Thesis

THE ASSESSMENT OF IRON STATUS IN THE CONTEXT OF INFECTION AND INFLAMMATION

A Review of the Current Literature for the Clinical Pilot Study: OLEOvital®Fe FEMINA

Submitted by

Sarah Maria Taschwer

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Under the guidance of

Assoz. Prof.in Priv.-Doz.in Mag.a Dr.in rer.nat. Sandra Holasek

MMag.a Dr.in Sonja Lackner

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Statutory Declaration

I declare on my honor that I have written this thesis independently and without assistance, I have not used other than the specified sources, and parts taken from other sources, verbatim or in substance, have been identified as such.

Graz, November 24th, 2021 *Sarah Maria Taschwer eh.*

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Table of contents

Abbreviations

ACD	anemia of chronic inflammatory diseases
AGP	α1-acid glycoprotein
aHR	adjusted hazard ratio
BMI	body mass index
BRINDA	Biomarkers Reflecting Inflammation and Nutrition Determinants of Anemia
CF	correction factor
CHr	reticulocyte hemoglobin content
CI	confidence interval
CK	creatine kinase
CKD	chronic kidney disease
CRP	C-reactive protein
DCT 1	divalent cation transporter 1
ELSA	enzyme-linked immunoassay
EP	erythrocyte protoporphyrin
EPDS	Edinburgh Postnatal Depression Scale
Fe2+	ferrous iron
Fe3+	ferric iron
FPN	ferroportin
Hb	hemoglobin
HCP 1	heme carrier protein 1
HDL-col	high-density lipoprotein cholesterol
ID	iron deficiency
IDA	iron deficiency anemia
IL	interleukin
LDH	lactate dehydrogenase
MCH	mean corpuscular hemoglobin
MCV	mean corpuscular volume
mRNA	messenger ribonucleic acid
NTBI	non-transferrin bound iron
OR	odds ratio
PRISMA-ScR	Preferred Reporting Items for Systematic Reviews and Meta-Analyses extension for Scoping Reviews

RC	regression correction
RCT	randomized controlled trial
SF	serum ferritin
sTfR	soluble transferrin receptors
T2D	type 2 diabetes
TBI	total body iron
TfR	transferrin receptor
TIBC	total iron-binding capacity
TNF-α	tumor necrosis factor alpha
TSAT	transferrin saturation
VO_2 max	maximal oxygen consumption
VO_2 peak	highest measured oxygen consumption
WHO	World Health Organization

Figures

Table directory

Zusammenfassung

Einleitung: Eisenmangel kommt in der heutigen Welt sehr häufig vor und ist durch verschiedenste Mechanismen verursacht. Trotzdem ist die richtige Diagnose eines Eisenmangels nicht immer einfach. Vor allem in Bezug auf Entzündungen sind die Biomarker des Eisenhaushaltes nicht immer klar zu deuten. Dies könnte besonders im Zusammenhang mit chronisch-entzündlichen Erkrankungen, dem metabolischen Syndrom und Depression ein Problem darstellen, unter Beachtung dessen, dass Zusammenhänge zwischen der Höhe der Eisenstatusparameter und diesen Erkrankungen gefunden wurden. Zusätzlich erweist sich Eisenmangel und dessen richtige Diagnose und Therapie auch im Bereich der Sportler*innen als ein häufiges und wichtiges Thema. Diese Diplomarbeit bezieht sich speziell auf diese Personengruppen.

Methoden: Diese Arbeit basiert auf den Methoden eines Scoping Reviews. Es wurde eine strategische Suche auf PubMed durchgeführt. Insgesamt wurden 26 Übersichtsarbeiten, Metaanalysen und randomisierte, kontrollierte Studien in diese Arbeit eingeschlossen, um eine Übersicht über die aktuellste Literatur zum Thema Eisenstatus im Bezug auf Entzündung und die Zusammenhänge mit häufigen klinischen Bedingungen, wie chronisch-entzündliche Erkrankungen, das metabolische Syndrom und Depression, sowie mit Athlet*innen, zu geben.

Ergebnisse: Insgesamt erfüllten 23 Studien von der Suche auf PubMed die Einschlusskriterien. Anhand sieben dieser Studien wurden die Ansätze einer richtigen Interpretation der Eisenmarker bei erhöhten Entzündungsmarkern erläutert. Serum Ferritin ist aktuell noch der meist verwendete Eisenmarker. Damit dieser jedoch auch bei systemischen Entzündungen zuverlässig verwendet werden kann, muss Serum Ferritin an erhöhte Entzündungsmarker angepasst werden oder in Kombination mit TSAT interpretiert werden. Aus Sicht der heutigen Forschung beziehen sich sehr wenige akademische Arbeiten auf neuere Eisenmarker, wie sTfR oder Hepcidin. Durch die Ergebnisse von zehn der Studien, die aus der Literatursuche hervorgingen, wurde das Problem der korrekten Eisenstatusdiagnostik unter verschiedenen klinischen Bedingungen analysiert, wobei sechs weitere Arbeiten eingeschlossen wurden, die die Relevanz dieses Themas bei Sportler*innen behandeln. Die Ergebnisse dieser Diplomarbeit zeigen, dass viele Studien einen Zusammenhang zwischen einem niedrigen Eisenstatus, Entzündung und den klinischen Zustandsbildern, die in dieser Arbeit im Mittelpunkt stehen (chronisch-entzündliche Erkrankungen, das metabolische Syndrom, Depression, Athlet*innen), finden

konnten. Die genauen pathophysiologischen Mechanismen dieser Zusammenhänge sind jedoch noch nicht endgültig geklärt.

Schlussfolgerung: Entzündungen haben eine große Bedeutung in der Diagnostik und schließlich auch in der Therapie von Eisenmangel. Obwohl die Verwendung von Serum Ferritin zur Erhebung des Eisenstatus bei erhöhten Entzündungsmarkern umstritten ist, wird es aktuell auch in diesem Zusammenhang noch hauptsächlich verwendet, obgleich auch andere Eisenmarker erforscht werden. Da sowohl Entzündungen als auch Eisenmangel in vielen sehr häufigen Krankheitsbildern der heutigen Welt, wie chronisch-entzündlichen Krankheiten, dem metabolischen Syndrom und Depression, und auch bei Sportler*innen eine erhebliche Rolle zu spielen scheinen, ist die korrekte Diagnostik des Eisenstatus unter inflammatorischen Bedingungen wesentlich. Um die effektivste Behandlung für alle Patient*innen mit Eisenmangel zu gewährleisten, werden zusätzliche Studien mit speziellem Augenmerk auf diese bestimmten Personengruppen benötigt.

Abstract

Introduction: Iron deficiency is very common in the world nowadays and caused by various mechanisms. Nevertheless, the correct diagnosis is not always easy. Especially in the setting of inflammation, the proper interpretation of the biomarkers of the body iron status is difficult. This problem may also be essential in detecting iron deficiency in individuals with chronic inflammatory diseases, the metabolic syndrome and depression, particularly as associations between the iron indicator levels and these diseases have been found. Additionally, iron deficiency and its correct detection and treatment is crucial for athletes. This thesis focuses on all these patient groups.

Methods: This thesis is based on the methods of a scoping review. A strategic search was done on PubMed. In total, 26 systematic reviews, meta-analyses and randomized controlled trials were included to give an overview of the most recent literature on iron status, inflammation and the correlation with common settings including chronic inflammatory diseases, the metabolic syndrome and depression as well as athletes.

Results: In total, 23 studies from the PubMed search met the inclusion criteria. Based on seven of these studies, the approaches for a correct interpretation of the iron indicators in the setting of inflammation were elucidated. Serum ferritin still is the most commonly used iron indicator, but in order to be reliable in the setting of inflammation, it has to be adjusted to increased inflammatory markers or interpreted in combination with TSAT. Regarding the contemporary research on this topic, rather few academic works focus on more recently considered markers of iron status, such as sTfR or hepcidin. The results of ten of the studies selected in the literature research were used to analyze the problem of the correct diagnosis of iron status in various clinical settings. Furthermore, six additional studies, which elaborate the relevance of this topic in athletes, were included. The results of this thesis reveal that numerous studies found associations between a low iron status, inflammation and the clinical settings that are in the focus of this work (chronic inflammatory diseases, metabolic syndrome, depression, athletes). Nevertheless, the exact pathophysiological mechanisms between these relations are still not entirely clear.

Conclusion: Inflammation is an important factor in the diagnosis and, hence, in the treatment of iron deficiency. Despite the use of serum ferritin in the detection of iron status in the setting of increased markers of inflammation is controversial, it is currently still the most employed marker in this context, even though other iron indicators are investigated. Since not only inflammation but also iron deficiency may play a crucial role in different very prevalent diseases of the Western world, such as chronic inflammatory diseases, the

metabolic syndrome and depression, and also in athletes, the correct diagnosis of iron status in the setting of inflammation is essential. In order to ensure the most effective treatment possible for all patients with iron deficiency, additional studies with special emphasis on these specific groups are necessary.

1 Introduction

Iron is a very important metal for the human body. A certain minimum amount of iron is needed for vital processes such as aerobic respiration. On the other hand, an iron overload leads to intoxication (1, 2). Iron deficiency is still a widespread problem of the world population. According to the World Health Organisation (WHO), there is a high prevalence of the lack of this essential trace element especially in preschool-aged children and women worldwide (3, 4).

1.1 Iron metabolism

The total amount of iron in the human body is ideally at about 3 to 4g. Around 70 percent of the total body iron is part of hemoglobin (5). The recommended daily intake of iron is 15mg for women and 10mg for men. The higher need in iron in females results from the monthly period and related blood loss in premenopausal women (6).

1.1.1 Iron absorption

Iron exists in food as heme iron, found in hemoglobin and myoglobin of meat, and non-heme iron, found in plant food such as legumes, greens like spinach, beet root, fruits like raspberries, strawberries, cassies and various seeds and spices, for example (6). In the duodenum, about 1.2mg non-heme iron and 0.4mg heme iron are absorbed per day (5). Since the main loss of iron in the body is caused by menstruation and other blood losses, an exact regulation of iron absorption is essential (1).

Heme iron is absorbed to the enterocytes through the duodenal heme carrier protein 1 (HCP 1). Intracellularly, the heme oxygenase splits heme iron into ferric iron (Fe3+), carbon monoxide and biliverdin. Fe3+ is reduced to ferrous iron (Fe2+) and bound to mobilferrin. In this state, the transport through the cell is possible.

Non-heme iron occurs as ferrous and ferric iron. Fe3+ forms salts and, therefore, it cannot be absorbed by the gastrointestinal tract. Fe2+, however, is absorbed by the duodenal mucosa. Specific ferrireductases (duodenal cytb) from the brush border of the enterocytes reduce Fe3+ to Fe2+ and thereby induce the absorption of non-heme iron (6). Ascorbic

acid, commonly known as Vitamin C, also reduces ferric to ferrous iron and, consequently, improves its absorption, too (7). Tannins, found in coffee or black tea, inhibit this reaction by building insoluble complexes with dietary iron (6). Proton pump inhibitors, antacids, tetracyclines and calcium, for example contained in dairy products, decrease the absorption of non-heme iron as well (1). The uptake of Fe^{2+} happens through the divalent cation transporter 1 (DCT 1). It is intracellularly bound to mobilferrin and transported through the cell (6). Ferroportin, a channel protein, facilitates the transport of ferrous iron through the basolateral membrane. Extracellularly, it is oxidized by a ferroxidase and bound to the transport protein transferrin (8).

One mechanism regulating iron absorption is the cysteine-rich peptide hepcidin. Hepcidin, which is produced by the liver, causes a higher lysosomal breakdown of ferroportin and, therefore, inhibits the intestinal iron absorption and the iron release out of macrophages. Through a negative feedback mechanism, the production of hepcidin is boosted by iron. Mediators of inflammation (Interleukin 6) further increase the production of hepcidin, whereas anemia and hypoxia reduce it (6).

Another regulation mechanism is illustrated by the crypt programming model. Hereby, it is assumed that the amount of iron in the crypt cells of the duodenum is related to the body´s iron storage. Depending on these values, a smaller or larger amount of iron is absorbed by the crypt cells becoming absorptive cells in the small intestine. Transferrin receptors (TfR1 and TfR2) regulate the uptake of transferrin-bond iron from the plasma into the crypt cells.

Finally, the iron absorption in enterocytes is mediated by influencing the expression of TfR1, DCT1 and ferroportin. This happens through cytosolic iron regulatory proteins, which react in response to the current intracellular iron concentration and are responsible for an upregulated or downregulated synthesis of the named transport proteins. An iron deficit increases the production of these, whereas a large amount of iron decreases them (2).

1.1.2 Iron homeostasis

In the body, iron can be divided into three main compartments, which can be evaluated each through different iron indicators. For iron stores, serum ferritin is used as the most

common biomarker (9). Main iron storage is found in the liver, spleen, bone marrow and in the reticuloendothelial system, more precisely in macrophages. Macrophages and hepatocytes store about 0,7g iron bound to ferritin (5, 1, 10). The second body iron compartment is transport iron. This part can be estimated through transferrin saturation, soluble transferrin receptor in the serum and protoporphyrin in the erythrocytes. Functional iron, as the third compartment, is measured through hemoglobin or hematocrit in the whole blood count (9).

