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REGULATION OF IRON METABOLISM AND EXERCISE

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Abstract

Iron is an essential element for all living cells. More in detail, iron is a key component for oxygen transport and storage, and is part of mitochondrial enzymes and cytochromes involved in the electron transport system. However, as the two sides of the same coin, iron is also involved in the production of reactive oxygen species and consequently requires a very fine regulation. In the context of exercise, and particularly in endurance disciplines, iron metabolism and its control is always a concern for physicians since exercise leads to iron losses and regularly produces hemolysis. In this article the complex molecular mechanisms governing iron homeostasis by the hepcidin-ferroportin axis are briefly reviewed. Afterwards the review is focused on the effect of endurance exercise on iron metabolism and its possible implications for physician and coaches. Overall, this review aims to stimulate the discussion and the research on iron metabolism and its important role in the athlete´s heath.

Key words: *anaemia, athlete, hepcidin, ferroportin, performance*

Introduction

Iron is part of the hemoglobin (Hb), the myoglobin, the mitochondrial enzymes and the cytochromes of the electron transport system. All together, this makes iron an essential element for all living cells. Performance in endurance exercise is determined by several factors depending of the characteristics of the effort [1], but the importance of [Hb] and the oxygen carrying capacity is well recognized and of major importance [2]. Iron deficiency therefore may reduce oxygen carrying capacity and/or decrease the oxidative capacity, affecting negatively exercise performance and working capacity [3-4]. In addition, iron deficiency also impairs the immune system and leads to other physiological dysfunctions [5]. The prevention of such effects is especially important in endurance athletes due to their regular training practices are related to an array of possible causes for iron losses such as intravascular hemolysis, inadequate dietary intake, gastrointestinal bleeding or sweating [6-11]. It is not surprising then why physicians and coaches are concerned about the importance of maintaining an adequate balance between the increased iron demand and iron absorption from the diet in their athletes.

Although iron is an essential element, it is also a transition metal that participates in Fenton and Haber-Weiss reactions. In other words, iron plays a key role in chemical reactions producing reactive oxygen species and cellular damage [12-13]. These two faces of the same coin have determined the evolution of complex mechanisms to control iron homeostasis very tightly. In the past decades the understanding of iron metabolism has enormously increased and nowadays it is recognized to be regulated by the hepcidin-ferroportin axis [12]. However, the signals affecting the hepcidin-ferroportin axis are so far not completely clear. Of major interest in exercise are the effects of inflammation, hypoxia or accelerated erythropoiesis on hepcidin and ferroportin expressions, but also the extent to which exercise *per se* may affect the hepcidin-ferroportin axis. Some recent publications have started to investigate the possible cross-talk between exercise and hepcidin expression, so this review will briefly introduce the mechanisms that regulate iron homeostasis [for further description see references 14,15-23] to subsequently review some of the available literature investigating the effect of exercise on the regulation of iron homeostasis. Overall, this review aims to encourage researches to further discuss and investigate the extent to which the mechanisms involved in the regulation of iron metabolism are affected by exercise and its implication for acute response or chronic adaptations to exercise.

Regulation of systemic iron homeostasis

Approximately, from 3-4 grams of iron present in the body, 70% is situated in the red blood cells as Hb. Iron from senescent erythrocytes is recycled in the spleen and deliver again to the bone marrow by transferrin (Tf). On the other hand, the 1-2 mg/day of

