# Iron considerations for the athlete: a narrative review

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Iron and the athlete.

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**Abstract**

Iron plays a significant role in the body, and is specifically important to athletes, since it is a dominant feature in processes such as oxygen transport and energy metabolism. Despite its importance, athlete populations, especially females and endurance athletes, are commonly diagnosed with iron deficiency, suggesting an association between sport performance and iron regulation. Although iron deficiency is most common in female athletes (~15-35% athlete cohorts deficient), approximately 5-11% of male athlete cohorts also present with this issue. Furthermore, interest has grown in the mechanisms that influence iron absorption in athletes over the last decade, with the link between iron regulation and exercise becoming a research focus. Specifically, exercise-induced increases in the master iron regulatory hormone, hepcidin, has been highlighted as a contributing factor towards altered iron metabolism in athletes. To date, a plethora of research has been conducted, including investigation into the impact that sex hormones, diet (e.g. macronutrient manipulation), training and environmental stress (e.g. hypoxia due to altitude training) have on an athlete's iron status, with numerous recommendations proposed for consideration. This review summarises the current state of research with respect to the aforementioned factors, drawing conclusions and recommendations for future work.

**Key words:** Iron deficiency, Anemia, Hepcidin, Exercise

**Abbreviations**

Change in Hemoglobin Mass (ΔHbmass)
Divalent Metal Transporter 1 (DMT-1)
Duodenal Cytochrome b (DCytB)
Erythroferrone (ERFE)
Erythropoietin (EPO)
Follicle Stimulating Hormone (FSH)
Gastro-Intestinal (GI)
Hemoglobin (Hb)
Hemoglobin mass ($Hb_{mass}$)
Hypoxia-Inducible Factor (HIF)
Interleukin-6 (IL-6)
Intravenous (IV)
Iron (Fe)
Iron Deficiency (ID)
Iron Deficiency Anemia; IDA
Iron Deficient Non-Anemia (IDNA)
Live High, Train Low (LHTL)
Low Carbohydrate, High Fat (LCHF)
Low Energy Availability (LEA)
Luteinising Hormone (LH),
Maximal Oxygen Uptake ($VO_{2max}$)
Messenger RiboNucleic Acid (mRNA)
Oral Contraceptive Cycle (OCC)
Oral Contraceptive Pill (OCP)
Recommended Dietary Intake (RDI)
Relative Energy Deficiency in Sport (RED-S)
Soluble Transferrin Receptor (sTfR)
Transferrin Receptor (TfR)
Transferrin Receptor-2 (TfR-2)
Velocity at Peak Oxygen Uptake ($vVO_{2peak}$)

## Introduction

Iron is a fundamental mineral used by the body for numerous processes such as oxygen transport and energy production at a cellular level (Beard 2001). Clearly, these processes are imperative in the support of athletic pursuit, hence, the provision, utilisation and storage of iron is extremely important to an athlete. Despite the biological importance, iron deficiency (ID) is a widely reported issue in athlete populations, with the documented prevalence reported at ~15-35% of female and ~3-11% of male athletes (Fallon 2008, 2004; Malczewska et al. 2001; Parks et al. 2017). However, smaller cohort studies report higher rates of compromised iron stores across a variety of sports settings, with the prevalence reported as >50% in female, and up to 30% in male athletes (Koehler et al. 2012, Tan et al., 2012).

Of note, female athletes tend to experience a greater incidence of ID (Beard and Tobin 2000), potentially a result of increased iron demand to account for menses (Pedlar et al. 2018). However, low energy intake, vegetarian diets and endurance exercise have also been proposed as potential factors impacting both male and female athletes' iron stores (Castell et al. 2018). The symptoms of compromised iron status include lethargy, fatigue and negative mood states (Pasricha et al. 2010; Patterson et al. 2001; Nielsen and Nachtigall 1998), with more severe cases (i.e. iron deficiency anemia; IDA) also compromising work capacity (Woodson et al. 1978). Such symptoms may impact the athlete's ability to train appropriately and to produce competitive performances (Garvican et al. 2011). Consequently, it is important that the iron status of an athlete is routinely monitored, and that appropriate action is taken should correction of a deficiency be required. However, to assess an athlete's iron status, and to determine an appropriate course of action, an understanding of the mechanisms that influence iron absorption is needed, since contemporary research has established a clear link between iron regulation and exercise. Furthermore, numerous research papers have explored the impact of diet, training and environmental stress on an athletes iron status over the past decade, with many

recommendations proposed for consideration. Therefore, this review will attempt to summarise the current state of play with respect to the aforementioned factors of interest, drawing conclusions and recommendations for future work in this area.

**How do we define an iron deficiency in athletes?**

Debate currently exists as to the most appropriate haematological variables (and their cut-off values) that should be measured in the assessment of an athlete's iron status; however, the reader is directed to a succinct summary of these various measures by (Clenin et al. 2015). Despite a plethora of variables available to practitioners, the current (minimum) routine clinical assessment of ID includes analysis of the blood markers; ferritin, hemoglobin concentration (Hb), and transferrin saturation. In an attempt to classify the various stages of ID using these three hematological variables, Peeling et al. (2007) proposed the following for athletic populations:

- ***Stage 1 - Iron deficiency (ID):*** Iron stores in the bone marrow, liver, and spleen are depleted (ferritin <35 µg/L, Hb >115 g/L, transferrin saturation >16%).
- ***Stage 2 - Iron-deficient non-anemia (IDNA):*** Erythropoiesis diminishes as the iron supply to the erythroid marrow is reduced (ferritin <20 µg/L, Hb >115 g/L, transferrin saturation <16%).
- ***Stage 3 - Iron-deficient anemia (IDA):*** Hb production falls, resulting in anemia (ferritin <12 µg/L, Hb <115 g/L, transferrin saturation < 16%).

Additionally, serum soluble transferrin receptor (sTfR) levels of 2.5 mg/L could be considered a reasonable threshold for identification of IDA (Koulaouzidis et al. 2009). Of note, it appears that depleted iron stores (Stage 1) may have a minimal impact on physical performance; however, early correction of iron depletion is likely to prevent the issue from further progressing into Stages 2 and 3. For example, despite unchanged Hb concentration in

IDNA, training and performance outcomes may be compromised (DellaValle and Haas, 2011). Furthermore, Garvican-Lewis et al (2016a) reported that supplementing athletes with low pre-altitude iron stores (<35 μg/L) with 210 mg supplemental iron per day over 3 weeks of moderate altitude exposure was associated with an increased hematological response compared to no supplementation; suggesting an essential role of iron in the adaptation process. Regardless, as noted by Clenin et al. (2015), a number of limitations are associated with the use of ferritin as a marker of iron status, such as its role as an acute phase protein, and the fact that ferritin levels are increased during periods of inflammation and after intensive exercise. Furthermore, measures of Hb are also affected by shifts in plasma volume, which when unaccounted for, may present issues such as pseudo-anaemia (see Bartsch et al. 1998): also commonly referred as sports anaemia (Sim et al. 2013), which does not appear to have any negative effects on performance. Considering training and/or heat adaptions can induce hypervolemia (Taylor. 2014; Voss et al. 2014), these factors should be considered to avoid underestimating the concentrations of plasma iron and red cell parameters. Additionally, blood collection standardisation is imperative prior to assessing an athlete's iron status, and consideration must be given to the time of day, the hydration state of the athlete, and their prior activity levels leading into the blood assessment (Castell et al. 2018). Of note, muscle damaging exercise (e.g. eccentric) should not be performed in the 2-3 days prior to the assessment, since this type of activity can induce high levels of systemic inflammation (Peake et al., 2005), which may impact on the blood picture captured. Ultimately, blood screening for such purposes should be collected in the morning, with the athlete in a rested (i.e. 24+ h post-training) and hydrated state (preferably assessed via waking urinary specific gravity of <1.025; Armstrong et al., 2010) after an overnight fast (*Figure 1*).

**What are the contributing factors to iron deficiency in athlete populations?**

Given the high incidence of ID reported in athletes, it is likely that exercise, and/or dietary/energy availability, can influence iron metabolism in this population. Previous reviews exploring the underlying mechanisms of iron deficiency in athlete populations have considered prospects such as hemolysis exacerbated by ground impact forces (e.g. foot strike; Telford et al. 2003) and muscle contraction (e.g. eccentric muscle damaging exercise; Theodorou et al. 2010), hematuria, gastro-intestinal (GI) bleeding, sweating, and inflammatory/iron regulatory hormone (hepcidin) responses (for review see Peeling et al. 2008). Over the past decade, a strong focus of the literature has been placed on this latter mechanism, with numerous papers showing that exercise has a transient impact on increasing levels of the master iron regulatory hormone, hepcidin (for 3-6 h post-exercise); likely a result of the well-documented exercise-induced inflammatory response and associated increases in the cytokine interleukin-6 (IL-6) (Peeling et al. 2008; Roecker et al. 2005; Newlin et al. 2012). Increases in hepcidin activity result in a decrease in iron absorption and recycling from the gut and scavenging macrophages, respectively. As such, it is likely that there exists a transient window of altered iron metabolism after exercise where nutrition strategies could be exploited to manipulate the outcome (i.e., strategic feeding times to avoid the window of decreased iron absorption). Regardless, it is understood that the function of hepcidin is homeostatic in nature, and therefore, it has been noted that the hepcidin response to exercise is attenuated in iron deficient athletes (Peeling et al. 2014; Peeling et al. 2017), likely a result of the body signalling an increased iron need. The problem exists that exercise-induced elevations in the hormone response are evident in athletes with 'healthy' iron stores, including those that present with values at the borderline of the abovementioned cut-off values (i.e. ferritin 30 µg/L). Therefore, athletes that tend to sit on the verge of having compromised iron stores seem to be in a vicious cycle of being unable to 'get

on top' of their iron status. As such, strategies to rapidly increase iron stores may be required to overcome this issue (discussed further in the supplement strategies section below).

In addition to these core mechanisms, there is also a likely link to the Relative Energy Deficiency in Sport (RED-S) concept (Mountjoy et al. 2014), whereby overall low energy availability (LEA) and energy intake in athlete populations may relate to either an overall deficit in dietary iron intake and/or dysfunction in its subsequent absorption. Such events may reduce an athlete's chance of meeting the increased iron demands, where an additional ~1-2 mg iron/day may be required to replenish exercise-related iron losses (Nielsen and Nachtigall 1998). Finally, there exists the added burden of menstrual blood losses in female athletes (Pedlar et al. 2018) and the decreased iron bioavailability in vegetarian diets (Venderley and Campbell 2006) that can add to the cumulative effect of compromised iron stores in athlete populations. As such, it is essential that practitioners are aware of, and assess, the multitude of factors that contribute towards ID when working with athletes who struggle to maintain iron balance. Furthermore, future research is required to explore these interactions and the best approaches to improving the iron status of athletes that struggle to maintain optimal iron stores.

