Factors Influencing Iron Metabolism in Female and Male Athletes

Cumulative Dissertation

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List of Publications

This cumulative dissertation is based on the following four publications:

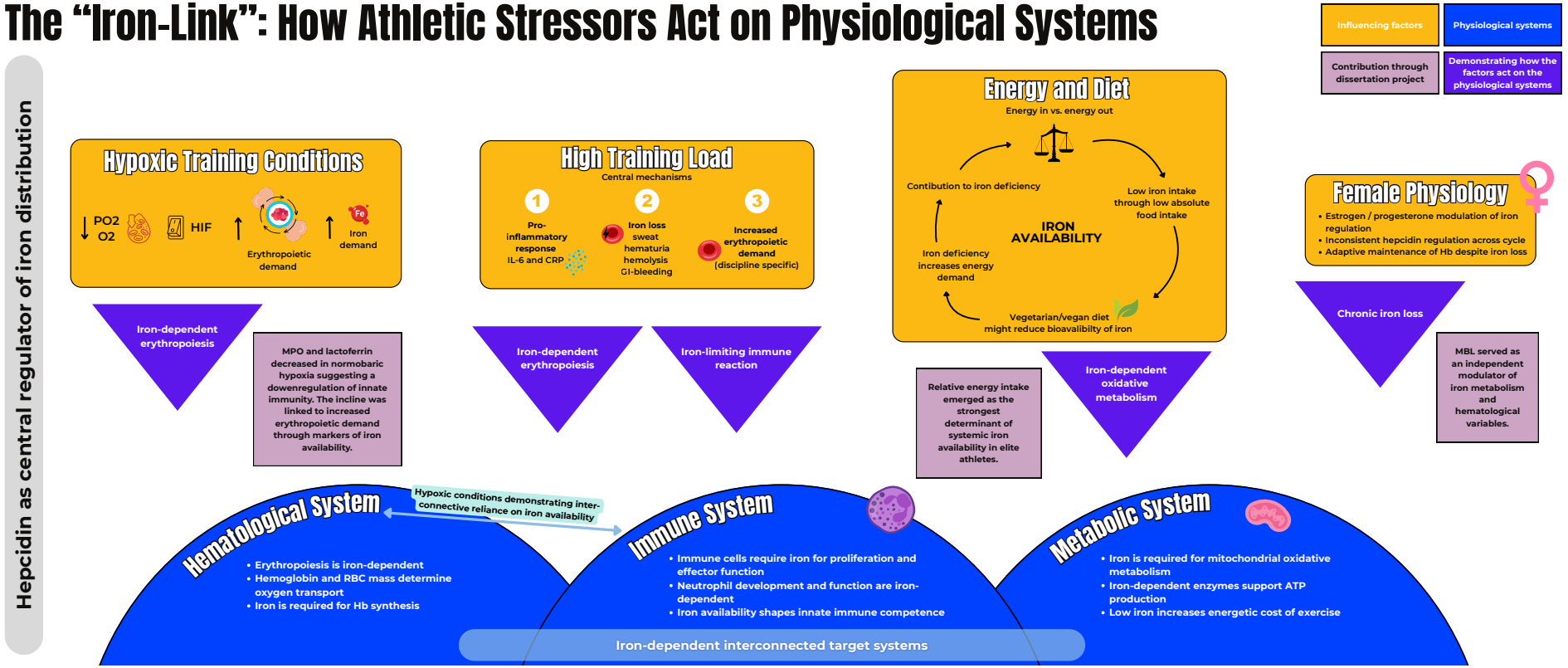
Year	Titel	Status	Authorts	Role
2024	Approaches to Prevent Iron Deficiency in Athletes	Published in Deutsche Zeitschrift für Sportmedizin (https://doi.org/10.5960/dzsm.2024.607)	**Nolte, S.**, Krüger, K., Hollander, K. & Carlsohn, A.	First author
2025	Training in normobaric hypoxia induces hematological changes that affect iron metabolism and immunity	Published in Scientific Reports (https://doi.org/10.1038/s41598-025-01542-w)	**Nolte, S.**, Malhan, D., Klemmer, A., Kastner, T., Walter, N., Fleckenstein, D., Keck, J., Klügel, S., Maier, C., Gebhardt, K., Stauber, T., Relógio, A., Krüger, K., & Hollander, K.	First author
2025	Menstrual blood loss as an initial trigger for adaptation of iron metabolism in eumenorrheic female athletes - An exploratory study	Published in Physiological Reports (https://doi.org/10.14814/phy2.70522)	**Nolte, S.**, Maier, C., Klügel, S., Weyh, C., Hacker, S., Badenhorst, C., & Krüger, K.	First author

2025	Relative Energy Intake as a Determinant of Iron Status in Elite Athletes	Pre-Print in ResearchSquare	**Nolte, S.,** Lenz, C., Fink, AK., Klügel, S., Hacker, S., Krüger, K.	Shared first author

Conference contributions related to the topic of the dissertation:

Year	Titel	Conference	Type of presentation
2025	Menstrual blood loss as an initial trigger for iron metabolism adaptation in female athletes.	27th Congress of the German Association of Sport Science (dvs), Münster	Oral Presentation
2025	FeMaLe Physiology - 'Low Hanging Fruits' in Gesundheit und Leistung?	FeMaLe Research Award – Bundesinstitut für Sportwissenschaft (BISp), Leipzig.	Oral Presentation & Poster
2024	Interaction of erythropoiesis, iron metabolism, and innate immunity in athletes during an altitude training camp under artificial hypoxic conditions	4th Young Investigators Symposium of the German Society of Sports Medicine and Prevention (DGSP), Freiburg.	Poster
2024	Interaction of erythropoiesis, iron metabolism and innate immunity in athletes training at artificial altitude.	16th Symposium of The International Society of Exercise and Immunology, Vienna.	Oral Presentation

Graphical Abstract



Conceptual graphical abstract of the cumulative dissertation. The figure illustrates iron that is shared between the hematological, immune and metabolic system. In athletes, specific influencing factors such as hypoxic exposure, menstrual blood loss, training load, and altered energy availability act as system stressors that challenge iron homeostasis. Through these stressors, the iron dependency of the individual systems visualizes, revealing how alterations in iron regulation spread across interconnected physiological areas. The schematic focuses solely on system interactions mediated by iron and does not aim to represent the full spectrum of physiological interconnections beyond iron-dependent pathways.

Abstract

“Athletes are at risk for iron deficiency” has long been the dominant narrative in sport science when bringing iron and athletic performance together. This perspective is not unsupported, prevalence data speak for themselves, but it seems to shrink a highly complex physiology into a single outcome. Iron metabolism represents a tightly regulated network that underlies complex regulatory mechanisms. Its involvement spreads across multiple physiological systems, including the hematological and immune system, energy metabolism and oxygen transport. This large involvement creates a platform that increases its susceptibility to influencing factors. Athletes with their extraordinary lifestyle place unique stress on iron homeostasis, often have elevated iron requirements and may therefore be particularly susceptible to iron deficiency. High training loads, exposure to hypoxia, altered energy availability, and sex-specific factors are only a few factors placing substantial influence on iron balance. Therefore, this dissertation project set out to investigate these interacting dynamics, rather than treating iron deficiency as an isolated endpoint. The aim was to advance the mechanistic understanding of iron regulation, and to inform evidence-based practical guidelines. To address this aim, the dissertation integrates evidence from a narrative review and multiple original empirical studies conducted in athletic populations.

The project opened with a narrative review synthesizing current knowledge iron-related challenges and practical prevention methods for iron deficiency in athletes. This background guided three original studies on distinct but interrelated stressors of iron homeostasis. Under controlled normobaric hypoxia, athletes demonstrated increased erythropoietic iron demand alongside alterations in immune-related markers, highlighting competition for iron between physiological systems. Menstrual blood loss appeared as a recurrent iron stressor in female athletes, showing that despite chronically lower ferritin concentrations, hematological function and oxygen transport capacity were maintained, indicative of adaptive iron redistribution. Finally relative energy availability was identified as a key determinant of systemic iron status in elite athletes, linking metabolic strain to reduced iron stores independent of dietary iron intake.

Taken together, this cumulative work places iron within the framework of network physiology. Iron emerges not as an isolated hematological variable, but as a dynamic mediator within an interconnected system of adaptations, in which influencing factors force a regulated symmetry under sustained demand. This perspective provides a conceptual basis for a broader yet more specific monitoring and management strategies that account systemic interactions.

List of Abbreviations

ATP	Adenosine Triphosphate
CHO	Carbohydrates
CRP	C-reactive protein
EA	Energy availability
EPO	Erythropoietin
ERFE	Erythroferrone
GF	Growth factor
GLUT	Glucose transporter
HAMP	Hepcidin antimicrobial peptide
HIF	Hypoxia Induced Factor
IDA	Iron-deficiency anemia
IDNA	Iron-deficiency non-anemia
IGF	Insulin-like growth factor
IL-6	Interleukin-6
LCHF	Low carbohydrate high fat
LEA	Low energy availability
LPS	Lipopolysaccharide
MBL	Menstrual blood loss
MCT	Monocarboxylate
OCP	Oral contraceptive pill
RBC	Red blood cell
sTfR	Soluble transferrin receptor
TfR1	Transferrin receptor 1
TNF-α	Tumor necrosis factor-alpha
TSAT	Transferrin saturation
VEGF	Vascular endothelial growth factor

Table of Content

1 Introduction – Scientific Background

1.1 Iron in Human Physiology

Iron is the fourth most abundant element and the second most abundant metal in the Earth's crust [1]. Its relevance, however, extends far beyond geology. Iron emerged shortly after the formation of the Universe and rapidly became embedded in the chemical reactions that underpin early biochemistry, giving it a foundational role in the evolution of life itself [2]. Because iron's utility antedates the rise of atmospheric oxygen, organisms have had to develop strategies to harness its benefits while protecting themselves from the potentially harmful interaction between iron and oxygen in biological systems [3].

Life across nearly all domains relies on metal cofactors, including iron, zinc, manganese, copper, and molybdenum [4]. Among these, iron holds a unique position. With the notable exceptions of Borrelia burgdorferi and certain lactobacilli, virtually all organisms utilize iron for structural functions, electron transfer, and a variety of enzymatic processes essential for cellular metabolism [5], [6]. Its capacity to participate in rapid redox cycling and to bind gaseous ligands has made it indispensable for biological energy production, respiration, and numerous metabolic pathways [3].

Despite its cosmic and biological abundance, iron presents a paradox in human physiology. It is the trace element with the highest prevalence of deficiency worldwide [7]. This discrepancy highlights the complex relationship between environmental availability and physiological accessibility. Under physiological conditions, iron's solubility is extremely limited, reducing its bioavailability [8], [9]. To ensure both adequate supply and protection against its potential toxicity, the human body maintains strict control over iron absorption, transport, storage, and recycling [10], [11].

1.1.1 Iron Metabolism

Following its entry into the body, iron is handled through a tightly coordinated network of absorption, cellular uptake, recycling, and hormonal regulation. All of which ensure sufficient availability for essential metabolic functions while preventing the toxicity associated with unbound iron. Under physiological conditions, humans absorb approximately 2 mg of dietary iron per day, an amount that generally matches the unavoidable basal losses through the shedding of epithelial cells, sweat, and minor bleeding [12]. Because the body lacks an active excretory pathway for iron, systemic balance is achieved almost entirely through the modulation of intestinal absorption. This process is directed by the size of iron stores and the iron requirements of erythropoiesis. Total-body iron levels serve as the primary determinant of absorptive efficiency for erythropoietic demand [13]. To keep these stores on an adequate level iron must be consumed through food. Dietary iron is consumed in two principal forms: heme iron, which is efficiently absorbed, and non-heme iron, which is less bioavailable because it is predominantly present as ferric iron (Fe^{3+}) and therefore requires reduction to the ferrous form (Fe^{2+}) prior to uptake by enterocytes [12].
Once absorbed, iron circulates predominantly bound to transferrin. Only small amounts of non–transferrin-bound iron appear under pathological conditions or when transferrin becomes saturated [14]. Transferrin delivers iron to tissues through binding to transferrin receptor 1 (TfR1), which is widely expressed across cell types. The transferrin-TfR1

complex is internalized by receptor-mediated endocytosis, after which vesicular acidification releases iron from transferrin, enabling its entry into the cytosolic labile iron pool [15].

Humans possess only a few grams of this reactive transition metal. Only 3 to 5 grams of iron can be found in the human body. This amount is distributed unequally across three pools. The first one is the *storage* that takes up to 10-12 mg Fe/kg bodyweight in men and 5 mg Fe/kg bodyweight in women. Iron is stored in the spleen, liver, bone marrow and muscle in either ferritin or hemosiderin. Secondly, the *functional* iron pool (approximately 36 mg Fe/kg bodyweight), which represents the rapidly available iron and is found in the erythrocytes as part of the oxygen transport protein hemoglobin, in the oxygen storage protein myoglobin of the muscles or in iron-containing enzymes [16]. The *storage* and the *functional* pool are connected through the much smaller *transport* pool. Only around 3 mg of total body iron are bound to transferrin and circulate in the blood and other extracellular fluids [17].

The largest proportion of body iron resides within erythrocytes, and their turnover provides a major source of recycled iron. After a lifespan of approximately 120 days, senescent red blood cells are removed by reticuloendothelial macrophages in a process termed erythrophagocytosis [18]. The iron released during hemoglobin degradation can be sequestered in ferritin for temporary storage or exported back into the circulation via the iron efflux transporter ferroportin. This recycling process supplies most of the iron required for ongoing erythropoiesis and contributes substantially to the stability of systemic iron availability [19].

The dynamic movement of iron within the body is tightly regulated because unbound iron can participate in Fenton and Haber-Weiss chemistry, generating reactive oxygen species that damage lipids, proteins, and nucleic acids [20], [21]. Maintaining the balance between iron sufficiency and protection against oxidative stress therefore requires a regulatory framework capable of responding to fluctuations in hypoxia, erythropoietic drive, and inflammatory signaling. Central to this regulatory system are the hormone hepcidin and its target, ferroportin. Hepcidin is a peptide produced in the liver and has been recognized as the main regulator of iron metabolism [22]. Since its discovery in blood [23] and in urine [24] the knowledge of its characteristics has increased rapidly. The hormone is systemically regulated by erythropoiesis, hepatic iron levels and inflammation [25] [26]. Hepcidin controls iron absorption in the gut and iron release from macrophages by binding, internalizing and degrading ferroportin and thereby reducing dietary iron absorption and restricting iron release from macrophages [27], [28], [29]. Ferroportin, the sole known cellular iron exporter that is dominantly expressed on enterocytes, hepatocytes and reticuloendothelial macrophages [30], [31]. Its stability on the cell surface dictates the rate at which iron enters the plasma [32], [33]. Through this system, the body integrates signals of iron status, oxygen demand, and immune activation to maintain iron homeostasis under both physiological and stress conditions [25].

While these mechanisms describe how iron is absorbed, transported, and regulated, their physiological relevance becomes evident only when considering the diverse biological systems that depend on iron availability.

1.1.2 Iron's Roles in the Body

In human physiology, iron fulfills multiple essential roles across several interconnected physiological systems. Rather than acting within a single functional domain, iron supports processes that span across oxygen transport, immune defense, cellular energy metabolism, and endocrine-regulated adaptations. Daily production of 200 billion new erythrocytes requires 20 mg of iron for hemoglobin which corresponds to nearly 80% of the iron demand in humans [34], making it a central player in hematological function.

Beyond its function in the hematological system, iron also plays a key role in immune competence. Immune cells depend on tightly regulated iron uptake to support proliferation, differentiation, and effector functions [35]. Accordingly, reduced cellular iron acquisition can impair immune responses even in the absence of marked anemia. Hypomorphic mutations in TfR1, which lower iron uptake by approximately 50%, cause a combined immunodeficiency with recurrent infections, neutropenia, and reduced T- and B-cell proliferation [36]. At the systemic level, iron availability is dynamically lowered during infection and inflammation via hepcidin-mediated hypoferremia as part of the acute-phase response [37], [38], [39]. While this response serves as host defense by restricting iron access for pathogens, it also illustrates how iron regulation links immune activation with hematopoiesis, contributing to anemia of inflammation [40] and can particularly impact granulopoiesis [41].

In addition to its role in oxygen transport, iron is indispensable for cellular energy metabolism. Numerous enzymes involved in oxidative metabolism depend on iron-containing cofactors, particularly iron-sulphur clusters [42] and heme groups [43], which are essential components of the mitochondrial electron transport chain. Through these functions, iron enables efficient oxidative phosphorylation and adenosine triphosphate (ATP) production, thereby supporting basal metabolic demands across tissues [44]. Disturbances in iron availability can consequently impair metabolic efficiency on the cellular level, potentially preceding detectable alterations in hematological parameters [45].

Iron does not directly regulate endocrine or reproductive function. However, endocrine signaling exerts a substantial influence on iron homeostasis. Sex hormones such as estrogen, progesterone, and testosterone modulate erythropoiesis, iron absorption, and iron distribution, thereby shaping systemic iron balance across the lifespan [46]. Physiological states characterized by cyclical or hormonally driven blood loss, such as menstruation, impose further recurrent demands on iron regulation [47].

The complexity and interdependence of these systems leave iron regulation sensitive to physiological stressors. This vulnerability becomes particularly relevant in populations exposed to repeated or intensified demands, such as athletes.

1.2 Iron in the Athletic Population

Athletes live in a state of continual physiological negotiation, much like a tightrope walker balancing on a narrow line. They operate close to the boundaries of their adaptive capacity, where training stress, recovery, and nutritional status must be carefully aligned to maintain homeostasis [48]. Within this narrow range, the body can achieve remarkable physiological adaptations; beyond it, the system can become vulnerable. This delicate balance creates the scene on understanding why the availability of specific nutrients can become a limiting factor in athletic performance. One of these potentially limiting nutrients is iron.

But why does iron in particular matter in athletic performance? One answer comes swiftly: iron is a key component of hemoglobin and, as such, is essential for the transport of oxygen to working muscles. Yet the physiological functions extend greatly beyond oxygen transport, and many of them take on a more prominent role in athletes. In the context of sustained training exposure, the body undergoes repeated cycles of elevated oxygen flux, mitochondrial remodeling, and increased erythrocyte turnover. These processes collectively increase the organism's reliance on an adequate iron supply but also influence the complex system [49].

1.2.1 Factors Influencing Iron Metabolism in Athletes

It is out of question by now: The exceptional lifestyle of athletes influences iron metabolism and therefore iron homeostasis. In the following chapters different physiological factors that impact iron metabolism in athletes are mechanistically discussed and will be worked out as factors that challenge this physiological system, marking adaptation and disruptions.

Effect of High Training Load

Chronic training regimes are an integral component of an athlete's lifestyle, setting them apart from the general population in both physiological demands and metabolic stress [50]. Strenuous exercise alters iron turnover through two principal pathways that are central to understanding the regulation of iron in athletic individuals.

First, exercise significantly increases iron loss and as the body is lacking the innate ability to replace this loss, a sufficient iron supply is necessary. There are different avenues on how iron is lost during exercise [51], [52], [53]. Mechanical hemolysis is a well-documented consequence of repeated foot strikes during running [54], while shear stress and the compression of blood vessels by contracting skeletal muscle further contribute to erythrocyte destruction [55], which makes the phenomena occur in non-weight bearing exercise as well. During hemolysis the hemoglobin of erythrocytes is released in the tissue [56]. Once released, free hemoglobin can promote oxidative tissue damage. To counteract this, the glycoprotein haptoglobin binds circulating hemoglobin to limit its reactivity [57]. In addition, the redistribution of blood flow during exercise further contributes to iron loss. Activation of the sympathetic nervous system and increased perfusion of skeletal muscle and skin decrease visceral blood flow substantially [58]. Reduced mucosal oxygenation may induce epithelial injury, leading to gastrointestinal micro bleeding and mucosal necrosis. Such gastric lesions have been suggested as a mechanism underlying exercise-related gastrointestinal bleeding [59], arising either from altered hemodynamics or diminished mucosal secretion [60].

Dermal iron loss represents another pathway through which iron is depleted. Exercise-induced sweating contributes to iron loss as part of normal thermoregulatory responses [61]. Although the absolute iron loss through sweat is relatively small, repeated and prolonged training sessions, particularly in hot environments, may cumulatively influence iron status [62].

A further contributor is exercise-induced hematuria. Mechanical and hemolytic stress within the kidneys can cause urinary loss of hemoglobin [52], [63]. In high-impact activities such as running, bladder wall microtrauma may also occur. Additionally

reduced renal blood flow at higher exercise intensities alters filtration dynamics, promoting hematuria [52], [64].

The second principal pathway by which strenuous training affects iron metabolism involves inflammation-driven changes in iron regulation [65].
Inflammation represents a rapid host response to physiological stress, marked by enhanced perfusion, fluid accumulation, and the recruitment of leukocytes together with cytokines and acute-phase proteins [66], [67]. One key biomarker of inflammation is interleukin-6 (IL-6), which is produced by several cell types, including activated monocytes and macrophages [68]. It can exert pro-inflammatory and anti-inflammatory effects [69] by T-cell activation, stimulation of acute-phase-proteins and B-cell differentiation (pro-inflammatory) [70] or inhibiting the pro-inflammatory cytokines like tumor necrosis factor-alpha (TNF-α) and IL-1ß (anti-inflammatory) [71].
In sport context, endurance exercise produces a robust acute-phase-like cytokine response with increases in other cytokines like TNF-α and IL-1ß [72] and a pronounced surge in IL-6 [73], to some extend comparable to trauma or infection [74]. IL-6 release during exercise predominantly originates from the contracting skeletal muscle, which its amount is determined by the mode, intensity and duration of exercise, rather than eccentric muscle damage alone [72], [75]. The marked post-exercise rise in IL-6 may contribute to hepcidin up-regulation, potentially limiting iron efflux from macrophages and reducing intestinal iron absorption [33]. This mechanism is increasingly discussed as a contributor to exercise-associated disturbances in iron homeostasis among athletes.
It has been suggested that with inflammation the rise in hepcidin causes a swift decrease of plasma iron levels, which when prolonged can spiral in iron deficient erythropoiesis and anemia at a later stage [25]. Research on hepcidin has been shaped largely by work in chronic disease, particularly in the context of inflammation-driven anemia. In clinical populations with chronic infections or severe inflammatory disorders, urinary hepcidin concentrations have been reported to rise up to 100-fold [31], highlighting the hormone's sensitivity to inflammatory signaling. Within sport science, IL-6 has similarly been recognized as a key mediator of hepcidin regulation, given its central role in inflammation-induced hepcidin synthesis. In humans, recombinant IL-6 infusion elicits an approximately 7.5-fold increase in urinary hepcidin, whereas IL-6-deficient mice fail to induce hepcidin in response to inflammatory stimuli [25]. Following lipopolysaccharide (LPS) administration, hepcidin peaks about six hours post-injection, roughly three hours after the IL-6 peak, while c-reactive protein (CRP) reaches its maximum much later, around 22 hours, coinciding with substantial declines in serum iron [76]. These kinetics illustrate why exercise-induced inflammation, characterized by a pronounced and rapid IL-6 surge, is increasingly regarded as an important driver of iron-regulatory processes in athletes. More recent work has extended this mechanistic perspective [77], [78], [79], [80], [81]. The meta-analysis of Fensham et al. included 17 studies and aimed to determine athlete and exercise characteristics that significantly influence the postexercise hepcidin response [82]. Although baseline ferritin, as previously proposed [83], remains a predictor of hepcidin elevation, its effect was smaller than anticipated. Evidence points to a ferritin-dependent threshold below which hepcidin expression is suppressed [84], potentially explaining observed sex differences, as many female participants exhibited lower ferritin concentrations (~30 $\mu g \cdot L^{-1}$) and may therefore have shown blunted hepcidin responses. Across studies, the most consistent finding was a doubled hepcidin concentration, corresponding to a 1.5–2.5-fold rise, approximately 3

hours post-exercise. IL-6 typically peaked around 2 hours after exercise and, like hepcidin, its magnitude was influenced by cardiorespiratory fitness level.
Importantly, the work of Fensham et al. also demonstrated that the absolute magnitude of the hepcidin response is shaped by a constellation of factors, including higher pre-exercise ferritin, lower VO_2max, male sex, longer exercise duration, and elevated post-exercise IL-6, highlighting that hepcidin secretion is sensitive to both physiological status and exercise characteristics [82].

Together, these findings reveal that homeostasis in iron metabolism can be interrupted. Whether erythrocytes can be "protected" from exercise-specific damage [85] or if there should be a specific timing for the consumption of iron-rich foods around training [86] has been the subject of early and modern research in this area.

Effects of Diet and High Energy Demands

Nutritional iron deficiency occurs when physiological requirements exceed the amount of iron that can be absorbed from the diet. In mixed diets, approximately one-third of daily iron intake originates from hemoglobin or myoglobin, although heme iron accounts for only 10–15% of total dietary iron content. Plant-based diets, by contrast, rely almost exclusively on non-heme iron, of which only ~10% is absorbed. Diets low in vitamin C or heme iron can therefore exhibit up to a three-fold lower bioavailability compared with diets rich in both enhancers [87]. As vegetarian and vegan dietary patterns have become increasingly popular among athletes, concerns regarding iron adequacy have grown, particularly since micronutrient demands in athletic populations exceed those of the general population[88]. However, recent research challenges the traditional view that plant-based diets inherently increase the risk of iron deficiency due to lower bioavailability: Nebl et al. reported that the expected disadvantages of non-heme iron absorption in plant-based eaters did not translate into poorer iron status when dietary patterns were well planned and nutrient dense [89]. Although most data originate from endurance athletes, similar dietary vulnerabilities may also apply to strength and team-sport athletes [90], [91].

Beyond the absolute amount of iron consumed, energy balance has emerged as an influential determinant of iron regulation. Bonilla et al. argue that the phenomenon often labelled "sports anemia" is driven less by excessive iron loss or diminished absorption and more by chronically low energy intake and suboptimal dietary patterns [92]. This positions overall energy availability (EA), not merely iron intake, as a central determinant of iron homeostasis.
Carbohydrates (CHO) are the main energy source [93] and further carbohydrate availability has also been explored as a potential modulator of iron metabolism [94]. This is primarily due to its role in glycogen restoration and the established link between low glycogen and elevated IL-6, which may results in an hepcidin elevation [95]. However, the current body of evidence suggests that CHO exerts only a limited regulatory influence. Studies by Badenhorst et al. [94] and Dahlquist et al. [96] demonstrated that CHO consumed immediately after exercise does not meaningfully alter IL-6 concentrations, likely because IL-6 has already peaked by the time post-exercise nutrition is provided. Investigations into CHO ingestion during exercise similarly reported no significant differences between CHO and placebo conditions in post-exercise hepcidin concentrations, either immediately or during 24 h recovery, although early studies lacked

sampling within the critical 3-6 h window of the hepcidin peak [97]. A follow-up trial using appropriate time-points again found no effect of CHO on IL-6 or hepcidin, a result likely explained by insufficient glycogen depletion due to the comparatively short trial duration [98].