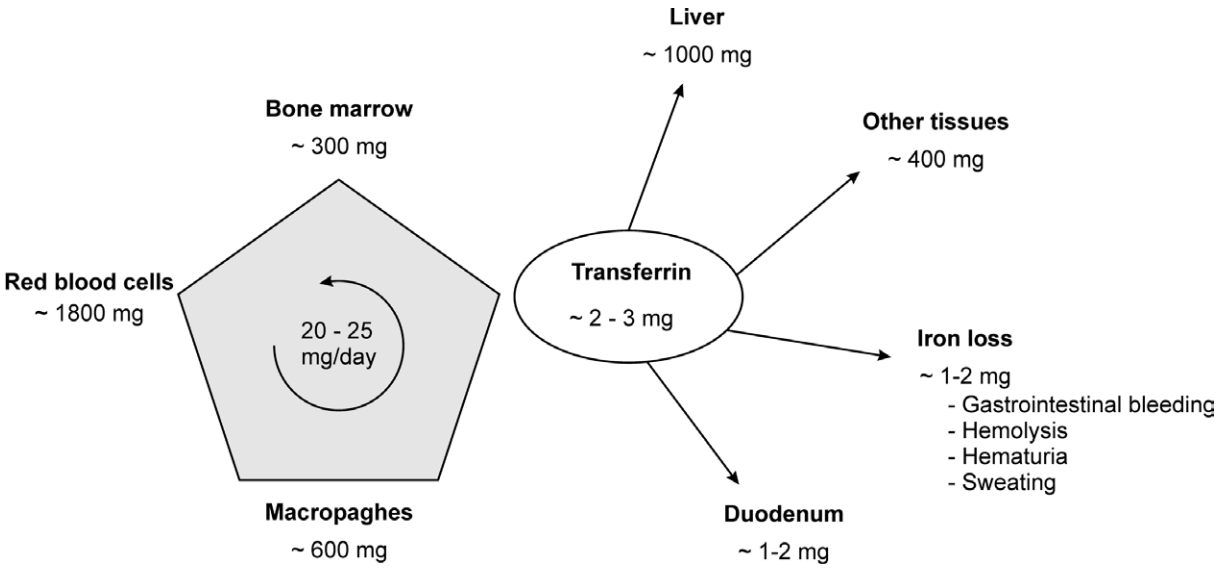

Figure 1. *General view of the traffic and distribution of iron. Iron losses and iron absorption are balanced. Liver and other tissues storage iron, while a continuous iron transfer take place between bone marrow, macrophages and red blood cells.*

