Hepcidin Response to Exercise: A Review

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Abstract

Given the multiple functions of iron in the body, any state of iron deficiency will induce a series of secondary effects that could compromise sports performance. Low serum iron levels are commonly observed in athletes during the course of a training period, especially in those performing aerobic exercises and resistance training. Sometimes, body iron levels will even fall below those detected in sedentary individuals, and we could go as far as to say that iron deficiency is the most frequently observed nutritional disorder among athletes of any sport. Hepcidin, a hormone secreted by hepatocytes whose principal mechanism of action is the degradation of ferroportin (the main iron exporter from macrophages and the basolateral membrane of duodenal enterocytes), has been proposed as the main regulator of the body's iron reserves. Thus, elevated serum hepcidin levels lead to diminished iron absorption and recycling, while lower levels of the hormone will cause greater iron absorption. Among the factors that affect the hepcidin response produced, we should highlight an individual's total iron levels, erythropoietic demands, state of hypoxia, dietary iron, inflammation and physical exercise. Given the important role played by iron regulatory mechanisms in physical performance, this report reviews our current understanding of the physiological response of hepcidin to different sports intensities and modalities. Turk Jem 2014; 18: 84-91

Key words: Iron, iron deficiency, inflammation, cytokines, aerobic exercise, hepcidin

Özet


Anahtar kelimeler: Demir, demir eksikliği, inflamasyon, sitokinler, aerobik egzersiz, hepcidin

Introduction

Iron deficiency is the most frequently observed nutritional disorder in athletes performing aerobic exercise and resistance training (1,2), especially affecting women (3,4) and adolescents (2,5). Estimates of the incidence of this disorder in men and women athletes run at 11% and 35%, respectively (6,7). Studies have shown that physical exercise modifies several variables related to iron metabolism (8), and mean haemoglobin and ferritin concentrations have been generally found to be lower in athletes than in untrained subjects (9,10). Significant variations have also been detected in such variables during the course of a sports season (11).
levels. A major goal for any athlete should therefore be to keep iron levels stable throughout an entire sports season (20). Although attempts have been made to relate the intake of iron in athletes to their body iron stores, numerous studies have detected no such correlation (21,22,23), mainly because absorption mechanisms may be affected by different physical activities (21). Thus, as a negative iron regulator in the duodenum (24) and the main iron metabolism regulator (25,26,27), understanding hepcidin response associated with physical exercise may help explain anaemia and iron deficiency that so frequently affect athletes, especially during resistance training (28).

In this report, we update the current state of the topic by reviewing the literature addressing the hepcidin response associated with exercise.

**Physiology of Hepcidin**

Hepcidin is a peptide hormone produced mainly by hepatocytes (29,30) and was given this name because its mRNA was found to be highly expressed in the liver and it showed weak microbicidal activity in vitro (31). Up to three different isoforms of hepcidin exist each with four disulphide bridges: one consisting of 25 amino acids (hepcidin-25) (27) and two smaller isoforms hepcidin-22 and hepcidin-20, comprising 22 and 20 amino acids, respectively (32,33). Blood and urine concentrations of hepcidin-20 and hepcidin-22 are generally low, and higher levels are only observed in some physiological processes associated with elevated hepcidin-25 concentrations, such as acute myocardial infarction, sepsis, anaemia of chronic disease, metabolic syndrome and chronic renal disease (34,35,36,37,38,39).

**Mechanism of Action**

According to Ganz (40), the main function of hepcidin is related to immunity since iron is essential for the survival of invading pathogens (41). In effect, a positive relationship has been observed between bacterial virulence and iron availability (42). Hepcidin regulates iron by hindering its absorption (30,43,44,45). Several studies have shown that overexpression of hepcidin leads to iron deficiency both in humans (46) and mice (47), while hepcidin deficiency gives rise to excessive deposition of iron (44,48). Thus, it seems that both iron deficiency (49) and iron overload (48) are influenced to a large extent by an individual’s hepcidin response. Hepcidin’s mechanism of action is based on degradation of ferroportin (50,51,52). In vitro, it has been observed that when hepcidin binds to its receptor ferroportin, the two proteins are degraded via endocytosis in lysosomes (53,54). As it is well-known, ferroportin exports iron from macrophages and the basolateral membrane of duodenal enterocytes (55,56,57). Hence, hepcidin besides reducing the absorption of dietary iron also blocks the recycling of iron released by haemolysis (53,54). Further, although still unconfirmed, it appears that hepcidin also exerts some effects on other means of iron transport, such as the divalent metal ion transporter (Figure 1).

