Diplomarbeit

Iron – risk or benefit? The role of imbalance in iron homeostasis in the pathogenesis of cardiovascular diseases and therapeutic options for iron overload and deficiency

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Graz, am 12.05.2020 *Christina Starchl eh.*

Danksagungen

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Zusammenfassung

Die Aufrechterhaltung der Eisen-Homöostase ist essentiell für zahlreiche physiologische Prozesse und unterliegt im menschlichen Körper hauptsächlich der Kontrolle der Hepcidin-Ferroportin-Achse und intrazellulären regulatorischen Proteinen. Herz-Kreislauferkrankungen sind eine häufige Konsequenz von Störungen des Eisenmetabolismus, sowohl von Eisenmangel, als auch von Eisenüberladung. Mehr als die Hälfte aller Patientinnen und Patienten mit chronischer Herzinsuffizienz weisen einen Eisenmangel auf, der als ernstzunehmende Komorbidität Symptome exazerbieren und die Prognose verschlechtern kann. Studien zeigen auch, dass der myokardiale Eisenmangel nicht nur durch gestörte Eisenaufnahme oder verminderte systemisches Eisen, sondern vielmehr durch intrinsische myokardiale Eisen-Deregulation wie die veränderte Expression von lokalem Hepcidin verursacht wird.

Im Gegensatz dazu resultiert eine Eisenüberladung entweder aus gestörter gastrointestinaler Absorption oder aus häufigen Bluttransfusionen und ist vergesellschaftet mit genetischen Erkrankungen wie den Thalassämien oder Hämochromatose. Letztere ist mit rund 15% der Bevölkerung als heterozygote Trägerinnen und Träger die am weitesten verbreitete genetische Erkrankung in Nord- und Zentraleuropa. Eisen kann oxidative Schäden hervorrufen und dadurch eine beachtliche Anzahl an Pathologien verursachen, wie beispielsweise Atherosklerose durch Lipidperoxidation. Darüber hinaus spielen Makrophagen eine wichtige Rolle im Eisenmetabolismus: Makrophagen sind nicht nur Teil des körpereigenen Eisendepots, sondern auch maßgeblich an der Entstehung, Progression und Destabilisierung von atherosklerotischen Plaques beteiligt.

Das Ziel dieser Diplomarbeit ist es, einen Überblick über den Eisenmetabolismus aufzubereiten, die Ursachen für eisenbezogene Störungen wie Eisenmangel oder -überladung aufzuzeigen, sowie die Mechanismen, die zu Atherosklerose, Kardiomyopathie und Herzinsuffizienz in Verbindung mit gestörter Eisenhomöostase führen, zu diskutieren. Der letzte Teil der Arbeit wird eine Beschreibung der aktuellen therapeutischen Möglichkeiten bei Eisenmangel mit oraler versus intravenöser Therapie, bzw. bei Eisenüberladung mit Chelatoren oder Aderlass bieten.

Abstract

The maintenance of the iron homeostasis is essential for several physiological processes and is mainly controlled by the systemic hepcidin and ferroportin axis, as well as intracellular regulatory proteins. It has been proven that heart disease is a common consequence of iron metabolism disorders, including both iron deficiency and iron overload. More than half of the patients with chronic heart failure suffer from iron deficiency, which - as a severe co-morbidity - can exacerbate symptoms and contribute to a poor prognosis. Studies show that myocardial iron deficiency is not only caused by defective iron absorption or decreased systemic iron levels, but rather by intrinsic myocardial iron deregulation, including altered expression of hepcidin in the failing heart.

On the contrary, iron overload can result from disordered gut absorption or frequent blood transfusions and is mainly observed in patients with genetic diseases like beta-thalassemia major or hemochromatosis. The latter is the most widely spread genetic disease in Northern and Central Europe, considering ~15% of the population are heterocygotic carriers. Iron can cause oxidative damage provoking a considerable number of diseases, including atherosclerosis by lipid peroxidation. Moreover, macrophages are important players in iron metabolism: They are not only part of the body iron deposit, but also play an important role in the onset, progression and destabilization of atherosclerotic plaques.

The aim of this thesis is to provide an overview of the iron metabolism, the causes behind iron disorders like iron deficiency and overload, as well as the mechanisms leading to atherosclerosis, cardiomyopathy and heart failure associated with imbalance of iron homeostasis. The final part of this thesis will discuss current treatment options for iron deficiency involving oral or intravenous supplementation and for iron overload phlebotomy or chelators from a clinical perspective.

Index

Glossary and Abbreviations

μ	Micro
μg	Microgram
Anemia	Decrease in red blood cells or hemoglobin
Antiapoptotic	Protection against self-destruction of cells
Apoptosis	Self-destruction of cells
Atherosclerosis	Disease of arteries due to the build-up of plaques
ATP	Adenosine triphosphate, universal energy provider
BMP	Bone morphogenetic proteins
Ca^{2+}	Calcium
Cardiomyopathy	Disease concerning the heart muscle
Ceruloplasmin	Protein that stores and carries copper
Clathrin	Coating protein for endocytosis
CNPase+	Enzyme that plays a role in myelination of oligodendrocytes
Creatinine clearance	volume of blood plasma that is cleared of *creatinine* per unit time
CRP	c-reactive protein
e.g.	Exempli gratia, for example
Endocytosis	cellular process of bringing substances into the cell
Epithelial cell	Cells from surfaces like skin, blood vessels, urinary tract or organs
ER	Endoplasmic reticulum
Erythrocytes	Red blood cells
Erythropoiesis	Synthesis of red blood cells
Erythropoietin	Mediates synthesis of red blood cells
Etc.	Et cetera
Fe^{2+}	Divalent iron
Fe^{3+}	Trivalent iron
Ferroprotein	Any protein that contains iron in a prosthetic group
FPN	Ferroportin
Glial cell	non-neuronal cells in the central and peripheral nervous system
HAMP	Hepcidin gene
HDL3-C	Subfraction of high density lipoprotein
Hematocrit	Cellular components of the blood
Hemochromatosis	Hereditary disease with inappropriate iron absorption
Hemoglobin	Iron-rich protein in red blood cells
Hemolysis	Rupturing of red blood cells
Hemopoietic cells	Blood producing cells
Hemosiderin	Iron storage complex
Hydroxyl radicals	Highly reactive, consisting of oxygen and hydrogen
ID	Iron deficiency
IDA	Iron deficiency anemia
IL-6	Interleukin 6, inflammatory marker

Insulin	Pancreatic derived hormone, that regulates blood sugar
IO	Iron overload
IOC	Iron overload cardiomyopathy
LDL	Low density lipoprotein
LDN	Low-dose naltrexone, inhibitor of BMP
Macrophages	White blood cells of the immune system
MCH	Mean corpuscular hemoglobin
MCV	Mean corpuscular volume
Metabolic syndrome	Clustering of several conditions that occur together, increasing risk of heart disease, stroke and type 2 diabetes.
Mitochondrion	Semiautonomous double-membrane-bound organelle found in many organisms
Myelination	Generating lipid-rich myelin in the central nervous system, for insulating nerves
Myoglobin	Iron- and oxygen binding protein in the muscle tissue
N-acetyl cysteine	Medication to treat paracetamol overdose or cystic fibrosis
NAD(P)H oxidase	Nicotinamide adenine dinucleotide phosphate oxidase, catalyzes production of superoxide free radicals
N-terminal pro-brain natriuretic peptide	Pro-hormone for diagnosis of congestive heart failure
Oligodendrocyte	Type of neuroglia, that support and insulate axons of the central nervous system
Oxidation	Chemical reaction by transfer of electrons
PET-CMR	Positron emission tomography–magnetic resonance imaging, functional imaging
Phagocytosis	process by which a cell uses its plasma membrane to engulf a large particle
Blood plasma	Liquid component of blood that carries cells and proteins, 55% of blood volume
SERCA2	ATPase, its major function is to transport calcium from the cytosol into the sarcoplasmic reticulum
Sideroblasts	Nucleated precursors of mature red blood cells with granules of iron
Thalassemia	Genetic diseases with diminished quantitative hemoglobin synthesis

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1 Introduction

Iron as an essential micronutrient is included in several physiological and vital processes such as oxygen transport and utilization, oxidative phosphorylation, mitochondrial function, DNA biosynthesis and ATP production. The role of iron homeostasis concerning cardiovascular disease, cancer and genetic diseases has been subject in many studies during the last decades and still implies several unknown molecular pathways.

The aim of this thesis is to provide an overview on iron metabolism associated with acute or chronic disease, putting a focus on cardiovascular diseases, especially atherogenesis, heart failure and cardiomyopathy.

1.1 Iron Metabolism

Key players in iron metabolism include:

- *ferritin*, a protein that stores intracellular, redox-inactive iron;
- *transferrin*, a serum transport protein for iron;
- *transferrin receptor 1*, a plasma membrane protein that enables cellular iron uptake by erythroblasts of the bone marrow or reticulocytes;
- *divalent metal transporter 1*, which absorbs iron in duodenal enterocytes;
- *ferroportin*, the only cellular iron efflux pump, that is responsible for the transportation of iron from the intestine into the portal blood and
- *hepcidin* – the most important regulator of the iron metabolism inhibits iron resorption by ferroportin degradation.(1)

The average iron loss is around 1 to 2 mg per day, due to epithelial cell desquamation or menstrual blood loss and is easily replenished by dietary ferric iron. *Heme iron* Fe^{2+}, which is found in hemoglobin or myoglobin and is absorbed more efficiently and *non-heme iron* Fe^{3+} are the two major forms of dietary iron. The iron store of the body in the average adult contains around 3 to 4 grams (g) of iron. Body iron consists mainly with around 70% of heme iron, 18% intracellular iron deposit ferritin or hemosiderin, 12% of the iron is to be found in bone marrow, myoglobin and enzymes and just around 0,1% is transferrin bound transport iron. However, iron in human organisms is perpetually recycled by erythrocytes and reticuloendothelial macrophages, as the macrophages phagocytose senescent erythrocytes and the iron of the hemoglobin is released back to the plasma to be reuptaken by reticulocytes in the bone marrow for erythropoiesis. (2–4)

Intracellular iron metabolism is balanced by each cell individually and controlled through absorption, loss, storage and mobilization. Absorption works via clathrin-mediated endocytosis by *holotransferrin* binding to transferrin receptor 1, following by reduction of Fe^{3+} to Fe^{2+} through a H^{+} pump that lowers the pH. Fe^{2+} can either leave the endosome via divalent metal transporter 1, is utilized for ferroprotein synthesis, uptaken by mitochondria for synthesis of iron-sulfur clusters, stored in iron-ferritin complexes or exported via ferroportin.(2)

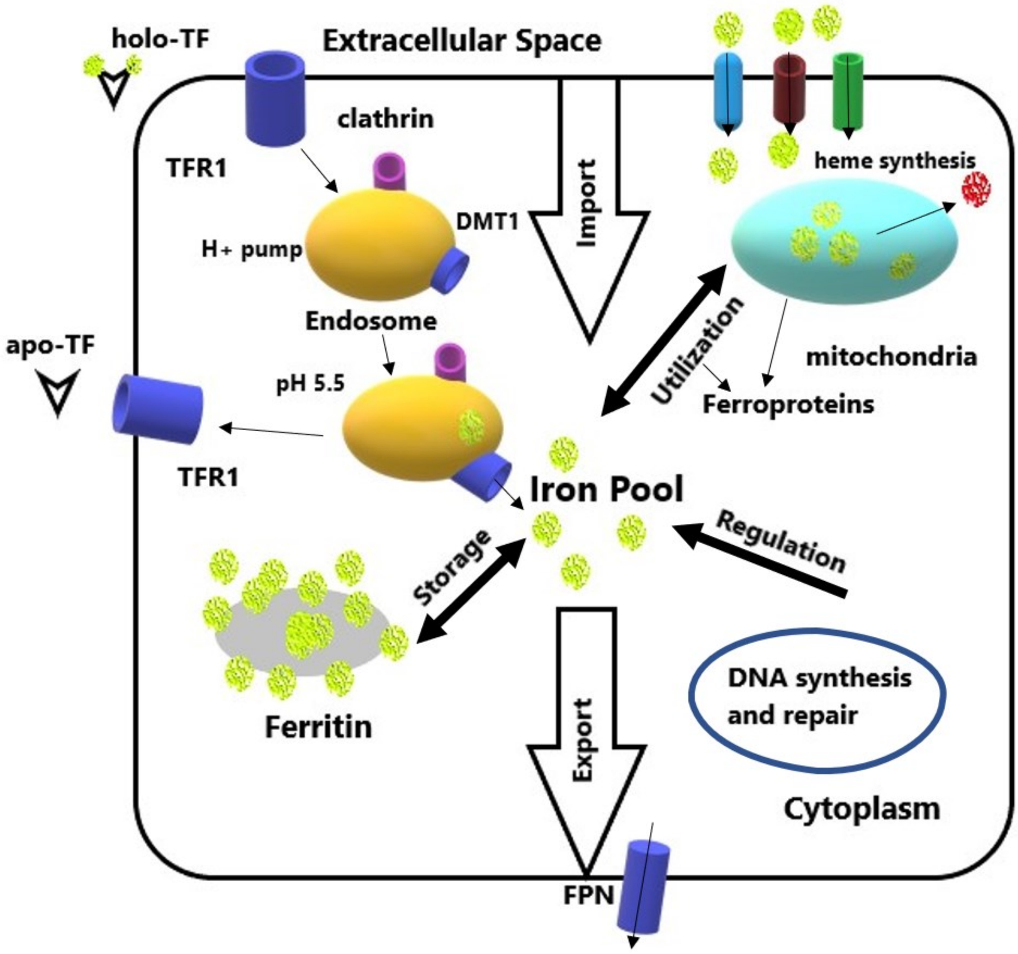

Figure 1: Cellular iron metabolism. Adapted from Zhang et al. (2019)

1.1.1 Hepcidin-ferroportin-axis

Hepcidin is the main hormone in iron homeostasis. It is built in the liver in response to an increased saturation of holo-transferrin and in combination with cellular iron export pump ferroportin (FPN) it controls iron fluxes from intestinal absorption, the iron delivery by macrophages and the release of liver iron stores.(4)

Hepcidin inhibits the dietary iron absorption of enterocytes by binding and internalization of FPN. By degradation of FPN into the lysosomes, only small quantities of iron can be released into the blood. (5)

Mechanisms behind hepcidin-release can be described starting with *apo-transferrin* (transferrin without iron), which cannot dissociate human hemochromatosis protein (HFE) from the transferrin receptor 1 (TFR1) when plasma iron is low. Thus, in absence of HFE protein iron-sensing complex does not form and hepcidin is not synthesized. As transferrin binds iron, it turns into *holo-transferrin* which can bind to TRF1 and releases HFE protein triggering a cascade of several iron regulating proteins expressing hepcidin gene HAMP.(2)

Disorders of this complex mechanism can lead to iron overload or deficiency. Especially after hemorrhage, hemolysis or other situations that trigger stress erythropoiesis, bone marrow is in need of an enhanced amount of iron to enable sufficient erythropoiesis. The hormone *erythroferrone* is produced from erythroblasts responding to erythropoietin. It suppresses hepcidin to facilitate stress erythropoiesis and subsequently helps to increase iron absorption and supports iron release from stores.(6)

The liver-derived hormone hepcidin is not only suppressed by erythroferrone but also by hypoxia and low transferrin saturation and it is stimulated by inflammation. Anemia of chronic disease is therefore thought to be a result of inhibited hepcidin due to inflammation.(7) Also, secondary iron overload in diseases with inefficient erythropoiesis, like ß-thalassemia, can be explained with regards to this mechanism.

Iron plays an crucial role in cardiovascular medicine as well and local cardiac hepcidin and ferroportin have been found to be even more vital for heart homeostasis than liver hepcidin. (5) Studies in mice with specific cardiac FPN knockout showed more serious cardiac dysfunction than in mice with systemic

hepcidin knockout, even though the hepcidin knockout caused a higher cardiac iron load. In the FPN knockout mice the iron was mainly concentrated in cardiomyocytes, whereas in the systemic hepcidin knockout mice, the iron was mainly seen in non-cardiomyocytes.(8) Other studies found that loss of cardiac hepcidin leads to fatal cardiac function, left ventricle hypertrophy, reduction in ejection fraction of rat hearts as well as apoptosis. (9)
This implies that the local homeostasis of hepcidin and FPN constitute a substantial homeostatic factor for cardiac physiology and mutations in cardiac hepcidin could result in previously unrecognized forms of cardiomyopathies.

1.1.2 Transportation and storage

Solubility of dietary non-heme Fe^{3+} iron is increased by acidic gastric secretions, subsequently it is absorbed in the proximal duodenum by divalent metal ion transporter 1 (DMT1), which takes up Fe^{2+} into the enterocyte from the apical membrane after it has been reduced by duodenal cytochrome b.(2)
During iron deficiency expression of DMT1 and duodenal cytochrome b is increased. DMT1 deletions result in poor iron absorption, anemia and decreased life expectancy.(3)
Fe^{2+} enters the cytoplasm and can leave the duodenal enterocyte at the basolateral membrane via FPN. Fe^{2+} is oxidized to Fe^{3+} by *hephaestin* and finally distributed through the body in form of holo-transferrin.(5)
On the contrary, heme iron is absorbed by heme/folate transporter 1 (HCP1). Once taken up into the cytoplasm it can either be oxidized by *hemeoxygenase* releasing Fe^{2+} or be transported into the plasma via heme transporter through the basolateral membrane.(2)
Beyond that, extracellular heme seems to be able to mediate neutrophil chemotaxis, stimulate endothelial cells to express adhesion molecules and activate macrophages via toll-like receptor 4. Heme can trigger numerous forms of cell death, including necroptosis, apoptosis and netosis (“neutrophil-extracellular-traps”-osis). Through oxidative harm to thick filament proteins, heme iron may also be able to cause contractile dysfunction in cardiomyocytes. Heme clearance in serum is provided by haptoglobin, albumin and hemopexin. Macrophages have the

ability to convert heme to less toxic metabolites via high levels of heme oxygenase 1. (10)

The distribution of iron through the body is provided by transferrin in the form of holotransferrin, containing around 3 mg of iron. The majority of iron – 1800 mg - is contained in erythrocytes as part of hemoglobin, 300 mg of iron are to find in hemopoietic cells in the marrow and 600mg are stored in macrophages, which are recycling senescent erythrocytes. 300 mg of iron are found in the skeletal muscles – known as myoglobin - and the main storage is the liver with around 1000 mg.(2)

Cytoplasmatic ferritin is relevant for systemic iron homeostasis as the ability of this compartment to store iron in enterocytes may be essential for controlled basolateral iron export. Fe^{2+} is conversed into an oxidized mineral form for storage.