Chronic CHO restriction, such as low-carbohydrate high-fat (LCHF) diets, may theoretically amplify hepcidin responses through reduced iron intake and persistently low muscle glycogen. However, findings remain mixed. One study reported higher post-exercise IL-6 and hepcidin under LCHF, yet ferritin differences between groups appeared to confound these results. When ferritin was matched, no differences in hepcidin were observed between LCHF and high-CHO athletes [99]. Long-term CHO restriction therefore does not consistently alter iron regulation, likely due to metabolic adaptation or the overriding influence of baseline iron status. Even strategies involving extremely high CHO intake do not improve iron-related outcomes beyond those achieved with more moderate CHO intake [79]. Collectively, these findings suggest that carbohydrate availability is not a primary regulator of iron metabolism. Instead, CHO intake becomes relevant mainly through its contribution to overall EA, which is frequently compromised in athletes at risk for low energy availability (LEA) [100].

A growing body of evidence supports LEA as an important modulator of hepcidin expression independent of inflammatory pathways. A four-day military training protocol producing an approximate 55% energy deficit resulted in elevated resting hepcidin, which were positively correlated with energy expenditure and negatively with energy balance, with no influence of macronutrient composition [101]. In endurance athletes, a three-day LEA intervention (18 kcal·kg FFM^{-1}) similarly increased resting hepcidin compared with adequate EA (52 kcal·kg FFM^{-1}), indicating that impaired EA alone is sufficient to alter iron-regulatory signaling. During the LEA condition, 75 min of running at 70% VO_2max produced markedly greater IL-6 elevations, likely driven by progressive glycogen depletion (−28% across three days). Yet despite higher IL-6, post-exercise hepcidin concentrations at 3 h remained comparable between conditions, suggesting that LEA may influence hepcidin through mechanisms distinct from IL-6-mediated inflammatory signaling [102].

Experimental work points to several potential candidates. Starvation conditions have been shown to induce a 5-fold increase in hepcidin antimicrobial peptide (HAMP) transcription and a subsequent 2-fold rise in hepcidin concentrations via CREBH-mediated gluconeogenic signaling [103]. Additionally, erythroferrone (ERFE), a hormone that suppresses hepcidin during erythropoietic stimulation, declines under nutrient restriction, thereby removing its inhibitory effect and permitting hepcidin elevation [104]. While these mechanisms remain speculative in athletic LEA, as most evidence stems from animal or starvation models, they support a framework in which hepcidin becomes sensitive to metabolic stress beyond classical inflammatory pathways.

At this point, the emerging bi-directional relationship becomes relevant. Although the connection between EA and iron regulation was initially understood as a unidirectional pathway, where LEA increases hepcidin and reduces iron availability, evidence now suggests the reverse may also hold true [105]. Low iron stores can impair mitochondrial ATP production because iron is essential for iron–sulphur proteins and heme-containing cytochromes in the electron transport chain [106]. When iron availability is insufficient, oxidative phosphorylation becomes less efficient and reliance on anaerobic metabolism increases. Consequently, the energetic cost of performing a given exercise intensity rises, effectively lowering an athlete's usable EA [107]. With this influencing factor in

mind, this has opened up a new way in the prevention and displays a relevant factor for the recovery process of iron-deficiency anemia (IDA) [108].

Specific Aspects of Female Physiology

Sex-based differences in iron metabolism begin to emerge with puberty [109]. During childhood, boys and girls present with comparable hemoglobin concentrations. However, the rise in testosterone during male adolescence stimulates erythropoiesis, contributing to greater hemoglobin and red blood cell (RBC) mass in males [110], [111]. Whether menstrual onset independently contributes to sex differences in erythropoiesis has been debated. Although this appears unlikely given that circulating erythropoietin (EPO) levels do not differ between males and females [112]. Whether the modest fluctuations in female testosterone across the menstrual cycle, from ~0.9 $nmol \cdot L^{-1}$ in low-hormone phases to ~1.34 $nmol \cdot L^{-1}$ around ovulation [113], meaningfully influence iron metabolism remains unstudied.

Given the additional hormonal variability across the menstrual cycle, attention has increasingly turned to the roles of estrogen and progesterone in iron regulation. Experimental models indicate that progesterone may enhance hepcidin expression, whereas estrogen tends to suppress it [114], [115], [116], [117]. Early human research reported a consistent pattern in which hepcidin concentrations were lowest during menstruation and increased as the cycle advanced [118], [119], implying cyclical modulation. More recent findings are less conclusive: hepcidin did not vary significantly across menstrual phases, although average values tended to be highest mid-cycle and lowest during the follicular phase [120]. Thus, despite mechanistic evidence for hormonal regulation, human data indicate a complex and inconsistent relationship between estrogen and hepcidin [121].

The IRONFemme study group provides the most comprehensive evaluation of iron regulatory dynamics across menstrual phases in endurance-trained, eumenorrheic women. Their work consistently shows that resting ferritin, serum iron, and transferrin saturation are lower in the early follicular phase, when estrogen is lowest, compared with the late follicular phase, when estrogen peaks. In contrast, hepcidin does not meaningfully differ between phases, and post-exercise hepcidin responses similarly appear unaffected by menstrual cycle–related hormonal fluctuations [122], [123].

Hormonal contraceptive use adds an additional layer of complexity. Oral contraceptive pill (OCP) users typically exhibit higher serum iron and transferrin concentrations than naturally menstruating athletes, whereas ferritin remains comparable between groups. Both groups, however, demonstrate reductions in serum iron and transferrin saturation during menstruation or withdrawal bleeding [124], reinforcing menstrual blood loss as a principal driver of fluctuating iron status regardless of endogenous or exogenous hormone profiles.

Beyond regulatory hormones, female athletes experience recurring iron loss through menstrual bleeding, typically amounting to approximately 10 mg of iron per cycle. Combined with dietary factors, this contributes to iron insufficiency rates of up to 30% in athletic women [125]. Low daily iron intake is also common in females [126], [127]. Diets such as ketogenic or plant-based patterns, prevalent in some athlete groups have historically been considered risk factors due to their limited iron bioavailability [128].

Despite sex-based differences in hemoglobin and RBC mass, and despite recurring physiological iron loss, females maintain adequate hemoglobin concentrations and

tissue oxygen delivery throughout the menstrual cycle [129]. This ability has prompted hypotheses of sex-specific adaptive mechanisms in iron handling and microvascular regulation. Such adaptations may be supported, at least in part, by interactions between female sex hormones and iron metabolism, although the precise regulatory pathways remain only partially understood. Still underlying mechanisms remain underexplored, especially regarding the protection of iron stores.

Effect of Training under Hypoxic Conditions

Altitude training camps have become a non-negotiable in many endurance sports and have spread even further into team sports [130]. The effect that has been proposed and is promised to the athletes undergoing such strenuous times, is the increase in endurance capacity mainly based on the rational of an increased total hemoglobin mass and therefore an optimized way of the body to transport more oxygen to the working muscle using less blood. The cornerstone of the production of hemoglobin and red blood cells is iron [34]. The trace element that may already be limited in athletes is now becoming the center to not only their performance enhancement but also their adaptation to a new environment. It has already been stated that training camps under hypoxic conditions are only successful, when iron stores have an adequate level [131], [132]. Under hypoxic conditions, the amount of oxygen in the aspirated air is reduced, or that partial pressure of the air is reduced. Both conditions signaling the body a state of reduced oxygen, which primarily sensed in the carotid body [133]. The next step is the activation of the hypoxia-induced factors (HIF) pathway, which primarily involves hypoxia-inducible factor 1 (HIF-1) and HIF-2. Under normoxic conditions HIF is ubiquiniered and intracellular levels remain low. While during hypoxic conditions HIF ubiquitination is inhibited, increasing its stability and transcriptional activity. Stabilized HIFs bind to hypoxia-responsive elements of target genes, markedly enhancing their transcription. This cascade induces a broad range of molecular responses that collectively aim to improve oxygen delivery to hypoxic tissues. Among the most relevant adaptations for athletes are increases in erythropoiesis and iron-handling capacity. HIF-2 regulates genes essential for iron uptake and hemoglobin synthesis, including transferrin and the EPO receptor. Upregulation of transferrin supports efficient iron transport from the bloodstream into cells via receptor-mediated endocytosis, while elevated EPO stimulates red blood cell production [134].

In addition, HIF activation enhances angiogenesis through increased expression of vascular endothelial growth factor (VEGF) and modifies metabolic pathways to favor glycolytic ATP production in low-oxygen states. HIF-1 regulates several glycolytic enzymes (e.g., phosphofructokinase, hexokinase, lactate dehydrogenase), glucose transporters (GLUT-1, GLUT-3), monocarboxylate transporters (MCT-1, MCT-4), carbonic anhydrase for pH regulation, nitric oxide synthase, and heme oxygenase, among others [135].

Together, these coordinated molecular adjustments enhance oxygen transport and utilization under hypoxic conditions. However, all erythropoietic adaptations remain iron-dependent, making robust iron stores essential for altitude training success. Without adequate iron, the HIF-driven increase in oxygen transport capacity cannot be fully realized [136], highlighting the need for systematic iron monitoring and targeted supplementation in athletes preparing for altitude exposure.

Together these factors influence iron metabolism and can limit iron availability. This becomes particularly visible in the prevalence rate of iron deficiency, which is stated to be 23% in collegiate athletes globally [137].

1.3 Stages and Diagnosis of Iron Deficiency in Athletes

The awareness of the factors influencing iron metabolism of athletes and potentially threaten their homeostasis aka the tightrope walker to trip and fall is crucial. To understand the impact of these factors, blood biomarkers are analyzed and used to classify stages to differentiate the severity. Therefore, a nuanced interpretation is essential, particularly in athletic populations whose iron demands and metabolic dynamics differ from those of the general population.

Ferritin is the classical and most widely used biomarker, as it reflects the size of the body's iron stores. Low ferritin concentrations indicate depleted reserves and thus are characteristic of absolute iron deficiency [138]. However, ferritin is also an acute-phase protein that increases in response to inflammation, infection, or cellular injury. Under such conditions, which are not uncommon in athletes due to repetitive mechanical and metabolic stress, ferritin may appear normal or elevated despite insufficient iron stores [139]. Consequently, ferritin alone cannot always reliably distinguish iron deficiency from inflammatory driven elevations.

To complement ferritin, transferrin and transferrin saturation (TSAT) provide information on the availability of circulating iron. Transferrin is the main plasma iron-transport protein, and TSAT indicates the fraction of transferrin molecules loaded with iron. A low TSAT signals that only a small proportion of transport capacity is utilized, implying reduced circulating iron supply to tissues [140].

A more inflammation-resistant parameter and suitable marker to diagnose iron deficiency in athletes is the soluble transferrin receptor (sTfR) [141]. This protein originates from the extracellular domain of the cellular transferrin receptor, which is upregulated when cells in particular erythroid precursors sense insufficient iron availability [142]. As sTfR concentrations increase when intracellular iron becomes limiting and are largely unaffected by inflammatory signaling, the marker provides valuable information about functional iron deficiency and tissue iron demand [143]. However, sTfR assays remain incompletely standardized, and elevated values may also reflect heightened erythropoietic activity, which can occur in endurance-trained athletes [141].

To overcome the limitations inherent in single parameters, the ratio of sTfR to the logarithm of ferritin (sTfr/logFerritin), commonly referred to as the sTfR-Ferritin Index, has gained attention. This index integrates information about iron storage and iron demand, thereby improving diagnostic accuracy for both absolute and functional iron deficiency, particularly in the presence of inflammation [144], [145]. Nonetheless, methodological inconsistencies across laboratories and the absence of universally accepted cut-off values currently limit its broader adoption [146].

A more recently applied biomarker in sports science is hepcidin, the central hormonal regulator of systemic iron homeostasis. Nonetheless, clinical use of hepcidin remains limited due to assay variability, circadian fluctuations, and the strong acute responsiveness of the hormone to training, illness, and other stressors [16].

There are numerous different classification systems to categorize iron status of athletes using blood-based biomarkers. Here the classification of McKay et al. for the athletic

population is used [147]. The authors differentiate between a sufficient iron status and three stages of deficiency.
Sufficient iron status is characterized by serum ferritin concentrations above 35 µg/L, hemoglobin values within the normal sex-specific range (>130 g/L in males; >120 g/L in females), and transferrin saturation above 16%. In this state, there is no evidence of impaired iron metabolism, and iron supply adequately meets the demands of erythropoiesis and cellular function.
Stage 1: Iron depletion marks the earliest shift away from equilibrium. Here, ferritin falls below 35 µg/L, indicating depleted iron stores in the liver, bone marrow, and spleen, while hemoglobin and transferrin saturation remain relatively preserved. Although performance may not yet be affected, the body has lost its buffer, leaving athletes vulnerable to further decline when training demands increase.
Stage 2: Iron-deficient non-anemia (IDNA) reflects a more pronounced disturbance. Serum ferritin levels drop below 20 µg/L and transferrin saturation falls below 16%, signaling reduced iron delivery to the erythroid marrow and compromised erythropoiesis. Hemoglobin may remain within the normal range, but functional iron deficiency is already present. At this stage, athletes often experience subtle but measurable reductions in aerobic capacity, increased fatigue, and impaired adaptation to training.
Stage 3: Iron-deficiency anemia (IDA) represents a complete breakdown of iron balance. With ferritin concentrations below 12 µg/L, transferrin saturation below 16%, and hemoglobin falling below normal thresholds (<130 g/L in males; <120 g/L in females), hemoglobin synthesis becomes insufficient, resulting in anemia. The physiological consequences are profound: oxygen transport is impaired, mitochondrial energy production is compromised, and performance capacity declines markedly.

The knowledge of the variety blood-based biomarkers and their adaptive responses through exercise display important insights in iron metabolism.

2 Aim of the dissertation

The primary aim of this dissertation project was to advance the understanding of iron metabolism in athletes, a population in which disturbances of iron homeostasis are highly prevalent.

Iron regulation represents a tightly controlled physiological process that extends beyond erythropoiesis and involves multiple iron-dependent systems, including immune, metabolic, endocrine, and hematological pathways. Accordingly, this cumulative dissertation adopted a system-oriented perspective to investigate how distinct physiological stressors relevant to athletic practice influence iron regulation under conditions of increased demand and loss. Specifically, the included studies examined selected influencing factors, such as hypoxic exposure, menstrual blood loss, and energy availability, that have the potential to challenge iron homeostasis in athletes.

A further aim was to generate evidence that may inform more differentiated monitoring and management strategies for athletic populations facing recurrent or combined iron-related stressors.

3 Synopsis

Despite extensive research on iron deficiency in athletes, iron metabolism is still predominantly interpreted and analyzed in isolation to the hematological system and variables. This synopsis synthesizes the findings of the present cumulative dissertation to reframe iron metabolism as a dynamic and potentially limiting mediator of adaptation. Which becomes particularly relevant when physiological stress challenges iron homeostasis across interacting systems in athletes.

3.1 Conceptual Framework of the Dissertation

Traditional approaches have mainly focused on erythropoiesis and hemoglobin-related outcomes when addressing iron metabolism in athletes. Although it is increasingly recognized that disturbances in iron homeostasis, most visible as iron deficiency, can extend far beyond the hematological system, this broader physiological relevance is often insufficiently integrated into diagnostic and monitoring strategies.

Based on this framework, the dissertation followed a stepwise approach in which distinct influencing factors, each potentially challenging iron homeostasis, were mechanistically examined across individual studies. This strategy allowed unravelling how specific physiological stressors altered iron regulation and how these alterations interact across interconnected physiological systems. And providing the conceptual foundation for a network-physiological interpretation of iron metabolisms that constitutes the central focus of this cumulative dissertation.

3.2 Overview of the Included Studies

To kick off the topic in a practical manner a narrative review in the German Journal of Sports Medicine was published, focusing on the prevention of iron deficiency in athletes. This review served as a translational framework, synthesizing current evidence on iron-related challenges in athletic populations and highlighting key physiological and practical factors that can compromise iron homeostasis [148]. This synthesis allowed the identification of specific influencing factors in the original studies included in this dissertation.

The following original studies therefore addressed the different factors in depth to investigate the underlying mechanism that contribute to changes in iron homeostasis in athletes and addressed factors that can potentially disrupt homeostasis.

The second original paper investigated iron metabolism and immune-related markers during controlled normobaric hypoxia, addressing iron allocation between erythropoiesis and innate immune function [149].

The third original paper examined menstrual blood loss as an initiating factor for adaptive iron regulation in female athletes [150].

The fourth and final original paper in a pre-print state investigated whether relative energy and nutrient intake predicted serum ferritin levels in elite athletes under real-world training conditions [151].

3.3 Integrated Interpretation of Findings

3.3.1 Hypoxia as a Model of Competing Iron Demands

Hypoxic exposure served as a controlled model to illustrate how iron mediates interactions between physiological systems. Activation of hypoxia-inducible signaling pathways increased erythropoietic iron demand, prioritizing oxygen transport capacity. Simultaneously, markers of innate immune function displayed sensitivity to alterations in iron distribution, indicating that enhanced erythropoiesis occurred in parallel with functional constraints in other iron-dependent processes.

These observations demonstrated that hypoxia amplifies competition for iron across systems, implying trade-offs between hematological adaptation and immune competence. Importantly, this model represented an example for the “iron-link” that connects physiological systems. A link that may become most visible under conditions of increased physiological, rather than under resting homeostasis.

3.3.2 Recurrent Iron Strain and Adaptive Regulation

Beyond hypoxia, the included studies identified recurrent physiological stressors that challenge iron homeostasis through different pathways. Menstrual blood loss represented a predictable, cyclical form of iron strain, while insufficient energy availability constituted a metabolically driven restriction on iron regulation. Although mechanistically distinct, both stressors imposed sustained demands on systemic iron availability.

Across these contexts, iron regulation appeared adaptive rather than fundamentally pathological. Female athletes maintained hemoglobin concentration and oxygen delivery despite chronically lower iron stores, suggesting dynamic redistribution and prioritization of iron under recurrent demand. In contrast, the energy availability study suggested a different scenario: low ferritin concentrations co-occurred with insufficient caloric intake, pointing toward a metabolic context in which adaptive capacity may become constrained.

3.3.3 Iron as a Central Integrator Across Physiological Systems

Viewed collectively, these findings support the concept that iron metabolism responds to both acute and chronic stressors through regulated allocation across co-existing, not to say competing systems. In this framework, reduced iron stores may represent a stabilized symmetry under sustained physiological demand, emphasizing the importance of longitudinal and system-level interpretation over static deficiency thresholds. Across all included studies, iron regulation consistently emerged as a central mediator linking multiple physiological systems rather than as an isolated hematological variable. Changes in iron availability were observed in response to diverse physiological stressors and were accompanied by system-specific adaptations across the hematological system, immune defense, and metabolic regulation.

Taken together, the findings of this dissertation support a network-physiological perspective in which the “iron-link” is positioned within interconnected regulatory systems.

4 Publications

4.1 Approaches to Prevent Iron Deficiency in Athletes

Nolte, S., Krüger, K., Hollander, K., & Carlsohn, A. (2024). Approaches to prevent iron deficiency in athletes. *Dtsch Z Sportmed*, 75(5), 195-202.

DOI: 10.5960/dzsm.2024.607

URL: https://www.germanjournalsportsmedicine.com/archive/archive-2024/issue-5/approaches-to-prevent-iron-deficiency-in-athletes/

Nolte S[1], Krüger K[1], Hollander K[2], Carlsohn A[3]

Approaches to Prevent Iron Deficiency in Athletes

Ansätze zur Prävention eines Eisenmangels bei Athleten

STATEMENT

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Summary

› **For athletes,** iron plays an important role in improving oxygen supply, energy production, muscle function, and cognitive performance. However, iron deficiency is a common problem in athletes, especially endurance athletes, due to factors such as increased iron loss due to exercise-induced sweating, hematuria, and gastrointestinal bleeding. Exercise induces hematological adaptations due to increased demand of oxygen transport as well as inflammation, which reduces the ability to absorb iron post-exercise. Iron deficiency can lead to fatigue, reduced performance, poorer recovery and increased susceptibility to infections.

› **Iron absorption** is tightly regulated to prevent toxicity, with hepcidin playing a central role. Elevated hepcidin levels, which are influenced by exercise-induced inflammation and circadian rhythms, can significantly reduce iron absorption. In addition, the bioavailability of dietary iron varies, with heme iron from animal products being more readily absorbed than non-heme iron from plant sources. Athletes on a vegetarian or vegan diet may require increased iron intake to meet their needs.

› **Monitoring iron status** through regular blood tests, including serum ferritin and hemoglobin levels, is critical for early detection and treatment of iron deficiency. Strategies to improve iron absorption include consuming iron-rich foods with promoters such as vitamin C, while inhibitors such as phytates and calcium should be avoided. In cases of significant deficiency, supplementation under medical supervision may be necessary. Understanding these factors and using appropriate nutritional and monitoring practices can help athletes maintain optimal iron levels and overall performance.

KEY WORDS:
Iron Metabolism, Heme and Non-Heme Iron, Dietary Iron, Hepcidin, Promotors and Inhibitors of Iron Absorption, Nutrient Timing

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Introduction

Iron plays a crucial role for health and performance of athletes by supporting oxygen transport and delivery, mitochondrial energy production, muscle function, immune response and cognitive function (2). Among others, iron is an essential component of hemoglobin and myoglobin, which are responsible for transporting oxygen in blood and muscles, respectively (21). Thus, iron deficiency may impair athletic performance by lack of energy and reduced work capacity, suppressed training adaptation, impaired recovery from exercise, fatigue and increased susceptibility to infections (15). In line with the importance of iron, athletes notice an iron deficiency by unspecific symptoms such as fatigue, dyspnea, lethargy and higher susceptibility to infections. Secondary symptoms include a reduction of training and competition outcomes, reduced adaptation to training load and overall work capacity (15).

Iron deficiency is one of the most common nutritional deficiencies worldwide. Athletes, particularly those involved in endurance sports, are at an even higher risk for iron deficiency with a prevalence of 15-35% of female athletes and 3-11% of male athletes compared to approximately 5% in the general population.

Risk factors for developing iron deficiency in athletes include

a) increased iron loss due to exercise-induced sweating, hematuria, and gastrointestinal bleeding,
b) exercise-induced hematological adaptations due to increased demand of oxygen transport and
c) exercise-induced inflammation, which reduces the ability to absorb iron post-exercise (20).

Regulation of Iron Absorption

Although iron is essential for health, well-being, and athletic performance it is also a highly reactive mineral that may be toxic to the human body when ingested in high dosages (26). Iron overload is discussed to be associated with different diseases such as hemochromatosis, cardiovascular diseases, cancer and type 2 diabetes mellitus (24).

Due to the toxicity of iron, its absorption is highly regulated and very limited with absorption rates ranging from only 2 to 35%. One of the key fac- ›

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Table 1

Promotors and inhibitors of iron absorption and dietary sources (adapted from Biesalski HK (3)).

INHIBITORS OF NON-HEME IRON ABSORPTION	PROMOTORS OF NON-HEME IRON ABSORPTION
Phytates (in whole grain cereals, bread, legumes, nuts and seeds)	Vitamin C (in fruits and vegetables, ask your sports dietitian for support to aim for ≥50 mg in a iron-rich meal)
Polyphenolic and phenolic compounds (in herbal and non-herbal tea, coffee, red wine, chocolate)	Carotenoids (in carrots, pumpkin, apricot, green leafs, tomatoes)
Calcium (in dairy products or in multi-mineral or calcium dietary supplements)	Fermented food such as sauerkraut, kimchi or fermented mixed pickels (fermentations reduces phytates)
Other minerals (zinc, manganese)	Cooking the food (reduces presence of phytate)

tors in the regulation of iron absorption is the hormone hepcidin. Briefly, increased hepcidin levels in the blood decrease iron absorption, and hepcidin increases due to exercise-induced temporary inflammation and following a circadian rhythm with higher levels later in the day (15).

In addition, many studies have shown an increase in inflammation markers (IL-6) and hepcidin levels in athletes in a state of low energy availability (LEA), and some have observed LEA-independent yet insignificant rises in hepcidin levels following glycogen-depleting exercises or exercises with low carbohydrate availability (16) (figure 1).

Degradation of senescent red blood cells also contributes to iron loss in humans, even though the human body has the capacity to recycle 90% of the iron from degraded erythrocytes. In a state of iron homeostasis, where iron losses are minimized to 1-2 mg/d the absorption of dietary iron (0,5-2 mg) is sufficient to counterbalance the losses. However, this counterbalance may be disrupted in competitive athletes.

It must be admitted that although iron deficiency is more common in females compared to males, the paucity of studies on iron and hepcidin metabolism in athletes have been conducted in males. Due to the gender data gap little is known about iron and hepcidin metabolism in female competitive athletes (10).

Iron Availability

The availability of iron is very limited depending on the dietary source and the presence of inhibitors and promotors of iron absorption in the meal. The typical absorption rate varies between 5 to 35% for heme iron (Fe^{2+}) and 2-20% for non-heme iron (Fe^{3+}) (4).

Heme iron is found in animal products such as red meat, poultry and fish, whereas non-heme iron is derived from vegetable foods like legumes, grains, and vegetables (13). The divalent heme (Fe^{2}+) iron is easier to absorb than the oxidized non-heme iron due to its distinctive absorption pathway, resistance to dietary inhibitors, and effective transport and utilization mechanisms within the body (22). Vegetarian and vegan athletes may struggle to meet their iron needs, as their diets primarily contain non-heme iron. Vegetarian athletes might ingest approximately 10% more iron than omnivore athletes to account for the lower iron absorption.

However, the absorption of non-heme iron in vegetarian diets may be increased by adding antioxidant-rich foods to the meal which helps to reduce the oxidized Fe^{3+} into Fe^{2+}. Promotors of iron absorption are antioxidants such as Vitamin C or carotenoids and are naturally abundant in fruits and vegetables. Inhibitors of iron absorption may be found in both plant-derived and animal-derived food. For example, phytates found in whole-grain cereals, legumes, nuts and seeds or polyphenols in tea, coffee or chocolate may reduce iron absorption by 60-70%, whereas dairies may reduce iron absorption by 50-60% (table 1). It should be noted that both inhibitors, as well as promotors, modify the absorption of non-heme iron. Adding antioxidants to animal-derived food such as meat or to medical products containing divalent iron does not promote iron absorption from these sources.

Thus, athletes need to be educated about adequate iron sources considering both total amount and availability of iron in the food or complete meal (table 2).

Challenges of Adequate Iron Status in Athletes

To achieve and maintain an individually adequate iron status is often challenging for athletes as they intensively use the oxygen-transporting system. In addition to the aforementioned factors, iron losses are also increased in athletes by foot-strike hemolysis or increased blood loss in marathon runners or martial artists, which can lead to depleted iron stores (7).

Regarding the immune response, hepcidin levels are elevated via secretion of interleukin-6 (IL-6) and peak 3 hours after exercise remaining elevated for 3 to 6 hours after cessation of exercise. As mentioned before, elevated hepcidin levels strongly inhibit iron absorption from food or supplements (9). The background of this mechanism is the body's defense against pathogens. Iron is essential for the survival of bacteria, limiting the availability is a physiological response to "starve" the pathogens. Simultaneously, training acts as a stressor to the athletes' body and pro-inflammatory cytokines are released. The principal cellular targets of hepcidin are enterocytes, macrophages and hepatocytes. In these cells, hepcidin leads to the internalization and degradation of ferroportin, preventing the release of iron stored in liver cells, the absorption of dietary iron, and the recycling of iron in macrophages (12) (figure 2).

Altitude training poses an additional challenge to the iron metabolism. The associated acclimatization and the desired adaptation process to the reduced oxygen partial pressure stimulate erythropoiesis to increase the physiological transport capacity for oxygen. When iron availability is insufficient, adaptation on the hematological level is limited. Altitude exposure was shown to decrease hepcidin levels which in response increases iron absorption and promotes altitude-induced erythropoiesis (figure 1). Therefore, securing adequate iron availability is a key in altitude adaptation (19).

