Are Serum Hepcidin Levels Chronically Elevated in Collegiate Female Distance Runners?

Xiaoya Ma, Kaitlyn J. Patterson, Kayla M. Gieschen, and Peter F. Bodary

The prevalence of iron deficiency tends to be higher in athletic populations, especially among endurance-trained females. Recent studies have provided evidence that the iron-regulating hormone hepcidin is transiently increased with acute exercise and suggest that this may contribute to iron deficiency anemia in athletes. The purpose of this study was to determine whether resting serum hepcidin is significantly elevated in highly trained female distance runners compared with a low exercise control group. Due to the importance of the monocyte in the process of iron recycling, monocyte expression of hepcidin was also measured. A single fasted blood sample was collected midseason from twenty female distance runners averaging 81.9 ± 14.2 km of running per week. Ten age-, gender-, and BMI-matched low-exercise control subjects provided samples during the same period using identical collection procedures. There was no difference between the runners (RUN) and control subjects (CON) for serum hepcidin levels ($p = .159$). In addition, monocyte hepcidin gene expression was not different between the two groups ($p = .635$). Furthermore, no relationship between weekly training volume and serum hepcidin concentration was evident among the trained runners. The results suggest that hepcidin is not chronically elevated with sustained training in competitive collegiate runners. This is an important finding because the current clinical conditions that link hepcidin to anemia include a sustained elevation in serum hepcidin. Nevertheless, additional studies are needed to determine the clinical relevance of the well-documented, transient rise in hepcidin that follows acute sessions of exercise.

The female athletic population is at a heightened risk for iron deficiency anemia (Beard & Tobin, 2000). Because iron is essential to produce adequate hemoglobin for erythropoiesis, iron deficiency anemia results in profound decrements in endurance performance (LaManca & Haymes, 1993; Risser et al., 1988; Beard & Tobin, 2000). Iron deficiency anemia is often diagnosed in females with ferritin levels below 12 μg/L accompanied by hemoglobin concentration below 12 g/dl. Low hematocrit, low mean corpuscular volume (MCV), and high transferrin receptor values are additional indicators of iron deficiency anemia (Killip et al., 2007). Maintaining adequate iron stores is a high priority to athletes and coaches; however, iron deficiency continues to be prevalent in this population.

Iron recycling is a highly regulated process. Iron depletion begins when iron losses continually exceed iron absorption and recycling. Several mechanisms of iron loss can be attributed to exercise including gastrointestinal blood loss (Stewart et al., 1984), hematuria (Abarbanel et al., 1990), and increased red cell turnover (Shaskey & Green, 2000). Menstruation also contributes to iron loss in females. Iron is absorbed by enterocytes in the duodenum and proximal jejunum; however, only about 5% (nonheme sources) to 25% (heme sources) of the iron in the diet is typically absorbed (Aspuru et al., 2011; Roughead & Hunt, 2000). The majority of the body’s iron stores are recycled from senescent red blood cells by splenic and hepatic tissue macrophages (Ganz, 2012). In addition, the iron recycled by macrophages after phagocytosis of senescent red blood cells represents the main iron supply for erythropoiesis in the bone marrow (De Domenico et al., 2008). Hormonal control contributes to iron homeostasis primarily through the macrophage and enterocyte (Andrews & Schmidt, 2007).