Plasma ferritin is a soluble, relatively iron-poor form of ferritin, primarily deriving from macrophages. Ferritin concentrations in serum often but not always correlate with stored iron; exceptions include hemochromatosis, thalassemia or inflammation where ferritin increases. (4,11)

Ferritin iron is also stored in the heart and it increases progressive with age, whereas mitochondrial iron, which mainly consists of iron-sulfur clusters and heme functional groups, increases rapidly through growth but remains stable in the adult heart. (12)

1.1.3 ROS and lipoprotein oxidation

Iron is able to create *reactive oxygen species* (ROS) along with the oxidation of biomolecules, generating toxic hydroxyl radicals via the *Fenton reaction*. (13)

ROS are originated from several endogenous derivations like the mitochondrial electron transport chain or the NAD(P)H oxidases and include hydrogen peroxide, lipid hydroperoxide, ozone, several free radicals like hydroxyl radical and a lot more oxygen-containing reactive species. Considering that ROS are able to cause harm to biomolecules such as proteins, lipids or nucleic acids, humans have evolved antioxidant defense mechanisms to protect vital biomolecules by scavenging ROS, inhibiting the formation of ROS or by repairing the damage caused by ROS. Well known antioxidants are the endogenous *superoxide dismutase* or *vitamin C* derived from dietary sources. ROS is a key player regarding inflammatory responses, activates cell signaling and releases proinflammatory cytokines. Oxidative stress occurs when ROS cannot be kept within the physiological borders by antioxidants, leading to oxidative damage. Moderate oxidative stress can lead to cell dysfunction like abnormal proliferation or dysregulated inflammatory responses, whereas massive oxidative stress unleashes cell death. Some drugs or their metabolites act as electrophiles and can lead to secondary oxidative stress.(14)

1.1.4 Contribution of iron to atherogenesis

The relationship between iron and cardiovascular diseases has been investigated by numerous studies. In 1992, a Finnish study showed that a high stored iron level, that was assessed by elevated serum ferritin concentration, constitutes a risk factor for coronary heart disease.(15) Later it became clear that the increased risk of myocardial infarction described in the Finnish study was due to consumption of processed meat, which contains many detrimental components that lead to an increased mortality risk.(16)

Iron as a redox active metal and powerful catalyst can contribute to the formation of ROS through the Fenton reaction by the transfer of electrons between Fe^{3+} and Fe^{2+}. Ferritin and hemoglobin storage can reduce the toxicity of iron but free iron can still cause tissue damage via formation of ROS. Oxidation of lipoproteins by ROS forming oxidized LDL supports endothelial dysfunction through inflammatory transcription factors and increases ROS even more. (13)

Oxidized LDL is eventually taken up by macrophages via the high-affinity LDL-receptor. Macrophages develop to foam cells, which promote proteolytic enzymes such as matrix metalloproteases, degrade extracellular matrix and lead to plaque rupture and myocardial infarction.(13,17)

Necrotic foam cells that cannot be cleared lead to plaque growth and eventually to the development of a necrotic core. *Ferroptosis* is an regulated form of cell death, that leads to an lethal iron-dependent accumulation of lipid hydroperoxides.(18) Ferroptosis might play an important role in plaque destabilization. This ironcatalyzed form of necrosis can be induced by *erastin*, which blocks intracellular antioxidants and leads to cell death due to excessive oxidation of membrane lipids.(19,20)

Nagy et. al revealed the endothelial cell cytotoxicity of plaque components and came to the conclusion that the inside of late stages of atheromas represents a pro-oxidant environment that leads to erythrocyte lyse, hemoglobin oxidation into ferri- and ferrylhemoglobin and that releases heme and iron, that initiate even more lipid oxidation. When hemoglobin is interacting with atheroma lipids, heme and hemoglobin increase lipid oxidation and seem to promote cytotoxicity to the endothelium. If heme is split by oxidation, iron is released and leads to an iron-driven plaque lipid oxidation. (21)

The intracellular enzyme *heme oxygenase-1* (HO-1) catalyzes the oxidation of heme into anti-inflammatory, antioxidant, antiapoptotic and antithrombotic products. HO-1 has gained attention as a protector against cell injury and the progression of atherosclerotic lesions. In animal models, lack of HO-1 leads to accelerated progression of atherosclerosis, whereas induction of the enzyme reduced atherosclerosis, improved heart function and survival after an infarction in animal models.(22)

Although several studies have pointed out that iron has a pro-oxidative effect on lipoproteins in vitro, the results on associations of systemic iron and progression of atherosclerosis in vivo is rather inconsistent. While some studies show an association between high serum ferritin levels and carotid atherosclerosis (23), others like a systematic review of twelve prospective reports involving 7800 subjects found no epidemiological associations between iron status and cardiovascular disease. (24)

Wang et al. found an association between hepcidin and arterial stiffness, independent of gender, age or other vascular risks. Additionally, an correlation between hepcidin and HDL3-C was found, which may indicate that hepcidin is involved in cholesterol efflux in peripheral arteries, being a promising target to prevent atherosclerosis by enhancing reverse cholesterol transport from macrophages.(25)

Moreover, iron seems to be involved into the pathomechanisms of insulin resistance, which can be considered as a potentiating factor in the development of atherosclerosis. Ferritin and transferrin have been shown to be independently predictive of hyperglycemia and hyperinsulinemia.(26)
A systematic review and meta-analysis of 31 studies regarding iron and diabetes suggests that either higher levels in ferritin as well as heme-iron intake are connected to a higher risk of diabetes mellitus type 2.(27)
Another study also came to the conclusion that not only increased iron stores, higher ferritin, but also iron transport, higher transferrin levels, at a baseline were associated with higher risk of hyperinsulinemia. These markers came with an increased risk for metabolic syndrome and were, after a 6-year-follow-up, associated with a poorer outcome.(28)
Cooksey at al. showed with targeted inactivation of hemochromatosis-gene in mice that iron excess leads to increased ß-cell oxidative stress, apoptosis of pancreatic ß-islet cells, subsequently desensitization of glucose-dependent insulin secretion and decreased insulin secretion capacity. However, in conclusion they stated this abnormal mechanisms are not barely sufficient to be the solitary cause of diabetes mellitus type 2.(29)
Autophagy dysregulations seem to play an important role in the development of cardiac insulin resistance. Studies investigated that iron, via a cascade of ROS production, attenuates autophagic flux and induces insulin resistance in cardiomyocytes. (30)
Moreover, haptoglobin genotype Hp 2-2 has been investigated and proved to be associated with an increased risk of developing vascular complications in diabetes patients. Haptoglobin has the main function of binding hemoglobin to reduce oxidative damage that would otherwise occur between heme iron and plasma, tissue and lipids. Therefore, haptoglobin is elevated especially in situations like hemolysis, when free hemoglobin plasma level increases. In Hp 2-2 genotype, antioxidant capacity of haptoglobin is reduced and levels of oxidative stress enhance, leading to oxidation of lipids and impaired lipoprotein function. As a result, accumulation of iron in tissues and plasma occurs. Recent clinical studies established the beneficial effect of vitamin E in this special diabetic genotype. (31)

1.1.5 Role of macrophages in inflammation and atherosclerosis

Macrophages are important for the maintenance of the systemic iron levels and FPN mediates the iron efflux from macrophages into the circulation.(32)
Atherosclerosis is an inflammatory disease promoted by macrophages taking up oxidized LDL and their transformation into foam cells. Although some specific mechanisms of how macrophages conduct inflammation and its resolution to repair tissue remain unclear, it can be suggested that macrophages play an essential regulatory role in iron homeostasis and in atherosclerotic progression. Within atherosclerotic plaques different subtypes of macrophages have been discovered. Macrophage apoptosis can contribute to atherosclerotic plaque development, e.g. initiated by oxidative or ER stress, depending on the stage of the plaque. In early stages, macrophage apoptosis is considered beneficial and limits plaque progression. However, taking into account that lipid peroxidation, intraplaque hemorrhage and iron deposition are a substantial part of advanced human plaques, Martinet et al. assume that macrophage ferroptosis has a major role in atherosclerotic plaque destabilization. Intracellular iron metabolism modification in macrophages are suggested to alter their inflammatory and lipid peroxidation responses and therefore might be a potential therapeutic target in cardiovascular diseases.(13,19)

Saeed et al. showed in an animal model that by suppression of liver hepcidin by an BMP signaling inhibitor LDN, expression of macrophage FPN is boosted, which leads to reduced intracellular iron and less oxidative stress within macrophages. By lowering the free intracellular iron in macrophages, cholesterol efflux transporters expression can be increased via reduced generation of ROS. LDN-treated mice had increased their lipid efflux and foam cell formation was decreased, leading to decelerated plaque lesion progression.(33)
Another study came to the similar conclusion that deletion of the hepcidin gene was a protective factor against atherosclerosis and reduced pro-inflammatory markers in macrophages in an animal model. (34)

On the contrary, in the *Large AtheroGene Study* the hepcidin levels of a total of 2198 patients who underwent coronary angiography between 1999 and 2004, were measured. No independent connection between hepcidin and future myocardial infarction risk or risk of cardiovascular death could be found, implicating a secondary, if any, risk prognostic role in patients with manifest cardiovascular diseases.(35)

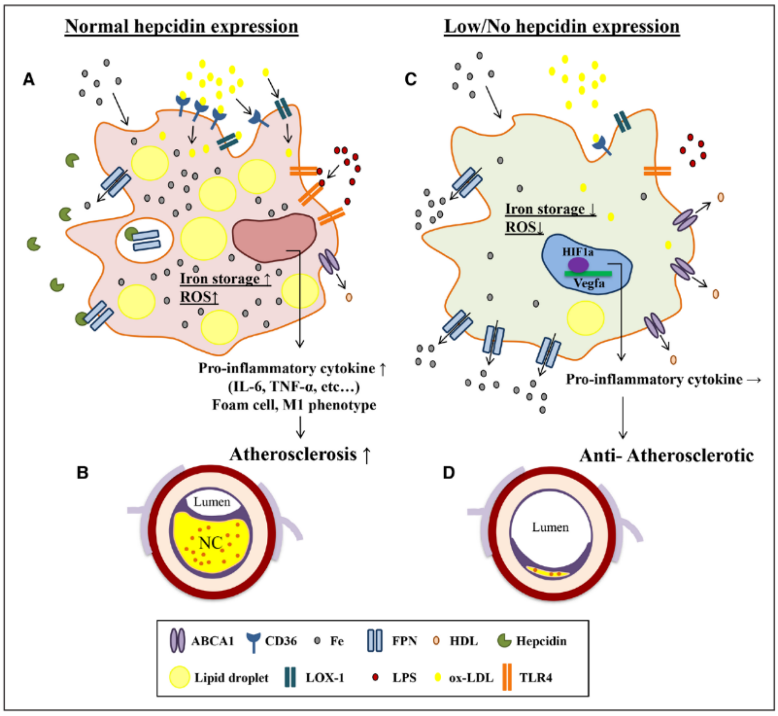

Figure 2: Effect of hepcidin on macrophages and role in the setting of atherosclerosis. Guo et al. 2019

In another study, apoE$^{-/-}$ FPN$^{wt/C3265S}$ knock-in mice with disrupted interaction of hepcidin and FPN have been observed regarding the effect of serum and tissue iron overload on atherosclerosis, caused by the continuous iron efflux into the blood. In this study, because of saturated transferrin capacity to bind iron, free iron came into the blood stream and aggravated via increased pro-inflammatory mediators and iron deposits in vascular media the progression of atherosclerosis.(36)

Guo et al., who also did a study on the role of hepcidin in atherosclerosis, came to the conclusion that hepcidin might have distinct effects in atherogenesis, depending on the stage of progression. Inhibiting hepcidin in an advancing plaque led to reduced macrophage proinflammatory enzymes and was associated with beneficial effects concerning atherogenesis. However, the effect of hepcidin in areas of intraplaque hemorrhage was suggested to be rather harmful.(32)

2 Methods

A systematic literature research with focus on the role of iron in cardiovascular disease was conducted, using MESH to search the terms "Iron"[Mesh]) AND "Cardiovascular Diseases"[Mesh] with the database “Pubmed” and using free text search in “Pubmed”. In the first round of selecting, 333 publications were selected and after processing all abstracts, 175 were finally used.
Additionally, ResearchGate, Google Scholar, onkopedia, the website of the World Health Organization as well as other reliable websites were searched to gather literature on the topic.
A focus was put on iron in association with cardiovascular disease to limit the search results and narrow the research question.
The collected publications were processed with regards to the research question, summarized and cited according to the guidelines.

3 Results

3.1 Iron Deficiency

Iron deficiency (ID) is the most widespread nutritional disorder, affecting approximately two billion people worldwide. Responsible for 80% of anemia it is the most common cause of anemia in the world, affecting over 30% of the world´s population. The WHO defines iron deficiency as a “public health condition of epidemic proportions”; it concerns more people than any other nutrient deficiency and ID is the only nutrient deficiency that significantly affects industrialized countries as well.(37–39)

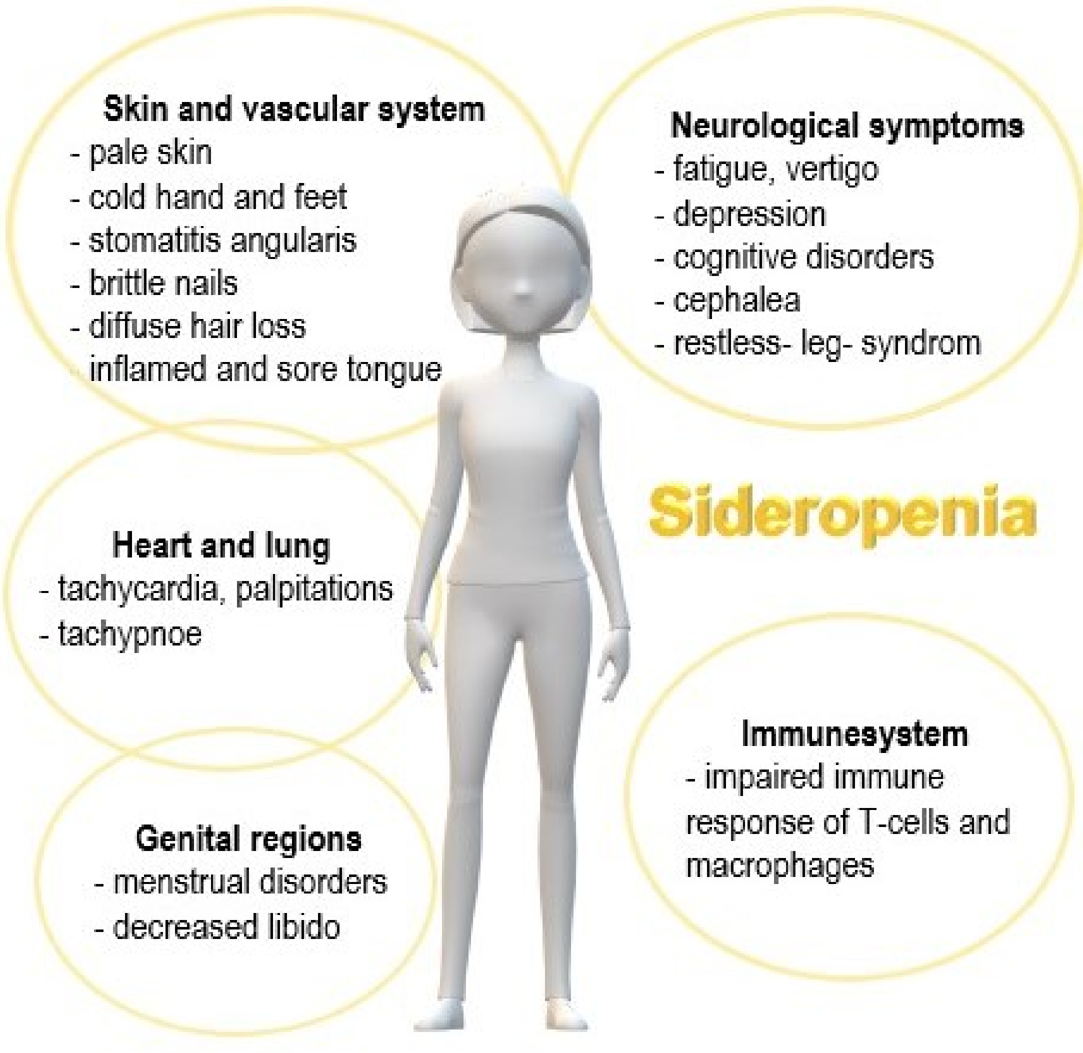

Figure 3: Symptoms of iron deficiency. Adapted from Ludwig H. (1998);25(suppl 7):2-6

Common symptoms of ID or *sideropenia* include pale skin, fatigue, weakness, tachypnoea and tachycardia, cold hand and feet, inflamed and sore tongue, angular stomatitis, brittle nails, menstruation and libido disorders, impaired immune response, as well as neurological problems like lacking concentration, cephalea, depression and restless-legs-syndrome. (38)

Anemia is characterized by a decreased number of erythrocytes, appearing with change in size or shape, that leads to impaired normal physiological capacity of oxygen transport through the blood.(39)
Most severe complications of iron deficiency anemia (IDA) include poor pregnancy outcome, impaired development of physical and cognitive performance, higher risk of child morbidity, decreased work productivity in adults and poorer outcome of chronic diseases. Additionally, in African mothers during postpartum period a strong relation between iron level and depression, stress and cognitive function has been found.(37,40) Iron supplementation is crucial in preventing such consequences.
However, concerns about adverse effects of iron increasing susceptibility to infections like malaria have emerged.(39)