Sex also influences iron status, with female athletes being at a higher risk for iron deficiency as menstruation can lead to significant iron losses, which, when combined with the increased iron demands of physical training, places female athletes at a higher risk for iron deficiency and anemia (25). The

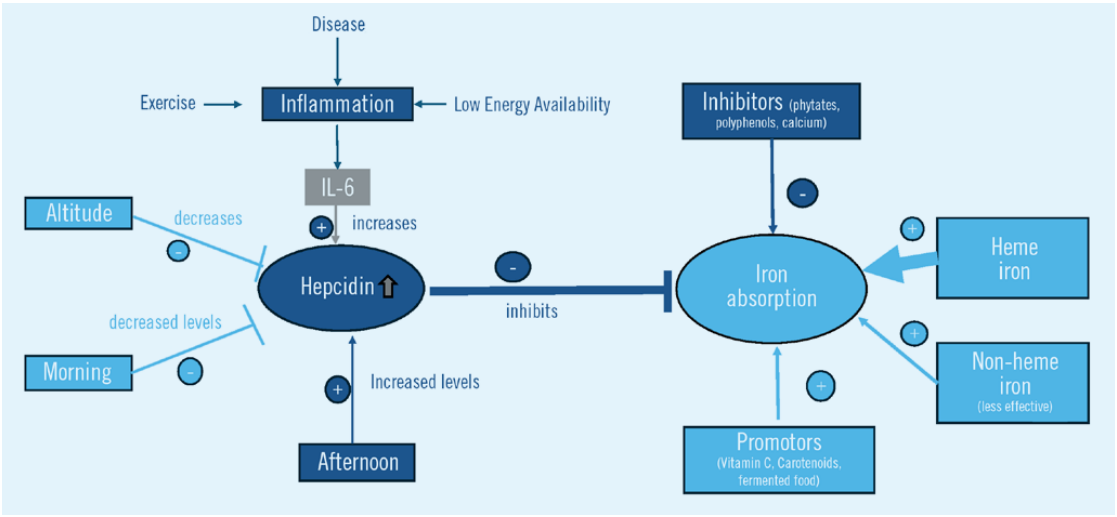

Figure 1

Regulation of iron absorption due to modifications in hepcidin levels. The figure illustrates why iron intake in the morning before or within 30 min after cessation of exercise is an evidence-based strategy to improve iron absorption. Preference of foods rich in heme-iron and promotors of iron absorption as well as avoiding co-ingestion of inhibitors such as phytates or polyphenols with iron-rich meals also supports iron absorption.

average iron loss during menstruation is estimated to be 0.55 mg/day with a huge inter-individual variety (5). Cohort studies have shown that female athletes, often have lower iron stores compared to their male counterparts (6). More high-quality studies are needed to determine whether sex hormones or monthly blood loss are the cause of differences in iron metabolism parameters.

Athletes at Risk for Iron Deficiency

Considering the above-mentioned factors that either increase iron loss, reduce total iron intake or reduce iron absorption from food athletes at risk for inadequate iron stores and the development of iron deficiency include:

- athletes with monthly blood and iron loss during menstrual cycles
- athletes with high exercise load such as endurance athletes (due to increased iron loss via sweat, hematuria and gastrointestinal bleeding)
- vegetarian and vegan athletes (as these diets are typically low in heme-iron which is more likely to be absorbed than non-heme iron)
- athletes who co-ingest high amounts of grains and cereal foods together with iron-rich foods to meet their carbohydrate requirements (as grains contain phytates that are inhibitors of iron absorption)
- athletes with low energy intake (as low overall food intake is associated with low intake of several micronutrients, including iron)
- athletes suffering from Relative Energy Deficiency in sports (RED-S) (as this may increase hepcidin levels due to lowered estrogen levels and possibly due to exercise-induced transient inflammation) (4, 15, 16).

Monitoring Iron Status

Early stages are referred to as non-anemia iron deficiency and are characterized by hemoglobin levels within the respective reference range but reduced iron stores (serum ferritin; sFer range<12 to 40 µg L^{-1}). Left untreated, non-anemia iron deficiency can develop into iron deficiency anemia where iron stores and iron transport are insufficient to sustain hemoglobin production (sFer<15 µg L^{-1} and [Hb]<11.5 g dL^{-1}) (15).

Peeling et al. suggested to distinguish between three different stages of iron deficiency (table 3). Using this model of an iron deficiency spectrum may be helpful for practitioners to involve more than only one parameter to assess iron status, as early stages of iron deficiency may be detected and adequately treated to prevent a progression of severity (18).

Regular blood tests (e.g. during annual pre-participation screening or more often for athletes at risk) are essential to monitor the iron status of athletes. Any history of iron deficiency (<24 months), irregular/excessive menses or amenorrhea, high training loads and volume, unexplained loss of performance, vegetarian or vegan diet, or history of RED-S/LEA represent indications for quarterly blood tests. Blood tests twice a year should be considered in female athletes with a previous history of iron deficiency. All other athletes should be screened for iron status annually (20). A minimum clinical screening for iron deficiency should include the following parameters: serum ferritin (sFer), hemoglobin and transferrin saturation to be able to monitor the progression between the different stages of iron deficiency (table 3). As an increased iron requirement is often accompanied by a systemic immunological reaction, inflammatory changes should always be monitored. Accordingly, the combined measurement of inflammatory cytokines, such as CRP and IL-6, provide more accurate information (20). Desirable markers which are less affected by training-induced inflammatory responses, are represented by soluble transferrin receptor (sTfR) and sTfR/log ferritin ratio (sTfR index) (14).

To reliably evaluate and interpret the results of blood testing for iron deficiency standardization prior to the measurements is crucial. The sports physician should obtain blood samples: ›

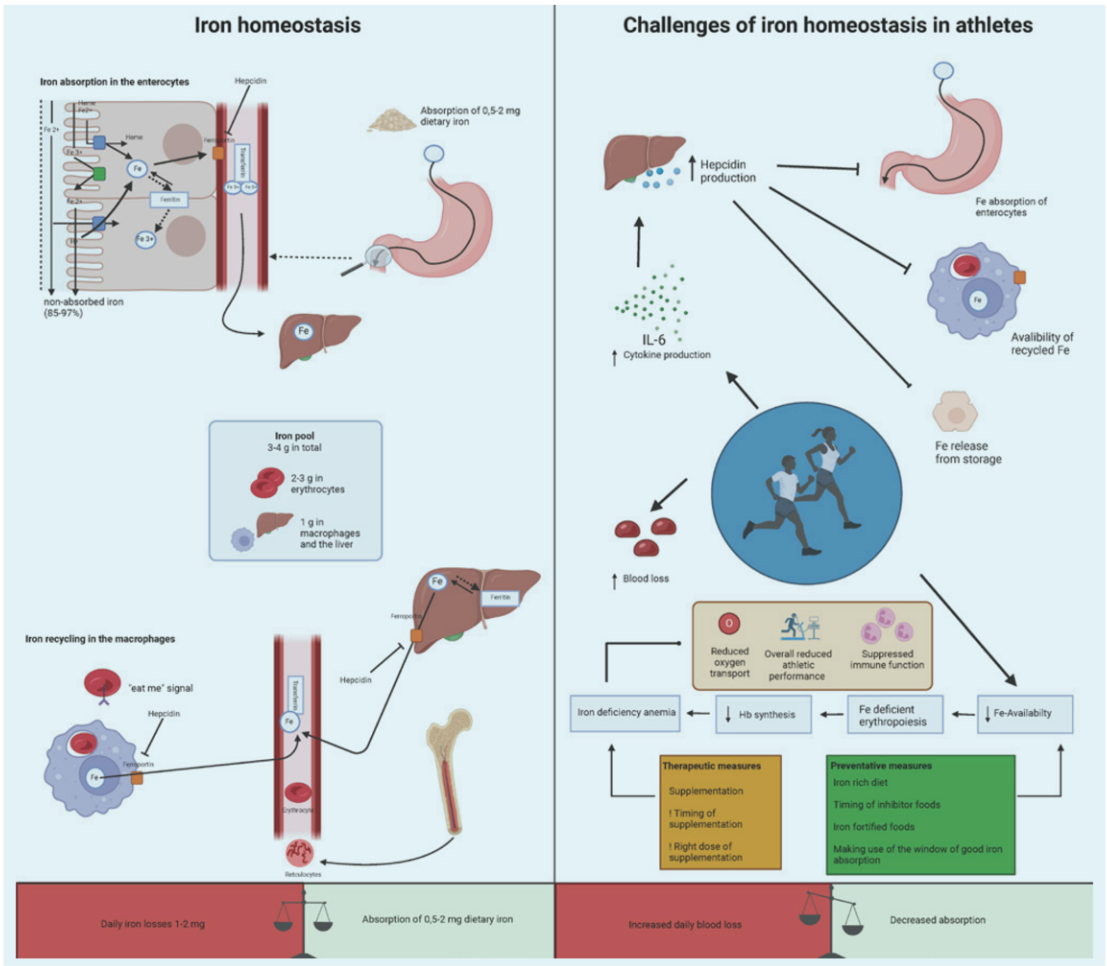

Figure 2

Regulation of iron (Fe) status and challenges of adequate iron status in athletes. The left side illustrates the regulation of iron homeostasis, showing how iron is absorbed in the intestine and recycled by macrophages. It highlights the complexity of iron balance with the focus on iron absorption, iron recycling and iron losses. The right side outlines the challenges athletes face in maintaining iron homeostasis. It indicates that factors such as increased hepcidin production and blood loss through gastrointestinal bleeding, hemolysis, and sweating can lead to reduced iron absorption and availability. This can result in impaired hemoglobin (Hb) synthesis, reduced oxygen transport, and overall decreased athletic performance and suppressed immune function. Created with BioRender.com.

1. At standardized time of the day (morning is to be preferred)
2. Prior to exercise (12-24 hours rest from exercise prior to the blood sample)
3. Considering hydration status (urinary specific gravity<1.025 to not influence concentrations of the iron status marker)
4. Avoiding blood sampling in ill or injured athletes or athletes reporting for muscle damage (e.g. following eccentric exercises), as ferritin is an acute phase protein that increases in response to stress and inflammation (validated by history, clinical exam and extended blood analysis)
5. During standardized time of the menstrual cycle in females (as during menstruation both hepcidin as well as ferritin may be modulated significantly)
6. Use of at least two markers of iron deficiency (e.g. hemoglobin and serum ferritin) to be able to diagnose the stage of iron absorption (1, 15, 20).

Key Approaches to Increase Iron Intake and Maximize Iron Absorption

To reduce the individual risk of becoming iron-deficient, athletes should endeavor to optimize iron absorption in their habitual diet. This includes adequate energy intake, as restricted food intake correlates with a lower intake of micronutrients such as iron. Athletes should at least aim for the recommended intake values for the general population, which have currently been elevated to 11 mg/day for males and 16 mg/day for adult females before menopause (8). As low energy availability and presumably glycogen-depletion may promote a hepcidin-mediated decrease in iron absorption, conditions of low energy availability should be avoided. Athletes at risk for LEA and RED-S require interdisciplinary support and treatment as quickly as possible and according to the IOC RED-S clinical assessment tool (17).

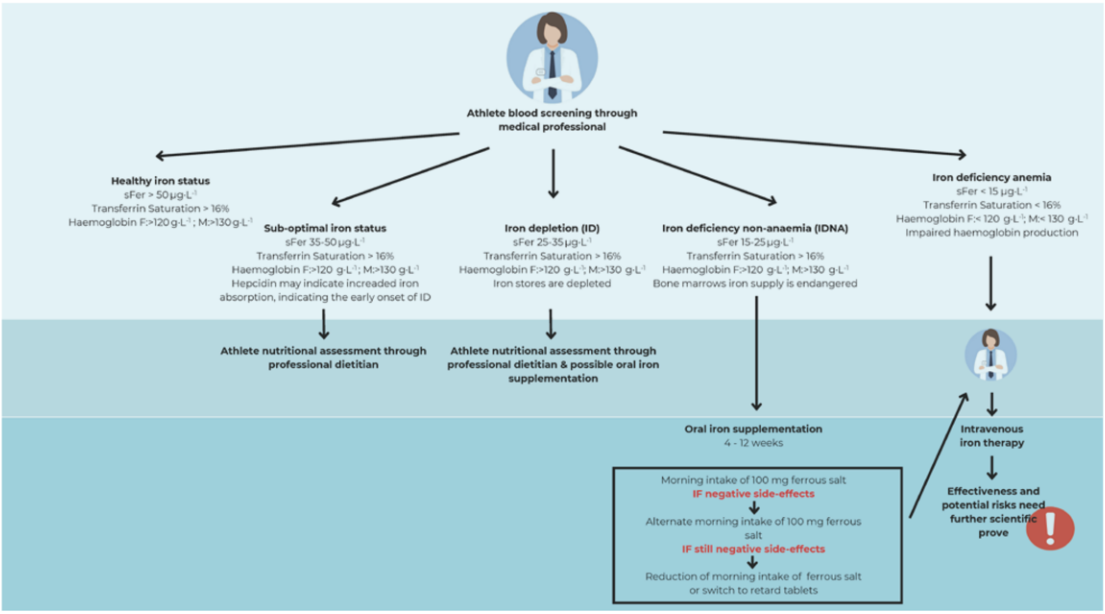

Figure 3

Flowchart to assist practitioners in the selection of optimal treatment protocols for iron-deficient athletes (F=female, M=male), diagnosed via hematological indices (serum ferritin (sFer, transferrin saturation, hemoglobin). This flowchart outlines the recommended steps for managing iron status in athletes, starting with an initial blood screening by a medical professional. If an athlete is identified with insufficient iron levels or iron deficiency, different interventions are proposed based on the severity of their condition.

Athletes at risk for iron deficiency might be encouraged to include animal sources of readily absorbable heme-iron into their weekly meal plan, where a maximum of 300 g of meat per week should not be exceeded due to its carcinogenic potential and sustainability aspects. However, combining small amounts of heme-iron with non-heme iron relevantly increases iron absorption from plant foods. Vegan athletes should be educated by sports nutritionists on how to prepare nutrient-dense foods containing high amounts of iron with promotors of iron absorption, for example combining whole-grain cereals with orange juice, wholegrain pasta with tomato sauce rich in carotenoids or steamed chickpea salad with a lime dressing. To maximize iron absorption, food and meals rich in iron should preferably be ingested in the morning rather than in the afternoon and before exercising or at least up to 30 minutes after cessation of exercise to avoid iron intake during the post-exercise hepcidin peak. Co-ingesting foods or drinks known to effectively inhibit iron absorption along with iron-rich foods should be avoided. Thus, coffee, tea or chocolate rich in polyphenols, milk and milk products rich in calcium or nuts and seeds rich in phytates should not be part of an iron-rich main meal. If preferred, those foods might be consumed as an evening post-exercise snack when iron absorption is reduced anyway.

Medical Treatment of Iron Deficiency

In cases where an adequate iron intake cannot be guaranteed through diet alone, a medical treatment using pharmacological iron may be necessary (figure 4). The dosage and duration of oral therapeutic iron administration should be taken under medical supervision and based on individual blood values to avoid overdosing and possible side effects. Recent research indicates that alternate-day oral iron supplementation is an effective strategy to replenish iron stores, enhance iron absorption, minimize gastric irritation, and increase hemoglobin levels similarly to daily supplementation (15).

Parenteral iron preparations delivered intramuscularly or intravenously (IV) offer another therapeutic solution for addressing an iron deficiency. An evident advantage of IV therapy lies in its rapid and substantial response, free from gastrointestinal discomfort, unlike oral administration. This advantage likely stems from these administration routes bypassing the gut, where absorption issues commonly occur (20). However, potential adverse events such as anaphylaxis need to be taken into consideration (11). Risks that could impair immune function (23) need further scientific proof and a parenteral preparation should be wisely considered. Current anti-doping regulations limit the amount of infusions to less than 100ml IV infusion in 12 hours, otherwise an anti-doping violation will result. With Iron(III) carboxymaltose or Iron(III) gluconat preparations lower volumes can be adminstered, otherwise a TUE (Therapeutic Use Exemption) has to be granted before treatment or the infusion is performed in a hospital setting.

Key Messages for Athletes and Practitioners

1. The main risk factors for iron deficiency in athletes are iron loss due to exercise-induced sweating, hematuria, and gastrointestinal bleeding, exercise-induced modifications in iron absorption and recycling, menstrual blood loss, low energy availability and vegetarian/vegan diets.
2. Standardized monitoring and documentation (e.g. during annual pre-participation screening) with at least serum ferritin, hemoglobin and inflammation marker (CRP), desirable sTfR and sTfR index following standardized protocols are recommended. ›

Table 2

Total iron content in typical foods and the amount available iron per serving (adapted from (12)). *Bioavailability with 500mg iron stores: Heme iron (mg)*23% + Non-heme iron (mg)*5%. The average bioavailable iron content of 55% was used for lamb and beef, 35% for seafood, fish and chicken.

TYPICAL FOOD AND SERVING SIZE	PREDOMINANT IRON FORM	IRON CONTENT (mg)	
		TOTAL	BIOAVAILABLE (ESTIMATED)*
85g Chicken liver	Heme	7.20	0.81
85g Beef liver	Heme	5.34	0.60
85g Beef roast	Heme	3.22	0.48
85g Tuna fish	Heme	2.72	0.31
85g Shrimp	Heme	2.63	0.30
1 tbsp Blackstrap molasses	Non-Heme	5.05	0.25
1/2 cup Breakfast cereals	Non-Heme	4.50	0.23
1/2 cup Potato	Non-Heme	2.75	0.14
1/2 cup Kidney beans	Non-Heme	2.58	0.13
80g Tofu	Non-Heme	2.30	0.12

Table 3

Stages of iron deficiency and suggested diagnostic cut-off values (20).

STAGE OF IRON DEFICIENCY	SERUM FERRITIN (µg/l)	HEMOGLOBIN CONCENTRATION (g/L)	TRANSFERRIN SATURATION (%)
I Iron deficiency	<35	>115	>16
II Iron-deficient non-anemia	<20	>115	<16
III Iron-deficient anemia	<12	<115	<16

3. Adequate iron intake considering total amounts of iron and its availability is most important in the prevention of iron deficiency. Consuming iron-rich foods in the morning before exercise and reducing coffee and tea promotes iron absorption. Seek support from a certified (sports) nutritionist.
4. If medically indicated (i.e. diagnosed iron deficiency), a medical iron therapy according to figure 3 should be approached. Finding a dosage and timing of iron administration that works for the individual and reduces the risk of adverse events is necessary to achieve improved iron availability.
5. The intake of dietary supplements (i.e. no medical iron products as mentioned in point 4) containing iron is strongly discouraged, especially for males and young athletes. Due to the toxicity of iron overload, the Federal Institute for Risk Assessment recommends a maximum dose of 6 mg/day and a warning on iron supplements that they are unsuitable for men and postmenopausal women. The food first approach (see key point 3) should be preferred as it seems safe with respect to risk of overdosage and doping aspects. ■

Conflict of Interest

The authors have no conflict of interest.

Summary Box

Iron deficiency is a common problem in athletes, especially endurance athletes, due to factors such as increased iron loss due to exercise-induced sweating, hematuria, and gastrointestinal bleeding. Monitoring iron status through regular blood tests, including serum ferritin and hemoglobin levels, is critical for early detection and treatment of iron deficiency.

Strategies to improve iron absorption include consuming iron-rich foods with promoters such as vitamin C, while inhibitors such as phytates and calcium should be avoided. In cases of significant deficiency, supplementation under medical supervision may be necessary.

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4.2 Training in normobaric hypoxia induces hematological changes that affect iron metabolism and immunity

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Training in normobaric hypoxia induces hematological changes that affect iron metabolism and immunity

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Altitude training is a method among endurance athletes to enhance performance via hypoxia-induced adaptations. However, individual responses vary significantly, with some athletes even showing performance decrements. Iron metabolism and immune function may influence these adaptations, as hypoxia-induced erythropoiesis increases systemic iron demand, potentially affecting immune cells reliant on iron. This study investigated the interplay between hematological, iron, and immunological variables under controlled normobaric hypoxia. 15 highly trained athletes participated in a 21-day live-high-train-low training camp in a normobaric altitude house. Blood samples were collected pre- and post-camp and at four intermediate time points to measure hematological variables, iron metabolism variables, and immunological variables. Pre- and post-performance was assessed via VO_2max tests. Statistical analyses included paired t-tests, Wilcoxon rank-sum test, Spearman correlations, and Granger causality analysis to explore systemic temporal interactions. VO_2max increased significantly ($p < 0.05$) with large interindividual variability (2.4 ± 3.5 ml/min/kg). Hemoglobin concentration, erythrocytes, and the soluble transferrin receptor (sTfR) showed significant increases over time ($p < 0.05$), while ferritin peaked early and declined post-camp. Myeloperoxidase and lactoferrin exhibited dynamic correlations with iron variables ($p < 0.05$), reflecting competition between erythropoiesis and immune function for iron. The structure of the Granger causality network places transferrin in a central role, highlighting iron metabolism as one key regulator of these adaptations. Normobaric hypoxia training induces systemic physiological changes involving hematological, iron, and immune systems. Controlled hypoxic conditions enable detailed exploration of these interactions, providing insights into optimizing altitude training strategies for endurance performance enhancement.

Keywords Neutrophils, Athletes, Hypoxia, Transferrin, Lactoferrin

Altitude training camps are a widely utilized method among elite athletes aiming to enhance endurance performance. These camps expose athletes to hypoxic conditions, which provides an additional stimulus to adapt the oxygen transport capacity. Accordingly, numerous adaptations of the hematological system have been shown to have a positive effect on performance in endurance sports[1].

However, the literature highlights a very individual response to hypoxia-induced altitude exposure. While some athletes experience substantial performance gains, others show minimal improvements or even suffer performance decrements[2–4], maybe due to an overreaching of their physiological capacity to adapt to these demanding conditions.

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One of the primary mechanisms proposed to underpin performance enhancement from altitude exposure is the increase in total hemoglobin mass (Hb_{mass}), which augments oxygen transport capacity[5]. However, the causal factors for driving Hb_{mass} elevation remain insufficiently understood, and the broader physiological consequences of these changes on other systems, such as iron metabolism and immune function, are inadequately explored. Investigating the system-physiological interactions may help better understand the variability in individual responses to altitude training[6].

Iron plays a pivotal role in the physiological adaptation to hypoxia, primarily by supporting erythropoiesis. Hypoxia-induced erythropoiesis significantly increases iron demand, which can deplete systemic iron stores[7]. This in turn could have consequences for other organs or tissues that are also dependent on iron and also have a physiological significance for performance. Studies that have used omics analyses have expanded the understanding of iron metabolism and its systematic consequences, such as the immune system[8]. It is well known that neutrophils rely on iron to maintain their antimicrobial functions. In particular, myeloperoxidase (MPO) production is highly iron-dependent and can be impaired by iron deficiency[9]. The heightened competition for iron between erythropoiesis and immune functions under physiological stress and hypoxic conditions represents a physiological challenge that can influence performance development as well as overall health.

In training camps in hypobaric hypoxia, where athletes usually train, it is very difficult to create controlled conditions. Camps in normobaric hypoxia provide more controlled conditions because hypoxia effects are isolated from confounding environmental variables such as UV exposure and changing weather conditions. In altitude houses, athletes train and reside under precisely controlled hypoxic conditions, enabling standardized investigations of the complex physiological networks of altitude adaptations[10].

The aim of our study was to investigate the interplay between hematological changes, iron metabolism, and the immune system under the controlled conditions of normobaric hypoxia in national level athletes. We hypothesized that training at altitude would lead to a competitive situation for iron between erythropoiesis and neutrophil function, which may partly explain the training response's variability. By elucidating these relationships, we aim to contribute to a more comprehensive understanding of the physiological adaptations to hypoxia and implement individualized altitude training strategies.

Methods

Study design

The study included three 21-days-training camps in normobaric hypoxia. In every camp, four to six athletes were monitored. All athletes (except one) supplemented iron in the lead-up to camp, which followed an individual approach that was monitored by medical staff and coaches. The altitude camp employed a semi-structured live-high-train-low approach with a minimum of 800 km·h of normobaric hypoxia (Höhenbalance GmbH, Going, Austria). Athletes spent a minimum of 14 h daily in normobaric hypoxia with training sessions either within the normoxic environment or outside the altitude facility. Six blood samples were collected, one a minimum of 7 days before (pre) and one 10–14 days after (post) the altitude training camp, and at four subsequent time points at altitude: 24 h (T1), 7 days (T2), 14 days (T3), and day 21 (T4) (Fig. 1).

Subjects

National team athletes were recruited from the German Athletics Federation (Deutscher Leichtathletik Verband – DLV). Fifteen highly trained endurance athletes (n = 15 [female (f) = 9; male (m) = 6]; age: 19.3 ± 1.4 years [f = 19.4 ± 1.7 years; m = 19.2 ± 1.0 years]; VO_2max of 61.40 ± 8.28 ml/min/kg [f = 56.95 ± 6.16 ml/min/kg; m = 68.08 ± 6.48 ml/min/kg]) from long- and middle-distance running disciplines and race walking were

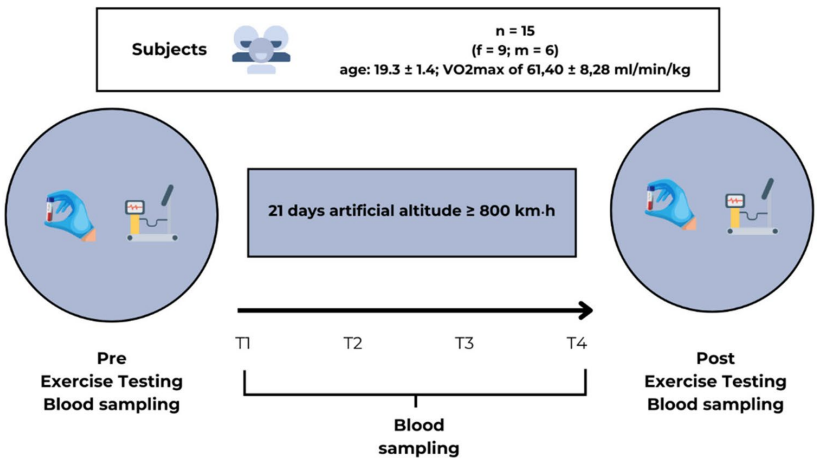

Fig. 1. Study design of the normobaric hypoxia training camp. The procedure applies to every group of athletes.

included in the study. Exclusion criteria were contraindications for exposure to altitude (anaemia, acute upper respiratory tract infections, chronic lung diseases (asthma only with adequate medication), cardiac insufficiency, myocarditis (in the last 6 months), epilepsy, cardiac arrhythmia). Athletes were recruited in close coordination with the coaches and were selected for the junior or perspective squad of the 2023 season. Prior to participation, all individuals were informed about the potential risks and benefits of the study and provided written informed consent, as per institutional guidelines.

The study was approved by the local ethical committees (MSH-2022/211) and was conducted following the Declaration of Helsinki for human research.

Exercise testing

At least 1 week before and after the training camp, performance diagnostics were conducted to determine the maximal oxygen consumption (VO_2max), the velocity where VO_2max is reached (VO_2max) and the velocity at volitional exhaustion (ramp test; 1 min stage duration; 0.15 m/s incline; 0% treadmill incline; individual starting speed according to performance level; test duration 6–10 min). The respiratory gases were recorded using the breath-by-breath method (MetaLyzer 3B-R2, CORTEX Biophysik GmbH, Leipzig, Germany).

