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INVITED REVIEW

Athletic induced iron deficiency: new insights into the role of inflammation, cytokines and hormones

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Abstract Iron is utilised by the body for oxygen transport and energy production, and is therefore essential to athletic performance. Commonly, athletes are diagnosed as iron deficient, however, contrasting evidence exists as to the severity of deficiency and the effect on performance. Iron losses can result from a host of mechanisms during exercise such as hemolysis, hematuria, sweating and gastrointestinal bleeding. Additionally, recent research investigating the anemia of inflammation during states of chronic disease has allowed us to draw some comparisons between unhealthy populations and athletes. The acute-phase response is a well-recognised reaction to both exercise and disease. Elevated cytokine levels from such a response have been shown to increase the liver production of the hormone Hepcidin. Hepcidin up-regulation has a negative impact on the iron transport and absorption channels within the body, and may explain a potential new mechanism behind iron deficiency in athletes. This review will attempt to explore the current literature that exits in this new area of iron metabolism and exercise.

Keywords Iron deficiency · Interleukin-6 · Hepcidin · Athlete

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Introduction

Iron is the functional component of hemoglobin and myoglobin, making it an essential nutrient for optimal oxygen delivery to the body's tissues. Furthermore, iron plays a key role in the electron transport chain, since mitochondrial enzymes and cytochromes are heme containing proteins that promote oxidative phosphorylation within the mitochondria (Williams 2005). Therefore, insufficient iron stores may lead to a decline in not only health and well-being, but also athletic performance (Schumacher et al. 2002; Wilkinson et al. 2002). Athletes are commonly diagnosed with iron deficiency, particularly those involved in endurance sports (Beard and Tobin 2000; Zoller and Vogel 2004). A number of exercise-generated mechanisms are proposed to influence the iron status of an athlete including hemolysis, hematuria, sweating and gastrointestinal bleeding (Babic et al. 2001; DeRuisseau et al. 2002; McInnis et al. 1998; Zoller and Vogel 2004). It is possible that frequent losses of iron from such mechanisms over the duration of a training program or competitive season may negatively affect iron status. Although these more traditional explanations of iron deficiency in athletes are commonly accepted, recent research investigating the influence of cytokines and hormones on the anemia of chronic disease may hold an interesting application to sports medicine, providing new explanations regarding potential mechanisms causing iron deficiency in athletes.

Iron metabolism

Approximately 3–5 g of iron is normally contained within the human body, of which 1–2 mg is lost daily (Nielsen and Nachtigall 1998). Iron losses are recovered by absorption of dietary iron within the small intestine. To maintain sufficient stores of iron, it is generally recommended that the daily intake (RDI) of dietary iron is 8 mg/day for adult men, and 18 mg/day for adult women (Food and Nutrition Board, Institute of Medicine, Washington, DC 2001). The higher RDI for women comes as a result of menstrual blood loss, which is estimated to equate to 1.5–2.1 mg/day of iron loss (Cole et al. 1971). The body absorbs approximately 6–12% of the total dietary iron consumed daily (Adamson 1999). Within the diet, iron is present in two forms, including non-heme (inorganic) iron and heme iron. Non-heme iron is derived from plant foods, and accounts for approximately 80% of the iron consumed within the standard diet (Craig 1994). The remaining ~20% of iron intake is comprised of heme iron, which is derived primarily from the hemoglobin and myoglobin of meat. The mechanism by which heme iron is absorbed differs to that of non-heme iron, making its absorption much more efficient and less affected by accompanying dietary factors. As such, approximately 5–35% of heme iron is absorbed from a single meal, as opposed to non-heme iron, where only 2–20% is absorbed (Beard and Tobin 2000).

Iron absorption, transport and storage

Iron is absorbed by duodenal enterocytes that line the absorptive villi close to the gastroduodenal junction (Andrews 2000). The apical surfaces of mature enterocytes contain an enzymatic ferric reductase, known as Duodenal cytochrome b (Dcytb) (McKie et al. 2001), which reduces Ferric iron (Fe^3) to its Ferrous form (Fe^2). Once in this ferrous form, the iron is transported across the brush border membrane and into the cytoplasm of the enterocyte by the protein divalent metal transporter 1 (DMT1) (Andrews 1999). Subsequently, the iron must pass through the enterocyte to the basolateral surface and into the circulation. This export pathway requires the protein transporter ferroportin, and a membrane-bound ferroxidase called Hephaestin (Wessling-Resnick 2006). The free iron that enters the plasma has a reduction-oxidation (redox) potential, which promotes free radical formation as a result of Fenton and Harber–Weiss reactions (Risom et al. 2005). Such reactions result in oxidative damage to tissues. Therefore, it is imperative that iron is transported around the body in a redox inactive form. For this to occur, iron is bound to a monomeric glycoprotein known as transferrin, which maintains the iron as soluble and non-toxic, and is therefore unable to engage in Fenton and Harber–Weiss reactions (Gomme et al. 2005).

