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Role of iron metabolism in heart failure: From iron deficiency to iron overload☆

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ABSTRACT

Iron metabolism is a balancing act, and biological systems have evolved exquisite regulatory mechanisms to maintain iron homeostasis. Iron metabolism disorders are widespread health problems on a global scale and range from iron deficiency to iron-overload. Both types of iron disorders are linked to heart failure. Iron play a fundamental role in mitochondrial function and various enzyme functions and iron deficiency has a particular negative impact on mitochondria function. Given the high-energy demand of the heart, iron deficiency has a particularly negative impact on heart function and exacerbates heart failure. Iron-overload can result from excessive gut absorption of iron or frequent use of blood transfusions and is typically seen in patients with congenital anemias, sickle cell anemia and beta-thalassemia major, or in patients with primary hemochromatosis. This review provides an overview of normal iron metabolism, mechanisms underlying development of iron disorders in relation to heart failure, including iron-overload cardiomyopathy, and clinical perspective on the treatment options for iron metabolism disorders.

1. Introduction

In the aging society, heart failure (HF) is a problem of epidemic proportions, which projected to rise in the next decades imposing tremendous societal and economic costs [1]. Major physiological functions of iron include oxygen transport as a component of hemoglobin in blood (and myoglobin in striated muscle), energy production through oxidative phosphorylation as an integral component of iron-sulfur cluster-containing enzymes such as cytochromes, NADPH, and succinate dehydrogenases, and as a component of peroxide- and nitric oxide-generating enzymes. Both iron deficiency and iron overload (primary hemochromatosis and secondary iron-overload) have high prevalence.

In the developed world, prevalence of iron deficiency in population vary from 10 to 16% depending on the sex, age, and race with pregnant women having the highest risk [2]. Prevalence of iron deficiency anemia vary from 2.6 to 5% [2]. Socio-economic status considerably influences prevalence of iron deficiency; for example, although iron deficiency prevalence in Northern and Western Europe was 4–18%, the prevalence was considerably higher in Eastern Europe (9–50%) [3]. Alleles causing primary hemochromatosis have considerably high frequency (about 10% in western Europeans) with approximately 1% population being affected [4–6]. Secondary iron overload associated with treatment of hemolytic anemias mainly thalassemias and sickle cell disease [7]. Thalassemias is caused by reduced synthesis of globin chains, and worldwide about 5% population carry globin variants with considerably higher allele frequency in Mediterranean, Middle East, North India, and South West Asia with some population reaching 100% for α-thalassemia variants [8–10]. Prevalence of sickle cell disease is high in sub-Saharan Africa, India, Middle East, and Mediterranean with about 300,000 babies born worldwide with sickle cell disease [11,12]. Both iron deficiency and iron-overload can lead to cardiomyopathy and ultimately heart failure (HF) [13,14]. Iron deficiency emerges as a major comorbidity in a large fraction of HF patients [15,16]. However, the mechanism linking HF and iron deficiency remains poorly understood. In this review, we highlight the key role of iron metabolism in cardiac function and illustrate the central importance of maintaining normal myocardial iron levels.

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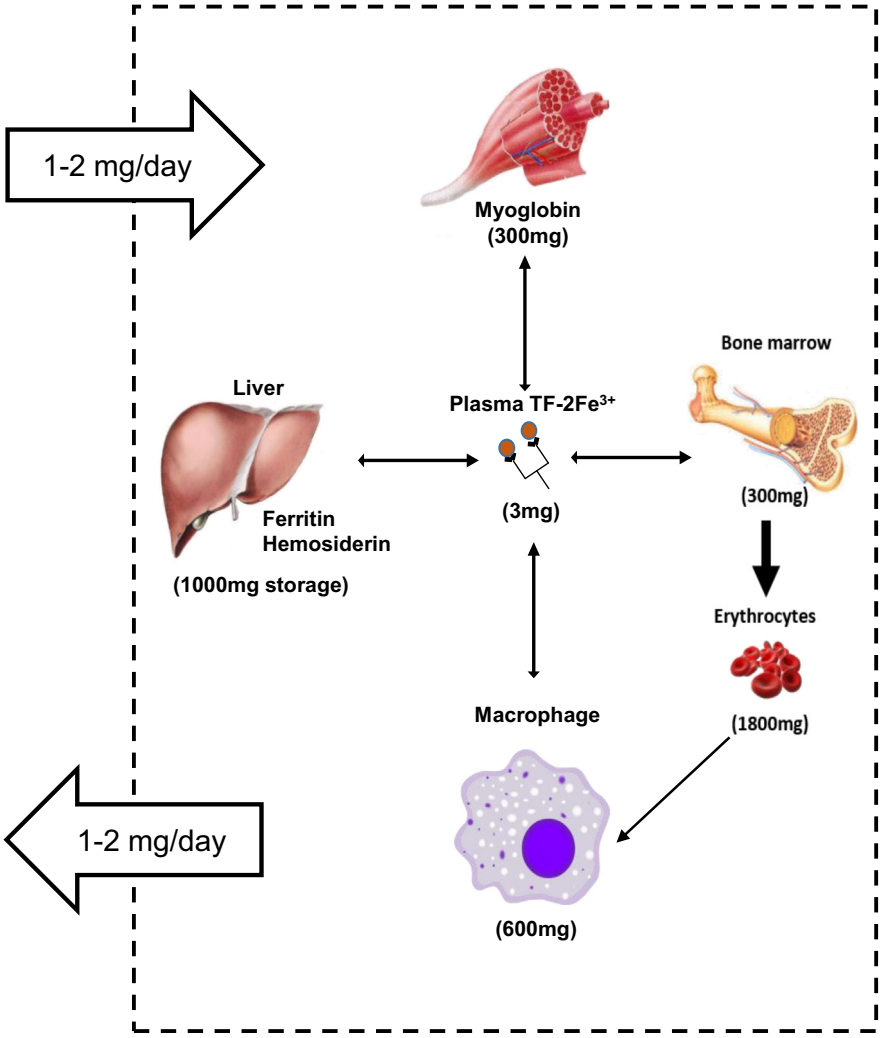

Fig. 1. Systemic iron distribution. The absorption and losses of iron are normally balanced with an average amount being 1–2 mg daily. Iron is distributed through the body by the plasma, which contains only small amount of iron (3 mg) in the form holotransferrin (TF-2Fe^{3+}). Majority of iron is contained in the erythrocytes (1800 mg) as a part of hemoglobin, hemopoietic cells in the bone marrow (300 mg), and macrophages (600 mg), which are recycling iron from aging erythrocytes. Liver acts as a major storage site for iron and contains about 1000 mg. Skeletal muscles contain about 300 mg of iron in the form of myoglobin.

2. Iron Metabolism

2.1. Distribution of iron through the body

Normally, male adults have 35 to 45 mg of iron per kilogram of body weight [4,17] while premenopausal women have somewhat lower amount due to recurrent menstruation. Out of total amount of iron in the body (about 3500 mg) erythrocytes (1800 mg) and liver (1000 mg) contain the highest amount of iron (Fig. 1A). Normally, in the absence of blood loss, only small amounts of iron (1–2 mg/day) are lost due to desquamation of epithelial cells, and these losses are replenished by the uptake of dietary iron. The iron present in the body is constantly recycled with the erythrocytes and macrophages being the major part of the recycling process. As erythrocytes age, they undergo phagocytosis by macrophages, and the iron contained in the hemoglobin of these erythrocytes is released back to the plasma to be reuptaken by bone marrow for the synthesis of new erythrocytes. Other organs uptake iron from plasma to synthesize iron-containing molecules (e.g., myoglobin) and extrude excesses of iron back to the plasma. Plasma contains only small amounts of iron (3 mg) bound to transferrin with liver acting as a main storage site of the excess of iron containing about 1000 mg (Fig. 1A). These depots ensure seamless iron recycling and erythropoiesis because under normal circumstances, < 10% of daily iron needs are supplied from diet. Lack of specialized extrusion pathway for iron makes regulation of the iron uptake to be especially important to ensure optimal iron metabolism.