Training load

The athletes completed a 3 week training block in normobaric hypoxia. Due to the high-performance sports setting, individual training and season planning, no intervention was applied in the training process. Training organisation remained the responsibility of the coach and the athletes. Guidance regarding training zones (based on prior performance diagnostics) was provided to the athletes for training monitoring. The training sessions were individually regulated using heart rate and lactate measurements. All athletes were at the camp during an early phase of the season (preparatory phase), with a primary focus on low-intensity training.

For every training session, the session-RPE (rating of perceived exertion) was calculated for the purpose of recording the individual training load of the athletes[11,12]. This method considers both the intensity and the duration of a training session, on a scale of RPE multiplied by the training duration. The calculated score was recorded for each athlete individually to determine the training load for each day and to calculate an average value over the duration of the stay in normobaric hypoxia. Furthermore, the 'session RPE' was calculated for the 4-week period preceding the altitude training to ascertain whether and to what extent training loads were reduced or increased during the altitude training. A ratio analogous to the acute:chronic workload ratio (ACWR) was calculated to correlate the 4-week training load prior to the training camp with the training load at altitude. The aforementioned rate of increase is incorporated into the analysis based on a factor, which is hereinafter referred to in a simplified manner as the "Training Load Ratio Altitude" (TLRA).

Blood sampling

All venous blood samples were taken between 8 and 10 a.m. In total, 17.5 ml of blood was collected. The EDTA blood tubes were processed immediately at the camp and centrifuged at 2500 g for 10 min at room temperature. Plasma was separated into aliquots and stored in Eppendorf containers, which were then stored on dry ice for transport and frozen at − 80 °C.

Hematological variables

To determine the number of erythrocytes, lymphocytes, thrombocytes, neutrophils, reticulocytes and hemoglobin concentration the small EDTA vacutainer was analyzed by routine clinical laboratory methods by SYNLAB Medical Care Center (Bad Nauheim, Germany). Erythropoietin (EPO) levels in 50-µl blood plasma samples were measured in duplicates by enzyme-linked immunosorbent assay (ELISA) using the Human EPO ELISA Kit (Thermo Fisher Scientific, Waltham, MA, USA) according to the manufacturer's instructions. Signal was detected with an Infinite M Plex plate reader (TECAN, Männedorf, Switzerland) at 450 nm excitation.

Iron metabolism variables

Ferritin, transferrin, transferrin saturation, hepcidin, soluble transferrin receptor (sTfr) and Ferritin index (sTfR/log ferritin) were analyzed using the serum gel vacutainer and were also analyzed by routine clinical laboratory methods by SYNLAB Medical Care Center (Bad Nauheim, Germany).

Plasma immune variables

CD163, TNF-alpha, VEGF, IL-6, IL-10, BDNF, IL-1 beta, IL-1ra, IL-8, S100A8, myeloperoxidase (MPO) and lactoferrin levels in 50-µl blood plasma samples were measured in duplicates by enzyme-linked immunosorbent assay (ELISA) using the magnetic Luminex assay (Bio-Techne Ltd, Abingdon, Oxon, UK).

Statistics

Before carrying out further statistical analysis the Shapiro–Wilk test was performed to test for normal distribution of all parameters. If the assumption of normality was violated ($p < 0.05$), a non-parametric test was performed. If normal distribution was present ($p > 0.05$), a parametric test was conducted.

A paired t-test was conducted to assess the change in VO_2max before and after (ΔVO_2max) the intervention and analyzed the effect size by calculating Cohen's d. This test was chosen to account for the fact that the same subjects were measured at both time points, allowing for the comparison of dependent samples.

To analyze the association between ΔVO_2max and TLRA a Spearman correlation was carried out. The Wilcoxon rank-sum test was performed to determine whether the parameters showed significant differences at different time points. Additionally, a Spearman correlation was carried out to analyze associations between

hematological, iron metabolism, and immune variables, as this method does not require the assumption of normality.

A Granger network analysis was conducted to explore temporal interconnections between the individual parameters, allowing for a deeper understanding of the causal relationships among the variables. The analysis was carried out in several steps. First, a representative time series for each sample was calculated using robust scaling within each parameter across all subjects to minimize potential influence of outliers. Next, stationarity of the time series was assessed using the Kwiatkowski-Phillips-Schmidt-Shin (KPSS) test. This test was critical for ensuring that the data met the necessary assumptions for Granger causality analysis, as non-stationary data could lead to misleading conclusions. This step aimed to determine whether incorporating part values of one parameter (time lag = 1) enhances the prediction of future values of another parameter, and vice versa. This approach allowed for the exploration of temporal relationships in both directions. the prediction of future values of one variable is increased by incorporating past values of another (time lag = 1), and vice versa, allowing for the exploration of temporal relationships in both directions. Finally, significant Granger causal connections were identified by determining whether incorporating the past values of one parameter improved the prediction of future values compared to using only the parameter itself. Only those relationships that showed statistically significant causal links after correction for multiple testing were retained for further analysis, providing a refined network of temporal associations among the parameters[13].

Results

Training characteristics, training load ratio and VO_2max

The average weekly training time was 14.52 ± 3.36 h. The number of training sessions per week was recorded at 12.98 ± 2.14 (including physiotherapy and recovery sessions), and strength training was reported at 1.32 ± 0.45 h. Specific training time (running and/or race walking) amounted to 6.01 ± 1.48 h per week, with a contribution of intensive training kilometres by 13.2 ± 12.0%. Additionally, athletes spent an average of 142 ± 88 min per day outside the altitude house. For TLRA, an average value, across all athletes, of 1.52 ± 0.41 was observed. The lowest ratio recorded was 0.98, and the highest was 2.35. This indicates that, during their time in normobaric hypoxia, the athletes increased their training load by an average of 52% compared to the 4 weeks before. However, individual variability was relatively high: one athlete maintained nearly the same load (− 2%), while another athlete increased their load by 135%. The effect on the development of performance at altitude was measured by the change in VO_2max (ΔVO_2max) between pre and post. The results showed that VO_2max increased by 2.1 ± 1.9 ml/min/kg ($p < 0.05$, d = 0.69) after 21 training days at altitude. However, subjects demonstrated high inter-individual differences between a 1.7 ml/min/kg decrease and an 4.6 ml/min/kg increase. No associations were found between the TLRA and ΔVO_2max.

Hematological variables

Number of neutrophils showed a marginal decrease at altitude (pre 2.85 ± 0.90, T1 2.49 ± 0.59 10^9/l), followed by an increase at T4 (3.05 ± 1.13 10^9/l) (Fig. 2a). In contrast, the number of lymphocytes demonstrated no statistically significant alterations over time (Fig. 2b). Significant changes over time were found for thrombocytes ($p < 0.05$) (Fig. 2c) and reticulocytes ($p < 0.05$) (Fig. 2d), showing an increase at altitude. Similarly, the number of erythrocytes increased (pre 4.63 ± 0.37, T2 4.92 ± 0.37, post 4.68 ± 0.35 10^{12}/l) without a significant time effect (Fig. 2e). Hemoglobin concentration presented a significant time effect ($p < 0.05$) (Fig. 2f) demonstrating an increase at altitude. EPO increased only within the first 24 h (pre 10.22 ± 4.59, T1 (15.16 ± 7.44) and dropped near baseline over time (T4 10.67 ± 4.62) (Fig. 2g).

Iron metabolism variables

Ferritin levels increased from pre-measurement (40.92 ± 24.49 ng/ml) to T1 (79.50 ± 86.43 ng/ml), followed by a moderate decrease until post (49.22 ± 35.74 ng/ml) (Fig. 3a). While mean serum transferrin levels remained constant over time (Fig. 3b) significant changes were found for concentrations of the soluble transferrin receptor ($p < 0.05$) and the ferritin index ($p < 0.05$) over time, (Fig. 3c,d).

Immunological variables

Both parameters of neutrophil activity, MPO and lactoferrin, showed a significant time effect ($p < 0.05$) (Fig. 4a,b). No significant changes were observed for plasma levels of CD163, TNF-alpha, VEGF, IL-6, IL-10, BDNF, IL-1 beta, IL-1ra, IL-8, and S100A8 (data not shown).

Associations between hematological variables, factors of iron metabolism and immunological parameters

Spearman correlations between MPO and ferritin index are demonstrated in Fig. 5. Negative associations were found between the ferritin index at pre-measurement and MPO at different time points pre (r = − 0.46, $p > 0.05$), T1 (r = − 0.62, $p < 0.05$), T2 (r = − 0.54, $p < 0.05$), T3 (r = − 0.54, $p < 0.05$) and T4 (r = 0.54, $p < 0.05$). Positive correlations were found between MPO at post-measurement and the ferritin index at T1 (r = 0.84, $p < 0.001$), T2 (r = 0.79, $p < 0.001$), T3 (r = 0.72, $p < 0.01$) and T4 (r = 0.71, $p < 0.01$).

The relationship between sTfR and lactoferrin differed across the various measurement points (Fig. 6). At altitude, positive correlations were found between sTfR levels at T1 (r = 0.58, $p < 0.05$), T2 (r = 0.59, $p < 0.05$), T3 (r = 0.57, $p < 0.05$), and T4 (r = 0.62, $p < 0.05$) with lactoferrin at the post-measurement. In contrast, a negative correlation was observed between pre-measurement sTfR and lactoferrin levels at the post-measurement (r = − 0.55, $p < 0.05$).

Associations between the number of neutrophils and the ferritin index are shown in Fig. 7. Negative correlations were observed between the number of neutrophils at T4 and the ferritin index at different time

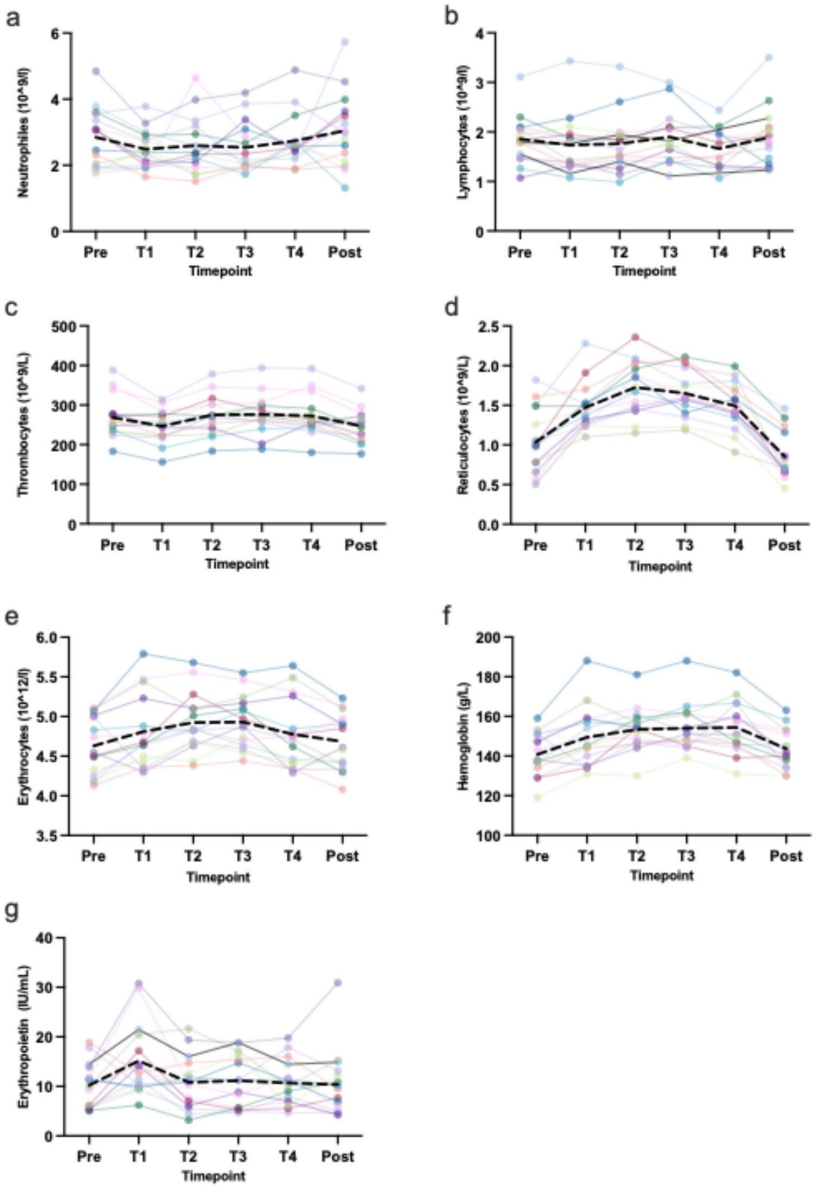

Fig. 2. Changes of hematological variables over time. Shown are individual progressions (colored lines) and the mean progression (black dotted line).

points, with r = − 0.52 for T1, r = − 0.26 for T2, r = − 0.42 for T3, and r = − 0.42 for T4 ($p > 0.05$). Conversely, the post-measurement neutrophil count showed positive correlations with the ferritin index at these same time points, with T1 (r = 0.63, $p < 0.05$), T2 (r = 0.68, $p < 0.01$), T3 (r = 0.55, $p < 0.05$), and T4 (r = 0.61, $p < 0.05$).

Granger causalities between hematological, iron metabolism and immune function variables

We applied the Granger causality test to see whether prior values of the measured parameters could predict future values of those. The analysis revealed significant unidirectional or bidirectional relationships between various hematological, immune, and iron metabolism parameters, as visualized in the network diagram (Fig. 8). Each arrow represents a Granger-causal connection, highlighting how these biological systems might interact. (Fig. 8). All reported influences are statistically significant.

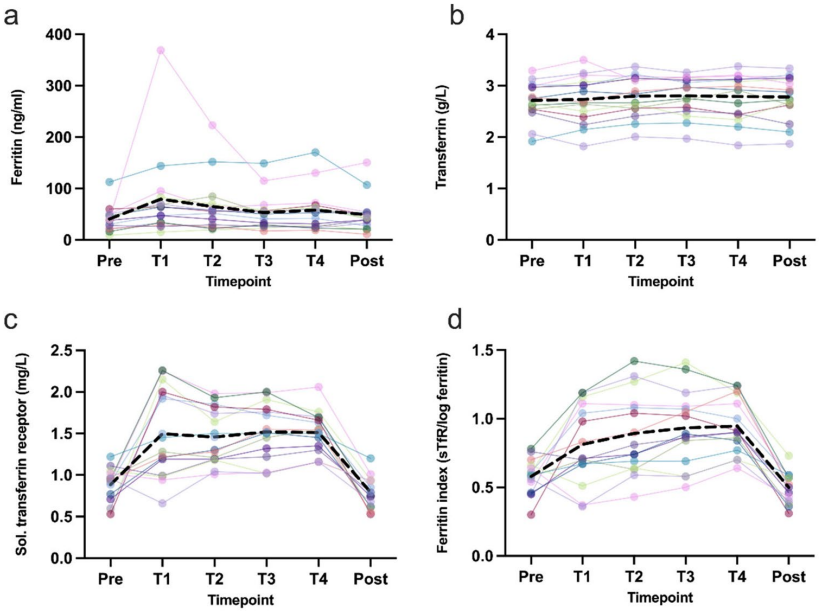

Fig. 3. Changes of iron metabolism variables over time. Shown are individual progressions (colored lines) and the mean progression (black dotted line).

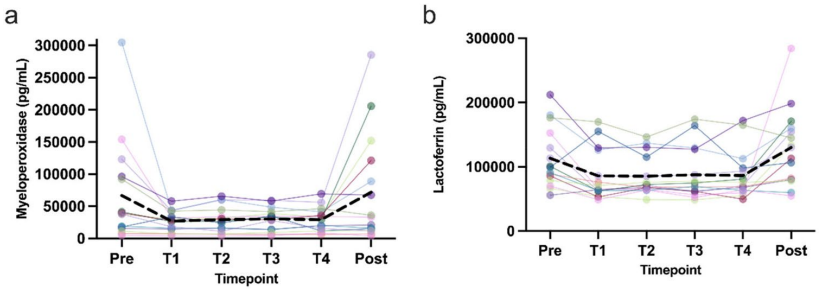

Fig. 4. Changes of products of neutrophil activity over time. Shown are individual progressions (colored lines) and the mean progression (black dotted line).

In the Granger causality network analysis, erythrocytes emerged as a significant predictor for lymphocytes ($F = 16.25$, $p < 0.01$), indicating an influence of erythrocytes on lymphocyte dynamics. Transferrin exerted a predictive effect on both thrombocytes ($F = 26.91$, $p < 0.001$) and erythrocytes ($F = 698.44$, $p < 0.001$). Ferritin was found to predict hemoglobin concentration ($F = 25.44$, $p < 0.001$), while ferritin itself was influenced by sTfR ($F = 14.14$, $p < 0.05$). Moreover, sTfR and transferrin demonstrated a bidirectional relationship (sTfR—>transferrin: $F = 13.81$, $p < 0.05$; transferrin—>sTfR: $F = 15.68$, $p < 0.05$), highlighting a mutual predictive influence between these two parameters. Neutrophils ($F = 46.06$, $p < 0.001$) and thrombocytes ($F = 21.58$, $p < 0.001$) both showed predictive effects on the ferritin index, indicating their involvement in ferritin index modulation. Additionally, myeloperoxidase (MPO) predicted lactoferrin ($F = 76.05$, $p < 0.001$), though notably, both MPO and lactoferrin are functionally isolated from the main network, suggesting a distinct subnetwork or independent regulatory pathway.

Discussion

After an average of 21 days of normobaric hypoxia, VO_2max increased significantly. However, large interindividual differences were observed and no correlation of VO_2max increases with training load.

Hematological variables showed marked adjustments, including increases in platelets, reticulocytes, and hemoglobin concentration during altitude, while erythropoietin only increased in the first 24 h. Regarding iron

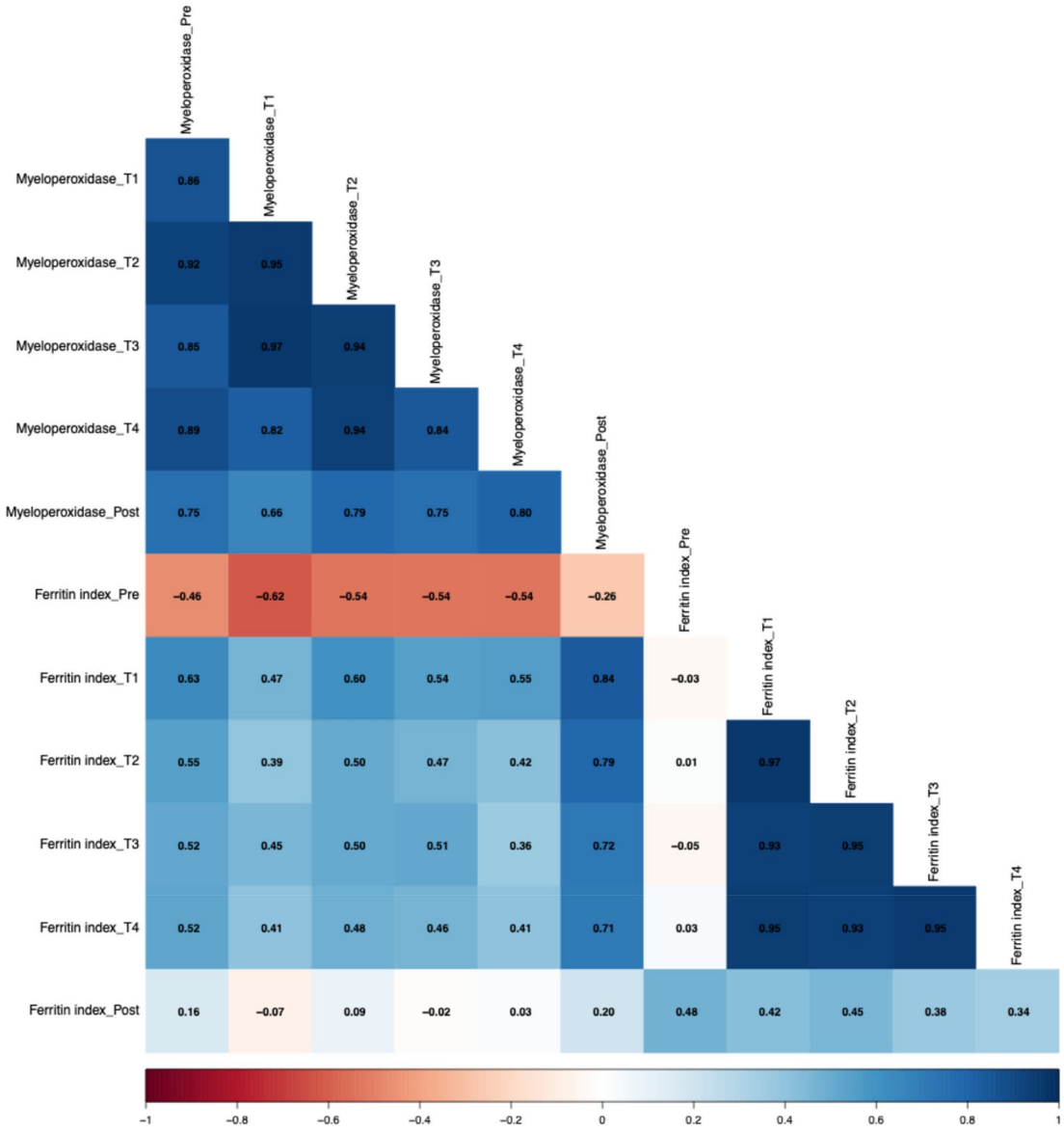

Fig. 5. Spearman correlations between the ferritin index and myeloperoxidase.

metabolism, ferritin concentration increased initially but dropped again by the end of the measurements, while sTfR showed a significant increase. MPO and lactoferrin decreased but showed correlations with iron metabolism variables. Finally, network and Granger causality analyses revealed bidirectional interactions between central hematological and metabolic parameters, with MPO and lactoferrin showing functional isolation within a subnetwork.

The performance of the athletes, assessed through VO_2max, increased following the training camp in normobaric hypoxia, though there was high interindividual variability. This variability has been widely documented in the literature and appears to depend on genetics, baseline fitness levels, nutritional status, and individual acclimatization capacity[2,14]. The extent to which the VO_2max changed is similar to that observed in comparable studies in hypobaric hypoxia suggesting that both, training camps in normobaric and hypobaric hypoxia, may elicit comparable adaptation effects on endurance performance[15,16].

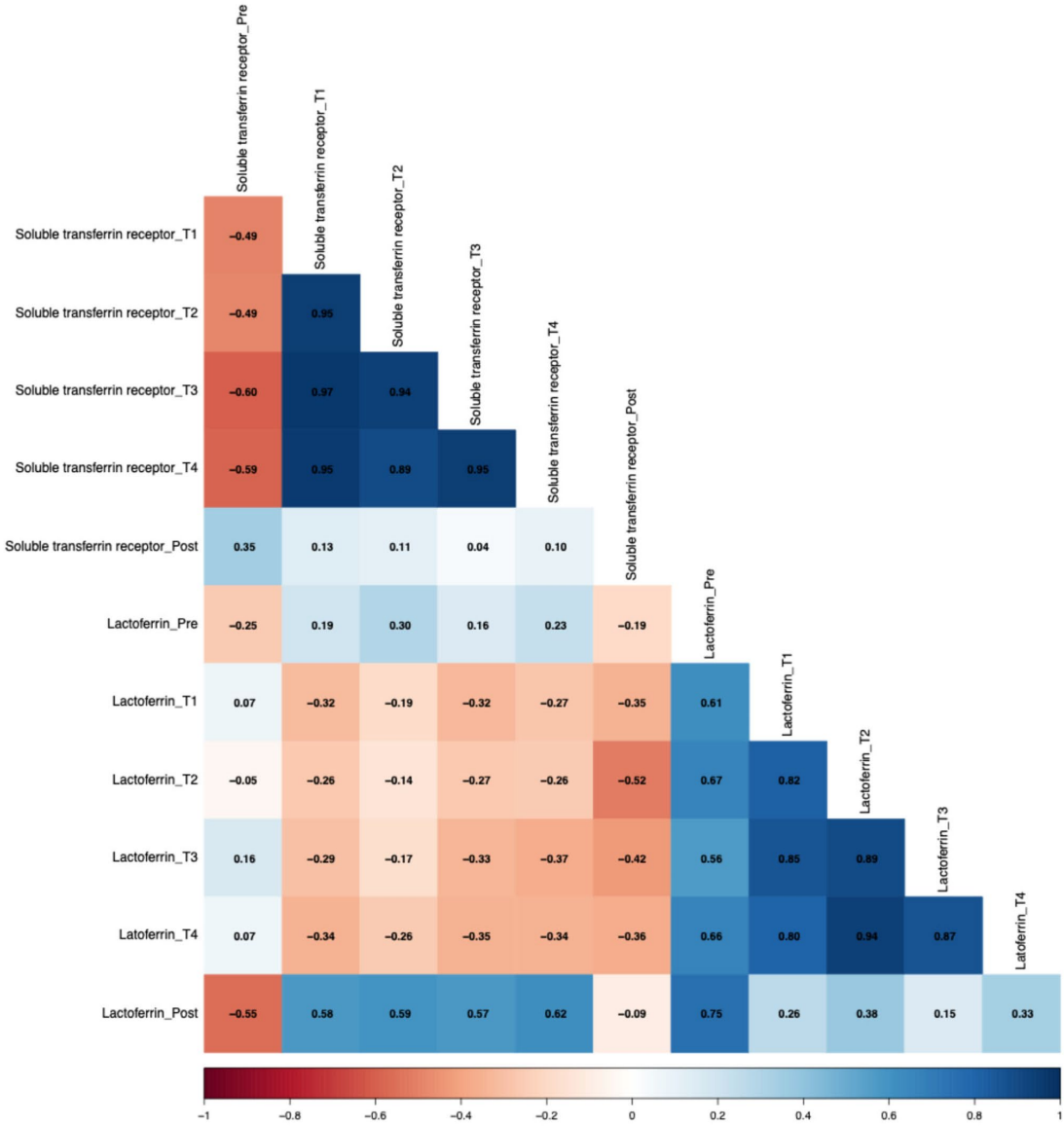

Fig. 6. Spearman correlations between soluble transferrin receptor and lactoferrin.

Hematological adaptations were also consistent with findings from studies in hypobaric hypoxia, showing an increase in hemoglobin concentration and erythrocytes[17]. The increase of sTfR and ferritin index ties erythropoiesis to adaptive responses in iron metabolism. Increased iron demand is met through suppression of hepcidin—the central regulator of systemic iron homeostasis[18,19]—mobilization of stored iron, and upregulation of intestinal iron transport. These mechanisms ensure the body has sufficient resources to sustain the hematological adaptations necessary for improved oxygen transport capacity. These findings are supported by detailed molecular and systemic studies, reinforcing the centrality of iron metabolism in hypoxic adaptation[7,20,21]. However, it must be taken into account that iron intake was not controlled in this study and that inter-individual differences may also be affected by this.