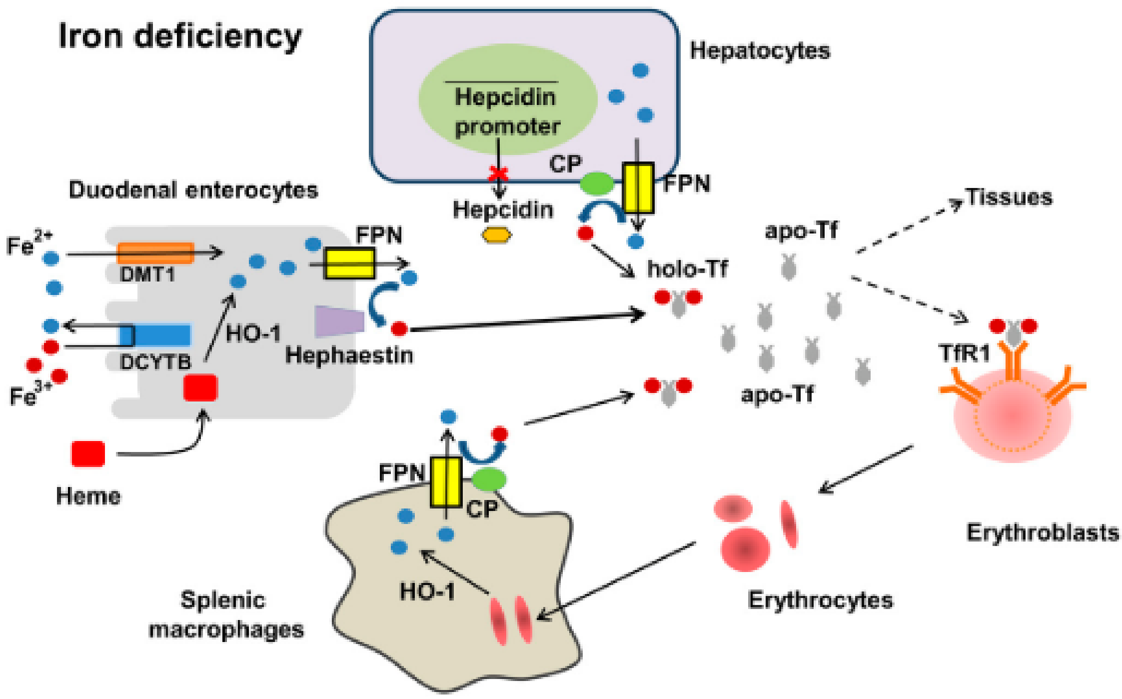

Figure 4: Altered iron metabolism in iron deficiency. Hepcidin expression is inhibited, FPN is upregulated and more iron gets absorbed. N. Ueda et al. (2018)

3.1.1 Diagnosis

First of all, a diligent history and physical examination, considering the main symptoms, can help to detect ID, IDA and potential causes.

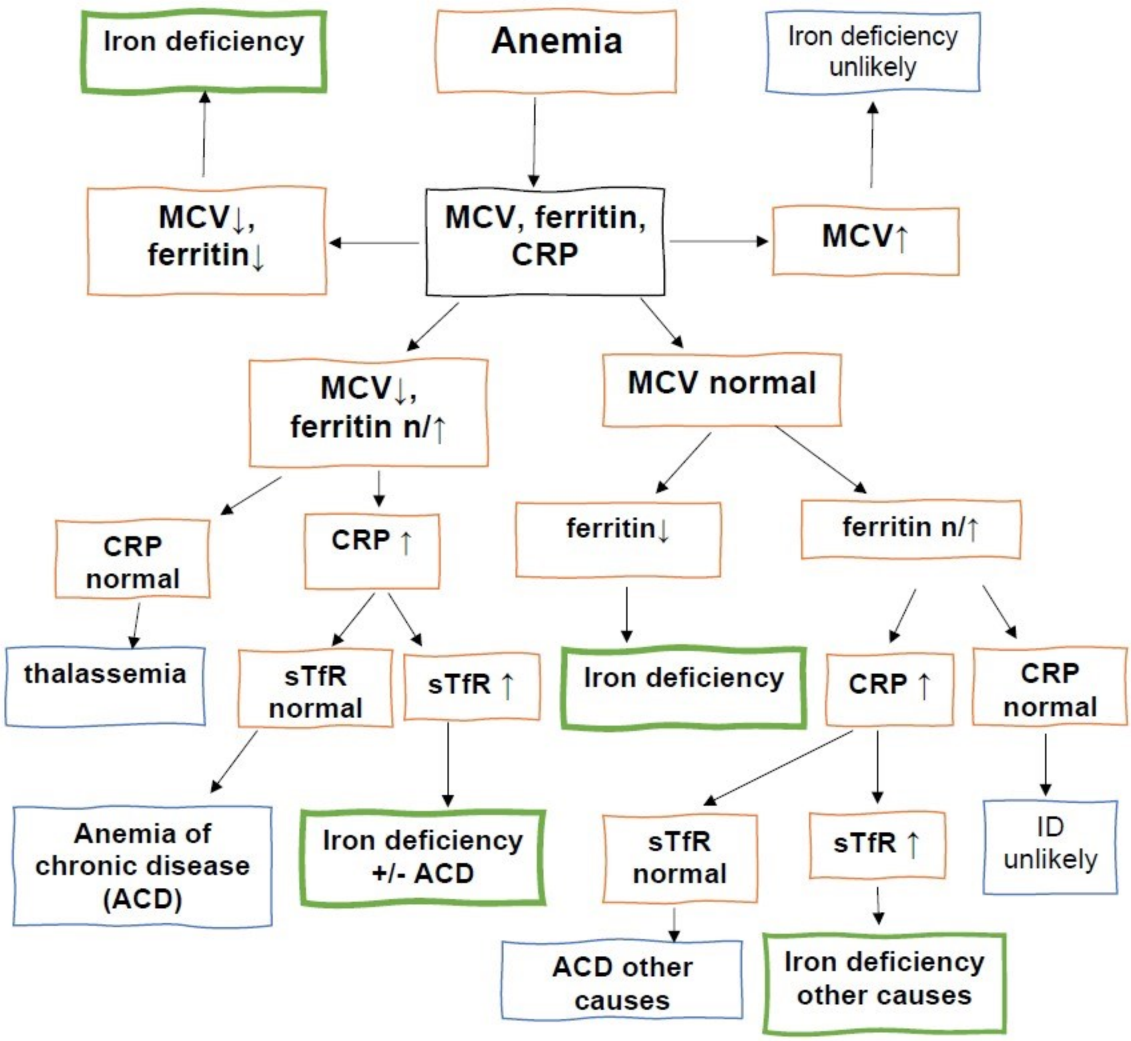

Figure 5: Algorithm for the diagnosis of ID. Onkopedia (2018) (41)

3.1.2 Laboratory parameters

Although bone marrow aspiration is the most accurate method to assess iron status, it is an invasive and not always available test. Laboratory blood tests are the preferred method for diagnosis and monitoring ID in clinical practice.(42)

According to laboratory parameters, four different stages of ID have been suggested.

1) ***Latent ID***: Serum ferritin level and bone marrow iron low but no signs of anemia (MCV and MCH normal)
2) ***Manifest ID 1***: Serum ferritin, bone marrow iron and transferrin saturation low
3) ***Manifest ID 2***: iron deficient erythropoiesis, soluble transferrin receptor (sTfR) high, reticulocyte hemoglobin <26 picograms and less than 15% sideroblasts in bone marrow
4) ***Manifest ID 3***: IDA, hemoglobin, erythrocytes and hematocrit low
    a. IDA is defined as a hyporegeneratory, microcytic, hypochromic anemia with anisocytosis due to iron deficient erythropoiesis and hemoglobin below standard level of 110 g/L (WHO).(38,43,44)

In the table below, iron indicators are ranked from the easiest and most economical to measure to the most expensive and invasive ones.

Table 1: Iron indicators. Burke et al. 2014

Biomarker	Advantages	Limitations	Norm. range/cut-off
Hemoglobin (Hb)	Easy, economical to measure, good screening tool for severe ID	Neither sensitive nor specific for iron status, better measure of function rather than status	• Pregnant women: anemia <11.0 g/dL (1./3. trimester) or <10.5 g/dL (2. trimester) • Newborns: anemia <13.0 g/dL (venous), <14.5 g/dL (capillary) • Infants 6–24 months: anemia <11.0 g/dL
Hematocrit	Easy to measure	Provides additional information above Hb	• Pregnant women: anemia <33% • Infants 6–24 months: anemia <32%
Mean cell volume (MCV) red cell distribution width (RDW)	Low MCV and increased RDW characteristic of iron deficient erythropoiesis, clinically useful	Late finding, not representative of iron status	MCV: • Pregnant and lactating women: cut-off <82 fl (femtoliters) • Infant reference ranges (age-dependent): Neonates: 100–112 fl <2 months: 85–98 fl 2–12 months: 73–84 fl 12–24 months: 72–85 fl RDW abnormal: <11.5%, >14.5%
Serum/ plasma iron	Measure of circulating iron	Easily contaminated by iron from other sources, variation by time of day, post-prandial, does not detect iron in Hb	• Adults: <40–50 µg/dL • Infants <24 months: <50–60 µg/dL
Serum ferritin (SF)	Sensitive indicator of ID, proportional to liver iron stores, responds well to iron interventions	Increases with acute phase response (not specific in presence of inflammation)	• Pregnant women: <12.0 µg/L (1. trimester) • Reference range (women): 0–230 µg/L (trimester-dependent) • Newborns: <34.0 µg/L (cord blood) • Infants 6–24 months: <12.0 µg/L
Transferrin saturation	Marker of circulating iron	Levels are depressed by inflammation	• Pregnant women: <16% • Infants <24 months: <10%
Transferrin receptor (TfR)	Less sensitive to inflammation than SF, useful in populations with high levels of background	Not very sensitive; levels change only late in ID not as specific as other measures; other	• Pregnant women: >8.5 mg/L or >4.4 mg/L

	infection	conditions may cause restriction of iron to RBCs	• Infants <24 months: >20 mg/L
TfR:SF ratio	• Proportional to stored iron or iron deficit • Sensitive indicator of response to iron supplementation	• Vulnerable to effects of inflammation on SF • Not validated in children or infants • Assay dependent (based on Ramco assay for TfR)	• Pregnant women: >500 consistent with iron deficiency or depleted iron stores • Can be used to calculate body iron stores: *-[log (TfR/ferritin ratio) −2.8229]/0.1207* o Negative values defined as tissue iron deficit
Total iron binding capacity	• More stable than other measures • Measures iron-binding sites on transferrin	• Changes only with depletion of iron stores • Not typically used in newborns	Adults: >400 µg/dL
Zinc protoporphyrin	• Sensitive indicator of severe iron deficiency, but not of moderate iron deficiency • Can be measured with very little blood volume	• Not specific as levels can be increased due to lead poisoning, inflammation, and other situations • Cut-off levels not well established for infant populations	• Pregnant women: >70 µg/dL RBCs (1T) • Infants <24 months: >70–80 µg/dL RBCs
Hepcidin	• Reflects iron homeostasis • May be measured in blood or in urine	• Also increases in conditions of inflammation • Normative levels not well defined	• Pregnant and lactating women: Mean levels immediately prior and following delivery have ranged 2.5–17.5 µg/dL • Newborns: Mean levels in cord blood have ranged 48.5–69.3 µg/dL
Reticulocyte hemoglobin	• Not affected by inflammation • Measure of iron availability to cells	Assay not yet widely available	• Adults: reference range 28–35 pg/L • Infants <24 months: reference range 23–35 pg/L
Stainable bone marrow	Gold standard for diagnosis of iron deficiency	Invasive, subject to observer error	Units: Observer assesses stained iron content according to a semi-quantitative scale

Measuring ferritin concentrations is recommended by most guidelines for the diagnosis of ID. The advantages of ferritin measuring are accessibility, low cost, sensitivity to variations of the iron store (ID, iron overload, iron supplementation); although some are weighed out by the fact that ferritin increases while inflammation, liver disease, alcoholism and certain metabolic syndromes. But conveniently, the only reason for low serum ferritin concentration is ID.(38,45)
Considering WHO guidelines, ID is defined as serum ferritin <120 µg/L.(44) Other research suggests levels of 100 µg/L and below.(42)
Reference values depend on age and sex, but Peyrin-Biroulet et al., who did a review on all available guidelines concerning ID worldwide, came to the conclusion that a *serum ferritin concentration cutoff of 100 µg/L* is reasonable for clinical practice. Considering the cell metabolism, the cell cannot produce ferritin in absence of cellular iron, whereas they decided for the cutoff of 100. *Transferrin saturation* (TSAT) is considered as an alternative diagnostic test for ID, recommended by almost half of all guidelines. Whereas TSAT can function as one of the earliest indicators of ID, it is not able to differentiate between functional or absolute ID. (45)

Hemoglobin and myoglobin are not optimally adequate for iron status assessment either, particularly during pregnancy. However, conventional tests for iron status imply hemoglobin, hematocrit, mean cell volume (MCV), serum ferritin, serum iron, transferrin, transferrin saturation and serum soluble transferrin receptor (sTfR).(46)
A Chinese study investigated the changes in iron metabolism during different periods of pregnancy to establish reference values, using conventional measurements and reticulocyte indices. Ferritin levels began to decrease with progressing pregnancy, reached the lowest point in third trimester and subsequently approached the level of first trimester. Similar trends could be observed in serum iron and transferrin saturation. On the contrary, sTfR began to increase in the first trimester, peaked in third and decreased postpartum again to the first trimester level. Reticulocytes concentration reached its highest point in second trimester and was at its lowest postpartum. Considering their results, the best combination of measurements during pregnancy are hemoglobin, sTfR and ferritin saturation, whereas the conventional gold standard for iron status

examination of bone marrow has limited usability in pregnancy and generally in clinical practice. (46)

3.1.3 Differential diagnoses

Obviously, differential diagnoses of ID must always be considered. First of all, *Anemia of chronic disease* (inflammatory, cancer or infectious origin) is mostly normochromic but potentially can present as a hypochromic anemia, defined as MCH lower than 28 pg. Secondary, pregnant women are not defined as anemic until hemoglobin is lower than 11 g/dL. Is hemoglobin >11 g/dL, it is *called dilutional anemia* or *hydremia of pregnancy*, because of the blood dilution as blood volume expands during pregnancy. Further, runners can develop a dilutional anemia similar to hydremia of pregnancy by higher increase of the plasma volume than of the hemoglobin mass. (38)

Diagnosis	Haemoglobin	Mean cell volume and mean cell haemoglobin	Serum ferritin µg/L	Transferrin or total iron binding capacity	Transferrin saturation†	Soluble transferrin receptor	Serum iron‡
Tissue iron deficiency without anaemia	Normal	Normal or low	< 15–30	Normal or high	Low-normal or low	High-normal or high	Low
Iron deficiency anaemia (IDA)	Low	Low (or normal in early IDA)	< 15–30 adult < 10–12 child	High	Low	High	Low
Anaemia of chronic disease or inflammation	Low	Normal (may be mildly low)	Normal or elevated (elevated ferritin does not imply elevated iron stores)	Normal	Low	Normal	Low
IDA with coexistent chronic disease or inflammation	Low	Low	Low or normal, but usually < 60–100 µg/L	Normal or high	Low	High	Low
Thalassaemia minor§	Low (or normal)	Low (or normal)	Normal or elevated	Normal	Normal or elevated	Normal or elevated	Normal
Iron overload	Normal	Normal	Elevated (correlates with body iron stores)	Normal to low	High	Normal	Normal to elevated

* Compared with laboratory reference range for age, sex and gestation if applicable. † Ideally performed on fasting morning sample.
‡ Serum iron is markedly labile with a significant diurnal variation, is low in both iron deficiency and inflammation, and should not be used to diagnose iron deficiency.
§ Includes β-thalassaemia minor and single or two alpha gene deletion thalassaemia minor. A thalassaemic condition and iron deficiency may coexist, particularly in pregnancy.

