How can sodium–glucose cotransporter 2 inhibitors stimulate erythrocytosis in patients who are iron-deficient? Implications for understanding iron homeostasis in heart failure

Milton Packer[1,2]*

[1]Baylor Heart and Vascular Institute, Dallas, TX, USA; and [2]Imperial College, London, UK

Many patients with heart failure have an iron-deficient state, which can limit erythropoiesis in erythroid precursors and ATP production in cardiomyocytes. Yet, treatment with sodium–glucose cotransporter 2 (SGLT2) inhibitors produces consistent increases in haemoglobin and haematocrit, even in patients who are iron-deficient before treatment, and this effect remains unattenuated throughout treatment even though SGLT2 inhibitors further aggravate biomarkers of iron deficiency. Heart failure is often accompanied by systemic inflammation, which activates hepcidin, thus impairing the duodenal absorption of iron and the release of iron from macrophages and hepatocytes, leading to a decline in circulating iron. Inflammation and oxidative stress also promote the synthesis of ferritin and suppress ferritinophagy, thus impairing the release of intracellular iron stores and leading to the depletion of bioreactive cytosolic Fe^{2+}. By alleviating inflammation and oxidative stress, SGLT2 inhibitors down-regulate hepcidin, upregulate transferrin receptor protein 1 and reduce ferritin; the net result is to increase the levels of cytosolic Fe^{2+} available to mitochondria, thus enabling the synthesis of heme (in erythroid precursors) and ATP (in cardiomyocytes). The finding that SGLT2 inhibitors can induce erythrocytosis without iron supplementation suggests that the abnormalities in iron diagnostic tests in patients with mild-to-moderate heart failure are likely to be functional, rather than absolute, that is, they are related to inflammation-mediated trapping of iron by hepcidin and ferritin, which is reversed by treatment with SGLT2 inhibitors. An increase in bioreactive cytosolic Fe^{2+} is also likely to augment mitochondrial production of ATP in cardiomyocytes, thus retarding the progression of heart failure. These effects on iron metabolism are consistent with (i) proteomics analyses of placebo-controlled trials, which have shown that biomarkers of iron homeostasis represent the most consistent effect of SGLT2 inhibitors; and (ii) statistical mediation analyses, which have reported striking parallelism of the effect of SGLT2 inhibitors to promote erythrocytosis and reduce heart failure events.

Keywords   SGLT2 inhibitors • Iron • Hepcidin • Ferritin • Transferrin receptor protein • Soluble transferrin receptor • Heart failure • Haemoglobin • Hematocrit • Ferritinophagy • Ferroptosis

One of the most striking features of treatment with sodium–glucose cotransporter 2 (SGLT2) inhibitors in heart failure is their effect to increase haemoglobin and haematocrit. The increase in red blood cell production produced by these drugs occurs within the first month of therapy and is triggered by an increase in erythropoietin.[1,2] The degree of erythrocytosis precedes, predicts and closely parallels the effect of SGLT2 inhibitors to reduce the risk of heart failure events in large-scale cardiovascular outcome trials.[3–5] Patients who develop striking erythrocytosis while receiving an SGLT2 inhibitor experience the most marked reduction in heart failure hospitalizations.

The enigma of erythrocytosis during sodium–glucose cotransporter 2 inhibition

The erythrocytosis seen with SGLT2 inhibitors is not seen with other drugs used for the treatment of heart failure, and it is particularly impressive, given the fact that ≈50% of patients with heart failure and a reduced ejection fraction fulfill current criteria for an absolute or functional iron deficiency, manifested as (i) a low serum ferritin level (<100 μg/L), or (ii) low transferrin saturation (<20%)

*Corresponding author. Baylor Heart and Vascular Institute, 621 North Hall Street, Dallas, TX, 75226, USA. Tel: +1 214 820-7500, Email: milton.packer@baylorhealth.edu

together with a serum ferritin level of <300 µg/L.[6,7] Iron deficiency indicates an impaired ability to deliver iron to erythroid precursors for adequate erythropoiesis or to cardiomyocytes to sustain optimal mitochondrial ATP production. Iron deficiency may result from (i) an absolute deficiency of iron, reflected by the absence of iron in the main storage depot in the liver or bone marrow and is typically due to poor dietary intake or gastrointestinal blood loss; or (ii) defective gastrointestinal absorption or impaired release of iron from hepatocytes and from macrophages involved in the recycling of senescent red blood cells, typically related to chronic inflammation. The relative importance of these two distinct aetiologies in patients with heart failure has been controversial,[8] since most studies have evaluated small highly selected cohorts and relied on diverse criteria for defining iron deficiency.