A systemic physiological connection between hematological changes and the immune system is indicated by the negative correlation between the ferritin index at pre-measurement and MPO over time. These associations might reflect the iron dependency on neutrophil function, indicated by MPO[22]. However, positive correlations were found between the ferritin index and MPO at all time points at altitude. These findings suggest that an

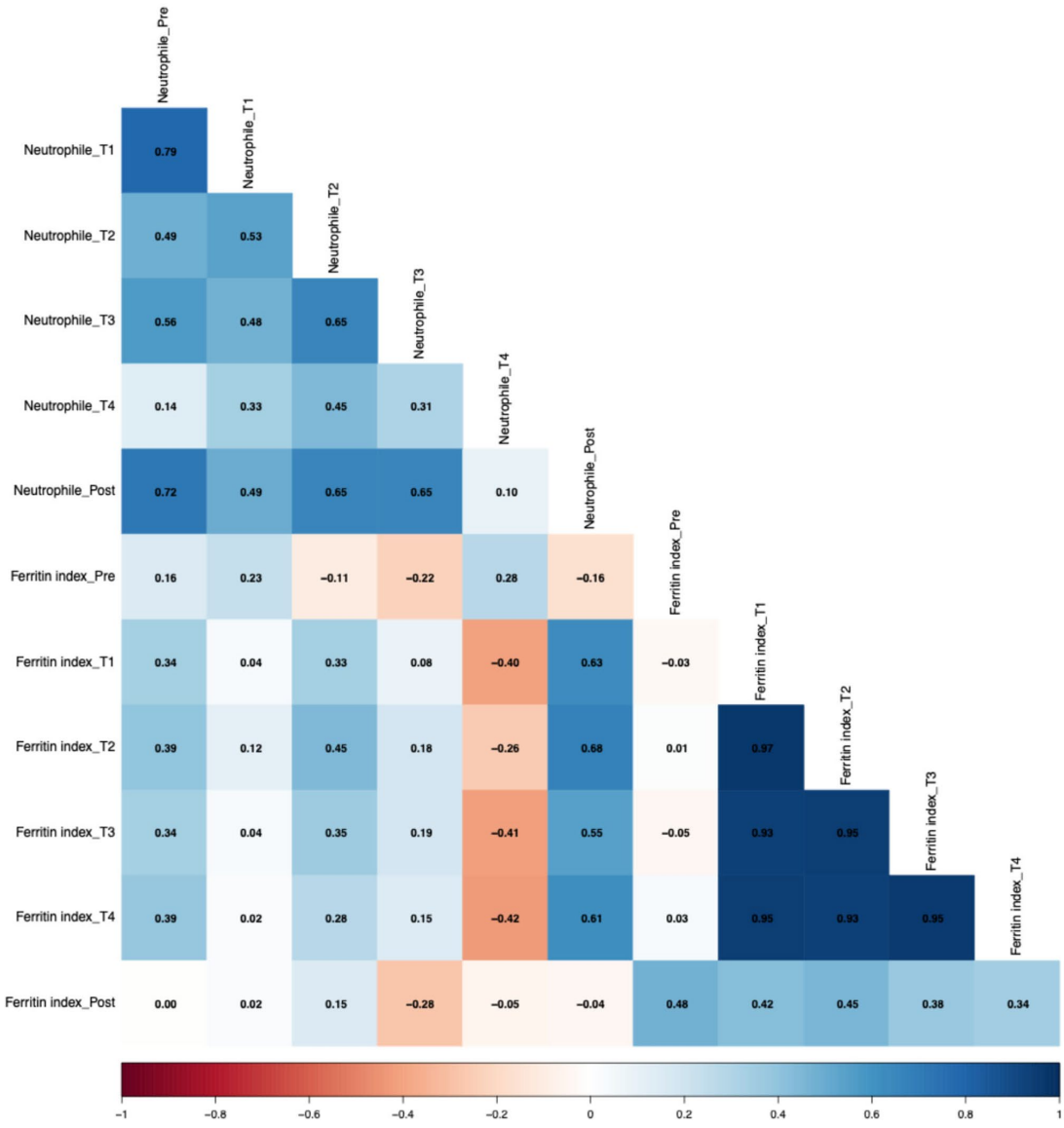

Fig. 7. Spearman correlations between the number of neutrophils and ferritin index.

increased iron demand at earlier time points may be linked to a subsequent rise in inflammatory activity, potentially as an adaptive response to altered iron availability[23]. To further understand how iron-related immune responses are modulated under hypoxic conditions lactoferrin was included in the analysis. As a neutrophil-derived glycoprotein with antimicrobial and iron-scavenging properties, lactoferrin offers insights into neutrophil-mediated iron handling. In parallel, sTfR correlates with the receptors expressed on cells that are "fishing" for iron. The expression of this receptor is upregulated when there is a higher iron demand in these cells[24]. Associations between these markers disclose an interesting interplay. Based on our data, two patterns were identified. The first is that lactoferrin at pre- and post-measurements show positive correlations with sTfr at altitude (T1–T4), with lactoferrin at post displaying moderately significant associations. The second pattern demonstrated that lactoferrin at altitude (T1–T4) shows only small but constant negative correlation with sTfr at altitude. The first pattern indicated that higher sTfR levels during altitude exposure correlated with an increase in lactoferrin levels at the end of the exposure period, reflecting adaptive changes in iron metabolism in response to altitude. This assumption is underlined through the fact that, even though correlations are not significant,

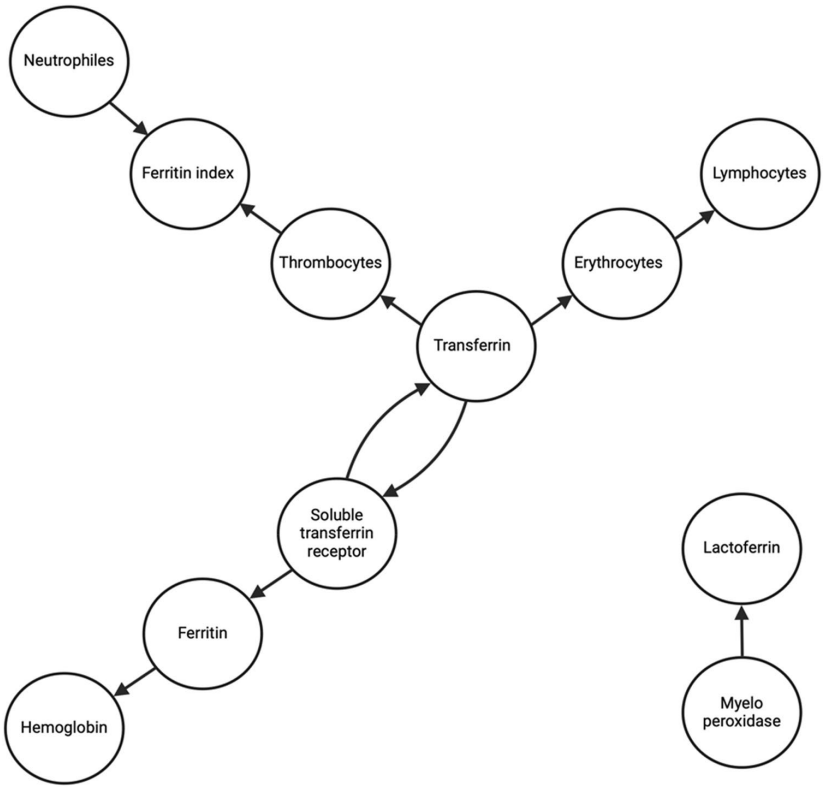

Fig. 8. Granger causality network of homological, iron metabolism and immunological variables.

there is also a negative correlation between pre-lactoferrin and sTfr at altitude. The second pattern suggests a shift in the relationship between these markers in normobaric hypoxia exposure. The interplay between those markers seems to be the same at sea level, only changing under normobaric hypoxia. The hypoxic conditions might reduce lactoferrin as a marker of neutrophil activity as a response to an increased cellular demand for iron. The positive correlations between the number of neutrophils at post-measurement and the ferritin index at altitude (T1–T4) might reflect that an increased number of neutrophils results in an increase of the ferritin index. Neutrophils also express transferrin receptors (TfRs) to ensure iron uptake for their activity in pathogen defense[22]. The increased sTfR values, consequently, result in an elevated ferritin index.

Some of the correlations can be explained more precisely by the Granger causality analysis, which provides a statistical framework that discloses potential directional influences between the parameters we measured. Granger analysis reveals a structure centered around transferrin, with three main branches extending from it and an additional subnetwork involving lactoferrin and myeloperoxidase. The central position of transferrin underscores the importance of iron availability in the body's adaptation to normobaric hypoxia, as transferrin is recognized as a key regulator for iron homeostasis[25].

The upper left branch illustrates the interplay between the innate immune response and iron metabolism (Fig. 8). Here, neutrophils and thrombocytes are shown to predict the ferritin index, suggesting the iron demands of both these cell types. This increased demand may drive the elevated expression of transferrin receptors, particularly on neutrophils, where they facilitate iron uptake and support neutrophil functions such as ROS production during pathogen defense[22]. Furthermore, thrombocytes are known to express transferrin receptor 2 (TfR2), which binds diferric transferrin and might influence platelet activation, count, and size[26]. Specifically, the inverse correlation between transferrin saturation and thrombocyte function may reflect a regulatory mechanism, as higher transferrin saturation has been associated with reduced platelet aggregation and activation. These findings highlight a coordinated role for transferrin in balancing iron metabolism across immune and hemostatic systems, linking iron availability with cellular function and homeostasis.

The lower left branch represents the hematological system's dependency on iron (Fig. 8). The bidirectional relationship between transferrin and soluble sTfR suggests a feedback mechanism where increased iron demand, reflected in elevated sTfR levels, stimulates transferrin production to facilitate iron transport[27,28]. sTfR predicts ferritin levels, as higher receptor expression indicates enhanced iron uptake at the cellular level, mobilizing ferritin-bound iron stores[29]. This mobilization suggests the prioritization of erythropoiesis during

iron deficiency or physiological demands, such as altitude adaptation, where ferritin predicts hemoglobin concentration by providing the iron reserves required for hemoglobin synthesis[30]. These findings emphasize the intricate regulatory mechanisms that govern iron metabolism, ensuring adequate erythropoiesis under varying physiological conditions.

The right branch highlights transferrin's critical role in predicting erythrocyte levels, emphasizing iron availability as a cornerstone of red blood cell production. Transferrin acts as the primary plasma iron transporter, delivering iron to erythroid precursors via TfRs, which are crucial for hemoglobin synthesis and erythropoiesis[31]. This relationship demonstrates a shared dependency of erythroid cells and lymphocytes on iron, as both require sufficient iron availability for optimal proliferation and function. At altitude, the prioritization of erythropoiesis to meet oxygen transport demands could explain the downstream effects on lymphocyte populations. This interplay is potentially mediated by EPO signaling, which activates both erythrocytes and lymphocytes through the EPO receptor (EPO-R). The response of these two very different cell types to the same growth signal could be explained by the need to maintain a relatively constant ratio even under extreme conditions, such as hypoxic stress. Erythroid precursors may exhibit a higher sensitivity for EPO, enabling them to respond more rapidly and efficiently to hypoxic stress, which aligns with the prioritization of erythropoiesis during hypoxia[32]. This dual influence of EPO on erythroid and lymphoid cells underscores the interconnected nature of the hematological and immune systems, especially under conditions of increased physiological demand, such as high altitude. Lastly, the subnetwork involving lactoferrin and MPO, with MPO predicting lactoferrin, aligns well with their respective roles in neutrophil granule function. MPO, as a component of the primary (azurophilic) granules, is among the first antimicrobial agents released during neutrophil activation and is critical for pathogen destruction but can also cause significant collateral damage and release free iron into the environment[33,34]. In contrast, lactoferrin, stored in the secondary granules, is released subsequently. This process mitigates the damage caused by MPO through its iron-binding capability, which sequesters free iron and contributes to microbial growth inhibition by depriving pathogens of this essential resource[35,36]. Thus, lactoferrin not only complements MPO's antimicrobial effects but also helps maintain immune balance by reducing potential tissue damage through iron binding.

All data and results are derived from experiments conducted under normobaric hypoxia. Therefore, any interpretation or translation to hypobaric hypoxia should be approached with caution. Due to the stringent scheduling to the training and competition planning of elite athletes, it was necessary to adhere to their individualized training programs rather than implementing a standardized training protocol during a camp in normobaric hypoxia. This may have introduced variability in training loads and adaptations across participants. Furthermore, logistical limitations restricted recruitment to three distinct groups of athletes, resulting in a relatively small sample size of 15 individuals, which may limit the generalizability of our findings. Additionally, total hemoglobin mass (tHb) was not measured due to practical and logistical constraints, representing a further limitation of this study. This may imply that hemoglobin concentration from our data could be influenced through plasma reduction which occurs in hypoxic conditions[37]. We acknowledge that hemoglobin concentration and changes in iron metabolism are also affected by other factors than normobaric hypoxia, such as iron status before the camp. To analyze this a control group would have been necessary, which we were not able to provide due to limited opportunities for intervention within the rigid seasonal planning of the athletes. We recognize the need for caution when interpreting the network due to several limitations. Granger causality cannot always distinguish direct from indirect effects and relies on temporal predictability rather than mechanistic proof. Additionally, the limited number of time points restricts the analysis to time lags of 1. Despite these limitations, it serves as an initial tool for gaining deeper and novel insights into the underlying mechanisms and for generating hypotheses for future studies.

Conclusion

The study highlights the importance of considering additional physiological systems, especially iron metabolism and the immune system, for a more comprehensive understanding of adaptation to normobaric hypoxia. The strong dependence of erythropoiesis on iron availability presents a significant challenge for maintaining adaptive immune function, which is crucial for athletes' health and performance. In practical sports applications, this highlights the importance of monitoring iron metabolism and immune function alongside hematology, training, and performance assessments. Additionally, ensuring continuous and sustainable iron availability for athletes is essential to support performance adaptations, particularly in endurance athletes using (normobaric) hypoxia to enhance performance. Future research should explore larger cohorts and incorporate measurements of total hemoglobin mass to better understand interindividual variability in adaptations. Moreover, mechanistic studies linking iron metabolism, immune function, and performance outcomes could refine altitude training protocols, contributing to more personalized approaches in sports science.

Data availability

All data is available upon request. Please reach out to Svenja.Nolte@sport.uni-giessen.de.

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Author contributions

Funding acquisition: K.H., K.K.; Conceptualization, S.N., K.H., K.K.; data collection: S.N., A.K., S.K., N.W., D.F., T.K.; data analysis: T.S., K.G., S.N., N.W., D.F., C.M.; data curation: S.K.; S.N.; statistical analysis: D.M., A.R., J.K., S.K., S.N.; visualization: D.M., A.R., J.K., S.N.; original—draft preparation, S.N.; writing—review & editing, K.H., K.K. ., D.F., T.K.; supervision: K.H, K.K.

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Declarations

Competing interests
The authors declare no competing interests.

Ethical approval and consent to participate
All authors approved the final version of the manuscript. The study was approved by the local ethics committees (MSH-2022/211). All subjects provided written informed consent to participate in the study.

Additional information
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4.3 Menstrual blood loss as an initial trigger for adaptation of iron metabolism in eumenorrheic female athletes - An exploratory study

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ORIGINAL ARTICLE

PHYSIOLOGICAL REPORTS

Menstrual blood loss as an initial trigger for adaptation of iron metabolism in eumenorrheic female athletes—An exploratory study

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Abstract

Iron deficiency is a risk factor for impaired performance and recovery. While exercise-related iron losses are well-documented, the role of menstrual blood loss (MBL) as a physiological trigger of iron regulation remains underexplored. This study examined whether MBL in eumenorrheic female athletes induces measurable hematological and iron-related responses, accounting for sex hormone fluctuations. Ten regional-level football players underwent menstrual cycle tracking and venous blood sampling in both the early follicular and mid-luteal phases. Hematological parameters, iron markers (ferritin and hepcidin), erythropoietic markers (erythropoietin and reticulocytes), and inflammatory markers (myeloperoxidase [MPO] and C-reactive protein [CRP]) were measured. MBL was assessed using the Pictorial Blood Loss Assessment Chart (PBAC). Analyses included descriptive statistics, correlation, and linear mixed modeling. MBL was significantly associated with ferritin ($\beta = -0.289$, $p = 0.001$), reticulocyte counts ($\beta = 0.004$, $p = 0.019$), and reticulocyte production index ($\beta = 0.004$, $p = 0.027$). MPO and CRP showed inverse correlations with MBL, suggesting potential immunomodulatory effects. No interaction between MBL and cycle phase was found. MBL appears to stimulate compensatory erythropoiesis in female athletes, largely independent of hormonal phase. Incorporating MBL assessment into athlete monitoring may support individualized strategies to maintain iron balance and optimize performance.

KEYWORDS

erythropoietic response, iron homeostasis, menstrual cycle monitoring, performance

1 | INTRODUCTION

Despite growing interest in sex-specific performance optimization, the physiological needs of female athletes remain underrepresented in sports science, particularly regarding factors that directly impact training adaptation and competitive performance. Iron deficiency is a striking example: iron plays a central role in oxygen transport, mitochondrial energy metabolism, and recovery, making it essential for endurance capacity and athletic output.

Yet female athletes are significantly more prone to iron depletion and deficiency, with prevalence rates ranging from 15% to 35%, compared to 3% to 11% in males (Sim et al., 2019). These figures highlight a critical and often overlooked lever for performance enhancement: addressing iron deficiency in female athletes represents a clear low hanging fruit. A physiologically meaningful yet readily addressable factor with the potential for substantial impact. Female-specific factors significantly influence iron status and the risk of deficiency (Sims et al., 2022). Despite this, these physiological and hematological factors remain underexplored in sports science. Targeted research in this area is urgently needed to support both health and performance in female athletes.

It is important to recognize that the hematological system and iron metabolism are under considerable strain in both female and male athletes engaging in exercise or high-performance sport training. The underlying causes are multifactorial and include increased iron loss through sweat, gastrointestinal micro bleeding, and hemolysis, as well as inadequate dietary intake (Peeling et al., 2008). In elite sports, this problem is particularly pronounced, as high training loads and intense exercise can accelerate iron losses, which are caused by multifactorial mechanisms (Damian et al., 2021). This negative iron balance can be exacerbated by changes to iron regulation in response to the transient acute inflammatory response following exercise. While moderate activity can promote anti-inflammatory effects, prolonged or intense exercise provokes a pro-inflammatory milieu, thereby intensifying the problem when the intensity is very high and recovery time is often insufficient. This includes elevated cytokine production, which stimulates hepatic expression of hepcidin, a peptide hormone that serves as the master regulator of iron homeostasis (Ganz, 2003). Hepcidin restricts dietary iron absorption and sequesters iron in storage sites, reducing circulating iron availability in a process termed nutritional immunity. This adaptive mechanism, while protective in the context of infection, may inadvertently limit iron availability for erythropoiesis and metabolic demands in athletes (Ganz & Nemeth, 2024).

Against this background, female athletes face a unique additional burden: menstrual bleeding as the main source of iron loss. Menstrual iron losses are estimated to range from 10 to 40 mg per cycle (Angeli et al., 2016) and can therefore significantly impact iron balance, especially when combined with exercise-induced losses and increased erythropoietic demands (Bruinvels et al., 2021). Despite its physiological relevance, menstrual status is frequently omitted in iron-related sports science research—indeed, 65% of studies on iron supplementation fail to report menstrual cycle phase or status (Smith et al., 2022). Preliminary evidence has suggested that iron regulation may vary across the menstrual cycle. Notably, the IronFEMME research group reported phase-dependent changes in iron status markers but did not observe consistent effects of estrogen on hepcidin levels or post-exercise iron responses, suggesting a more complex interplay (Alfaro-Magallanes et al., 2022; Barba-Moreno et al., 2020). However, it is important to note that participants in these studies generally exhibited low baseline iron status and stores, a factor that may have confounded the interpretation of hormonal influences on hepcidin. Given that iron status itself is considered one of the strongest predictors of hepcidin activity, it is plausible that fluctuations in hepcidin in these cohorts were primarily driven by iron demand, with secondary modulation by other factors such as inflammation, altitude, or sex hormones. In this context, menstrual blood loss, characterized by acute iron loss and increased iron demand, may exert a stronger influence on hepcidin regulation than fluctuations in sex steroid hormones alone.

Therefore, in this study we investigate menstrual blood loss as a potential trigger for acute hematological and iron-regulatory adaptations. Specifically, we examine whether menstruation in eumenorrheic female athletes induces a compensatory erythropoietic response, potentially mediated by shifts in iron metabolism. A better understanding of these dynamics could help inform individualized strategies for iron monitoring and supplementation in female athletes.

2 | METHODS

2.1 | Subjects

Ten female football players (age: 24.5 ± 4.4 years; BMI: 22.8 ± 1.4 kg/m) from two regional football clubs were recruited. All athletes had been playing competitively for several years and were actively competing in the third and fourth tiers of the German women's football league system at the time of data collection. Included participants met the following criteria: (a) healthy adult females between 18 and 35 years; (b) no acute or chronic infection; (c) no consumption of medication; (d) regular menstrual cycles, defined by Elliott-Sale et al. (2021) as a cycle length of 21–35 days; (e) females with eumenorrhea (no hormonal contraception or implants for at least 6 months prior to initial tracking). To confirm eligibility, participants completed a self-reported questionnaire on contraception. The study received approval from the local ethics committee of the University of Giessen (No. 2024-0014) and was conducted following the Declaration of Helsinki for human research. Prior to participation, all individuals

were informed about the potential risks and benefits of the study and provided written informed consent, as per institutional guidelines.

2.2 | Menstrual cycle tracking

Menstrual cycle tracking followed a strict, three-step protocol described in Peinado et al. (2021). Prior to initiating the tracking process, all athletes completed a questionnaire assessing their use of hormonal contraception or implants, the onset of menarche, history of pregnancy, and the perceived regularity of their menstrual cycles. Five months prior to the scheduled blood sampling, all participants were trained in the symptothermal method for cycle tracking. This method combines daily monitoring of basal body temperature, cervical mucus consistency, and other fertility signs such as cervical position to estimate ovulation and determine fertile and infertile phases of the menstrual cycle. From that point onward, they completed a standardized self-report questionnaire documenting these fundamental cycle characteristics, which was then manually summarized by the study teams. The collected data were used to determine the optimal timing for luteinizing hormone (LH) testing, which was initiated after 3 months of tracking. Participants received email notifications from the study team with instructions on when to begin and conclude LH testing and were required to submit a photograph of their positive test result. The study team determined whether the LH test result according to the photograph should be considered positive. This combined procedure—symptothermal tracking and LH testing—was conducted over two consecutive cycles. In the third and fourth cycles, participants were scheduled for blood sampling at two time points within the menstrual cycle while continuing to adhere to both tracking steps (symptothermal and LH-testing) (Figure 1).

2.3 | Blood sampling and analysis

Participants underwent two blood draws: the first during the early follicular phase (EFP; cycle days 3–5), and the second during the mid-luteal phase (MLP), scheduled 7–9 days after a positive urinary luteinizing hormone (LH) test. Athletes were classified as eumenorrheic if they exhibited a positive LH test, met the single time point progesterone threshold ($\geq 16\,nmol \cdot L^{-1}$), and had a luteal phase that was longer than 10 days (Elliott-Sale et al., 2021).

All blood samples were collected between 7:00 and 9:00 A.M. after an overnight fast. Participants were instructed to refrain from intense exercise the day before each sampling and to ensure a minimum of one rest day following their most recent competition. A brief questionnaire was administered to assess recent training load and current health status to rule out subjective feelings of an acute illness or infection. Three venous blood samples were collected in 7.5 mL Serum Gel, two 7.5 mL EDTA, and 2.7 mL EDTA tubes (Sarstedt, Nümbrecht, Germany). The 7.5 mL EDTA samples were immediately centrifuged at 2500*g* for 10 min at room temperature. Plasma was aliquoted into Eppendorf tubes, placed on dry ice for transport, and subsequently stored at −80°C until analysis.

Hormonal parameters—including testosterone (measured once in EFP), follicle-stimulating hormone (FSH), luteinizing hormone (LH), estradiol, and progesterone—were analyzed via chemiluminescent immunoassay (CLIA) from plasma samples by an accredited medical laboratory (SYNLAB Medical Care Center, Bad Nauheim, Germany).

Hemoglobin, hematocrit, and red blood cell count were measured using automated impedance and photometric methods (ADVIA systems). Ferritin, transferrin, and soluble transferrin receptor (sTfR) were quantified using immunoassays (ELISA); the ferritin index (sTfR/log ferritin) was calculated accordingly, and hepcidin was

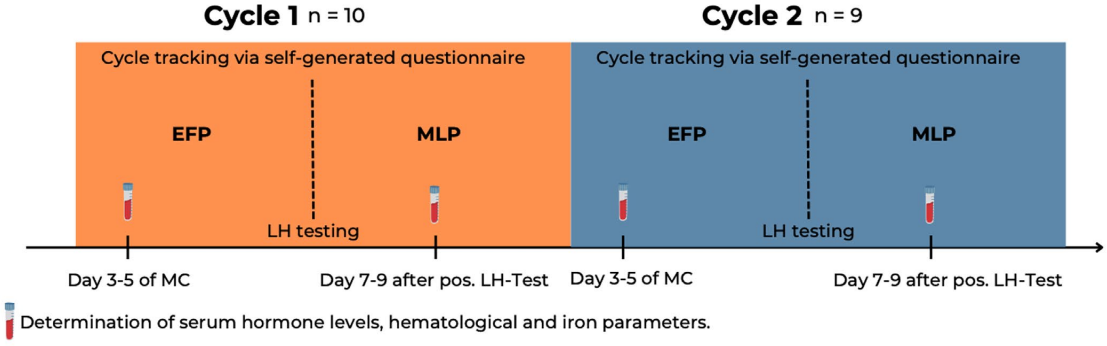

FIGURE 1 Study design.

analyzed by liquid chromatography coupled with mass spectrometry (LC–MS). In addition, markers of neutrophil activation (myeloperoxidase (MPO) and lactoferrin) were quantified from 50-μL plasma samples in duplicates using a magnetic Luminex ELISA assay (Bio-Techne Ltd., Cat# LXSAHM-08, Abingdon, Oxon, UK).

2.4 | Menstrual blood loss validation

To validate the assessment of menstrual blood loss (MBL), a pictorial blood loss assessment chart (PBAC) was used. The PBAC serves as a noninvasive and cost-effective tool for estimating MBL by recording the usage and degree of saturation of single-use sanitary products. Based on the PBAC entries, the Higham Score was calculated, which serves as an objective and standardized measure of MBL (Higham et al., 1990; Zakherah et al., 2011).

Participants were provided with the same brand of single-use sanitary products to minimize variability in absorbency and instructed to document their menstrual bleeding using the PBAC over the course of the two cycles where venous blood samples were collected. As part of the PBAC, participants were asked to report the number of tampons and pads used, along with their degree of saturation (light, moderate, or fully soaked). Additionally, they were asked to document the occurrence and frequency of menstrual clot passage. The completed PBAC was returned to the study team at the time of the blood draw during the EFP.

2.5 | Statistical analysis

All statistical analyses were conducted using Microsoft Excel (Microsoft Corporation, Redmond, WA, USA) and JASP (Version 0.17.2.1, University of Amsterdam, Netherlands). Data are presented as mean ± standard deviation (SD), unless otherwise stated. Normality of distribution was assessed using the Shapiro–Wilk test. To evaluate the consistency of repeated measurements across two consecutive menstrual cycles, intraclass correlation coefficients (ICCs) were calculated. The results indicated moderate-to-high agreement for most variables, supporting the use of both cycles in the statistical models. This approach ensured the inclusion of biologically plausible variation while improving statistical reliability and power.