2.2. Iron absorption and its regulation

Iron is available to biological system in two distinct forms: organic form (heme iron) and inorganic form (ionic iron). Though the amount extracted from diet is tiny (1–2 mg/day), dietary ferric iron (Fe^{3+}) is the major source of total iron intake [4]. Epithelial cells of small intestine that are responsible for dietary iron uptake are capable to absorb both inorganic and heme forms of iron via two distinct mechanism (Fig. 2A). Inorganic form of iron is absorbed at the apical membrane of duodenal epithelial cells after duodenal cytochrome *b* (DTYCB) reduces Fe^{3+} to ferrous form (Fe^{2+}), which is transported by divalent transporter 1 (DMT1) across the plasma membrane. After Fe^{2+} enters cytoplasmic pool, it can leave the epithelial cell at the basolateral membrane via iron transporter (ferroportin, FPN) [17]. Once Fe^{2+} leaves the cell via ferroportin (FPN), it is oxidized by hephaestin to Fe^{3+}, which is bound by transferrin (TF), and distributed through the body as transferrin-iron complex (holo-transferrin or Holo-TF; Fig. 2A). The organic form of iron (heme) is absorbed via heme/folate transporter 1 (HCP1) at the apical membrane [18–20]. Once absorbed, heme can leave the

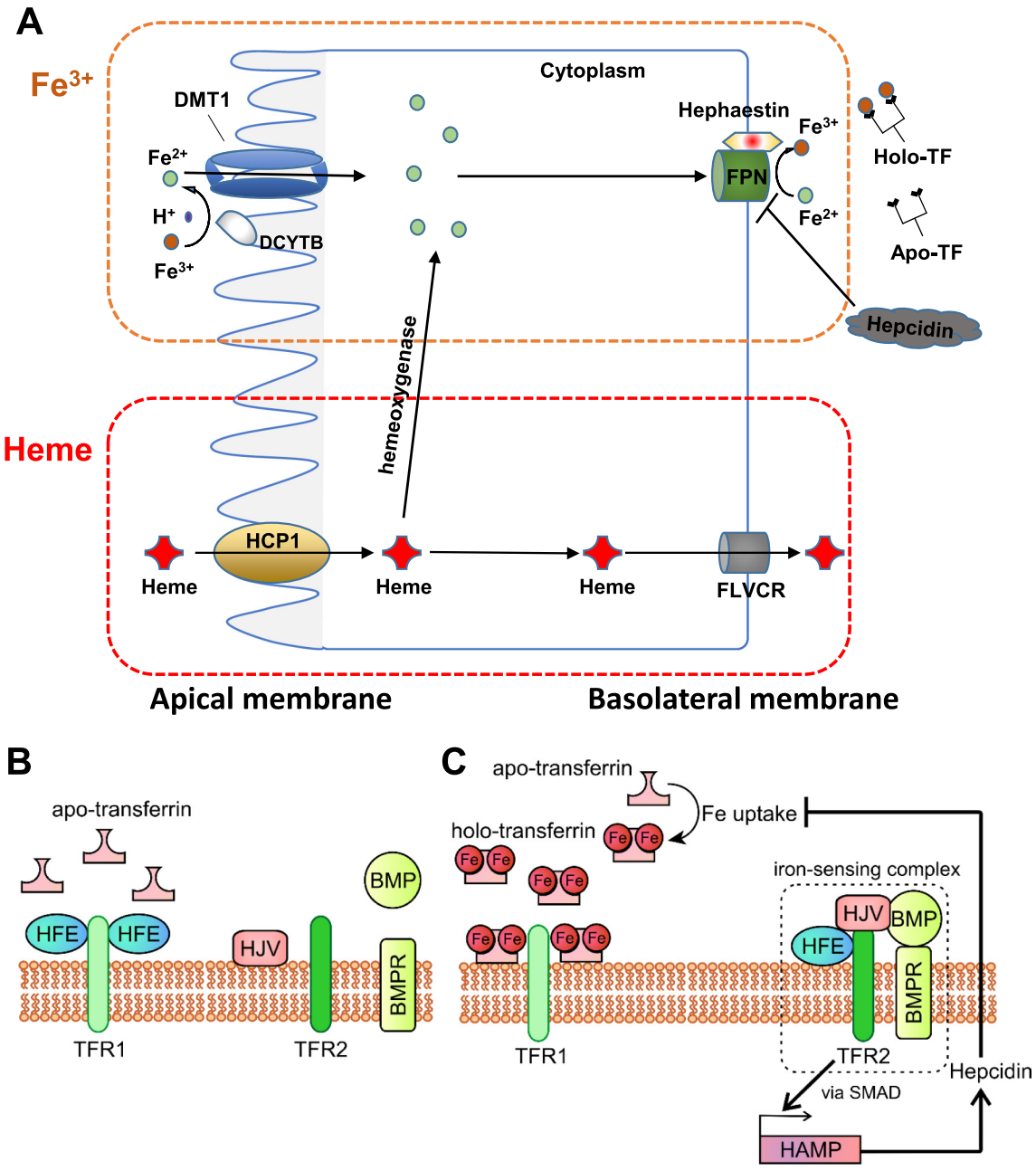

Fig. 2. Iron absorption and systemic regulation of iron absorption. A. Iron absorption in the enterocyte. Iron is uptaken via enterocyte in inorganic (Fe^{3+}) and organic (heme) form. *Absorption of inorganic form of iron.* At apical membrane, Ferric iron (Fe^{3+}) is reduced by duodenal cytochrome *b* (DCYTB) to ferrous form (Fe^{2+}), which is transported by divalent metal transporter 1 (DMT1) in to the cytoplasm. At basolateral membrane, Fe^{2+} is exported by ferroportin (FPN) and then immediately oxidized by closely linked enzyme hephaestin to Fe^{3+}, which is bound by apotransferrin (Apo-TF) to form holotransferrin (Holo-TF). *Absorption of organic form of iron.* Organic form of iron (heme) is absorbed by heme/folate transporter (HCP1). Once in the cytoplasm, heme can be oxidized by hemeoxygenase releasing Fe^{2+}, or be transported out in to the plasma by heme transporter (FLVCR) in the basolateral membrane. B. Sensing of systemic iron levels and systemic regulation of iron absorption. Systemic iron is assessed by hepatocytes via sensing transferrin saturation levels. If circulation iron is low, plasma contains mainly apo-transferrin (no iron), which cannot displace human hemochromatosis protein (HFE) from its complex with transferrin receptor 1 (TFR1), so in the absence of HFE iron sensing complex cannot be formed and hepcidin production cannot be initiated. If circulating iron is high, plasma contains holo-transferrin (iron containing form), which binds to TFR1 displacing HFE. Released HFE forms iron sensing complex with transferrin receptor 2 (TFR2), hemojuvelin (HJV), bone morphogenetic protein (BMP), and bone morphogenetic protein receptor (BMPR). Iron-sensing complex activates expression of hepcidin gene (*Hamp*) via SMAD pathway leading to hepcidin production and release. Circulating hepcidin suppresses iron uptake inhibiting ferroportin in the enterocytes (see panel **A**).

cell via heme transporter (feline leukemia virus type C receptor, FLVCR) in the basal membrane or be oxidized by heme oxygenase releasing Fe^{2+} to cytoplasm [20,21] (Fig. 2A). Only inorganic iron uptake is regulated by hepatic hormone hepcidin-25, which binds to ferroportin inhibiting iron transfer across basolateral membrane and promoting ferroportin degradation due to internalization [22]. Hepcidin is produced in the liver in response to increase in saturation of transferrin (higher concentration of holo-transferrin, Holo-TF; Fig. 2B). When the iron content of the plasma is low, apo-transferrin (transferrin without iron) is unable to disassociate human hemochromatosis protein (HFE) from transferrin receptor 1 (TFR1). Without HFE protein iron-sensing complex cannot be formed, so no hepcidin is produced (Fig. 2B). In the absence of hepcidin, enterocytes are absorbing dietary iron. As iron absorbed, it binds to transferrin turning transferrin in to holo-