Figure 6: Interpreting laboratory test results to assess iron status. Pasricha et al. 2012*

3.1.4 Uncomplicated iron deficiency

Absolute ID is defined by serum ferritin concentrations less than 15 µg/L, elevated sTfR and TSAT lower than 20%. Infants, children and women in childbearing age, but also blood donors are at highest risk for simple ID or absolute ID. The recommended daily iron intake depends on individual conditions, but in general children need 8 to 10 mg, teenage girls as well as menstruating women need 15 mg and teenage boys 12 mg per day. Pregnant women need 30 mg daily and adult men as well as women older than 50 years need 10 mg daily.(37,38)

In patients with absolute ID iron storages are empty due to 4 different reasons:

1) ***Deficient iron supply***: Mostly infants, children and vegetarians are afflicted.
2) ***Inadequate iron resorption***: Chronic inflammatory bowel diseases (CEDs, celiac disease,…) and malassimilation syndromes, achlorhydria and gastrectomy can cause impaired iron absorption.
3) ***Increased requirements***: Pregnant women, or women during lactation period, children in growth periods and athletic people are in higher need of iron.
4) ***Iron loss***: In many patients, iron loss is the main cause for ID. Women with menorrhagia suffer from heavy and prolonged menstrual bleeding and lose more blood than they can compensate with nutrition and hematopoiesis. Other causes include gastrointestinal bleeding due to ulcers, erosive gastritis, esophageal varices and cancer, traumatic or iatrogenic blood loss through operations. Frequent blood donors as well as hemodialysis patients are at risk for ID.(38)

Further, ID and IDA can result from infection with helminths (schistosomiasis or hookworm), malaria, tuberculosis or human immunodeficiency virus (HIV). Many pathogens require iron for survival and virulence, therefore concerns have been raised regarding iron interventions in malaria-endemic regions, as they might possibly increase the risk for malaria infection, especially in iron replete children. Nutritional deficiencies including vitamins A, B12 and C as well as folate and genetic disorders such as thalassemia and sickle cell anemia are ancillary able to provoke ID.(39)

3.1.4.1 Iron deficiency in children

ID is common in young children in developing and most western countries, although large disparities in prevalence exist, due to ethnicity, socioeconomic status and dietary intake. In 2011 approximately 300 million children worldwide suffered from anemia; South Asia and Central/West Africa are hit hardest. (39,47)

In Western Europe IDA prevalence is low, but in Eastern Europe it is still a common disease, as well as in developing countries - almost half of preschool children between 0 and 5 years there are estimated to be anemic.(37,44)
Risk for anemia in children starts during gestation. Infants require iron to produce erythrocytes in the first months postnatal, using the iron stored during the last gestational period. At around 4 to 6 months of age their stores become low or empty and this situation aggravates when iron stores are insufficient due to low birth weight.(39)
In preschool age, sufficient iron supply is required due to rapid growth velocity. Among school-age children, iron deficiency has been associated with impaired cognitive functions and supplementation of iron showed beneficial effects.(39)
A Netherlandish study in a population of healthy children aged 0.5 to 3 years showed that Children that visit preschool or daycare and/or get formulas are less likely to develop ID, while children with a high intake of more than 400 mL cow´s milk are more vulnerable to ID. In children who received breastfeeding and no formula before the age of six months were at a significantly higher risk for ID than children who received formula before the age of six months. However, therapeutic range of iron is small and in children with replete iron stores iron supplementation always entails the risk of bringing forward infections, impairing growth and cognitive development.(47)

3.1.4.2 Obesity alters the iron metabolism

Several studies investigated the impact of obesity on the iron metabolism. Obesity is connected to hypoferremia, lower body iron stores and reduced gut iron absorption despite normal dietary iron intake. The swiss researchers Stoffel et al. came to the conclusion that central obesity increases the risk for ID. Body fat distribution in obese and overweight women alters the iron metabolism and inflammatory response, considering that adipose tissue is infiltrated by more macrophages producing interleukin-6 (IL-6) than peripheral fat and waist circumference is a predictor for plasma IL-6 concentrations. Obesity is linked to high-circulating IL-6 and serum hepcidin level. Released adipocytokines have stimulatory effects on hepcidin expression and thus contribute to higher risk of ID and IDA in women with central adiposity compared to normal-weight women.

Weight loss in overweighed women with ID reduces not only systemic inflammation but also increases dietary iron absorption. (48–50)

3.1.4.3 Iron deficiency in pregnancy and effects on newborns

ID is the most frequent nutrient deficiency in pregnancy and consequences range from maternal anemia to reduced iron stores in the newborn, weakness, fatigue, reduced immune response and increased risk of complications in delivery. It has been subject of many studies that especially pregnant women are vulnerable to ID, regarding the higher iron demands due to increased hematopoiesis, plasma volume expansion and fetal-placental growth.(46,51,52)

In Europe, approximately 10% and in developing countries every second woman of childbearing age is estimated to suffer from IDA, contributing to 20% of all maternal deaths. The incidence of iron deficiency without anemia in pregnant women is unknown. (37,53)

Iron supplementation during pregnancy can be vital, however, numerous potential mechanisms behind adverse birth outcomes in relation to excess iron intake or high iron status can be thought of, including oxidative stress, impaired systemic inflammation response, infection, alteration in the maternal microbiome or increased blood viscosity. (54)

Chełchowska et al. measured lower hepcidin concentrations in the cord blood of newborns of smoking mothers, that correlated with high levels of erythropoietin (EPO) and sTfR. Presumably, this is a result of chronic exposure to monoxide due to the smoking mother, inducing hypoxia through limited hemoglobin availability in the fetus and inducing fetal erythropoiesis. Hepcidin correlated negatively with EPO, sTfR and hemoglobin, but positively with iron and ferritin. (55)

Canadian researchers asserted that conditions leading to cardiovascular or metabolic disorders may be induced and programmed by exposure to stressors in utero. Such programming stressors include nutrient deficiencies or excesses, maternal psychosocial stressors or hypoxia. ID is suggested to be the most significant stressor, considering the high global prevalence. (56–58)

ID in combination with an high fat Western diet contributes to cardiovascular and metabolic disorders in the offspring. Bourque et al. demonstrated in an animal model that iron restriction during pregnancy caused severe IDA in dams as well as in the newborns, which showed a reduction of 15% in birth weight. Iron deficient offspring that was fed with Western diet additionally presented with reduced physical activity, increased energy intake, accumulation of visceral adipose tissue, increased blood pressure and waist circumference and they tended to have an enhanced sensitivity for salt. The ID and Western diet offspring exhibited an altered metabolic phenotype that was on a higher risk for obesity in later life. On the other hand, the ones with normal iron intake did not show such effects. Maternal perinatal iron restriction was identified as a fetal stressor that is able to program metabolic and cardiovascular dysregulation. ID can cause persistent effects independent on caloric and macronutrient intake of the mother, suggesting that metabolism depends on the balance of the iron status per se.(59)

Furthermore, low serum iron concentrations in 10 to 14 gestational weeks seem to be significantly associated with twofold higher risk of pregnancy-induced hypertension, compared with women in the highest iron quartile. Mechanisms behind low serum iron and higher risk of preeclampsia, maybe can be explained by the trophoblast development in this period. (60)

Interestingly, a study in South Africa concluded that iron depletion and IDA in the middle of pregnancy, as well as iron deficiency erythropoiesis (IDE) in late pregnancy led to a higher birth weight of 205 to 207g more. But IDE in mid-pregnancy caused a 3.6 times higher risk of premature birth and low Hb in early pregnancy was also significantly linked to preterm birth, in comparison to the pregnant women with the highest Hb. Despite regular iron supplementation, iron depletion, anemia and erythropoiesis increased during pregnancy.(61) Recent studies suggest U-shaped associations between maternal hemoglobin, serum ferritin and low birth weight, respectively preterm birth. (54,61)
This U-shaped curve concerning the risk of adverse birth outcomes seems to be confirmed, although relations must be differentiated by trimester. Accordingly, the link to detrimental birth outcome is more significant for low hemoglobin in early pregnancy. In the first trimester, low Hb concentration is significantly associated with low birth weight, but in second trimester, low Hb showed no association with low birth weight. However, a high Hb concentration in the second trimester was

significantly associated with low birth weight. In the third trimester, a weak link between low Hb and higher risk for low birth weight, preterm birth or small-for-gestational-age was found. When measuring hemoglobin in second or third trimester, relations more and more weaken. High hemoglobin and undesired birth outcomes were connected in all three trimesters. In addition, high iron status that was assessed by low sTfR in late pregnancy is associated with smaller birth size. (54)

The described inverse associations between maternal iron status and birth weight in respect of regular iron supplementation may be caused by protective effects of antenatal iron supplementation in iron depleted women. But routine iron administration in a mixed population of both deficient and replete women may lead to adverse effects on fetal growth in iron replete women, considering high iron being associated with lower birth weight on the right side of the U-shaped curve. (61)

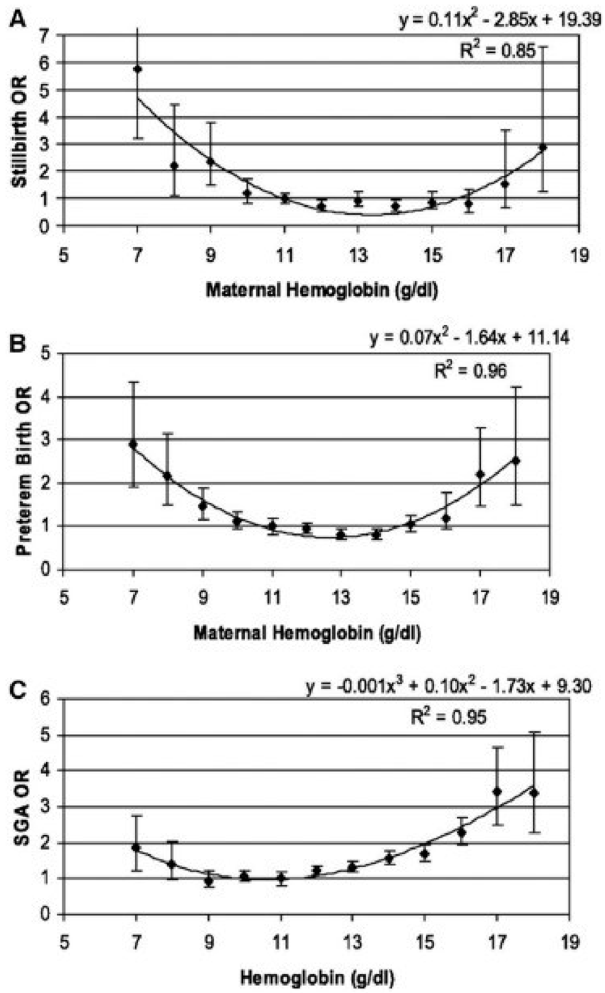

Figure 7: Odds Ratio (OR) for stillbirth (A), preterm birth (B) and Small-for-gestational-age (C), by maternal Hb. Dewey, Oaks et al. (2017)

However, the data existing on the influence of maternal iron status during gestational period on children´s iron status is rather conflicting. Interestingly enough, Uijterschout et al. investigated the associations between decreased iron supply to the fetus and ID in a well-defined, healthy population of children aged 0.5 to 3 years. They found that in a population with low prevalence of maternal anemia iron status in children is not affected by maternal risk factors like anemia, diabetes, hypertension or smoking during pregnancy, although MCV values in mother and child correlated. Their explained the similar MCV values by genetic and shared environmental factors, considering familial tendencies. Despite maternal decreased iron transport during pregnancy having a negative influence on iron status in children between 0.5 to 1 year of age, this impact is not powerful enough to cause ID. After reaching the age of 1 year, ID in children is most efficiently prevented by using formulas and sufficient iron intake from complementary diet. In contrast to previous studies Uijterschout et al. found no differences in hemoglobin, MCV or ferritin status between children and mothers with ID or IDA during gestation and children born to mothers without ID or IDA. One attempt to explain could be that most studies regarding ID were performed in developing countries with much higher prevalence of maternal anemia than in industrialized countries. (47,62)

ID has serious intergenerational effects on global health, considering the high prevalence and tendency to concern pregnant women. However, the effort to prevent ID would be minimal in relation to other fetal stressors.

3.1.4.3.1 Iron deficiency and brain development

Further research with rats on a low-iron diet but without developing severe IDA during pregnancy, mimicked a realistic clinical situation that would not raise concern in human clinical settings, as the absence of severe anemia would not require intervention and may even remain undetected. Lower body weights, as well as functional neurodevelopment impairments, that could be explained by decreased myelination, were found in offspring, using the Auditory Brainstem Responses test to analyze neural conduction velocity. Brain development was profoundly compromised, which was demonstrated by an increased auditory brainstem response time. Suboptimal iron levels particularly affect early embryonic cells, like the glial population and neuronal embryonic progenitor cells. Fetal negative central nervous system effects were more serious when ID was initiated at the onset of the pregnancy, in comparison to initiating ID previous to the beginning of pregnancy.(63)

Guitart et al. evaluated effects of developmental ID on oligodendrocyte complexity throughout different brain areas. Iron deficient mice at first increased their CNPase+ cells with domination of immature oligodendrocytes, but in later development accommodated by selective cell death of immature oligodendrocytes. Consequently, this led to impaired development behavior concerning body balance, sensorimotor functions and muscle response. The expression of transcriptional factors that play a rule in maturation of oligodendrocytes were studied and results provided evidence that correlates with an adverse oligodendrocyte maturation. Overall, they demonstrated that low iron promotes hypomyelination by leading immature oligodendrocytes to specific cell death and inhibiting their maturational path. (64)

Further study demonstrated that early-life ID can lead to DNA methylation – an important epigenetic mechanism to control gene expression – and subsequently resulted in neural gene dysregulation. Datasets exhibited altered similar functional networks mediating nervous system development and cell-to-cell signaling, vital for learning and behavior, which can lead to lasting abnormalities in cognitive function and affective behavior in adulthood. Whole genome bisulfite sequencing of iron deficient rat hippocampus was performed to identify gene loci with altered DNA methylation. On postnatal day 15 iron demand for axonal and dendritic growth in the hippocampus is maximal; that is when the measurements were taken. Differential methylation was increased at intergenic regions in the ID hippocampus, which might represent enhancers or cis-regulatory areas for gene expression. Signaling pathways underlying neurobehavioral abnormalities were identified, demonstrating that DNA methylation at genes mediating cAMP- and protein-kinase-A-signaling was particularly altered in iron deficient rat hippocampus. Results were consistent with previous studies that suggested abnormal dendritogenesis and synaptogenesis in iron deficient rats, as well. Additionally, altered reelin signaling pathway in ID hippocampus was revealed, implicating pathogenesis of schizophrenia, bipolar disorders and autism, considering all of them have been associated with ID in early life. (65–68)

All this evidence emphasizes the importance of monitoring the iron status, even beyond the level of anemia, especially in pregnant women. However, limitations of using animal models and directly transferring knowledge to humans must be recognized.

3.1.4.4 Iron deficiency in blood donors

High-frequency whole blood donors have lower body iron stores, lower oxidative stress and increased vascular function in comparison to low-frequency donors.(69) Iron depletion, referred to as low storage iron but normal hemoglobin, is a common condition in whole blood donors, as well as an increasing number of donations

entail lower ferritin and hepcidin distributions. Ceruloplasmin (acting as a ferroxidase) and erythropoiesis upregulate as a result of blood donation, which in turn downregulates hepcidin expression. (70,71)
Increased hepcidin has been associated with increased arterial stiffness in previous studies. (25) Taking into account, that increased iron and labile iron pool (LIP) in macrophages are associated with a higher risk of cardiovascular events, depleted iron stores could on the other hand have protective effects against atherosclerosis. Frequent blood donation has been associated with reduced risk of atherosclerotic cardiovascular disease and reduced all-cause mortality. (72)
On the other hand, a study in the Netherlands investigated whether frequent whole blood donation decreases the risk of cardiovascular disease but did not find an impact of blood donation on the magnitude of subclinical atherosclerosis. They rather discarded the beneficial effect of frequent whole blood donations on atherosclerosis.(70)
Moreover, Riško et al. surprisingly found significantly higher labile iron pool (LIP) levels in the donors than in the healthy control group, implying that active iron turnover may increase macrophages. However, no correlation between LIP levels and atherosclerosis could be found.(71)

3.1.5 Complex iron deficiency

Complex ID can be described as functional ID, which means the iron storage is full, but not available. Generic causes of functional ID are inflammatory or cancer-associated conditions.(38) Missing iron results in low hepcidin whereas iron overload or inflammatory cytokines like IL-6 increase expression of the regulator-hormone hepcidin, inhibiting the iron absorption by enterocytes and iron secretion by macrophages and hepatocytes. Therefore in inflammatory diseases, functional ID is a common finding.(73)

Complex ID is associated with serum ferritin concentrations higher than 100 μ/L and TSAT lower than 20%. (45) In functional ID iron cannot be mobilized for erythropoiesis despite adequate iron stores and it is a common finding in patients with chronic kidney disease and several other chronic diseases like inflammatory bowel disease, chronic heart failure and cancer. Those are commonly complicated by ID, with or without anemia, which is considered to be a serious comorbidity. Functional ID also contributes to anemia in patients with rheumatoid arthritis. (43)

3.1.5.1 Iron deficiency in the elderly

Not only infants, children and women suffer from consequences of ID. Especially older people have to face poorer outcome, higher mortality and impaired quality of life, if they present with low iron status. Anemia is the most common hematological problem in elderly but is not referred to as “normal” senescence or process of aging. Consequently, reaching standard values is as relevant as in young people, considering that anemia at an older age is an independent risk factor for 5-year mortality. Anemia in older people is additionally associated with increased frailty, higher risk for falls, loss of cognitive function, higher risk of osteoporosis and depression. The causes for anemia in elderly include dietary deficiencies like iron, folic acid or vitamin B12, functional anemia due to chronic diseases, renal insufficiency as well as toxic drug effects, endocrine or hematological disease. (38)

A retrospective study showed that low serum iron levels, particularly in older patients, are an independent predictor of in-hospital mortality of critically ill patients. A negative association between serum iron levels and inflammatory parameters like CRP, as well as with the use of vasoactive drugs was observed.(74)

Hsu et al. published in 2013 that according to their study in Taiwan, iron-deficient elderly people in long-term care facilities had a higher risk of cardiovascular disease and all-cause mortality. (75)
Another common reason for ID in older people is that around 30% of them suffer from achlorhydria, as gastric acid is essential for ferric iron absorption.(76)

3.1.6 Role of iron in chronic heart failure

Heart failure (HF) is a common disease in which the heart is unable to provide the tissues with the needed amount of blood. Due to lack of oxygen this results in impaired organ function, venous congestion and reduced exercise capacity. HF is caused by structural and/or functional cardiac defects, like myocardial abnormality

or impairment of conduction or rhythm, valves, endocardium or pericardium ultimately leading to reduced cardiac output, potentially accompanied by elevated intracardiac pressures. The underlying disease should be identified to initialize specific therapy. (77,78)
HF is characterized by symptoms like breathlessness, ankle swelling, fatigue, elevated jugular venous pressure and pulmonary crackles. Patients can present asymptomatic before clinical symptoms become apparent with systolic or diastolic left ventricular (LV) dysfunction, which are important precursors of HF. (77,78)

Terminology distinguishes between ejection fraction, heart time volume, location (left/right ventricle, global insufficiency), NYHA functional classification I-IV and time course (acute, chronic).