To examine differences between the early follicular phase (EFP) and the mid-luteal phase (MLP), paired *t*-tests were applied for normally distributed variables; otherwise, the nonparametric Wilcoxon signed-rank test was used.

A quotient of serum ferritin (sFer) to MBL was calculated to explore the relationship between iron stores and individual bleeding volume. This index was developed to facilitate a more practical interpretation of iron status in athletes.

To assess associations between variables, Pearson correlation coefficients were calculated for normally distributed data. These correlations were used to explore relationships between menstrual blood loss (MBL), reticulocyte (%), quotient (sFer/MBL), and EPO and hepcidin.

All tests were two-tailed, and statistical significance was defined as $p < 0.05$.

Figures and visualizations were generated using R (Version 4.5.0, R Foundation for Statistical Computing, Vienna, Austria) and JASP.

In a final exploratory step, potential associations between variables of iron metabolism, hematological parameters, cytokines, hormones, and menstrual blood loss (MBL) were analyzed using linear mixed models (LMM). The models of LMM accounted for both fixed and random effects, allowing for the dependency of repeated measurements within participants to be appropriately modeled. The dependent variables were grouped into four categories: iron metabolism parameters, hematological parameters, cytokines, and hormones. Fixed effects included menstrual cycle phase and menstrual blood loss (MBL). Additionally, an interaction term between cycle phase and MBL was included to test whether the impact of cycle phase on the dependent variables varied according to the extent of menstrual blood loss. This interaction was specified based on the hypothesis that greater blood loss during menstruation might influence iron status, as well as hematological and immunological parameters, in a cycle-phase-dependent manner. To control for interindividual differences, a random intercept was included in the model. Maximum likelihood estimation (ML) was used, and likelihood ratio tests assessed the significance of model terms. Unstandardized regression coefficients (B) were reported for the fixed effects, reflecting the practical relevance of findings in the original measurement units of the dependent variables. The significance level was set at $\alpha = 0.05$.

3 | RESULTS

3.1 | Hormonal and cycle characteristics

Table 1 displays menstrual cycle characteristics, including the cycle length, luteal phase length, and the Higham score for all participants collected over 2 months. Estrogen, progesterone, LH, and FSH concentrations in both the EFP and MLP, measured over two cycles, are summarized in Table 2. Testosterone was measured in

TABLE 1 Menstrual cycle characteristics across two cycles.

Descriptive	Cycle length (days)	Luteal phase length (days)	Higham-score
Mean (Median)	29.37	13.89	(97.5)
SD (IQR)	4.73	3.56	(57.25)

TABLE 2 Hormonal profiles of two cycle phases across two cycles.

Cycle phase	Descriptive	Testosterone (ng/mL)	Estrogen (pg/mL)	Progesterone (ng/mL)	LH (mU/mL)	FSH (mU/mL)
EFP	Mean	0.45	36.89	0.41	4.71	7.11
	SD	0.10	16.84	0.19	1.12	1.33
MLP	Mean		127.28	13.56	4.84	3.51
	SD		49.48	5.92	2.30	1.49

the EFP of the first cycle. A significant time effect for estrogen, progesterone, and FSH ($p < 0.001$) between EFP and MLP was found. LH showed no significant time effect across the two cycles.

3.2 | Changes of hematological and iron metabolism parameters in different cycle phases

Figures 2 and 3 illustrate differences in hematological and iron metabolism parameters between the EFP and MLP. Erythropoietin and reticulocytes were higher in the EFP compared to the MLP, whereas erythrocytes, hemoglobin, and hematocrit were higher in the MLP compared to the EFP. However, none of these differences were statistically significant. For ferritin and MPO, nonsignificantly higher values were found in the EFP compared to MLP. Hepcidin, lactoferrin, sTfR, and transferrin were higher in the MLP compared to the EFP without reaching statistical significance. Only hepcidin showed a significant time effect ($p < 0.05$).

A significant correlation was found between reticulocytes and menstrual blood loss (Higham Score) ($r = 0.64$, $p < 0.05$), as demonstrated in Figure 4a. In contrast, a nonsignificant association was observed between the percentage of reticulocytes and the quotient sFer/MBL ($r = -0.61$, $p = 0.09$) (Figure 4b). While no correlation was found between menstrual blood loss MBL and EPO, a significant negative correlation was identified between hepcidin and EPO in the EFP ($r = -0.71$, $p < 0.05$) (Figure 4c).

3.3 | Linear mixed model

Associations between variables of iron metabolism, hematological parameters, cytokines, hormones, and MBL are demonstrated in Figure 5. Ferritin and the ferritin index showed significant associations with MBL. Specifically, there was a significant negative association between MBL and ferritin levels ($\beta = -0.289$, SE = 0.085, 95% CI [−0.460, −0.118], $p = 0.001$), indicating that higher menstrual blood loss was linked to lower ferritin concentrations. In contrast, no significant effects of cycle phase or the interaction between cycle phase and MBL were observed for ferritin. Similarly, there was a significant positive association between MBL and the ferritin index ($\beta = 0.005$, SE = 0.002, 95% CI [0.001, 0.009], $p = 0.010$), suggesting that increased menstrual blood loss corresponded to higher ferritin index values. Again, neither cycle phase nor its interaction with MBL showed significant effects on this parameter. All other analyzed markers of iron metabolism did not reveal any significant associations.

Regarding hematological parameters, we found a significant positive association between MBL and erythrocyte levels ($\beta = 0.002$, SE = 0.001, 95% CI [0.0001, 0.004], $p = 0.040$), and between MBL and reticulocyte levels ($\beta = 0.004$, SE = 0.002, 95% CI [0.001, 0.008], $p = 0.019$). Furthermore, a significant positive association was found between MBL and the reticulocyte production index ($\beta = 0.004$, SE = 0.002, 95% CI [0.001, 0.007], $p = 0.027$). For all three hematological parameters, neither cycle phase nor the interaction between cycle phase and MBL demonstrated significant effects. No further associations were detected in the remaining hematological markers.

Cytokine analysis revealed that higher MBL was significantly and negatively associated with MPO levels ($\beta = -69.30$, SE = 10.98, 95% CI [−91.55, −47.05], $p < 0.001$), indicating that greater blood loss corresponded to lower MPO concentrations. Neither cycle phase nor its interaction with MBL had a significant effect on MPO. Similarly, MBL showed a significant negative association with CRP levels ($\beta = -0.001$, SE = 0.000439, 95% CI [−0.002, −0.0003], $p = 0.003$). No significant effects were

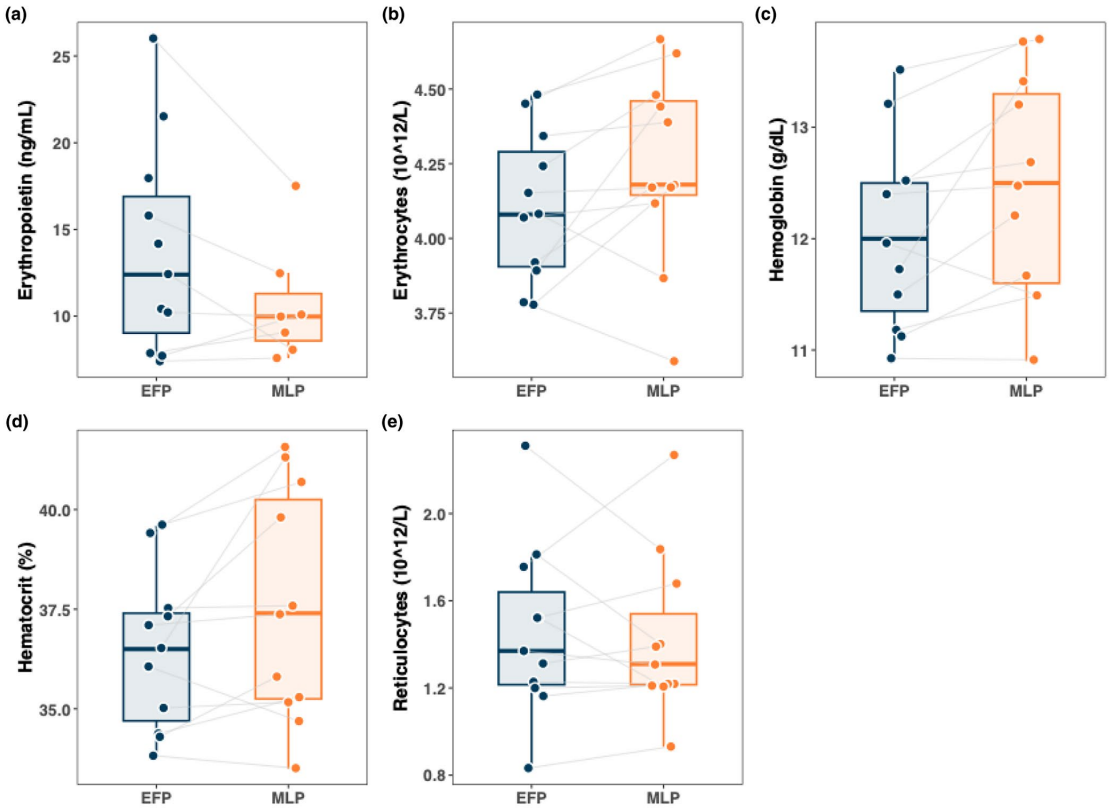

FIGURE 2 Comparison of hematological parameters between EFP and MLP.

found for cycle phase or its interaction with MBL in relation to CRP.

For hormonal parameters, estradiol and progesterone were significantly influenced by cycle phase. Estradiol levels were significantly lower in the early follicular phase (EFP) compared to the mid-luteal phase (MLP) ($\beta = -46.69$, SE = 12.78, 95% CI [−72.53, −20.84], $p < 0.001$). However, no significant associations were observed between estradiol levels and MBL, nor was there a significant interaction between cycle phase and MBL. Similarly, cycle phase was a significant predictor of progesterone levels ($\beta = -6.43$, SE = 1.48, 95% CI [−9.44, −3.41], $p < 0.001$), with lower progesterone concentrations found in the EFP compared to the MLP. Again, no significant associations were observed between progesterone levels and MBL, nor was there a significant interaction effect.

4 | DISCUSSION

Our findings demonstrate that MBL is significantly associated with key hematological and iron-related parameters. Specifically, greater MBL correlated with lower ferritin concentrations, elevated reticulocyte counts, and higher reticulocyte production index. Such results would suggest a compensatory erythropoietic response to iron loss during menstruation. Moreover, a negative association between MBL and inflammatory markers such as MPO and CRP was found, indicating potential immunological adjustments alongside hematological changes that occur during menstruation.

These findings align with prior evidence that iron metabolism in female athletes is shaped by both training-related demands and menstrual blood loss (Badenhorst et al., 2021; Pedlar et al., 2018). The novel contribution of this study lies in the observation that even within athletes, variation in MBL can modulate iron availability and erythropoietic activity, independent of cycle phase. This finding aligns with growing research indicating that the magnitude of iron regulation, particularly hepcidin activity, is primarily influenced by iron status and iron demand. Regulating factors, such as sex hormone levels, exert a secondary effect (Sim et al., 2019). Importantly, the lack of interaction effects between cycle phase and MBL suggests

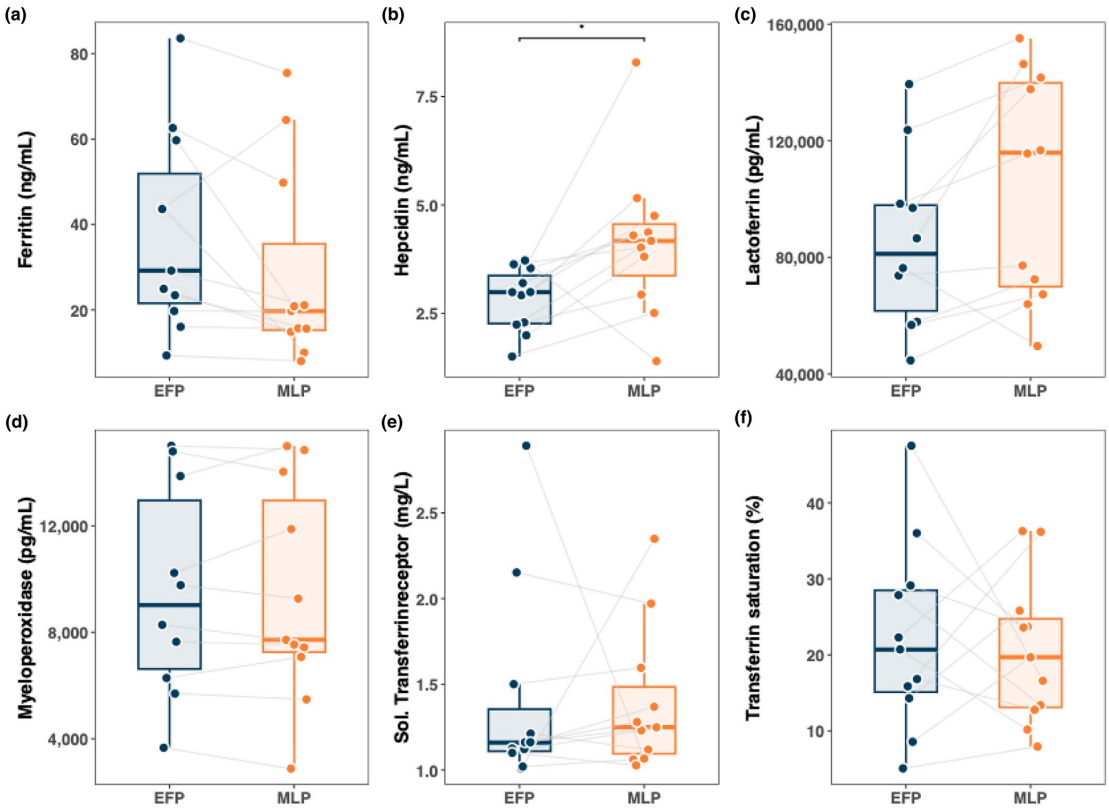

FIGURE 3 Comparison of iron metabolism parameters between EFP and MLP. Asterisks indicate statistically significant differences ($p < 0.05$).

that iron-related adaptations are more directly linked to the magnitude of blood loss than to fluctuating sex hormone levels per se.

4.1 | Menstrual cycle characteristics and hormonal fluctuations

The mean cycle length, luteal phase duration, and Higham Score fall within established reference ranges (Skiba et al., 2019), suggesting our participants had regular menstrual cycles and typical menstrual bleeding patterns.

Estrogen, progesterone, and FSH showed significant differences between the early follicular phase (EFP) and the mid-luteal phase (MLP), which reflects the characteristic hormonal fluctuations of the menstrual cycle, low ovarian hormone levels during the EFP and elevated estrogen alongside peak progesterone during the MLP (Guo et al., 2015; Rishpon-Meyerstein et al., 1968). These hormonal fluctuations must be considered when interpreting downstream physiological processes such as erythropoiesis and iron regulation. In this study, the cohort displayed eumenorrheic sex steroid hormonal concentrations. Previous research has reported variations in iron status throughout the eumenorrheic cycle, with suggested contributions from sex steroid hormone concentrations (Alfaro-Magallanes et al., 2022). For instance, some studies have indicated that fluctuations in estrogen and progesterone levels may influence iron metabolism and erythropoietic activity (Hamad et al., 2020; Li et al., 2016). However, the associations described in this study appear to be largely independent of the menstrual cycle phase. Despite substantial variations in circulating estrogen and progesterone levels across the cycle, the relationship between cycle phase and iron metabolism was not observed in this data. This suggests that the regulatory mechanisms governing erythropoiesis and iron mobilization in response to menstrual blood loss may operate independently of cyclical hormonal changes. Our results differ from previous findings in that they highlight the primary role of blood loss in modulating iron availability and erythropoietic activity, rather than the secondary

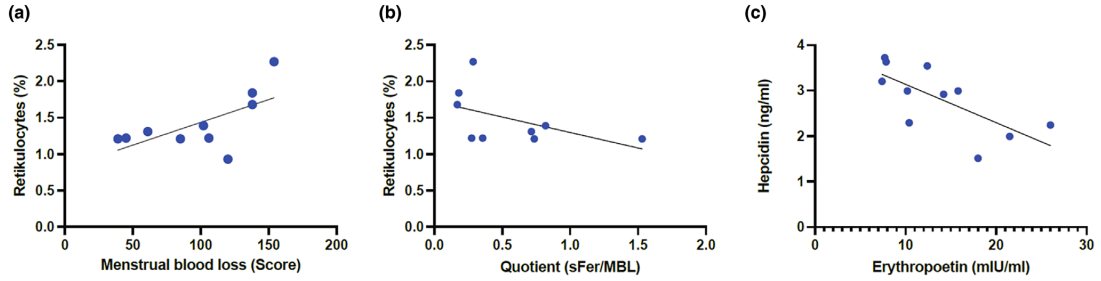

FIGURE 4 Correlations between hematological variables, menstrual blood loss, and iron metabolism variables.

effects of sex hormone fluctuations. These findings are consistent with previous research demonstrating that menstrual phase-related hormone fluctuations did not alter erythropoietic responses in women living at high altitude (Reeves et al., 2001). Nevertheless, it is important to acknowledge that physiological systems are highly interconnected, and subtle or indirect interactions cannot be entirely ruled out.

4.2 | Hematological and iron regulatory adaptation to menstrual blood loss

Despite cyclical hormonal fluctuations and menstrual blood loss, hemoglobin, hematocrit, and red blood cell (RBC) counts remained stable across the menstrual cycle, indicating that oxygen transport capacity is maintained. This suggests that, in athletes with sufficient iron reserves, menstrual blood loss does not immediately impair hematological parameters.

However, mean ferritin (37.83 ± 23.08 ng/mL) and hemoglobin levels (12.05 ± 0.86 g/dL) measured in the EFP were close to thresholds indicative of suboptimal iron status (Nolte et al., 2024). While these values were sufficient to maintain stable hematological parameters, they suggest a generally lower iron reserve in this athletic cohort, potentially increasing their relative susceptibility to iron deficiency. This is consistent with prior findings showing a higher prevalence of iron deficiency in the female athletic population (Pengelly et al., 2025).

A positive correlation between MBL and reticulocyte count was observed, particularly in the MLP, indicating a compensatory increase in red blood cell production over time. This aligns with the findings from Mullen et al., who showed that reticulocyte percentage was significantly lower in the follicular and ovulatory phase (Mullen et al., 2020). Since erythropoiesis is a time-dependent process, this temporal relationship supports the physiological mechanism of delayed hematological response to blood loss. The association was further influenced by iron availability: when the ratio of ferritin to MBL was low—suggesting depleted iron stores relative to blood loss—reticulocyte counts were higher. This pattern reflects an increased erythropoietic drive in response to iron stress and emphasizes that iron-deficient individuals may require a stronger compensatory response to maintain hematological balance. Such a response is characteristic of stress erythropoiesis, a form of emergency red blood cell production triggered by acute or chronic blood loss, iron deficiency, or other physiological stressors (Ruan & Paulson, 2023). In contrast to steady-state erythropoiesis, stress erythropoiesis is associated with increased EPO activity and enhanced proliferation of erythroid progenitors, often under conditions of limited iron availability (Paulson et al., 2011, 2020). This mechanism appears to be relevant in female athletes, whose physiological baseline includes recurrent blood loss through menstruation combined with athletic training loads. Given the predictable and cyclical nature of menstrual bleeding, it is conceivable—albeit speculative—that such erythropoietic flexibility may reflect an evolutionary adaptation to recurring hematological stress in women. While direct evidence for this hypothesis is currently lacking, the regular activation of erythropoietic mechanisms in response to menstrual blood loss could represent a biologically conserved strategy to preserve oxygen-carrying capacity and physiological resilience under conditions of repeated iron loss.

Changes in hematological and iron metabolism markers across the menstrual cycle were relatively subtle, with hepcidin being the only parameter to show a significant time effect, higher in the MLP. Beyond temporal effects, several associations emerged independently of the cycle phase. Reticulocytes, erythrocyte numbers, and the reticulocyte production index all showed significant positive associations with MBL. MBL was also positively associated with red blood cell counts, while ferritin and ferritin index were negatively associated, regardless of cycle phase, indicating that the physiological response to MBL operates independently

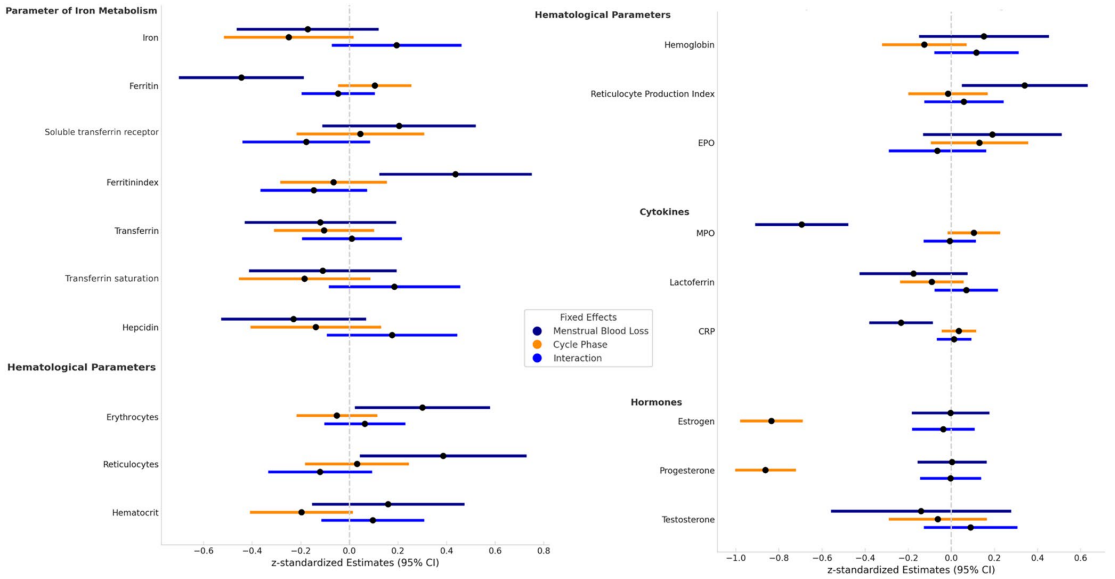

FIGURE 5 Forest plot illustrating the results of the linear mixed model analyses examining the associations between menstrual blood loss (MBL), menstrual cycle phase, and their interaction with parameters of iron metabolism, hematological markers, cytokines, and hormones. Displayed are standardized regression coefficients (β) and their 95% confidence intervals (CI) for each predictor across outcome variables.

of fluctuating sex hormones. These findings support the concept that menstrual blood loss acts as a key driver of adaptive hematological responses. Iron status and demand thus appear to be the primary regulators of iron metabolism, overriding cyclical hormonal influences. This is consistent with the well-established notion that iron deficiency is primarily caused by acute or chronic blood loss, with menstruating women representing a high-risk group (Charlton & Bothwell, 1982; Hallberg & Rossander-Hultén, 1991). The observed negative association between MBL and ferritin highlights the depletion of iron reserves during menstruation, while a concurrent increase in the ferritin index suggests an attempt to preserve erythropoiesis under iron-limited conditions (Infusino et al., 2012). Supporting this hypothesis, soluble transferrin receptor (sTfR) concentrations may also rise, reflecting increased cellular demand for iron to support red blood cell production (Harms & Kaiser, 2015). In summary, the combined evidence of stable hemoglobin alongside rising reticulocyte counts, declining ferritin levels, and phase-independent associations with MBL supports a coordinated erythropoietic adaptation to menstrual blood loss in female athletes. These adjustments are consistent with physiological compensation mechanisms and point to the importance of iron availability in sustaining erythropoiesis during recurring blood loss (Silvestri & Nai, 2021).

4.3 | Regulation of hepcidin and erythropoiesis

Focusing specifically on the hormonal regulation of iron metabolism, our data revealed a distinct phase-dependent pattern in the interplay between EPO and hepcidin. In the EFP, when menstrual blood loss likely triggers compensatory erythropoiesis, we observed a significant negative correlation between EPO and hepcidin levels. This aligns well with the physiological model in which EPO stimulates erythropoiesis and concurrently suppresses hepcidin to facilitate iron mobilization, a key mechanism in stress erythropoiesis (Paulson et al., 2020; Ruan & Paulson, 2023).

Interestingly, this regulatory relationship appeared to shift in the MLP. While EPO levels declined, hepcidin concentrations were significantly higher compared to the EFP. This pattern suggests that the erythropoietic drive, and thus the suppressive effect of EPO on hepcidin, diminishes as the cycle progresses. The rise in hepcidin during the MLP may therefore reflect a return to baseline regulation once the acute need for iron mobilization subsides.

One possible contributing factor is the concurrent increase in progesterone levels during the luteal phase, which has been shown to upregulate hepatic hepcidin expression (Li et al., 2016). However, our data do not

support a direct association between hepcidin levels and menstrual cycle hormones; suggesting that the observed regulation may occur independently of progesterone fluctuations.

Taken together, these findings support the hypothesis that female athletes may undergo cyclical, low-grade states of stress erythropoiesis in response to menstrual blood loss, requiring finely tuned hormonal (EPO) and iron regulatory mechanisms to maintain oxygen transport capacity. The elevated hepcidin levels in the MLP, while initially counterintuitive and in contrast to previous literature reporting suppressed hepcidin under erythropoietic stress (Yang et al., 2012), can thus be understood as part of a temporally dynamic regulatory system, influenced by erythropoietic signals.

4.4 | Inflammatory regulation and immune modulation

Additionally, we found a negative association between myeloperoxidase (MPO), a marker of neutrophil activity, and MBL. Importantly, blood samples were obtained at rest to avoid exercise-induced transient changes in MPO levels, which have been previously described in the literature (Rooney et al., 2018). By controlling for this factor, we aimed to investigate whether interindividual differences in resting inflammation relate to MBL, rather than short-term exercise effects. This finding may reflect an iron-dependent impairment of neutrophil function, as sufficient iron availability is crucial for optimal immune cell activity (Kuźmicka et al., 2022; Maneva & Taleva, 2009).

Furthermore, C-reactive protein (CRP), a marker of systemic inflammation, has previously been reported to fluctuate across the menstrual cycle, with inverse associations to estrogen and positive associations with progesterone (Wander et al., 2008). In our study, however, CRP was negatively associated with MBL but showed no significant variation according to menstrual cycle phase. It is speculated that the reason for this inverse relationship is that a reduction in CRP—and thus in systemic inflammation—may represent a regulatory strategy to suppress hepcidin levels. Since hepcidin inhibits iron absorption and release, its downregulation would facilitate iron availability for erythropoiesis in the context of increased iron demand (Ganz, 2003), here in response to MBL.

Together, these associations point to a possible role of resting inflammatory tone in iron regulation, independent of acute inflammatory events in menstruating female athletes.

4.5 | Limitations

While these findings are promising, several limitations should be acknowledged. The relatively small sample size, primarily resulting from logistical challenges and strict methodological criteria, particularly regarding menstrual cycle classification, may limit statistical power and generalizability. Additionally, given the exploratory nature of the linear mixed model, no correction for multiple testing was applied, increasing the potential risk of type I errors. As such, results should be interpreted with appropriate caution and regarded as hypothesis-generating. Moreover, the current findings are limited to a specific subgroup, eumenorrheic, hormonally non-contracepting athletes, and may not be directly transferable to nonathletic populations, individuals with menstrual irregularities, or those using hormonal contraception. Nonetheless, the application of z-standardization across outcomes and visualization of standardized effect sizes in forest plots enhances interpretability and provides a strong foundation for future confirmatory research.