Iron uptake is responsible for a correct iron homeostasis (10). The larger part of iron is recycled after hemolysis. Only a small amount of total body iron is provided through diet. Both ways, ferric iron – non-heme iron – is bound to transferrin and mainly transported to the bone marrow, where it is reused for the synthesis of hemoglobin. However, a certain percentage of transferrin-bound iron also enters immune cells and hepatocytes. The normal saturation of transferrin is at 20 to 40 percent. Specific transferrin receptors are expressed by the target cells to ensure iron uptake. TfR1 are found on each cell of the body, whereas TfR2 are more specific and expressed only by enterocytes, hepatocytes and erythrocytic progenitor cells. The iron-transferrin complexes, coated with clathrin and called siderosomes in this form, are absorbed by the cells. Intracellularly, Fe^{3+} is separated, reduced to Fe^{2+} and the transferrin-TfR complex is recycled (1, 2).

The usual occurrence of heme iron is within red blood cells, in the complex of hemoglobin. Iron is required for aerobic respiration in this form (1). In body fluids, the chaperone protein haptoglobin binds hemoglobin and prevents it from being broken down into its parts. Likewise, free heme iron is bound to the protein hemopexin (11).

It is very important that proteins such as transferrin, haptoglobin and hemopexin exist, since free iron reacts toxically to the tissue by producing free radicals, which in turn lead to oxidative damage in the tissue (12).

If there is an imbalance in iron homeostasis due to a variety of causes, two main severe disorders can be named, iron deficiency and iron overload.

1.2 Iron deficiency

An insufficient iron supply, higher loss or need of iron can result in iron deficiency (ID). This issue is associated with a variety of clinical symptoms and affects human health in different ways, including changes in the energy metabolism or reduced immune function even before reaching an anemic state (13).

Iron deficiency may be caused by different factors. Firstly, growth increases the need for iron, which is the reason for a higher incidence of iron deficiency among children. Secondly, the demand for iron is higher in pregnant women and post-bleeding recovery, as an increased erythropoiesis is necessary in these settings. Thirdly, iron deficiency can result from a diminished dietary supply, for instance due to malnutrition, but also because of the consumption of previously mentioned inhibitors of iron absorption or malabsorption. Finally, increased blood loss, for example during menstruation or chronic bleeding, can be responsible for a non-sufficient iron supply (1).

In line with the three main iron compartments, iron deficiency can be divided into three different stages. Iron depletion, also named latent ID, is the first stage, in which iron stores are reduced. It can be detected through low serum ferritin. At this stage, erythropoiesis and hemoglobin concentration are not yet affected. If iron transport is limited, it will first be shown in erythropoiesis. Therefore, the second stage of iron deficiency is called iron-deficient erythropoiesis or storage ID. The amount of hemoglobin is not remarkably lowered in this stage (9). The laboratory markers which are important for iron-deficient erythropoiesis are primarily transferrin saturation (TSAT), soluble transferrin receptors (sTfR) and the concentration of erythrocyte protoporphyrin (EP). In storage ID, TSAT is low and sTfR and EP show an increase (14). Iron deficiency anemia, as the third stage of ID, is marked by lowered hemoglobin concentration. At this stage, in otherwise healthy people, all three main iron compartments are reduced. This is reflected in low serum ferritin and low hemoglobin concentration, as well as in higher iron transport markers to increase iron supply (9).

In addition to that, there is another state called functional ID, when iron stores, e.g. serum ferritin, do not show a depletion but there is no sufficient iron supply to the bone marrow for erythropoiesis, which for example is displayed by low TSAT or a low reticulocyte hemoglobin content. This occurs due to inadequate mobilisation of iron out of the

macrophages, for example in anemia of chronic inflammatory diseases (ACD) as will be explained later in this report (1).

1.3 Iron overload

Too much iron is toxic for the human body. This is due to the fact that if there are not enough iron-binding proteins available, free iron accumulates in vital organs, such as the liver or the myocardium, and causes damage to the tissues (11). Therefore, it is important to diagnose and treat iron overload properly. There are many different causes for iron overload disorders. There are two types of iron overload, primary iron overload syndromes, which are hereditary forms of hemochromatosis, and secondary iron overload.

The different hereditary forms that lead to primary iron overload syndromes stem from mutations in the genes responsible for the production of iron-metabolism regulating proteins or iron-binding proteins. The most common form is the autosomal recessive Type 1 hereditary haemochromatosis HFE. Hereby, a mutation in the HFE gene is responsible for smaller iron uptake in duodenal crypt cells from plasma. Consequently, the crypt programming model is impaired, and more and more iron is absorbed by the gut.

Secondary iron overload is caused by the following disorders. In case of iron loading anemias, for instance in thalassaemia, ineffective erythropoiesis and hyperplastic erythroid marrow are stimulating iron absorption. Also the setting of chronic blood transfusion, e.g. in children with beta thalassaemia, can lead to iron overload. Another form of secondary iron overload is African iron overload. Initially, this form of iron overload in African people was thought to be caused by a high dietary iron intake due to a traditional home brewed beer. Today, also a genetic cause is discussed, but the exact reason for African iron overload remains unclear. At last, iron overload occurs in chronic liver diseases such as alcoholic liver disease or non-alcoholic steatohepatitis as well. This may be due to different causes, such as decreased transferrin synthesis or decreased uptake of iron into erythrocytes in cirrhosis (2).

1.4 Biomarkers of iron status

Since too little iron in the body results in severe complications including iron deficiency anemia (5), and iron overload also leads to serious problems because of accumulation in tissues, e.g. the liver or myocardium (15), it is important to evaluate a precise iron status. Some iron markers are affected by different settings (16, 14). Therefore, a range of biomarkers is needed to detect iron deficiency or iron overload under any circumstances.

1.4.1 Iron stores – Ferritin

15-25% of the whole body iron are stored in ferritin and hemosiderin. One ferritin molecule holds approximately 4500 ferric iron atoms. In contrast to the soluble ferritin, hemosiderin, a degradation product of ferritin, is insoluble and stored in lysosomes. Both protein complexes provide iron for processes in which it is needed, e.g. for heme-synthesis. Two main subunits, L- and H-subunits, form a variety of different isoferritins. In cells which have a high need for iron, such as cells of erythropoiesis or cardiac muscle cells, ferritins with mostly H-subunits are found, since these have a better iron uptake due to ferroxidase activity. Iron storing cells, especially in the liver and spleen, contain L-rich ferritins (8).

Stainable bone marrow iron is the gold standard to estimate iron stores, but it is a rather invasive intervention (9). Therefore, serum ferritin (SF) is the most commonly used iron indicator reflecting iron stores, since there is a proven correlation between this parameter and iron storage (16). The main laboratory methods to detect serum ferritin are enzyme-linked immunoassays (ELSA). The normal concentration of serum ferritin is between 15-300µg/l. Children and premenopausal women have lower serum ferritin values than men. In a healthy population, less than 15µg of ferritin is defined as iron deficiency with a sensitivity of 82% and specificity of 95%. In pregnancy, women should already take iron supplements if they display a ferritin concentration of less than 20µg. For the diagnosis of the early stage of primary iron overload, hemochromatosis, serum ferritin is not a reliable predictive parameter since it remains normal in this phase. However, it can be used in secondary iron overload to display the treatment effect, as it mirrors not only iron stores but also liver damage. Hereby, SF should get below 1000µg/l. As will be explained later in

this paper, serum ferritin concentrations vary in certain clinical settings. Therefore, these values only apply to otherwise healthy people (16).

1.4.2 Transport iron

There are different indicators that reflect transport iron availability. Transferrin saturation, erythrocyte protoporphyrin and the concentration of soluble transferrin receptors are the ones mainly used (9).

1.4.2.1 Transferrin saturation (TSAT)

In the plasma, the larger part of iron is bound to the transport protein transferrin. Every transferrin molecule can hold two iron atoms. The amount of transferrin in the blood varies under certain conditions. For example, transferrin production increases during iron deficiency to provide a more efficient iron supply (14). To receive a reference value of the amount of transport iron in the blood, which is also known as transferrin saturation, there are different ways of calculation dependent on the measured indicators (9). The following markers can be used for estimation of the transferrin saturation. The first marker, the serum iron concentration, detected either by colorimetric principles or by direct measurement with a spectrophotometer, usually lies between 50 and 120µg per dl serum. The second parameter is the total iron-binding capacity of transferrin. Hereby, the number of binding sides of transferrin is measured in micrograms per decilitre serum (14). It is also possible to directly quantify transferrin itself. Finally, the unsaturated iron-binding capacity can be displayed by measuring only the free binding sides of transferrin. There are three ways of calculating the percentage of the transferrin saturation. Either the ratio of serum iron to total iron-binding capacity or to transferrin can be computed, or serum iron may be divided through the sum of unsaturated iron-binding capacity and serum iron (9). The usual TSAT counts between 35 and 45% (14).

1.4.2.1 Erythrocyte protoporphyrin

In hemoglobinsynthesis, ferrous protoporphyrin and globin react to hemoglobin in progenitor cells of erythrocytes, such as reticulocytes. If there is a shortage in the iron supply, zinc binds to protoporphyrin instead of iron. Therefore, in the state of iron deficiency, when too little iron reaches the bone marrow, there is an increase in zinc protoporphyrin. By measuring this parameter directly, the availability of transport iron can be displayed. Another method is the chemical extraction of free erythrocyte protoporphyrin as a remnant from zinc protoporphyrin. Erythrocyte protoporphyrin (EP) is the generic term for both variants and it is given as µg/dl blood or µg/dl erythrocytes. In a healthy population, EP concentration measures less than 40-50µg/dl erythrocytes. EP is indirectly proportional to the relative iron supply and shows a direct proportion to erythropoietic activity (14).

1.4.2.2 Soluble transferrin receptor (sTfR)

The transferrin receptor (TfR) is a membrane receptor for iron uptake from transferrin-bound iron in the plasma to the cells. With exception of mature red cells, all body cells have this receptor on their surface. Since most of the circulating iron is needed for erythropoiesis, the largest amount of TfR is found in the bone marrow. Soluble transferrin receptor is a form of TfR circulating in plasma in a small amount but proportional to the cellular TfR (17). Erythroblasts are the main producing source of TfR using iron for hemoglobin production (14). Hence, sTfR concentration mirrors erythropoietic activity and abnormal red blood cell production can be detected by it. For instance, in a current state of anemia, erythropoiesis is upregulated and sTfR concentration is high. That means that soluble transferrin receptor concentration itself is not specific enough for the evaluation of the iron status. Consequently, this parameter is only meaningful if other iron indicators, such as serum ferritin, are low (17). The laboratory methods of measuring sTfR are based on enzyme linked immunosorbent assays (ELISA). Since no standardized assay exists, it is important to compare the values with given references of each laboratory (14).

1.4.3 Functional iron – red blood cell parameters

Many processes in the human body are dependent on a sufficient iron supply. Erythrocytes need a large percentage of the functional iron pool. So, to identify if a functionally relevant iron deficit is present, red blood cell parameters have to be measured in addition to previously mentioned iron indicators. In case of iron deficiency anemia, for example, erythropoiesis is disturbed and erythrocytic parameters are abnormal. The following biomarkers should be used to interpret the iron status only in connection with other iron indicators (18).

1.4.3.1 Hemoglobin

Hemoglobin is the most important protein-binding functional iron for oxygen transport in red blood cells. A normal hemoglobin concentration indicates that the third stage of iron deficiency has not been reached yet (10). The following limits of hemoglobin are defined by the World Health Organization for different phases of life and sexes (19):

Table 1: Limits of hemoglobin level (19)

Patient group	Hemoglobin level limit
Children 6 months to 59 months	110 g/l
Children 5–11 years	115 g/l
Children 12–14 years	120 g/l
Non-pregnant women (above 15 years of age)	120 g/l
Pregnant women	110 g/l
Men (above 15 years of age)	130 g/l

Nevertheless, there is a large variation of hemoglobin concentration in healthy people. The reason for that is the adaption of red blood cell production to less oxygen supply in the lungs. For example, smokers or people who exercise at high altitudes produce more erythropoietin, a hormone which in turn causes a larger number of erythrocytes. As a result, they show higher values of hemoglobin than non-smokers and individuals living at sea level. This must be kept in mind in the interpretation of red blood cell parameters. Otherwise, hemoglobin concentration is a reliable biomarker for anemia, but it is important to differentiate between iron deficiency anemia and anemia of other causes. If hemoglobin

is low and other parameters of iron status such as SF or TSAT are deviating from their normal values, this is a strong indicator for iron deficiency anemia (18).

1.4.3.2 Other red blood cell parameters

In addition to hemoglobin, there also are other red blood cell parameters which can be examined to evaulate the functional iron status. The hematocrit is the percentage of red blood cell volume to the whole volume of blood (6) [p225]. This parameter does not provide additional information to hemoglobin to detect anemia (18). Mean corpuscular volume (MCV) is defined as the ratio of hematocrit to the number of erythrocytes per blood volume and expressed in femtolitre, whereas the ratio of hemoglobin concentration to the number of erythrocytes per blood volume displays mean corpuscular hemoglobin (MCH), expressed in picogramme (6) [p231]. Both values are low in iron deficiency anemia, mirroring a microcytic, hypochromic anemia. Although these ratios were used to diagnose iron deficiency in the past, they are not specific enough for it. Since a reduction of MCV and MCH occurs in other diseases, for example in thalassaemia as well, other iron indicators are more suitable to detect anemia (18).

1.4.4 Additional iron indicators

As many commonly used iron markers are affected by certain clinical settings and a sway in iron concentration in the body shows severe consequences, plenty of research is done to reliably detect the iron status of the individuals. Hence, there is a variety of additional iron indicators available to diagnose either iron deficiency or iron overload (9, 13).