Once bound to transferrin, iron is transported from the bloodstream to be released into a variety of cells. This is accomplished by iron loaded transferrin binding to one of two specific cell surface receptors. These receptors include transferrin receptor 1 (TfR1), commonly found on red blood cells, erythroid cells, hepatocytes, monocytes and the blood–brain barrier, or transferrin receptor 2 (TfR2), expressed predominately on liver cells (Gomme et al. 2005). The transferrin-transferrin receptor (Tf-TfR) complex undergoes endocytosis, allowing iron to be released from transferrin and transported across the endosomal membrane by DMT1 (Papanikolaou and Pantopoulos 2005). A number of factors regulate the conformational changes of transferrin that are critical to iron release. These include receptor binding, temperature, chelator concentration, ionic composition of the supporting buffer and pH (He et al. 2000; Zak et al. 1997). Once inside the cell, iron is incorporated into iron-containing proteins, and excess intracellular iron is converted into Ferritin, a stored form of iron.

Iron deficiency and athletic performance

Peeling et al. (2007) suggested that iron deficiency can be categorised into three stages of severity: Stage One-Iron Depletion: Iron stores in the bone marrow, liver, and spleen are depleted (SF: <35 μg L^{-1}, Hb: >115 g L^{-1}, Transferrin Saturation: >16%). Stage Two-Iron Deficient Erythropoiesis: Erythropoiesis diminishes as the iron supply to the erythroid marrow is reduced (SF: <20 μg L^{-1}, Hb: >115 g L^{-1}, Transferrin Saturation: <16%). Stage Three-Iron Deficient Anemia: hemoglobin production falls, resulting in anemia (SF: <12 μg L^{-1}, Hb: <115 g L^{-1}, Transferrin Saturation: <16%). It has been well established that the more severe cases of iron deficiency, resulting in anemia are responsible for a decline in work capacity (Nelson et al. 1994; Schumacher et al. 2002) as a result of a decreased hemoglobin concentration and associated oxygen carrying capacity to the working skeletal muscles (Lukaski et al. 1991). However, the effect of iron deficiency without anemia (Stage one, which is more common in endurance athletes than iron deficiency-anemia) is unclear. In order to investigate how this less severe form of insufficient tissue iron store affects exercise performance, many studies have utilised a period of iron supplementation. By implementing pre- and post-exercise tests, comparisons of athletic performance of both iron deficient and iron replete populations, which act as their own control have been possible. However, much debate still exits as to

whether tissue iron deficiency without anemia actually affects performance.

For example, past findings from our own research group have shown that a course of intramuscular iron injections (5 × 2 mL of Ferrum H over an 8-day period) significantly increased the serum ferritin levels from 22 to 61 μg/L, with no effect on the performance of a vertical jump test, a 5 × 6 s cycle ergometer repeat sprint test, or a 20 m multistage shuttle run in elite female netballers (Blee et al. 1999). Although these results showed no effect of iron supplementation on athletic performance, the exercise tests used were primarily tests of anaerobic power, and possibly not sensitive, or specific enough to exploit the potential benefits of iron supplementation on athletic performance. It would be expected that any changes in performance would be most apparent in exercise tasks that predominately utilise the aerobic energy system. However, the results do show that iron supplementation might not enhance tasks that stress the anaerobic energy system.

Given these outcomes, a further study by our research group (Peeling et al. 2007) assessed the effect of a similar iron injection protocol (5 × 2 mL of Ferrum H over a 10-day period) on sub-maximal economy, $\dot{V}O_{2\,max}$, and time to fatigue at the $\dot{V}O_{2\,max}$ intensity in 16 iron deficient, non-anemic, female runners. Again, a significant increase in serum ferritin levels (from 19 to 65 μg/L) was seen, however the increase in body iron stores had no beneficial effect on the tests of aerobic capacity implemented. Despite this outcome, it was postulated that although the increased ferritin levels did not seem to benefit the aerobic running performance of iron depleted women, supplementation should not be discouraged since the decline of iron stores beyond that of stage one iron depletion, may be attenuated (Peeling et al. 2007).

These findings support those of Klingshirn et al. (1992) who also showed that 8 weeks of oral iron supplementation (100 mg of ferrous sulfate/day) significantly improved serum ferritin levels in 18 iron deficient, non-anemic female runners, but with no improvements in run time to exhaustion during a constant speed treadmill run (which was run at a speed 2–3% lower than each subjects most recent 10 km race time). However, it should be considered that in both the Peeling et al. (2007) and the Klingshirn et al. (1992) investigations, a running test timed to exhaustion might not be an appropriate test of performance, since athletic performance is often based on time to complete a given task, rather than how long an athlete can continue exercising for. Secondly, in the case of the Klingshirn et al. (1992) study only, it should be noted that despite a significant increase in serum ferritin within the supplemented group, the post-supplementation value for this variable was still only 23.44 μg L^{-1}. Although this value is above that characterised by the authors as iron deficient (<12 μg L^{-1}), previous research has used serum ferritin cut-off values for athletes between 12 and 40 μg L^{-1} to characterise iron deficiency (Blee et al. 1999; Fogelholm et al. 1992; Hinton et al. 2000; Klingshirn et al. 1992; Peeling et al. 2007). Furthermore, it has been recommended that oral iron supplementation should be prescribed for athletes when their serum ferritin levels fall below 35 μg L^{-1} (Nielsen and Nachtigall 1998), suggesting that the iron needs of an athlete are possibly greater than those of the normal population. Hence, the lack of effect of supplementation on performance seen in many studies may be a result of the duration of supplementation, and an inadequate increase in iron to levels which could affect performance.