- Regarding ***ejection fraction,*** heart failure with preserved, mid-range and reduced ejection fraction can be differentiated. Ejection fraction less than 40% (defined as stroke volume per end-diastolic ventricle volume) is referred to as *HF-REF* (heart failure with reduced ejection fraction). *HF-mrEF* (heart failure with midrange ejection fraction) is a grey area between 40 and 49% ejection fraction of the LV. *HF-PEF* (heart failure with preserved ejection fraction) is characterized by EF more than 50%, whereas this is referred to as normal EF. HF-PEF is more challenging to diagnose, as patients that suffer from this condition present with normal systolic function and normal size of the LV. Increased wall thickness of the LV or increased left atrium (LA) can be signs of increased filling pressures.
  The differentiation between ejection fractions is therapeutically relevant, as most clinical trials are based on it.
- Determination based on heart time volume can be subdivided into ***Low-output-failure*** (which is by definition HF) and ***High-output-failure*** (insufficient oxygen supply despite enhanced heart time volume, according to ESC guidelines no HF)
- HF can be described as ***left heart, right heart or global insufficiency***, whereas isolated right heart insufficiency is rather rare (e.g. cor pulmonale, pulmonal artery emboli,…). More often, left heart insufficiency subsequently

leads due to blood congestion in lung and lung veins to right heart insufficiency.

- HF can be characterized related to the time course. If patients have had HF for months or years it is normally referred to as ***chronic HF*** and if symptoms have remained unchanged for at least one month HF is called "***stable***". Chronic HF can exacerbate, which is referred to as "decompensated HF". New onset or ***acute HF*** can result from decompensated chronic HF and is defined as rapid decline and can be a result of acute myocardial infarction (MI).
- According to the New York Heart Association subjective complaints and symptoms can be categorized into four stages from ***NYHA I to IV***. Whereas I means no impairment and normal physical capacity and IV is defined as apparent symptoms also at rest or during light activities.

(38,78)

ID is very common in HF and up to 50% of patients with HF suffer from systemic ID. Leading to anemia and skeletal muscle dysfunction, ID is associated with a worse prognosis and impaired exercise capacity.(77,78)
Besides anemia, prevalence of ID in HF patients increases with female sex, higher NYHA class, higher N-terminal pro-brain natriuretic peptide and inflammation. (79)
Beyond that, HF patients show skeletal muscle myopathy and severity increases with ID. Melenovsky et al. investigated whether energetic abnormalities in skeletal muscles of HF patients are influenced by ID and the effect of IV iron on these patients with magnetic resonance spectroscopy. Maximal isometric muscle strength seems to be related to Hb and ferritin in HF patients. The presence of ID leads to bioenergetic deficits and greater acidosis during exercise. Acidification of the muscle during exercise is consistent with metabolic shift towards anaerobic glycolysis, maybe being the reason for early muscle fatigue. HF patients with ID generally present with graver symptoms, lower peaks in muscle strength and larger energetic depletion than patients without ID. To improve skeletal muscle function, especially in older HF patients with ID, a longer treatment with ferric carboxymaltose may be required, compared with normal repletion of internal iron stores. Novel markers for the iron content of the tissue would be needed to ensure adequate therapy. (80)

ID has been recognized as a severe comorbidity in HF-REF, as it entails impaired outcomes including death, heart transplantation or worsening of HF symptoms, nonetheless there is an underuse of iron supplementation in current clinical practice.

The RAID-HF follow-up study revealed that only 9% of patients with HF-REF received iron supplementation therapy, mostly because of existing anemia and only 4.7% received intravenous therapy. Prospectively, particularly effort should be put in patients with HF-REF without anemia to identify those patients at higher risk of death. (81)

A retrospective analysis including 109 patients by Mistry et al. led to the conclusion that ID during hospitalizations for HF is not being adequately and consequently evaluated, neither being treated properly. In this American study only around 1.5% of patients with HF got proper iron assessment and if diagnosed with ID, only 21% of the patients received intravenous treatment and despite data recommending against oral iron for ID in HF, 18 patients received oral iron. (82)

Intravenous iron supplementations such as ferric carboxymaltose in patients with HF-REF and ID have been shown to improve functional status, symptoms and exercise capacity in several studies. Also, in patients with HF-REF ID was related to reduced long-term survival as well as quality of life. (81,83–86)

Anemia, defined as hemoglobin lower than 13.0 g/dL in men and lower than 12.0 g/dL in women, frequently occurs in patients with HF, especially in hospitalized patients. The prevalence is even higher in women, elderly and patients with renal disorders. Anemia in HF leads according to ESC Guidelines 2016 to a higher number of hospitalizations, myocardial remodelling, advanced symptoms, worse functional status and reduced survival. (78) Interestingly, in the RAID-HF follow-up study ID in patients without anemia was a prognostic marker for mortality, but not in patients with anemia.(81)

Tkaczyszyn et al. investigated the relation between anemia and ID in association to long-term prognosis and came to the conclusion that in patients with HF, ID constitutes not only another cause of anemia but an “equivalent comorbid condition”, potentially occurring without hematological abnormalities like red cell indices (RCI) or hemoglobin concentration. They claimed that the detrimental impact of ID is independent of decreased RCI regarding long-term survival of HF patients. Screening for ID irrespective of the existing of anemia or abnormal red cell indices should be performed routinely in patients with HF. (87)

J. Petrak et al. dedicated their investigation to the genesis of myocardial iron deficiency (MID). Overall, they concluded that MID is caused by intrinsic myocardial iron deregulation and not by iron absorption disorders or reduced systemic iron levels. In their established rat model of HF, altered cardiac expression of hepcidin as well as other iron-related genes were observed. The liver-derived peptide hormone hepcidin is a negative regulator of iron homeostasis. It triggers degradation of iron transporter FPN and thus reduces iron efflux from enterocytes, iron recycling from iron stores in hepatocytes and macrophages. Hepcidin expression is mediated by hypoxia, iron status and inflammation; anemia of chronic disease is therefore thought to be a result of inhibited hepcidin due to inflammation. However, local hepcidin expression was among others observed in cardiomyocytes, specifically when exposed to stress. (5,7,8,58,66)

Petrak et al. created HF with volume overload by inflicting aorto-caval fistulas in male rats. They controlled iron status of their rat models nutritionally and simulated mild iron deficit, physiological iron status and mild iron surplus. All animals with aorta-caval fistuals developed HF through across all iron status cohorts, as well as cardiac hypertrophy, increased lung weights, relative wall thinning, LV dilatation, increased cardiac output and reduced LV systolic function. Animals with HF fed with low iron diet developed more concentric remodeling, probably due to the higher blood load because of anemia, compared to normal fed HF animals. The high iron supplementation cohort had a significantly improved survival compared to the normal iron group, whereas the survival of the low iron group did not differ much from the normal iron cohort. (77)

Myocardial iron decreased progressively with HF and increased only mildly with nutritional iron substitution, but if there was an diet-induced increase of myocardial iron it led to longer survival. Across all groups, there was an *inverse correlation between myocardial iron and cardiac dysfunction*. In this animal model, HF had no significant effect on hepatic hepcidin expression, but ID led to downregulation of hepcidin. However, hepcidin expression in the heart was not significantly changed by diet iron but it was significantly and markedly upregulated in all animals with HF as a response to cardiac stress, knowing that HF leads to lower myocardial iron content. Furthermore, the expression of many iron-related genes was upregulated in the presence of HF, whereas diet iron did not seem to have an impact. Strategies to increase myocardial iron in chronic HF, involving parenteral iron administration, could be a therapeutic target.(77)

Other studies proved that mitochondrial dysfunction contributes to cardiac ageing, as well as the pathogenesis of HF through decreased biogenesis increased ROS and impaired substrate utilization. In patients with HF local MID or disorders of iron homeostasis in the heart can lead to reduced mitochondrial functions. MID in HF entails reduced activity of mitochondrial aconitase and citrate synthase as well as decreased ROS-protecting enzymes. (89,90) Although the mechanisms of altered myocardial iron homeostasis are poorly understood, local dysregulation of iron in the heart apparently plays a role in pathophysiologic pathway of HF and may be used as a therapeutic target. (77,89)

In conclusion, ID seems to be of highest clinical relevance for prognosis of HF, irrespectively of the presence of anemia and it is crucial to identify patients at higher mortality risk for iron supplementation. Many studies above demonstrate the distressing gap between guidelines and newest research and practical approach in the real world when dealing with patients with ID and HF.

3.1.7 Kidney function affecting iron status

Chronic kidney disease (CKD) is generally defined as glomerular filtration rate lower than 60 mL/min/1.72m², with or without albuminuria. In hemodialysis patients, malnutrition and abnormal iron status is a common problem, linked to poorer morbidity and mortality. Also, HF and chronic kidney disease often coexist, sharing many risk factors like hypertension, diabetes and hyperlipidaemia and both together tend to worsen prognosis. As patients with renal dysfunction have been systemically excluded from clinical trials there is a lack of evidence based ID therapies for them.(78,91)

Causing increased mortality in CKD, particularly IDA is a major problem. In Patients without inflammation ferritin and hepcidin are robust markers for the prediction of iron status and responsiveness to therapy. Due to inflammatory processes that are common in CKD ferritin and hepcidin increase independently of iron status, reducing iron availability and reducing the predictive value of those two parameters. Subsequently this leads to functional ID, where iron is “trapped” within macrophages and hepatocytes and iron absorption and efflux are impaired. The common inflammation in hemodialysis patients also entails hyporesponsiveness to iron therapy and erythropoiesis stimulants by increasing hepcidin and ferritin. Beyond that, in patients with end-stage renal disease aggressive intravenous (IV) iron therapy additionally enhances inflammation, contributing to further disbalancing of iron metabolism. IV iron therapy in patients with high inflammation marker additionally implicates the possibility of iron accumulation, increasing the mortality risk in CKD patients. (91–94)

A retrospective study showed that transferrin levels are a predictive marker of the adequacy of hemodialysis, but also a parameter of inflammatory response. Transferrin has been recognized as a reliable marker of inflammation and ferritin levels when adjusted for CRP.(91)

Inflammation, malnutrition and iron blockade due to hepcidin tend to reciprocally intensify and aggravate each other. Therefore many researchers point out the importance of adjusting iron status in CKD patients to delay the onset of this vicious cycle.(91)

Most important differential diagnoses include renal anemia, characterized by normochromic, normocytic erythrocytes and hyporegeneratory marrow. Renal anemia appears in most patients with creatinine clearance below 30 ml/min, decreases quality of life and is a risk factor for higher mortality. The main cause is erythropoietin deficiency due to renal insufficiency, whereas ID is a common comorbidity but not the cause of renal anemia.(38)

3.2 Iron overload

Iron is a toxic heavy metal, that can cause reactions including the Fenton reaction leading to formation of oxygen radicals. In turn, ROS are able to harm DNA, tissue and organs. Many studies demonstrated the important role of ROS in the pathogenesis of atherosclerosis and cardiovascular disease. (13,17,21,95)

Iron overload (IO) is characterized by ferritin levels above 1000 ng/mL and the main causes are hereditary hemochromatosis, exogenous supply through transfusions or excessive supplementation in dialysis patients to stimulate erythropoiesis. To investigate IO in organs performing liver biopsy, magnetic resonance tomography and echocardiography can be helpful.(38,95) Via cardiac T2 magnetic resonance imaging myocardial siderosis, e.g. in thalassemia major patients at risk of heart failure and arrythmia, can be identified. Cardiac T2 is preferable to ferritin and liver iron for early assessment and therapy of patients at risk.(96)

Higher iron status has been associated with increased risk of stroke, especially cardioembolic stroke, as well as increased ferritin concentrations seem to be a marker of an overall adverse risk factor profile. (97,98) Interestingly, although with different pathophysiological mechanisms underlying, either ID and IO have been correlated with increased thrombotic risk. Higher iron status and increased risk of venous thromboembolism positively correlate. But the role of iron in atherosclerotic disease remains controversy as high iron has also been associated with a reduced risk of carotid plaque disease, suggesting even a protective role of higher iron status in some forms of atherosclerosis, requiring further investigations in this field. (99,100)

In children, three quarters of Western European children present iron replete and excess iron stores do not give reason for concern. However, definition of iron excess in children remains a knowledge gap. (44)

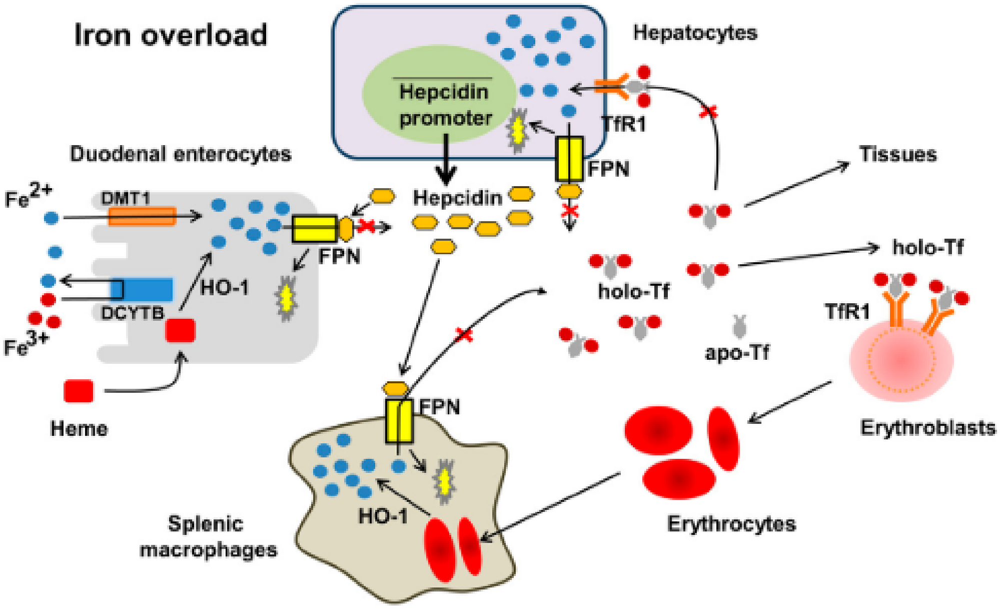

Figure 8: In iron overload high hepcidin binds to FPN and inhibits iron export from enterocytes, hepatocytes and macrophages by triggering internalization and degradation of FPN, leading to reduction of iron storage. Ueda et al. (2018)

3.2.1 Secondary iron overload

Secondary iron overload is a complication of hematological diseases or hemodialysis. Every erythrocytic concentrate used in blood transfusions contains between 200 and 500 mg of iron, depending on literature. If frequent blood transfusions are needed, these patients can have a up to 10 fold higher intake of iron than normal adults, leading to secondary iron overload.(2,95)
Systemic iron is elevated by either blood transfusions as well as IV iron supplementation, bypassing the adjusting mechanisms of iron homeostasis. High systemic iron consecutively leads to higher saturation of transferrin until capacity utilization is reached and non-transferrin bound iron emerges, inducing oxidative stress and resulting in tissue damage. (2)

Diseases that are associated with at higher risk for secondary IO –due to frequent blood transfusions – include:

- *Hemodialysis dependent CKD*: Despite hemodialysis patients suffer from blood loss and ID, IO is increasingly being recognized as an eligible clinical concern. In end-stage renal disease erythropoiesis-stimulating agents and IV iron are used to treat anemia, creating a twin risk. On the one hand it arouses ID (due to blood loss) and on the other hand IO can occur (due to possibility of excessive supplementation). Studies demonstrated a link between IO and the use of IV iron products, despite prescribed and given at doses within current guidelines. Other studies suggested an association between higher IV iron doses in dialysis patients and increased cardiovascular risk and mortality. The iron-mediating hormone hepcidin is regarded as a key player, as it leads to activation of macrophages in atherosclerotic plaques and high levels of hepcidin are linked to cardiovascular events in dialysis patients. (101–103)

- *Myelodysplastic syndrome*: Whereas the number of red blood cells is diminished, resulting in anemia, more than half of these patients are reliant on blood transfusions.
- *Myelofibrosis*: Due to progressive fibrotization of hematopoietic bone marrow, blood cells reduce, leading to anemia.
- *Thalassemia*: Impaired synthetization of hemoglobin due to genetic disorders leads to anemia, IO of organs and dependence on blood transfusions.
- *Others: Sickle-cell anemia, aplastic anemia, Diamond-Blackfan-Anemia, leukemia* etc.(95)

3.2.1.1 Thalassemia

Thalassemia is one of the most common hereditary, autosomal recessive disorders, resulted by a quantitative – not qualitative – dysregulation of hemoglobin synthesis. This reduced or absent synthesis of the globin chains in the hemoglobin tetramer results in profoundly different clinical appearances from severe anemia to clinically asymptomatic phenotypes. (38,104)

In the world´s population there is a total of 270 million carriers of hemoglobinopathies of which 30% are carriers of ß-thalassemia. Due to a partial resistance against malaria thalassemia provides selective advantage in endemic areas. The high frequency of hemoglobinopathies is suggested to be a result of the combination of natural selection, consanguineous marriages in many countries, together with epidemiological alterations. In people originating from Mediterranean regions, thalassemia and ID are the most common causes for hypochromic anemia.(38,105,106) A meta-analysis on cardiac complications in beta thalassemia major patients showed a considerable geographical difference in cardiac iron overload, identifying the highest prevalence in Africa, presumably due to worse access to transfusion and/or chelation therapy.(106)

There are two main types of thalassemia:

- **α-thalassemia**: The synthesis of hemoglobin α-chains is reduced, due to either partial (α+) or total (α0) deletions. As there are four copies of α-globin-genes, there are four different clinical pictures:
    - α-thalassemia minima: three copies intact, clinical and hematological normal
    - α-thalassemia minor: two copies intact, clinical inapparent, anemia and microcytosis possible
    - HbH-disease: one copy intact, unstable Hb, hemolytic anemia
    - Hb-Barts: all copies defect, hydrops fetalis

- **β-thalassemia**: More than 250 known mutations of the β-globin-gene with impaired (β+) or lacking (β0) production of β-globin-chains lead to anemia, ineffective erythropoiesis and intramedullary hemolysis.
    - **β-thalassemia minor**: heterozygosity, hypochromic, microcytic anemia
    - **Thalassemia intermedia**: mild homozygote or mixed heterozygote
    - **β-thalassemia major**: homozygosity, severe hemolytic anemia, transfusion-dependent, leading to hepatosplenomegaly, growth disturbance, extramedullary haematopoiesis, skeletal abnormalities (due to ineffective erythropoiesis), organ damages via secondary hemosiderosis (38)

Due to anemia, frequent blood transfusion and dose of red cell concentrate are indicated, involving adverse effects of IO such as iron accumulation in several organs. The major cause for morbidity and mortality in transfusion-dependent thalassemia patients is cardiac iron overload and cardiovascular complications.