1.4.4.1 Total body iron

Total body iron (TBI) is calculated on the base of the serum ferritin to sTfR ratio independently from the hemoglobin concentration. Cook et al (20) found that there seems to be a good relation between body iron estimated with this method and more complicated calculations (20). The advantage of including these two parameters is that the severity of iron deficiency in every stage can be diagnosed, as serum ferritin is sensitive for iron

storage and sTfR reliably indicates emptied iron stores. TBI, expressed as mg/kg body weight, is an estimation of body iron stores, not a quantitative measurement. If iron stores are depleted, TBI shows values below 0, which can be interpreted as functional iron shortage that has to be refilled before iron can accumulate again in the stores of the body (9).

1.4.4.2 Hepcidin

Hepcidin, as a regulator of iron uptake from diet and iron release from macrophages, mirrors the iron status through a negative feedback mechanism (6). In individuals with iron deficiency or iron deficiency anemia, the hepcidin concentration decreases, whereas patients with iron overload show a massive increase in hepcidin. Since hepcidin also works as an acute phase reactant, it may be relevant in the distinction between iron deficiency anemia, with a low hepcidin concentration, and anemia of chronic diseases, which displays a higher hepcidin concentration. The influence of certain clinical settings in hepcidin concentration has to be considered when using it for diagnostic issues (21).

1.4.4.3 Non-transferrin bound iron

Non-transferrin bound iron (NTBI) is a biomarker mirroring the amount of iron in the plasma that is not bound to iron-binding proteins. This form of iron is known for its toxicity due to acting as a free radical and, thereby, catalysing different oxidative reactions, which in turn result in tissue damage (22). Consequently, it is useful to measure NTBI to detect the extent of iron overload and its severity. Since NTBI also appears in patients receiving iron supplementation shortly after the administration, this biomarker may be of importance not only for tracing the efficiency of the treatment of iron overload but also for controlling the therapy of iron deficiency. An important subtype of NTBI is labile plasma iron (LPI), which is the term for the free plasma iron that is redox active. As the equivalent of the labile cell iron, this fraction is a relevant marker for reflecting therapeutic chelation in patients suffering from iron overload (23). Considering the fact that there is a variety of chemical forms of NTBI, there are many different methods to measure NTBI concentration. Due to this, the different assays do not show a good comparability (9).

1.4.4.4 Reticulocyte indexes

Additionally, reticulocyte indexes are another useful indicator for detecting functional iron deficiency (9). Reticulocyte hemoglobin content (CHr) reflects the iron status of the bone marrow and, hence, whether it contains enough functional iron for an efficient erythropoiesis. Especially in the early state of functional iron deficiency, CHr is a useful parameter since reticulocytes remain only one to two days in the blood (24). Functional iron deficiency can be defined with a CHr below 28pg (1). As will be mentioned in the next section of this thesis, CHr is an important parameter in diagnosing the kind of iron deficiency via the Thomas-plot (13). Otherwise, reticulocyte cell volume is sensitive for evaluating and monitoring anemia (24).

1.4.4.5 Thomas-plot

The Thomas-plot is a diagnostic tool to differentiate between normal iron status, latent iron deficiency, storage ID and functional ID combined with the anemia of chronic diseases. This diagnostic plot uses a CHr cut-off of 28pg to distinguish normal iron status/latent ID from storage ID/functional ID with ACD. The ratio of sTfR and logarithm of ferritin (sTfR/log ferritin) is taken to discriminate normal iron status and functional ID from the states in which iron stores are depleted. The cut-off values of this ratio depend on the existence of an acute-phase reaction measured with C-reactive protein (CRP). In case of a CRP value of ≤0.5mg/dL, the sTfR/log ferritin cut-off lies at 1.5, whereas a CRP of >0.5 mg/dL requires a cut-off of 0.8 (25).

The following table provides an overview of the biomarkers of the iron status and the recommended cut-offs for defining iron deficiency.

Table 2: Biomarkers of the iron status

Biomarker	Recommended cut-off
Ferritin	15 - 300µg/l
Transferrin saturation	35-45%
Erythrocyte protoporphyrin	<40-50µg/dl erythrocytes
Soluble transferrin receptor	no standardized values

Hemoglobin	women: >120g/l, men: >130g/l
Total body iron	>0mg/kg body weight
Hepcidin	no standardized values
Non-transferrin bound iron	no standardized values
Reticulocyte hemoglobin content	>28pg

1.5 Patient groups affected by iron deficiency

Iron deficiency has a high prevalence around the world. Since pathologies such as chronic inflammatory diseases, the metabolic syndrome including obesity, and depression are health problems that occur with increasing frequency today and seem to be linked to a disturbed iron homeostasis, more and more research is conducted about these relations. In this review, the most recent literature on the cause, diagnosis, and therapeutic approaches of the pathologic iron status in patients suffering from the following diseases will be discussed: chronic diseases, metabolic syndrome/obesity and depression.

The anemia of chronic inflammatory diseases (ACD) is a well-known problem patients are suffering from additionally to their underlying disease. In individuals with chronic diseases such as infections, cancer, and autoimmune diseases, cytokines and immune cells are responsible for a disturbed iron homeostasis, resulting in hypoferremia and anemia. Nevertheless, it has to be mentioned that the dysregulation of body iron is only one possible cause for ACD. Inhibited erythropoietic proliferation and reduced production of erythropoietin constitute other mechanisms for the pathogenesis of ACD (26). As Weiss and Goodnough (26) reviewed in their paper, it has been observed that in a state of chronic inflammation, cytokines induce an iron shift into macrophages by the transport protein DCT1. On the one hand, the upregulation of DCT1 is done by cytokines such as interferon-γ, lipopolysaccharide, and TNF-α, on the other hand these cytokines also downregulate ferroportin, so that iron is trapped in the macrophages (27). Moreover, hepcidin has been discovered to play an important role in the pathophysiology of the anemia of chronic diseases. As mentioned above, the acute-phase protein is upregulated by interleukin-6 and lipopolysaccharide and leads to a diminished iron absorption in the duodenum as well as to a reduced release from macrophages (28). Consequently, not only a hypoferremia in the

blood can be observed, but also an iron overload in tissues (29). These changes in iron homeostasis are named functional iron deficiency, since iron storages remain high, but the iron supply for erythropoiesis is limited. Due to the disturbed iron homeostasis, iron supplementation, oral or intravenous, is not effective in patients suffering from chronic diseases and it is only recommended if an absolute iron deficiency (SF <100ng/mL) was diagnosed or if a therapy with erythropoietic agents did not succeed (26).

Metabolic syndrome is a generic term for a group of cardiovascular risk factors with an increased risk for type 2 diabetes. These risk factors include abdominal obesity, hypertension, dyslipidemia with high fasting triglycerides and low high-density lipoprotein cholesterol (HDL-col) and altered glucose metabolism, resulting in increased insulin-resistance (30). In individuals with visceral obesity, the adipocytes of the adipose tissue produce cytokines (TNF-α, IL-6), which lead to an increase of the CRP concentration in the blood, oxidative stress in the tissue and, eventually, to a low-grade systemic chronic inflammation (31). Due to this pro-inflammatory state, especially overweight and obesity appear to have a strong impact on iron metabolism, similar to the hypoferremia in chronic diseases. The main changes in the iron homeostasis are an increase of DCT1 (iron uptake) and hepcidin, and reduced ferroportin for the release of iron from cells. Therefore, functional iron deficiency and, consequently, anemia are expected to have a high prevalence in obese subjects (32).

Recently, also research on the impact of iron status on a very common psychiatric disorder, depression, has been done. According to the WHO, depression is defined by the following symptoms: sadness, loss of interest, low self-worth, feelings of guilt, tiredness, poor concentration and changes in sleep behaviour or appetite (33). Since iron is needed as a cofactor for several enzymes to ensure an impeccable brain function, specifically for oxidation-reduction and different neurotransmitter metabolisms, an association between this psychiatric disorder and a low iron status seems not far-fetched (34). This possible relation aroused interest especially regarding postpartum depression in women and may reflect another cause for the importance of adequate iron supplementation (35).

Furthermore, iron homeostasis plays a very important role in athletes. Due to the high prevalence of iron deficiency occurring in elite athletes, particularly women (36), the pathogenesis and difficulties in supplementation will be reviewed in this article. Additionally, the gender aspects in the included studies will be analysed.

2 Material and methods

A review of the current literature was done in order to analyse the impact of inflammation on the most common iron indicators and summarise the existing research about the relevance of this problem in different clinical settings, especially regarding three rather frequent disorders in the industrialized world: chronic diseases, the metabolic syndrome and depression. Search results were limited to publications in English from July 2015 to July 2020 to find the most recent articles on this topic. To give a precise overview solely over scientific research of this field, search results were also restricted to meta-analyses, randomized controlled trials and systematic reviews. To guarantee high quality, mainly literature published in journals showing an impact factor ≥3.5 were considered in this thesis. Other selection criteria were that studies had to be based on human individuals and that they included an inflammatory state. Exclusion criteria were: 1) animal studies, 2) studies that had been published before 2015, 3) cross-sectional studies 4) papers that had been published in other languages than English.

In order to gain a first overview over the topic, PubMed was searched on July 27th, 2020 using the following search terms: “iron”, “iron deficiency”, “low ferritin”, “ferritin”, “ferritin blood level”, “inflammation”, “infection”, “depression”, “obesity” and “supplementation”. Moreover, reference lists of relevant articles were reviewed to find additional literature.

A second literature search in PubMed was conducted on October 8th, 2020 using the following, more exact search term combinations “iron/iron deficiency”, “inflammation/infection” and “ferritin” (n=58), “iron/iron deficiency”, “inflammation/infection” and “soluble transferrin receptor” (n=18) and “iron/iron deficiency”, “inflammation/infection” and “total-body iron stores” (n=1) to answer the first research question:

- What are the effects of the acute-phase response of inflammation on the interpretation of common iron indicators and innovative parameters?

The terms “iron/iron deficiency” and “anemia of chronic diseases” (n=1), “iron/iron deficiency” and “obesity” (n=53), “iron/iron deficiency” and “depression” (n=21) and “iron/iron deficiency” and “metabolic syndrome” (n=6) were used for the second research question:

- What is the relevance of this problem in different clinical settings, especially regarding the metabolic syndrome and depression?

To complete the gathering of information about the topic, a third database search was done on October 26th, 2020 employing the search terms: “iron/iron deficiency” and “athletes” (n=16) and “iron/iron deficiency”, “inflammation” and “supplementation” (n=30).

This literature review was conducted based on the PRISMA-ScR recommendations for scoping reviews. The Preferred Reporting Items for Systematic Reviews and Meta-Analyses extension for Scoping Reviews provides a checklist as a guidance for the implementation of a valid scoping review. 26 experts established 20 items plus 2 optional items, which should be included in this kind of research to ensure structure and traceability (37). The aim of a scoping review is to give an overview over existing literature about a specific topic rather than providing a concrete result to an exact question (38).

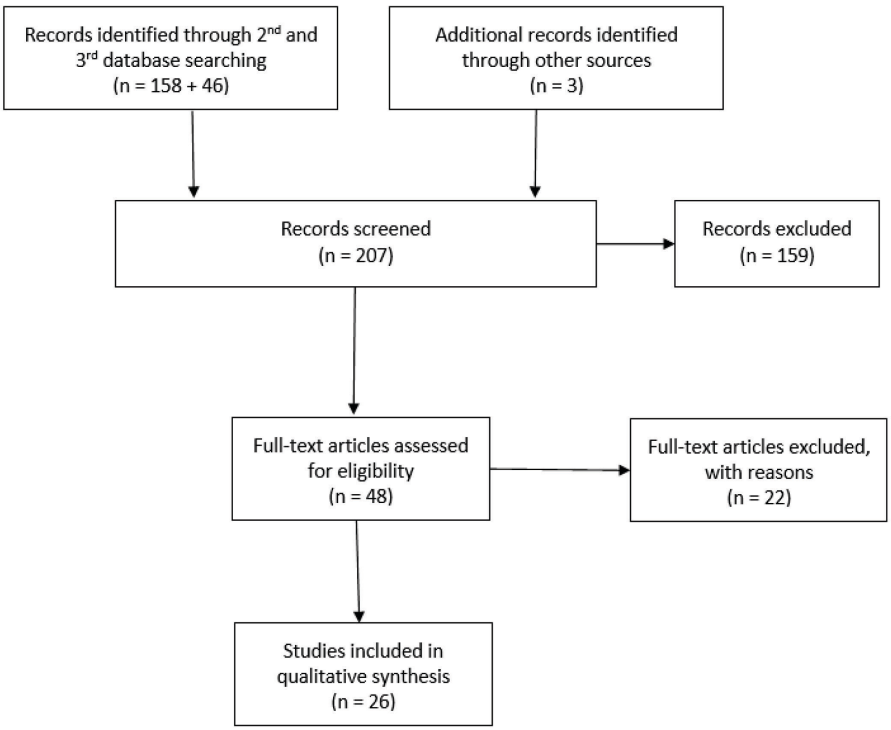

Figure 1: Flow diagram

As displayed in the flow diagram above, the second PubMed search with the already mentioned keywords resulted in 158 hits, whereas the third search counted in total 46 systematic reviews, meta-analyses and RCTs. However, only 23 articles meeting the inclusion criteria were selected for this review after the exclusion of papers investigating

iron deficiency in specific clinical settings such as hemodialysis or multiple sclerosis, as well as studies that considered malaria and articles about the relation between the maternal and the infantile iron status. A first selection of the papers was based solely on the titles. A more exact investigation of possibly qualified papers was made by consulting their abstracts or full-text, respectively. Additionally, four papers were referred to as standard literature to make the following information more comprehensible. These literature sources were used mainly to explain the basics of the pathophysiological mechanisms.