**Hepcidin Determination**

No reference standard exists to determine hepcidin levels and neither is there a valid calibrator for its measurement, hindering the comparison of results emerging from the studies conducted to date on this peptide (27). Notwithstanding, there appears to be no great difference in the methods usually employed, and for both serum and urine samples, mass spectrometry and immunochemical techniques are equally effective (58). We should, however, bear in mind the following basic principles:

- Hepcidin determination does not accurately reflect serum levels although correlation exists between the two variables both in healthy individuals and subjects with an iron metabolism disorder (27). We should also consider that being highly susceptible to oxidation (59), hepcidin-25 cannot be distinguished from the other isoforms of hepcidin in urine samples (58). Further aspects that impair the interpretation of urine hepcidin measurements are: their dependence on the glomerular filtration rate, tubular reabsorption and on the hepcidin production capacity of tubular epithelial cells (60).

- Serum hepcidin concentrations are affected by circadian rhythm (61,62,63,64,65). No differences have been detected according to age in men (63), nor according to sex (34,61,62,64,65,66), with the exception of women of fertile age. This population subset shows lower hepcidin levels than both postmenopausal women and men of the same age range (63). Good correlation has been reported between serum hepcidin and ferritin levels (63).

**Regulating Hepcidin Synthesis**

Given hepcidin’s main role as a regulator of body iron homeostasis (67), in vitro studies have suggested the presence of iron sensors in hepatocytes along with the transduction apparatus required to modulate hepcidin synthesis (68). The different mechanisms controlling hepcidin synthesis proposed so far are:

a) **Iron Status**

Circulating transferrin can be detected as a hepatocellular complex, including the transferrin receptor protein transferrin 1 (TfR1), transferrin receptor protein 2 (TfR2) and human hemochromatosis protein (HFE). It has been proposed that reduced abundance of TfR2 and HFE receptors will lead to a drop...
in hepcidin levels via extracellular signals mediated by kinases (ERK/MAPK) and bone morphogenetic protein/Drosophila (BMP/SMAI) (27). This regulation of hepcidin synthesis serves to explain why serum hepcidin levels correlate negatively with transferrin levels (63,69). Accordingly, individuals with low total body iron levels show high hepcidin concentrations (70), while iron overload has been linked to low hepcidin concentrations (48).

In an effort to correlate serum ferritin concentrations with hepcidin response to iron supplementation, Borronne et al. (71) stratified a group of athletes according to their serum ferritin levels into: < 20 µg/dl; 20-30 µg/dl; 30-50 µg/dl or > 50 µg/dl. The results of this study revealed that subjects in the group featuring levels lower than 30 µg/dl of serum ferritin showed a diminished hepcidin response compared to the remaining groups. Thus, it seems that this level of 30 µg/dl of serum ferritin is the threshold below which iron reserves play an important role in regulating hepcidin synthesis.

b) Erythropoietic Demands

It has been proposed that stimulation of the bone marrow to upregulate erythropoiesis leads to increased iron absorption, driven by a reduced hepcidin response (40). In a cross-sectional study designed to obtain reference hepcidin values, it was observed that premenopausal women with higher erythropoietic demands due to menstrual blood loss showed significantly lower hepcidin levels than postmenopausal women (63). Similarly, other authors have reported that erythropoiesis inducing agents reduce hepcidin production (72,73). The induction of erythropoietin has also been linked to a drop in hepcidin levels. Thus, administration of testosterone (enhancing erythropoiesis) to mice blocks hepcidin mRNA synthesis, which in turn, leads to increased iron import into red blood cells (74). Hence, the intake of erythropoiesis stimulating agents seems to reduce the hepcidin response (72,73). This is also true of anabolic substances (74), indicating a mechanism of action that improves aerobic performance in athletes consuming such performance-enhancing drugs.