Especially β-thalassemia major presents with a higher risk of cardiac iron overload than other subtypes. (106–108)

IO results primarily from regular blood transfusion but is also caused by hemolysis and enhanced dietary absorption. Russo et al. confirmed in their investigations that the most considerable life-limiting complications of β-thalassemia are heart failure and arrhythmias due to myocardial iron overload. In earlier disease stage the occurrence of highly symptomatic noncomplex arrythmias was observed and high confidence of sudden cardiac death in β-thalassemia major patients has been reported. (104)

Currently, allogeneic hematopoietic stem cell transplantation is recognized as the sole curative treatment method for patients with β-thalassemia major, thus survival has been significantly improved.(109)

3.2.2 Hemochromatosis

Hemochromatosis is a hereditary disease including four different types that entail high iron absorption and storage in parenchymal cells in the liver, pancreatic ß-cells, pituitary and myocardium. (38)

Diagnostic tests include measurement of cytoplasmatic storage protein ferritin. IO can be suspected if serum ferritin exceeds 200 µg/L in women or 300 µg/L in men. Further measurements of transferrin saturation are required to undoubtedly confirm the diagnosis. Transferrin saturation is calculated by division of serum iron level by the total iron binding capacity. While in women a higher saturation of 45%, in men a saturation of higher than 50% stands for iron overload. Normal transferrin saturation rules out hemochromatosis. Nevertheless, it should be considered that hemochromatosis type 4 does not elevate TSAT.(110)

Liver biopsy can indicate iron accumulation status and ultrasound of the liver should be performed regularly for the early diagnosis of possible hepatocellular carcinomas. Additional genetic test for HFE can be performed, but incomplete penetrance must be considered. Genetic testing is only reasonable combined with a coherent clinical picture and iron parameters. (38)

Table 2: Classification of hemochromatosis (38)

Type	Gene mutation	Age of manifestation	Prevalence	Organs affected
1	HFE	30-50a	1:1000	Liver (cirrhosis) Pancreas (diabetes) Heart (heart failure) Joints (arthralgia) Pituitary (hypogonadism)
2a	HJV (hemojuvelin)	10-20a	1:1 million	Heart (heart failure) Hypophysis (hypogonadism) Liver (cirrhosis)
2b	HAMP	5-15a	Rarity	Heart (heart failure) Hypophysis (hypogonadism) Liver (cirrhosis)
3	TFR 2 (transferrin receptor 2)	10-50a	Rarity	Heart (heart failure) Hypophysis (hypogonadism) Liver (cirrhosis)
4	SCL11A3 (ferroportin-1-gene)	10-50a	1:1 million	Liver (cirrhosis) Bone marrow (anemia) Spleen (iron deposit)

Type 1 hemochromatosis is the most common autosomal recessive hereditary disease, over 90% of the patients are homozygote for C282Y-mutation in the HFE-gene. But the disease penetrance is low and only around one quarter of them develops manifest hemochromatosis. Men are more susceptible to symptomatic hemochromatosis than women, but susceptibility can also be affected by lifestyle habits like alcoholic consumption or diet as well as hepatitis C. Juvenile hemochromatosis is a rare disease including iron overload previous to the age of 30 years. Thirdly, neonatal hemochromatosis is hallmarked by intrauterine cirrhosis, with the newborn being dependent on liver transplantation within the first three months of life. (38,110)

In hereditary hemochromatosis the maintenance of iron homeostasis is dysbalanced. Hepcidin expression is impaired or reduced, as a result of gene mutation in HFE, hemojuvelin and TFR2, that are involved in iron sensing and hepcidin expression. In hemochromatosis type 1, 2a, 2b and 3 despite IO serum hepcidin is inappropriately reduced, leading to excessive iron absorption in the gut. In type 4, which affects FPN, hepcidin levels are elevated in response to IO. (110)

Common manifestations of hemochromatosis are caused by accumulation of iron in several organs and include cirrhosis and increased risk for hepatocellular carcinoma. Hepatic dysfunction often occurs in early stages, indicated by

increased serum alanine aminotransferase levels. Patients often suffer from diabetes mellitus, also called bronze diabetes because of the significant dark cutaneous pigmentation, which especially occurs in the armpit area. Iron overload cardiomyopathy and arrythmias occur in severe iron excess, can lead to heart failure and increase mortality. Endocrine disorders including hypogonadism are possible. (38,111,112)
Suggesting a proatherogenic effect of iron overload, hemochromatosis patients seem to be at a higher risk of atherosclerotic cardiovascular disease in some studies, but others claim the opposite.(72,113)

3.2.3 Iron overload cardiomyopathy

Iron overload is connected to numerous processes that contribute to heart failure. The high incidence of cardiomyopathy among patients with either secondary or genetically determined IO implicates a major role of iron accumulation in the pathogenesis of cardiomyopathy. Altered iron homeostasis leads to iron deposition in the heart, provoking progressive tissue damage via noxious ROS, formed to highly reactive hydroxyl radical. Particularly the heart is susceptible to ROS-mediated damage, as it is rich in mitochondria and consuming vast amounts of oxygen. (114)
Cardiomyopathy due to iron overload is the most common reason for death among patients with secondary iron overload or with early onset of genetic hemochromatosis. However, the precise mechanisms by which iron overload leads to heart disease are not fully understood. (30,96,115)

Iron overload cardiomyopathy (IOC) tends to be overlooked, due to its progressive silent disease patterns. Its prevalence is expected to increase with the improvement in life expectancy of hematologic disorders and IOC is a predictor of prognosis for patients with hereditary hemochromatosis and hematologic diseases.(116)

Non-transferrin bound iron has been associated with the mechanisms underlying IOC, as its presence in the plasma accelerates accumulation of iron in other tissues. Particularly excitable tissues like the heart seem to be susceptible, since cardiac tissue contains many voltage-gated Ca^{2+} channels, that also bring Fe^{2+} into the cells. Divalent transporters ZIP8 and ZIP14 provide entry for iron as well. Via Haber-Weiss and Fenton reaction labile fraction of non-transferrin bound iron produces ROS and contributes to injure proteins, nucleic acids and lipids. Oxidative stress has several effects on cardiac tissue: Firstly, ROS lead to *reduced excitation contraction* coupling, as well as *suppressed SERCA2 function* resulting in *increased cytoplasmatic calcium*, impaired relaxation and in later stages impaired contractility. Secondly, *lipid peroxidation* also involves mitochondrial membranes, reducing ATP production and inhibiting oxidative phosphorylation. Thirdly, oxygen radicals can *directly damage DNA and mitochondrial DNA*. Apart from cellular harm, non-transferrin bound iron is able to directly activate proliferation and differentiation of fibroblasts to myofibroblasts, leading to progressive fibrosis of cardiac tissue. Beyond that, ferroptosis has been identified to contribute to iron-mediated cell-death dependent on ROS and potentially is conducive to further damage in IOC. However, current knowledge of ferroptosis is resting upon cell-culture experiments. (117–124)

Lakhal-Littleton et al. reported that cardiac ferroportin is essential for intracellular iron homeostasis and that the severity of consequences of cardiac IO is highly dependent upon the site of iron deposition. In ferroportin knockout mice, iron accumulates predominantly within cardiomyocytes, whereas in hepcidin knockout mice (model of hemochromatosis) iron is found in non-cardiomyocytic cells. Cardiac ferroportin upregulation is therefore suggested to be a new therapeutic target in IO disease. (8)

3.2.4 Iron, coronary artery disease and acute myocardial infarction

Due to the increased use of advanced imaging techniques, including PET-CMR and CMR, biological processes within a myocardial infarction (MI) and diversity of risks between patients surviving a ST-segment elevation myocardial infarction (STEMI) can be evaluated on a superior level. (10)

In cardiogenic shock, unbound ferric iron – also called catalytic iron – is linked to higher all-cause mortality and high levels of catalytic iron on day three after MI are associated with bleeding and high troponin levels. (125) In addition, taking into account that degradation of hemoglobin products has toxic effects, myocardial hemorrhage after STEMI can lead to adverse remodeling and heart failure. Presence of hemorrhage is associated with poorer infarct healing, due to iron-containing blood substances stimulating chronic inflammation. Persistent iron within a MI core 6 months after STEMI is associated with adverse 3-fold increased risk of major cardiac events, 4-fold likelihood of all-cause death or heart failure and with increased LV end-diastolic volume and decreasing LV ejection fraction.(10,126)

Kaluza et al. proposed in a prospective study that heme iron, but not non-heme iron intake correlates with a higher risk of fatal acute MI. Men with higher heme iron intake of 2.33 mg/day had a 51% higher risk of fatal MI than men with a daily heme iron intake of less than 1.28mg. As meat is the main source of heme iron, between men in the highest and men in the lowest quintiles of heme iron intake was 638 g of red meat per week. Also, the positive association between heme iron intake and fatal acute MI was especially significant in men who had low intake of calcium, zinc and magnesium. It is suggested that those minerals are able to inhibit the DMT1 and decrease iron bioavailability.(127)

Statins and some angiotensin-converting enzymes inhibitors stimulate heme oxygenase 1, which converts heme iron into less noxious metabolites, adding further endorsement for the use of these agents after MI. Beyond that, de-chelation therapy could be beneficial, if balanced against the risk of IDA.(10)

3.2.5 Iron overload and endocrinopathies

Frequent blood transfusions result in iron overload and can promote endocrine disorders including impairment of growth, sexual development and fertility, abnormal bone mineralization, diabetes mellitus, hypothyroidism, hypoparathyroidism and hypocortisolism. Endocrine dysfunctions are amongst the most common complications of thalassemia, as 40% of adult transfusion dependent thalassemia patients present endocrinopathy, leading to higher health-care resource utilization, costs and significant morbidities among those patients.(128,129) Mean serum ferritin levels and liver iron concentration tend to be higher among patients with endocrine complications. (129)

Hypogonadism is the most frequent endocrine complication and is mainly caused by iron accumulation in the pituitary gonadotropic cells, as well as by iron deposits in the gonads. Another theory suggests, that hypogonadism is a result of low leptin levels, that are often found in thalassemic patients. Leptin acts as an signal to initiate puberty and low leptin levels could cause delayed sexual development. (129)

Another endocrine complication is osteoporosis with a prevalence of 20% and was significantly associated with diabetes mellitus and hypogonadism in a study by Tat et al.. They found no association between osteoporosis and vitamin D levels in thalassemic patients. (129) Yang et al. found lower bone mineral density in even 42.9% of women and 23.1% of men, as well as higher fracture risk. (130) Recent findings propose as well that hypothyroidism, higher HbA1c and lower adrenocorticotropin are predictors of abnormal bone mineral density in thalassemic patients. Other related factors include PTH, FGF23, IGF-1 and testosterone levels. Whether the correction of these factors improves bone mineral density warrants further research.(130)

Additionally, thyroid dysfunction is a common endocrine complication of thalassemia major and the incidence is directly related to the degree of iron overload. The most common cause of hypothyroidism seems to be central hypothyroidism(129), and autoimmunity apparently plays no role in the pathogenesis of thalassemic hypothyroidism.(131)

Hypocalcemia due to hypoparathyroidism is a late and relatively rare complication, mainly as a result of iron overload. Even et al. observed subclinical hypoparathyroidism in their clinical study with thalassemia major patients in almost 100% of them. However, the study only investigated 13 patients.(132) Literature on prevalence of overt hypoparathyroidism in thalassemic patients ranges from 3.6% (133)to 20%.(134) Limited data suggests that early supplementation of vitamin D or calcitriol treatment for three months is sufficient to normalize plasma calcium and phosphate levels.(135,136)

Early detection, appropriate transfusion regimen and intensification of chelation therapy are essential for the treatment of these patients.

3.3 Therapy

3.3.1 Therapeutic options for iron deficiency

In the seventeenth century iron replacement therapy was commenced and until now, relatively slow progress has been made. Either oral and intravenous (IV) iron formulations are still thought to be suboptimal and particularly IV iron products are prejudiced with regards to tolerability and safety issues. In practice they are safe and efficient, including only a minimal risk of adverse effects. Pharmacology attempts to minimize adverse effects with new formulations, which is challenging with respect to the substantially different pharmacokinetic of oral and parenteral iron. (137)

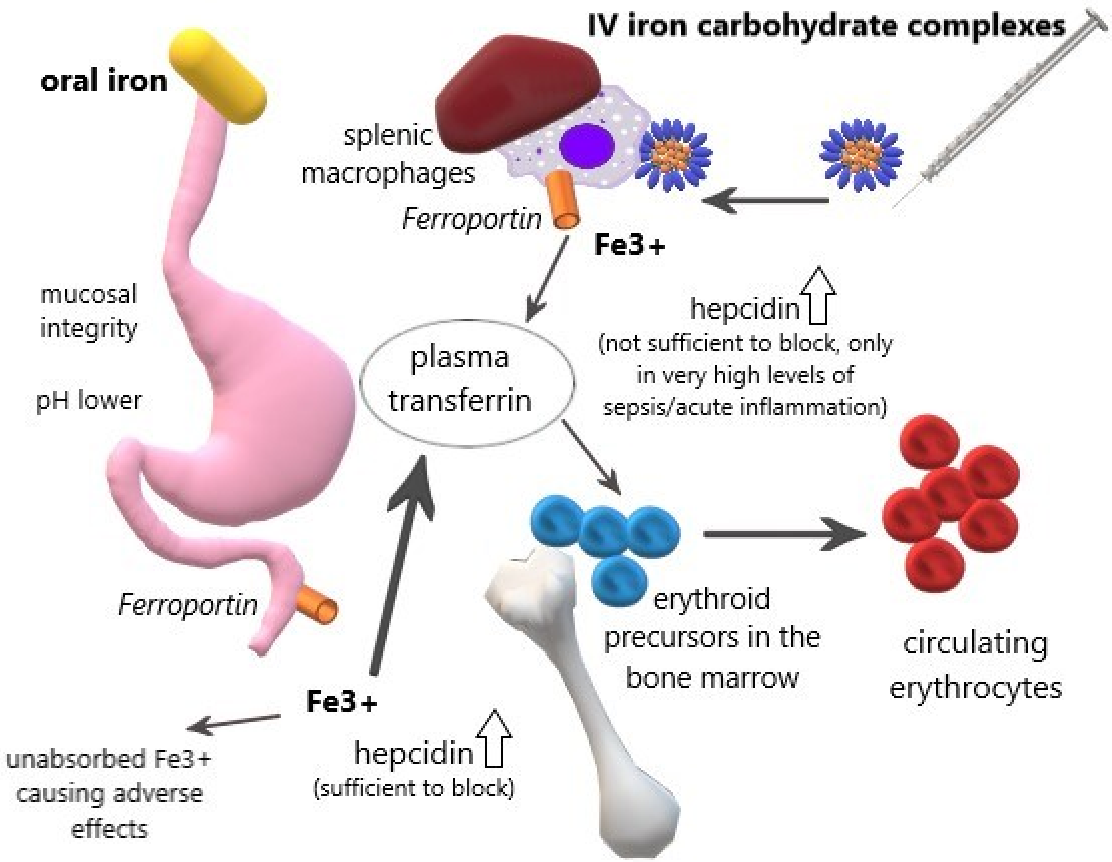

Figure 9: Different pharmacokinetic of oral and IV iron.