3 Results

From the PubMed search, 23 studies met the inclusion criteria, of which seven studies were chosen to review the diagnostic approaches to displaying the iron status in an inflammatory state correctly. Another ten studies were included to show the importance of these diagnostic methods in clinical settings with a high prevalence in the modern world, such as obesity, the metabolic syndrome and depression. In addition, six studies pointing out the correlation between iron homeostasis and athletic training were incorporated. Including the standard literature, a total of 26 papers were considered for answering the research questions of this thesis. Table 3 and 4 provide an overview over the included studies and their main outcomes in chronological order, classified by the two main research questions.

Table 3: Included studies - iron indicators (randomized controlled trial (RCT), systematic review (SR), meta-analysis (MA))

Author and Year of Publication	Study design	Subject of study	Population	Main Outcome
Kasvosve et al 2006	RCT	sTfR and inflammation	Zimbabwean children (n=208)	Increased sTfR during inflammation without relation to iron status
Peyrin-Biroulet et al 2015	SR	Diagnosis of iron deficiency in different clinical settings	26 guidelines	To diagnose ID, different cut-offs for SF are recommended in different settings; SF cut-off of 100µg/L and 20% of TSAT are appropriate in most clinical settings
Thurnham et al 2015	MA	Serum ferritin and inflammation	30 studies, all age groups and both genders (n=8,796)	Correction factors (0.77/ 0.53/ 0.75) for adjusting SF concentrations to inflammation in the incubation phase, early and late convalescence
Namaste et al 2017	MA	Serum ferritin and inflammation	BRINDA datasets (~26,000 premenopausal women, ~30,000 preschool children)	Use of a regression correction approach for SF to CRP or AGP to detect iron deficiency most precisely
Rohner et al 2017	MA	sTfR and inflammation	BRINDA datasets (~26,000 premenopausal women, ~30,000 preschool children)	Use of a regression correction approach for sTfR to AGP to detect iron deficiency most precisely
Mei et al 2017	MA	TBI and inflammation	BRINDA datasets (~26,000 premenopausal	Adjustment of TBI for inflammation by using a

			women, ~30,000 preschool children)	regression correction approach
Jorgensen et al 2017	RCT	Ferritin and inflammation in postpartum women	Postpartum women (n=114)	SF is not rising with elevated CRP or AGP concentrations in postpartum women
Garcia-Casal et al 2018	SR	Serum Ferritin in different clinical settings	Non-healthy participants of 38 studies (n=2,572)	The mean SF was 82.43µg/L in iron-depleted, non-healthy individuals
Cacoub et al 2019	SR	TSAT and inflammation	41 studies	SF and TSAT should be used to diagnose ID, especially to detect functional ID

Table 4: Included studies – different clinical settings (randomized controlled trial (RCT), systematic review (SR), meta-analysis (MA))

Author and Year of Publication	Study design	Subject of study	Population	Main Outcome
Zhao et al 2015	MA	Iron deficiency in overweight/obese	26 studies (13,393 overweight/obese, 26,621 non-overweight)	Overweight/obese show a higher overall risk of iron deficiency
Cepeda-Lopez et al 2015	RCT	Iron absorption in overweight/obese	Premenopausal women (n=62)	Ascorbic acid could not improve iron absorption in overweight/obese as good as in normally weighted individuals
Liu et al 2016	SR	Hepcidin and anemia of chronic diseases	129 references	Hepcidin antagonists are a potential therapy for anemia of chronic diseases
Li et al 2017	MA	Depression and iron intake	3 studies; 1 included women only, 2 both sexes (n=6,854)	Negative association between iron intake and risk of depression
Sheikh et al 2017	RCT	Depression and iron intake	Women (n=70)	Iron supplementation achieved better improvement of postpartum depression than placebo
Ishibashi et al 2017	RCT	Iron supplementation and its effect on hepcidin during exercise	Male runners and triathletes (n=14)	Iron supplementation increases hepcidin after 3 days of training
Suarez-Ortegon et al 2018	SR+MA	Ferritin and metabolic syndrome	26 studies (21 for MA), no information about	The metabolic syndrome was positively associated with high ferritin levels

			the size of population	
Hidese et al 2018	MA	Depression and IDA	Individuals with depression (n=1,000; 499 women), without depression (n=10,876, 5,185 women)	Positive association between self-reported history of depression and history of IDA
Rubeor et al 2018	SR	Iron supplementation and athletic performance in non-anemic ID	12 studies; non-anemic iron deficient athletes (n=273; 257 female)	No clear results as to whether iron supplementation is beneficial or not
Jiang et al 2019	MA	Ferritin and type 2 diabetes	15 studies (n=77,352; 24,629 female)	Linear correlation between SF levels and risk for type 2 diabetes
Heffernan et al 2019	SR	Iron supplementation and athletic performance	29 studies (n=945; 776 female)	Positive effect of iron on iron status and athletic performance when SF levels are low
Pompano, Haas 2019	RCT	Iron supplementation´s impact on performance	73 non-trained women	Iron supplementation in the untrained group showed a trend for higher VO2 peak than the placebo
Cordova et al 2019	RCT	Iron supplementation and its effect on blood parameters in athletes	Male cyclists (n=18)	Significant differences in serum iron, SF, Hb, hematocrit, cortisol
McCormick et al 2019	RCT	Time of exercise and its effect on hepcidin and iron absorption	Runners (n=16; 6 female)	Iron absorption is higher when conducting exercise in the morning
Zhang et al 2020	RCT	Iron storage markers and metabolic syndrome	Chinese rural children aged 6-12 years (n=1,333)	Positive correlation only between SF and HDL-cholesterol and diastolic blood pressure, not with other components
Teng et al 2020	MA	Weight loss and iron status	Overweight/obese children, adolescents, adults (n=879)	Loss of bodyweight through hypocaloric diet/physical activity can improve iron homeostasis by increasing functional iron parameters
Lee et al 2020	MA	Depression and IDA	Taiwanese population (n=58,191; 76.77% female)	Positive association between depression and IDA compared to the non-IDA individuals

All these studies contributed to the findings of this thesis. In the following section, the results of these papers will be elucidated more extensively. At first, the correlation between

iron homeostasis and inflammation will be discussed, then, the iron status in different clinical settings will be examined, and finally, relevant gender-related aspects will be taken into account.

3.1 Iron status indicators and inflammation

As Ross reviewed in her paper 2017 (10), the iron metabolism is affected by the hepatic acute phase response to infection and inflammation. This is due to the fact that several proteins involved in iron homeostasis also react as acute phase proteins. These include transferrin, ferritin, ceruloplasmin, haptoglobin, hemopexin and hepcidin to decrease the availability of free iron. Soares and Weiss reported in their paper 2015 (39) that this is an intelligent mechanism to withhold iron, and thereby an essential micronutrient, from pathogen microbes and diminish their virulence. Moreover, there is a difference in the methods that deprive the pathogens of iron which depends on the location of the infectious bacteria. As shown in Figure 1, if the host is infected with microbes intracellularly, mechanisms to export iron from the cells are activated and vice versa.

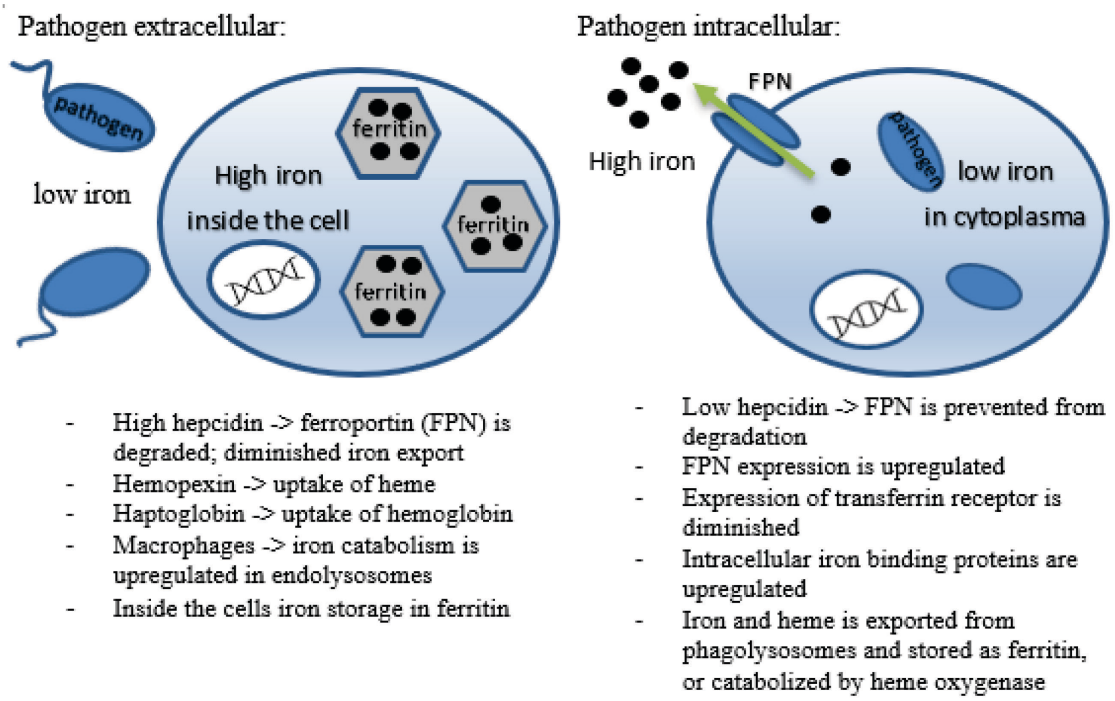

Figure 2: Mechanisms of the body to withhold iron from the pathogens (adapted from (10)).

Regarding this finding, it has to be considered that traditional indicators of iron status are changing in the presence of infection and inflammation. Therefore, the assessment of the

body´s iron status shows several difficulties in these settings and possible adjustments in evaluating the laboratory values during an inflammatory state have to be examined.

All included studies used C-reactive protein (CRP) or α1-acid glycoprotein (AGP) or both to diagnose inflammatory status. Thurnham et al (40) determined certain factors to adjust iron indicators in different phases of inflammation in 2015. The following phases were defined by the measurement of CRP and AGP: incubation (CRP >5 mg/L, AGP ≤1 g/L), early convalescence (CRP >5 mg/L, AGP >1 g/L), and late convalescence (CRP ≤5 mg/L, AGP >1 g/L).

In 2016 and 2017, a series of papers, inter alia discussing the problem of the difficulties in diagnosis of iron deficiency in the state of inflammation, was published by the research group "Biomarkers Reflecting Inflammation and Nutrition Determinants of Anemia" (BRINDA). They included cross-sectional data out of preschool-aged children datasets (about 30,000 subjects, aged 6-59 months) and women of reproductive age datasets (about 26,000 subjects, aged 15-49 years), which are considered to be high-risk groups for anemia. BRINDA displayed the relation between inflammation markers, such as C-reactive protein (CRP) and α1-acid glycoprotein (AGP), and iron indicators like ferritin, sTfR and TBI, as well as anemia biomarkers, such as hemoglobin (41).

According to Suchdev et al (41, 42) not only serum ferritin, but also sTfR concentration and total body iron change due to inflammation. When adjusting these values for the indicators of inflammation, CRP and AGP, with the regression-correction approach, a clear rise in the prevalence of iron deficiency can be seen.

3.1.1 Serum ferritin

As mentioned earlier, ferritin is the most commonly used indicator for the body iron status. More specifically, it reflects the iron storage and thereby, it is relevant for diagnosing manifest iron deficiency. Nevertheless, ferritin also increases in settings of inflammation or infection, as it works as an acute phase protein and the synthesis of ferritin is higher due to cytokines of the immune system. Therefore, if inflammation is not taken into account, diminished iron stores are likely to be missed (42).

In 2015, a systematic review of different guidelines on how to diagnose iron deficiency in various clinical settings was done by Peyrin-Biroulet et al (43). In total, 26 guidelines on nine different specialities mostly by American and European experts were selected and analysed. All of them recommended serum ferritin as the marker for defining the body iron status. Ten of the included guidelines mentioned TSAT as an alternative for or in addition to SF, whereas four guidelines referred to sTfR for the diagnosis of ID. The changes of iron markers in an inflammatory state were considered by only eight of these guidelines. However, different cut-offs for SF were recommended in different settings. A 12-15µg/L or 25-30µg/L cut-off was proposed in 19 guidelines, especially for women, children, and patients with chronic kidney disease (CKD). Otherwise, a 100µg/L cut-off was recommended in 12 guidelines: in five for patients with CKD, in two guidelines each for individuals with heart disease, active inflammatory bowel disease, anesthesia, and for patients with an estimated possible ID. A 200µg/L, 500µg/L or even 800µg/L cut-off was proposed by five guidelines in total, for patients with CKD or chemotherapy-induced anemia, particularly with reference to functional ID. Finally, Peyrin-Biroulet and his colleagues concluded that in most clinical settings, a SF cut-off of 100µg/L is recommended (43).