Iron homeostasis is regulated by hepcidin, a small peptide hormone produced primarily by the liver (Nemeth & Ganz, 2009). It is encoded by the HAMP gene which results in the production of the 80 amino acid prepro-hormone (Ganz, 2003). Numerous proprotein convertases can cleave preprohepcidin to the 25 amino acid active peptide (Schranz et al., 2009). HAMP is known to be induced by iron overload and inflammation, and hepcidin regulates the exclusive cellular iron exporter, ferroportin (Nemeth et al., 2003; Pigeon et al., 2001). Hepcidin inhibits iron export from macrophages and enteric cells by binding to and inducing degradation of ferroportin causing sequestration of iron in these cells. This reduces both intestinal iron absorption and iron release from macrophages into the circulation (Ganz, 2005). Moreover, hepcidin is proposed to be increased in states of inflammation as a protective mechanism to reduce free iron in the presence of possible bacterial infection (Krause et al., 2000; Park et al., 2001; Pigeon, et al., 2001). This response is mediated by the action of...
various cytokines including the proinflammatory cytokine interleukin-6 (IL-6), which has been shown to induce HAMP expression (Nemeth et al., 2004). In addition to liver-derived hepcidin, the monocyte and macrophage have been demonstrated to secrete hepcidin and attenuate iron release through an autocrine mechanism (Peyssonnaux et al., 2006; Theurl et al., 2008). Thus, monocyte hepcidin has the potential to be detrimental to health by reducing the amount of iron available for erythropoiesis, increasing the intracellular macrophage iron content, and increasing the proinflammatory state of the cell (Ganz & Nemeth, 2009).

A significant elevation in hepcidin has been observed in specific groups with anemia associated with inflammation and/or chronic diseases (Andrews, 2004; Cheng et al., 2011; Ganz, 2003; Ganz & Nemeth, 2009; Nemeth et al., 2004; Nicolas et al., 2002; Theurl et al., 2009). In these conditions, anemia is secondary to an upregulation of hepcidin induced by chronic inflammation (Theurl et al., 2009). Exercise training-induced inflammation, possibly through activation of the acute phase response and/or elevation in circulating IL-6, has been speculated to promote a similar set of symptoms including elevated hepcidin and iron deficiency anemia (Banzet et al., 2012; Newlin et al., 2012; Peeling et al., 2008). Several studies have described a transient elevation in serum hepcidin levels approximately three hours after exercise (Newlin et al., 2012; Sim et al., 2012, 2013). However, many of the published studies have focused on urinary hepcidin and/or the response to a single exercise stimulus (Newlin et al., 2012; Peeling et al., 2009a, 2009b, 2009c; Robson-Ansley et al., 2011; Roecker et al., 2005). Despite the growing literature on the topic, the clinical significance of the hepcidin response to exercise and its relevance to iron deficiency anemia have yet to be established.

The purpose of this study was to determine whether a chronic hepcidin elevation is evident during periods of high training in female runners. This was investigated through measurement of serum hepcidin levels and other hematologic indicators in a cohort of collegiate female distance runners and low exercise control subjects. In addition, isolated monocytes were examined to investigate a potential influence of training on inflammatory and iron regulatory gene expression.

**Methods**

**Subjects**

A cohort of 20 female runners (RUN) was recruited from a NCAA Division I Varsity Cross Country team with cross-country 5 km personal best times between 17 and 20 min. A control group (CON) was comprised of 10 age- and BMI-matched female subjects with low levels of exercise (Table 1). The study was approved by the Institutional Review Board of the University of Michigan. Subjects were informed of the requirements of the study before consenting to participation. All subjects were female between 18 and 23 years of age. None of the subjects were pregnant or lactating, and there was no evidence or history of cardiovascular or metabolic disease.

A preliminary questionnaire was conducted before the blood collection to gather information regarding vitamin and iron supplementation, training level, lifestyle trends, and menstrual history. RUN reported average training distance per week which was converted to minutes per week. Minutes of weekly training in RUN also includes biweekly weight lifting sessions. Each subject’s blood collection date was standardized for menstrual cycle in regularly cycling subjects, occurring between the 15th and 19th day of the cycle. On the day of blood sample collection, subjects reported the most recent exercise session, iron supplementation, and recent use of anti-inflammatory medications. Subject height and weight were measured to calculate BMI. A 20 ml blood sample was collected from the antecubital vein following a 12-hr overnight fast.