3.3.1.1 Oral iron therapy

In the general population, oral iron supplementation reasonably constitutes the therapy of first choice, being easy, cheap and effective in mild ID with hemoglobin > 11 g/dl; (137) even though the success of the treatment remains suboptimal. In uncomplicated IDA, the classically prescribed dose is 100 to 200 mg of elemental iron daily, mostly in the form of 2 to 3 tablets of ferrous sulfate. However, oral iron has failed to improve functional ID. For example, in patients with CKD, oral iron was unable to adjust appropriate serum ferritin levels, whereas parenteral IV iron supplementation did. The final goal and optimal response to oral iron treatment involves an increase in hemoglobin of 2 g/dl after 3 weeks and more importantly, repletion of iron stores reflected in ferritin levels higher than 100 µg/L. A common error in clinical practice is stopping the treatment too early, particularly in young premenopausal women. On the other hand, refractoriness can occur, which is defined as an increase less than 1 g/dl after 3 weeks despite adequate compliance. (45,137,138)

3.3.1.1.1 Pharmacokinetic

The pharmacokinetic of oral iron demands the integrity of intestinal mucosa, as well as a mildly acidic medium to solubilize iron. Mucosa integrity may be impaired by the presence of malabsorption syndromes, such as celiac disease or chronic gastritis, which has to be clarified before administering oral iron supplements. Aside from that, antacids like proton pump inhibitors have been found to decrease absorption of oral iron, particularly in elderly patients. If absorbed properly, iron then is released by enterocytes and taken up by plasma transferrin. During oral iron supplementation the maximum absorption that can be accomplished is 25 to 30 mg per day, which constitutes a 10 to 20 fold higher absorption than the normal 1 to 2 mg per day. Only 10 to 20 % of divalent iron is resorbed by the intestine, trivalent iron even less. Iron that remains unabsorbed in the lumen, frequently leads to gastrointestinal side effects. (38,45,137)

Hepcidin affects the absorption of oral iron, as it is normally suppressed in patients with uncomplicated IDA, promoting maximal absorption through FPN. But in situations like chronic inflammatory processes (chronic HF, CKD) or after transient stimulation by a first dose of oral iron, hepcidin levels appear slightly increased, impairing intestinal FPN. In comparison, in macrophages, FPN expression is higher than in enterocytes and they require much more elevated hepcidin to be suppressed. Therefore, in IV iron supplementation adjustment to physiological levels is more efficient and faster.(137)

3.3.1.1.2 Oral iron preparations

Oral iron preparations that are currently available in a variety of pharmaceutical forms have heterogenous chemical compositions. Tolerability of iron depends on formulation of absorbable iron. Divalent ferrous iron Fe^{2+} and trivalent ferric iron Fe^{3+} either as iron salts or iron polysaccharide complexes represent the two forms of oral iron, whereas ferric iron is not as bioavailable as ferrous iron. Non-heme dietary iron is mainly ferric and highly insoluble, it needs to be reduced by duodenal cytochrome b. Therefore, divalent iron salts seem to be the best form for iron supplementation, including *ferrous gluconate*, *ferrous fumarate* and *ferrous sulfate*. Particularly ferrous sulfate is considered as generally available by all guidelines. (45,137,139,140)

Recommended dose of oral iron is 50 mg of divalent ferrous iron two times a day. It is crucial to check the content of elemental iron per tablet, because proportion can vary by manufacturer. (38) However, daily recommendations vary between 50 and 200 mg. Some guidelines suggest not to split the daily iron dosage because hepcidin increases regulatory after oral iron intake and impairs gut absorption for around 24 hours. Maximal absorption of oral iron can be achieved by assuming on an empty stomach. If patients have gastrointestinal side effects it can be helpful to take the tablet during or after meal, however, this can lead to poorer absorption. Therefore, those patients should get higher doses. To assess the success of therapy, after one week hemoglobin and reticulocytes ought to show increasement. Possible causes for inefficient increase include inefficient therapy (lack of compliance), malabsorption, wrong diagnosis (anemia of other origin) or persistent bleeding. To reach the target for serum ferritin of more than 100 µg/L, oral substitution is required for three to six months after vanishing of anemia. (38,41,137)

In the past two decades, new formulations of iron in a *delayed-release polysaccharide complex* (PIC) have gained attention. In this combination of around 150 mg of elemental iron (ferric iron) and a low-molecular weight polysaccharide, endogenous iron carriers are closely resembled and constitute effective and stable iron supplements. In addition, PIC seems to minimize gastrointestinal adverse effects due to retarded iron release and has higher bioavailability. In an animal model a new low molecular weight PIC made of one of the most common green algae *Enteromorpha prolifera* was synthesized and investigated. The therapeutic effects turned out to be promising, as hematological indices and organ coefficients of ID anemic rats improved significantly when treated with the algae iron complex. Further studies proposed a novel polysaccharide-iron(III) complex extracted from fungal species *Auricularia auricular*, that showed high efficiency in the treatment of ID anemia in rats, as well as high antioxidant activity. (42,141–143)
On the other hand, Powers et al. compared in their randomized clinical trial effects of ferrous sulfate with PIC on hemoglobin concentration in infants and children with nutritional ID anemia. They proposed that ferrous sulfate group had a higher increase in hemoglobin and ferritin than the PIC group as well as fewer reports of gastrointestinal disturbances.(144) Likewise, Taiwanese researchers exhibited that delayed release of iron from PIC leads to slower improvement of ferritin and hemoglobin and therefore entails slower recovery from ID anemia than with an equivalent daily dose of ferrous fumarate.(145)

Sucrosomial® iron (SI) is an innovative formulation consisting of ferric pyrophosphate, protected by a phospholipid bilayer, mainly sunflower lecithin, and a sucrester matrix (sucrosome or liposome). Sucrester is a surfactant made of sucrose esters and enhances absorption due to its ability to reduce intestinal barrier resistance. Sucrester seems to protect the trivalent iron pyrophosphate against enzymatic reduction and owing to this structure SI is mostly absorbed as a vesicle-like structure via para-cellular as well as trans-cellular absorption routes. Bypassing DMT1, the conventional iron absorption pathway, it prevents inflammation in the intestines and shows not only high gastrointestinal absorption but also higher bioavailability than iron salts and at the same time low incidence of side effects. Microfold cells of the Peyer's patches (M cells) seem to support the intestinal absorption of SI, facilitated by macrophages that take it up directly into the lymphatic circulation. The liposome is carried to the hepatocytes, where lysosomal enzymes make the iron available. (146,147) In Austria, SI is available under the name of Oleovital® Eisen Classic/Femina/Forte (12mg/21mg/30mg), combined with vitamins.

SI technology has been proven to 3- to 3.5-fold increase ferritin iron accumulation within enterocytes, compared to ferrous sulfate or ferric pyrophosphate.(148)

In piglets, Si improved all red blood cell indices, similar to iron dextran, but no excess iron accumulation was observed in organs. Due to the resorption of SI through the lymphatic system, hepcidin levels increased not significantly. (149)

In oncological as well as in patients with celiac disease or irritable bowel syndrome, SI led to results comparable to IV iron sucrose concerning red blood indices and showed great gastrointestinal tolerance. Thus, SI should be considered as an alternative therapy for the management of ID.(146)

In CKD patients with anemia, SI was compared to IV ferrous gluconate and led to significantly fewer adverse effects and similar mean Hb levels. However, mean ferritin increase was greater in IV ferrous iron group than in oral SI group and Hb concentrations remained stable in the IV but not in the oral SI group after treatment discontinuation, suggesting that less iron enters stores from the SI form. Nevertheless, oral liposomal iron is not inferior to IV iron gluconate to correct anemia in CKD, although the repletion of iron stores and maintenance of Hb values was lower than in the IV group. But the few side effects, practicality and lower cost support SI as first approach to treat anemia in uncomplicated CKD patients. (147)

Recently, Riccio et al. did a cost-minimization analysis of oral SI iron versus IV iron gluconate in CKD patients and concluded that the use of SI instead of IV iron could potentially save around 1200€ per patient per circle, considering direct and indirect costs.(150)

3.3.1.1.3 Adverse effects of oral iron

Adverse effects of oral iron treatment are frequent and mainly include gastrointestinal disorders such as metallic taste, vomiting, heartburn, nausea, diarrhea, epigastric pain or constipation. Aside from that, studies showed an decrease of beneficial bacteria and change in the gut microbiome as a side effect of oral iron.(137,151)

Up to 30 to 70% of patients are expected to have gastrointestinal upset. Due to its toxic mucosal effects, iron may exacerbate preexisting gastrointestinal disorders like gastritis or esophageal erosion.

These adverse effects often tend to worsen compliance and in combination with required prolonged treatment, oral iron treatment in ID anemia patients often leads to significant undertreatment.(137)

Particularly in children and in suicidal tendency the risk of lethal overdose is constituted, leading to life threatening intoxications. Lethal dose for children is 3 g of Fe^{2+}-sulfate; this is why iron preparations should be kept safe out of reach for children. However, deferoxamine is a potent antidot.(38)

3.3.1.2 Parenteral iron supplementation

To administer parenteral iron, either intramuscular or intravenous injections can be performed. Intramuscular injections are painful and possibly lead to siderosis – iron accumulation in tissue – and a higher risk of intramuscular neoplasm.

Intravenous (IV) injections are able to rapidly counterbalance iron levels bypassing the gastrointestinal tract. To adjust optimal administration dosage, considering body weight and hemoglobin levels, *Ganzoni´s equation* can be used:

$$dose = body\ weight(kg) * \left(15 - actual\ hemoglobin\ \left(\frac{g}{dl}\right)\right) * \ 2.4 + 500\ \mathrm{mg}$$

IV ferric carboxymaltose proved to have beneficial effects on symptoms, quality of life, functional capacity and completes iron stores in HF patients. (2)

IV iron administration is associated with less gastrointestinal side effects and better drug tolerance than oral iron and is better suitable for functional ID. In severe IDA, meaning that hemoglobin is < 8 mg/dl, the agreement on using new IV iron as first-line therapy is increasing, taking into account that they are more efficient and have a prompter effect.(137)

3.3.1.2.1 Pharmacokinetic

Contrary to oral iron, which has to be taken up by enterocytes, IV iron has a substantially different pharmacokinetic. Once injected into the circulation, bypassing intestinal absorption and possible problems lying there, IV iron is mainly taken up by macrophages. Macrophages subsequently slowly release them into the bloodstream via ferroportin. (137)

3.3.1.2.2 Parenteral iron preparations

All IV preparations are colloidal solutions and consist of a polynuclear core containing Fe^{3+} hydroxide particles bounded by a carbohydrate shell. Differences between variant preparations are constituted of differences in the chemistry of the carbohydrate shell, immunogenicity and of the different strength of bonds between the shell and the iron core. IV iron should possibly be administered within high-molecular stabile complexes that are free of dextran. (38,137)

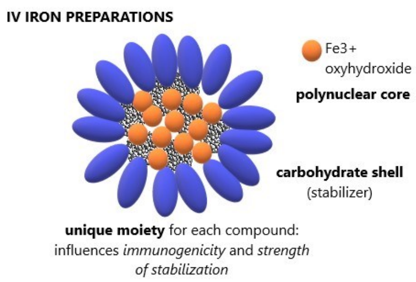

Figure 10: Intravenous iron preparations.

Those major components of the iron carbohydrate complex constitute the limiting factor of the maximum dose of iron that can be given with a single infusion. Nevertheless, complexes that are not stable enough enable ferric iron to go directly into the blood, leading to the noxious labile-free iron, when transferrin is saturated. The more stable the complex, the less adverse effects are to expect.(137)

IV iron has to be given slowly, preferably as a short infusion of 100 ml 0,9% saline. Of note, IV iron should never be administered simultaneously with reducing compounds such as ascorbic acid.

Total requirements of iron in ID is around 500 mg and in IDA 1000-2000 mg. Therapeutic success is indicated by adjustment of hemoglobin, serum ferritin (target value is 100 µg/L and TSAT 20-45%). Measurement previous to 8 to 12 weeks after last IV iron leads to incorrect high values. (38)

Table 3: Currently used IV iron preparations available in Austria. Girelli et al. (2018)/Gasche et. al(2011)

Drug	Fe- (III)-dextran	Fe- (III)-sucrose	Fe-(III)-carboxymaltose	Fe-(III)-isomaltoside	Ferumoxytol
Brand name	Cosmofer®	Venofer® FerMed® Ferrologic®	Ferinject®	Monofer®	Feraheme®
Stability	High	Moderate	High	High	High
Maximum single dose	1000 mg	200 mg	1000 mg	20 mg Fe/Kg	510 mg
Total replacement dose in single infusion (1-1.5g)	Yes	No (repeated treatment needed)	Yes	Yes	Yes for ID No for anemia
Minimum administration time (min)	4 – 6 hours	30	15	15	15
Available in Austria	Yes	Yes	Yes	Yes	Yes

3.3.1.2.3 Adverse effects of IV iron

Infusion reactions mainly consist of mild effects like a rash, palpitations, myalgias, dizziness and chest discomfort, occurring in about 1:200 administrations. Mostly, the reactions resolve themselves quickly after stopping the infusion without treatment. Side effects such as cephalea, nausea, vomiting and collapse as well as thrombophlebitis and anaphylactoid reactions, including respiratory symptoms and hypotension, can occur. High-Molecular weight dextran iron (removed from markets in 2009) particularly caused severe hypersensitivity reactions, which led to great caution when prescribing IV iron and nurtured the prejudice against IV iron. (38,137)

However, a recent meta-analysis of more than 10,000 patients that were treated with various IV iron preparations and more than 7000 patients treated with oral iron or placebo, worked out that adverse effects in IV iron are exceedingly rare and were not more prevalent than in the control group. Only minor reactions were observed and no fatal reaction ore true anaphylaxis was reported. (137,152)

Nevertheless, the currently recommended dose of IV carboxymaltose iron for HFrEF patients has been exhibited to have a decisive hypophosphatemic effect via FGF-23 increase, involving dropping of serum phosphate and 1,25-OH vitamin D levels. (153)

3.3.1.3 Iron supplementation in infants and children

Daily oral iron supplementation for anemia treatment and prevention is commonly recommended but debate remains about efficacy and safety. A systematic review and meta-analysis of randomized controlled trials investigated benefit and safety on iron supplementation in children and came to the conclusion that it effectively reduces anemia but adverse effects on development remain uncertain. (154)

According to this, infants and children between 4 and 23 months who received daily iron supplementation reduced their risk for IDA, critical outcomes of anemia and ID in general. Contrary to expectations, there was no difference in growth between the iron supplementation and placebo group.(39,154)

Further studies investigated effects of iron supplementation in children aged 2 to 5 years, coming to the conclusion that it increased ferritin and hemoglobin but found no difference in terms of final height and final weight between children receiving daily iron compared to those receiving placebo.(155) In children aged 60 months and older, studies found that receiving daily oral iron supplements increases the adverse outcomes of anemia, ID and IDA. Additionally, there was a small but significant difference in final height but not final weight between school-age children. receiving iron and placebo.(156)

The WHO strongly recommends daily iron supplementation in infants and children living in areas with high prevalence (40% and higher) of anemia to prevent ID and anemia and improve iron status as a public health intervention.

Concerning long-term harm, for example on overdose, not enough data are available, particularly for iron replete children.(39)

Table 4: WHO Guideline (2016) for daily iron supplementation in infants and children living in areas where anemia is highly prevalent.

Target group	**Infants and young children (6-23 months)**	**Preschool-age children (24-59 months)**	**School-age children (5-12 years)**
Supplement	10-12.5 mg elemental iron	30 mg elemental iron	30–60 mg elemental iron
Supplement form	Drops/syrups	Drops/syrups/tablets	Tablets or capsules
Frequency	Daily		
Duration	3 consecutive months per year		

Table 5: Normal ranges for Hb, MCV, Ferritin. Mattiello et al. (2020)

Age	Hemoglobin (g/L)	MCV (fl)	Ferritin (μg/L)
0–7 days	135–200	95–115	153–1092
8–30 days	100–160	85–100	247–692
1–3 months	95–145	85–100	148–744
4–9 months	95–135	75–95	21–240
9–24 months	105–135	75–85	10–168
2–16 years	115–150	77–85	10–99
> 16 years (females)	120–160	78–95	18–103
> 16 years (males)	130–170	78–95	16–213

In most children with IDA oral substitution therapy is effective and should be initiated after laboratory results diverge from the norm. (157)

Before iron therapy is started, the solution of any other nutritional cause for ID or anemia is crucial. In infants, reducing the intake of cow´s milk and in children that are receiving formula or are being breastfed, introduction of solid nutrition is helpful. By the age of 4 months, the breast milk´s iron content is permanently decreasing. In adolescents, reducing tea, soft drink or phytate/oxalate supplement consumption is recommended when initiating iron supplementation. Mattiello et al. proposed slightly different recommendations on iron intake than the WHO:

- ***Oral Fe^{2+} supplementation***: One or two doses of 2 to 3 mg/kg of elemental Fe^{2+} iron half an hour before or half an hour after meal in combination with juice or water are recommended.
- ***Oral Fe^{3+} supplementation***: one or two doses of 3 to 5 mg/kg of elemental Fe^{3+} iron with meals and obligatory in combination with juice or water, as polymaltose is dissolved in gastric fluid to make iron accessible in the small intestine.

For infants and young children, either capsules or tablets and liquid iron are available and in older children, also Fe^{2+} tablets that get resorbed in the intestines are considerable. (157)

3.3.1.4 Therapeutic options for iron deficiency in women

Women are an especially susceptible group when it comes to ID. Adolescent girls are particularly vulnerable to ID in comparison to their male counterparts, as they cope with menstrual blood loss during rapid growth periods with higher iron demands of tissues and expansion of the red cell mass.(158,159)

Menstruating women and adolescent girls decrease their risk for ID and critical outcomes of anemia if they receive daily iron supplements, compared to women who receive placebo. Further, hemoglobin levels of iron supplemented women are significantly higher than in the placebo group. However, as iron produces changes in bowel function, iron supplements showed increased risk for gastrointestinal adverse effects. (160)

Pregnant women must mobilize around 1.200 mg of iron during gestational period and produce 35% more erythrocytes than before. Net requirements during pregnancy including additional demands and savings due to absence of menstruation add up to around 600 mg increased demand. With increasing demand during pregnancy, the efficiency of iron absorption capacity is advancing, too. Beyond that, risk factors such as multiparity or heavy uterine bleeding add up to the prevalence of ID.