**How does iron deficiency impact athletic performance?**

Iron-dependent metabolic pathways involve (i) Hb and myoglobin for oxygen transport to the exercising skeletal muscle; and (ii) the oxidative production of adenosine triphosphate at the electron transport chain that is highly reliant on non-heme iron sulfur enzymes and heme-containing cytochromes (Beard and Tobin 2000). Iron deficiency is typically associated with impaired aerobic power, with the magnitude of the expected performance reduction related to the severity of the ID. Specifically, aerobic performance is likely to be most severely affected when iron stores are depleted and Hb production is compromised (e.g. IDA; Stage 3 of ID) (Myhre et al. 2016). Consequently, reduced oxygen transport to the exercising skeletal muscle

may place higher demands on anaerobic metabolism (Gardner et al. 1977), which could negatively influence performance (e.g. lower blood pH, depletion of muscle glycogen). Given the reliance on aerobic metabolism and high prevalence of ID in endurance athletes, this population is often studied (Rubeor et al. 2018).

Despite the importance of iron, supplementing iron to athletes without ID does not appear to improve endurance performance (Garvican et al. 2014; Powell and Tucker 1991; Tsalis et al. 2004). Furthermore, evidence for the negative influence that IDNA (Stage 1 and 2) has on endurance performance is also equivocal, with both no effect and negative effects reported (Rubeor et al. 2018; Burden et al. 2015a). Specifically, as oxygen transport capacity is unchanged in IDNA (Stages 1 and 2), any negative influence that IDNA has on endurance performance may be associated with impaired function of oxidative enzymes and respiratory proteins (Haas and Brownlie 2001). However, as we cannot exclude other potential negative effects that IDNA has on health, preventing further declines to iron status must be prioritised. A combination of regular screening tests alongside dietary assessments should be scheduled periodically throughout the year to identify the need for iron supplementation (discussed in other sections). Such propositions have led research to investigate the role of iron supplements on endurance performance in IDNA athletes. For instance, in 165 IDNA female rowers (Hb >120 g/L), lower ferritin stores (<20 µg/L vs. ≥20 µg/L) was retrospectively associated (2-3 months prior) with slower (~21 s) 2 km rowing TT ergometer performance (DellaValle and Haas 2011). In a follow-up randomised controlled trial conducted in a similar population (n=31, IDNA female rowers), despite unchanged 4 km rowing TT performance, daily iron supplements (100 mg/d) over 6 weeks were shown to decrease energy expenditure and increase energy efficiency during the TT (Dellavalle and Haas 2014). The authors hypothesised that physiological adaptions (e.g. increase in oxidative enzymes, red cell volume) typically associated with endurance training may only be maximised in the presence of adequate iron

stores. For example, greater iron bioavailability may favour adaptations leading to better exercise economy. However, such propositions remain to be confirmed by future research.

Similar sentiments to those above were echoed in a meta-analysis reporting that iron supplementation in IDNA athletes could lead to improved aerobic performance (Burden et al. 2015a). Here, Burden et al. (2015a) explored the efficacy of iron treatments (injection or oral supplements) on iron status and aerobic capacity (assessed by maximal oxygen uptake; $VO_{2max}$) in IDNA individuals (12 studies, n=443, 82% female). Here, iron treatment had a large effect on ferritin, and a moderate effect on both Hb and $VO_{2max}$. However, no improvements in $VO_{2max}$ were reported across eight studies, while the greatest changes in aerobic power were observed in studies with less-trained individuals ($VO_{2max}$ <40 mL/kg/min). Another recent meta-analysis (18 trials and 2 companion papers, n=1170 individuals) by Houston et al. (2018) reported that iron supplementation in IDNA adults (combing both trained and untrained populations) did not improve physical capacity (assessed by $VO_{2max}$ and other timed-exercise tests) despite improved perceived measures of fatigue. However, such findings may be different if only well-trained or elite endurance athletes were considered. Accordingly, Rubeor and colleagues concluded in their systemic review (12 studies, n=283 participants, 91% female) that 50% of studies found no performance improvements for IDNA athletes after iron supplementation (Rubeor et al. 2018). However, unlike the meta-analysis where aerobic power was assessed using $VO_{2max}$, performance was quantified using a range of tests differing in their metabolic demands (e.g. shuttle run, 3000 m run, time to fatigue), which complicates the interpretation of results. Of note, when ferritin stores are substantially compromised (≤20 µg/L) in IDNA athletes, Rubeor and colleagues (2018) reported that iron supplementation can improve performance. Collectively, these meta-analyses highlight that a definitive relationship between IDNA and compromised performance remains ambiguous in elite athlete populations,

although it is perhaps the bioavailability of iron (as indicated by ferritin) that is likely to be a contributing factor when performance decrements are evident.

Alternatively, for IDA, the benefits of iron supplements to improve iron status and performance are well-documented and widely recognised. IDA is estimated to affect up to 18% and 7% of female and male athletes across a variety of sports (See Parks et al. 2017 for review). However, the extent of IDA could be higher during intense physical preparation, with 29% of elite female soccer players (from a single team) diagnosed with IDA six months prior to the FIFA Women's World Cup (Landahl et al., 2005). Of interest, a case study of an elite female endurance athlete (19 years of age) presenting with anemia (ferritin 9.9 µg/L, Hb 88 g/L) found that an initial intramuscular injection (100 mg Fe), followed by twice daily oral supplements (100 mg, elemental Fe) over 15 weeks substantially improved iron status (ferritin 27.0 µg/L, Hb 130 g/L) (Garvican et al. 2011). Here, a marked and rapid increase in absolute hemoglobin mass ($Hb_{mass}$; measured via the carbon monoxide rebreathing method; Schmidt and Prommer, 2005) was recorded in the first ~2 weeks after the iron injection (389 g to 580 g) and continued to rise over the next 13 weeks (710 g). Improved iron status enabled the athlete to gradually increase her training load, which likely contributed to a 3000 m personal best run time ~8 weeks after iron treatment commenced. Although results from case studies cannot be generalised, this work highlights the benefits of a well-devised iron supplementation framework. Under similar scenarios, the aforementioned framework should be considered by support staff (i.e. sports medicine, nutrition and physiology) when dealing with athletes presenting with IDA.

In summary, despite a lack of definitive evidence that IDNA compromises performance, the iron status of athletes should be monitored consistently throughout the training year. Early detection of low iron stores and subsequent supplementation may reverse (or limit) further declines in iron status (e.g. the progression to IDA), where a range of other negative effects (e.g. compromised immune function, lethargy, weakness) can exacerbate

performance declines. Furthermore, it is possible that compromised iron stores could have other unknown negative influences on numerous training (e.g. enzymes involved in energy production, muscle physiology/function) and recovery adaptions in athletes. Such prospects require further investigation by future work. Finally, as highlighted in *Figure 1*, blood screening needs to consider a range of factors (discussed subsequently) and should be performed (i) annually for athletes with no symptoms (or history of ID), or (ii) quarterly (or at least biannually) for 'at risk' individuals.

**Are there any sex differences relevant to iron deficiency?**

An estimated 11% of male and 35% of female athletes suffer from ID (Malczewska et al. 2001; Dubnov and Constantini 2004). In the general population, IDA is estimated to affect 3-5% of women and <1% of men. For IDNA, this affects approximately 12-16% of premenopausal adult women and 2% of men (Looker et al. 1997). Active women are estimated to be twice as likely to present with IDNA compared to sedentary women (Sinclair and Hinton 2005), with 24-47% of exercising women experiencing IDNA (Rowland 2012). In 193 elite young (<25 years) German athletes (mean age 16.2 years, ~50% female) across 24 different sports, the prevalence of low ferritin (<35 ug/L) was almost double in females compared to males (57% vs. 31%) (Koehler et al. 2012). However, the incidence of low Hb (<120 g/L or <130 g/L for females and males, respectively) was similar (6.2% vs 7.3%). Although sex-specific cut-points for biomarkers related to ID have been proposed (Looker et al. 1997), such methods have been met with criticism. Specifically, as current practice used to establish reference ranges (for serum ferritin, Hb and transferrin saturation) for women contained a large proportion of individuals with suboptimal iron status from the general population, the lower limits may be set too low (Rushton et al. 2001). Therefore, the universal cut-points established in the iron deficiency definition section above, are generally used for athlete populations.

With the aforementioned guidelines in mind, IDA as defined by Dubnov and Constantini (2004) (ferritin <12 μg/L, transferrin saturation <16%) was observed among 7% of elite Basketball players (n=103, 64% male). Of the IDA athletes, 3% were male and 14% were female. These findings highlight the frequency of poor iron status in athletic populations, which may be related to a combination of factors including: (i) the aforementioned exercise-induced mechanisms; (ii) inadequate dietary iron and/or energy intake; (iii) an influence of sex hormones on iron metabolism; and (iv) menses. For example, in regularly menstruating women, ~30-50 mL of blood is lost during menses (Dasharathy et al. 2012), with ~40 mL of blood resulting in an average loss of 1.6 mg of iron (Jacob and Blanche, 1965). Of concern, consecutive menstrual blood loss of greater than 60 mL can compromise iron stores (Andrade et al. 1991). Given the differences in sex hormones between males and females, this section will focus on the interaction between sex hormones, hepcidin and iron parameters that may contribute towards impaired iron metabolism.

The predominant circulating gonadal sex steroid hormones after puberty are androgens in males and oestrogens in females (Sims and Heather 2018). Both testosterone and oestrogen can influence iron metabolism via their suppressive effects on the hepcidin-ferroportin axis (Bachman et al. 2010; Bachman et al. 2013; Hou et al. 2012; Yang et al. 2012). For athletes, it is possible that high training loads may alter an individual's hormonal profile (Warren and Perlroth 2001), thereby suppressing gonadotrophin-releasing hormone (GnRH); a precursor for sex hormones. In women, this can lead to suppressed luteinising hormone (LH), follicle stimulating hormone (FSH; to a lesser extent) and consequently oestrogen. Such events likely contribute to secondary amenorrhea, which has previously been reported in recreational runners (De Souza et al. 1998).

Few studies to date have investigated the specific effects of sex hormones such as oestrogen, progesterone and testosterone on hepcidin and iron status in exercising populations.