c) Hypoxia

Hypoxia seems to be another factor able to modulate hepcidin, diminishing its serum levels (75,76). In exercise physiology, exposure to a high altitude has been the classic method of improving erythropoietin levels (EPO), as well as enhancing reticulocytes, haematocrit and haemoglobin (77,78,79). However, it is claimed that for a beneficial effect on the different haematological variables, a set of conditions has to be met including exposure to altitudes higher than 2000-2200 metres for at least 12 hours per day (80).

Serum testosterone levels are thought to be one of the factors responsible for the haematological modifications produced at high altitude. Several studies have observed elevated testosterone levels after a period at high altitude (81,82), probably attributable to its attenuating effects on altitude-related hyperventilation and respiratory alkalosis. Based on the finding that exogenous testosterone intake reduces hepcidin levels (83) by interfering with mRNA synthesis (74), it could be speculated that the hypoxia response of improved haematological indicators is due in part to a decrease in hepcidin synthesis as a consequence of the testosterone response to high altitude.

d) Dietary Iron

As may be predicted, iron ingested through diet may also affect the hepcidin response such that a transient increase in its urine levels is produced 4-8 hours after iron intake (34,62). In subjects given dietary iron supplements, Lin et al. (84) observed an increase in urine hepcidin clearance proportional to serum transferrin saturation.

e) Inflammation

Inflammation has been proposed as another factor that modulates the synthesis of hepcidin (85). Interleukin-6 (IL-6) is thought to play an important role in this process (86,87,88,89), along with BMP-2 (90).

In many chronic diseases, moderate anaemia is produced by inflammation. This anaemia of chronic disease is characterized by low serum iron concentrations (91). In patients on dialysis, alterations have been observed in levels of hepcidin, interleukin-6 and C-reactive protein (92). These same modifications have been observed in acute stage malaria (93), tuberculosis (94), inflammatory disorders (62, 95), multiple myeloma (90), Hodgkin's disease (96), Castleman's disease (88), and in some patients with tumours (97).

Obesity is another disorder in which there is inflammation (98) and, a direct relationship has been noted between obesity and anaemia (99). In a group of morbidly obese subjects, it was observed that a reduction in the body mass index of 47.5 kg/m² to 39.5 kg/m² led to improved inflammation variables as well as hepcidin and haemoglobin levels and haematocrit (100).

f) Exercise

Exercise affects several of the factors mentioned above given; it promotes erythropoiesis (101) and induces an acute inflammatory response -similar to that observed in infection or inflammatory states (102). It has also been well established that free iron levels rise during post-exercise recovery (103,104,105,106,107,108,109). In the pursuit of ergogenic effects, athletes often take iron supplements (110,111,112). In addition, several studies have shown that exercise affects the hepcidin response increasing its synthesis or release (3,4,41,113,114,115,116,117,118,119,120).

Since inflammation is one of the factors that mediate the hepcidin response (34,86,88,89) and given that exercise directly affects cytokine levels (121), it has been proposed that the hepcidin response to exercise is a consequence of the inflammatory and haemolytic state associated with exercise (122,123). Although the regulation of hepcidin synthesis by means of modulating the levels of the variables related to inflammation has been confirmed in patients with chronic inflammatory processes (100), it has not been possible to reproduce this response following exercise (41,118). Recently, Sim et al. (119) observed differences in serum levels of interleukin-6 when running at 85% VO2max versus the same intensity and volume of exercise performed on a cycle ergometer, though no changes in hepcidin levels were observed. These results suggest that other factors associated with physical exercise act as precursors of the hepcidin response to exercise, and it is unlikely that the inflammatory response induced by exercise plays a major contributing role.
Hepcidin Response to Exercise