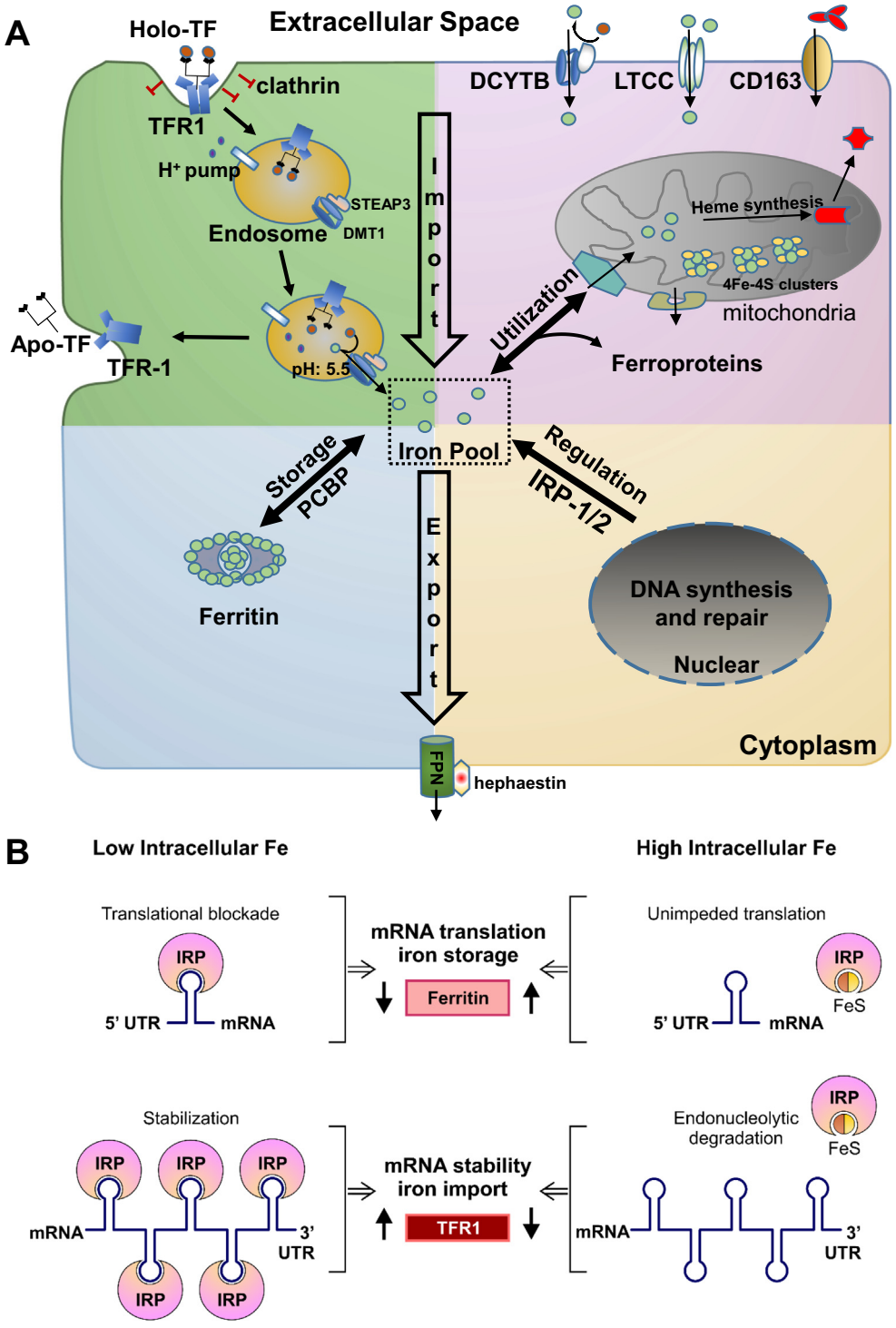

Fig. 3. Cellular iron metabolism and its post-translational regulation. A. Cellular iron metabolism. Iron is absorbed via clathrin-mediated endocytosis of holo-transferrin (holo-TF) bound to transferrin receptor 1 (TFR1). After formation of endosome, H^+ pump lowers pH to facilitate reduction of Fe^{3+} to Fe^{2+}, which can leave the endosome for cytoplasm via divalent metal transporter 1 (DMT1). Once in the cytoplasm Fe^{2+} is utilized for ferroprotein synthesis, uptaken to mitochondria to form iron-sulfur clusters (4Fe−4S) of oxidative-phosphorylation proteins, stored in iron-ferritin complexes with poly-(rC)-binding proteins (PCBP) acting as chaperons, or exported out of the cell via ferroportin. B. IRP/IRE post-translational regulation of cellular iron metabolism. Iron response elements are "hairpin" structure on the untranslated regions (UTR) of mRNA that can bind iron response proteins (IRPs). In the low intracellular iron state, IRP binding to IRE promotes translational blockade of storage proteins (e.g., ferritin) and stabilization of mRNA of iron import proteins (e.g., TFR1) mobilizing iron from storage and facilitating import of iron, respectively. The opposite process happens in the high intracellular iron state. IRPs are bound to iron-sulfur clusters (4Fe−4S) preventing them from binding to IREs. In the absence of IRP binding to IRE, translation of storage proteins (e.g., ferritin) is disinhibited, whereas mRNA of import proteins (e.g., TFR1) is no longer stabilized and undergoes endonucleolytic degradation resulting in facilitation of storage and inhibition of iron import, respectively.

transferrin, which can bind to TFR1 and displace HFE protein. Once HFE protein is released, it will promote a formation of iron-sensing complex consisting of transferrin receptor type 2 (TFR2), HFE protein, hemojuvelin (HJV), bone morphogenic protein (BMP), and bone morphogenic protein receptor (BMPR). Formation of iron-sensing complex activates SMAD signaling pathway triggering expression of hepcidin gene (*Hamp*), which leads to production of hepcidin to reduce iron uptake via enterocytes (Fig. 2B) [23–26]. Disruptions in this intricate machinery lead to dysregulation of iron uptake resulting in iron overload or iron deficiency.

2.3. Cellular iron metabolism

Intracellular iron content is maintained as a balance of absorption, loss, storage, and mobilization of iron. Absorption of iron happens via several distinct mechanisms such as transferrin-mediated endocytosis, divalent metal transporter 1 (DMT1), voltage-gated Ca^{2+} channels (mainly L-type Ca^{2+} channels, LTCC), and heme/folate transporter (HCP1) (Fig. 3A). Transferrin-mediated endocytosis and DMT1 are ubiquitous mechanisms of iron absorption, [27–29] whereas entry of Fe^{2+} via voltage-gated Ca^{2+} channels is limited to excitable cells like myocytes and neurons mainly under iron overload conditions [5]. CD163 is mainly expressed in monocytes/macrophages, microglia, and

some neurons [30,31] making it important for iron recycling [32–34] and neuronal damage due to hemorrhage and iron overload [30,31]. Transferrin-mediated endocytosis occurs when holo-transferrin binds to transferrin receptor 1 (TFR1). This binding triggers formation of clathrin-coated endosome containing TFR1-holo-transferrin complexes, H^+ pumps, metalloreductases STEAP3, and DMT1s. H^+ pumps decrease endosomal pH to 5.5 facilitating dissociation of Fe^{3+} from transferrin and conversion of Fe^{3+} to Fe^{2+} by metalloreductase STEAP3. Fe^{2+} leaves endosome for cytoplasm via DMT1. Following this, endosome re-integrates with plasma membrane completing transferrin cycle (Fig. 3A) [27–29]. Inside the cells, Fe^{2+} is used for synthesis of metalloproteins in the cytosol and uptaken to mitochondria via mitoferrin for utilization mainly in iron-sulfur clusters of oxidative-phosphorylation proteins, heme synthesis, and DNA repair enzymes [35,36] (Fig. 3A). Excess of iron is stored intracellularly by complexing iron with ferritin with the help of poly-(rC)-binding proteins acting as chaperons (PCBP) [37] or removed from the cell via iron transporter (ferroportin, FPN; Fig. 3A) [17].