Of these hormones, only the role of testosterone is well-established in erythropoiesis. For example, in young strength-trained healthy males (n=12), weekly testosterone injections (600 mg) resulted in a ~10% increase in Hb concentration over 20 weeks (Bhasin et al. 2001). Potential mechanisms including erythropoietin (EPO) secretion and the stimulation of erythroid progenitor cells have been proposed (Moriyama and Fisher 1975). An investigation in males of varying ages (aged 19–35 years, n=53; 59–75 years, n=56) reported that testosterone enanthate supplementation (using a range of doses 25, 50, 125, 300, and 600 mg) over 20 weeks, suppressed basal serum hepcidin concentration by more than 50% (Bachman et al. 2010). In younger men (19-35 years), hepcidin suppression was more pronounced, while appearing dose-dependent in older individuals (59-75 years). The proposed mechanisms for hepcidin down-regulation by testosterone appears multifactorial, including its stimulatory effect on erythropoiesis, modulation of bone morphogenetic protein signalling by the androgen receptor, and alterations in epidermal growth factor receptor signalling (Li et al. 2016; Guo et al. 2013; Latour et al. 2014). Lower circulating (~40%) and production rates of testosterone (~20-30%) resulting from altered hypothalamic-pituitary-testicular axis has been demonstrated in endurance-trained men compared to age-matched sedentary controls (Hackney et al. 2003). Unsurprisingly, endurance exercise completed over extended periods has also been implicated with lower circulating testosterone levels in men (Hackney 1996). Albeit an extreme example of acute endurance exercise, testosterone levels were reduced by ~58% in 38 trained males (aged ~32 years) after completing an Ironman triathlon race (swim 3.8 km, cycle 180 km and run 42.2 km) (Ginsburg et al. 2001). Comparable findings have also been reported after less demanding exercise, as reviewed previously (Hackney 2001). Consequently, chronic suppression of testosterone may be linked to higher hepcidin levels in male athletes, potentially impairing iron regulation, and thereby helping to explain the incidence of ID among this sex. In women, although lower levels of testosterone are present, its importance in iron metabolism

should not be ignored. For example, higher testosterone levels have been associated with lower risk for anemia in both healthy older women (n=509) and men (n=396) (Ferrucci et al. 2006). Of note, the primary female sex hormones oestrogen and progesterone (and their synthetic forms oestradiol and progestogens) have been implicated in hepcidin and iron regulation (Sim et al. 2014).

Currently, evidence exists for oestradiol supplementation to down-regulate hepcidin production (Hou et al. 2012; Yang et al. 2012). Specifically, a link between oestrogen and hepcidin mRNA in mice has been uncovered, where oestrogen deficiency resulted in lower iron stores (in the liver and spleen) and higher transcription of hepcidin mRNA (Hou et al. 2012). Another investigation examined the relationship between oestrogen and hepcidin in women undertaking in-vitro fertilisation treatment who had their oestrogen suppressed using buserelin (GnRH agonist) (Lehtihet et al. 2016). In this study, when a FSH injection was provided to stimulate oestrogen production, hepcidin levels decreased by 40% (median hepcidin: 4.85–1.43 ng/mL). These results indicate that large amounts of endogenous oestrogen can suppress basal hepcidin levels. When examining synthetic sex hormones, positive effects of an oral contraceptive pill (OCP) use on iron status have been reported; as OCP users have higher serum ferritin, iron, total iron binding capacity and lower menstrual blood loss (~50%) compared to non-users (Frassinelli-Gunderson et al. 1985; Larsson et al. 1992). Evidence also exists that after commencing with OCP use, ferritin increases (~21-29%) in women with poor iron stores (ferritin <10 µg/L) (Larsson et al. 1992).

Although ID is often studied in female athlete populations, the interaction of the aforementioned sex hormones with hepcidin and iron metabolism remain unclear. For example, besides an inverse link between progesterone and IL-6 (a primary mediator of hepcidin) (Angstwurm et al. 1997), relatively little is known about the potential role of progesterone (and its synthetic form progestogens) on iron metabolism. To our knowledge, only one study has

examined the role of oestradiol in combination with progestogens for their acute effects on hepcidin and iron metabolism in exercising young women (Sim et al. 2015). In that study, 10 active females on OCP performed two separate 40 min run trials at 75% of the velocity attained at peak oxygen uptake ($v\dot{V}O_{2peak}$) during specific phases of the oral contraceptive cycle (OCC): (a) Day 2–4, representing a hormone-free withdrawal period (D-0); (b) Day 12–14, representing the end of the first week of active hormone therapy (D+7). Exercise performed during the different phases (D-0 vs. D+7) did not alter exercise-induced IL-6 or hepcidin production. Specifically, serum hepcidin concentration was significantly elevated 3 h post-exercise in both trials. Of interest, serum iron remained significantly elevated 3 h post-exercise as compared to baseline only during D−0 (not D+7). Of note, higher oestradiol levels are hypothesised to reduce oxidative stress (Stirone et al. 2005), which is known to exacerbate hemolysis (indicated by serum iron), thereby likely explaining why serum iron levels return to baseline by 3 h post-exercise at D+7, but not D−0.

Similar basal serum iron, hepcidin, IL-6 and transferrin saturation levels have also been reported during different phases of an OCC (e.g. D-0 vs. D+7) in young active women (Sim et al. 2017). Only serum ferritin was significantly higher at D+7 compared to D-0 (69.4 vs. 61.1 µg/L), suggesting that a combination of oestradiol and/or progestogens from the OCP can influence iron stores. Considering the high prevalence of ID in female athletes and active women, numerous questions remain regarding the potential interaction between oestrogen, progesterone and iron metabolism. Since favourable links between oestradiol (or oestrogen) and iron regulation exist, future studies should examine both the acute post-exercise hepcidin response and its longitudinal influence on markers of iron status in OCP users vs non-users (including different phases of the menstrual cycle). This work should also consider the influence that sex hormones might have on menstrual blood loss and oxidative stress-induced hemolysis, and the implications for exercise-induced ID.

**What is the impact of diet on the iron status of athletes?**

The current recommended dietary intake (RDI) for elemental iron is 8 mg for males and 18 mg for females (Trumbo et al. 2001), with the higher intake in women attributed to the iron losses associated with menses (McClung 2012). However, given the aforementioned mechanisms of iron loss that occur as a result of exercise, it is likely that athletes have a higher iron requirement than the general population. For instance, despite consuming iron at the RDI (13-18 mg/day), a block of intensified training was shown to reduce ferritin concentrations by 25-40% in a group of international-level endurance athletes (McKay et al. 2018). This suggests that the current iron recommendations for the 'general population' may not be sufficient for athletes, supporting the case for athlete-specific recommendations to be developed (Thomas et al. 2016).

Developing appropriate RDIs for athletes is important, since iron cannot be synthesised by the body and is attained solely from dietary sources, primarily in the form of wholegrain cereals, fish, poultry and meat. For reference, a comprehensive list of the iron content for various foods can be found on the United States Department of Agriculture Food Composition Database (USDA, 2015), while a condensed list of iron rich food has been prepared by the British Dietetic Association (Gill, 2017). The timing, amount and source of dietary iron, in combination with the overall iron composition of the diet are all important factors for practitioners to consider, since they collectively influence total iron absorption. For instance, it is well-known that heme iron sources (from meat) exhibit greater absorptive capacity (~5-35%) than non-heme sources (~2-20%) from a single meal (Beard and Tobin 2000). Furthermore, the presence of various dietary components such vitamin C, meat, poultry and fish can enhance non-heme iron absorption, whereas substances such as polyphenols, phytates, or calcium, that are part of tea, coffee, whole grains, legumes and dairy products, can decrease

the amount of non-heme iron absorbed from a given meal (Saunders et al. 2013). With this in mind, it is clear that athletes should be working with trained dietitians and nutrition experts when planning their meal composition to optimise dietary iron absorption.

Notwithstanding the dietary composition, it might also be considered that the aforementioned transient increases in hepcidin activity may require strategic thinking relevant to the timing of post-exercise iron intake for athletes, with impaired iron absorption likely to occur during the post-exercise window. However, to add another layer of complexity, hepcidin activity also shows a strong diurnal variation, with the lowest levels observed in the morning, and a steady increase reported throughout the day (Schaap et al. 2013). Therefore, it may be that optimal iron ingestion should occur as far away from exercise as possible, in an attempt to maximise its absorption, with a preference for the morning, compared to evening consumption. However, the impact of exercise, coupled with typically higher breakfast calcium intake, and the diurnal variation of hepcidin presents a logistical challenge for the elite athlete population, who often complete multiple training sessions on each day, and likely consume their highest iron containing meals in the evening, when hepcidin levels are naturally elevated. As a result, literature is lacking when it comes to the appropriate timing of iron consumption for the elite athlete population. Interestingly, recent work from our laboratory (McCormick et al., 2019) indicates a potential open window of opportunity if the iron is consumed within 60 min of completing exercise in the morning, likely a result of the iron reaching the gut within the 3-hour period before transient hepcidin elevations take effect. However, further work is required to fully elucidate the prospects of such a window, and whether this strategy is applicable to elite-level athletes with full training schedules.

**What is the influence of contemporary nutritional strategies on iron regulation?**

Recent interest in iron metabolism has focused on nutritional strategies where select

exercise sessions are deliberately undertaken with low muscle glycogen stores, with the primary goal of increasing transcriptional activation of enzymes involved in carbohydrate (CHO) and fat oxidation, as well as greater mitochondrial biogenesis (Burke et al. 2018; Impey et al. 2018). While these strategies may promote endurance adaptation, they may also interfere with post-exercise iron metabolism. Given IL-6's role as an energy sensor for contracting muscle, this explains augmented post-exercise IL-6 concentrations when training under conditions of low CHO availability (Hennigar et al. 2017). Furthermore, as IL-6 is a key cytokine involved in the up-regulation of hepcidin levels post-exercise, training with low muscle glycogen stores may also amplify hepcidin levels following exercise. Such events potentially prolong the post-exercise period of impaired iron absorption and metabolism.

Investigations of acute CHO supplementation have found minimal influence on iron-regulation (Dahlquist et al. 2017; Robson-Ansley et al. 2011; Sim et al. 2012), however, it appears diet-training strategies that target depleting muscle glycogen stores may have a greater impact. For instance, Badenhorst et al. (2015) demonstrated both IL-6 and hepcidin concentrations were elevated in response to exercise after 24 h of a low (3 g/kg), compared to high CHO (8 g/kg) diet. Interestingly, when this diet was extended across 7 days, no differences in iron-regulation were evident (Badenhorst et al. 2016). However, the programing of a rest day prior to the exercise test on day 7, combined with the increased protein consumption in the low CHO condition likely minimised any differences in muscle glycogen stores between dietary conditions. Further research in this area, using elite athletes adhering to a low CHO high fat (LCHF) diet for 3 weeks (<50 g CHO / day), observed increased IL-6 and hepcidin levels (3 h post-exercise) following a 2 h bout of race walking, compared to athletes consuming a CHO-rich diet (McKay et al. 2018). However, differences in the baseline iron status of the athletes in this investigation may have been a confounding factor when interpreting changes to hepcidin concentrations (McKay et al. 2019; Peeling et al. 2014). In light of these recent

findings, it is evident that the macronutrient composition of an athletes' diet may impact on post-exercise iron metabolism. As a result, we currently suggest that the use of nutritional approaches that restrict CHO should be carefully considered, particularly for athletes with increased iron requirements (e.g. growth, altitude, females or endurance athletes) or those consuming low dietary iron intakes (e.g. vegetarians, weight category athletes). Finally, a greater understanding of the relationship between the dietary macronutrient composition, current iron status, inflammatory responses to exercise, and subsequent hepcidin activity is warranted to further our understanding of the true influence of contemporary nutritional strategies on iron metabolism.