ID is the most common cause of anemia during pregnancy and is correlated to higher maternal and fetal morbidity and mortality. Oral iron is recommended as first-line therapy but is sometimes inefficient and tolerated poorly. (41,53)

General recommendations that work for most pregnant women suggest to begin iron substitution – preferably starting at 50 mg daily oral iron - at the latest when serum ferritin declines below 30 µg/L. This dose can be increased to up to 200 mg.(41)

Eating habits tend to influence the iron status of pregnant women. Thus, rare coffee consumption, regular consumption of fish and legumes as well as low milk consumption during the intervals between food intake are conditions for optimization of iron status during gestational period. (161)

For pregnant women living in settings of high prevalent anemia the *International Nutritional Anaemia Consultative Group* recommends a twice daily dose of 65 mg elemental iron supplementation. On the other hand, the WHO recommends a daily dose of 60 mg. A randomized clinical trial on the subject investigated whether the once or twice daily dose of ferrous sulphate was the adequate treatment of ID in pregnancy. In summary, they proposed that once daily a 65 mg dose was as effective as a twice daily 65 mg dose regimen in prevention of anemia in pregnancy. However, the once daily dose came along with significantly less adverse effects including nausea, vomiting and epigastric pain and guaranteed better compliance. (162)

Daily iron supplementation is strongly recommended by the WHO as a public health intervention in settings where anemia is highly prevalent (more than 40% anemia prevalence) in menstruating adult women and adolescent girls as well as in postpartum women. In postpartum women, iron should be provided either alone or in combination with folic acid 6 to 12 weeks after childbirth for reducing risk of anemia in areas of prevalent gestational anemia. However, quality of evidence for iron supplementation on postpartum women is rather low.(158,159)
IV iron substitution in pregnancy should be considered in cases when there is lack of compliance, oral iron is not tolerated or in progressive anemia during second or third trimester. Evidence supports early intervention with IV iron. The iron preparation of choice is either Fe^{3+}-sucrose (Venofer®) or iron carboxymaltose (Ferinject®), although studies suggested the latter to cross placental barrier and have an impact on fetal skeletal development. However, other studies abandoned this theory. During the first trimenon, administration of IV iron is strictly contraindicated, due to lack of safety data. (41,53)

Table 6: WHO Guidelines (2016) for iron substitution in women.(41,158,159)

Target group	Menstruating adult women and adolescent girls (non-pregnant in reproductive age)	Pregnant women	Postpartum women
Supplement	30–60 mg elemental iron	50-200 mg elemental iron oral with or without folic acid or IV	30–60 mg elemental iron with or without folic acid
Supplement form	Tablets	Tablets or IV	Tablets
Frequency	Daily	Daily or weekly	
Duration	3 consecutive months in a year		6-12 weeks following delivery

3.3.1.5 Iron substitution in heart failure

ID is an indication for iron supplementation in symptomatic HFrEF patients, regardless of the hemoglobin level. Intravenous carboxymaltose has proven to be safe and effective for iron repletion, whereas oral supplementation in patients with HF is not recommended by ESC guidelines. Although oral ferrous fumarate, gluconate or sulphate are cheap and easily administrable options, they failed to improve clinical outcomes in HF patients, implicating that ID in HF patients is a more sophisticated ID. The IRONOUT HF study investigated the effect of high-dose oral iron on exercise capacity in HFrEF patients over 16 weeks and concluded that there was no improvement. (42,163)

ID constitutes a common comorbidity in HF and anemia in HF is an independent predictor of mortality and morbidity. According to ESC guidelines for heart failure, in patients with HFrEF and ID, defined as serum ferritin <100 µg/L or ferritin 100-299 µg/L and transferrin saturation <20%, ID should be treated with intravenous ferric carboxymaltose in order to alleviate symptoms and improve quality of life. Erythropoietin stimulating agent darbepoetin alfa on the contrary is not recommended as it did not prove to be effective in treatment of HFrEF patients with anemia, but rather increased thromboembolic events.(42,78)

The FAIR-HF study was an assessment of iron repletion therapy with IV Ferinject® in patients with ID and chronic HF with or without anemia, conducted in 2009. 50% of the patients receiving ferric carboxymaltose reported improved Patient Global Assessment, higher distance in the 6-minute walk test and better quality of life. Concerning death rate and side effects there were no differences between study and control groups.(164) Following, the CONFIRM-HF was conducted in 2014 and aimed to evaluate the benefits and safety of long-term IV iron in HF patients. Treatment with ferric carboxymaltose significantly improved the 6-minute walk test, reduced markedly the risk for hospitalization for worsening HF and improved not only the NYHA class, but also Patient Global Assessment, quality of life and Fatigue Score. However, the mortality and adverse effects were similar in the IV-iron and control group, as well.(83)

Evidence from randomized controlled studies shows that patients who received intravenous iron sucrose had lower NT-pro-brain natriuretic peptide, fewer hospitalizations and better functional capacity in comparison to patients who received normal saline. Other studies observed as well significant improvements of NYHA functional class and Patient Global Assessment.(42)

3.3.2 Therapeutic options for iron overload

Human beings have not established endogenous mechanisms to remove excess iron from the body, which is why therapies for iron overload should decrease systemic iron levels or inhibit iron from entering into tissues. (2) Iron overload is driven by hemochromatosis, as well as by secondary iron overload such as thalassemia or sickle cell anemia, due to their transfusion dependence. With each blood transfusion, 200 to 250 mg of iron are transferred to the patient, gradually leading to accumulation and reaching noxious levels in organs such as liver and heart. Iron overload cardiomyopathy results from iron accumulation in the myocardium, constitutes a major comorbidity in hemochromatosis and is the most prevalent cause of death in patients with secondary iron overload. Studies suggest that iron chelation therapy can be clinically beneficial in treating various forms of heart disease. (117,165,166)

First step in the treatment of iron overload involves reduction of dietary iron intake, which can be potentiated by drinking black tea during meals. Besides this fundamental component of therapy, there are two ways to lower systemic iron: Phlebotomy or bloodletting and iron chelation therapy. Furthermore, intracellular iron can be lowered with amlodipine by blocking L-type calcium channels and antioxidants like resveratrol is able to reduce oxidative stress caused by iron overload. Therapeutic target value is decreasing serum ferritin below 50 µg/L. (2,38)

3.3.2.1 Phlebotomy

Phlebotomy is primarily applied for the treatment of hereditary hemochromatosis and is only used when erythropoiesis is not affected and hemoglobin is within the normal range. In patients with IO but simultaneous anemia, iron chelation therapy is used. (2) With every phlebotomy around 500 mL of blood containing 250 mg of iron are removed. Initially, weekly phlebotomies should be performed until the patient presents with microcytic anemia. Afterwards maintenance therapy is required until serum ferritin level of around 50 µg/L is reached. Contraindications for phlebotomy include heart failure or anemia. (38)

An alternative treatment is erythrocyte apheresis, a red blood cell exchange by extracorporeal blood separation. (2)

3.3.2.2 Iron chelation therapy

Application of chelation therapy has improved survival in iron overload patients. Nevertheless, long-term observation showed persistently high and premature cardiac mortality. (166)

Well-known iron chelators are *deferoxamine* (Desferal®, parenteral use), *deferasirox* (Exjade®, oral), as well as *deferiprone* (Ferriprox®, oral) in reserve. (38)

Indications for chelation therapy include juvenile hemochromatosis or secondary siderosis and contraindicated phlebotomy.

The benefits of chelators are often hindered by adverse effects that are mainly related to IV administration or neurotoxicity in deferoxamine, infections or by poor compliance. Deferoxamine can lead to neurotoxic adverse effects such as labyrinthine hearing loss, tinnitus and impaired vision following retina damage. Deferasirox on the other hand can possibly elevate creatinine and transaminases or lead to gastrointestinal disorders and rash. (38,166)

Iron chelation therapy is the first-line treatment for iron excess in thalassemia patients. However, its cardioprotective influence is controversial and severe adverse effects remain concerning.(121)

Deferiprone seems to be superior in therapy of myocardial siderosis, whereas, the combination therapy of deferoxamine and deferiprone given simultaneously or sequentially showed synergistic effects as they eliminate iron via different pathways. (166,167)

On the other hand, iron status affects vascular endothelial inflammation and deferoxamine is able to inhibit inflammation and atherosclerotic lesion development in experimental mice. Additionally, deferoxamine inhibits TNFα-induced expression of adhesion molecules in human aortic endothelial cells. (168)

Deferoxamine has been proven to extend survival and diminish cardiac complications. Furthermore, deferoxamine has been theorized to remove non-heme iron, thus reduce radical formation and lipid peroxidation, as well as it induces neuroprotective effects. (169) Nevertheless, those findings mostly rely on animal models.

Generally speaking, iron chelation therapy should be based on the patient´s individual needs and requirements, considering indications and contraindications. Whereas deferasirox offers flexibility as it is administered oral once a day, deferiprone and deferoxamine need to be given three times per day, subcutaneous or IV, leading to worse compliance.(166)

3.3.2.3 Treatment and prevention of iron overload cardiomyopathy

Intracellular calcium dysregulation is a main issue in pathophysiology of iron overload cardiomyopathy.(170) Iron uptake in the heart is facilitated through L-type calcium channels, providing a basis for the idea of using calcium channel blockers to reduce cardiac iron accumulation. (2)

The use of calcium channel blockers like *amlodipine* is a new concept that is in early stages of investigation for its application in reducing myocardial iron overload. Amlodipine potentially provides benefits in IO and represents an optimal calcium channel blocker to be used in patients with iron-overload cardiomyopathy. Due to its balancing act between calcium channel blockade and avoiding excessive sino-atrial or atrio-ventricular nodal blockade, it avoids arrhythmias, common complications of IO related diseases. (2)

However, amlodipine has shown promising results in animal studies but for preventing cardiomyopathy in beta-thalassemia patients evidence is insufficient and randomized trials are warranted. A review on two randomized controlled trials came to the conclusion that there was no significant reduction in myocardial iron in people with transfusion-dependent beta-thalassemia. However, tendencies for this were seen.(171)

On the contrary, a pilot study that investigated the use of amlodipine in thalassemia major patients found attenuation of cardiac IO in combination with standard chelation therapy. They suggested calcium channel blockers as complementary treatment in addition to standard chelators to improve efficacy of heart iron removal and reduce serum ferritin for patients with IO.(172)

Kumfu et al. compared therapeutic efficacy of iron chelators and dual T- and L-type calcium channel blockers on cardiac and mitochondrial function in mice. They found that either amlodipine, efonidipine as well as all commercial iron chelators (deferoxamine, deferasirox and deferiprone) reduced cardiac iron accumulation and improved mitochondrial and cardiac dysfunction in wild-type and beta-thalassemic mice. Only efonidipine but all iron chelators were able to reduce liver iron deposition, leading to the conclusion that efonidipine provides all benefits as good as commercial iron chelators. Kumfu et al. suggested efonidipine as an alternative therapy for iron overload patients unable to take chelators.(121)

Additionally, verapamil significantly attenuated cardiac iron uptake in hemojuvelin knockout mice. Besides, the same study revealed that vitamin D-depletion leads to higher liver but not cardiac iron accumulation.(173)

Although the calcium blocker verapamil effectively inhibits iron entry into cardiomyocytes, its effect on myocardial fibrosis in iron overloaded hearts is poorly understood. Studies suggest that the efficacy of calcium channel blocker on myocardial fibrosis is marginally better than iron chelators. (174)

In beta-thalassemia patients, studies showed beneficial effects of deferoxamine in the treatment and prevention of cardiac iron overload as well as in reducing the total body iron. (166)

Another study revealed that deferoxamine, deferiprone, deferasirox or N-acetyl cysteine alone improved cardiac calcium homeostasis similarly, but only the combination of N-acetyl cysteine and deferiprone led to restoring of the left ventricular contractility in IO rats.(170)

In murine IO models cardiac IO, myocardial fibrosis, altered calcium homeostasis and high oxidative stress led to heart disease. *Resveratrol* is a natural polyphenol that acts as an activator of SIRT1/FOXO1 pathway, resulting in reduced oxidative stress and higher stress resistance via altered redox state. Resveratrol appeared to be beneficial by reducing remodeling and improving heart function, as well as by restoring myocardial SERCA2a (myocardial sarcoplasmic reticulum calcium ATPase) levels and correcting calcium homeostasis in iron overload hearts.(117)

In a further study, resveratrol therapy led to complete normalization of iron overload related diastolic and systolic dysfunction in hemojuvelin knockout mice. Likewise, SERCA2a levels were restored and iron-mediated oxidative stress and myocardial fibrosis were diminished by resveratrol.(175)

Interestingly, *Salvia miltiorrhiza (Danshen)*, a traditional Chinese medicinal herb that has been used for hundreds of years to treat cardiovascular diseases, has been proven to exert activities to impair iron overload induced myocardial fibrosis. *Salvia miltiorrhiza* not only inhibits iron deposition but also collagen metabolism and oxidative stress.(174)

4 Discussion

Iron homeostasis is of the utmost importance for physiological processes including oxygen transport, DNA synthesis and ATP production. Both iron deficiency and iron overload have been associated with higher cardiovascular mortality and morbidity and therefore need to be treated carefully and efficiently. (73)

Iron deficiency is the most common nutrient deficiency. Even in industrialized countries around 20% of the population are estimated to suffer from ID without anemia. (53)

Studies provide evidence that ID, particularly in chronic heart failure, is underdiagnosed and undertreated in clinical practice. The RAID-HF follow-up study revealed that only 9% of patients with HF-REF received proper iron supplementation therapy, mostly because of existing anemia and only 4.7% received intravenous therapy.(81) ID is of high clinical relevance for prognosis of HF, irrespectively of the presence of anemia and it is crucial to identify patients at higher mortality risk for iron supplementation. Many studies demonstrate the gap between guidelines and newest research and practical approach in current clinical practice in patients with HF.

In pregnant women, ID is suggested to be the most significant fetal stressor, considering the high global prevalence. ID can lead to numerous adverse birth outcomes, including consequences that range from maternal anemia to reduced iron stores in the newborn, weakness, fatigue, reduced immune response, impaired brain development and increased risk of complications during delivery. Maternal perinatal iron restriction has even been identified as a fetal stressor that is able to program metabolic and cardiovascular dysregulation.(37,59)

For pregnant women iron supplementation can be vital, even though, numerous potential mechanisms behind adverse birth outcomes related to excess iron intake or high iron status can be thought of, including oxidative stress, impaired systemic inflammation response, infection, alteration in the maternal microbiome or increased blood viscosity. (54)

Nonetheless, the existing data on the influence of maternal iron status during gestational period on children´s iron status is rather conflicting. Inverse relations of maternal iron status and adverse birth outcomes seem to exist. However, routine iron administration in a mixed population of both deficient and replete women may lead to adverse effects on fetal growth in iron replete women, considering high iron being associated with lower birth weight on the right side of the U-shaped curve. (56–59,61)

Evidence emphasizes the importance of monitoring the iron status, even beyond the level of anemia, especially in pregnant women. However, limitations of using animal models and directly transferring knowledge to humans must be considered.

Human beings have not established endogenous mechanisms to remove excess iron and if full physiological iron capacity is utilized, it can gradually cause accumulation and reach toxic levels in organs such as liver, heart and endocrine organs. (2) Iron overload cardiomyopathy due to siderosis is the most common reason for death among patients with secondary iron overload or with early onset of genetic hemochromatosis.(170) Beyond that, numerous studies investigated the impact of iron on atherogenesis through oxidation of lipoproteins by ROS, forming oxidized LDL and supporting endothelial dysfunction through inflammatory transcription factors. While some studies show an association between high serum ferritin levels and carotid atherosclerosis (23), others found no associations between iron status and cardiovascular disease. (24) However, the iron chelator deferoxamine has been proven to inhibit inflammation and progression of atherosclerosis in mice experiments, providing a proof-of-concept that iron is a key player in atherogenesis. Potential benefits of chelation in iron overload or restricted iron intake in humans as complementary therapy target in atherosclerosis requires further investigation.(168)

Previous work on cardiac iron accumulation leading to cardiac dysfunction by disbalanced calcium metabolism suggests the use of calcium channel blockers as a novel target in the therapy of iron-overload cardiomyopathy.(121)

In conclusion, the field of diseases associated with iron deficiency and iron overload is highly complex and often poorly understood. Many questions remain unanswered and further studies are urgently warranted because of the high prevalence of these conditions and their high impact on affected individual's health.

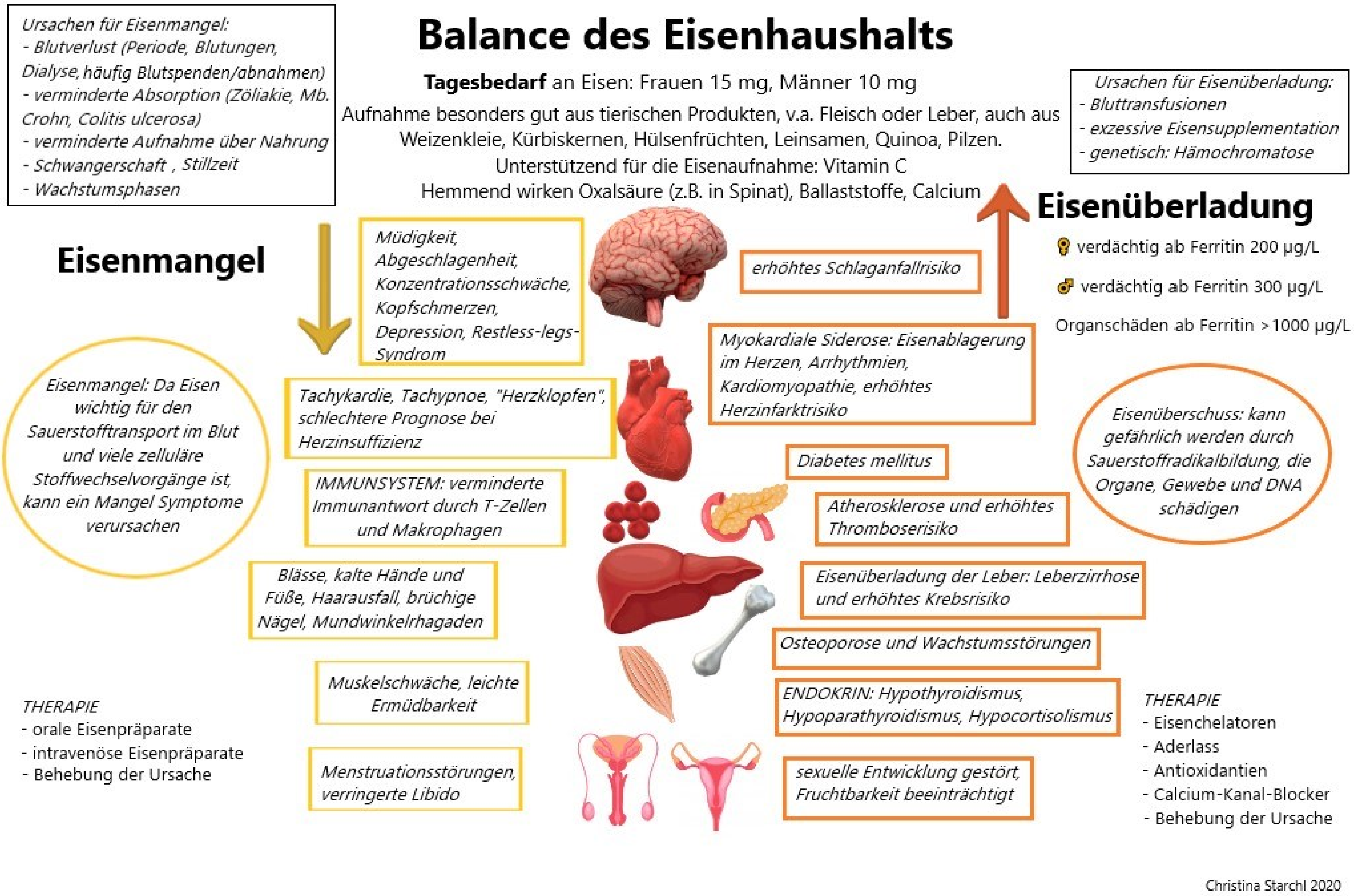

Figure 11: Iron homeostasis.

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