Thurnham et al (40) defined so-called correction factors (CF) for the adjustment of serum ferritin to the following phases of inflammation: incubation (CRP >5 mg/L, AGP ≤1 g/L), early convalescence (CRP >5 mg/L, AGP >1 g/L), and late convalescence (CRP ≤5 mg/L, AGP >1 g/L). In order to obtain these correction factors, Thurnham and his colleagues collected data from 22 published studies, which encompassed 7,848 subjects in total, and conducted four-group analyses according to the mentioned phases of inflammation. The studies mirrored different population groups, and men, women and the different phases of life were considered. Nearly all included studies (except for one) measured CRP and AGP as well as ferritin. The CFs were calculated on SF values of a reference group not affected by inflammation (CRP ≤5 mg/L, AGP ≤1 g/L). CFs of 0.77 for the incubation phase, 0.53 for early convalescence and 0.75 for late convalescence were determined (40).

In the BRINDA study (44), different approaches were compared to assess serum ferritin appropriately despite inflammation and, thus, to minimize the cases in which iron deficiency is not detected. 24 data sets, including 27,865 preschool children and 24,844 women of reproductive age, were used to obtain information about the relation between

serum ferritin and inflammation. Inflammation was defined with CRP >5 mg/L and/or AGP >1 g/L. The first approach was to raise the serum ferritin limit for diagnosing ID. The cut-off of 12µg/L for iron deficiency in preschool children and 15µg/L SF in women was heightened to 30µg/L for both groups. In one examination, BRINDA set the higher cut-off for the whole population group, which entailed a large increase in the estimated percentage of ID, and in another examination only for individuals showing increased inflammation markers. When adjusting only for those with inflammation, the mean of the prevalence of ID in preschool children was 12.2% higher, whereas in women it was 3.0% higher than without any adjustment of SF. Secondly, the exclusion approach was applied by solely using data of individuals without elevated CRP or AGP to calculate the prevalence of ID. However, this resulted in a relevant loss of the sample size (26-68% of the children and 14-34% of the women were excluded) (44). The third approach was to use an arithmetic correction factor for the different phases of inflammation, as introduced by Thurnham et al (40). The cut-offs for these phases were defined as previously mentioned. Three different calculated correction factors (CF) were applied by the BRINDA study: the Thurnham CF, the BRINDA CF and an internal CF based on survey-specific data. The BRINDA study group suggests using the internal CF whenever the sample size is big enough to calculate an internal CF. Otherwise, an age-specific external CF, such as the BRINDA CF, should be used, as Thurnham CFs are generalised and do not differentiate between age groups or genders. The last approach to detecting the prevalence of iron deficiency in the world population correctly was a regression correction (RC) approach. This method is based on a linear regression to get an adjusted ferritin value for each CRP and each AGP concentration (44). According to Suchdev et al (42), this approach is the most appropriate one for mirroring iron storage in presence of inflammation, since already small inflammatory activity, reflected as low CRP and AGP, affects the SF concentration. Additionally, as the estimated prevalence of ID rises more strongly in preschool children than in reproductive women in each of the used approaches, the results of the BRINDA study suggest a better correlation between inflammation and ferritin in preschool-aged children than in women (44).

In 2018, a systematic review on studies assessing ferritin cut-offs in different settings was published by Garcia-Casal et al (45). 108 studies that provided data on serum ferritin were included in this analysis. Additionally, these studies displayed information either on the iron content in the bone marrow, the gold standard to define iron depletion (iron in bone

marrow = 0) or iron repletion (iron in bone marrow +1 to +3), or on the liver iron concentration to detect iron overload (>3.2 mmol/100mg dry liver weight). The results of this systematic review revealed a mean SF concentration of 15.1µg/L for otherwise healthy individuals with depleted iron stores and 70.4µg/L for individuals with iron repletion according to the bone marrow assessment. As for non-healthy individuals, the mean SF concentration varied widely between different clinical settings, separated into the following groups: rheumatoid arthritis, alcoholism, liver cirrhosis and diverse chronic diseases for different age groups (mean SF for iron depletion: 33.6-158.3µg/L, iron repletion: 171.6-565.7µg/L). From 38 studies in total, a mean SF concentration of 82.43µg/L was calculated for depleted iron stores (1,023 individuals) and 381.61µg/L for iron repletion (1,549 individuals) in non-healthy patients. However, no inflammatory markers were considered in this systematic review, although most of the mentioned clinical settings are accompanied by an inflammatory condition. Therefore, the association between high ferritin and elevated inflammatory markers is not clearly reflected in this study and can only be guessed in this context (45).

In the postpartum period, on the other hand, serum ferritin shows a different correlation to elevated inflammatory markers, as the randomized controlled trial of Jorgensen et al suggests (10, 46). Since several studies, among them one by Groer and his colleagues (47), revealed a high prevalence of increased inflammatory markers in women postpartum, this RCT was conducted to display the relation between ferritin and CRP as well as AGP, respectively. By investigating 114 lactating women at two weeks and 17 weeks postpartum, Jorgensen and his colleagues found that serum ferritin does not rise with elevated CRP or AGP concentrations, in contrary to children, men, and nonpregnant women. Median CRP and AGP were lower in the 17th week than in the 2nd week postpartum. Furthermore, no significant correlations between ferritin and the inflammatory markers (P=0.48 for CRP and P=0.51 for AGP) were found in the 2nd week postpartum, whereas in the 17th week postpartum, there was a higher trend to decreased ferritin in women with elevated CRP or AGP than in women not showing increased inflammatory markers. Nevertheless, this negative correlation was not significant (P= 0.18 for both) (46).

3.1.2 Transferrin saturation and soluble transferrin receptor

The transferrin saturation or the concentration of soluble transferrin receptor are used as an alternative to ferritin to display iron status in settings with increased inflammatory markers. In their systematic review over 41 studies, Cacoub et al (48) revealed the advantages of TSAT over SF, especially in individuals suffering from chronic inflammatory diseases. The following cut-offs for the diagnosis of ID were used in the included studies: Serum ferritin <100–300µg/L in the presence of inflammation and <16–100µg/L for individuals without inflammation, TSAT <15-20% for both. The results of the analysis showed that published studies used SF or SF and TSAT for the diagnosis of absolute and functional ID, whereas no publication referred to TSAT alone when diagnosing ID. The majority of the included studies (18/24) used SF and TSAT to show functional ID. In contrast to SF, TSAT is low not only in absolute, but also in functional iron deficiency, since it displays the iron availability for erythropoiesis and is no acute phase protein. Therefore, it is suggested to measure both, SF and TSAT, for the diagnosis of ID (48). Similar results were found in the previously mentioned review of Peyrin-Biroulet and his colleagues (43), as it showed that ten of the included guidelines mentioned TSAT either as an alternative or additional biomarker for iron deficiency, even though different cut-offs for TSAT were defined to diagnose ID across different indications. A 15-16% cut-off was recommended in three guidelines for inflammatory bowel disease, CKD, and the general population. Most of the guidelines, seven in number, proposed a 20% cut-off, although it was applied to the same population groups. Rarely, cut-offs of 25%, 30%, or even 50% were proposed by one guideline each for CKD or chemotherapy-induced anemia in case of functional ID. Therefore, it was concluded that a cut-off level of 20% for TSAT should be considered in most settings (43).

The use of soluble transferrin receptor, on the other hand, is more controversial. As sTfR is no acute phase protein either, it is thought not to be affected by an inflammatory state (41). In his review from 2002, Beguin (17) reported that an increased sTfR concentration may be a promising test for the diagnosis of iron deficiency in individuals showing inflammation, because sTfR remains low in several different clinical disorders, such as HIV infection or in chronic liver disorders. On the contrary, other studies that were reviewed by Beguin found that sTfR concentrations did not help to distinguish iron deficiency from non-iron deficient patients in certain clinical settings (17).

Apart from that, Kasvosve et al (49) showed in their study on 208 Zimbabwean children that increased sTfR concentration exists during inflammation (defined through CRP, IL-6 and neutrophils) without a relation to iron status, erythropoiesis, or hypoxemia. The BRINDA study (50) also displayed an almost linear increase in sTfR concentration in individuals with high CRP and/or AGP values. This relation appears to be stronger with AGP than CRP. Elevated CRP and cytokines, present during inflammation, are responsible for limited erythropoietin, erythropoiesis and, consequently, diminished sTfR concentrations in the plasma (17, 50). Therefore, the BRINDA group adjusts sTfR values only for AGP and not for CRP. When using an adjustment of sTfR concentration for inflammation, a decrease in the prevalence of iron-deficient erythropoiesis can be observed (50). As sTfR concentration seems to be affected even by slightly elevated inflammation markers, Suchdev et al (42) suggest to use a regression-correction approach for this biomarker as well.

3.1.3 Total body iron

Total body iron was the third iron indicator the BRINDA research group (51) investigated regarding inflammation. This value, calculated from serum ferritin and sTfR, represents an estimation of the overall iron status in individuals. Since unadjusted ferritin decreases the estimated prevalence of iron deficiency in an inflammatory state and sTfR increases it, it can be assumed that these changes in the biomarker concentrations due to inflammation neutralize each other. However, the BRINDA study shows that there is a correlation between TBI and CRP or AGP, the markers of inflammation. High concentrations of CRP and AGP are related to an increase in total body iron. Therefore, the prevalence of iron deficiency might be underestimated when using unadjusted TBI values in case of inflammation. As in the studies adjusting ferritin and sTfR for inflammation (44, 50), it is also suggested to use a regression-correction approach for correcting total body iron (51).

3.2 Iron status in different clinical settings

16 publications about the issue of iron status in the following settings were included from the database search: chronic diseases, metabolic syndrome, depression, and athletes.

Additionally, studies that are either considered as standard literature on this topic or appear in the reference lists of selected papers were consulted.

3.2.1 Chronic diseases

As previously mentioned in this review, hepcidin was recently found to play an important role in the development of disturbed iron homeostasis and the consequent anemia of chronic diseases. Figure 3 shows important functions of hepcidin in the iron homeostasis in the body.

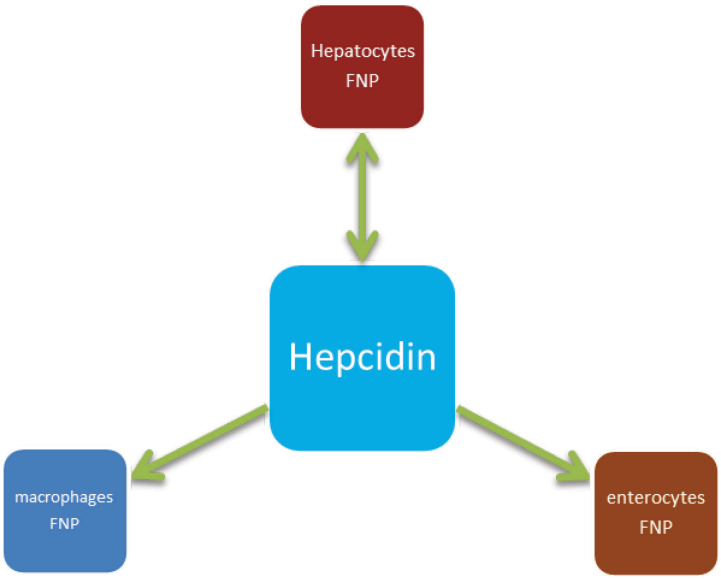

Figure 3: Functions of hepcidin: degradation of ferroportin (FNP), reduction of iron release from macrophages, hepatocytes, and enterocytes; hepcidin is produced by hepatocytes (adapted from (52))

In their systematic review, Liu and his colleagues (52) investigated the role of hepcidin inter alia in the anemia of inflammation and the possible advantages of using hepcidin as therapeutic intervention. The 72 included references reported a change in hepcidin levels in the anemia of inflammation. Liu et al concluded that, based on contemporary literature, the higher hepcidin levels are induced by different signalling pathways through IL-6, IL-1β and IL-22 due to chronic inflammation. Therefore, while the current therapy is based on iron supplements, erythropoiesis-stimulating agents, and erythrocyte transfusions, hepcidin antagonists were revealed as potential therapeutics to regulate iron homeostasis in chronic diseases. Several different methods are reported for this new therapeutic approach, including suppressing hepcidin by targeting hepcidin mRNA or protein, as well as the IL-6 pathway (52).

3.2.2 Metabolic syndrome

There are different hypotheses about how the metabolic syndrome and iron status correlate. Suárez-Ortegón et al (53) discussed the relation between ferritin and the metabolic syndrome in their systematic review and meta-analysis. They reviewed studies published by 2018 that focused on the adult human population. Most of these selected studies adjusted ferritin values for BMI and/or inflammatory markers and defined the cut-offs for high ferritin as lower than 200µg/L (women) and 300µg/L (men). The odds ratio (OR) for metabolic syndrome in individuals with high ferritin levels was 1.78 (95% CI: 1.60-1.97) in comparison to individuals with normal SF levels. Especially high triglycerides and high glucose showed a strong correlation with high SF levels, but also other components of the metabolic syndrome, such as low HDL-cholesterin and high blood pressure, tended to be associated with high SF. Otherwise, this meta-analysis presented a reduced correlation between the metabolic syndrome and high SF when adjusting for BMI and/or liver damage, whereas the adjustment of SF values for CRP increased the relation between SF and high triglycerides. Additionally, the laboratory method of measuring SF has an impact on the intensity of the correlation between SF and the metabolic syndrome is. Nevertheless, other studies included in this thesis did not find a significant association between high SF and the metabolic syndrome. The recent randomized controlled trial by Zhang et al (54) examined the relationship between different iron storage markers and the metabolic syndrome in 1,333 Chinese rural children (6-12 years old, 658 female). They defined the components of the metabolic syndrome as following: obesity: waist circumference or BMI $\geq 95^{th}$ percentile, hypertension: blood pressure $\geq 95^{th}$ percentile, dyslipidemia: HDL-cholesterol <1.03mmol/L or triglycerides ≥1.47mmol/L, hyperglycemia: fasting glucose ≥5.6mmol/L. Iron deficient individuals (SF <12µg/L) were excluded from the study. Zhang et al´s analysis suggests that there is no association between the markers of iron storage (whole blood iron, SF, TBI) and the metabolic syndrome itself, although a significant positive association was displayed between many components of the metabolic syndrome (obesity, hypertension, low HDL-cholesterol, hyperglycemia) and whole blood iron. Regarding ferritin, however, a positive relation was only found with low HDL-cholesterol and diastolic blood pressure, whereas the component of the metabolic syndrome that was significantly associated with TBI was solely HDL-cholesterol (54).