**Is there an impact of overall energy availability?**

Low energy availability occurs in athletes when a mismatch between energy intake and energy expenditure is evident (Loucks et al. 2011). RED-S is an umbrella term used to describe the health and performance consequences associated with LEA in athletes. Of note, ID is an associated haematological outcome of RED-S (Mountjoy et al. 2018), with a retrospective questionnaire-based investigation of 1000 female athletes reporting that LEA was associated with greater risk (64%) for hematologic dysfunction (characterised by a history of anaemia, low Hb, iron or ferritin) (Ackerman et al. 2018). Recent work supports these findings, with significantly higher $Hb_{mass}$ (7.9%) recorded in eumenorrheic (n=22) versus amenorrheic (n=13) elite females runners; indicating the importance of long-term energy availability for relevant health outcomes (Heikura et al. 2018). One explanation is that athletes with LEA are in energy deficit and are not reaching the RDI for dietary iron. A typical Western diet is shown to provide ~6 mg of iron per ~4200 kJ (Beard and Tobin 2000). Therefore, reduced energy intake may result in low dietary iron consumption, subsequently having negative implications for an athlete's iron status. Additionally, many negative health outcomes associated with RED-S can

be exacerbated by ID. For example, low iron stores are known to perturb thyroid function, decrease appetite and impair metabolic efficiency; alterations of which can lead to reduced energy intake, increased energy expenditure and potentially contribute to LEA in athletes (Petkus et al. 2017). Therefore, it appears that ID can be a negative consequence of LEA; however, ID itself may also amplify some of the other undesirable outcomes (e.g. weakness and extreme fatigue) associated with RED-S.

With this in mind, studies directly linking LEA and ID are lacking, and further research is required to understand this relationship. Perhaps, low iron stores may be an early indication of LEA and supplementation should be considered to minimise other health consequences associated with RED-S. However, first and foremost, targeted nutrition counselling focusing on adequate energy and nutrient intake should be emphasised to correct energy availability. Notably, future work examining the implications of RED-S and/or LEA on hepcidin expression and iron metabolism is warranted. Such work could provide direct evidence for the mechanistic interaction between RED-S and ID. Finally, since the physiology of elite athletes, in combination with high training loads, make such populations unique, the inclusion of healthy recreationally active controls as part of this work should be considered.

**What are the strategies to address an iron deficiency?**

Given the increased iron demand placed on an athlete, an adequate exogenous iron supply is imperative to maintaining appropriate iron levels. With the inability to endogenously replace taxed iron stores, supplemental sources of iron are an important consideration for athletes and their support team. When faced with an ID, there are three primary strategies for iron supplementation (Castell et al. 2018); these include (a) increasing dietary iron intake, (b) supplemental oral iron, or (c) parenteral iron administration. In determining the most appropriate strategy, the athlete and support team must first consider the severity of the ID, the

typical individual response to a given supplement preparation, the time required for iron repletion via the strategy chosen (generally in context of the training phase), and of course, any legalities of supplement choice in respect to the sport's governing body and anti-doping authority. Furthermore, it is important to ascertain and treat any potential causes of ID (e.g. underlying pathophysiology), as this could facilitate the efficacy of iron supplementation and prevent future reoccurrence.

The initial and most conservative strategy to approaching an ID is for a full dietary assessment by a qualified sports dietitian, with a subsequent eating plan that focuses on increasing dietary iron intake from food. Considering the aforementioned superior absorption of heme compared to non-heme iron, vegetarian athletes presenting with compromised iron stores are a more complex case when approaching the issue via this strategy. An additional complexity when considering diet as a primary means to address an ID are the concurrent absorption enhancers and/or inhibitors that may be consumed with the iron source (Saunders et al. 2013). As such, careful planning is required when manipulating the diet to increase iron absorption, and factors other than total iron intake must be considered.

The second strategy to addressing compromised iron stores requires the use of oral iron supplements, commonly consumed as either tablet or liquid preparations. Oral iron supplements are generally provided in the ferrous form (fumarate, sulphate or gluconate), however, ferric preparations are also available; although the GI tolerance for ferric iron supplements appears low (Hoffman et al. 2000). Of note, in a comparative review of ferrous versus ferric oral iron formulations (Santiago 2012), slow-release ferrous sulphate preparations remained the established and standard treatment of ID, resulting from their acceptable tolerability, adequate bioavailability and overall efficacy of effect. However, it should be noted that a generally high incidence of GI-disturbance from oral iron supplementation is commonly

reported (Tolkien et al. 2015); and in such cases, consideration of iron polymaltose preparations or using enteric tablet coating may be helpful.

Generally, the overall response to oral iron supplementation in athlete cohorts appears positive (40-80% increases to ferritin) when consumed over an 8-12 week time frame, utilising doses of ~100 mg per day (Garvican et al. 2014; Dawson et al. 2006). However, alternate day supplementation may increase the efficacy of effect via an improvement in the fractional absorption of iron from a given dose, which over time, results in a greater cumulative response (Stoffel et al. 2017). Such regimens, in combination with iron absorption enhancers such as vitamin-C, should be considered. Furthermore, under extreme environmental stress (e.g., exposure to altitude), a greater dose of oral iron may be required to sustain iron stores and assist in the haematological adaptation (as discussed in detail below).

The final method of addressing an iron deficiency comes in the form of parenteral iron preparations via intramuscular or intravenous (IV) administration. Previous literature has shown both approaches to be very effective at improving an athlete's iron status, with 200-400% increases in ferritin levels reported from 300-550 mg of iron delivered over a 1 to 42-day period (Dawson et al. 2006; Garvican et al. 2014; Woods et al. 2014; Burden et al. 2015b). Modern advances in IV preparations (Macdougall, 2009) have increased the safety and accessibility of IV supplementation, with injections or infusions commonly delivered in an out-patient setting. As such, IV administration has largely supersceded intra-muscular administration that is commonly associated with soreness and staining around the injecion site. The clear benefit of an IV supplement strategy is the speed and magnitude of the response, without GI upset, in comparison to the aforementioned approaches of dietary modification or oral supplementation. This is likely a result of such administration methods by-passing the gut where key issues of iron absorption exist. With this in mind, parenteral supplement methods may be of great importance when a situation requires rapid improvement in iron stores, or when

gut issues appear to render more traditional methods of iron therapy impractical and ineffective. Regardless, the use of these adminstration methods do come with some risks of adverse reactions, which may manifest as a mild rash through to anaphlyaxis (in very rare cases; Rampton et al. 2014).

Also worth considering is the concept of maximising iron stores through supplementation during periods of lower activity (e.g. off-season). Inevitably, as training load and iron demands increase during the competitive season, higher iron reserves may limit the negative influence that exercise training has on the bioavailability of iron. In conclusion, the decision to undertake, and the supplement administration strategy should be made by the sports physician, in consultation with the support team (i.e. dietician). Furthermore, in deciding to pursue this avenue, care should be taken to ensure that the method of supplement delivery is acceptable and consistent with the sport's governing body and the anti-doping authorities.

**Does iron play a significant role in adaptation to hypoxic environments?**

Prolonged (>2 week) exposure to low (1000–2000 m) and moderate (2,000–3000 m) simulated or terrestrial altitudes (Bärtsch et al. 2008) is associated with several hematological (increased $Hb_{mass}$) and non-hematological adaptations (increased iron-dependent oxidative and glycolytic enzyme concentrations) that assist aerobic exercise performance (see Hahn and Gore, 2001) for a comprehensive review). The magnitude of the change in $Hb_{mass}$ during live high, train low (LHTL) altitude training depends upon the hypoxic dose (defined in kilometre hours (km/h) = (m/1,000) × h), and is characterised by an exponential increase, followed by an eventual plateau after ~4 to 5 weeks (Garvican-Lewis et al. 2016b). Although large intra- and inter-individual variability exists in the $Hb_{mass}$ response (Siebenmann et al. 2015), hypoxic exposure of 1000 km/h (e.g. 21 days at 2,000m) is associated with a ~3-4% increase in $Hb_{mass}$. Of note, altitude training places a large demand on an athlete's iron stores, since, in addition to

the aforementioned ~1-2 mg iron/day required to replenish exercise-related iron losses (Nielsen and Nachtigall 1998), altitude exposure increases erythroid iron demand by 3- to 5-fold (Reynafarje et al. 1959). Therefore, low iron availability during prolonged altitude exposure may blunt hematological adaptations (Stray-Gundersen et al. 1992; Garvican-Lewis et al. 2018), in turn decreasing the potential performance benefits that may be gained from altitude exposure.

**How is iron metabolism regulated in hypoxia?**

Oxygen sensing and the regulation of iron metabolism during altitude exposure are linked by three distinct isoforms of hypoxia-inducible factor (HIF-1α, HIF-2α and HIF-3α), with the HIF-2α isoform stimulating EPO production (Kapitsinou et al. 2010). Continuous hypoxic exposure causes plasma EPO to increase within ~90 min (Rodríguez et al. 2000), peaking usually within 48 hours and returning to baseline levels (or below) after one week (Garvican et al. 2012). EPO principally acts upon receptors located on erythroid progenitor cells in the bone marrow, stimulating the differentiation of proerythroblasts into basophilic, polychromatic and orthochromatic forms. Iron supply to differentiating erythroid cells is met almost entirely by diferric transferrin (Leimberg et al. 2008). Proerythroblasts then enucleate to form reticulocytes, which are expelled into the blood plasma as mature red blood cells. These adaptations typically manifest as measurable changes in $Hb_{mass}$ after approximately 10 days (Garvican et al. 2012).