In contrast, Zhu and Haas (1998) showed that 8 weeks of oral iron supplementation (135 mg of ferrous sulfate/day) in 37 iron deficient, non-anemic, physically active women, was able to significantly increase the serum ferritin levels from 14.3 to 36.9 μg L^{-1}. The result of this positive change in iron status showed a decrease in the energy expended (by 2.0 kJ min^{-1}) and the fractional utilisation of peak oxygen consumption (by 5.1%) during a 15 km running time trial performance, when compared to a placebo controlled group. Additionally, Hinton et al. (2000) showed that 6 weeks of iron supplementation (100 mg ferrous sulfate/day) in 42 iron deficient, non-anemic, physically active women was enough to significantly increase serum ferritin levels from 10.4 to 14.5 μg L^{-1}, and serum iron from 12.2 to 19.4 μmol L^{-1}. As a result of such improvements, the authors saw not only an increased energy efficiency of performance by 0.6 kJ W^{-1}, but also significantly improved the performance time of a 15 km cycle time trial by 6.7%.

Although a positive finding, the improvements in exercise efficiency found by Zhu and Haas (1998) are better explained by the changes occurring in the placebo, rather than the supplemented group. The energy expenditure during the time-trial remained the same from pre to post-supplementation testing in the iron-supplemented group, but significantly increased in the placebo group over the course of the intervention. To help explain this finding, it should also be noted that the placebo group showed a significant 5.1 g L^{-1} decrease in Hb concentration from baseline to post-treatment blood analysis. The significance of such an outcome is explained by the study of Hinton et al. (2000), who showed total energy expended during the time trial was significantly and negatively associated with changes in Hb concentration. They reported that for every 1 g L^{-1} increase in Hb, there is a 1.04 kcal decrease in energy expenditure. As a result, the 5.1 g L^{-1} decrease in the placebo groups Hb concentration in the Zhu and Haas study would have been equal to a 5.3 kcal increase in exercise energy expenditure. Hence, the between group

differences in exercise efficiency are more likely due to the responses of the control group, rather than iron supplementation. However, it is possible that such an outcome indicates the iron supplementation may have been responsible for avoiding such a negative Hb response in the iron supplemented group. As such it is evident that iron supplementation in stage one iron deficient athletes should occur to prevent the progression of the problem to more advanced stages, even if the current research is equivocal on athletic performance during stage one.

Additionally, Friedmann et al. (2001) showed that the $\dot{V}O_{2\,max}$ and sub-maximal $\dot{V}O_2$ (measured on a separate days) of 40 iron deficient, junior elite athletes from a range of sports (such as swimming, triathlon, athletics and rowing) were improved during a incremental exercise test to exhaustion and a maximal accumulated oxygen deficit (MAOD) test respectively, after 12 weeks of iron supplementation (2×100 mg of ferrous-glycine-sulfate/day). The iron supplement period was responsible for raising the serum ferritin levels from 15.8 to 35.9 μg L^{-1}, and decreasing the transferrin levels from 2.8 to 2.6 g L^{-1}. However, these improvements in performance occurred without any significant increases to red blood cell volume. It was concluded that the maximal aerobic capacity of elite athletes could be improved through iron supplementation without increasing oxygen-carrying capacity. The supplementation was proposed to be responsible for an increase in muscle oxidative capacity (although not measured), and it was therefore suggested that the improvements might occur at the level of the mitochondria rather than as a result of improved oxygen transport. However, such a proposition is yet to be verified since no research to date has looked at the effect of iron supplementation at the muscle level.

Finally, Lukaski et al. (1991) induced 11 healthy female volunteers into a state of iron deficiency (without anemia), over an 80-day period through the administration of a low iron diet and phlebotomy. During this iron depletion phase, the participants serum ferritin status was taken from 26.0 to 6.0 μg L^{-1}, however, Hb levels were a healthier 120 g L^{-1}. Prior to and at the completion of the iron depletion phase, participants undertook a continuous graded exercise test to exhaustion with O_2 consumption and CO_2 output determined via indirect calorimetry with an automated Sensormedic system. Subsequent to this, all subjects underwent a 100 day iron repletion phase, with a diet containing 0.25 mmol of iron for each 8.3 MJ of food consumed, and an additional ferrous sulfate supplement of 0.9 mmol of iron during the last 14 days. At the completion of this repletion phase, ferritin levels were returned to 10.0 μg L^{-1}, and Hb to 126 g L^{-1}, and a final graded exercise test was completed.