**Hematologic Measures**

A 2 ml blood sample was collected into a commercially available Acid Citrate Dextrose tube (BD vacutainer, Franklin Lakes, NJ); complete blood count was performed by an Advia 120 Hematology System analyzer according to manufacturer instructions (Bayer Diagnostics, Tarrytown, NY). Whole blood was collected into a Plus Plastic Serum tube (BD vacutainer, Franklin Lakes, NJ). The sample was kept at room temperature for 30 min and was then centrifuged at 3000 × g for 10 min. Serum was stored in a -80 °C freezer until cytokine assays were performed. Serum iron, transferrin, and transferrin saturation were determined using the Total Iron Binding Capacity (TIBC) assay (Pointe Scientific Inc., Canton, MI). Serum ferritin concentration was determined using the DRG ferritin immunoassay (DRG International, Inc., Mountainside, NJ). Hepcidin was measured by a commercially available enzyme immunoassay (EIA) kit (Peninsula Laboratories, San Carlos, CA).

**Table 1  Subject Information**

<table>
<thead>
<tr>
<th></th>
<th>Age (years)</th>
<th>BMI (kg/m²)</th>
<th>Training level (min/week)</th>
<th>Percent Iron supplemented</th>
</tr>
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<tbody>
<tr>
<td>CON (n = 10)</td>
<td>19.5 ± 1.35</td>
<td>20.5 ± 2.27</td>
<td>51.5 ± 39.7</td>
<td>0%</td>
</tr>
<tr>
<td>RUN (n = 20)</td>
<td>19.5 ± 1.07</td>
<td>20.5 ± 1.32</td>
<td>441.8 ± 64.2</td>
<td>85%</td>
</tr>
</tbody>
</table>
Isolation of Monocytes

Peripheral blood mononuclear cells were freshly isolated from whole blood by Histopaque-1077 (Sigma-Aldrich; St. Louis, MO) density gradient centrifugation as previously described (Feldman & Mogelesky, 1987). Monocytes from 6 ml whole blood were prepared for RNA isolation and reverse transcription Polymerase Chain Reaction (rtPCR). Monocytes isolated from an additional 3 ml whole blood were prepared for cell cytokine examination.

Monocyte CD163 Determination

Following monocyte isolation, the monocyte cell pellet was homogenized with 400ul RIPA buffer (Boston Bioproducts, Ashland, MA) to produce a cell lysate. The total protein of the cell lysate was determined by Bradford assay. CD163 concentration of the cell lysate was then measured by an immunoassay kit obtained from R&D (Quantikine HS ELISA kit; Minneapolis, MN) and normalized to total protein.

Determination of Human Monocyte Gene Expression

Total RNA was extracted from monocytes using the RNaseous kit (Life Technologies, Grand Island, NY) according to the manufacturer’s instructions. Total RNA was quantified by NanoDrop (NanoDrop Technologies, Wilmington, DE), and equal amounts of RNA (2 μg) were reverse transcribed into cDNA with High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, CA). Quantitative PCR (qPCR) was used to amplify the cDNA with gene specific primers using Fast SYBR green method (Applied Biosystems, Grand Island, NY). Different monocyte gene primers were constructed by the Perl Primer program (Marshall, 2004). GAPDH was used as a housekeeping gene. Table 2 shows the genes and sequences of primers used in this study. qPCR was carried out by StepOne Plus software (Applied Biosystems, Foster City, CA). Results were analyzed by 2^(-ΔΔCT) method described previously (Livak & Schmittgen, 2001; Yang et al., 2009).

Statistical Analysis

The statistical analysis was carried out using the SPSS statistics package (IBM SPSS statistics 19). Results are expressed as Mean (± SD). Each dependent variable was tested for evidence of a normal distribution by use of a Q-Q plot. For measures that were normally distributed, we used an independent t test to compare group means. For serum hepcidin, which had a nonnormal distribution, we performed a log-transformation of the data. An independent t test was then applied to the normally distributed log-hepcidin data. The nonparametric Mann-Whitney U test was used for other variables with a nonnormal distribution. P-values less than 0.05 were considered statistically significant.