Several changes in systemic iron metabolism occur to support accelerated erythropoiesis in hypoxia, including increased iron efflux from storage sites (reticuloendothelial macrophages, hepatocytes and enterocytes), increased iron transport to the erythron, and increased intestinal iron absorption (see Chepelev and Willmore (2011) for review). Hypoxic stress has been shown to strongly inhibit liver hepcidin production within 15

h of exposure (Ravasi et al. 2018), thereby promoting iron release from (predominantly) reticuloendothelial macrophages in the bone marrow to support erythropoiesis. Hepcidin suppression in hypoxia is not directly mediated by EPO (Canali et al. 2016); instead, the hormone erythroferrone (ERFE) is released by proerythroblasts and acts to suppress hepcidin expression (Kautz et al. 2014). Simultaneously, during early hypoxic exposure, several growth factors in the bone marrow (but not the spleen or liver) may also play a role in hypoxic hepcidin suppression (Ravasi et al. 2018). However, since these growth factors and ERFE are down-regulated within 48 h, alternative mechanisms have been suggested to regulate the later phases of hepcidin suppression in hypoxia (Ravasi et al. 2018). These mechanisms include erythroid iron demand as indicated by the current plasma transferrin receptor-2 (TfR-2) concentration. TfR-2 is highly expressed by erythroblasts and involved in sensing plasma diferric transferrin concentration, in turn modulating EPO receptor signalling. In addition to hepcidin suppression, hypoxic stabilisation of HIF-2α up-regulates divalent metal transporter 1 (an iron import protein; DMT-1) and duodenal cytochrome b (a ferric reductase; DCytB) expression on duodenal enterocytes, thereby increasing intestinal iron absorption (Goetze et al. 2013) from ~15-20% in normoxia to ~20-25% in hypoxia (Reynafarje et al. 1959). Additionally, hypoxic stabilisation of HIF-1α promotes increased iron transport to the erythron by increasing TfR expression within ~16-40 h of hypoxic exposure (Piperno et al. 2010).

**Are there specific iron supplementation strategies at altitude for an optimised outcome?**

Athletes need sufficient iron stores to support accelerated erythropoiesis and to replenish exercise-related iron losses associated with daily training during prolonged altitude exposure. Athletes typically receive oral iron supplements before and during altitude exposure to maintain iron balance (Garvican-Lewis et al. 2018). However, despite several different serum ferritin cut-off values being used to guide iron supplementation during altitude exposure

(Constantini et al. 2017; Garvican-Lewis et al. 2018; Hauser et al. 2018), the optimal iron supplementation strategy, including timing, dose and administration route (i.e. oral *versus* intravenous) to maximise hematological adaptations is still emerging.

The decision to provide iron supplementation to athletes undertaking altitude exposure should be made by a sports physician following a review of their pre-exposure blood profile. Blood screening should be performed three-to six weeks before exposure, and should include a full blood count (but preferably $Hb_{mass}$ via the carbon monoxide-rebreathing method, since it is not affected by the plasma volume shifts that occur at altitude), iron profile (including serum iron, serum ferritin and transferrin saturation) and C-reactive protein assessment (reflecting systemic inflammation over the previous ~24-48 h). This enables the size of the red cell compartment, iron storage pool and the presence of inflammation, to be quantified, respectively. Based upon their pre-exposure serum ferritin concentration, athletes are usually prescribed iron supplements to avoid the development of an ID and to support erythropoiesis (Govus et al. 2015). On a cautionary note, pre-exposure serum ferritin concentration appears to be a weak predictor of $Hb_{mass}$ changes at altitude in individuals with otherwise healthy iron stores (Hauser et al. 2018; Ryan et al. 2014). Other factors, such as the rate of iron efflux from bone marrow and splenic macrophages, in addition to the rate of iron transport to the erythron (indicated by the transferrin saturation) during early adaptation, likely exert a greater influence on the resulting haematological adaptation. In practical terms, the adequacy of an athlete's iron storage pool for a prolonged altitude sojourn likely depends both on the overall hypoxic dose (Garvican-Lewis et al. 2016b) (with larger hypoxic doses, such as longer duration and higher elevations requiring more iron owing to the potentially greater hematological response), the volume of training performed, and the preparation time available.

Of note, oral iron supplements remain the most common form of iron used for altitude training, and are typically administered daily for two to six weeks before, and throughout

exposure, in order to ensure that athletes can maintain a sufficient iron balance over time. In a retrospective analysis of athletes undertaking simulated LHTL altitude training, $Hb_{mass}$ was improved even in athletes with low pre-exposure iron stores (considered by the authors as serum ferritin: <35 μg/L) who were supplemented with oral iron supplements for two weeks before, and for the duration of the altitude exposure (Govus et al. 2015). In fact, athletes with a pre-exposure serum ferritin concentration <20 μg/L, who were supplemented with 210 mg elemental iron/day, not only increased their $Hb_{mass}$ (+4.0%), but also increased their iron stores, indicating that the oral iron dose exceeded bone marrow iron uptake. As such, hypoxic-mediated changes in iron metabolism during altitude exposure (e.g. suppression of hepcidin, increased iron absorption, transport and the mobilisation of iron from storage sites) may improve the efficacy of oral iron supplementation; assisting in the hematological adaptation to the environmental stress.

The timing and dose of oral iron supplements during prolonged altitude exposure may affect intestinal iron absorption and subsequent incorporation by the erythron, in turn affecting haematological adaptations to prolonged altitude exposure. For example, Hall et al. (2018) found that a single nightly dose of oral iron supplement (200 mg elemental iron/d delivered nightly), rather than a split dose of oral iron supplement (2 × 100 mg elemental iron/day delivered in the morning and evening, respectively), was associated with a higher $Hb_{mass}$ response in athletes exposed to 2106 m terrestrial altitude for ~3 weeks. Of note, both doses allowed athletes to maintain or increase their pre-exposure serum ferritin concentration. However, GI discomfort, a common side effect of oral iron supplements, was higher in the single compared with the split dose group; decreasing by the third week of exposure. As such, a single dose of oral iron supplement may be superior to a split dose (of the same concentration) during prolonged altitude exposure to maximise $Hb_{mass}$ adaptations.

In contrast to oral iron supplements, IV iron preparations allow athletes to rapidly increase their iron stores and are not associated with GI discomfort, instead delivering iron directly into the splenic macrophages (Girelli et al. 2018). We recently reported that both IV iron supplementation and oral iron supplementation augment $\Delta Hb_{mass}$ (%) in endurance-trained athletes who undertook 3 weeks of simulated LHTL altitude training (Garvican-Lewis et al. 2018). Notably, the $\Delta Hb_{mass}$ (%) was not greater than the coefficient of variation for the CO rebreathing method (1.5%) in the non-iron supplemented group. Of note, $Hb_{mass}$ decay was less rapid post-altitude exposure in the IV group as compared to the oral group. Therefore, IV iron supplementation may be useful to prepare athletes for prolonged sojourns at moderate altitudes (~2000-3000 m) when athletes have little time to adequately prepare for the altitude exposure. However, given the greater logistical (i.e. medical assistance required), financial (IV iron is more expensive than oral iron) and ethical concerns (some sports have a "no needle policy") associated with IV iron supplements, oral iron supplementation should be considered as the first choice iron supplementation strategy to prepare athletes for prolonged altitude sojourns.

**Summary**

This review summarises evidence regarding the regulation and increased demands of iron in athletic populations. We have covered key topics related to athletes' iron regulation including (i) the effects and severity of ID on performance; (ii) sex and the implications of sex hormones on iron parameters; (iii) diet, including macronutrient manipulation and RED-S; and finally, (v) the demands and influence of hypoxia as part of altitude exposure. Collectively, we also highlight the multifactorial causes of exercise-induced ID and prevention strategies (e.g. dietary intake or supplements), and provide suggestions for future work and practical information that should be considered by athletes and their support staff (sports dietitians, physiologists and physicians). We propose that the iron status of athletes are monitored closely

throughout the training year, and that particular attention should be paid in situations linked with increased iron loss and/or demands; such as chronically high training loads and environmental factors (altitude), respectively. Although the influence of IDNA on athletic performance remains unclear, early detection of low iron stores and subsequent supplementation may reverse (or limit) further declines to iron status. As detailed in *Figure 1*, we propose that blood screening should be performed regularly for athletes, while also considering a diverse range of circumstances. On a positive note, substantial progress has been made in understanding the role of iron, mechanisms of its regulation, and the strategies used to correct a deficiency/optimise adaptation; however, continued research is required to further our ability to reduce the burden of an iron deficit in athlete populations.

**Conflicts of interest**

The authors have no conflicts of interest to disclose.

**References**

Ackerman KE, Holtzman B, Cooper KM, Flynn EF, Bruinvels G, Tenforde AS, Popp KL, Simpkin AJ, Parziale AL (2018) Low energy availability surrogates correlate with health and performance consequences of Relative Energy Deficiency in Sport. Br J Sports Med:bjsports-2017-098958

Andrade AT Souza JP Shaw ST Jr Belsey EM Rowe PJ . Menstrual blood loss and body iron stores in Brazilian women. Contraception . 1991. 43:241–249.

Angstwurm MWA, Gärtner R, Ziegler-Heitbrock HWL (1997) Cyclic plasma IL-6 levels during normal menstrual cycle. Cytokine 9 (5):370-374

Armstrong LE, Pumerantz AC, Fiala KA, Roti MW, Kavouras SA, Casa DJ, Maresh CM (2010) Human hydration indices: acute and longitudinal reference values. IInt J Sport Nutr Exer Metab 20 (2):145-153

Bachman E, Feng R, Travison T, Li M, Olbina G, Ostland V, Ulloor J, Zhang A, Basaria S, Ganz T (2010) Testosterone suppresses hepcidin in men: a potential mechanism for testosterone-induced erythrocytosis. J Clinl Endocrinol Metab 95 (10):4743-4747

Bachman E, Travison TG, Basaria S, Davda MN, Guo W, Li M, Connor Westfall J, Bae H, Gordeuk V, Bhasin S (2013) Testosterone induces erythrocytosis via increased erythropoietin and suppressed hepcidin: evidence for a new erythropoietin/hemoglobin set point. J Gerontol Series A: Biomed Sciences Med Sciences 69 (6):725-735

Badenhorst C, Dawson B, Cox G, Laarakkers C, Swinkels D, Peeling P (2015) Acute dietary carbohydrate manipulation and the subsequent inflammatory and hepcidin responses to exercise. Eur J App Physiol 115 (12):2521-2530.