The results of this investigation showed no impairment to exercise duration, $\dot{V}O_{2\,peak}$, or cardiovascular function as a result of tissue iron deficiency. However, there was a significant increase in peak CO_2 production and respiratory exchange ratio, as well as reductions in the rate of O_2 utilisation, total oxygen uptake and energy output during exercise. Furthermore, tissue iron deficiency showed reduced oxygen uptake and utilisation, in association with a significant increase in post-exercise blood lactate concentration, indicating a greater dependence on glycolytic metabolism (Lukaski et al. 1991). Although this investigation highlights a mix of positive and negative results, one may argue that the lack of impairment to $\dot{V}O_{2\,peak}$ and exercise duration may have been a result of the pre-investigation serum ferritin levels being low enough to be considered as stage 1 iron deficient before depletion phase even began, and that the iron-repletion protocol had little effect on improving the iron status of the participants. As such, the authors were likely comparing an already iron deficient population to a further iron deficient group, rather than one that was fully iron loaded initially.

The results of the research presented above suggests that tissue iron deficiency without anemia may have a negative impact on both oxygen transport and utilisation, which in turn might influence athletic performance. However, contrasting results between a number of investigations do not allow conclusive statements to be made, highlighting the importance of continued research in this field. Furthermore, it is also important that we understand the mechanisms by which athletes lose iron during exercise so as to possibly reduce the severity of this condition.

Avenues of iron loss during exercise

Iron has many important roles that bear direct application to athletic performance. As such, a significant loss of this mineral commonly occurs with exercise. Despite this, the body has no innate mechanism to replace the iron losses incurred by physical activity; thereby a sufficient dietary intake is essential for athletes in periods of heavy training. The mechanisms of exercise-induced iron loss include such processes as hemolysis, hematuria, sweating and gastrointestinal bleeding (Babic et al. 2001; DeRuisseau et al. 2002; McInnis et al. 1998; Zoller and Vogel 2004), and may also now extend to inflammation and hormone activity, based on recent research (Roecker et al. 2005).

Gastrointestinal bleeding

During exercise, blood flow to the gastrointestinal tract (GIT) is sacrificed for increased blood flow to the muscles and skin (Otte et al. 2001; Peters et al. 2001). The visceral blood flow can be reduced up to 56% during exercise,

resulting from an increased sympathetic nervous system activity that is driven by exercise intensity (Osada et al. 1999; Rowell 1974). As a result, the epithelial cells of the GIT may be deprived of oxygen and metabolic substrates, leading to necrosis and mucosal bleeding (Peters et al. 2001). It is possible that gastric lesions may in part explain the occurrence of GI-bleeding. In fact, pre and post-run endoscopic examination of long-distance runners completing a moderate paced (exact intensity was not quantified) endurance run (ranging from 18 to 50 km) showed a high incidence of histological lesions to the gastric antrum mucosa (Gaudin et al. 1990). The observed gastric lesions were of two types, including those possibly due to hemodynamic changes in the gastric wall, and lesions occurring as a result of a decrease in mucosal secretions (Gaudin et al. 1990). Therefore, repeated episodes of training and competition induced blood loss through the GIT may contribute to iron deficiency and anemia within the athlete (Nielsen and Nachtigall 1998).

Hematuria

Hematuria is the presence of blood in the urine, and is found to occur as a result of physical activity. It is suggested that mechanical trauma and hemolysis are indicated in the glomerulus (McInnis et al. 1998), since the resultant excess of hemoglobin is lost in the urine (Rosse and Gutterman 1970). Furthermore, the movement of the bladder during running activities may cause bleeding due to microscopic lesions of the interior wall (Blacklock 1977). McInnis et al. (1998) suggest that the intensity of exercise is the causal mechanism underlying hematuria, since renal blood flow is decreased proportionally to exercise intensity, resulting in an increased filtration fraction and glomerular filtration rate.

Sweating

Exercise promotes the up-regulation of sweating as a mechanism to assist in thermoregulation. However, sweating is also a mechanism by which the body may lose iron. The average sweat iron concentration of a combined male and female population during cycling for 60 min at 50% $\dot{V}O_{2\,max}$ in a temperature controlled environment was 0.22 mg L^{-1} (Waller and Haymes 1996). However, it was also found that the sweat rates during the first 30 min of exercise were significantly greater than that of the second 30 min (0.31 and 0.14 mg L^{-1}, respectively). Therefore, sweat iron concentration may decline over time, possibly due to the washing of cellular debris and external contamination from the sweat pores into the initial sweat collected (Waller and Haymes 1996). Iron losses from the skin are a result of sweat and desquamated epithelial cells; hence, sweat may be contaminated by iron that originates from the ducts of the sweat glands, cellular debris, and external contamination (Brune et al. 1986; Waller and Haymes 1996). As a result, to determine sweat iron losses with minimal contamination from external sources, an extensive skin cleaning procedure in association with a period of profuse sweating is commonly used prior to collecting sweat samples for analysis (Brune et al. 1986). As such, the sweat iron concentration reported during the second 30 min of exercise during the Waller and Haymes (1996) investigation is possibly more representative of the average iron loss through sweating during exercise. With this in mind, it is possible that 0.14 mg L^{-1} of iron in sweat may not be a significant enough amount to explain an athlete's iron deficiency. However, athletes exercising for prolonged time periods, over multiple training sessions in the heat may incur a cumulative debt, which could ultimately impact on body iron status.