2.4. Post-translational regulation of cellular iron metabolism

Post-translational regulation of the expression of the proteins involved in the iron metabolism is achieved via iron response elements (IREs) and iron regulatory proteins (IRPs). IREs are highly conserved hairpin structures of mRNAs found in 5′ and 3′ untranslated regions [38–40] (Fig. 3B). H- and L-ferritin mRNA contains single IRE in the 5′ untranslated region, [38,39] whereas TFR1 mRNA has five IREs in the 3′ untranslated region [39,40]. IREs act as binding sites for IRPs that either prevent translation (IRP binding to IRE in 5′ untranslated region) or stabilize mRNA preventing endonucleolytic degradation (IRP binding to IRE in 3′ untranslated region) (Fig. 3B) [41,42]. Capability of IRPs to bind to IRE depends upon presence of iron (Fe^{2+}) in the cell. In the low intracellular Fe^{2+} environment IRPs do not have iron-sulfur clusters (4Fe−4S) bound to them, so IRPs bind to IRE leading to suppression of translation of mRNAs of ferritins (storage proteins) and stabilization of mRNA of TFR1 (iron-uptake protein). The resultant action is decrease in storage proteins facilitating release of iron from storage and increase in cellular iron uptake (Fig. 3B). As concentration of Fe^{2+} rises in the cell, more iron is incorporated into the iron-sulfur clusters (4Fe−4S), which bind to IRPs preventing IRP-IRE interaction [43–45]. In the absence of IRP's inhibitory action on storage proteins and stabilizing action of iron-uptake proteins, translation of storage proteins (ferritins) increases, and translation of iron-uptake proteins decreases (TFR1) resulting in more available iron storage and less iron uptake (Fig. 3B). A number of other proteins were identified that are subject of IRP/IRE regulation, e.g., DMT1, ferroportin, 5-aminolevulinic acid synthase 2 (ALAS2; heme biosynthesis), hypoxia inducible factor 2 alpha (HIF2α), and some others (for review see [42]).

3. Iron deficiency

3.1. Iron deficiency: definition, impacts and relation with anemia

Iron depletion frequently results from inadequate dietary intake or chronic blood loss. However, iron deficiency, which is the focus of this review, is inability to utilize the circulating iron or failure to meet excessive iron demands. The accepted criterion of systemic iron deficiency is serum ferritin < 100 μg/L, and functional iron deficiency defined as serum ferritin 100–300 μg/L with transferrin saturation (*TSAT*) < 20% [46,47]. Use of ferritin by itself as diagnostic criterion of iron deficiency is unreliable because inflammation and oxidative stress may increase ferritin levels independent of iron status [46,47]. Similarly, use of transferrin saturation by itself to define iron deficiency lacks sensitivity, because catabolism and malnutrition may lower serum transferrin levels artificially inflating transferrin saturation despite actual low iron levels [46,47]. Another approach in defining iron deficiency is to use combination of low serum hepcidin (< 14.5 ng/mL, the 5th percentile in healthy peers) as a reflection of depletion of iron stores and high serum soluble transferrin receptor (> 1.59 mg/L, 95th percentile in healthy peers) as a reflection of unmet cellular requirements [47]. Interestingly, systemic and myocardial iron markers are poorly correlated indicating different regulatory mechanisms [48]. The relationship between systemic iron deficiency and myocardial iron deficiency remains unclear. However, clinically, iron deficiency is present in up to 66% of HF patients and leads to reduced exercise tolerance, progressive HF, and increased mortality [13,49–51].

Anemia that is intuitively seems to be related to iron deficiency is diagnosed primarily based on hemoglobin concentration (< 130 g/L in men and < 120 g/L in non-pregnant women) in clinical practices [52]. Anemic conditions lead to changes in corpuscular cell volume and hemoglobin content. By volume of red blood cells, anemia could be microcytic (< 82–98 fL), normocutic (82–98 fL), and macrocytic (> 82–98 fL). By hemoglobin content per erythrocyte, hypochromic (< 27–31 pg), normochromic (27–31 pg), and hyperchromic (> 27–31 pg) [53]. However, counter-intuitively, iron deficiency and anemia coexist infrequently (only in 17% of patients) [15]. Interestingly, anemia itself did not affect mitochondrial functions; nor the correction of anemia (e.g., using erythropoietin-stimulating agents) was associated with significant improvement of HF symptoms, though the risk of thromboembolic events was increased [48,54–57].

3.2. Role of IRP1 and IRP2 in the development of iron-deficient HF

Iron deficiency defined as depleted iron stores and unmet cellular iron demand was present in 37% of patients with chronic HF and up to 66% of patients with acute HF [13,49–51]. HF is associated with low IRP activity and reduced tissue iron that is potentially detrimental since iron is critical element for energy production [45]. Cardiac-specific deletion of both IRP1 and IRP2 in mice led to non-anemic and normal mice at baseline conditions; however, cardiac function in these mice was vulnerable to dobutamine challenge due to failure to increase mitochondrial respiration in response to higher workload. These mice also developed more severe LV dysfunction in response to myocardial infarction. Iron supplementation was able to restore both mitochondrial and contractile function in these mice (Fig. 4A) [45]. In another mouse model of anemia, cardiac-specific knockout of *Tfr1* had more severe phenotype: mice were dying in the second week due to cardiomegalia, poor cardiac function, failure of mitochondrial respiration, and ineffective mitophagy. Similarly to IRP1/2 knockout model, *Tfr1* knockouts were rescued by aggressive iron supplementation (Fig. 4A) [58]. Currently, several ongoing clinical trials explore iron supplementation to restore intracellular cardiac iron levels (Fig. 4B) as an option for treatment of HF in the patients with iron deficiency [59–62]. For instance, the double-blinded IV injections of either ferric carboxymaltose or saline to 459 HF patients with iron deficiency for 6 month showed notable improvement in the experimental arm on primary endpoints (patient global assessment and NYHA class) and secondary endpoints (6-min walking distance and quality-of-life assessments), but not on hard endpoints (rate of re-hospitalization and mortality), with no statistical difference between anemic and non-anemic patients [63]. Iron dextran, iron sucrose, and iron gluconate are other popular IV iron supplements, with similar compound structure: iron (core) and carbohydrate (coat). Though the data on their efficacy and safety profiles are currently incomplete, anaphylaxis, which is triggered by the carbohydrate coat, remains as the major concern [64–66]. More comparative clinical data on the long-term safety of IV iron therapy are needed. Another possible mechanism for development of iron deficiency associated with HF in humans proposes that excessive levels of catecholamines and aldosterone down-regulate expression of *Tfr1* and *Tfr2* resulting in reduced iron uptake by cardiomyocytes [67]. In this regard, use of β-blockers and mineralocorticoid receptor antagonists (MRA) not only a good conventional HF treatment, [68,69] but may also benefit