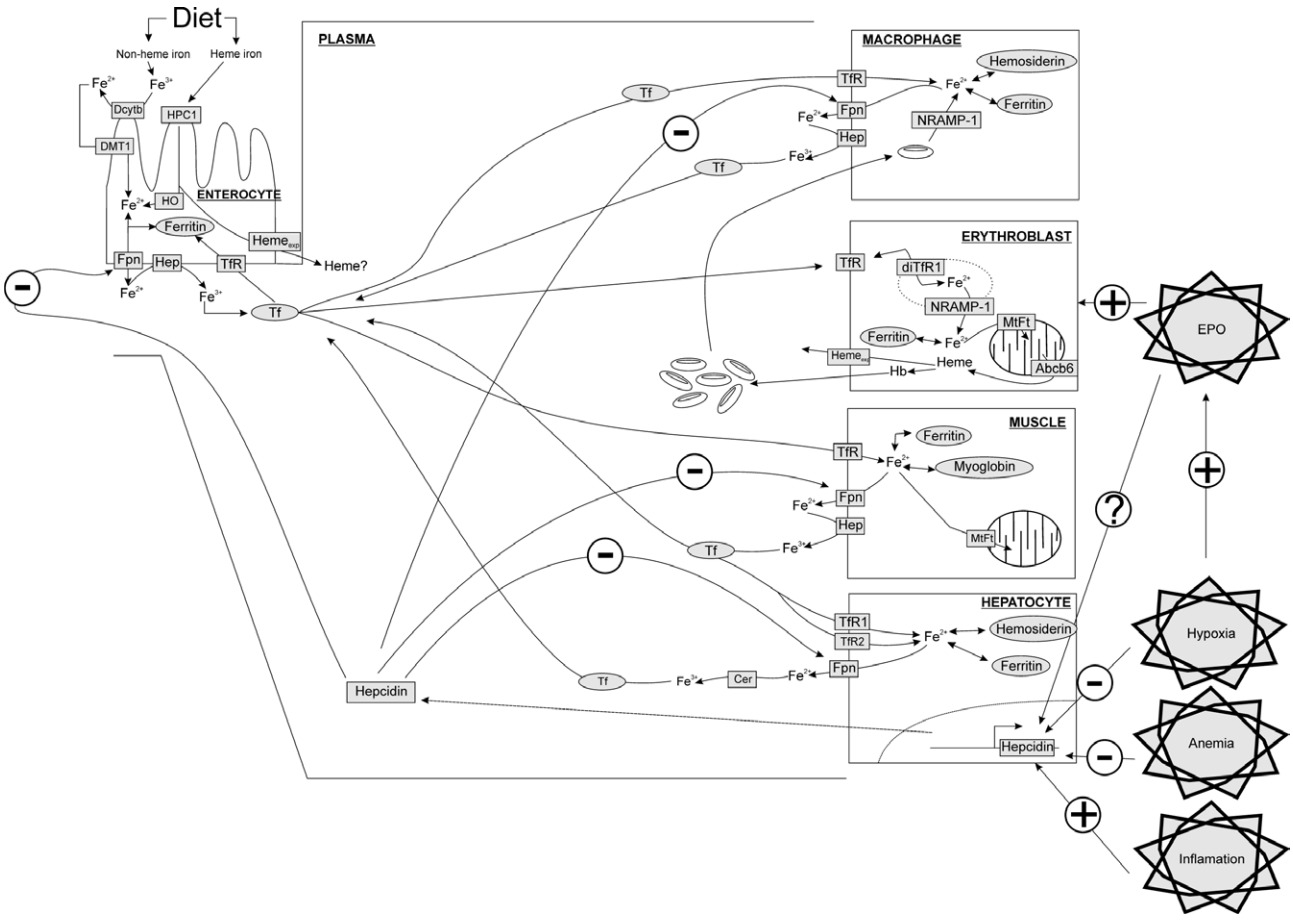

Figure 2. *Molecular mechanisms controlling iron homeostasis and signals affecting liver-hepcidin expression. Iron is exported into plasma through Fpn, which is negatively regulated by hepcidin. On the other hand, hepcidin expression increases or decreases in response to different signals as hypoxia, anemia or inflammation. Dcytb: duodenal cytochrome b; DMT1: divalent metal transporter; HPC1: heme protein carrier 1; HO: Heme oxygenesa; Fpn: ferroportin; Hep: hephaestin; TfR: transferrin receptor, Heme$_{exp}$: heme exporter; Tf: transferrin; NRAMP-1: natural macrophage resistance protein; diTfR1: diferric transferring receptor complex 1; MtFt: mitoferrin; Abcb6: mitochondrial iron exporter; Cer: ceruloplamin; EPO: erythropoietin.*