Hemolysis

Exercise has been shown to increase the rate of red blood cell (RBC) destruction, otherwise known as exercise-induced hemolysis (Miller et al. 1988; Telford et al. 2003). Hemolysis has been reported during a range of exercise modalities such as swimming, cycling and resistance training (Selby and Eichner 1986; Telford et al. 2003). During these non-weight bearing activities, it is suggested that the hemolysis may result from the compression of the blood vessels caused by the vigorous contraction of the muscles involved in performance (Selby and Eichner 1986). However, the high-impact, weight bearing nature of running is associated with the greatest degree of hemolytic activity, due primarily to the impact forces that result from foot-strike (Telford et al. 2003). Hemolysis is implicated as a mechanism of iron loss, since the destruction of a RBC's membrane allows the hemoglobin (Hb) and associated iron held within the cell, to be released into the surrounding plasma, which may then cause oxidative tissue damage (Reeder and Wilson 2005). To ensure this oxidative potential is limited, the glycoprotein haptoglobin (Hp), with its strong affinity for free Hb, is utilised to 'clean up' the lost contents of a hemolysed RBC (Giblett 1968). As such, the result of a hemolytic episode would be demonstrated in human blood via an increased plasma free Hb concentration, in association with a decline in serum Hp levels.

Such blood profile responses are common in runners, and have led to a number of conclusions with respect to

running and hemolysis over the past three decades. For example, Hanson et al. (1978) suggested that occult hemolysis was a common response to recreational running, as marked by a decrease of serum Hp levels in physically active males, after a three-week period of alternate day running on a concrete surface at 75% $\dot{V}O_{2\,max}$, to levels similar of that seen in well-trained distance runners. It is plausible to suggest that the change in serum Hp levels seen by Hanson and colleagues may have been due to plasma volume expansion resulting as a training effect in the recreational running group. However, a number of more recent studies have also shown the same response of serum Hp to exercise in trained populations. For instance, Miller et al. (1988) showed a force dependent relationship between heel-strike and the degree of hemolysis experienced during running. Significantly greater changes to serum Hp and plasma free Hb levels were noted during downhill running when compared to an equivalent duration and gradient uphill run. Supporting such findings, Telford et al. (2003) showed that an acute, 1 h bout of continuous running at 75% peak $\dot{V}O_2$, was responsible for a fourfold increase in the levels of plasma free Hb when compared to an intensity and duration equivalent bout of cycling. After accounting for such factors as circulatory stress, these authors concluded that heel strike was the major cause of hemolysis during running.

To this end, it is possible that a force-dependent relationship between heel-strike and hemolysis may be directly affected by the ground surface type that the athlete is training on, or the intensity at which the session is completed at, since these variables are subjected to differing levels of ground impact force upon heel-strike. However, no research group has directly explored the effect of these variables on hemolysis. Furthermore, little is known about the effect of such a stimulus over the course of multiple training sessions, but it has been suggested that a chronic hemolytic stimulus (i.e. consecutive training bouts) might have a cumulative effect on hemolysis and iron deficiency (Telford et al. 2003).

Hormones and cytokines and the inflammatory response

The aforementioned mechanisms of exercise-induced iron loss are well established. As such, during periods of heavy training demand, these avenues may pose a significant challenge to an athlete's iron status. However, recent advances in iron metabolism and chronic disease have uncovered a new iron-regulatory hormone called Hepcidin, which may have an impact on iron metabolism at the conclusion of an exercise bout (Roecker et al. 2005).

Hepcidin

Hepcidin is a liver produced, 20-, 22-, or 25-amino acid peptide that is derived from a larger precursor protein of 84 amino acids (Nicolas et al. 2002). Hepcidin was discovered by two independent research groups that were able to isolate this hormone in blood (Krause et al. 2000) and in urine (Park et al. 2001). Since its discovery, Kemna et al. (2005a) have advanced the ability to measure Hepcidin through surface-enhanced laser desorption/ironisation time-of-flight mass spectrometry (SELDI-TOF-MS) in urine. However, advances in serum measurement have been hampered by technical difficulties Kemna et al. (2005b).

Hepcidin has been suggested as the key regulator of iron metabolism (Kemna et al. 2005c). Hepcidin levels undergo homeostatic regulation by hepatic iron levels, the demand for erythropoiesis sensed by liver oxygenation, and in response to inflammation (Nemeth et al. 2004b). In fact, it is suggested that the inflammatory mediated rises in hepcidin ultimately result in rapid decreases in plasma iron concentrations, creating a bottleneck for hemoglobin synthesis that can eventually result in anemia (Nemeth et al. 2004a). Hepcidin regulates iron metabolism through its interaction with the body's only known cellular iron exporter, Ferroportin (Fpn). Ferroportin channels are expressed in a number of cell types, such as duodenal enterocytes, hepatocytes and reticuloendothelial macrophages (Lymboussaki et al. 2003; Nemeth et al. 2004b). The function of these channels is to transport iron across the cell membrane during such processes as intestinal iron absorption and macrophage iron release (Lymboussaki et al. 2003). Hepcidin acts to internalise and degrade the Fpn export channels, ultimately by inhibiting the absorption of iron from the diet at the site of the duodenal enterocytes, and by blocking the release of iron from macrophages that have collected senescent erythrocytes (Nemeth et al. 2004a, b).