Results

Questionnaire

Forty percent of RUN reported that they had been previously diagnosed with anemia, and 85% of RUN were regularly taking an iron supplement in the form of ferrous sulfate. Regular menstrual cycles were reported in 80% of CON and 70% of RUN. Approximately 75% of subjects across both groups reported two or less servings of red meat per week. Consistent with our recruitment strategy for CON and RUN, there was a distinct difference in the amount of weekly exercise between groups (Table 1).

Serum Hepcidin and Training Level

Hepcidin levels fell within a range of 1.3–41.2 ng/ml in RUN and 0.9–41.1 ng/ml in CON. These values are consistent with a recently published normal reference range for 18–24 year old females (1.95–29.28 ng/ml) where a median value of 7.25 ng/ml was observed (Galesloot et al., 2011). As the measurement was not normally distributed, a log-transformation was performed and independent t test was used to compare the groups (Figure 1). There was no significant difference for serum hepcidin concentration between CON and RUN (p = .159). The average weekly running distance in RUN ranged from 56.3 to 104.6 km and the average distance ran in the 24 hr

<table>
<thead>
<tr>
<th>Table 2 Primer Sequences for Monocyte Gene Expression</th>
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<tbody>
<tr>
<td><strong>Forward (5'→3')</strong></td>
</tr>
<tr>
<td>GAPDH</td>
</tr>
<tr>
<td>HAMP</td>
</tr>
<tr>
<td>HMOX1</td>
</tr>
<tr>
<td>SLC40A1</td>
</tr>
<tr>
<td>CCL2</td>
</tr>
<tr>
<td>IL6</td>
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<tr>
<td>CD163</td>
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before the blood collection was 10.5 ± 6.4 km and ranged from 0 to 19.3 km. The training session preceding the blood sampling was a continuous run at a moderate aerobic effort (corresponding to a running pace of 12.9–13.7 kph). To examine whether these factors related to the variation in serum hepcidin concentration, we performed correlational analyses using the Spearman’s rank-order correlation. However, there was no relationship evident between serum hepcidin and the weekly training distance or the running distance in the 24 hr preceding the blood sample (Figure 2a and 2b). To investigate any impact of previous anemia, RUN was divided into two groups: 8 with and 12 without a self-reported history of anemia. However, there was no difference in serum hepcidin based on the history of anemia (p = .173; Figure 2c).

**Monocyte Gene Expression**

Gene expression studies were successfully performed on 27 of the 30 subjects (CON: n = 10; RUN: n = 17). No differences were observed between RUN and CON in the primary iron export protein, ferroportin (SLC40A1). Similarly, no differences were evident for the heme oxygenase gene, HMOX1, the inflammatory related genes, CCL2 and IL-6, or the hepcidin gene (HAMP) between RUN and CON. However, gene expression of the cell surface iron scavenger, CD-163, was significantly higher in RUN than CON (p < .05; Table 3).

**Monocyte CD163 Protein**

To further investigate the observed increase in monocyte CD163 gene expression, we used monocyte lysates to determine the monocytic CD163 protein level in RUN and CON (n = 10 per group). However, there was no significant difference between RUN and CON for monocytic CD163 protein (CON: 20.6 ± 10.4 ng/mg, RUN: 23.8 ± 5.33 ng/mg; p = .399)

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**Figure 1** — Serum hepcidin. Log-transformed serum hepcidin levels in both CON (n = 10) and RUN (n = 20). The individual subject values are provided for each group adjacent to the mean value. Subjects without iron supplementation are depicted by gray diamonds while iron supplemented subjects are depicted with black diamonds.