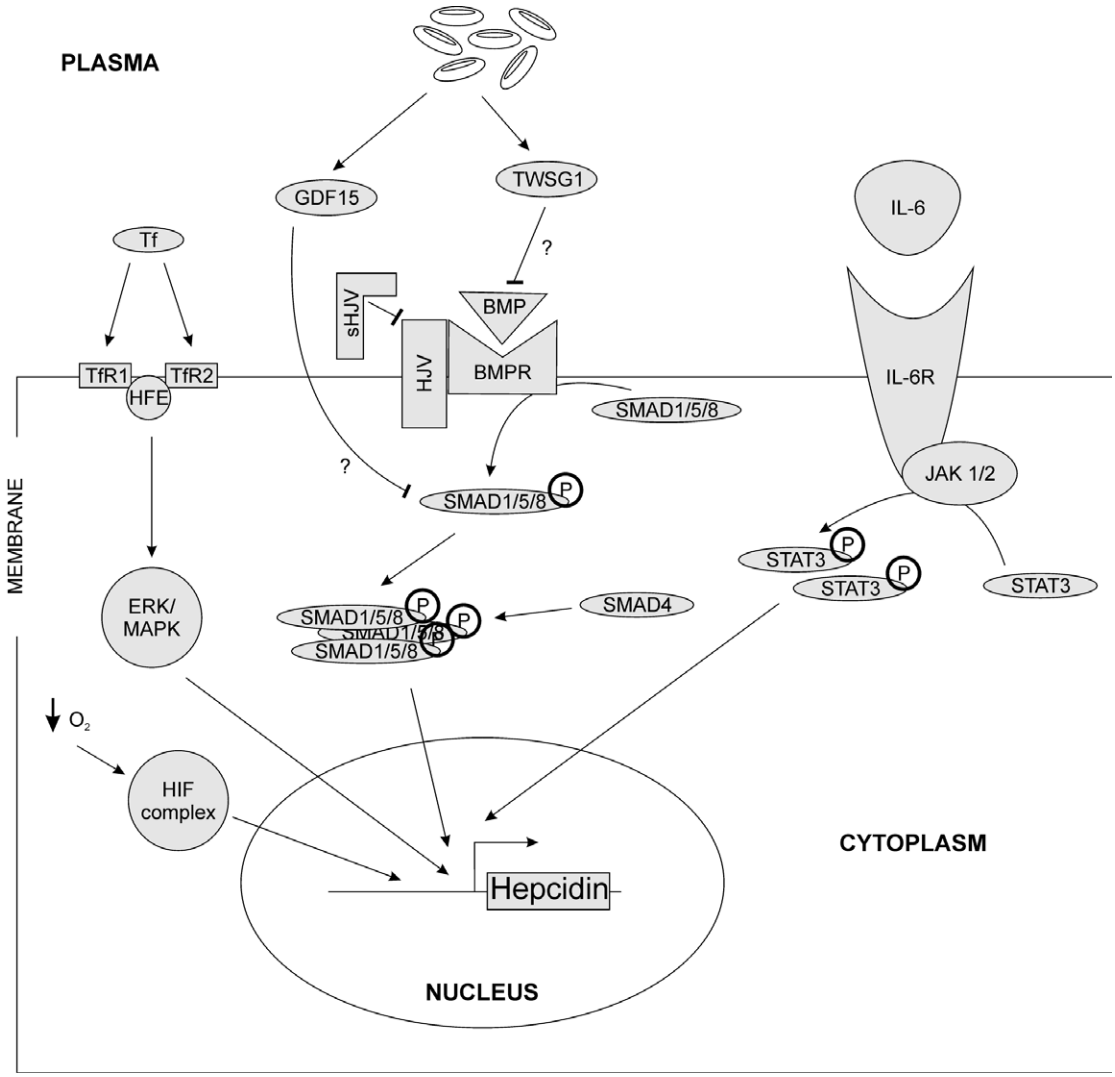

Figure 3. *Different signals involved in the control of liver-hepcidin expression. Hepcidin is regulated by systemic iron levels. High levels of transferrin (Tf) induce hepcidin expression via ERK/MAPK signaling. Hypoxia increase hypoxia inducible factor (HIF) complexes stabilization and affect hepcidin by reducing its expression. Erythroid precursors release GDF-15 and TWSG-1 that in turn modulate hepcidin expression via bone morphogenetic protein (BMP) pathway and hemojuvelin (HJV), a BMP´s coreceptor. Both induce to the phosporiation of receptor-activated SMAD and the subsequent modulation of hepcidin expression. Finally, IL-6 can bind to its receptor and through the Janus kinase (JAK) activates the signal transducer and activation of transcription (STAT) to stimulate hepcidin expression.*

iron loosed are compensated by duodenal absorption (Fig. 1). It is important to note two important facts that explain why iron metabolism requires fine regulation mechanisms. First of all, the organism has no ability to excrete the excess of iron; and secondly, several tissues are involved in storage, consumption and recycling iron, which determines the need of communication among them (Fig. 1).

Figure 2 represents how iron is stored, distributed and recycled, as well as how the communication between the involved tissues is possible. Apart from the complexity of the molecular mechanisms depicted in this figure, it is important to note that tissues can be classified in two general categories based on its ability to storage or use iron. Thus, enterocytes, macrophages, muscle and hepatocytes can storage iron as opposed to erythroblasts, which are pure consumers. In addition, those tissues able to export iron (i.e. deliver iron into plasma for later use in other tissues) always use the same pathway through the only known iron exporter, the protein ferroportin (Fpn). Fpn is in turn negatively regulated by hepcidin (Hpc), a 25-aminoacids peptide expressed and secreted in the liver which binds to Fpn inducing its internalization and subsequent digestion in the cytoplasm [22]. Therefore, the harmonization and control of systemic iron homeostasis is mediated by the Hpc-Fpn axis.

Thus, when iron is present in excess, Hpc expression increases and degrades Fpn reducing iron absorption and mobilization. In the opposite situation, when iron demand increases (e.g. anemia or accelerated erythropoiesis), the expression of Hpc is inhibited allowing the stabilization of Fpn and the increase of iron exportation from cellular cytoplasm into plasma. Since iron is exported as Fe^{2+}, it must be oxidized by hephaestin (Hep) or ceruloplasmin (Cer) prior to be

transported by Tf. Once in plasma, Tf binds to its receptor (TfR) and iron can be incorporated and storage in the cellular cytoplasm mainly as ferritin, as well as be transported into the mitochondria to participate in heme synthesis or incorporated in enzymes and cytochromes of the electron transport system.