Recent literature would suggest that the primary mediator for the up-regulation of hepcidin activity is the inflammatory cytokine Interleukin-6 (IL-6) (Nemeth et al. 2004a). To emphasise the role of this cytokine, Nemeth et al. (2004a) compared the effect of an inflammatory stimulus in healthy human subjects to IL-6 deficient mice. The healthy subjects were injected with recombinant human IL-6 at a rate of 30 μg/h, which resulted in a 7.5 fold increase in urinary hepcidin excretion. However, the IL-6 knockout mice were injected with a turpentine solution to produce the same inflammatory response, which resulted in the suppression of hepcidin mRNA. Nemeth and colleagues suggested that the attenuation of hepcidin mRNA in the absence of IL-6 was possibly due to suppressive effects on hepcidin by other acute-phase cytokines

stimulated by the inflammation. To further explore how IL-6 influences hepcidin activity, Kemna et al. (2005c) traced the time course of cytokine, acute phase protein and hepcidin activity in 10 healthy individuals injected with Lipopolysaccharide (LPS) at a rate of 2 ng/kg of body mass to produce an inflammatory response. These authors showed that the peak in urinary hepcidin occurred approximately 6 h post-LPS injection, which lagged the peak response time of IL-6 by 3 h. Additionally, Kemna et al. (2005c) showed that the acute phase C-reactive protein (CRP) peaked 22 h after the LPS injection, in conjunction with a significant decline in serum iron.

Current research investigating the role of hepcidin in iron metabolism is primarily focused in the area of chronic disease and the anemia of inflammation. For instance, hemochromatosis patients suffer a chronic mal-accumulation of iron within various tissues of the body (Goswami and Andrews 2006). Explanations suggest that a lack of hepcidin expression may be responsible for the increased intestinal iron absorption and a reduced macrophage iron store, which results in plasma iron increases greater than the transferrin binding capacity. Non-transferrin-bound iron will then accumulate in various tissues, including the heart and the pancreas (Nicolas et al. 2001). Conversely, urinary analyses of patients with chronic infections or severe inflammatory diseases have shown up to 100-fold increases in the excreted levels of hepcidin (Nemeth et al. 2003). The result of this infection/inflammatory-mediated up-regulation in hepcidin levels is for iron sequestration in macrophages and decreases in the absorption of iron from the small intestine (Ganz 2003), eventually resulting in anemia.

Through such epidemiological studies and mouse-model investigations, there has been a rapidly growing understanding of how hepcidin operates in the metabolism of iron, but to date, only one attempt to consider the function of this hormone in response to exercise has occurred (Roecker et al. 2005). It is possible that hepcidin might be a new mechanism behind the high incidence of iron deficiency amongst athletes. However, the dearth of research is surprising, since long duration, or high intensity exercise commonly results in a high degree of inflammation, and as previously mentioned, inflammation is a key regulator of hepcidin activity (Nemeth et al. 2004b). To understand how hepcidin may be up-regulated in response to exercise, the inflammatory process that is evoked by physical activity must be considered.

Inflammation: cytokine and acute-phase proteins

Inflammation is an acute phase response of the body to stressful stimuli (Kataranovski et al. 1999). Acute phase inflammation is characterised by an increased blood flow, in association with the accumulation of fluid, leukocytes, and inflammatory mediators known as cytokines and acute phase proteins (Feghali and Wright 1997; Gabay and Kushner 1999). Acute-phase proteins are distinguished by plasma protein concentration increases (positive acute-phase proteins) or decreases (negative acute-phase proteins) of at least 25% during inflammatory disorders (Morley and Kushner 1982). Such proteins are produced by hepatocytes in the liver, and function in many mechanisms of innate immunity, such as pro- and anti-inflammatory activity, wound healing and phagocytic activity (Ceciliani et al. 2002). Common acute phase proteins measured during inflammation include CRP, Serum amyloid A, α_1 Antitrypsin, Fibrinogen and Haptoglobin (Gabay and Kushner 1999; Semple et al. 2006). Cytokines on the other hand, are a group of cell-derived polypeptides that exhibit both pro- and anti-inflammatory characteristics to mediate the inflammatory response (Feghali and Wright 1997). Cytokines are produced by a variety of cells, and act as signalling compounds by binding to high-affinity cell surface receptors at the site of inflammation (Chung 2001). These proteins react in both an autocrine (acting on itself and similar cell types) and a paracrine (acting on local surrounding cells) fashion that produce effects on various target cells (Feghali and Wright 1997). Several cytokines are important in mediating acute inflammatory reactions, such as interleukin (IL)-1, IL-6, IL-8 and tumour necrosis factor alpha (TNF-α) (Feghali and Wright 1997).