5 | CONCLUSION

This study supports the notion that iron status and demand are the primary modulators of iron metabolism. In females, MBL may be considered a primary relevant modulator of iron status and demand as higher levels of MBL will negatively affect iron status and increase iron demand; as a result, influence iron metabolism. By quantifying MBL in this population for the first time, we demonstrated that greater blood loss is associated with lower ferritin levels and increased hematological signs of stimulated erythropoiesis, such as elevated reticulocyte and erythrocyte counts. These findings suggest that erythropoietic adaptation to blood loss occurs independently of cyclical sex hormone fluctuations, underscoring MBL as a direct contributor to hematological regulation. Importantly, this study highlights the value of integrating MBL quantification into female athlete health monitoring. Conventional markers like ferritin and hemoglobin may not sufficiently capture ongoing physiological demands or early stages of iron depletion, particularly in the absence of anemia or hypoxia. In contrast, a combined evaluation of MBL, iron storage indicators, and markers of erythropoietic activity such as reticulocyte counts offers a more individualized and physiologically sensitive approach for female athletes.

The findings of this study underscore the value of incorporating MBL as a physiological parameter in athlete health and performance monitoring. Acknowledging

individual variability in MBL enables more timely and tailored interventions, ranging from nutritional strategies to optimize iron intake and absorption to temporary modifications in training load during periods of increased physiological strain. Notably, while MBL is a key determinant of iron status, it remains a subjective experience, often misjudged by athletes. The use of standardized tools such as the PBAC can enhance objectivity and facilitate a more accurate assessment in both clinical and applied sports settings.

Rather than being viewed solely as a clinical symptom, MBL should be recognized as a relevant physiological stimulus with direct implications for erythropoiesis and iron metabolism. Future research and athlete care models should integrate MBL assessment into personalized monitoring strategies to safeguard iron homeostasis, support recovery, and optimize long-term performance in female athletes.

AUTHOR CONTRIBUTIONS

K.K. was involved in supervision. S.N. and C.M. were involved in conceptualization. S.N., S.K., and C.M. were involved in data collection, data analysis, and data curation. S.N. and C.W. were involved in statistical analysis. S.N., S.H., and C.W. were involved in visualization. S.N. was involved in original—draft preparation. S.N., K.K., C.B., and C.M. were involved in writing—review and editing. All authors approved the final version of the manuscript.

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CONFLICT OF INTEREST STATEMENT

There is no conflict of interest.

DATA AVAILABILITY STATEMENT

All data are available upon request.

ETHICS STATEMENT

The study was approved by the local ethics committee of the University of Giessen (No. 2024-0014).

INFORMED CONSENT

All subjects provided written informed consent to participate in the study.

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4.4 Relative Energy Intake as a Determinant of Iron Status in Elite Athletes (Pre-print)

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Relative Energy Intake as a Determinant of Iron Status in Elite Athletes

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Research Article

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Abstract

Background

Iron deficiency is highly prevalent in elite athletes and can impair performance even without anemia. While iron intake is essential, emerging evidence suggests that energy availability can critically affect iron metabolism. This study investigated whether relative energy and nutrient intake predict serum ferritin levels in elite athletes under real-world training conditions.

Methods

One hundred forty-nine national squad athletes (80 females, 69 males) from nine sports disciplines completed 3-day food protocol, analyzed against individualized recommendations. Iron status was classified by serum ferritin and hemoglobin; fractional absorption was estimated using a predictive model. Multiple linear regression identified predictors of ferritin concentration.

Results

Most athletes failed to meet recommendations for calories (87%), carbohydrates (95%), and iron (39%). Biochemical iron deficiency (iron depletion or iron-deficient erythropoiesis) was present in 42% of athletes, with a marked female predominance. Relative caloric intake, iron supplementation, and age significantly predicted ferritin concentration ($R^2 = 0.34$, $p < 0.001$), whereas carbohydrate intake and sex showed non-significant trends. Low energy intake was associated with higher risk of iron deficiency and altered absorption profiles.

Conclusion

Relative energy intake emerged as the strongest determinant of systemic iron availability in elite athletes. Integrated nutrition strategies that address both energy sufficiency and iron intake are essential to prevent subclinical deficiency and sustain high-performance capacity.

Key Points

1. Energy availability emerges as one of the primary determinant of systemic iron status in elite athletes. Despite iron intakes within recommended ranges, 42% of athletes exhibited iron deficiency or impaired erythropoiesis. Relative caloric intake, age and supplemental iron were the strongest predictors for ferritin levels.
2. Suboptimal fueling contributes to subclinical iron dysregulation and may impair performance. Widespread deficits in energy and carbohydrate intake were linked to low ferritin and potential

suppression of iron absorption. These combined deficiencies may compromise oxygen transport, recovery, and adaptation, underscoring the physiological interdependence of macronutrient and iron balance.

3. Ferritin represents both a marker of iron status and an indicator of insufficient energy uptake. Low ferritin concentrations should be interpreted not solely as iron deficiency but as a possible early warning sign of low energy availability (LEA). Integrating ferritin screening into athlete monitoring may enable proactive identification of metabolic strain and support food-first, individualized nutrition strategies.

Introduction

Iron is a critical micronutrient for oxygen transport, mitochondrial function, and cellular energy metabolism, all of which are essential for athletic performance and recovery. Among elite athletes, iron deficiency is a prevalent concern, particularly in endurance sports and among female athletes, due to increased physiological demands, dietary restrictions, and iron losses through hemolysis, sweating, and menstruation (Peeling et al., 2014; Sim et al., 2019). Maintaining sufficient iron stores is therefore challenging and fundamental to optimizing oxygen-carrying capacity and aerobic performance.

Recent evidence highlights that iron status is not solely determined by absolute dietary iron intake but is intricately linked to broader nutritional status and energetic factors (Burden et al., 2015). Among these, energy availability has emerged as a critical modulator of iron homeostasis, influencing both systemic iron regulation and erythropoietic capacity (McCormick et al., 2020). The condition of low energy availability (LEA) has been shown to dysregulate the hepatic synthesis of hepcidin, a central hormone that controls iron absorption and distribution by inducing the degradation of ferroportin, the only known iron exporter in enterocytes and macrophages. Paradoxically, in the context of LEA, hepcidin concentrations may remain elevated despite depleted iron stores, thereby reducing iron bioavailability and impairing adaptive responses to training. In addition, chronic energy deficiency can compromise erythropoiesis by limiting the availability of key substrates and by downregulating the expression of iron transport proteins such as transferrin (McKay et al., 2020). These mechanisms are particularly relevant in the context of the Relative Energy Deficiency in Sport (REDs), where sustained energetic mismatch may lead to systemic alterations in iron metabolism and hematological function (Logue et al., 2018).

Despite growing recognition of the interplay between energy availability and iron metabolism, few studies have systematically quantified the relationship between relative energy intake, macronutrient composition, and iron status in elite athletes under real-world training conditions (Gibson-Smith et al., 2020; Hertig-Godeschalk et al., 2023). In particular, the combined influence of caloric intake, carbohydrate availability, and dietary iron intake on systemic iron stores remains poorly understood. This gap is critical, given that nutritional interventions aimed at preventing iron deficiency must account not only for micronutrient supply, but also for the energetic and metabolic context in which absorption and utilization occur (McKay et al., 2020). Therefore, the aim of this study was to examine the prevalence of insufficient caloric, carbohydrate, and iron intake in elite athletes and to determine how these nutritional

insufficiencies relate to biochemical markers of iron status. We specifically hypothesized that inadequate energy availability would be highly prevalent, particularly among female athletes, and that it would constitute a central determinant of iron deficiency beyond dietary iron intake alone. Moreover, we expected relative caloric intake to emerge as an independent predictor of serum ferritin concentration and that athletes with iron deficiency would display compensatory increases in fractional iron absorption, insufficient to restore normal iron status under conditions of low energy intake.

Methods

Participants and study design

The participating athletes were drawn from nine distinct Olympic disciplines: 3x3 basketball, artistic gymnastics, rhythmic gymnastics, trampoline gymnastics, bobsleigh, skeleton, ice hockey, modern pentathlon, table tennis, and volleyball. Elite athletes were characterized as active members of German national teams (junior, perspective, or Olympic squads) in Olympic-level sports, representing endurance-oriented (e.g., modern pentathlon), strength/power-based (e.g., skeleton), and skill-focused (e.g., table tennis) performance profiles. The final dataset comprised 149 athletes (69 male, 80 female), with a median age of 19.0 years (range: 14.2–38.6 years). All athletes provided written informed consent, and the study was approved by the Ethics Committee of the Justus Liebig University Giessen (ethical approval number: AZ 55/22; approval date: 10 May 2022).

Nutritional assessment

Dietary intake was assessed via 3-day food protocol, including details on all meals, beverages, supplements and medication including the art and quantities of each. Nutritional protocols were analyzed using the software DGE Expert (Version 2.0.45.1) based on the German Food Code and Nutrient Database (BLS) developed by the German Nutrition Society. The quality and comprehensiveness of each protocol was carefully evaluated. Only those protocols that were found to be adequately completed were selected for further analysis.

For each athlete, the energy requirement was calculated based on a physical activity level (PAL) of 2.0. Macronutrient targets were set in accordance with DGE guidelines, with 50% of energy intake derived from carbohydrates. Nutrient intakes were expressed as percentages of individual recommendations. Relative values were computed for total caloric intake, carbohydrate intake, and iron intake (including supplements).

Iron status classification and blood sampling

Three venous blood samples of the athletes were collected in 7.5 mL Serum Gel and 7.5 mL and 2.7 mL EDTA tubes (Sarstedt, Nümbrecht, Germany) at the respective training centers. No standardized fasting

or activity restrictions were applied prior to sampling. Serum was separated via centrifugation and analyzed in a certified clinical laboratory (SYNLAB Medical Care Center, Bad Nauheim, Germany). Ferritin and hemoglobin concentrations were measured using standard clinical chemistry methods. Serum ferritin was determined via electrochemiluminescence immunoassay (ECLIA). Hemoglobin was measured using a photometric cyanmethemoglobin method. All values were interpreted based on reference ranges and classification criteria by Peeling et al., 2008. This model differentiates between: Iron depletion (ferritin < 35 µg/L, normal hemoglobin), Iron-deficient erythropoiesis (ferritin < 20 µg/L, transferrin saturation < 16%, normal hemoglobin), Iron-deficiency anemia (ferritin < 12 µg/L, hemoglobin < 115 g/L). Nine athletes with serum ferritin levels > 100 µg/L were excluded from analyses of iron absorption, as this exceeds the validity range of absorption estimation models. To ensure full transparency regarding the real-life setting, all athletes were included in the descriptive presentation (Tables 1 and 2). To estimate predictive individual iron absorption, a model developed by Fairweather-Tait et al., 2017 was applied. This tool estimates fractional absorption based on serum ferritin concentration and sex-specific parameters. By combining these datasets, we calculated the estimated amount of iron absorbed (mg) for each participant.

Statistical analysis

Data preprocessing was performed in Microsoft Excel 365, all statistical analyses were conducted using JASP (Version 0.16.2.0) and figures were generated in R version 4.5.1 (R Core Team, 2025) using Positron version 2025.09.0 (Posit Software, PBC, 2025). Descriptive statistics are presented as means ± standard deviations (SD) for normally distributed variables or as medians with interquartile ranges (IQR) for non-normally distributed variables. Normality was assessed by the Shapiro-Wilk test in combination with visual inspection of Q-Q plots.

Group differences in relative caloric intake and relative carbohydrate intake between participants with iron deficiency (ID), iron deficient erythropoiesis (IDE), and non-deficient (ND) were analyzed using one-way analysis of variance (ANOVA). The dependent variable was relative caloric intake or relative carbohydrate intake and the independent variable was iron status (ID, IDE, ND). Assumptions of normality and homogeneity of variances were tested prior to analysis. In the case of a significant main effect, post hoc pairwise comparisons were performed using Holm adjustment. For actual iron absorption, where normality assumptions were not met, the Kruskal-Wallis test was applied.

Associations between nutritional and hematological variables were examined using Pearson's correlation coefficient for normally distributed data and Spearman's rank correlation coefficient otherwise. Statistical significance was set at $\alpha = 0.05$ (two-tailed).

To identify predictors of serum ferritin concentration, we performed multiple linear regression analyses including relative caloric intake, relative carbohydrate intake, relative iron intake, age, and sex as independent variables.

Results

Dietary intake and nutrient sufficiency

To evaluate the adequacy of nutrient intake, relative iron, carbohydrate, and caloric intake were assessed against individualized recommendations derived from DGE standards. Nutrient intake ≥ 100% of the recommended value was classified as sufficient, values < 100% were considered insufficient.

In the total cohort (n = 149), the majority of athletes failed to meet recommended intake levels across all categories. Specifically, 87.25% of athletes consumed insufficient calories, 95.30% failed to meet carbohydrate targets, and 38.93% demonstrated inadequate iron intake. When stratified by gender, 130 athletes exhibited insufficient caloric intake (60 males, 70 females), and 142 athletes fell short of carbohydrate recommendations (66 males, 76 females). Regarding iron, 58 athletes had insufficient intake, with a notable disparity between sexes: 50 of these were female, representing 86.21% of all iron-insufficient athletes (Table 1).

Table 1
Prevalence of insufficient nutrient intake by sex.

Nutrient	Total with insufficient intake (n, %)	Males (n)	Females (n)	Proportion of females among insufficiently supplied (%)
Energy	130 (87.25%)	60	70	53.85%
Carbohydrates	142 (95.30%)	66	76	53.52%
Iron	58 (38.93%)	8	50	86.21%

Classification of iron status

Ferritin and hemoglobin concentrations were used to classify iron status according to Peeling et al. (2008). Out of the 149 athletes, 86 (57.72%) presented no indication of iron deficiency. 37 athletes (24.83%) were classified with iron depletion (ID), and 26 athletes (17.45%) exhibited iron-deficient erythropoiesis (IDE). No cases of iron-deficiency anemia (IDA) were observed (Table 2). Sex-specific analysis revealed a disproportionate prevalence of iron dysregulation among female athletes. Of those with iron depletion, 56.76% were female, within the IDE group, 80.77% were female. By contrast, iron sufficiency was more evenly distributed, with 55.81% of the non-deficient (ND) athletes being male and 44.19% female (Table 2).

Table 2
Iron status classification based on ferritin and hemoglobin concentrations according to Peeling et al. (2008)

Iron Status Category	Total (n, %)	Males (n)	Females (n)	Proportion of females within category (%)
No iron deficiency	86 (57.72%)	48	38	44.19%
Iron depletion (ID)	37 (24.83%)	16	21	56.76%
Iron-deficient erythropoiesis (IDE)	26 (17.45%)	5	21	80.77%
Iron-deficiency anemia (IDA)	0 (0%)	0	0	-

Estimated iron absorption and intake

Using the predictive tool of Fairweather-Tait et al., results revealed that 67 athletes exhibited an estimated absorption rate between 10–15%, which is considered physiological for iron-repleted individuals. Notably, 8 athletes showed reduced absorption (< 5%), while only one athlete demonstrated exceptionally high absorption (35–40%) (Table 3). The total mean iron intake over three days was 16.33 (± 6.78) mg in this cohort, and the calculated mean actual iron absorption was 2.31 (± 1.2) mg.

Table 3
Model-based estimates of fractional iron absorption across the cohort. Estimates based on the predictive model by Fairweather-Tait et al. (2017)

Fractional iron absorption	n (out of 149)	% of cohort
Low (< 5%)	8	5.37%
Physiological (10–15%)	67	44.97%
High (35–40%)	1	0.67%

Intersection of nutritional and hematological data

To identify potential links between dietary insufficiencies and iron status, overlap analyses were conducted. Among the 130 athletes with insufficient caloric intake, 61 athletes were simultaneously classified as ID or IDE. A similar pattern emerged for carbohydrate intake: of the 142 athletes with suboptimal carbohydrate intake, 62 were diagnosed with ID or IDE. Further analysis revealed that 32 athletes overlapped between insufficient iron intake and biochemical evidence of iron deficiency, indicating a convergence of nutritional and physiological vulnerability.

Comparison of relative caloric intake revealed a significant overall group effect ($p < 0.01$). Post hoc analyses indicated significantly lower intake in the ID group (69.85 ± 16.25%) compared with the ND group (81.42 ± 21.8%; $p < 0.05$). The IDE group (71.23 ± 18.34%) did not differ significantly from the ND group nor the ID group ($p > 0.05$). However, the difference between IDE and ND approached significance ($p = 0.052$), suggesting a potential trend toward lower intake in the IDE group (Fig. 1).

Iron status and iron absorption

Actual iron absorption differed significantly between groups ($p < 0.001$). Post hoc analyses revealed higher absorption in the ID group (2.56 ± 1.02 mg) compared to the ND group (2.05 ± 1.0 mg; $p < 0.05$), and in the IDE group (3.48 ± 0.91 mg) compared with the ND group ($p < 0.001$) (Fig. 2). Additionally actual iron absorption was significantly higher in the IDE compared to the ID group ($p < 0.001$). No significant difference in actual iron absorption was observed between female (2.41 ± 1.23 mg) and male (2.49 ± 0.93 mg) athletes ($p > 0.05$). However, a Mann-Whitney U test indicated a significant sex difference in relative iron intake, with lower values in female athletes (94.69 ± 45.55%) compared with male athletes (153.31 ± 50.15%; $p < 0.001$).

Relative Energy Intake Predicts Iron Status in Elite Athletes

Serum ferritin levels and relative caloric intake showed a significant positive correlation ($r = 0.24$, $p < 0.01$) (Fig. 3A). To investigate whether ferritin levels were positively associated with carbohydrate intake another correlation analysis was carried out and showed a non-significant positive correlation between ferritin and relative carbohydrate intake ($r = 0.01$, $p > 0.05$) (Fig. 3B). To examine whether higher caloric intake translated into higher iron intake, correlation analysis revealed a positive correlation between relative iron intake (diet and supplements) and relative caloric intake ($r = 0.57$, $p < 0.001$) (Fig. 3C).

A multiple linear regression analysis was conducted to examine predictors of serum ferritin levels in athletes. The model included relative caloric intake, relative carbohydrate and iron supplementation, age, and gender as independent variables. The overall model was statistically significant and explained 34% of the variance in serum ferritin levels ($R^2 = 0.34$, adjusted $R^2 = 0.32$, $F(5,143) = 14.73$, $p < 0.001$). Three variables emerged as significant positive predictors: relative caloric intake ($B = 0.64$, $p = 0.0018$), iron supplementation ($B = 0.11$, $p = 0.050$), and age ($B = 2.11$, $p < 0.001$). In contrast, relative carbohydrate intake ($B = -0.42$, $p = 0.06$) and female gender ($B = -10.1$, $p = 0.09$) showed non-significant trends towards a negative association with ferritin (Table 4).

Table 4
Multiple linear regression Model examining predictors of Serum Ferritin Levels in Athletes.

Predictor	β (standardized)	95% CI	p-value	Significance
Age	+ 0.32	[+ 0.18, + 0.46]	< 0.001	***
Relative caloric intake	+ 0.28	[+ 0.11, + 0.44]	0.0018	**
Iron supplementation	+ 0.14	[+ 0.00, + 0.28]	0.050	*
Relative carbohydrate intake	−0.12	[−0.25, + 0.01]	0.06	(ns)
Female gender	−0.10	[−0.21, + 0.01]	0.09	(ns)

Discussion

The present study provides novel evidence that systemic iron status in elite athletes is shaped not only by dietary iron intake but also by overall energy intake. On average, athletes achieved iron intakes within the recommended reference range. However, biochemical classification revealed that 42% displayed ID or IDE. Consistent with physiological regulation, athletes with reduced iron stores exhibited higher estimated absorption, reflecting adaptive upregulation of iron uptake under conditions of insufficiency. Beyond intake and absorption, we identified a moderate but significant correlation between relative caloric intake and relative iron intake, indicating that greater total food consumption increases the likelihood of meeting iron requirements. Importantly, relative caloric intake also emerged as the strongest nutritional predictor of ferritin levels, surpassing both supplemental iron and carbohydrate intake in explanatory power. Despite similar absorption rates between sexes, female athletes were disproportionately represented in the ID and IDE groups, 56.8% and 80.8%, respectively, largely due to lower relative iron intake compared with males.

Dietary intake, iron status, and estimated absorption

The high prevalence of insufficient energy, carbohydrate, and iron intake observed in this cohort reflects a pronounced imbalance between nutritional intake and physiological requirements, which aligns with previous observations (Burke et al., 2019; Jenner et al., 2019; Lundstrom et al., 2025). These findings indicate that a substantial proportion of athletes fail to meet fundamental dietary recommendations, which may have implications for recovery, adaptation, and overall metabolic stability (Heaton et al., 2017; Mountjoy et al., 2023).

The distribution of iron status categories further supports the notion of widespread subclinical iron dysregulation. While the absence of overt anemia may suggest a generally adequate hematological profile, the considerable proportion of athletes with iron depletion indicates that early stages of deficiency are common. The current data reflect this pattern, with a relatively high prevalence of iron deficiency but no cases of clinically manifest anemia (Thompson et al., 2025). This discrepancy likely reflects the impact of regular screening and timely intervention practices, which help prevent further

progression to anemia. Nonetheless, adopting a preventive approach remains preferable, as it may reduce the need for therapeutic supplementation, which is frequently associated with gastrointestinal side effects and variable adherence.

Applying the predictive model of Fairweather-Tait et al. (2017) provided an additional dimension to these observations by estimating functional iron absorption (Fairweather-Tait et al., 2017). The majority of athletes exhibited physiological absorption rates, yet the presence of both markedly reduced and compensatory elevated absorption patterns suggests the body's ability to adapt and to compensate, hepcidin downregulation and upregulated expression of iron transporters, such as divalent metal transporter 1 (DMT1) in the enterocytes are likely the underlying physiological pathways for this adaptation (Vogt et al., 2021). The heterogeneity in absorption rate again highlights the complexity of the bodies physiological systems working intertwine and being dependent on an umbrella of different variables influencing iron availability (Peeling et al., 2014; Sim et al., 2019).

Implications for performance and health

The observed interplay between energy intake and iron status carries important implications for both athletic performance and health. Iron deficiency, even in the absence of anemia, has been consistently associated with impaired oxygen transport, reduced mitochondrial function, and diminished endurance capacity (Solberg & Reikvam, 2023). Our findings suggest that insufficient caloric intake not only jeopardizes overall energy balance but also indirectly exacerbates the risk of iron insufficiency, thereby amplifying physiological strain (Petkus et al., 2017). In line with this, the substantial prevalence of combined caloric and iron insufficiency in our cohort highlights a potentially underappreciated mechanism contributing to iron deficiency in high-performance sport: suboptimal energy availability may impair gastrointestinal iron absorption through suppression of hepcidin-regulating pathways (Areta & Hopkins, 2018; McClung et al., 2014). This mechanism could attenuate the efficacy of supplementation strategies and underscores that nutritional interventions cannot be reduced to isolated micronutrient replacement.

Building upon the broader physiological implications of energy balance, our findings delineate a specific nutritional vulnerability within the athletic population: a high prevalence of insufficient dietary intake of energy, carbohydrates, and iron. Despite the well-established role of carbohydrates as the primary substrate for high-intensity and prolonged exercise (Burke et al., 2011) over 95% of athletes failed to meet carbohydrate recommendations. Such widespread under-consumption may compromise glycogen resynthesis, reduce training quality, and elevate infection risk through impaired immune function (Walsh, 2019). It should be acknowledged, however, that dietary assessments relying on self-reported food records are prone to underreporting, particularly in athletes with high energy expenditure (Capling et al., 2017). While this methodological limitation may partly inflate the apparent prevalence of insufficient carbohydrate intake, the magnitude of the discrepancy observed in our cohort strongly suggests that suboptimal fueling represents a genuine and prevalent challenge rather than a mere artifact of reporting bias. Notably, although we observed no direct association between carbohydrate intake and iron status, inadequate carbohydrate availability may indirectly interact with iron metabolism by promoting glycogen

depletion, elevating systemic stress and inflammatory responses, and altering hepcidin dynamics, thereby compounding the risk of functional iron insufficiency (McKay et al., 2020). Furthermore, experimental evidence by Hennigar et al. (2020) indicates that energy deficit can upregulate hepcidin even in the absence of inflammation, providing a mechanistic link between low energy availability and impaired iron absorption (Hennigar et al., 2021).

Although LEA is not limited to female athletes, our sex-stratified data revealed a higher prevalence of insufficient intake in women, particularly regarding iron, underscoring the need for sex-specific monitoring strategies (Mountjoy et al., 2018). Biochemical assessment substantiated these findings: ~42% of athletes showed depleted iron stores or impaired erythropoiesis, with a marked overrepresentation of females. This aligns with previous work linking female vulnerability to menstrual iron losses, lower dietary intake, and possible hepcidin-mediated regulatory differences (Ponorac et al., 2020). Importantly, dietary intake alone proved to be an insufficient predictor of systemic iron status. Several athletes with apparently adequate iron intake exhibited depleted ferritin, while others with inadequate intake-maintained sufficiency, suggesting that absorption efficiency, iron turnover, and hepcidin regulation critically shape individual status. Thus, integrated screening approaches combining nutritional assessment with biomarker evaluation are required (Maughan et al., 2018; Peeling et al., 2008). Notably, no cases of overt anemia were observed, yet more than 40% of athletes displayed subclinical iron dysfunction, iron depletion or iron-deficient erythropoiesis, that may compromise oxygen transport capacity and performance. These findings highlight the need to assess iron status as a continuum, rather than relying solely on anemia-based cut-offs that risk missing performance-limiting deficits (Peeling et al., 2008; Solberg & Reikvam, 2023).

The convergence of dietary insufficiencies and impaired iron status in this cohort indicates a systemic mismatch between physiological demand and nutritional supply. Sixty-one athletes with suboptimal caloric or carbohydrate intake also presented with biochemical iron deficiency, suggesting that inadequate energy availability may act as a mechanistic driver of iron dysregulation (Areta & Hopkins, 2018; Mountjoy et al., 2018). Beyond absolute iron intake, the overlap between insufficient nutrient consumption and low ferritin points to a multifactorial etiology involving impaired gastrointestinal absorption, hepcidin-mediated regulation, and elevated metabolic demands. A particularly vulnerable subgroup was identified in the 32 athletes with both insufficient dietary iron and biochemical deficiency, highlighting the compounded risk when nutritional factors occur.

Multivariate modeling confirmed relative caloric intake, iron supplementation, and age as the most robust predictors of ferritin levels, collectively explaining one-third of the variance. The positive association between energy intake and ferritin aligns with recent literature emphasizing the critical role of energy availability in supporting erythropoiesis and regulating hepcidin suppression (Burden et al., 2015). The fact that carbohydrate intake and sex showed only non-significant trends suggests that the energetic environment may override more specific macronutrient or sex-based effects on iron homeostasis when considered in aggregate. These findings lend mechanistic support to the REDs model, in which low energy availability disrupts iron turnover and impairs recovery, performance, and

adaptation (Mountjoy et al., 2023). Model-based estimates of iron absorption corroborated this conclusion. While most athletes exhibited physiological absorption rates (10–15%), a small subgroup showed markedly reduced absorption (< 5%), which may reflect either impaired regulatory control or suppression of absorption in the context of elevated ferritin and increased hepcidin activity. Conversely, one athlete demonstrated compensatory hyperabsorption (> 35%), indicative of advanced iron depletion. Although numerically limited, these extreme phenotypes may hold disproportionate relevance for individualized monitoring and intervention strategies.