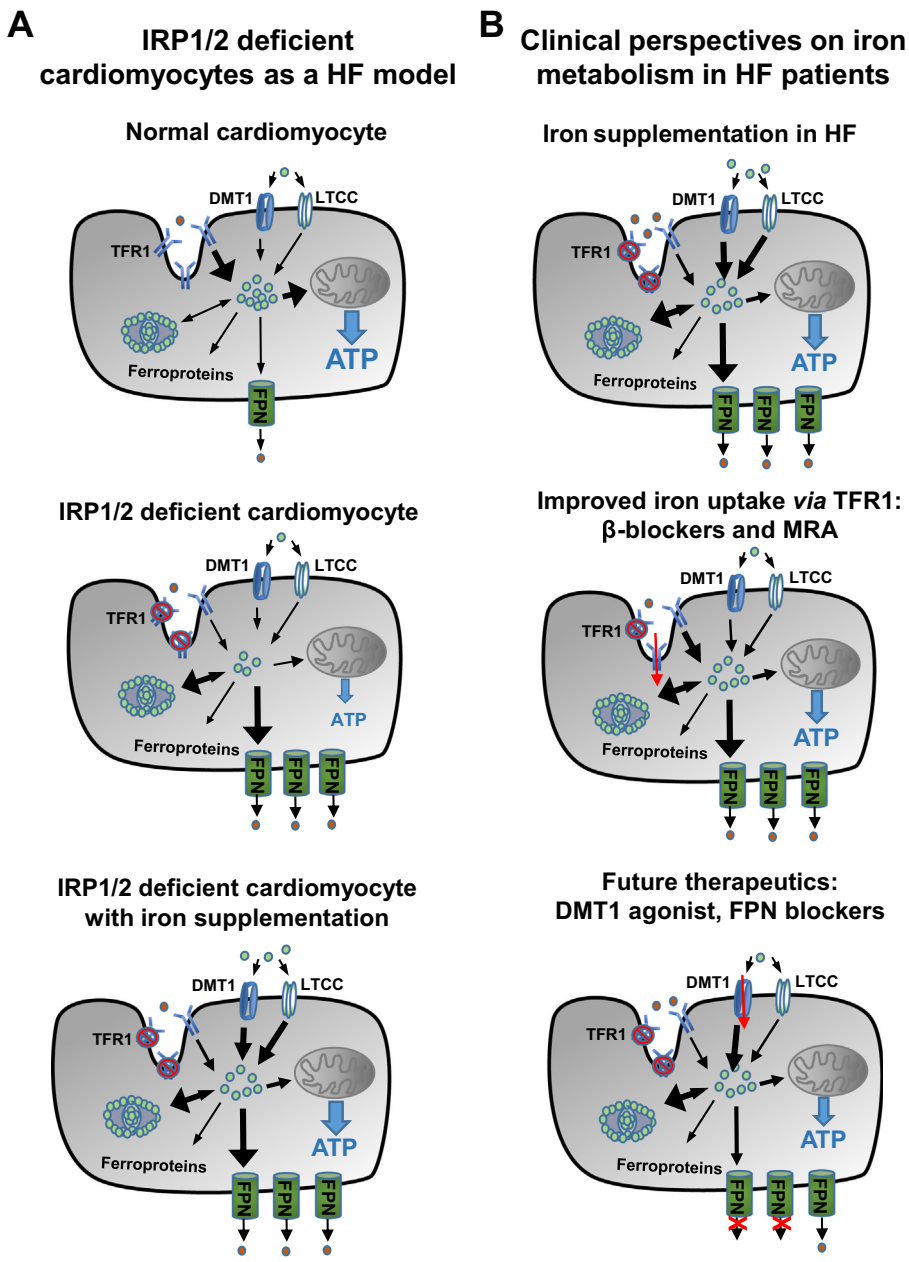

Fig. 4. Iron deficiency in animal model and patients. A. IRP1/2 deficient cardiomyocytes as a HF model. Cardiomyocyte-specific deletion of IRP1 an IRP2 leads to reduction in iron uptake due to TFR1 deficiency and increase of iron efflux via overexpressed ferroportin (FPN) resulting in low intracellular iron, which compromises energy (ATP) production. The model can be rescued by iron supplementation, which raises intracellular iron and normalizes ATP production. B. Clinical perspective on iron metabolism in HF patients. Direct iron supplementation has been proposed to normalize intracellular iron concentration and ATP production. Improved iron uptake via TFR1 due to β-blockade and mineralocorticoid receptor antagonist (MRA). Future therapeutics to consider are DMT1 agonist and ferroportin (FPN) blockers to facilitated iron uptake and block iron efflux, respectively.

cardiac iron metabolism by improving iron uptake via TFR1 (Fig. 4B) due to normalization of *Tfr1* and *Tfr2* expression [35]. Future approaches to treatment of iron deficiency in HF may consider upregulation of alternative import routs for iron entry (e.g., DMT1 agonists) or inhibition of iron export via ferroportin (FPN) by blockers or suppression of ferroportin expression (Fig. 4B) [35].

3.3. Role of hepcidin in the development of iron-deficient HF

The heart has the second highest expression levels of hepcidin [70]. Systemic hepcidin produced by the liver is known to inhibit iron transfer by ferroportin and to promote internalization of ferroportin [22]. Recent work by Robbins group proposed a similar role for cardiac hepcidin in regulation of iron efflux from cardiomyocytes [71]. Cardiomyocyte-specific ablation of hepcidin in mice led to a large reduction in whole-heart levels of hepcidin and lack of hepcidin immunofluorescent staining in the cardiomyocytes. Mice lacking hepcidin production in the heart exhibited shortened lifespan (~30% survival to the 1-year age), systolic dysfunction (ejection fraction about 45%), and cardiac hypertrophy. Strangely, cardiac tissue iron levels were not changed, but direct measurement of $^{55}Fe^{2+}$-efflux confirmed an increased iron loss. Cardiac-specific hepcidin knockouts also had reduced complex I and complex IV levels suggesting mitochondria-dependent metabolic dysfunction. Similarly to IRP1/2 knockout model, supplementation of iron to cardiac-specific hepcidin knockouts rescued mitochondrial function [71]. However, lack of change in iron levels in the cardiac tissue still leave a possibility that function of cardiac hepcidin is not limited to control of iron levels. Hepcidin has been shown to have anti-apoptotic, anti-hypertrophic and anti-fibrotic effects in HF models [72–74].

3.4. Translational insights in management of iron-deficient HF patients

Currently, the therapeutic emphasis is gradually shifting from alleviating HF symptoms to managing the co-morbidities exacerbating HF, including iron deficiency, as reflected in the newest European Society of Cardiology (ESC) Guidelines for multidisciplinary management of HF [69]. In HF patients, iron deficiency is highly prevalent and is an

independent predictor of clinical outcomes and exercise intolerance, even in the absence of anemia, necessitating the use of oral or parenteral iron supplementation [13,49–51].

Oral iron supplements, such as ferrous fumarate, ferrous gluconate, and ferrous sulphate, are easily administrable and cheap option, but may lead to drug intolerance (mainly gastrointestinal discomfort), impaired diversity of microbiota, and counteractive action of hepcidin may prevent effective absorption of additional iron [75–78]. Moreover, efficacy of dietary iron absorption can be impeded by many other factors, such as food (e.g., polyphenols and phytates in tea and coffee), [79] medications (e.g., Ca^{2+} supplements and proton pump inhibitors), [80,81] and co-morbidities like intestinal edema and inflammation [82,83]. However, oral iron supplementation failed to improve clinical outcomes for HF patients in a randomized clinical trial [51] suggesting that iron deficiency in HF is more complex problem than a mere lack of dietary iron, which can be prevented by iron supplements (e.g., use of "sprinkles" and iron-fish ingots in South West Asia) [84,85].

Parenteral iron supplementation is achieved by intramuscular or intravenous injections. Intramuscular injections, although easier to perform, are painful and are associated with siderosis (iron deposition in tissues) and a higher risk of intramuscular neoplasm [86,87]. Intravenous injections rapidly correct iron levels bypassing gastrointestinal absorption and all problems associated with this route of administration. Each administration can be accurately tailored to the current body weight and hemoglobin levels using Ganzoni's equation (dose = body weight (kg) $*$ [15 − actual hemoglobin (g/dL)] $*$ 2.4 + 500 mg) [88]. Clinically, intravenous supplementation of HF patients with ferric carboxymaltose improved heart functions and other HF symptoms in patients with iron deficiency [63,67]. This approach is associated with less gastrointestinal distress and drug intolerance than oral regimens and is more suitable for those with functional iron deficiency [63,67]. The elucidation of signaling pathways of iron regulation in HF continues to be a priority for developing heart-oriented delivery of iron to optimize myocardial iron contents, which remains a bottleneck in practical application. Besides that, more research is required to improve monitoring of cardiac iron status in HF.