**Figure 2** — Correlation between serum hepcidin and training levels, recent training, and history of iron deficiency anemia in RUN (n = 20). A) The relationship between weekly running distance and log-transformed serum hepcidin ; B) The relationship between distance run in 24 hr before the blood sample collection and log-transformed serum hepcidin; C) Bar graph of log-transformed serum hepcidin in runners with (n = 8) and without (n = 12) history of iron deficiency anemia. Black diamonds represent iron-supplemented runners (n = 17), gray diamonds represent runners without iron supplementation (n = 3).
Serum Hepcidin Levels in Female Distance Runners

Hematology

Complete blood count and iron status measures are shown in Table 4. White blood cell (WBC) count and several WBC subpopulations, such as leukocytes and eosinophils, were significantly lower in RUN ($p < .05$). This is consistent with previous studies regarding exercise and immune function (Gabriel & Kindermann, 1997). Monocyte count tended to be lower in RUN ($p = .064$), but there was no significant difference between RUN and CON (Table 4). However, mean corpuscular volume (MCV) was significantly higher in RUN versus CON ($p = .015$). This may be indicative of increased hemolysis in the runners (Eichner, 1985) and is not consistent with the microcytosis present in iron deficiency anemia. However, all subjects fell within the normal MCV clinical range of 80–99 fL. In both groups, the hemoglobin levels were relatively low across all subjects (range: 10.5–13.3 g/dl) compared with a typical normal range of 12.1–15.1 g/dl, with no significant difference between RUN and CON. Mean serum ferritin concentration was not significantly different between groups (Table 4).

Discussion

This is the first study using female collegiate distance runners with sustained high-level training that investigates resting hepcidin levels. Evidence of elevated hepcidin at around 3 hr following moderate to high intensity exercise is well documented. However, the question of an additive or cumulative effect of multiple acute excursions of hepcidin in response to everyday training on elevating resting levels is still unclear. The main finding of this study, based on a single measure, was that highly trained female athletes did not have significantly higher resting hepcidin levels compared with control subjects, suggesting that there is no cumulative effect of chronic, daily endurance training. A chronic elevation of hepcidin in response to training would represent an important finding due to hepcidin’s critical role in iron homeostasis and the relatively high prevalence of iron deficiency anemia in female athletes. However, we observed that serum hepcidin levels in highly trained female runners did not differ significantly from an age- and BMI-matched low exercise control group in a single resting measure. This

Table 3 Iron- and Inflammation-Related Monocyte Gene Expression

<table>
<thead>
<tr>
<th></th>
<th>CON ($n = 10$)</th>
<th>RUN ($n = 17$)</th>
<th>$p$</th>
</tr>
</thead>
<tbody>
<tr>
<td>HAMP (AU)</td>
<td>1.00 ± 0.39</td>
<td>1.11 ± 0.61</td>
<td>.635</td>
</tr>
<tr>
<td>IL6 (AU)*</td>
<td>1.00 ± 0.68</td>
<td>0.62 ± 0.34</td>
<td>.083</td>
</tr>
<tr>
<td>CD163 (AU)</td>
<td>1.00 ± 0.17</td>
<td>1.18 ± 0.21*</td>
<td>.039</td>
</tr>
<tr>
<td>CCL2 (AU)*</td>
<td>1.00 ± 0.67</td>
<td>1.01 ± 0.61</td>
<td>.711</td>
</tr>
<tr>
<td>SLC40A1 (AU)</td>
<td>1.00 ± 0.19</td>
<td>0.98 ± 0.25</td>
<td>.834</td>
</tr>
<tr>
<td>Hmox1 (AU)</td>
<td>1.00 ± 0.24</td>
<td>1.02 ± 0.23</td>
<td>.843</td>
</tr>
</tbody>
</table>