Regulation of hepcidin expression

The regulation of Hpc expression has been of great interest since it was recognized as the master regulator of iron metabolism. Liver-hepcidin expression is regulated by different signals (Fig. 3) as iron levels, inflammation, hypoxia or anemia [24]. Hereafter some of the signals involved in the regulation of Hpc expression and important in the context of exercise are briefly exposed.

Exercise is known to increase several markers of inflammation [25-27]. During inflammation, increased interleukin 6 (IL-6) binds to its receptor and the subsequent downstream cascade leads to an upregulation of Hpc expression, which is thought to be an old mechanism to reduce iron availability for virus and infections [28]. The relationship between inflammation, exercise and Hpc activity has been recently addressed in different works presented below in greater detail.

Hypoxia is a strong stimulus for erythropoiesis and therefore its use is relative common among athletes in order to increase their total Hb mass. The overall result of a hypoxic challenge is the increase of iron demand which needs to be compensated by a reduction of Hpc expression. Since hypoxia elevates erythroid activity, the regulation of Hpc expression turns complex due to the superimposition of different signals. In a very recent study performed during a expedition to the Himalaya, Piperno *et al.* [29] have shown that altitude exposure leads to a marked reduction in Hpc activity. Such decrease was primary associated to a reduction in serum ferritin while the importance of signals as erythropoietin was considered less important. Hypoxia has also been demonstrated to reduce iron content in the skeletal muscle [30], while in a later study on humans treated with recombinant human erythropoietin (hypoxic stimulus was not present), a single high dose of the drug inhibited Hpc levels before any change in haematocrit occurred [31]. This and an *in vitro* study where a direct effect of erythropoietin on Hpc through the erythropoietin receptor was reported [32] suggest a possible direct effect of erythropoietin in Hpc expression, although a proof of concept *in vivo* is still required. In addition, the importance of each signal affecting Hpc expression needs to be further investigated since the regulation of iron homeostasis by hypoxia and erythropoiesis has been revealed to be more complex than expected.

Nonetheless, it is important to note that the aforementioned studies may account for changes in iron mobilization accompanied or not by an increase in duodenal iron absorption. Recent publications point out the importance of the hypoxic inducible factors (HIF-1α and HIF-2α) in the regulation of iron metabolism [33]. It has been shown an upregulation of HIF-1α in the liver subsequent to a reduction of iron content in the diet [34]. Additional experiments from the same group and others have revealed that iron uptake in the mucosal side of the enterocytes is under the control of HIF-2α [35-36]. Since stimulation of erythropoiesis is accompanied by a delayed response of Hpc [37] and erythropoietin *per se* seems unable to affect iron absorption [38], it is thought that a rapid response to a decreased iron content in the diet or an increased iron demand is primary mediated by HIF-2α in the duodenum, while HIF-1α would coordinate the later response to harmonize absorption, mobilization of iron storages and erythropoiesis. Therefore, the cross-talk between erythropoietin, hypoxia, iron uptake in the mucosa, iron absorption in the basolateral side of the enterocytes, and mobilization of iron storages supposes a research avenue that will be further investigated in the next years.

Iron status and performance

Endurance athletes are frequently diagnosed of iron deficiency [7,39-40], display lower iron levels compared to athletes participating in anaerobic disciplines [3,39,41] or untrained subjects [42]. Moreover, iron deficiency has been associated with a reduced exercise capacity [3-4] or adaptation to training [43]. Consequently, there has been a great interest to investigate the effect of iron supplementation on performance. In order to replete iron storages, it is possible to use oral or intramuscular injections as both treatments have been reported to be effective increasing ferritin levels [44], and medical control of iron levels together with regular oral supplements prevent anemia in professional athletes through several consecutive seasons [45]. However, the extent to which iron storages repletion improve exercise performance is not clear. Hereafter some of the studies in which iron deficiency subjects were treated with iron are reviewed.