The acute-phase response involves the production and distribution of cytokines into the site of inflammation, which in turn facilitates the influx of lymphocytes, leukocytes and other antigen clearing cells (Ostrowski et al. 1999; Pedersen and Hoffman-Goetz 2000). Additionally, leukocytes such as neutrophils and monocytes have the ability to synthesize large quantities of cytokines and growth factors that modulate the inflammatory response (Cassatella 1999; Zallen et al. 1999). As such, the increased number of leukocytes and macrophages at the site of inflammation further increases the production of cytokines, which in turn stimulate an increase in the level of acute-phase protein production by hepatocytes (Gabay and Kushner 1999). To this end, the severity of inflammation can be detected in the blood through the measurement of plasma cytokines and positive acute-phase proteins.

A commonly measured cytokine in the assessment of inflammation is IL-6. The production of this cytokine is generated by a number of different cell types, inclusive of other cytokines, monocytes and macrophages at the site of inflammation (Gabay 2006). Interleukin-6 plays a number of pro and anti-inflammatory roles throughout the body (Villarino et al. 2004). The pro-inflammatory activity of

IL-6 incurs T-cell activation, B-cell differentiation, and the stimulation of acute-phase protein production by the hepatocytes in the liver (Heinrich et al. 1990; Van Snick 1990). Conversely, IL-6 is anti-inflammatory in nature since it directly inhibits the expression of the pro-inflammatory cytokines IL-1β and TNF-α (Pedersen et al. 2001). Furthermore, IL-6 indirectly inhibits the activity of IL-1, a pro-inflammatory cytokine that promotes the production of inflammatory mediators (Biasucci et al. 1999), by stimulating the production of IL-1 receptor antagonist (IL-1Ra), which binds to IL-1 cell surface receptors without activating target cells (Arend 1991; Biasucci et al. 1999). To this end, it is the multi-factorial function of IL-6 that makes its presence during periods of inflammation so abundant, and thereby a logical measure to quantify the severity of inflammation.

Inflammatory response to training

Endurance exercise has been shown to evoke an acute phase response resulting in post-exercise cytokine levels comparable to those seen during bacterial infection, surgery, burns and inflammatory disease (Fallon 2001; Margeli et al. 2005). As such, it is suggested that strenuous exercise induces a 2–3 fold increase in the pro-inflammatory cytokines TNF-α and IL-1β (Ostrowski et al. 1999), and a dramatic increase in the inflammation responsive cytokine IL-6 (Pedersen and Hoffman-Goetz 2000). In fact, IL-6 is said to be the largest contributor to this systemic cytokine increase (Pedersen et al. 2001), with plasma levels rising up to 100 times greater than that recorded pre-exercise (Helge et al. 2003).

With this in mind, the acute phase response to exercise has recently been studied in a number of investigations. For instance, Ostrowski et al. (1998) showed that at the completion of a competitive marathon (mean run time of 3 h: 17 min:03 s $\pm$ 0 h:07 min:39 s), there was a significant increase in the circulating levels of TNF-α (two-fold increase) and IL-1β (1.5 fold increase), in association with a 63 fold increase in plasma IL-6, and a 10 fold increase in the muscle enzyme Creatine Kinase (a blood marker used to indicate muscle damage). Further to this, these authors also showed that IL-6 mRNA was detectable in post-exercise muscle biopsies, leading to the conclusion that IL-6 is produced locally in the skeletal muscle in response to prolonged exercise with a large eccentric component. More recently however, it has been suggested that eccentric exercise is not associated with a more marked increase to plasma IL-6 levels when compared concentric muscle contractions, and that it is the combination of exercise mode, intensity and duration that determines the magnitude of the increase in plasma IL-6 (Fischer 2006). In support of these findings, Helge et al. (2003) had their participants perform knee extension exercise using both legs at 25% of maximal power for 45 min, and then simultaneously with one leg at 65% and the other leg at 85% of maximal power for 35 min. Their results also showed that IL-6 was released from the working muscle, and that the rate of cytokine production was related to the intensity of exercise.

In addition to intensity, repeated bouts of exercise completed on the same day have also been shown to influence the inflammatory response. Ronsen et al. (2002) compared a training day that included two cycling sessions separated by 3 h, to that of a single session day. The cycling exercise was the same for each training bout, and included 65 min of work at 70% of the $VO_{2\ max}$ intensity. The results showed that IL-6 was significantly (69%) more pronounced after the second bout of training during the two session day than after a single bout of exercise during the one session day. It was suggested that the increased cytokine levels were likely due to the limited amount of time (3 h) for muscle glycogen resynthesis between the two sessions (Ronsen et al. 2002), since it has been established that IL-6 production in the contracting muscle increases when in a glycogen depleted state (Steensberg et al. 2001).