Badenhorst CE, Dawson B, Cox GR, Sim M, Laarakkers CM, Swinkels DW, Peeling P (2016) Seven days of high carbohydrate ingestion does not attenuate post-exercise IL-6 and hepcidin levels. Eur J App Physiol 116 (9):1715-1724

Bartsch P, Mairbaurl H, Friedmann B (1998) Pseudo-anemia caused by sports. Ther Umsch 55 (4):251-255

Bartsch P, Saltin B, Dvorak J (2008) Consensus statement on playing football at different altitude. Scand J Med Sci Sports 18:96-99

Beard J, Tobin B (2000) Iron status and exercise. Am J Clin Nutr 72 (2):594S-597S

Beard JL (2001) Iron biology in immune function, muscle metabolism and neuronal functioning. J Nutr 131 (2):568S-579S

Bhasin S, Woodhouse L, Casaburi R, Singh AB, Bhasin D, Berman N, Chen X, Yarasheski KE, Magliano L, Dzekov C (2001) Testosterone dose-response relationships in healthy young men. Am J Physiol-Endocrinol Metab 281 (6):E1172-E1181

Burden RJ, Morton K, Richards T, Whyte GP, Pedlar CR (2015a) Is iron treatment beneficial in, iron-deficient but non-anaemic (IDNA) endurance athletes? A systematic review and meta-analysis. Br J Sports Med 49 (21):1389-1397

Burden RJ, Pollock N, Whyte GP, Richards T, Moore B, Busbridge M, Srai SK, Otto J, Pedlar CR (2015b) Effect of intravenous iron on aerobic capacity and iron metabolism in elite athletes. Med Sci Sports Exer 47 (7):1399-1407

Burke LM, Hawley JA, Jeukendrup A, Morton JP, Stellingwerff T, Maughan RJ (2018) Toward a common understanding of diet-exercise strategies to manipulate fuel availability for training and competition preparation in endurance sport. Int J sport Nutr Exer Metab 28 (5):451-463

Canali S, Vecchi C, Garuti C, Montosi G, Babitt JL, Pietrangelo A (2016) The SMAD pathway is required for hepcidin response during endoplasmic reticulum stress. Endocrinology 157 (10):3935-3945

Castell LM, Nieman DC, Bermon S, Peeling P (2018) Exercise-induced illness and inflammation: can immunonutrition and iron help? Int J sport Nutr Exer Metab (in press).

Chepelev NL, Willmore WG (2011) Regulation of iron pathways in response to hypoxia. Free Radic Biol Med 50 (6):645-666

Clenin G, Cordes M, Huber A, Schumacher YO, Noack P, Scales J, Kriemler S (2015) Iron deficiency in sports-definition, influence on performance and therapy. Swiss Med Wkly 145:w14196

Constantini K, Wilhite DP, Chapman RF (2017) A clinician guide to altitude training for optimal endurance exercise performance at sea level. High Alt Med Biol 18 (2):93-101

Dahlquist DT, Stellingwerff T, Dieter BP, McKenzie DC, Koehle MS (2017) Effects of macro- and micronutrients on exercise-induced hepcidin response in highly trained endurance athletes. App Physiol Nutr Metab 42 (10):1036-1043

Dasharathy SS, Mumford SL, Pollack AZ, Perkins NJ, Mattison DR, Wactawski-Wende J, Schisterman EF (2012) Menstrual bleeding patterns among regularly menstruating women. Am J Epidemiol 175 (6):536-545

Dawson B, Goodman C, Blee T, Claydon G, Peeling P, Beilby J, Prins A (2006) Iron supplementation: oral tablets versus intramuscular injection. Int J Sport Nutr Exer Metab 16 (2):180-186

De Souza MJ, Miller BE, Loucks AB, Luciano AA, Pescatello LS, Campbell CG, Lasley BL (1998) High frequency of luteal phase deficiency and anovulation in recreational women runners: blunted elevation in follicle-stimulating hormone observed during luteal-follicular transition. J Clin Endocrinol Metab 83 (12):4220-4232

DellaValle DM, Haas JD (2011) Impact of iron depletion without anemia on performance in trained endurance athletes at the beginning of a training season: a study of female collegiate rowers. Int J Sport Nutr Exer Metab 21 (6):501-506

Dellavalle DM, Haas JD (2014) Iron supplementation improves energetic efficiency in iron-depleted female rowers. Med Sci Sports Exer 46 (6):1204-1215

Dubnov G, Constantini NW (2004) Prevalence of iron depletion and anemia in top-level basketball players. Int J Sport Nutr Exer Metab 14 (1):30-37

Fallon KE (2004) Utility of hematological and iron-related screening in elite athletes. Clin J Sport Med 14 (3):145-152

Fallon KE (2008) Screening for haematological and iron-related abnormalities in elite athletes-analysis of 576 cases. J Sci Med Sport 11 (3):329-336.

Ferrucci L, Maggio M, Bandinelli S, Basaria S, Lauretani F, Ble A, Valenti G, Ershler WB, Guralnik JM, Longo DL (2006) Low testosterone levels and the risk of anemia in older men and women. Arch Int Med 166 (13):1380-1388

Frassinelli-Gunderson EP, Margen S, Brown JR (1985) Iron stores in users of oral contraceptive agents. Am J Clin Nutr 41 (4):703-712

Gardner GW, Edgerton VR, Senewiratne B, Barnard RJ, Ohira Y (1977) Physical work capacity and metabolic stress in subjects with iron deficiency anemia. Am J Clin Nutr 30 (6):910-917

Garvican-Lewis LA, Govus AD, Peeling P, Abbiss CR, Gore CJ (2016a) Iron supplementation and altitude: decision making using a regression tree. J Sports Sci Med 15 (1):204-205

Garvican-Lewis LA, Sharpe K, Gore CJ (2016b) Time for a new metric for hypoxic dose? Am J Physiol-Heart Circ Physiol 121 (1):352-355

Garvican-Lewis LA, Vuong VL, Govus AD, Peeling P, Jung G, Nemeth E, Hughes D, Lovell G, Eichner D, Gore CJ (2018) Intravenous iron does not augment the hemoglobin mass response to simulated hypoxia. Med Sci Sports Exer 50 (8):1669-1678

Garvican LA, Martin D, Quod M, Stephens B, Sassi A, Gore C (2012) Time course of the hemoglobin mass response to natural altitude training in elite endurance cyclists. Scand J Med Sci Sports 22 (1):95-103

Garvican LA, Saunders PU, Cardoso T, Macdougall IC, Lobigs LM, Fazakerley R, Fallon KE, Anderson B, Anson JM, Thompson KG (2014) Intravenous iron supplementation in distance runners with low or suboptimal ferritin. Medicine and science in sports and exercise 46 (2):376-385

Garvican LA, Lobigs L, Telford R, Fallon K, Gore CJ (2011) Haemoglobin mass in an anaemic female endurance runner before and after iron supplementation. Int J Sports Physiol Perform 6 (1):137-140

Garvican LA, Saunders PU, Cardoso T, Macdougall IC, Lobigs LM, Fazakerley R, Fallon KE, Anderson B, Anson JM, Thompson KG, Gore CJ (2014) Intravenous iron supplementation in distance runners with low or suboptimal ferritin. Med Sci Sports Exer 46 (2):376-385

Gill S (2017) British Dietitics Association food fact sheet for iron. Retrived from: https://www.bda.uk.com/foodfacts/iron_food_fact_sheet.pdf.

Ginsburg GS, O'Toole M, Rimm E, Douglas PS, Rifai N (2001) Gender differences in exercise-induced changes in sex hormone levels and lipid peroxidation in athletes participating in the Hawaii Ironman triathlon: Ginsburg-gender and exercise-induced lipid peroxidation. Clinica Chimica Acta 305 (1-2):131-139

Girelli D, Ugolini S, Busti F, Marchi G, Castagna A (2018) Modern iron replacement therapy: clinical and pathophysiological insights. Int J Hematol 107 (1):16-30

Goetze O, Schmitt J, Spliethoff K, Theurl I, Weiss G, Swinkels DW, Tjalsma H, Maggiorini M, Krayenbuhl P, Rau M (2013) Adaptation of iron transport and metabolism to acute high altitude hypoxia in mountaineers. Hepatology 58 (6):2153-2162

Govus AD, Garvican-Lewis LA, Abbiss CR, Peeling P, Gore CJ (2015) Pre-altitude serum ferritin levels and daily oral iron supplement dose mediate iron parameter and hemoglobin mass responses to altitude exposure. PLoS One 10 (8):e0135120

Guo W, Bachman E, Li M, Roy CN, Blusztajn J, Wong S, Chan SY, Serra C, Jasuja R, Travison TG (2013) Testosterone administration inhibits hepcidin transcription and is associated with increased iron incorporation into red blood cells. Aging Cell 12 (2):280-291

Haas JD, Brownlie T (2001) Iron deficiency and reduced work capacity: a critical review of the research to determine a causal relationship. J Nutr 131 (2):676S-690S

Hackney AC (1996) The male reproductive system and endurance exercise. Med Sci Sports Exer 28 (2):180-189

Hackney AC (2001) Endurance exercise training and reproductive endocrine dysfunction in men alterations in the hypothalamic-pituitary-testicular axis. Curr Pharmaceu Design 7 (4):261-273

Hackney A, Szczepanowska E, Viru A (2003) Basal testicular testosterone production in endurance-trained men is suppressed. Eur J Appl Physiol 89 (2):198-201

Hahn AG, Gore CJ (2001) The effect of altitude on cycling performance: a challenge to traditional concepts. Sports Med 31 (7):533-557

Hall R, Peeling P, Nemeth E, Bergland D, McCluskey WT, Stellingwerff T (2018) Single versus split dose of iron optimizes haemoglobin mass gains at 2106 m altitude. Med Sci Sports Exerc (in press)Hauser A, Troesch S, Steiner T, Brocherie F, Girard O, Saugy JJ, Schmitt L, Millet GP, Wehrlin JP (2018) Do male athletes with already high initial haemoglobin mass benefit from 'live high–train low'altitude training? Exp Physiol 103 (1):68-76

Heikura IA, Uusitalo AL, Stellingwerff T, Bergland D, Mero AA, Burke LM (2018) Low energy availability is difficult to assess but outcomes have large impact on bone injury rates in elite distance athletes. In J Sport Nutr Exer Metab 28 (4):403-411

Hennigar SR, McClung JP, Pasiakos SM (2017) Nutritional interventions and the IL-6 response to exercise. FASEB J 31 (9):3719-3728

Hoffman R, Benz E, Silberstein L, Heslop H, Weitz J, Anastasi J (2000) Disorders of iron metabolism: iron deficiency and overload. In: Hematology: basic principles and practice. Harcourt Brace & Co, NY: Churchill Livingstone

Hou Y, Zhang S, Wang L, Li J, Qu G, He J, Rong H, Ji H, Liu S (2012) Estrogen regulates iron homeostasis through governing hepatic hepcidin expression via an estrogen response element. Gene 511 (2):398-403

Houston BL, Hurrie D, Graham J, Perija B, Rimmer E, Rabbani R, Bernstein CN, Turgeon AF, Fergusson DA, Houston DS (2018) Efficacy of iron supplementation on fatigue and physical capacity in non-anaemic iron-deficient adults: a systematic review of randomised controlled trials. BMJ Open 8:e019240

Impey SG, Hearris MA, Hammond KM, Bartlett JD, Louis J, Close GL, Morton JP (2018) Fuel for the work required: A theoretical framework for carbohydrate periodization and the glycogen threshold hypothesis. Sports Medicine 48 (5):1031-1048

Jacob A Butler E Blanche M . Menstrual blood loss in iron deficiency anemia. Lancet . 1965; 407:1102–1107.