Roecker et al. (117) were the first to report changes in urine hepcidin levels in women who had completed a marathon. The main findings of studies analysing the urine and/or serum hepcidin response to different exercise protocols are summarized in Tables 1 and 2. The main observation in all these investigations, except for the study by Troadec et al. (120), is that hepcidin levels rise significantly after exercise (3,4,41,113,114,115,116,117,118,119,120). Below we summarize the results obtained from different studies that have examined the effects of several exercise-related variables on the hepcidin response.

a) Exercise Intensity
- An intensity of exercise corresponding to 60% of the heart rate reserve or under does not seem to be associated with a significant increase in blood or urine hepcidin levels as a response to exercise (120).
- Exercise conducted at an intensity corresponding to 65% of VO_{2\text{max}} seems to be linked to an increased serum hepcidin concentration (119).
- Interval exercise performed at 85% VO_{2\text{max}} does not produce a greater response of serum hepcidin than that recorded for a continuous exercise protocol at 65% VO_{2\text{max}} (113).
- The same exercise volume (10 km) performed continuously at a relative intensity of 70% VO_{2\text{max}} induces a reduced response compared to the same volume performed at 90%-95% VO_{2\text{max}} in intervals (114). In this study, it was also observed that while at the lower intensity (70% VO_{2\text{max}}) hepcidin levels returned to normal after 12 hours, in the high intensity (90%-95% VO_{2\text{max}}) elevated levels of the hormone persisted for 24 hours.

In summary, exercise performed at relative intensities of 65% VO_{2\text{max}} or greater induces an increase in serum hepcidin levels (113,119), with maximum levels reached when intensities approach the individual’s VO_{2\text{max}} (90%-95% VO_{2\text{max}}) (114).

b) Volume
In the only study that has examined the hepcidin response to training volume, it was observed that 120 minutes of exercise performed at a relative intensity of 65% VO_{2\text{max}} on a treadmill by physically active women triggered a significantly higher response.

<table>
<thead>
<tr>
<th>Reference</th>
<th>Subjects</th>
<th>Experimental conditions (C)</th>
<th>Procedure</th>
<th>Main results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Roecker et al.</td>
<td>14 female runners</td>
<td>Marathon</td>
<td>Pre and post-run, 24 h of recovery. Hepcidin</td>
<td>Hepcidin increased at 24 h of recovery*. 6/14 classified as non-responders.</td>
</tr>
<tr>
<td>Peeling et al.</td>
<td>10 highly-trained triathletes</td>
<td>C1. 10 km at 70% VO_{2\text{max}} C2. 10 km at 70% VO_{2\text{max}} + 10 x 1 km at 90% VO_{2\text{max}} (12 h recovery).</td>
<td>Pre, post-run and 3 h, 24 h of recovery. Hepcidin, IL-6, serum ferritin, SF, Hapt.</td>
<td>Hepcidin increased at 3 h of recovery*, IL-6 post-run* and 3 h of recovery*. Greatest increases produced in 10 x 1 at 90% VO_{2\text{max}}. No cumulative effect. Fes*, Fe* and Hapt* increased in post-run and 3 h of recovery (same as hepcidin and IL-6), but with a cumulative effect.</td>
</tr>
<tr>
<td>Peeling et al.</td>
<td>10 trained male runners</td>
<td>C1. 10 km at 75-80% VO_{2\text{max}} on grass. C2. 10 km at 75-80% VO_{2\text{max}} on asphalt. C3. 10 x 1 km at 90-95% VO_{2\text{max}} on grass.</td>
<td>Pre, post-run, 3 h, 24 h of recovery. Hepcidin, IL-6, free Hb; Hapt.</td>
<td>Hepcidin increased at 3 h of recovery*. No differences between groups. IL-6 and free Hb: increases post-run*, no differences between C1 and C2, but C3 showed an increase * versus C1 and C2. Hapt: post-run reduction*.</td>
</tr>
<tr>
<td>Peeling et al.</td>
<td>11 trained male runners</td>
<td>60’ Cr: 15’ at 75-80% peak HR + 45’ at 85%-90% peak HR.</td>
<td>Pre, post-run, 3 h, 6 h, 12 h, 24 h of recovery. Hepcidin, IL-6, PCR, serum ferritin, serum Fe.</td>
<td>Hepcidin: increases at 3 h*, 6 h*, 12 h* and at 24 h of recovery*. Peak at 3 h and 6 h. IL-6: increased at post-run* and 3 h of recovery*. Post peak. CRP: increased at 6 h*, 12 h* and 24 h of recovery*. Peak at 24 h of recovery. Ferritin and serum Fe: increased at post-run <em>. 3 h of recovery</em> lowest vs Pre.</td>
</tr>
<tr>
<td>Troadec et al.</td>
<td>14 sedentary men</td>
<td>45’ HRR cycle ergometer.</td>
<td>Pre, post-exercise, 0.5 h, 1 h, 2 h, 4 h, 6 h, 12 h, 24 h of recovery. Hepcidin, IL-6, serum ferritin, serum Fe, Hapt, CRP.</td>
<td>No changes detected in any variable examined.</td>
</tr>
</tbody>
</table>