4. Iron overload

4.1. Iron overload: definitions

Iron-overload is an accumulation of iron in the organism in the excessive amounts. The excessive accumulation can be divide in two main types: (1) primary hemochromatosis (genetic mutations in genes responsible for iron absorption and regulation of absorption) and (2) secondary iron overload (therapeutic interventions like repetitive blood transfusion to treat hemolytic anemias and excessive iron supplementation to stimulate erythropoiesis in dialysis patients).

4.2. Primary hemochromatosis

Primary hemochromatosis is caused by mutations in five genes coding various iron-transporting and iron-sensing proteins. Loss-of-function mutations in the genes coding for the proteins of iron-sensing complex, such as human hemochromatosis protein (*Hfe*), hemojuvelin (*Hjv*) (formerly known as *Hfe2*), and transferrin receptor 2 (*Tfr2*) lead to development of hemochromatosis types 1, 2A, and 3, respectively (Fig. 5A). Disruption of iron-sensing complex disables production of hepcidin in response to rising transferrin saturation in the blood. In the absence of suppressive action of hepcidin, iron continues to accumulate eventually rising above binding capacities of transferrin and ferritin resulting in appearance of non-transferrin bound iron (NTBI) or free iron (Fig. 5A). Increase in transferrin saturation above 45% leads to appearance of NTBI, and transferrin saturation above 80% allow formation of special type of NTBI in the plasma, labile plasma iron, which can produce reactive oxygen species (ROS) [89–91]. Hepcidin deficiency may also arise due to loss-of-function mutations in the gene coding for hepcidin (*Hamp*) disrupting production of hepcidin while mutations in the hepcidin lead to hemochromatosis type 2B. Similarly to disruption in iron-sensing complex, loss of hepcidin production also leads to the excess of iron due to unchecked uptake of iron (Fig. 5A) and development of hemochromatosis. Hemochromatosis types 1, 2A, 2B, and 3 are all similar in a sense that they lead to hepcidin deficiency [91]. On the other hand, gain-of-function mutations of the gene coding for ferroportin (*Slc40a1*), which is classified as hemochromatosis type 4 (ferroportin-associated hemochromatosis or type 4B), is in essence hepcidin resistance [91] because ferroportin loses the ability to interact with hepcidin [92]. Lack of hepcidin-mediated down regulation of ferroportin results in an increase in iron uptake via enterocyte also producing systemic iron accumulation (Fig. 5A). Loss-of-function mutations of ferroportin produce conditions that share some features of hemochromatosis and classified as ferroportin disease (also classified as type 4A hemochromatosis). In case of ferroportin disease, there is no systemic iron accumulation, and accumulation of iron is limited to storage and recycling sites, mainly macrophages [93].

Clinically, hemochromatosis is characterized by fatigue, joint pain, hyperpigmentation, impotence, diabetes, osteoporosis, hepatomegaly (sometimes with cirrhosis or hepatocellular carcinoma), and cardiac symptoms, such as rhythm disturbances and heart failure. Onset and severity of symptoms vary depending on the type of hemochromatosis. Type 1 has long asymptomatic phase reaching clinical manifestation by 30–40 years in men and up to 10 years later in women. Types 2A, 2B, and 3 have early onset of the disease with clinical expression before 20–30 years. These types of hemochromatosis usually have more severe symptoms including cirrhosis, heart failure, and endocrinal insufficiencies. Surprisingly, Type 4 hemochromatosis typically is only mildly symptomatic despite a high degree of iron overload [91].

4.3. Secondary iron overload

Secondary iron overload arises as a complication of hematological or hemodialysis disorders. Hematological disorders such as hereditary hemolytic anemias (e.g., thalassemia and sickle cell disease) and acquired hemolytic anemias (e.g., autoimmune hemolytic anemia and myelodysplastic syndrome) lead premature destruction (hemolysis) of red blood cells or its precursors [7,94,95]. These conditions require repetitive blood transfusions of 2 units per month resulting in 400–500 mg of iron per transfusion or about 20 g in 4 years, which is 10 fold more than normal iron content of the body for adult [7,94,95]. Transfusion-induced iron overload is especially dangerous in patients with myelodysplastic syndrome due to the advanced age and co-existing heart disease [7,94,95]. Continuous accumulation of iron in the body promotes liver and heart damage due to iron toxicity. Until recently, it was believed that risk of iron overload for dialysis patients was quite low due to use of erythropoiesis-stimulating agents instead of blood transfusions to manage anemia; however, now, iron overload is increasingly recognized as a valid clinical concern [96,97]. Patients suffering from end-stage renal disease are given erythropoiesis-stimulating agents in combination with parenteral or intravenous (IV) iron to counteract anemia that arises from inescapable blood loss due to dialysis, gastrointestinal bleeding, and blood sampling [96,98]. This creates twin risks of iron deficiency (from blood loss), on the one hand, and iron overload (from potentially excessive iron supplementation), on the other hand. Both of these risks must be carefully controlled. Epidemiological studies suggested a link between higher IV iron doses and increase in cardiovascular events as well as higher mortality in dialysis patients [99–101] prompting an update of recommendations on use of IV iron for hemodialysis patients [102]. However, iron overload that may arise in dialysis patients is mostly limited to liver with heart being relatively unaffected [103–105]. Both blood transfusions and iron supplementation increase systemic iron directly, bypassing normal regulatory mechanisms of iron homeostasis. This increase in systemic