*Nonparametric Mann-Whitney U test; *$p < .05$

Table 4 Complete Blood Cell Count and Hematologic Results for CON and RUN

<table>
<thead>
<tr>
<th></th>
<th>CON ($n = 10$)</th>
<th>RUN ($n = 20$)</th>
<th>$P$</th>
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</thead>
<tbody>
<tr>
<td>WBC $\times 10^3$ (cells/μl)</td>
<td>6.3 ± 1.64</td>
<td>5.1 ± 0.82*</td>
<td>.009</td>
</tr>
<tr>
<td>Lymph $\times 10^3$(cells/μl)</td>
<td>2.3 ± 0.47</td>
<td>1.8 ± 0.39*</td>
<td>.005</td>
</tr>
<tr>
<td>Mono $\times 10^3$(cells/μl)</td>
<td>0.4 ± 0.15</td>
<td>0.3 ± 0.11</td>
<td>.064</td>
</tr>
<tr>
<td>Eos $\times 10^3$(cells/μl)</td>
<td>0.2 ± 0.10</td>
<td>0.1 ± 0.06*</td>
<td>.006</td>
</tr>
<tr>
<td>RBC $\times 10^6$ (cells/μl)</td>
<td>3.9 ± 0.31</td>
<td>3.8 ± 0.24</td>
<td>.283</td>
</tr>
<tr>
<td>Hgb (g/dl)</td>
<td>11.9 ± 0.76</td>
<td>11.8 ± 0.71</td>
<td>.737</td>
</tr>
<tr>
<td>MCV (fL)</td>
<td>90.8 ± 4.43</td>
<td>94.52 ± 3.45*</td>
<td>.015</td>
</tr>
<tr>
<td>Serum iron (μg/dl)</td>
<td>113.3 ± 30.62</td>
<td>101.8 ± 34.65</td>
<td>.384</td>
</tr>
<tr>
<td>UIBC (μg/dl)</td>
<td>200.2 ± 70.36</td>
<td>213.8 ± 82.70</td>
<td>.659</td>
</tr>
<tr>
<td>TIBC (μg/dl)</td>
<td>313.5 ± 55.66</td>
<td>315.7 ± 72.46</td>
<td>.933</td>
</tr>
<tr>
<td>Transferrin saturation (%)</td>
<td>37.3 ± 12.25</td>
<td>33.4 ± 11.36</td>
<td>.384</td>
</tr>
<tr>
<td>Serum ferritin (ng/ml)*</td>
<td>25.0 ± 29.27</td>
<td>32.5 ± 24.23</td>
<td>.120</td>
</tr>
</tbody>
</table>

*Nonparametric Mann-Whitney U test; *$P < .05$
result is in agreement with recently published training studies investigating serum hepcidin concentration where there was no significant increase in resting hepcidin following weeks of training (Auersperger et al., 2012; Karl et al., 2010).

Studies have demonstrated that low iron stores directly suppress hepcidin concentration (Krijt, Frydlova, Kukackova, Fujikura, Prikryl, Vokurka, & Necas, 2012; Nemeth et al., 2004). In the current study, low serum ferritin levels were evident in both control and experimental groups compared with population reference standards. Therefore, the consistent level of serum hepcidin may be attributed to the low iron stores evident in both RUN and CON. Even an inflammatory stimulus that would normally increase hepcidin (e.g., exercise-induced IL-6) may be inhibited in the presence of low iron stores (Nicolas et al., 2002). This concept is not without precedent as previous exercise studies have speculated that the transient rise in hepcidin in response to a single exercise session is suppressed in athletes with low serum ferritin (Peeling et al., 2009b) or low serum iron (Newlin et al., 2012). The cause of the low iron stores in the current study is also of interest. Although extensive dietary records were not collected, both CON and RUN subjects self-reported low intake of red meat. Red meat represents a source of heme iron which is most readily absorbed from the gut. A limited consumption of this type of dietary iron may have contributed to the observed low serum ferritin in both groups.

The monocyte, as a precursor to the macrophage, plays important roles in both inflammation and iron homeostasis. There is evidence of a twofold elevation of hepcidin (HAMP) gene expression from the circulating monocytes of ACD patients versus non-ACD controls (Theurl et al., 2008). As high intensity endurance exercise has been hypothesized to promote hepcidin and induce an inflammatory response similar to ACD, we isolated monocytes from RUN and CON and determined HAMP gene expression. However, despite the high training level of RUN (on average more than 80 km per week), we did not observe a significant increase in HAMP gene expression compared with CON. This result is consistent with other ACD-related measures in our study where we observed no difference between RUN and CON including serum levels of iron, ferritin, and hepcidin. Overall, the results from our study do not suggest an ACD-like phenotype resulting from the rigors of collegiate distance running in female athletes.