Iron injections have been demonstrated to increase oxidative damage and decrease exercise tolerance in mice [46]. In humans, a negative effect of iron supplementation has never been observed and the direct extrapolation of animals studies to human beings would be inappropriate. In fact, five intramuscular iron injections containing 2 mL of Ferrum H over the course of 8 days did not increase, nor decrease, performance in exercise tests primary involving anaerobic metabolism [47]. The same treatment over the course of 10 days did not enhanced performance in female runners during a time to exhaustion test

[48], although in both studies ferritin levels increased above 60 μg/L. When the iron repletion was carried out by oral supplementation, Klingshirn *et al.* [49] did not report improvement in run time to exhaustion in 18 female athletes diagnosed of iron deficiency. Similarly, a group of 37 iron deficiency, non-anemic, women treated with 135g of ferrous sulfate per day did not improve performance in 15 km time trial, thought the fractional utilization of maximum peak oxygen consumption ($\dot{V}O_2$max) decreased in the treated subjects compared to placebo group. In contrast, Friedmann *et al.* [50] reported improvements in $\dot{V}O_2$max, individual lactate threshold and anaerobic performance (measure by maximal accumulated oxygen deficit test) compared to placebo group after 12 weeks of treatment with 100 mg ferrous iron twice a day. Of note, the improvements were independent to red blood cell volume changes, which remained stable through the study, and it was suggested that exercise performance improvements may be mediated by enhanced oxidative capacity within the muscle. In the same line, Hinton *et al.* [51] have reported positive effects of oral iron suplemmentartion on time to complete a 15 km cycling time trial, thought the same group suggested that rather than an increase in exercise performance, iron repletion improves the adaptation to training [43].

Overall, the previous studies suggest that iron deficiency may affect exercise performance, but it is not clear if iron repletion by oral or intramuscular injections has a positive effect on exercise performance. The controversy may be due to the different cut-off values used as inclusion criteria or different tests used. Of note, except in the work from Fogelholm *et al.* [48] in which [Hb] increased without affecting performance during an incremental ergometer test, those studies that reported positive effects of iron supplementation also failed to find any change in red blood cell mass or [Hb] [52], suggesting that iron-mediated exercise improvements might be related with other factors as oxygen utilization by the mitochondria. This represents an interesting challenge and requires further investigation that nowadays it is possible since mitochondrial function can be evaluated *in situ* using high resolution respirometry [53].

How athletes loose iron?

Although increasing iron storages levels may not be beneficial nor detrimental for exercise performance, iron deficiency in athletes is still a concern for coaches and physicians since it may affect the immune system among others [5]. In fact, it has been recently reported that endurance athletes present lower white blood cells count that counterparts enrolled in anaerobic sports [54]. Thought it may signify a higher susceptibility in athletes to suffer from infectious diseases, it is also possible to be the result of chronic adaptations to exercise and regular training [55]. Therefore, the cause-consequence relationship between iron biology and immune system in athletes deserves further investigation. To that end, the knowledge of the mechanisms by which athletes use to loss iron is required and some are briefly reviewed below.

The presence of Hb in the urine, termed hematuria, is suggested to be a source of iron losses in athletes, however no all the athletes experienced symptoms of hematuria after a marathon [56]. A more recent study investigating the effect of intensity and exercise mode (running or cycling) showed that hematuria was positively related to the intensity of exercise and running, suggesting a relation between weight-bearing activities and hematuria [9]. The extent to which repeated iron losses by hematuria (if occurring) may lead to iron deficiency need to be discussed and investigated in follow-up studies.

A second source of iron losses is sweating. Exercise elevates core temperature resulting in a increased sweating rate as countermeasure [57]. The rate of iron loss by sweating has been estimated in recreational cyclists who exercised at submaximal intensity (50% of $\dot{V}O_2$max) during 2 hours. The exercise was performed under normal conditions of temperature and humidity and iron losses were found to be 0.060 mg/m^2/h and 0.042 mg/m^2/h during the first and second hour of exercise respectively [8]. Assuming a constant rate of sweating over the time of the competition, a hypothetical triathlete of 70 kg and 180 cm (1.89 m^2 of body surface calculated by DuBois and DuBois formula) competing in an ironman would loss between 0.79 and 1.134 mg of iron after 10 hours of competition. Taking into account that iron losses and absorption are 1-2 mg per day (Fig. 1), endurance exercise may overweight the balance in favors of the losses. However, it is important to note that an acute response to exercise may not necessarily appear as a chronic effect of training. Indeed, regular exercise in the heat has been shown to elicit adaptation and reduce sweating iron levels [58].