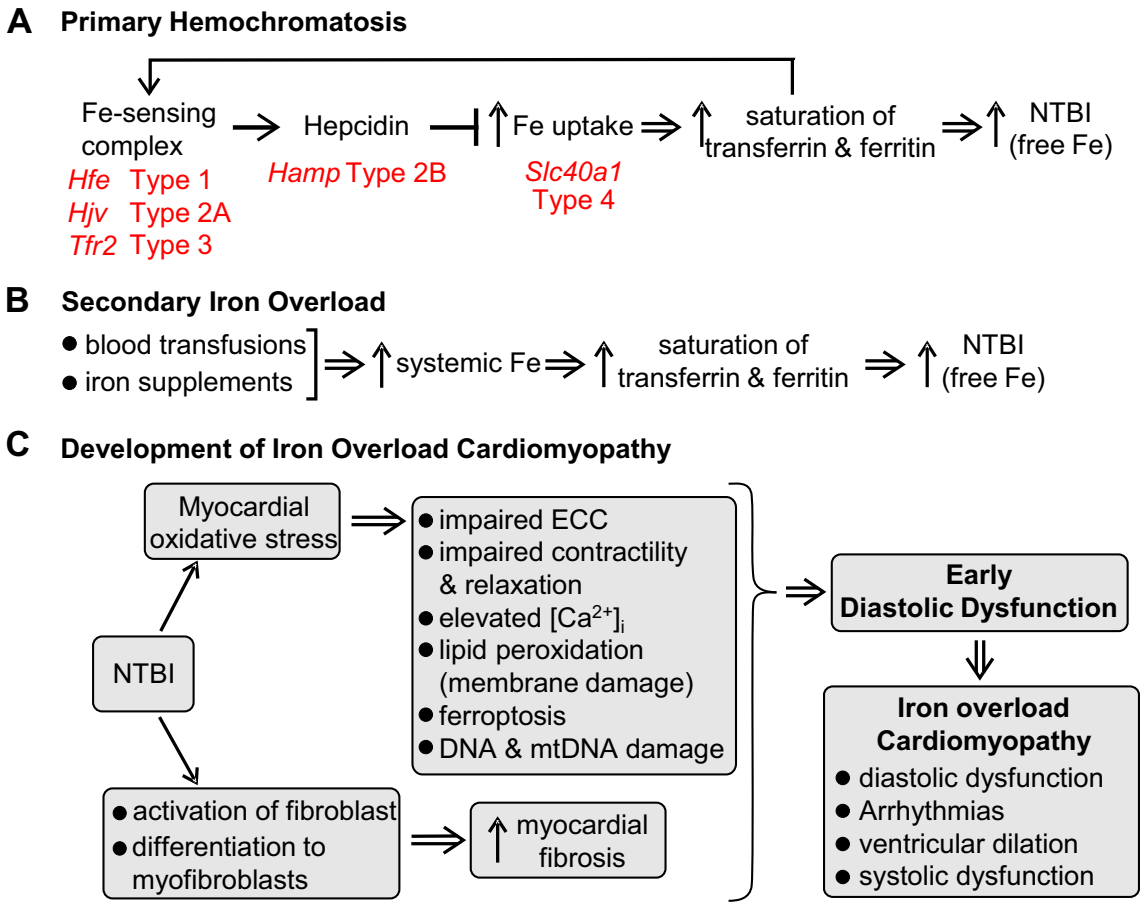

Fig. 5. Mechanism of development of iron overload. A. Mechanism of development of different types of primary hemochromatosis. Mutations in genes coding for iron-sensing complex (*Hfe*, Type 1; *Hjv* Type 2A; *Tfr2*, Type 3), hepcidin (*Hamp*, Type 2B), and ferroportin (*Slc40a1*, Type 4). B. Mechanism of development of secondary iron overload. Increased iron entry into the organism combined with the lack of iron extrusion mechanisms lead to gradual accumulation of systemic iron. C. Mechanism of development of iron-overload cardiomyopathy due to accumulation of non-transferrin bound iron (NTBI).

iron leads to increase in saturation of transferrin and ferritin, and eventually appearance of non-transferrin bound iron (NTBI; Fig. 5B), which is responsible for tissue damage in the case of hemochromatosis and iron overload.

4.4. Mechanism of development of iron-overload cardiomyopathy

Non-transferrin bound iron (NTBI) is mainly bound to low molecular weight molecules (such as citrate and acetate) and albumins [106]. Appearance of labile fraction of NTBI in plasma accelerates deposition of iron in other tissues, especially, excitable tissue that contain Ca^{2+} channels, which are known to conduct Fe^{2+} ions into the cells [5,107,108]. Since cardiac tissue contains high levels of functional voltage-gated Ca^{2+} channels, that makes cardiac tissue especially susceptible to iron overload. Divalent transporters such as ZIP8 and ZIP14 also provide pathway for Fe^{2+} entry [109–111]. Accumulation of high levels of NTBI in cardiac cells leads to multiple detrimental effects (Fig. 5C). Oxidative stress is a main factor of iron-overload injury [7,26,91]. Labile fraction of NTBI produces reactive oxygen species (ROS) via Haber-Weiss and Fenton reaction, [112] and once ROS production exceed capacity of endogenous anti-oxidant systems, ROS start to accumulate and damage multiple proteins, nucleic acids, and lipids. In cardiac tissue, oxidative stress results in (i) impaired excitation contraction coupling, (ii) suppressed SERCA2 function leading to elevated cytoplasmic Ca^{2+} ($[Ca^{2+}]_i$) and impaired relaxation and later contractility, (iii) lipid peroxidation leading to membrane damage including mitochondrial membranes causing inhibition of oxidative phosphorylation and ATP deficiency, and (iv) direct DNA and mitochondrial DNA (mtDNA) damage (Fig. 5C) [14,26,95]. Newly identified mechanism of iron-mediated cell death, ferroptosis (for review see [113,114]), which is dependent on ROS production, can potentially contribute to the cell death in iron-overload cardiomyopathy [115,116] and iron-mediated liver damage [117]. However, further research is needed to elucidate the extent of ferroptosis contribution in iron-overload cardiomyopathy in animal models and human tissue since our current understanding of ferroptosis is based primarily on cell-culture experiments. Besides cellular damage, NTBI can directly activate fibroblasts promoting their proliferation and differentiation to myofibroblasts resulting in increased fibrosis of cardiac tissue [14]. Evidence also emerges that matrix metalloproteinases and tissue inhibitors of metalloproteinases may play a role in development of iron-mediated injury [118]. Accumulation of NTBI-mediate damage in cardiac tissue leads to the development of diastolic dysfunction at early stages, and as accumulation of NTBI-related damage continues, diastolic dysfunction is followed by arrhythmias, ventricular dilation, and systolic dysfunction (Fig. 5C) [14,26,95,119].

4.5. Pre-clinical models of iron overload

Multiple pre-clinical models have been used to generate iron overload in order to elucidate the basis of iron metabolism and understand the mechanism of iron-mediated tissue injury (Table 1). The simplest approach for generating iron overload is to use wild-type (normal genotype) animals in combination with injections of iron dextran [14,111,120–124]. This approach avoids the use of genetically modified animals and allows for a high degree of tissue iron overload

Table 1
Pre-clinical acquired and genetic models of iron-overload.

Model	Phenotype	Ref
Wild-type gerbils (injections)	Cardiac iron 2.9 mg/g (20 weeks after subcutaneous injection [121]); cardiac iron 4.3 mg/g and hepatic iron 12 mg/g (10 weeks after subcutaneous injection [122]); cardiac iron 0.3 mg/g and hepatic iron 5.5 mg/g (15 IP injections followed by 9 months vehicle), systolic dysfunction, arrhythmias [123]; hepatic iron 48, 63, and 73 mg/g (14, 16, and 18 weeks after IV injection, respectively [111])	[111,121–123]
Wild-type rats (IP injections)	After 7 weeks, hepatic iron 2.3 mg/g	[120]
Wild-type rats (iron-rich diet)	After 5–7 weeks, hepatic iron 1.35 mg/g [125] and 4.2 mg/g [120]	[120,125]
Wild-type mouse (IP injections)	After 12 weeks, cardiac iron 0.4 mg/g and hepatic iron 12 mg/g [124]; cardiac iron 9 mg/g with diastolic dysfunction and preserved systolic function [14]	[14,124]
Wild-type mouse (iron-rich diet)	Systolic dysfunction (30-day iron-rich diet) [107]; cardiac iron 0.15 mg/g and hepatic iron 1.2 mg/g with systolic dysfunction (120-d iron-rich diet) [108];	[107,108]
Thalassaemic mouse ($mu\beta^{th\text{-}3/+}$) (iron-rich diet)	Systolic dysfunction (30-day iron-rich diet) [107]; cardiac iron 0.15 mg/g and hepatic iron 1.2 mg/g with systolic dysfunction (120-d iron-rich diet) [108];	[107,108]
Hfe$^{-/-}$ whole-body mouse (normal diet)	At 7 weeks, hepatic iron 1.3 mg/g [126]. At 14 month, heart dilation and systolic dysfunction [133]	[126,133]
Hjv$^{-/-}$ whole-body mouse	At 7 weeks with normal diet, hepatic iron 1.7 mg/g and cardiac iron 0.2 mg/g [127] At 12 months with iron-rich diet, cardiac iron 3.5 mg/g, diastolic and systolic dysfunctions, dysregulation of Ca^{2+} handling, hypertrophy, and fibrosis [134]	[127,134]
Hamp1$^{-/-}$ whole-body mouse (normal diet)	At 6 months, hepatic iron 1.5 mg/g [128] and 2.2 mg/g [131]; plasma iron 48 μmol/L [128] and 60 μmol/L [131,132]	[128,131,132]
Hamp1$^{-/-}$ hepatocyte-specific mouse (normal diet)	At 6 months, hepatic iron 2.5 mg/g [131], plasma iron 60 μmol/L [131,132]	[131,132]
Tfr1-R654A (enhanced HFE-TRF1 binding) mouse (normal diet)	At 8 weeks, NTBI was 0.27 and 0.055 mg/g in liver and heart, respectively (compare to 0.15 and 0.04 mg in liver and heart, respectively, for WT	[143]
Tfr2$^{-/-}$ whole-body mouse (normal diet)	Hepatic iron 1.7 mg/g (26-week age [129]) and 1.5 mg/g (5-week age [130])	[129,130]
Tfr2$^{-/-}$ liver-specific mouse (normal diet)	At 26 weeks, hepatic iron 2 mg/g	[129,130]
Tfr2$^{-/-}$/*Hfe*$^{-/-}$ whole-body mouse (normal diet)	At 5 weeks, hepatic iron 2.3 mg/g	[130]
Timp3$^{-/-}$ whole-body (IP injections) mouse	After 12 weeks of IP injections, both WT and *Timp3*$^{-/-}$ same cardiac iron (20 mg/g), whereas hepatic iron was 30 mg/g and 60 mg/g for WT and *Timp3*$^{-/-}$, respectively. Iron overload produced diastolic, but not systolic dysfunction in WT animals and systolic and diastolic dysfunctions in *Timp3*$^{-/-}$ animals	[118]