Studies examining the acute hepcidin response to exercise in urine. Cr: continuous running. HR: heart rate. Fe: iron; h: hour. Hapt: haptoglobin, Hb: haemoglobin, Il-6: interleukin-6, CRP: C-reactive protein, HRR: heart rate reserve, *: statistical significance (p<0.05)
to that observed by the same women performing the same intensity of exercise (65% VO2max) for a duration of 60 minutes (113). These results suggest that training volume may also influence the hepcidin response to exercise.

c) Exercise Modality

To determine if the modality of aerobic resistance exercise could affect the hepcidin response, Sim et al. (119) compared this response in a group of triathletes performing two forms of exercise (cycle ergometry vs running) at two different relative intensities (65% VO2max vs 85% VO2max). Results revealed no significant differences according to exercise modality or intensity despite the detection of some differences in serum iron or interleukin-6 levels. In a study performed in runners, Peeling et al. (115) compared the hepcidin response to footstrike as a cause of haemolysis in a protocol carried out at a fixed intensity of 70% VO2max on different running surfaces (grass vs. asphalt). Results indicated no significant differences in serum hepcidin levels between the two conditions.

Thus, the limited data available suggest that neither the exercise modality (running vs cycling) nor the running surface (grass vs. asphalt) significantly affect the hepcidin response to exercise.

Adaptations of the Hepcidin Response to Exercise

Although attractive, the working hypothesis suggesting a possible relationship between modified hepcidin levels and the onset of anaemia or iron deficiency in athletes (28) has not yet provided conclusive results, and few studies have addressed this hypothesis to date (3,4). In an initial study, Auersperger et al. (3) investigated the effects of long-term endurance exercise on hepcidin concentrations and inflammation and iron status parameters. These authors designed a training protocol consisting of two 3-week progressive overload periods, each followed by a week’s recovery and runners were assigned to either a continuous or interval training group. As the study’s main finding, significant correlation (p<0.05) was detected between total body iron levels and C-reactive protein or serum hepcidin concentrations. Also, higher hepcidin levels were observed after the first/second overload period compared to baseline and a significant decrease was produced after the first/final recovery period. In a later study by the same authors (4), an increased incidence of modified iron metabolism-related variables was noted in response to a similar training program (from 5/10 to 7/10), including reduced