Exercise also elevates the blood flow to muscle and decreases it to the gastrointestinal track [59-60]. Additionally, gastrointestinal problems, which etiology is not fully understood, are frequently reported in athletes participating in endurance and intense activities [61]. Therefore, it is thought that small but repetitive exercise-induced gastrointestinal bleeding is also a source of iron losses [62]. Gaudin *et al.* [63] reported a higher incidence of lessons in the intestinal mucosal after exercise, however the mechanisms producing these injuries are so far unknown and could be related with acidosis [64], mechanical stress due to the type of exercise [61] or hypoxic conditions in the mucosal [65].

Another consequence of exercise related to iron losses is the destruction of red blood cells or exercise-induced hemolysis. The assessment of exercise-induced hemolysis is usually performed by measuring plasma and/or serum concentration of the protein haptoglobin, a Hb scavenger that reduces the oxidative potential of free Hb. Although the primary cause of hemolysis seems to be the footsrike [10] in a force-dependent manner [66], it has been also reported in swimmers [67], suggesting a multifactorial phenomenon non only reduced to the mechanical stress. Therefore, the study of the mechanism(s) involved in the exercise-induced hemolysis is assure in the next years by for example investigating how exercise affect the membrane of the erythrocytes, or the different red blood cells turnover of each subpopulation of erythrocytes in athletes compare to normal population [68-69].

Role of exercise in hepcidin activity

All the aforementioned factors eliciting iron losses do not provide a mechanistic link between exercise and iron deficiency, however the understanding of the mechanisms regulating iron homeostasis by Hpc-Fpn axis [22] has provided new possibilities to explain how exercise induces iron deficiency. Accordingly, several studies, which are reviewed during the next lines, have investigated the effect of exercise intensity or exercise modality on Hpc levels.

The first report studying the relationship between exercise and Hpc was published in 2005 by Roecker *et al.* [70]. In this investigation designed as a pre-post study, urinary Hpc was measure in 14 female runners who participated in a marathon. Hpc levels were normalized to urinary creatinine and found to increase after the race in more than 70% of the runners (10 out 14 designated as "responders"). The highest increase in Hpc levels was found one day post-race and varied between 4- to 27-fold times compared to pre-race values. Authors point the known increase of IL-6 during exercise [25-27] as one possible explanation. However they also suggest that previous training status may influence the extent to which exercise affects Hpc levels, since "responders" runners had already lower pre-competition Hpc values.

In later studies, the response of Hpc to different exercise intensities or training surface has been investigated. In 6 men and 5 women moderately trained runners, Peeling *et al.* [71] showed that 60 min of exercise (running 15 min at 75% plus 45 min at 95% of the speed corresponding to the $\dot{V}O_2$max) produces an increase in IL-6 immediately after exercise and 3 hours post-exercise accompanied by an elevation of urinary Hpc 3- and 6-hours post-exercise. These authors also reported an increase of serum iron after completion of exercise, suggesting that exercise may affect Hpc increasing iron availability and/or IL-6. The same research group has investigate the effect of exercising on different surfaces on hemolysis, inflammation and Hpc [72]. In this work, ten male runners performed a 10 km run at 75-80% of the peak $\dot{V}O_2$max speed on a soft (grass) or hard (road) surface. In addition, participants carried out a complementary session on the grass consisting of 10 x 1 km interval running at higher intensity (90-95% of peak $\dot{V}O_2$max) in a 2:1 work-to-rest ratio. As indicators of hemolysis, free Hb and haptoglobin were measured. Both parameters changed independently of the surface and free Hb increased while haptoglobin decreased, suggesting that the occurrence of hemolysis may not be prevented by running on a softer surface. In addition, increased exercise intensity during the interval running session produced a higher variation of both parameters compared to grass condition, pointing out the importance of the intensity. Similarly, serum iron levels increased after all sessions. Regarding inflammation, all conditions were associated with an increase in IL-6 levels, although interval exercise induced the highest values. Finally, urinary Hpc levels were accordingly increased 3 hours post-exercise in all the session. Authors concluded that the degree of hemolysis and inflammation is related to the exercise intensity rather than the surface, but not associated with acute changes in Hpc expression which was similar elevated in all the conditions.