In the meta-analysis by Jiang et al (55), the relation between serum ferritin concentration and the risk of type 2 diabetes (T2D), a possible pathologic consequence of the metabolic syndrome, was investigated. They used 15 prospective cohort studies published between 2004 and 2017. The dose-response analysis was based on ferritin levels subdivided in categories of different SF concentration with a 100µg/L difference between them. Then the association with T2D risk was estimated. Men and women in the highest SF category showed a 54% higher risk for T2D than individuals in the lowest category. Moreover, a linear correlation between SF levels and risk of T2D was found. The meta-analysis suggests that higher SF levels are stronger associated with T2D in women (45% increase per category) than in men (20%).

As further components of the metabolic syndrome, overweight, defined as body mass index (BMI) of 25-29.9, and obesity, defined as a BMI of 30-39.9, display other difficulties regarding iron status. Zhao et al´s meta-analysis (56) discusses the problem of hypoferremia in obese patients by comparing overweight/obese groups to normal weight individuals. Their study included 21 cross-sectional studies and 5 case-control studies. Serum iron and TSAT were significantly lower in the overweight/obese population, whereas sTfR did not show any significant difference between the groups. Zhao et al calculated a higher overall risk of iron deficiency in overweight/obese patients than in the normal weight group (odds ratio: 1.31). Regarding the ID diagnosis method, there was no significant correlation between iron deficiency and overweight/obesity observed when using ferritin to diagnose ID. Otherwise, with ID diagnosis by TSAT, serum iron or sTfR values, a significant association between ID and overweight/obesity was detected. Only four studies investigated the relation of obesity and iron deficiency anemia, and Zhao et al could not observe significant results regarding this relation. Additionally, it was shown that the strength of the association between overweight/obesity and the risk of ID depends on age, ethnicity, as well as on the method used to diagnose overweight or obesity. This meta-analysis study, for instance, revealed a greater relation between obesity and the risk of ID in individuals under the age of 18. Furthermore, the results of this study propose not to use SF as an indicator for iron deficiency in the obese population (56).

Cepeda-Lopez et al (7) undertook a randomized controlled trial on 62 premenopausal, non-pregnant, apparently healthy women, who were separated into three groups according to their BMI (normal weight 18.5-24.9, overweight 25-29.9 and obese 30-39.9). The

comparison of the three groups resulted in finding that hemoglobin concentration, SF and sTfR were similar in all three BMI groups, but markers of inflammation and hepcidin showed differences between the groups, such as presenting higher values in individuals with higher BMI. The absorption of supplied iron was measured with and without adding ascorbic acid. Since the overweight and obese groups showed similar percentages of absorption, these two groups were combined. The percentage of absorbed iron was 12.9% in this combined group and an average of 19.0% in the normal weight women without addition of ascorbic acid. The addition of ascorbic acid to the meal resulted in an increase of iron absorption to 29.5% in the normal weight group, but only 16.6% in the overweight/obese group. In this study, a negative correlation between the percentage of body fat and serum iron as well as the total iron-binding capacity (TIBC) was displayed, whereas there was a positive association between body fat and SF, the inflammatory markers (CRP, IL-6, AGP) and hepcidin. No correlation was found between body fat and sTfR. Therefore, this study suggests using sTfR rather than SF or hepcidin as a predictor of the iron absorption in individuals with different BMIs. Moreover, an increase in hepcidin concentrations in the groups with higher BMI values was observed (normal weight: 9.2ng/mL, overweight: 11.36ng/mL, obese: 12.48ng/mL). Thus, these results support the theory that hepcidin is a negative predictor of iron absorption and could be one reason for decreased iron absorption in overweight and obese women, as well as responsible for a reduced effect of ascorbic acid in the enhancement of iron absorption (7).

Teng et al´s research (57) investigated the effect of weight loss on iron status (hemoglobin, TSAT, SF, serum iron) in overweight and obese patients. Their meta-analysis included 14 studies (longitudinal studies and RCTs) which encompassed research on all in all 879 overweight and obese children, adolescence, and adults who were losing weight through a programme with hypocaloric diet or physical activity, which they were following for at least a month. In individuals who achieved a significant weight loss with a mean of -4.60kg and -1.32 kg/m², a trend for increased hemoglobin levels was observed, although not significant, whereas a significant elevation of TSAT (1.68%) was found in the whole group as well as in the different intervention subgroups. Additionally, no significant correlation was seen in the whole group between weight loss and SF as well as serum iron. However, significantly higher serum iron levels were observed in physical activity and lifestyle modification programmes. Due to these results, the study suggests that a reduction of bodyweight through hypocaloric diet or physical activity can improve iron homeostasis

by increasing functional iron parameters (hemoglobin, TSAT). This may be of special relevance, since, as mentioned above, overweight/obese patients tend to suffer from functional ID.

3.2.3 Depression

In the following, the findings about the association between iron status and depression are depicted. Wenninger et al (58) found a correlation between low iron status and low tryptophan in their study. Since tryptophan is metabolized to serotonin in the central nervous system and there is evidence that the deficiency of serotonin accounts for the development of depressive symptoms (59), the connection between iron and tryptophan is a possible pathophysiological mechanism to explain the following epidemiological data, as illustrated in figure 4.

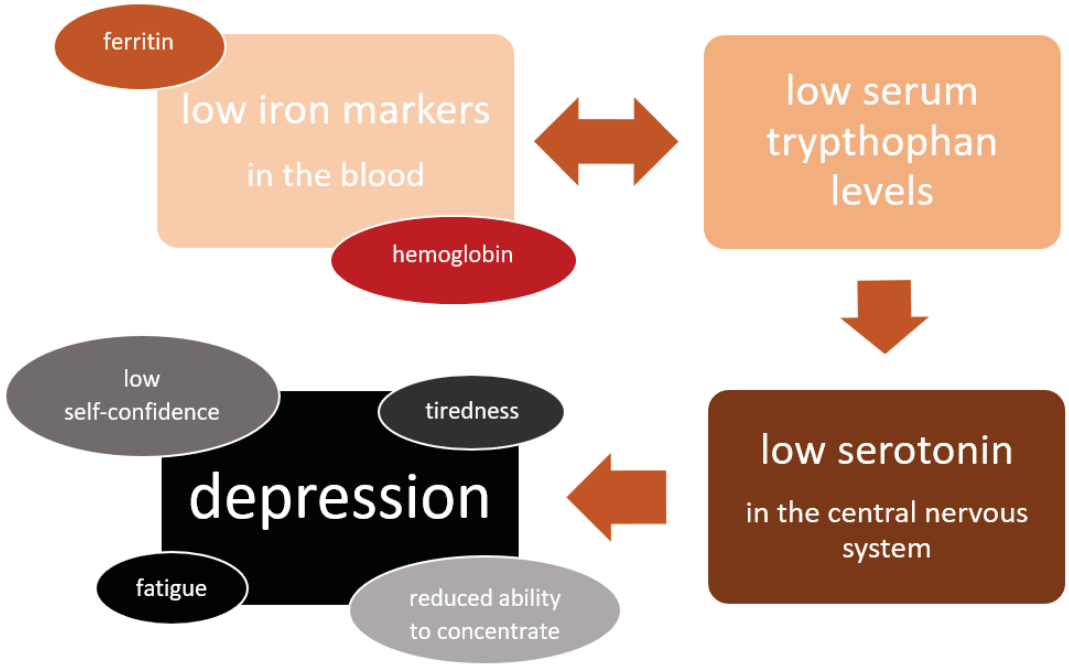

Figure 4: Possible mechanism linking iron status and depression

The association between iron deficiency anemia (IDA) and depression was recently investigated by Hidese et al (60). In total, 11,876 Japanese individuals who were users of an online platform, the Health Data Lab, participated in their study. 1,000 individuals revealed a self-reported history of depression, half of which were women (n=499). In this web-based cross-sectional study, a logistic regression analysis suggests a significant

positive association between the self-reported history of depression and the history of IDA (OR: 1.58, 95% CI: 1.33-1.88). Since the evaluation of these diseases was only based on the claim of the participants, no blood parameters were available to prove the statements. When using the 6-item Kessler scale, a psychological distress scale, in the control group, the score was significantly higher in self-reported IDA patients than in non-IDA individuals. Thereby, a higher score suggests a higher risk for mental illness. Hence, it can be assumed that there is a correlation between IDA and mental health issues (60).

Other publications examined the risk for depression in patients considering iron intake (61, 62). Li et al´s meta-analysis (61) researched the association between dietary iron intake and risk of depression. Three studies from a systematic literature search were included in this meta-analysis. A significant negative association between iron intake and risk for depression was found, as the relative risk of depression for study subjects with the highest dietary iron intake compared to those with the lowest intake was 0.57 (95% CI: 0.34-0.95). However, it has to be mentioned that two of the included studies found a significant association, whereas one study did not find any correlation between depression and iron intake (61). The web-based study by Lee et al (62) examined the risk of psychiatric disorders in general in patients with IDA, as well as their association with iron supplementation. The Taiwanese subjects were selected by use of the Longitudinal Health Insurance Database 2005, whereby 38,794 individuals without IDA were matched (age, sex, index year, year of IDA diagnosis) at a 2:1 ratio with IDA patients. However, no criteria for the diagnosis of IDA were mentioned in this study. The adjusted (age, gender, income level, comorbidities, iron supplementation, living area) hazard ratio (aHR) of psychiatric disorders in general suggests a higher occurrence in the IDA group in comparison with the non-IDA group (aHR: 1.52, 95% CI: 1.45-1.59). A subgroup analysis revealed a significant positive association between depression and the IDA patients compared to the non-IDA individuals with an adjusted HR of 1.49 (95%CI: 1.33-1.66). Moreover, a significantly higher risk for psychiatric disorders was found in IDA patients receiving no iron supplementation than those receiving iron supplementation (aHR: 0.85, 95% CI 0.80-0.90), although this association was not significant for depression only (62).

In accordance with these findings, Sheikh et al´s randomized controlled trial (63) revealed similar results in women suffering from postpartum depression. Thereby, 70 women (aged 20-40 years) who had delivered in a hospital in Tehran, Iran, were examined on the 7th day

postpartum. To be included in this study, the otherwise healthy mothers had to have delivered a healthy child by a complication-free caesarean section and showed a probable postpartum depression diagnosed through the Edinburgh Postnatal Depression Scale (EPDS≥11) as well as through a psychiatric interview. A randomly chosen intervention group got 50mg iron (ferrous sulfate tablets) per day for 6 weeks, whereas the control group received a placebo pill. The results displayed a significant decrease of the mean EPDS score in the intervention group, also in comparison to the control group, which did not show a significant decrease. Additionally, at the end of the intervention, a comparison between still depressed mothers and non-depressed mothers revealed a significant association between lower ferritin levels in case of depression (median SF: 41.8 mg/dl) and higher SF levels in non-depressive women (median SF: 67mg/dl). It is important to mention, however, that anemic mothers were excluded from this study, and iron deficiency without anemia was diagnosed only in 37.1% of mothers with postpartum depression. Thus, the majority of patients with postpartum depression were not considered in this RCT (63).

3.2.4 Athletes

Iron deficiency and iron deficiency anemia are very common problems among athletes as well. In 1992, Weaver and Rajaram reviewed possible explanations for the high prevalence in athletes, as shown in figure 5 (64).