Kapitsinou PP, Liu Q, Unger TL, Rha J, Davidoff O, Keith B, Epstein JA, Moores SL, Erickson-Miller CL, Haase VH (2010) Hepatic HIF-2 regulates erythropoietic responses to hypoxia in renal anemia. Blood 116 (16):3039-3048

Kautz L, Jung G, Valore EV, Rivella S, Nemeth E, Ganz T (2014) Identification of erythroferrone as an erythroid regulator of iron metabolism. Nat Genet 46 (7):678-687

Koehler K, Braun H, Achtzehn S, Hildebrand U, Predel H-G, Mester J, Schänzer W (2012) Iron status in elite young athletes: gender-dependent influences of diet and exercise. Eur J Appl Physiol 112 (2):513-523

Landahl G, Adolfsson P, Börjesson M, Mannheimer C, Rödjer S (2005) Iron deficiency and anemia: a common problem in female elite soccer players. Int J Sport Nutr Exer Metab 15(6):689-694

Larsson G, Milsom L, Lindstedt G, Rybo G (1992) The influence of a low-dose combined oral contraceptive on menstrual blood loss and iron status. Contraception 46 (4):327-334

Latour C, Kautz L, Besson-Fournier C, Island ML, Canonne-Hergaux F, Loréal O, Ganz T, Coppin H, Roth MP (2014) Testosterone perturbs systemic iron balance through activation of epidermal growth factor receptor signaling in the liver and repression of hepcidin. Hepatology 59 (2):683-694

Lehtihet M, Bonde Y, Beckman L, Berinder K, Hoybye C, Rudling M, Sloan JH, Konrad RJ, Angelin B (2016) Circulating hepcidin-25 is reduced by endogenous estrogen in humans. PLoS One 11 (2):e0148802

Leimberg MJ, Prus E, Konijn AM, Fibach E (2008) Macrophages function as a ferritin iron source for cultured human erythroid precursors. J Cell Biochem 103 (4):1211-1218

Li X, Rhee DK, Malhotra R, Mayeur C, Hurst LA, Ager E, Shelton G, Kramer Y, McCulloh D, Keefe D (2016) Progesterone receptor membrane component-1 regulates hepcidin biosynthesis. J Clin Invest 126 (1):389-401

Looker AC, Dallman PR, Carroll MD, Gunter EW, Johnson CL (1997) Prevalence of iron deficiency in the United States. JAMA 277 (12):973-976

Loucks AB, Kiens B, Wright HH (2011) Energy availability in athletes. J Sports Science 29 (1):S7-S15

Macdougall IC (2009) Evolution of iv iron compounds over the last century. J Renal Care 35:8-13

Malczewska J, Szczepańska B, Stupnicki R, Sendecki W (2001) The assessment of frequency of iron deficiency in athletes from the transferrin receptor-ferritin index. Int J Sports Nutr Exer Metab 11 (1):42-52

McClung JP (2012) Iron status and the female athlete. J Trace Elem Med Biol 26 (2-3):124-126

McCormick R, Moretti D, McKay AKA, Laarakkers CM, van Swelm R, Trinder D, Cox GR, Zimmerman MB, Sim M, Goodman C, Dawson B, Peeling P (2019) The impact of morning versus afternoon exercise on iron absorption in athletes. Med Science Sport Exer (in-press).

McKay AK, Peeling P, Pyne DB, Welvaert M, Tee N, Leckey JJ, Sharma AP, Ross ML, Garvican-Lewis LA, Swinkels DW (2018) Chronic adherence to a ketogenic diet modifies iron metabolism in elite athletes. Med Science Sports Exer (in press)

McKay AK, Peeling P, Pyne DB, Welvaert M, Tee N, Leckey JJ, Sharma AP, Ross ML, Garvican-Lewis LA, van Swelm RP (2019) Acute carbohydrate ingestion does not

influence the post-exercise iron-regulatory response in elite keto-adapted race walkers. J Science Med Sport (in press)

Moriyama Y, Fisher JW (1975) Effects of testosterone and erythropoietin on erythroid colony formation in human bone marrow cultures. Blood 45 (5):665-670

Mountjoy M, Sundgot-Borgen J, Burke L, Ackerman KE, Blauwet C, Constantini N, Lebrun C, Lundy B, Melin A, Meyer N (2018) International Olympic Committee (IOC) consensus statement on relative energy deficiency in sport (RED-S): 2018 update. Int J sport Nutr Exer Metab 28 (4):316-331

Mountjoy M, Sundgot-Borgen J, Burke L, Carter S, Constantini N, Lebrun C, Meyer N, Sherman R, Steffen K, Budgett R (2014) The IOC consensus statement: beyond the female athlete triad— relative energy deficiency in sport (RED-S). Br J Sports Med 48 (7):491-497

Myhre KE, Webber BJ, Cropper TL, Tchandja JN, Ahrendt DM, Dillon CA, Haas RW, Guy SL, Pawlak MT, Federinko SP (2016) Prevalence and impact of anemia on basic trainees in the US air force. Sports Med-Open 2 (1):23

Newlin MK, Williams S, McNamara T, Tjalsma H, Swinkels DW, Haymes EM (2012) The effects of acute exercise bouts on hepcidin in women. Int J sport Nutr Exer Metab 22 (2):79-88

Nielsen P, Nachtigall D (1998) Iron supplementation in athletes. Sports Med 26 (4):207-216

Pasricha SR, Flecknoe-Brown SC, Allen KJ, Gibson PR, McMahon LP, Olynyk JK, Roger SD, Savoia HF, Tampi R, Thomson AR, Wood EM, Robinson KL (2010) Diagnosis and management of iron deficiency anaemia: a clinical update. Med J Aust 193 (9):525-532

Patterson AJ, Brown WJ, Roberts DC (2001) Dietary and supplement treatment of iron deficiency results in improvements in general health and fatigue in Australian women of childbearing age. J Am Coll Nutr 20 (4):337-342

Peake J, Nosaka KK, Suzuki K (2005) Characterization of inflammatory responses to eccentric exercise in humans. Exer Immunol Rev11, 64-85

Pedlar CR, Brugnara C, Bruinvels G, Burden R (2018) Iron balance and iron supplementation for the female athlete: A practical approach. Eur J Sport Science 18 (2):295-305

Peeling P, Blee T, Goodman C, Dawson B, Claydon G, Beilby J, Prins A (2007) Effect of iron injections on aerobic-exercise performance of iron-depleted female athletes. Int J sport Nutr Exer Metab 17 (3):221-231

Peeling P, Dawson B, Goodman C, Landers G, Trinder D (2008) Athletic induced iron deficiency: new insights into the role of inflammation, cytokines and hormones. Eur J Appl Physiol 103 (4):381

Peeling P, McKay AK, Pyne DB, Guelfi KJ, McCormick RH, Laarakkers CM, Swinkels DW, Garvican-Lewis LA, Ross ML, Sharma AP (2017) Factors influencing the post-exercise hepcidin-25 response in elite athletes. Eur J Appl Physiol 117 (6):1233-1239

Peeling P, Sim M, Badenhorst CE, Dawson B, Govus AD, Abbiss CR, Swinkels DW, Trinder D (2014) Iron status and the acute post-exercise hepcidin response in athletes. PLoS One 9 (3):e93002.

Petkus DL, Murray-Kolb LE, De Souza MJ (2017) The unexplored crossroads of the female athlete triad and iron deficiency: a narrative review. Sports Med 47 (9):1721-1737

Piperno A, Galimberti S, Mariani R, Pelucchi S, Ravasi G, Lombardi C, Bilo G, Revera M, Giuliano A, Faini A, Mainini V, Westerman M, Ganz T, Valsecchi MG, Mancia G, Parati G (2010) Modulation of hepcidin production during hypoxia-induced erythropoiesis in humans in vivo: data from the HIGHCARE project. Blood 117 (10):2953-2959

Powell PD, Tucker A (1991) Iron supplementation and running performance in female cross-country runners. Int J Sports Med 12 (05):462-467

Rampton D, Folkersen J, Fishbane S, Hedenus M, Howaldt S, Locatelli F, Patni S, Szebeni J, Weiss G (2014) Hypersensitivity reactions to intravenous iron: guidance for risk minimization and management. Haematologica 99 (11):1671-1676

Ravasi G, Pelucchi S, Buoli Comani G, Greni F, Mariani R, Pelloni I, Bombelli S, Perego R, Barisani D, Piperno A (2018) Hepcidin regulation in a mouse model of acute hypoxia. Eur J Haematol 100 (6):636-643

Reynafarje C, Lozano R, Valdivieso J (1959) The polycythemia of high altitudes: iron metabolism and related aspects. Blood 14 (4):433-455

Robson-Ansley P, Walshe I, Ward D (2011) The effect of carbohydrate ingestion on plasma interleukin-6, hepcidin and iron concentrations following prolonged exercise. Cytokine 53 (2):196-200

Rodriguez FA, Ventura JL, Casas M, Casas H, Pages T, Rama R, Ricart A, Palacios L, Viscor G (2000) Erythropoietin acute reaction and haematological adaptations to short, intermittent hypobaric hypoxia. Eur J Appl Physiol 82 (3):170-177

Roecker L, Meier-Buttermilch R, Brechtel L, Nemeth E, Ganz T (2005) Iron-regulatory protein hepcidin is increased in female athletes after a marathon. Eur J Appl Physiol 95 (5-6):569-571

Rowland T (2012) Iron deficiency in athletes: an update. Am J Lifestyle Med 6 (4):319-327

Rubeor A, Goojha C, Manning J, White J (2018) Does iron supplementation improve performance in iron-deficient nonanemic athletes? Sports Health 10 (5) 400-405

Rushton DH, Dover R, Sainsbury AW, Norris MJ, Gilkes JJ, Ramsay ID (2001) Why should women have lower reference limits for haemoglobin and ferritin concentrations than men? BMJ 322 (7298):1355-1357

Ryan BJ, Wachsmuth NB, Schmidt W, Byrnes WC, Julian CG, Lovering AT, Subudhi AW, Roach RC (2014) AltitudeOmics: rapid hemoglobin mass alterations with early

acclimatization to and de-acclimatization from 5260 m in healthy humans. PLoS One 9 (10):e108788