relatively quickly, which can result in an advanced iron overload with systolic dysfunction and ventricular arrhythmias [123]. Use of iron-rich diet in wild-type animals usually yields fairly low cardiac and hepatic iron load [107,108,120,125].

In murine thalassaemic models, systolic dysfunction (reduction in cardiac contractility and stroke volume) was reported, but was not matched by diastolic changes (relaxation was not impaired) [107,108]. Genetically-modified mouse models (*Hfe*$^{-/-}$, *Hjv*$^{-/-}$, *Hamp1*$^{-/-}$, and *Tfr2*$^{-/-}$) take at least 6 months to reach substantial hepatic iron-overload (Table 1) [126–132]. The *Hfe*$^{-/-}$ model developed systolic dysfunction at an advanced age (14 month) even without use of iron-rich diet; however, cardiac and hepatic iron levels were not measured in this model [26,133]. *Hjv*$^{-/-}$ model with iron-rich diet did not develop systolic dysfunction at 6 months [14] but required 11 months on iron-rich diet to achieve it [134]. In this model, iron-overload-induced systolic and diastolic dysfunction were accompanied by fibrosis, hypertrophy, and heart-failure-like remodeling of Ca^{2+} cycling proteins. The detrimental effects of iron overload were rescued by 3-month resveratrol treatment without an effect on accumulated cardiac iron [134]. Combining knock out of two regulatory proteins (*Tfr2*$^{-/-}$/*Hfe*$^{-/-}$) considerably increased rate of hepatic iron accumulation [130].

Alternative approach in generating a mouse model with systolic dysfunction is to use a genetic model that already prone to develop more severe HF under stressful conditions (e.g., tissue inhibitor of metalloproteinase 3 knock out, *Timp3*$^{-/-}$) and to use iron injections as another stressful condition. In comparison to iron-injected WT mice, iron-injected *Timp3*$^{-/-}$ mice were characterized by comparable cardiac iron accumulation with increased hepatic iron accumulation. Diastolic and systolic dysfunctions, myocardial fibrosis, and hypertrophy were worsened despite comparable cardiac iron levels. Worsened cardiac and hepatic iron-mediated injuries were associated with increased matrix metalloproteinase activity and inflammation suggesting that TIMP3 plays an important role in the protection against iron-mediated pathology [118].

4.6. Treatment of iron-overload cardiomyopathy

Since humans have no endogenous mechanisms to remove excess iron, treatments of iron overload need to lower systemic iron levels or prevent iron entry into tissues. Phlebotomy (bloodletting) and iron chelation are two ways to lower systemic iron (Fig. 6). Phlebotomy is used when hemoglobin is in the normal range (no anemia) and

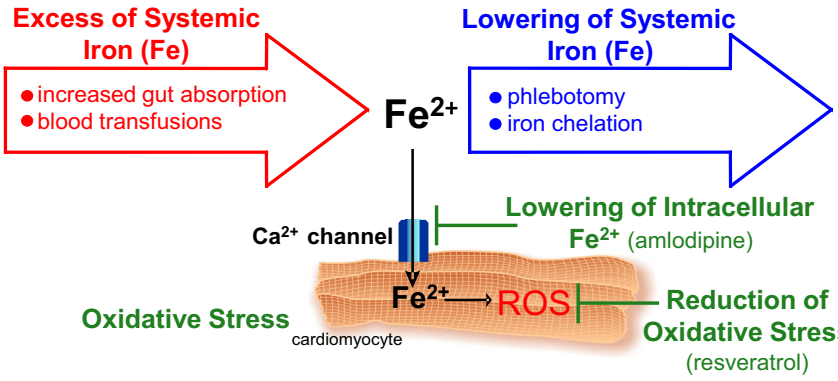

Fig. 6. General concept of treatment of iron-overload cardiomyopathy. Treatment of iron-overload cardiomyopathy has three main approaches: (1) lowering of systemic iron by phlebotomy or iron chelation, (2) lowering intracellular Fe^{2+} by block of Fe^{2+} entry via L-type Ca^{2+} channels (e.g., amlodipine), and (3) direct reduction of oxidative stress by anti-oxidants (e.g., resveratrol).

erythropoiesis is not affected. This approach mainly employed to treat primary hemochromatosis. In other cases, when iron-overload patients are anemic, iron chelation is used to remove excess iron from the body. The observation that iron uptake into the heart (and other excitable tissues) occurs through LTCC is the basis for the use of LTCC blockers, like amlodipine and verapamil, to reduce iron accumulation in the heart and prevent the associated heart disease in mice with iron-overload (Fig. 6) [119]. Verapamil treatment reduced cardiac iron levels in the hemojuvelin knockout mice, a genetic model of hemochromatosis [135]. In humans, several case reports have demonstrated that LTCC blockers have had therapeutic effects in patients with secondary iron overload and heart disease [136]. An early phase I clinical study with amlodipine has shown positive results in patients with thalassemia major [137], and a recently completed phase II trial showed therapeutic effects of amlodipine in addition to standard therapy in patients with secondary iron overload [138]. Amlodipine is likely to provide benefits in iron-overload cardiomyopathy as a blocker of LTCC mediated Fe^{2+} entry and an anti-oxidant counteracting oxidative stress. Amlodipine represents an ideal Ca^{2+} channel blocker to be used in patients with iron-overload cardiomyopathy due to its ability to provide a delicate balance between blockade of ventricular LTCC and avoiding excessive sino-atrial and atrio-ventricular nodal blockade that may exacerbate Brady-arrhythmias, a well-known complication of iron-overload [95,119]. Additional benefits of amlodipine as LTCC blocker could involve vascular relaxation and thus improved myocardial microvascular perfusion [139,140]. Besides LTCC blocking properties, amlodipine also an anti-oxidant, [141] and given the pivotal role of oxidative stress in iron-overload cardiomyopathy, [119,142] amlodipine may have therapeutic benefits independent of the inhibition of cardiac LTCC. Finally, future work may need to consider how other potential mechanisms for iron transport contribute along with LTCC to the cardiomyopathy induced by iron-overload, such as the divalent metal transporter (DMT1), transferrin receptors and ferroportin [119].

Transparency document

The Transparency document associated with this article can be found, in online version.

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Disclosures

None.

Hamp1, gene for hepcidin; *Hfe*, gene for human hemochromatosis protein; *Hjv*$^{-/-}$, gene for hemojuvelin; IP, intraperitoneal injection; IV, intravenous injection; mu$\beta^{th\text{-}3/+}$ heterozygous of β-globin knockout; *Tfr1*, gene for transferrin receptor 1; *Tfr2*, gene for transferrin receptor 2; WT, wild type.

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