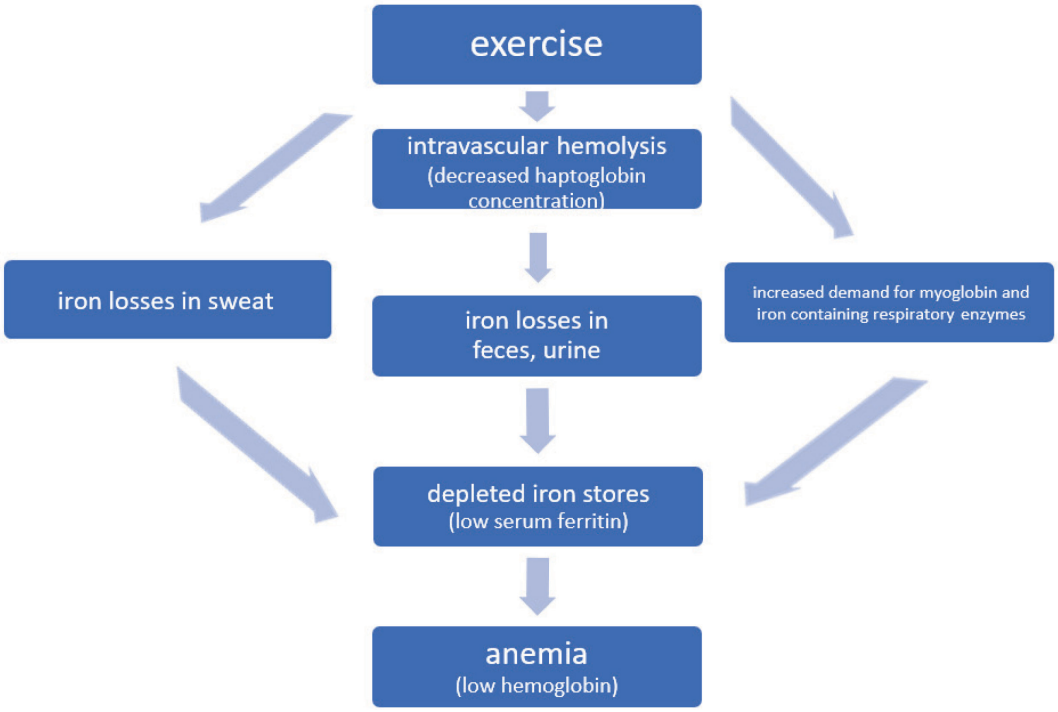

Figure 5: Mechanisms for iron deficiency in athletes (adapted from (64))

The literature search on PubMed on this topic resulted in 16 hits, of which six studies (2 systematic reviews, 4 randomized controlled trials) were considered for this thesis. The main question across these publications was how iron supplementation affects athletic performance. Rubeor et al (65) included 12 studies (283 participants: 257 women, 26 men) in their systematic review to conclude if iron supplementation is beneficial in athletes showing iron deficiency but no anemia. Only RCTs which investigated exercise performance after iron supplementation in non-anemic, iron deficient athletes in comparison to a control group receiving no supplements were selected by Rubeor and her colleagues. Since 6 studies showed an improvement in performance and, conversely, 6 studies did not, this systematic review could not unambiguously answer the question whether iron supplements are superior to no treatment in iron deficient, non-anemic athletes regarding their athletic performance. However, the measurements of performance as well as the ferritin cut-offs to define iron deficiency and the therapeutic approaches of iron supplementation (administration, dose, frequency, length of treatment) differed in the included studies (65). A second systematic review, which focused on publications dealing with the effects of mineral and trace element supplementation on athletic performance, was published by Heffernan et al (66) in 2019. For iron, they chose 29 studies (946 participants: 776 women, 170 men). The results of this study suggest that the current

ferritin level in athletes is crucial for the positive effect of iron supplements on performance. Most of the included publications display an improvement of iron status and the athletic performance when low iron ferritin is measured prior to the intervention. Nevertheless, again some studies did not find any advantages of iron supplementation regarding performance in sportsmen and women (66).

In 2018, a randomized controlled trail was conducted by Pompano and Haas (67) in order to investigate whether iron supplementation increases endurance performance in untrained women. 73 non-anemic (Hb >110g/L), iron deficient (ferritin <25 µg/L) women were included in this study. These women were randomly divided into four groups: receiving iron supplements (100mg ferrous sulfate, 2x/d) or placebo and aerobic training (25min pedaling, 5d/wk) or no training for eight weeks. The body composition of the participants did not differ significantly, nor did the women show inflammation (CRP ≤5 mg/L, AGP ≤1 g/L). In their paper, Pompano and Haas found a significant effect on the VO_2 peak (highest measured oxygen consumption) after week eight in the iron supplemented trained group as well as in the placebo trained group in comparison to the placebo untrained group. Additionally, the iron supplemented untrained group showed a trend for higher VO_2 peaks than the placebo untrained group. To clarify, the VO_2 peak is the highest measured oxygen consumption during exercise, which can be measured near maximal performance. However, these effects could not be mirrored in the estimated maximal oxygen consumption (eVO_2 max). In accordance with other authors, regarding submaximal exercise, a significant improvement was shown in the ventilatory threshold as percentage of the VO_2 peak in the iron supplemented trained as well as untrained groups in comparison to the placebo untrained women (67). Another study by Córdova et al (68) investigated the effects of iron supplementation on iron and hematologic parameters, biomarkers of muscle damage and cortisol, in non-iron deficient cyclists during a 3-week race. The study included 18 male cyclists participating in the Vuelta a Espana race (3,300km), who were randomly allocated to two groups: the study group received iron supplements (2x40mg/d in 800mg iron protein succinylate in total), whereas the control group did not. The two groups neither differed significantly in age, parameters of body composition, maximum oxygen uptake and measured blood parameters at the beginning of the study (T1), nor was there a difference in the average daily energy and micronutrient intake during the study. Blood samples were taken one week before the start of the race (T1) and at the end of the race (T2), so the study lasted for four weeks. The following iron

biomarkers and hematologic parameters showed a statistically significant difference between the groups at T2: transferrin saturation, serum iron, ferritin, red blood cells, hemoglobin and hematocrit. Additionally, a significant increase in ferritin, hemoglobin and hematocrit was observed in the iron supplemented group over the course of the study. When taking into account biomarkers of muscle damage, such as creatine kinase (CK), lactate dehydrogenase (LDH) and myoglobin, as well as the stress hormone cortisol, no significant difference between the two groups was found for the muscle damage markers, but there was for cortisol. Nevertheless, a tendency for a decrease of CK, LDH and, slightly, myoglobin between T1 and T2 was observed in the men receiving iron supplements, while these markers remained the same in the control group. In the control group, the cortisol level significantly increased, as opposed to the iron supplemented group, which showed a tendency for a cortisol decrease. Furthermore, a significant negative association between the hematologic biomarkers and CK, LDH, myoglobin and cortisol was found (68).

Ishibashi et al´s RCT (69) studied the effect of iron supplementation on the acute phase protein hepcidin in the context of physical exercise. For this study, fourteen male long-distance runners and triathletes (age: 19-22 years) with similar body compositions and fitness levels (in terms of VO_2 max) were randomly allocated to an iron supplemented group or to a placebo group. The study was conducted over three days of endurance training, which was executed twice a day at the same time to the same extent (75 minutes of running) with the same intensity (75% VO_2 max) by each participant. In the iron group, 12mg of iron were supplemented before and after the first training each day (in total 24mg/d). On each of these training days and on the day following the last training day (day 4), blood samples were taken from the fasting athletes at 8am. The results of the study suggest no significant differences between the groups regarding the training sessions (heart rate, running distance) and the dietary intake (except iron). No significant interactions between the iron supplementation and the measured iron parameters and hemoglobin levels in contrast to the placebo were found within the four days. The same applies in respect to muscle damage parameters (myoglobin, CK) and inflammatory parameters (CRP and IL-6). However, there was a significant interaction between hepcidin and iron supplementation. Although the hepcidin levels did not significantly differ during the training days, on day four, the iron supplemented group showed a significantly higher hepcidin level (mean: 12.6 ng/mL) than the placebo group (mean: 6.9 ng/mL) (69). In

another RCT, scientists McCormick et al (70) compared the effects of morning exercise on iron absorption, considering inflammatory markers, hepcidin and iron absorption parameters to those of afternoon exercise. In this study, 16 runners (10 men, 6 women) were examined on two experimental trials. One trial included a 90 minutes-run (65% VO_2 max) in the morning (after an overnight fast) and the other trial the same exercise in the afternoon. The participants, all showing a suboptimal iron status, received a stable iron isotope twice, one for the simulation of breakfast and another for the simulation of dinner. For each trial, blood samples were taken 30 minutes before the run, immediately after and three hours post-exercise. The iron status, the dietary analysis as well as the drawn parameters of the exercise (such as the heart rate) did not show significant differences among the athletes. The study revealed that fractional iron absorption was significantly higher from breakfast than from the dinner meal ($p=0.011$) when the exercise was conducted in the morning. Contrarily, no significant difference in iron absorption between the trials was seen when the athletes ran in the afternoon. Additionally, the results suggest that iron absorption from the meals (from breakfast as well as from dinner) is higher in general when doing exercise in the morning rather than in the afternoon. However, hepcidin levels rose significantly during both times of exercise and showed a higher increase post-exercise in the morning than when there was no exercise done in the morning. Therefore, McCormick and her colleagues proposed that other mechanisms than hepcidin controlling iron uptake during athletic training may play an important role for the iron homeostasis in athletes. Hence, to optimize their iron status, these mechanisms have to be further investigated (70).

3.3 Gender- and development-related aspects

The studies reviewed for this thesis focus on different gender-related aspects and specifics across the lifecycle of iron status. Especially in premenopausal women and children, there is a high prevalence of iron deficiency. In both, this may be due to the higher need for iron, because of menstrual bleeding in women and rapid growth in children (41). Ten of the included studies considered solely these risk groups in their research (7, 41, 44, 46, 49–51, 63, 67), whereas 14 studies included women and men. In six of the studies on the risk groups, children were particularly highlighted ((41, 44, 49–51, 54) and two studies included postpartum women only (46, 63). Conversely, solely two papers about iron and

exercise display data about men alone (68, 70, 69). However, in nearly all publications regarding both genders, a higher percentage of women was noticeable. In the analysis by Thurnham et al, 11 studies considered female and 3 studies male data (40). Zhao et al included 8 studies about women only and 11 studies on both genders (56). Li et al also considered one study exclusively on female individuals and two studies on both genders (61). In Teng et al´s meta-analysis, 82% of all participants were female (57), in the analysis by Lee et al about 77% (62). Also in the systematic reviews about exercise, the vast majority of study subjects were women (rate women:men - 257:26 and 776:179) (65, 68, 66). Six of the included papers did not mention gender ratio in their studies. Only one study, investigating the association of IDA and depression, evaluated about 50% women and 50% men (60), whereas the RCT by McCormick et al was the only included trial that assessed more men than women (10:6) (70).

4 Discussion

This thesis focuses on the issue of the body iron status in correlation with inflammation defined as increased inflammatory markers, in particular CRP and AGP. It summarises the most recent literature and gives an overview on this topic by considering the two research questions “What are the effects of the acute-phase response of inflammation on the interpretation of common iron indicators and innovative parameters?” and “What is the relevance of this problem in different clinical settings, especially regarding the metabolic syndrome and depression?”. In the following, the results of this thesis will be reflected and discussed.

4.1 The effects of the acute-phase response of inflammation on the interpretation of common iron indicators and innovative parameters

In accordance with a variety of other studies, the results of this thesis reveal that determining the body iron status in a state of inflammation and, therefore, in various clinical settings may involve several difficulties. All included studies are congruent regarding serum ferritin when inflammatory markers are increased. Serum ferritin as the standard biomarker for diagnosing iron deficiency should be used with caution when it comes to inflammatory or infectious settings. Since a state of inflammation is accompanied by higher serum ferritin concentrations regardless of the body iron status, an alternative way to diagnose iron deficiency in these patients has to be used. The literature suggests either an adjustment of serum ferritin to the inflammatory markers (CRP and AGP) or the usage of other iron biomarkers for the diagnosis of ID. For SF adjustment, the extensive BRINDA study proposes the regression correction approach, because the increase of SF correlates with the increase of CRP and AGP (44). Nevertheless, simply using different cut-off levels for SF in patients with and without inflammation seems to be easier for laboratory practise, although the prevalence of iron deficiency cannot be detected as accurately. Especially in children there seems to be a strong correlation between inflammation and ferritin, for which reason the cut-off level approach to diagnosing iron deficiency may prohibit a reliable detection of this disease in young individuals (44). When considering the results of the BRINDA study, it has to be kept in mind that only women and preschool-aged children were included in this study and that the recommended adjustments of the iron indicators have to be applied cautiously when it comes to other patient groups. Particularly in postpartum women, studies showed that, regardless of the

high prevalence of increased CRP and AGP, SF concentration does not rise with these inflammatory markers. There seems to be even a trend to decreased SF in women after giving birth (46). Further studies are needed to discover the pathophysiological mechanism behind these findings and to give recommendations for detecting iron deficiency in this phase of the life.

Several studies suggest using TSAT, sTfR concentration or total body iron in addition to SF to diagnose ID in patients with elevated inflammatory markers. Especially in individuals with chronic inflammatory diseases, TSAT appears to be a useful tool to detect functional iron deficiency (43, 48). These alternative markers of iron status are thought to be less affected by inflammation and infection. However, Kasvosve et al (49) falsified this hypothesis by detecting increased sTfR concentrations during inflammation without a relation to iron status in children. This may be explained with a possible different pathophysiological mechanism in young individuals. However, additionally, the BRINDA study found a significant increase of sTfR in particular in relation to increased AGP and, therefore, suggests to adjust sTfR to the AGP concentration as well with a regression correction approach (50). Since elevated AGP levels are characteristic for the early and late convalescence interval, this adjustment should be used especially in these phases. On the other hand, Pfeiffer and Looker criticize the use of sTfR for determining the iron status by pointing out that a standardized use has not been established yet and, hence, no consistent default values for sTfR concentrations have been defined so far (9). Also for TBI, the BRINDA study suggests an appropriate adjustment for inflammatory markers (51).