As a further part of this investigation, Ronsen et al. (2002) also looked at the effect of two training sessions in the one day, separated by 6 h recovery, rather than just 3 h. In this instance, it was found that the IL-6 levels were not significantly more elevated than they were after only 3 h of recovery. In fact, it was shown that the elevated post-exercise IL-6 levels had returned to baseline levels after 4 h of recovery. The results of this investigation are very applicable to high level athletes, since multiple training sessions in the one day are common practice. However, it is rare that the exercise intensity and even modality between the two sessions is the same. Furthermore, the duration of time between sessions may also extend over 8–12 h. As such, further research is warranted into the effect of multiple daily training sessions on the inflammatory response.

When considering the abovementioned research detailing the response of inflammation to exercise, it is plausible to suggest that the exercise-induced rise in IL-6 may have an effect on the up-regulation of hepcidin. Should this occur, iron uptake by macrophages in response to hemolysis would not be retainable due to the internalisation of the macrophage surface ferroportin channels. Furthermore, the absorption of iron from post-exercise feedings may be limited for a prolonged time period, as a result of the effect on duodenal enterocytes within the gut. Therefore, it is possible that exercise-induced, inflammatory up-regulation of hepcidin activity might potentially be a new mechanism causing iron deficiency in athletes.

Hepcidin and exercise

To date, only one investigation has explored the influence that exercise may have on the expression of hepcidin in humans. Roecker et al. (2005), collected urine samples from 14 female runners prior to, at the completion of, and at 24 and 72 h following a competitive marathon (42.2 km). The mean data of the group showed that the urinary levels of hepcidin at 24 h post-run were significantly (2.5 fold) greater than those measured pre-race, and at 72 h post-race. The authors suggested that a possible increase in inflammatory cytokines and CRP, caused by the trauma of running for a prolonged period of time were the predominant mechanisms behind the increase in hepcidin levels, but these blood markers were not measured. Furthermore, a closer analysis of the results showed that only 8 of the 14 subjects responded with an increase in their urinary hepcidin levels at the 24 h post-exercise time point, and that six of the subjects were classed as 'non-responders'. Caution should be applied when interpreting such results, since the frequency of urine measurement does not consider what might be occurring during this initial 24 h period. Hence, it is possible that the hepcidin levels of those athletes classed as 'non-responders' may have peaked and recovered earlier than those that were classified as 'responders'. Roecker et al. (2005) concluded from this investigation that in some female athletes, chronic increases in hepcidin could potentially lead to the development of iron deficiency.

Although the investigation by Roecker and colleagues was not thorough in the frequency of urine collection or the blood markers measured, the outcome was positive in showing an effect of exercise on hepcidin activity, and therefore a possible effect on the iron status of an athlete. Research to accurately describe the time course activity of hepcidin within the initial 24 h of exercise cessation is needed, since it has been shown that peak hepcidin activity occurs in lipopolysaccharide injected mice within 6 h of injection, which was followed by a significant decrease in serum iron levels (Kemna et al. 2005c). Furthermore, blood markers such as IL-6 and CRP need to be measured post-exercise to gauge the degree of inflammation associated with the rise in hepcidin. Additionally, the effect of hepcidin activity on serum iron 24 h post exercise should be evaluated. Finally, the intensity of exercise and the number of training sessions completed in a given period of time need to be controlled to accurately assess the effect of this hormone.

Conclusion

Iron is an essential dietary element, responsible not only for the efficient delivery of oxygen to the working muscle, but also for the production of energy at the level of the mitochondria. These roles of iron are critical to athletic performance, however it is common for athletes to be iron deficient as a result of iron losses occurring during training from hemolysis, hematuria, sweating and gastrointestinal bleeding (Babic et al. 2001; DeRuisseau et al. 2002; McInnis et al. 1998; Zoller and Vogel 2004). In addition to these well known mechanisms of iron loss, it is also possible that the activity of the inflammatory driven, iron regulatory hormone, hepcidin may have an impact on the iron status of an athlete. It is well known that during chronic disease, the circulating levels of IL-6 are increased, resulting in an up-regulation of hepcidin activity and subsequent decreases in serum iron levels that may eventually lead to anemia (Nemeth et al. 2004a). Since the inflammatory symptoms experienced after high intensity exercise are similar to those seen during chronic disease (Fallon 2001; Margeli et al. 2005), it is possible that the hepcidin activity is also similar, thereby providing new insights into athletic-induced iron deficiency. However, to date, only one investigation has attempted to show such an association (Roecker et al. 2005), yet in doing so, these authors failed to consider a number of associated variables. As a result, a more accurate observation of hepcidin activity is required within the 24 h post-exercise, in addition to the associated blood profiles of IL-6, CRP and serum iron. Furthermore, the effect of training intensity, duration and number of sessions completed should be assessed. Finally, the association between hepcidin activity and exercise induced hemolysis should be considered, since the iron lost from senescent RBC's may be lost to macrophages as a result of the hepcidin activity on ferroportin channels. In light of such conclusions, it is evident that a great deal of research is required to shed light on this currently grey area of iron metabolism in athletes.

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