Two more studies has been performed to elucidated i) the effect of several training session on Hpc expression [73] and, ii) the effect of submaximal intensity exercise on the daily regulation of hepcidin [74]. In the first work, ten highly trained endurance athletes performed two training sessions with 12 hours rest in between or a single training session as control condition. Exercise task were composed of 10 km running at 75-80% of peak $\dot{V}O_2$max speed (first training session) or 10 x 1 km interval running at a 90-95% of intensity (second and single training sessions). The degree of hemolysis was greater due to the cumulative effect of training sessions but not accompanied by further increases in markers of inflammation (IL-6 levels), urinary Hpc or serum iron levels, suggesting that hemolysis is affected by consecutive training stimuli but not affecting Hpc, which rather than training volume, would response to training intensity. In the second study, Troadec *et al.* 45 min of cycling at 60% of the heart rate reserve has no influence on daily variations of serum iron or urinary Hpc in 14 healthy volunteers. However, these authors identified that the urinary and serum circadian cycle of Hpc are different, which may be an important issue in future research. Nonetheless, these studies point out the importance of exercise intensity and IL-6 as modulators of Hpc activity [75], providing a better understanding of the mechanisms that may elicit iron deficiency in athletes.

Additional considerations

It is worth to include here some additional considerations concerning iron status and exercise. Until know, it has been shown that exercise imply iron losses by several mechanisms and athletes are frequently diagnosed of iron deficiency. However, it has been also reported that low levels of ferritin are not necessarily accompanied by a reduction or impairment of the red blood cell production [76]. In this context, Peeling *et al.* [48] have recently proposed the next stages of severity in the assessment of iron status in athletes: Stage one-iron depletion (Ferritin: <35 μg/L, Hb: >115 g/L, Transferrin Saturation: >16%), stage two-iron deficient erythropoiesis (Ferririn: <20 μg/L, Hb: >115 g/L, Transferrin Saturation: <16%), and stage three-iron deficient anemia (Ferririn: <12 μg/L, Hb: <115 g/L, Transferrin Saturation: <16%). However, earlier works propose more "flexible" limits since stage one or iron-deficient stores are defined only by plasma ferritin <12 μg/L [77]. Therefore, and due to the wide range of available cut-off values, a clear and specific definition for athletes seems mandatory.

Moreover, some of the publications previously reviewed [48,71-75] may alert to physicians and coaches about the importance of the conditions in which iron status is assessed, since Hpc is affected by inflammation and presents a circadian cycle through the day. This is also important because the World Antidoping Agency recently funded a project aiming to use Hpc levels as a candidate against doping practices (http://www.wada-ama.org/en/Science-Medicine/Research/Funded-Research-Projects/). Similar to the already recommended cautions to assess hematological indices in athletes [78], standardized conditions to analyze Hpc and iron status in athletes is required.

Conclusions and future directions

In this review, the fine and complex mechanisms governing iron homeostasis have been briefly presented. Subsequently, the manuscript is focused in the relationship between iron status and performance, the mechanisms eliciting iron losses in athletes and the review of the most recent literature investigating how exercise may modulate Hpc activity. Taking all together, it is concluded that:

- Iron homeostasis is finely harmonized and regulated by Fpn-Hpc axis.
- Hpc expression is affected by several and different signals which interactions are not completely understood. Some of these signals involved in the modulation of Hpc activity, as hypoxia or inflammation, are important in the context of exercise and physicians and coaches should take these factors into account to control iron status in their athletes.
- Due to their regular training practices, athletes are exposed to several factors that may increase iron losses. Therefore, diagnosis of iron deficiency in athletes is more frequent than in normal subjects. The treatment of iron deficiency with intramuscular or oral iron recovers ferritin levels successfully, but not necessarily increases performance in iron deficient athletes.
- Exercise may modulate Hpc activity via increased IL-6 and inflammation. Moreover, higher exercise training seems to be related with a greater increase in Hpc levels and submaximal exercise intensities may not affect daily variations of Hpc.

Together with the increased understanding of the mechanism regulating iron metabolism and its relationship with exercise, new questions have raised and the new investigations are guaranteed in the next years in order to elucidate:

- The mechanisms by which iron repletion has positive effects on performance in iron deficient subjects and the role of oxygen utilization and mitochondria in said improvement.
- The mechanisms underlying exercise-induced hemolysis and the extent to which small but repetitive iron losses may chronically affect iron homeostasis.
- How Hpc activity is modulate throughout the course of one or several seasons and by different training regimes.
- The exercise-induced changes in Hpc activity in already iron deficient athletes and it's response after iron supplementation.

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Declaration of interest

The authors reports no conflicts of interest.

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