Since in an inflammatory setting an alteration is proposed for all these biomarkers, it can be discussed if the alternative biomarkers show beneficial effects in terms of the diagnosis of ID. In socially privileged countries, it is common practise to identify various indicators of iron status anyway. However, it has to be taken into account that not only the effort to analyse these biomarkers, but also the financial situation might limit the diagnosis of ID by use of a variety of different biomarkers in many developing countries. Since these countries in particular show high prevalence of iron deficiency as well as inflammation, it would be beneficial if ID could be diagnosed reliably with as few iron indicators as possible. Nevertheless, in order to not underdiagnose a functional iron deficiency, the results of this thesis propose to use the combination of TSAT and SF to define iron status in the presence of inflammation (43, 48). Additionally, more research on the more recently

detected biomarkers would be needed to more precisely scrutinize the potentially beneficial effects on using these iron markers in addition to or instead of SF.

As Garcia-Casal et al mentioned in their systematic review as well, the current recommendations for diagnosing iron deficiency do not take into consideration differences in age groups and various clinical conditions. Their study showed that the mean SF concentration in non-healthy individuals with absent bone marrow iron was much higher (82.43µg/L) than the serum ferritin cut-off recommended by the WHO for children with inflammation (30µg/L) (45). To not accurately diagnose and treat iron deficiency in children may lead to severe complications as this trace element is essential for their development.

Since iron homeostasis is a very complex mechanism, all these variations should be kept in mind when diagnosing iron deficiency in each individual patient. The included studies in this thesis imply that the acute-phase response of inflammation has a considerable impact on common iron indicators as well as innovative parameters. In terms of serum ferritin and transferrin saturation, these relations have already been researched in greater detail and there are recommendations for how to interpret these biomarkers with regard to inflammation markers. However, these suggestions differ slightly across studies. Apart from that, rather few references exist that focus on more recently considered markers of iron status, such as sTfR or hepcidin. The literature is not consistent about using these indicators to diagnose iron deficiency and no standardised values have been defined so far.

4.2 The relevance of iron status in different clinical settings

Various clinical settings are associated with high inflammatory markers, which is relevant for diagnosing iron deficiency in a vast number of patients. As mentioned above, the detection of ID in individuals with increased indicators of inflammation bears different difficulties which may have to be considered in the situations discussed in the following.

4.2.1 Chronic diseases

The iron homeostasis in chronic diseases constitutes a complex setting, since the anemia of chronic diseases is partly due to functional iron deficiency. As previously mentioned, this state is caused by the iron withhold in macrophages and other tissues. Functional ID leads

not only to a decreased availability of iron for erythropoiesis and, in further consequence, to anemia, but also to an iron excess in tissues and, thereby, to damage due to the toxicity of free iron (26). This complicated setting can lead to difficulties regarding therapy. As the established treatment for iron deficiency, iron supplementation, may worsen the iron overload in tissues, other therapy options are investigated. One recent target in the therapy of functional ID is hepcidin. Due to the regulating function in iron homeostasis through ferroportin degradation, medications that antagonise hepcidin on its receptors, diminish hepcidin expression, or intervene in any other way in the hepcidin pathway promise to be a useful causal treatment for the disturbed iron homeostasis in patients with chronic inflammatory diseases (52).

Recently, a considerable amount of research has been done on the correlation between iron status and very common pathological settings in the western world, such as the metabolic syndrome and depression. Especially regarding the metabolic syndrome, the changes of iron indicator concentrations in an inflammatory setting seems to be of special importance for detecting iron deficiency. In case of depression, on the other hand, these correlations are not as thoroughly investigated yet.

4.2.2 Metabolic syndrome

When it comes to the metabolic syndrome, Suárez-Ortegón et al found a significantly higher risk for the condition in individuals with high ferritin levels. There are several components, such as high triglycerides and high glucose, that may be associated with iron biomarkers, such as serum ferritin (53). Regarding glucose metabolism, another meta-analysis confirms this finding by detecting a higher risk for type 2 diabetes in individuals with elevated serum ferritin levels compared to those with normal SF levels (55). These findings are in accordance with the results of Fernandez-Real et al´s study that displays several mechanisms connecting iron and glucose pathways (71). However, Zhang et al only found an association between high SF levels and dyslipidemia and hypertension, but no correlation between SF and glucose levels in their study of 6-12 years old children. In spite of that, a significant correlation was observed between whole blood iron and other components of the metabolic syndrome (54). Nevertheless, the mechanisms of all these associations seem not entirely clear yet, but the results of the named studies imply that there could be a diagnostically relevant correlation between SF concentration and some

components of the metabolic syndrome. The research by Suárez-Ortegón et al particularly confirms this theory with the adjustment of SF values to the BMI, which still showed a connection between iron status and the metabolic syndrome (53). The association between overweight/obesity as a main component of the metabolic syndrome and high SF levels is proved best. There is convincing evidence that subclinical inflammation produced by adipocytes is responsible for a disturbed iron homeostasis in overweight and obese individuals. On the one hand, this state of low inflammation in obesity has been found to lead to an increase of the acute phase protein ferritin. Therefore, as Zhao et al propose, SF is not appropriate for the diagnosis of iron deficiency in an overweight/obese setting, because SF appears to be not sensitive enough for the determination of iron status in this case. In contrast, when using other iron indicators such as TSAT, serum iron or sTfR to diagnose ID, a positive correlation between ID and overweight/obesity was found. The recommendation for not using SF concentration to diagnose ID in obese individuals was also made by Khan et al based on a cross-sectional, observational study investigating iron and inflammatory markers in obese individuals (72). On the other hand, it is likely that the higher levels of hepcidin and inflammatory markers in obese/overweight subjects lead to a higher risk for iron deficiency (54, 72). This can be explained by the fact that hepcidin, which is observed at higher concentrations in obese individuals, is responsible for iron sequestration in the macrophages as well as a diminished iron absorption from the duodenum (72). Cepeda-Lopez et al also confirm a positive relation between the BMI and inflammatory markers and hepcidin (7). Furthermore, due to the worse absorption of iron from diet in an inflammatory state, it has to be kept in mind that oral iron supplementation will not be as effective in overweight or obese as in normal weighted people. Additionally, Htet et al found that with an administration of vitamin A together with iron supplements in a state of sub-clinical inflammation, an improvement in the effectiveness of the therapy can be observed (73). In contrast, other studies, such as the examination of Ferrari et al, could not find a connection between the BMI and the occurrence of iron deficiency when using sTfR concentration for diagnosis. Moreover, the hypothesis that individuals suffering from obesity are more likely to develop ID due to their imbalanced diet could not be confirmed by Ferrari et al (74). However, these inconsistent findings may be traced back to the already mentioned unstandardised laboratory assessment and values of sTfR. Since the majority of the studies suggest that the metabolic syndrome and its components have a significant impact on the iron status, the iron indicators, especially SF, have to be

interpreted critically when diagnosing ID in obese patients and individuals showing the metabolic syndrome.

4.2.3 Depression

However, the role of iron status in patients with depression and depressive symptoms is not as clear as in the previously mentioned settings. Recently, a possible correlation between iron deficiency as well as iron supplementation and depression has been investigated. Due to overlapping syndromes of iron deficiency and depression, such as fatigue, it seems not far-fetched that there may be an association between the two conditions. Several studies could find a connection in this setting. Therefore, it can be assumed that depression is associated with iron deficiency. Additionally, a lower risk for depression was found when consuming iron supplements. However, it has to be mentioned that it is not clear how ID was detected by the included studies. Moreover, no consistent definition for depression could be found across the studies. Two of the included publications were web-based and the majority of the population was investigated retrospectively. In the web-based study of Hidese et al (60), not even one specific item was used to determine depression, so the depression diagnosis was based on self-report only. In contrast, Lee et al´s (62) study population was selected by the ICD-9 criteria for depression. It is important to state that Hidese et al included solely Japanese people in their study, whereas Lee et al considered the Taiwan population only. Therefore, in addition to the fact that these results are based on self-reported data and web-data, respectively, it has to be kept in mind that merely the population of certain countries, specifically in East Asia, were examined. Hence, future research should prove if these associations are valid also for other parts of the world. Furthermore, confounders which may be responsible for iron deficiency, such as chronic inflammatory diseases, should be considered when proving this relation. When it comes to depression it seems to be difficult to reliably show a correlation between the disease and iron deficiency, since there are no direct laboratory markers to detect depression. Similar to the case of the components of the metabolic syndrome – except for overweight –, the mechanism of this association is not entirely clear.

One possible explanation for the correlation between iron status and depression could be a connection between iron metabolism and tryptophan. A recent study indicated that iron deficient individuals tend to present lower tryptophan concentrations in the serum (58).

The amino acid tryptophan is metabolized to serotonin in the central nervous system and there is evidence that the deficiency of serotonin accounts for the development of depressive symptoms. The mechanisms of this correlation have not been ascertained yet and more exploration seems to be necessary to completely understand it. Notwithstanding, some possible explanations for the association of low tryptophan with a low iron status exist. For instance, the relation has been assumed to be connected to apoferritin or inflammation (59). Furthermore, the association between these nutritional components may be attributed to the diet. It is possible that tryptophan-bearing foods, for example legumes, soya, nuts and seeds, amaranth, millet, etc., also bear an essential amount of iron and, consequently a lack of these food items in the diet could be responsible for the simultaneous occurrence of low tryptophan and low iron in the body. To prove this theory, further research including a detailed nutritive assessment is necessary. In addition to that, the direction in which the correlation between iron and tryptophan works is still unclear, since it is not known whether the depression causes the iron deficiency or vice versa. So far, it appears as if iron affects tryptophan and is affected by tryptophan simultaneously. Additionally, this relation may be responsible for depressive symptoms (i.e. fatigue and tiredness) during iron deficiency without anemia even without an actual depression. Of course, there may be other relevant mechanisms for this correlation and there seems to be a lot of potential for further research. As already referred to, there also exists the hypothesis that the pathophysiology of depression is linked to chronic, low grade inflammation. This can be explained by neurodegeneration due to chronic stress and the resulting continuous activation of the immune system (75). According to the systematic review by Labaka et al, a correlation between higher CRP levels or other inflammatory markers and more severe symptoms of depression was found especially in women (76). As a result, the determination of the individual´s iron status may bear similar difficulties in the setting of depression as it does in case of obesity. Since about two times more women than men are diagnosed with depression and the prevalence of iron deficiency is much higher in women as well, the information about the relation between inflammatory markers and depression seems very essential for assessing iron status in the female population particularly. As mentioned previously in this thesis, an inflammatory setting can lead to changes in iron status indicators, for example increased serum ferritin concentration in the blood. Therefore, this connection could cause difficulties in detecting iron deficiency and, as a result, treat it properly, especially in women suffering from depression. However, the associations between iron deficiency and depression are not proved well enough and these

hypotheses require further research in the future, as does the relevance of the gender aspect in the setting of depression.

4.2.4 Athletes

A variety of studies are also dealing with the problem of iron status in athletes. Especially females frequently face a disturbance in iron homeostasis due to intensive and extensive training. There are several hypotheses on how sports activity affects the iron metabolism. One of these is the already mentioned increased iron loss in athletes that leads to iron deficiency and finally to iron deficiency anemia, which in turn causes a reduction of the athletic performance. Additionally, Bruinvels et al´s study showed that up to 50 percent of all exercising women suffer from heavy menstrual bleeding and many of them are either currently facing iron deficiency or anemia or have experienced it in the past (77). Another hypothesis for a disturbed iron homeostasis in athletes is based on the fact that physical exercise favours a pro-inflammatory milieu, resulting in higher hepcidin levels and sequestration of iron (78). The most recent literature focuses on the effect of iron supplementation on athletic performance especially. The studies included in this thesis described a well demonstrated positive effect of supplemented iron on physical exercise in athletes displaying an actual iron deficiency anemia. Apart from that, it is not clear if a supplementation is favourable in non-anemic, iron-deficient athletes. Nevertheless, Heffernan et al found that the current ferritin level is important for the impact of iron supplementation on the athletes´ performances (66). These outcomes may correspond with the findings of Peeling and his colleagues, which imply that hepcidin levels show no significant increase three hours post-exercise in athletes with SF lower than 30µg, while higher hepcidin levels were found post-exercise in all the other groups with more than 30µg SF. The higher hepcidin levels prevent a sufficient absorption of iron in the enterocytes. Since the main food intake mostly takes place post-exercise, this increase of hepcidin may be one reason for less iron uptake and, consequently, a lower iron status and a lack of improvement of the performance (79). McCormick et al created the hypothesis that in addition to hepcidin, also other mechanisms regulate the iron uptake in athletes based on the results of their study (70). It is essential that these possible mechanisms should be further investigated to guarantee an effective treatment of iron deficiency also for physically very active people.

5 Conclusion

In conclusion, inflammation plays a very important and challenging role in diagnosing iron deficiency as well as in its efficient treatment. Despite the consideration of employing other iron biomarkers, especially in the setting of inflammation, serum ferritin remains the most commonly used indicator for diagnosing iron deficiency, although the interpretation of serum ferritin in that setting is controversial. The role of inflammation in various diseases, not least in some very prevalent diseases of the Western world, is crucial for a proper diagnosis of iron deficiency. The importance is widely proved in the settings of chronic inflammatory diseases and the metabolic syndrome. However, not all mechanisms have been thoroughly explored yet. In case of depression as well as very active people/athletes, the association of an inflammatory setting and iron deficiency needs further investigation. All in all, it can be stated that plenty of high-quality research has been conducted in this field of study that has already yielded valuable results, but in order to ensure the most effective treatment possible for all patients with iron deficiency, additional studies with special emphasis on certain patient groups, such as athletes and people suffering from chronic inflammatory diseases, the metabolic syndrome or depression, is necessary.

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