here, subsequent to the inflammatory response, the urinary hepcidin levels were significantly increased at 3, 6, and 24 hr postrun when compared with the pre- and immediate postrun values (1.7–5.2 times greater). Similar to the current study, Roecker et al. (2005) found significant increases in urinary hepcidin compared with preexercise levels 24 hr after the completion of a marathon run. It was suggested by those authors that the increases in urinary hepcidin at the conclusion of the marathon may have been caused by the well-documented postexercise increase in IL-6. However, no serum IL-6 levels were measured. Therefore, the results of the current investigation add to the current understanding of exercise-induced changes in hepcidin production, confirming that these changes occur subsequent to an exercise-induced increase in IL-6.

Previously, postexercise measurements of urinary hepcidin have been limited to preexercise, immediately postexercise, and 24 and 72 hr postexercise (Roecker et al., 2005). However, Kemna, Pickkers, et al. (2005) showed that urinary hepcidin levels were at their greatest 3 hr after the peak in IL-6 activity, when the inflammatory response was induced by lipopolysaccharide injection rather than exercise. As such, the most active postexercise period of hepcidin synthesis during the initial hours of recovery is to date unknown. The data of the present investigation agree with the hepcidin time-course findings of Kemna, Pickkers, et al. (2005) showing that postexercise hepcidin levels were significantly elevated 3 hr after the peak in IL-6, with the highest mean values occurring at 6 hr postrun. However, there were no significant differences in the hepcidin levels measured from 3 hr postrun to those recorded 6 or 24 hr postrun. With this in mind, it is possible that Roecker et al. may have seen a greater peak in hepcidin production in the early phases of recovery from the marathon, which represents a greater exercise load than the 60-min run performed here.

In addition to changes in hepcidin levels from preexercise, the current investigation also compared the response with that of a control condition. When considering the group as a whole (N = 11), no postexercise hepcidin levels were significantly greater than in the rest trial, despite there being strong trends to suggest a tendency for elevated levels (p = .06). Initially, we thought that these results were in agreement with the findings of Kemna et al. (2007), because we saw a large variation in urinary hepcidin levels at all measured time points, which potentially contributed to the lack of significant trial differences between the run and the rest conditions. Furthermore, diurnal variation in hepcidin levels has previously been observed (Kemna et al., 2007), which may have also contributed to this variation, especially at the 6-hr postintervention time point. On further analysis of the blood results, however, it was evident that 3 of the initial 11 participants recruited for this study had serum ferritin levels that met the criteria of Stage 1 iron depletion in athletes (as based on the criteria listed by Peeling et al., 2007).

When these 3 iron-deficient participants were removed from the analysis, it became evident that the magnitude of hepcidin response was greater compared with preexercise levels and compared with the rest trial. In fact, the urinary hepcidin levels after 3 hr of recovery were significantly greater than in the control condition.
Furthermore, the 6-hr postrun levels of hepcidin showed a strong trend to being greater than in the rest trial (2.3 times greater, ES .80), but this data point failed to reach significance (p = .072), possibly as a result of a slight diurnal increase in the 6-hr hepcidin levels of the rest trial, as suggested by Kemna et al. (2007). To this end, it would appear that the 3 iron-deficient participants seen here were what Roecker et al. (2005) classed as nonresponders to exercise-induced increases in hepcidin production. However, the other 8 participants, with normal iron levels, showed an increase in hepcidin levels postrun, despite marked variation in the magnitude of this rise.

To help explain what may be occurring in the iron-deficient participants, it must be considered that the typical response of the body to low hepatic iron levels is to decrease hepcidin synthesis, which in turn increases iron absorption from the diet and iron release from macrophages (Nicolas, Viatte, et al., 2002). In contrast, the response to chronic illness and its associated inflammation causes patients to up-regulate hepcidin expression, causing decreased iron absorption and retention of iron in the macrophages, resulting in a reduced level of serum iron (Ganz, 2003; Nemeth et al., 2003). To date, the response of hepcidin to acute inflammation produced by exercise in participants with insufficient iron stores has not been investigated. However, it is possible that reduced liver iron levels in the 3 iron-deficient participants of this study may have attenuated the up-regulation of hepcidin in response to exercise-induced inflammation. If this is correct, this mechanism may explain why there were 6 nonresponders in Roecker et al.'s (2005) investigation. Because iron parameters were not measured, it is possible that these 6 athletes were iron deficient before the commencement of the marathon run. Furthermore, it should be noted that the 3 iron-deficient participants in our investigation were all women, as were the iron-deficient participants of Roecker et al. As such, these observations should be used as a basis for further investigation using a larger sample of iron-deficient athletes to confirm such a difference in response and to elicit possible gender differences.

**Serum Iron and Ferritin**

The current study showed a significant elevation in serum iron levels at the immediate completion of the run trial. Buchman et al. (1998) suggested that the increased serum iron markers postexercise may be reflective of red-blood-cell destruction, otherwise known as hemolysis. Exercise-induced hemolysis is the destruction of the red blood cells as a result of the circulatory stress and/or the physical impact incurred during activity. Telford et al. (2003) showed running to be associated with the greatest degree of hemolysis, primarily because of the impact forces that occur at the heel during foot strike. In the event of a hemolytic episode, macrophages engulf cellular debris and free iron released from the hemolyzed red blood cells (Bessis & Breton-Gorius, 1957). Macrophages usually recycle iron in the liver via ferroportin channels expressed on the cell surface (Lymboussaki et al., 2003). However, up-regulation of hepcidin causes internalization and degradation of ferroportin, thereby inhibiting the release of iron from the macrophages that was collected from hemolyzed and senescent red blood cells (Nemeth, Tuttle, et al., 2004). In addition to inflammation, hepatic iron levels are implicated in the regulation of hepcidin production (Nemeth, Tuttle, et al., 2004). As previously mentioned, increases in
serum iron result in increased hepcidin production to reduce iron absorption and recycling in the gut and from macrophages (Ganz, 2003; Nemeth et al., 2003). To this end, the postexercise increases in hepcidin production seen here and in previous investigations may be homeostatic in nature, acting to return serum iron levels back to baseline during the initial 3 hr of recovery via reduced absorption of iron in the gut and reductions in iron recycling from macrophages. Hence, the combination of inflammation and hemolysis add to the current body of knowledge with respect to the mechanisms that explain iron metabolism during exercise.

**Conclusion**

This study has shown that urinary hepcidin levels are significantly elevated postexercise relative to prerun levels and compared with a rested control condition in moderately trained athletes with serum ferritin levels >35 µg/L. The increase in hepcidin levels occurred approximately 3 hr subsequent to an increase in IL-6 and serum iron, suggesting that it is the combination of inflammatory and iron-regulated changes to hepcidin levels that may influence iron metabolism in athletes. However, it would appear that the elevation in hepcidin levels was attenuated in female iron-deficient participants, warranting further investigation in the future on the influence of current iron status and specific gender differences in hormone response to exercise.

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**References**