Santiago P (2012) Ferrous versus ferric oral iron formulations for the treatment of iron deficiency: a clinical overview. Sci World J 2012:846824

Saunders AV, Craig WJ, Baines SK, Posen JS (2013) Iron and vegetarian diets. Med J Aust 199 (4):11-16

Schaap CC, Hendriks JC, Kortman GA, Klaver SM, Kroot JJ, Laarakkers CM, Wiegerinck ET, Tjalsma H, Janssen MC, Swinkels DW (2013) Diurnal rhythm rather than dietary iron mediates daily hepcidin variations. Clin Chem 59 (3):527-535

Schmidt W, Prommer N (2005) The optimised CO-rebreathing method: a new tool to determine total haemoglobin mass routinely. Eur J Appl Physiol 95 (5-6):486-495

Siebenmann C, Cathomen A, Hug M, Keiser S, Lundby AK, Hilty MP, Goetze JP, Rasmussen P, Lundby C (2015) Hemoglobin mass and intravascular volume kinetics during and after exposure to 3,454-m altitude. J Appl Physiol 119 (10):1194-1201

Sim M, Dawson B, Landers G, Swinkels DW, Tjalsma H, Yeap BB, Trinder D, Peeling P (2015) Oral contraception does not alter typical post-exercise interleukin-6 and hepcidin levels in females. J Sci Med Sport 18 (1):8-12

Sim M, Dawson B, Landers G, Swinkels DW, Wiegerinck E, Yeap BB, Trinder D, Peeling P (2017) Interleukin-6 and hepcidin levels during hormone-deplete and hormone-replete phases of an oral contraceptive cycle: A pilot study. Ann Nutr Metab 70 (2):100-105

Sim M, Dawson B, Landers G, Trinder D, Peeling P (2014) Iron regulation in athletes: exploring the menstrual cycle and effects of different exercise modalities on hepcidin production. Int J Sport Nutr Exerc Metab 24 (2):177-187

Sim M, Dawson B, Landers G, Wiegerinck ET, Swinkels DW, Townsend M-A, Trinder D, Peeling P (2012) The effects of carbohydrate ingestion during endurance running on post-exercise inflammation and hepcidin levels. Eur J Appl Physiol 112 (5):1889-1898

Sim M, Dawson B, Peeling P, Trinder D. (2013). Encyclopedia of exercise medicine in health and disease. In F. C. Mooren (1st Ed.), Sports Anemia. Springer, New York, USA.

Sims ST, Heather AK (2018) Myths and Methodologies: Reducing scientific design ambiguity in studies comparing sexes and/or menstrual cycle phases. Exp Physiol 103 (10):1309-1317

Sinclair LM, Hinton PS (2005) Prevalence of iron deficiency with and without anemia in recreationally active men and women. J Am Diet Assoc 105 (6):975-978

Stirone C, Duckles SP, Krause DN, Procaccio V (2005) Estrogen increases mitochondrial efficiency and reduces oxidative stress in cerebral blood vessels. Mol Pharmacol 68 (4) 959-965

Stoffel NU, Cercamondi CI, Brittenham G, Zeder C, Geurts-Moespot AJ, Swinkels DW, Moretti D, Zimmermann MB (2017) Iron absorption from oral iron supplements given on consecutive versus alternate days and as single morning doses versus twice-daily split dosing in iron-depleted women: two open-label, randomised controlled trials. Lancet Haematol 4 (11):e524-e533

Stray-Gundersen J, Alexander C, Hochstein A, deLemos D, Levine BD (1992) Failure of red cell volume to increase to altitude exposure in iron deficient runners. Med Sci Sports Exer 24 (5):S90

Tan D, Dawson B, Peeling P (2012) Hemolytic effects of a football-specific training session in elite female players. Int J Sports Physiol Perform 7(3):271-6.

Taylor NA (2011) Human heat adaptation. Comprehen Physiol4 (1):325-365

Telford RD, Sly GJ, Hahn AG, Cunningham RB, Bryant C, Smith JA (2003) Footstrike is the major cause of hemolysis during running. J Appl Physiol 94(1):38-42

Theodorou AA, Nikolaidis MG, Paschalis V, Sakellariou GK, Fatouros IG, Koutedakis Y, Jamurtas AZ (2010) Comparison between glucose-6-phosphate dehydrogenase-deficient and normal individuals after eccentric exercise. Med Science Sport Exer 42(6):1113-1121

Thomas DT, Erdman KA, Burke LM (2016) Position of the academy of nutrition and dietetics, dietitians of canada, and the american college of sports medicine: nutrition and athletic performance. J Acad Nutr Diet 116 (3):501-528

Tolkien Z, Stecher L, Mander AP, Pereira DI, Powell JJ (2015) Ferrous sulfate supplementation causes significant gastrointestinal side-effects in adults: a systematic review and meta-analysis. PLoS One 10 (2):e0117383

Trumbo P, Yates AA, Schlicker S, Poos M (2001) Dietary reference intakes: vitamin A, vitamin K, arsenic, boron, chromium, copper, iodine, iron, manganese, molybdenum, nickel, silicon, vanadium, and zinc. J Am Diet Assoc 101 (3):294-301

Tsalis G, Nikolaidis MG, Mougios V (2004) Effects of iron intake through food or supplement on iron status and performance of healthy adolescent swimmers during a training season. Int J Sports Med 25 (04):306-313

United States Department of Agriculture, Agricultural Research Service Food Composition Databases for Iron (2015). Standard reference release 28, United States of America.

Venderley AM, Campbell WW (2006) Vegetarian diets. Sports Med 36 (4):293-305

Voss S, Alsayrafi M, Bourdon P, Klodt F, Nonis D, Hopkins W, Schumacher Y (2014) Variability of serum markers of erythropoiesis during 6 days of racing in highly trained cyclists. Int J sports Med 35 (02):89-94Warren MP, Perlroth NE (2001) The effects of intense exercise on the female reproductive system. J Endocrinol 170 (1):3-11

Woods A, Garvican-Lewis LA, Saunders PU, Lovell G, Hughes D, Fazakerley R, Anderson B, Gore CJ, Thompson KG (2014) Four weeks of IV iron supplementation reduces perceived fatigue and mood disturbance in distance runners. PLoS One 9 (9):e108042

Woodson RD, Wills RE, Lenfant C (1978) Effect of acute and established anemia on $O_2$ transport at rest, submaximal and maximal work. J Appl Physiol Respir Environ Exerc Physiol 44 (1):36-43

Yang Q, Jian J, Katz S, Abramson SB, Huang X (2012) 17β-Estradiol inhibits iron hormone hepcidin through an estrogen responsive element half-site. Endocrinology 153 (7):3170-3178

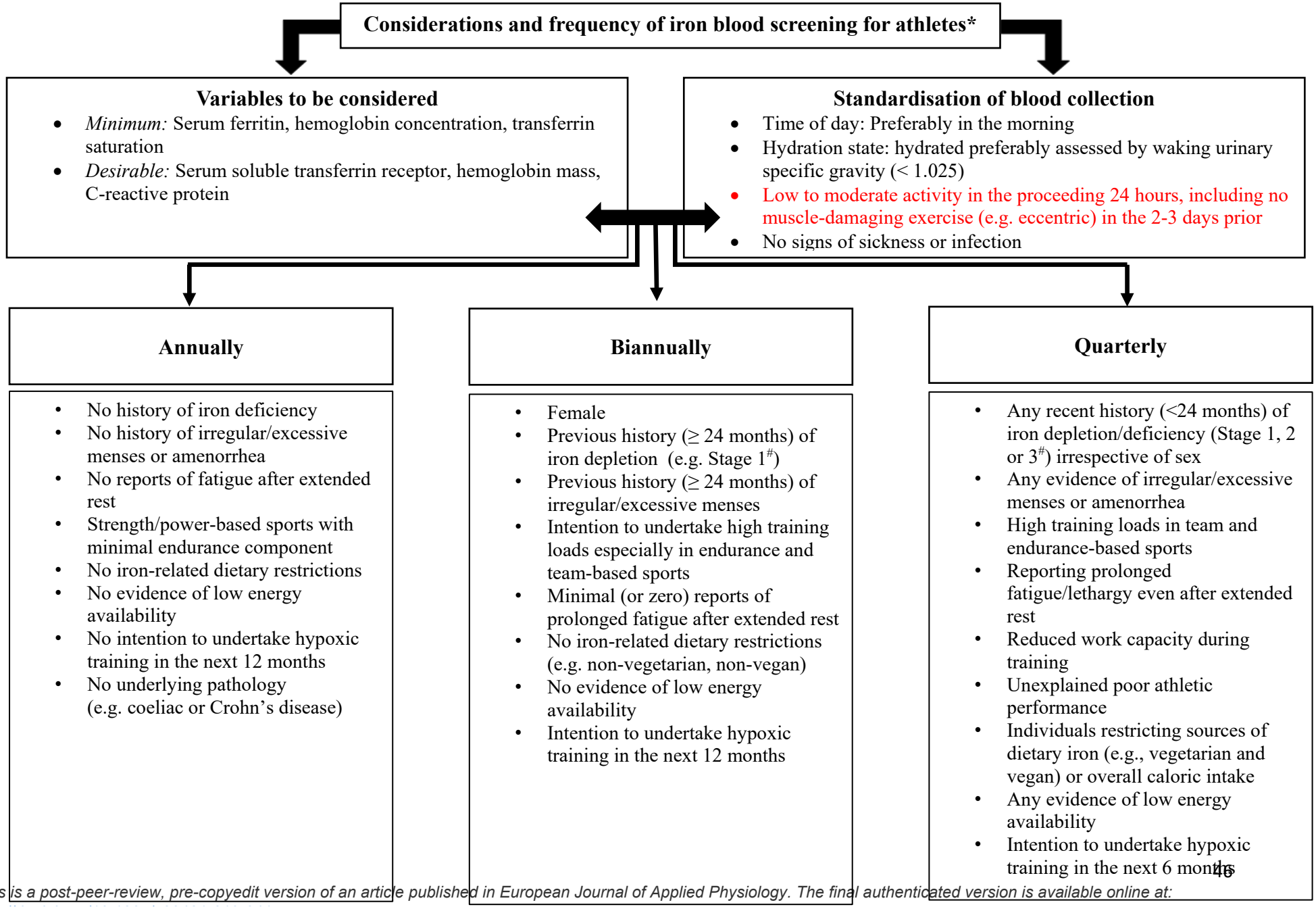






**Figure 1.** Framework of considerations for the frequency of iron blood screening in athlete populations. *This framework requires the expertise of trained professionals including sports physicians, dietitians and physiologists. #Stages of iron deficiency are defined by Peeling et al. (2007).