In addition to the measure of HAMP expression from our isolated monocytes, we also determined several other iron- and inflammation-related genes expressed in monocytes. Consistent with our lack of difference in HAMP, we did not observe differences in the gene expression of ferroportin (SLC40A1), IL-6, or CCL2 between RUN and CON. However, we did observe a modest increase in monocyte gene expression of CD163 in RUN. CD163 is expressed in monocytes and macrophages and is responsible for scavenging the hemoglobin-haptoglobin complex (Andrews, 2004) as well as free hemoglobin (Schaer et al., 2006). This is especially interesting since others have established that increased intravascular hemolysis in runners generates hemoglobin-haptoglobin complexes and free hemoglobin (Davidson, 1964; Magnusson et al., 1984; Peeling et al., 2009c). In addition, CD163 promotes an anti-inflammatory phenotype in monocytes and macrophages that promotes iron recycling (Kowal et al., 2011; Ugocsai et al., 2006). Although intriguing, this potential of an upregulation of CD163 is tenuous in the current study, as the effect was small and was not confirmed in our measure of monocytic CD163 protein. Nevertheless, we speculate that this may be an interesting iron-regulating response in the monocyte resulting from endurance exercise training.

Our study design had several limitations. One limitation is that we solicited blood samples from just one collegiate cross-country team at one time point in the middle of their competitive season. Therefore, our study had a small sample size and a limited power for detecting differences between the RUN and CON group. Another limitation was the partial confounding resulting from a high prevalence of iron supplementation in the athletes. Although the intent was for exercise volume to be the only variable that differed between RUN and CON, most of RUN (85%) were taking an oral iron supplement (ferrous sulfate) while none of CON were supplemented. However, the evidence to date suggests that iron supplementation results in an increase rather than a decrease in hepcidin concentration (Berglund et al., 2011; Nemeth et al., 2004). In addition, comparing the hepcidin level between the athletes that had or did not have iron supplementation did not provide evidence of differences in hepcidin level due to supplementation. Therefore, the presence of the iron supplementation should increase the likelihood of detecting higher serum hepcidin in the athletes rather than conceal a difference between the groups.

Our study was also limited by variable sample collection schedule. Blood sampling was carefully scheduled to control for menstrual cycle phase and the time of day of collection. However, athletes were not asked to alter their training plan. As a result, the time between the last training session and the blood collection was either 16 or 23 hr in subjects training the previous day. There was variable distance of their most recent run as well as three subjects who did not train on the day preceding the blood collection. We expect that this was not problematic, as our goal was to determine the cumulative effect of training at a single time point rather than the transient effect of training, which has been previously established (Newlin et al., 2012; Peeling et al., 2009a; Peeling et al., 2009b; Roecker et al., 2005). In addition, we found no correlation between hepcidin level and the distance of the most recent training bout or the total average weekly training volume. Nevertheless, our study design would have benefitted from additional time points and/or additional control over the training sessions of athletes.
Conclusion and Clinical Relevance

We have evaluated the resting serum hepcidin concentrations of collegiate female distance runners and observed no difference compared with a well-matched low exercise control group. Although hepcidin has been demonstrated to be transiently increased with strenuous exercise, we did not observe evidence of an elevation of resting hepcidin in highly trained female athletes with low iron stores. In addition, studies of monocyte gene expression resulted in higher CD163 gene expression in the athletes compared with controls, suggesting a potential anti-inflammatory response in the monocyte resulting from endurance training. Further research should be conducted to determine the importance of the exercise-induced, transient elevation in hepcidin to the incidence of iron deficiency anemia. Moreover, iron deficiency in female distance runners is a widespread and multifactorial problem, which will likely benefit from studies beyond the realm of the exercise-induced hepcidin response.

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Conflict of Interest

This research involves no professional relationships with companies or manufacturers who will benefit from the results presented; furthermore, the authors have no conflicting interests.

References


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