Table 2. Studies examining the acute hepcidin response to exercise in serum

<table>
<thead>
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<td>Troadec et al.</td>
<td>14 sedentary men</td>
<td>45° HRR cycle ergometer</td>
<td>Pre, post-exercise, 0.5 h, 1 h, 2 h, 4 h, 6 h, 12 h, 24 h of recovery. Hapt, Hepcidin, IL-6, serum ferritin, serum Fe.</td>
<td>No change* in any variable examined.</td>
</tr>
<tr>
<td>Robson-Ansley</td>
<td>9 trained men</td>
<td>120° Cr at 60% VO2max+5 km trial C1. Personalized hydration using a drink enriched with CH. C2. Control.</td>
<td>Pre, post-run, 24 h of recovery. Hapt, IL-6, serum Fe.</td>
<td>Hepcidin: elevated in C1 and C2 post-run*, no differences between groups. IL-6: increase post-run* and C2* greater than C1.</td>
</tr>
<tr>
<td>Sim et al.</td>
<td>11 highly-trained triathletes</td>
<td>90° Cr at 75% VO2max. C1. Personalized hydration using a drink enriched with CH. C2. Control.</td>
<td>Pre, post-run, 3 h, 24 h of recovery. Hapt, IL-6, free Hb; serum ferritin, serum Fe. Trans.</td>
<td>Hepcidin and IL-6: post-run increase*, no differences between C1 and C2. Hapt: post decrease*. Free Hb: post decrease*. Serum ferritin, serum Fe and Trans. post-run increase* and decrease 24 h of recovery* vs Pre.</td>
</tr>
<tr>
<td>Newlin et al.</td>
<td>12 physically active women</td>
<td>C1. 60° Cr at 65% VO2max. C2. 120° Cr at 65% VO2max.</td>
<td>Pre, post-run, 3 h, 6 h, 9 h, 24 h of recovery. Hapt, IL-6.</td>
<td>Haptid: increases post-run* and 3 h of recovery*. C2 greater* vs C1. IL-6: post-run increase*.</td>
</tr>
<tr>
<td>Sim et al.</td>
<td>10 highly-trained triathletes</td>
<td>C1. 60° Cr at 60-65% peak VO2. C2. 60° cycle ergometer at 60%-65% peak VO2.</td>
<td>Pre, post-exercise, 3 h of recovery. Hapt, IL-6, serum ferritin, serum Fe.</td>
<td>Hepcidin: post-exercise increase* in all groups with no differences between groups. IL-6: post-exercise increase* in all groups and increase* in C4 versus C3. Serum ferritin: post increase* in C3 and C4 versus C1 and C2. Serum Fe: post increase* in C1, C3 and C4.</td>
</tr>
</tbody>
</table>

Studies examining the acute hepcidin response to exercise in serum. Cr: continuous running. HR: heart rate; Fe: iron; h: hour, Hapt: haptoglobin, Hb: haemoglobin, CH: carbohydrates, IL-6: interleukin-6, CRP: C-reactive protein, Trans. transferrin, HRR: heart rate reserve, *: statistical significance (p<0.05)
serum hepcidin concentrations at the end of the study. This finding was attributed to a need to increase iron reabsorption to avoid compromising iron stores. However, it was observed that mean serum ferritin levels in the group of subjects without iron deficiency was 28 µg/dl. According to Borrione et al. (71), beyond 30 µg/dl of serum ferritin, the hepcidin response to dietary iron and probably also to exercise may be modified. Hence, the group initially classified as "without iron deficiency" could be considered equally deficient in physiological terms.

Among the limitations of studies addressing hepcidin adaptations to exercise, we should mention that if baseline samples are taken after 24 h of rest following exercise, hepcidin concentrations are likely to be normal considering that it is an acute phase hormone.

Conclusions

- The hepcidin response to exercise seems to be dependent on a minimum intensity of exercise (~65% VO2max) with maximal levels of the hormone recorded in response to intensities approaching VO2max (~90%-~95% VO2max). Exercise duration and load also seem to affect the hepcidin response, and higher hepcidin concentrations have been detected for longer exercise durations.

Finally, although only scarcely addressed, it does not seem that the modality of exercise is too important in the hepcidin response to exercise.

- The few investigations that have tried to examine possible adaptations of serum hepcidin levels to training have not provided sufficiently clear results to draw any firm conclusions. The reason for this is interference with other variables (diet, iron status) that have shown an effect on hepcidin regulation.

Future Studies

Given the key role played by hepcidin in whole body iron regulation and that exercise is able to modulate hepcidin synthesis, the response of hepcidin to exercise (modality, intensity, duration, frequency) needs to be further examined in detail. The information emerging from future studies should help modify training loads and/or establish dietary-nutritional regimens designed to maintain adequate body iron levels during training periods.

Conflicts of Interest

There are no conflicts of interest.

References