Limitations

Some limitations should be acknowledged. First, no standardized fasting or activity restrictions were imposed prior to blood sampling. While this reflects real-world conditions, it may have introduced variability in serum ferritin concentrations due to diurnal or exercise-related fluctuations (Sim et al., 2019). Second, although the applied models explained a relevant portion of variance in iron status, unmeasured confounders, such as inflammation, may have influenced individual outcomes. Moreover, hepcidin concentrations were not measured, as assay implementation was not feasible within the current study setting, which limits mechanistic interpretation regarding the regulation of iron metabolism under energy deficit conditions. Finally, the cross-sectional design precludes causal inference; longitudinal and interventional studies are needed to confirm these associations and to guide evidence-based strategies for iron optimization in elite sport.

Conclusion

Taken together, our findings underscore how far the wings of inadequate calorie intake, not to say energy availability, spread into the complexity of the bodies physiological systems. Implicating either how far athlete monitoring must go or how important athlete education is. Fostering self-responsible and independent athlete may be key in health and performance of this vulnerable group.

Importantly, our data reflect athletes in real-world conditions, within strained systems, under-staffed support structures, and, for younger athletes, often guided by unpaid or volunteer coaches. Consequently, the balance of responsibility between athlete and coach (or the broader system) may require recalibration toward the athlete's own accountability.

While practical implications can easily be formulated, their implementation remains challenging: the hurdles are high, and systemic change is slow. Monitoring strategies that encourage shared responsibility, between system and athlete, are therefore essential. Integrating simple blood markers such as ferritin and hemoglobin, which indicate an athlete's iron metabolism status, with practical tools like the one proposed by Fairweather-Tait, could empower athletes to better understand and manage their nutritional needs. Such approaches may help athletes at all levels assume greater ownership of their health and performance, especially since high-standard monitoring, if achieved, remains largely confined to the elite sphere of high-performance sport.

Perspective

In sports medicine, serum ferritin is the standard biomarker for assessing iron stores and is routinely employed in athlete health screening (Hennigar et al., 2021). Owing to its central role in iron homeostasis, strong clinical validity, and ease of measurement, ferritin remains the first-line indicator of iron status, preceding more specialized markers such as hepcidin or soluble transferrin receptor (Sim et al., 2019). Our findings demonstrate a clear association between low ferritin concentrations and low caloric intake, suggesting that inadequate energy intake can compromise iron metabolism independently of dietary iron or vice versa as discussed by McKay et al., 2020 (McKay et al., 2020). This real-world evidence aligns with laboratory findings by Hennigar et al., 2020, showing that energy deficit upregulates hepcidin and reduces iron absorption (Hennigar et al., 2021), thereby providing a mechanistic explanation for the observed link between low energy intake and depleted ferritin levels. Therefore, low ferritin values should be regarded as an early warning sign for potential LEA, prompting targeted evaluation of energy intake and a general food first approach (Nolte et al., 2024), rather than instantly initiating iron supplementation. This approach positions ferritin as both a marker of iron status and a practical indicator of broader metabolic stress in athletes.

Abbreviations

LEA
Low Energy Availability
REDs
Relative Energy Deficiency in Sport
PAL
Physical Activity Level
ND
Non-deficient
ID
Iron Depletion
IDE
Iron-deficient Erythropoiesis
IDA
Iron-deficiency Anemia
ANOVA
Analysis of Variance
CI
Confidence Interval

Declarations

Conflict of interest disclosure

Svenja Nolte, Claudia Lenz, Ann-Kathrin Fink, Simon Klügel, Sebastian Hacker and Karsten Krüger declare that they have no competing interests.

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Author contributions

SN, CL and KK designed research. SN, CL, SH and AKF conducted research. SN, CL, AKF, SH, SK and KK wrote the original draft, performed statistical analysis and created figures. SN, CL, AKF, SH, SK and KK reviewed and edited the paper. KK were responsible for project administration and acquisition of funding. All authors read and approved the final manuscript and agree with the order of presentation of the authors.

Data availability statement

Data described in the manuscript will be made available upon request pending (application and approval).

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Figures

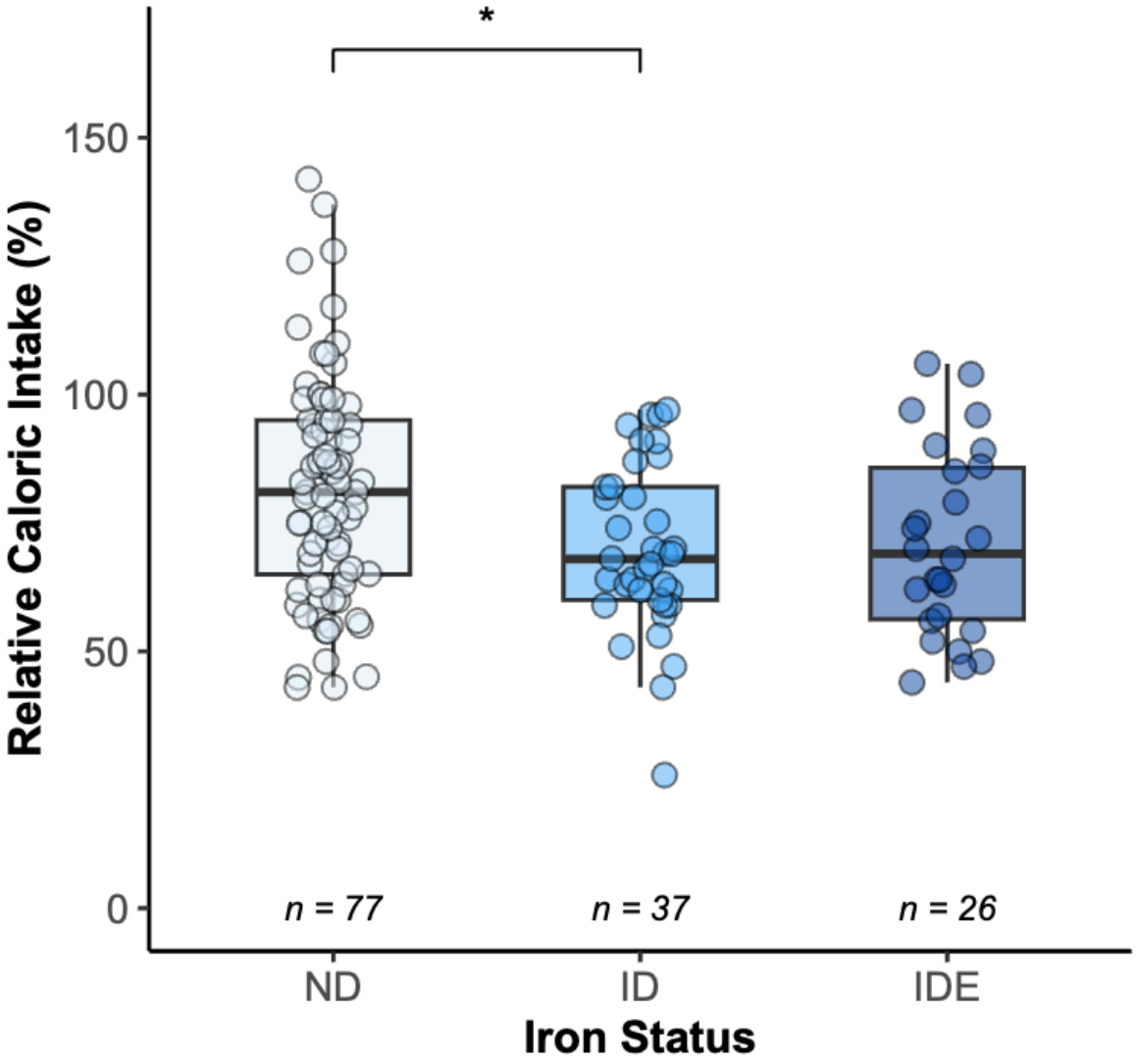

Figure 1

Relative caloric intake (%) across the groups (ND = non-deficient, ID = Iron Depletion, IDE = iron deficient erythropoiesis defined by iron status from McKay et al., 2020.

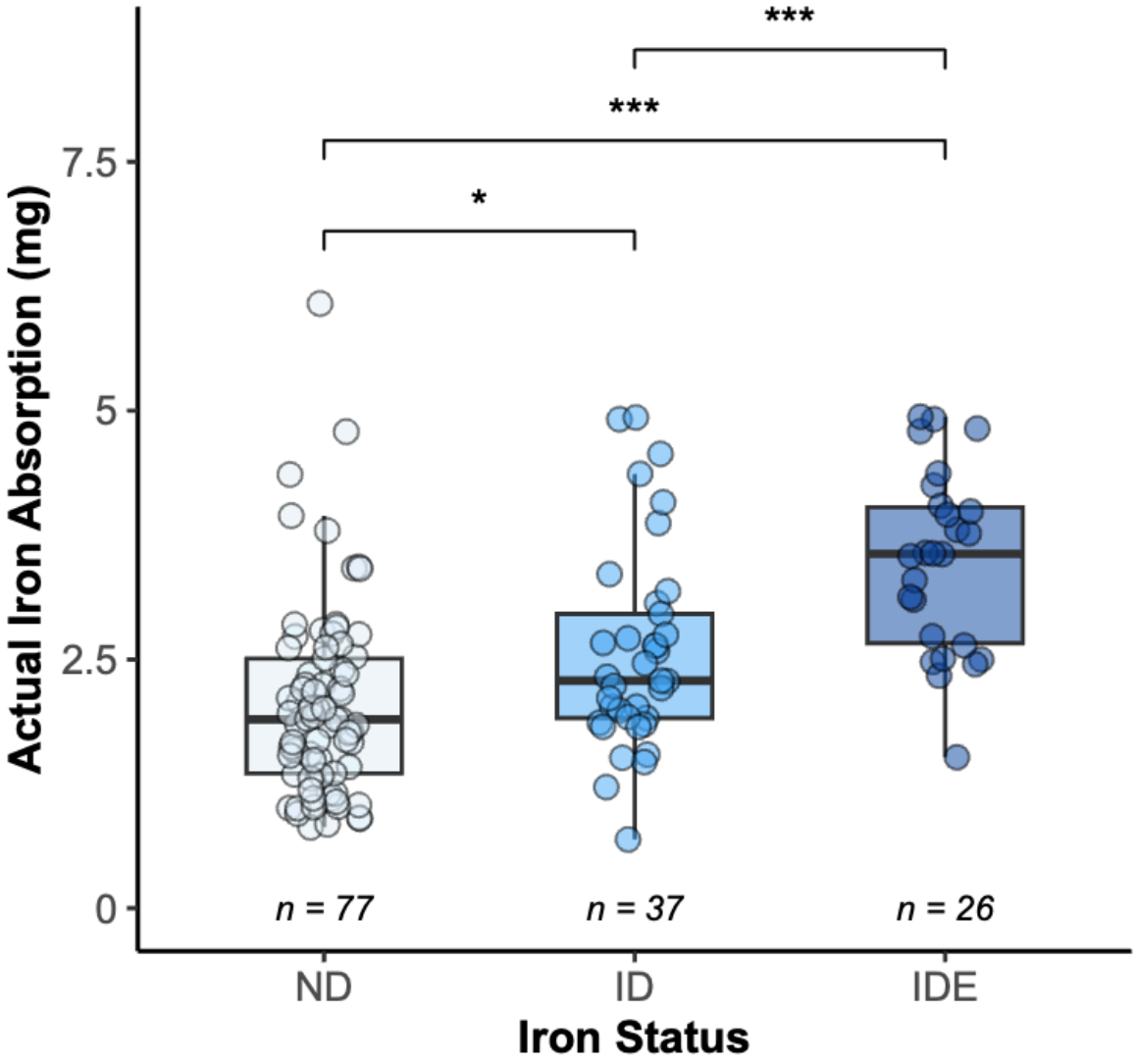

Figure 2

Actual iron absorption (mg) across the groups (ND = non-deficient, ID = Iron Depletion, IDE = iron deficient erythropoiesis defined by iron status from McKay et al., 2020.

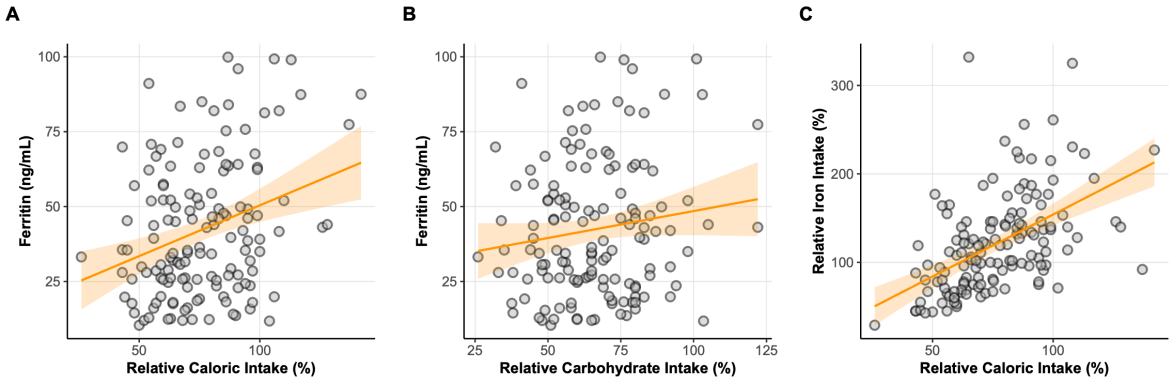

Figure 3

Associations between nutrient intake and serum ferritin or relative iron intake. Each dot represents an individual participant; the solid blue line indicates the unadjusted linear regression fit, and the shaded area represents the 95% confidence interval. (A) Positive association between relative caloric intake (%) and ferritin (ng/mL). (B) Positive association between relative carbohydrate intake (%) and ferritin (ng/mL (C) Strong positive association between relative caloric intake (%) and relative iron intake (%), indicating that higher energy intake is accompanied by higher relative iron intake. Axes are labeled with corresponding units. The plots display univariate, descriptive associations (not adjusted for covariates); causal inference cannot be drawn.

5 Discussion

The present cumulative dissertation set out to examine factors influencing iron metabolism in female and male athletes. While each included study addressed a distinct physiological stressor, the findings collectively point toward a broader conceptual implication: iron metabolism in athletes cannot be adequately understood when interpreted in isolation from the interacting physiological systems in which it is embedded.

Athletic training represents a state of continual physiological negotiation. Repeated exercise-induced stress, environmental challenges, sex-specific factors and nutritional constraints require the coordinated regulation of hematological, immune, metabolic, and endocrine processes to maintain functional homeostasis. Due to its involvement in several physiological systems, it offers a platform for disruptions in the context of athletic stress.

Accordingly, the purpose of this discussion is not merely to restate the findings of the individual publications, but to integrate them within a system-physiological framework. By synthesizing the results across studies, this chapter aims to demonstrate how different influencing factors join in iron metabolism, how disturbances in iron homeostasis spread across interconnected systems, and what this implies for the interpretation of iron-related biomarkers and practical monitoring strategies in athletes.

5.1 Iron Metabolism in the Athletic Population: From Isolated Markers to System Interactions

Interdependency, feedback-driven, non-linear and complex. These are words that can be used to describe network physiology [152], which is brought to the surface by physical exercise, where an orchestra of reactions take place: acute adaptation. Something that happens on daily basis in athletes and forces the network of systems to the level of chronic adaptations [50].
Within such framework, disturbances may not necessarily manifest within the system in which they originate. An alteration in one area may instead become apparent in another, compensating in an adaptive manner.

Projecting this concept onto iron metabolism illustrates its unique systemic relevance. Multiple physiological systems, including erythropoiesis, immune defense, mitochondrial energy metabolism, and endocrine regulation, depend on adequate iron availability for proper function [35], [45], [142]. Conversely, when iron availability becomes constrained, the consequences may spread across systems rather than remaining confined to hematological parameters alone [153].

Evidence for such system interactions becomes particularly apparent under conditions of elevated physiological strain, as exemplified by the hypoxia study included in this dissertation. At the mechanistic level, HIF signaling provides a unifying link between oxygen sensing, iron availability, and immune regulation [154]. Iron acts as a cofactor for HIF-degrading enzymes, such that reduced iron access stabilizes HIF signaling and modulates immune responses, including neutrophil and inflammatory activity. This illustrates how hypoxia and iron availability jointly shape immune function and

underscores that immune-related alterations observed under hypoxic stress are not secondary phenomena but integral components of iron-mediated system interactions [155]. Within this context, immune-related markers provided insight into how non-hematological systems participate in iron regulation and illustrate the systemic connection. Lactoferrin, a neutrophil-derived glycoprotein with antimicrobial and iron-binding properties, illustrates how immune function and iron metabolism are intrinsically linked. As a molecule involved in both host defense and iron sequestration, lactoferrin reflects neutrophil-mediated iron handling and highlights that immune cells actively contribute to iron redistribution when systemic demand increases [156].

Building on this systemic perspective another interconnection between iron and the metabolic system has recently been discussed, where iron deficiency may serve as an early indicator for LEA [105]. The findings of the final study of this dissertation project support this. Together, these results point to a bidirectional “iron-link” between iron and the metabolic system, opening another integrative: the directionality of interaction. This connection is further underlined by the observation that iron deficiency can impair thyroid function [157] and of insulin-like growth factors (IGF) and growth factor (GF) [158].

5.2 From Risk Factors to Regulated Symmetry: Iron Homeostasis Under Athletic Stress

In the athletic population, factors traditionally described as “risk factors” for iron deficiency [159] may be more appropriately conceptualized as physiological stressors that challenge iron homeostasis and necessitate regulatory adaptation. Rather than exerting linear, unidirectional effects on iron status, these stressors interact with existing regulatory networks and provoke compensatory responses aimed at preserving essential physiological functions.
Hypoxic exposure provides a clear example of such an adaptive stressor. The increase in erythropoietic drive under hypoxia substantially elevates iron demand. However, this demand is not met passively. Instead, regulatory mechanisms adjust to facilitate iron availability, including suppression of hepcidin, thereby enhancing intestinal iron absorption and mobilization from stores [160]. This response illustrates that iron regulation under stress is not merely disrupted but actively reconfigured to meet prioritized physiological demands.

A comparable pattern emerges when examining menstrual blood loss (MBL) in female athletes. Although MBL constitutes a recurrent and predictable iron loss [125], findings from the present dissertation demonstrated that reduced iron stores do not necessarily translate into impaired hemoglobin concentration or diminished oxygen transport capacity. This suggests that iron regulation adapts to cyclical iron loss through redistribution and prioritization mechanisms, allowing preservation of key performance-related functions. In this context, lower ferritin concentrations may reflect regulated iron strain rather than overt dysregulation.

However, adaptive capacity is not unlimited. The findings of the energy availability study included in this dissertation indicate that insufficient energy intake constitutes a critical contextual modifier of iron regulation. Low ferritin concentrations frequently co-occurred with indicators of inadequate energy availability, suggesting that iron homeostasis is tightly coupled to the broader metabolic environment. Unlike hypoxia or menstrual blood

loss, where regulatory responses facilitate iron redistribution, energy deficiency may constrain the system's ability to mount such compensatory adjustments.

From a systems perspective, low energy availability may therefore act not as a primary driver of iron loss, but as a factor that reduces regulatory flexibility. Under such conditions, iron-related adaptations that are otherwise effective may become diminished, increasing the likelihood that recurrent iron strain progresses toward functional limitation. The bodies inability to adjust to prolonged low energy availability is presented in several current studies, viewing the widespread of this phenomena in the athletic population [161], [162]

Collectively, these observations support the interpretation that influencing factors should not be viewed as isolated risks but as interacting stressors whose impact depends on the system's adaptive reserve. Iron homeostasis in athletes thus reflects not only exposure to specific challenges but also the metabolic context in which these challenges occur.

5.3 Re-interpreting Low Ferritin in Athletes: Deficiency, Adaptation, or Constriction?

If iron homeostasis reflects regulated adaptation under sustained physiological stress rather than isolated deficiency, inevitably this challenges how commonly used biomarkers are interpreted in athletic populations.

Whether reduced iron stores directly impair athletic performance remains a topic of ongoing debate. Several lines of evidence indicate that serum ferritin alone is an imperfect predictor of performance outcomes, particularly in iron-deficient non-anaemic athletes [163], [164]. A systematic review by Rubeor et al. (2018) demonstrated that iron supplementation does not consistently improve performance when baseline ferritin concentrations exceed approximately 20 $\mu g \cdot L^{-1}$. Importantly, all studies reporting performance benefits included athletes with ferritin concentrations ≤ 20 $\mu g \cdot L^{-1}$, suggesting the existence of a lower threshold below which iron availability becomes functionally limiting. In contrast, supplementation above this threshold frequently improved biochemical markers of iron status without translating into measurable performance gains [165].
At the same time, reduced ferritin concentrations have been proposed as an early contributor to fatigue and impaired exercise tolerance, even when hemoglobin remains within reference ranges [166]. This raises the possibility that iron deficiency initially manifests through subtle functional impairments, such as increased perceived exertion, impaired recovery, or reduced training quality [167], [168], rather than overt decrements in competition performance [169]. Such changes may be perceived by athletes during daily training long before they are detectable through conventional performance testing.
From a systems perspective, these seemingly inconsistent findings may reflect the adaptive capacity of the physiological network. Moderately reduced iron stores may represent a regulated equilibrium under sustained demand rather than overt pathology. In this context, compensatory mechanisms across hematological, metabolic, and immune systems may temporarily preserve oxygen transport and performance despite constrained iron availability. This framework provides a plausible explanation for the pronounced interindividual variability observed in athletic populations: while some

athletes with low ferritin continue to train and compete successfully, others exhibit clear functional limitations. Factors such as baseline iron stores, training load, energy availability, inflammatory burden, hypoxic exposure, and sex-specific influences likely modulate whether reduced iron stores translate into functional constraint.
Consistent with this interpretation, pooled analyses indicate that iron supplementation reliably improves markers of iron status, including serum ferritin, serum iron, and transferrin saturation, and moderately improves hemoglobin concentration and aerobic capacity, even when effects on performance outcomes remain equivocal [164]. Similarly, recent reviews have concluded that while iron supplementation effectively restores iron status, its effects on training adaptation and performance are heterogeneous and context dependent [170]. Collectively, these findings underscore that biochemical repletion does not automatically equate to functional benefit.

From a practical standpoint, several authors have therefore argued that iron requirements in athletes may exceed those of the general population. An early study from Nielsen and Nachtigall (1998) proposed initiating oral iron supplementation at ferritin concentrations below 35 $\mu g \cdot L^{-1}$, a threshold notably higher than that applied in many intervention studies [127]. Others have suggested that null findings in supplementation trials may reflect insufficient treatment duration or failure to restore iron availability to levels that meaningfully support erythropoiesis, mitochondrial function, and training adaptation [171]. Taken together, these considerations highlight that low ferritin should not be interpreted as a binary marker of deficiency, but rather as an indicator of physiological strain whose functional relevance depends on system context, adaptive capacity, as well as individual demand and consequences might be reflected in another connected system first.

5.4 Limitations

Adopting a network-physiological perspective enables a more integrative understanding of iron metabolism in athletes but is inherently associated with methodological limitations. These limitations apply both to the conceptual framework and to the individual studies included in this cumulative dissertation. Given the complexity of human physiology, it is neither reasonable nor methodologically realistic to capture all interacting systems simultaneously. The investigated influencing factors therefore represent selected entry points into a broader regulatory network of iron homeostasis.
Interpretation of iron-related biomarkers in athletes is further complicated by the dynamic nature of training and adaptation. Continuous exposure to fluctuating stressors, including training load, recovery, energy availability, inflammation, and hypoxic exposure, can induce temporary changes in iron parameters. Consequently, single time-point measurements may inadequately reflect longer-term regulatory states.
Methodological accuracy in blood sampling is therefore critical. Pre-analytical factors such as recent exercise, timing of blood collection, circadian variation, dietary intake, and acute inflammatory responses substantially influence iron-related markers [147]. In female athletes, additional variability arises from menstrual cycle-related hormonal fluctuations. The methodological framework proposed by McKay et al. provides practical guidance for blood sampling in sport science research, including a checklist addressing timing, training context, menstrual cycle phase, and nutritional influences, thereby reducing the risk of misinterpretation [147].

An additional layer of limitations arises from this system-based adaptive perspective and inevitably comes with the question of how to distinguish between adaptive and maladaptive alterations in iron status. Determining whether a given change reflects functional adaptation or a dysregulation requires broad diagnostics. Next to the bespoken systems, additional variables of added systems may need to be included and analyzed in a longitudinal approach. Across the studies of this project and within existing literature interindividual variability was evident, underscoring that reference ranges may serve as useful orientation but must be interpreted cautiously and in relation to complementary markers. It may be useful to move beyond objective markers and include subjective measure such as symptoms that are reported by athletes and also observation of coaches to identify the "tipping point", at which compensatory mechanisms are exhausted and reduced iron availability can no longer be buffered by systemic adaptation.

Further limitations include limited sample sizes due to the demands of controlled experimental designs and limited causal inference at the system level, particularly for multifactorial influences such as energy availability. Moreover, not all studies included direct performance outcomes, limiting conclusions regarding the immediate functional relevance of observed alterations in iron metabolism to athletes.

5.5 Conclusion

The findings of this cumulative dissertation demonstrate that iron metabolism in athletes cannot be adequately interpreted when reduced to isolated hematological markers. Instead, iron emerges as a dynamically regulated resource embedded within a network of interacting physiological systems, whose relevance becomes most apparent when homeostasis is challenged by sport-specific stressors.

Across the included studies, factors such as hypoxic exposure, menstrual blood loss, and constrained energy availability acted not as isolated risks but as influencing forces that continuously shape iron regulation. In this sense, iron metabolism in athletes resembles a tight-rope walker operating under changing conditions: balance is maintained not in the absence of disturbance, but through constant adjustment to external forces such as wind or shifting load. Reduced iron stores may therefore reflect a regulated symmetry under sustained demand rather than immediate dysfunction.

This perspective helps contextualize the ongoing debate surrounding low ferritin concentrations in athletes. While performance impairments appear most consistently when iron stores fall below critical thresholds, moderately reduced ferritin levels may coexist with preserved performance through compensatory adaptations across hematological, metabolic, and immune systems. However, such compensation may increase physiological strain over time and should not be interpreted as physiological neutrality. Future research should therefore move beyond static deficiency thresholds and adopt longitudinal, system-oriented monitoring strategies that integrate iron biomarkers with training load, energy availability, inflammatory status, and sex-specific factors.

Interventions may benefit from exploiting system interconnections, for example by restoring energy availability, optimizing supplementation timing, or targeting periods of

elevated erythropoietic demand. Multi-functional compounds such as lactoferrin represent a promising avenue for such approaches but require further investigation in athletic populations.

In conclusion, this dissertation advances the understanding of factors influencing iron metabolism in female and male athletes by reframing iron not as an isolated hematological variable, but as a central mediator within an adaptive physiological network. Recognizing iron metabolism as a process of continuous balance under influence provides a more physiologically grounded basis for interpretation, monitoring, and intervention in athletic practice. A systemic approach capable of identifying individual “tipping-points” where adaptive compensation transitions into maladaptation should be the overarching aim of sport science in this area.

Plainly speaking: the body is a wonder of complex, most flexible (inter-)reaction and adaptation, where constant changes are the norm. This dissertation and therefore iron metabolism can merely serve as an example, presenting one puzzle piece in a universe of pieces, only scratching on understanding something so manifold.

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