Many iron-deficient patients with heart failure are anaemic, suggesting that most are not able to deliver sufficient iron to support adequate red blood cell production, regardless of whether the deficiency is absolute or functional. Yet, SGLT2 inhibitors produce an erythrocytosis even when patients are deemed to be iron-deficient before treatment, and the magnitude of the increase in red blood cell mass following SGLT2 inhibition is not impaired as compared with patients who are deemed to be iron-replete.[9] Even the presence of anaemia prior to treatment does not attenuate the magnitude of the increase in haemoglobin following treatment with SGLT2 inhibitors in patients with heart failure.[10]

These findings are surprising since the depletion of systemic iron stores typically blunts the response to erythropoietin mimetics, thus necessitating intravenous iron supplementation to minimize the development of hyporesponsiveness or resistance to these drugs.[11,12] Furthermore, treatment of patients with heart failure with SGLT2 inhibitors typically aggravates conventional metrics of iron deficiency, as these drugs act to lower both serum ferritin and transferrin saturation. In a large-scale trial, SGLT2 inhibition increased the risk of patients fulfilling conventional criteria for iron deficiency by 70% after 12 months of therapy.[10] Yet, despite the presence of iron deficiency before treatment and the apparent exacerbation of iron deficiency during treatment, the erythrocytosis produced by these drugs is sustained and does not become attenuated over time.

Why does iron deficiency not blunt responsiveness to the increased synthesis of erythropoietin produced by SGLT2 inhibitors? Similarly, why does the apparent exacerbation of iron deficiency during SGLT2 inhibition not negate the beneficial effects of these drugs on the myocardium to reduce the risk of major heart failure events?

Regulation of iron homeostasis in heme-synthesizing cells

Iron enters the bloodstream when dietary iron is absorbed from the gastrointestinal tract or when it is released from hepatocytes involved in iron storage or from macrophages that are engaged in the recycling of senescent red blood cells (*Figure 1*). These points of entry are inhibited by hepcidin,[13] and suppression of hepcidin promotes the ingress of iron into the circulation, where it is bound to and carried by transferrin as ferric ion (Fe^{3+}).

Transport of iron into heme-producing cells

To exert its effects in the heart and bone marrow, iron must enter cells involved in the synthesis of heme and iron−sulfur clusters. This transport is achieved when transferrin attaches to transferrin receptor protein 1 (TfR1), forming a complex that is internalized by endocytosis (*Figure 1*). Movement of Fe^{3+} out of the endosome and its reduction by a ferrireductase leads to the release of ferrous iron (Fe^{2+}) into the cytosol, which is the highly reactive form of weakly-bound iron that is utilized by mitochondria in the synthesis of heme and iron−sulfur clusters,[14] which are essential to the production of haemoglobin (in erythrocytes) or to the generation of ATP (in cardiomyocytes). It has been estimated that this bioreactive pool of cytosolic iron represents 5−10% of the cellular iron content.[15]

Because Fe^{2+} is highly reactive, cytosolic levels of Fe^{2+} are tightly controlled to ensure an adequate supply for heme synthesis, while minimizing deleterious excesses. A decrease in cytosolic Fe^{2+} is sensed by iron regulatory proteins (IRP1 and IRP2), which promote the expression of TfR1,[16] thus facilitating the entry of iron into cells (*Figure 1*). In addition, in states of cardiomyocyte iron depletion, hepcidin is selectively upregulated within cardiomyocytes to retard the egress of iron through ferroportin.[16,17] The coordinated actions of TfR1, IRP1/IRP2 and hepcidin in the heart act to preserve the levels of cytosolic iron in the face of systemic iron deficiency, potentially explaining why cardiac iron stores are not depleted even when iron is absent in the bone marrow.[18–20] Accordingly, experimental cardiac-specific deletion of TfR1, IRP1/IRP2 or hepcidin leads to cytosolic iron deficiency and attenuation of iron-dependent heme-driven synthesis of ATP, thus impairing the heart's response to stress and leading to cardiomyopathy.[17,21,22]

Intracellular sequestration of iron in heme-producing cells

Homeostatic mechanisms in heme-producing cells ensure that intracellular concentrations of highly reactive cytosolic iron do not rise to unhealthy levels. If the level of cytosolic Fe^{2+} increases beyond the capacity for mitochondrial utilization, Fe^{2+} can interact with oxidized lipids (especially in states of glutathione depletion) to promote an iron-dependent apoptosis-independent form of programmed cell death known as ferroptosis. Ferroptosis is prevented by iron chelation and by cellular mechanisms (e.g. glutathione peroxidase 4) that control lipid peroxidation.[23] Ferroptosis contributes to the development of cardiomyopathy due to both ischaemic and non-ischaemic causes, even in clinical states that are not characterized by systemic or myocardial iron overload.[24,25]

To prevent excess levels of bioreactive cytosolic Fe^{2+}, cells involved in heme synthesis utilize intracellular ferritin as a nanocage, which sequesters excessive iron in a non-reactive ferric form (Fe^{3+}) for storage and subsequent release when needed.[26] The level of ferritin responds to deleterious changes in cytosolic Fe^{2+}; as cytosolic Fe^{2+} levels increase and lead to oxidative stress, ferritin is synthesized to enhance the degree of intracellular iron sequestration.[27] Accordingly, the cardiac-specific loss of ferritin
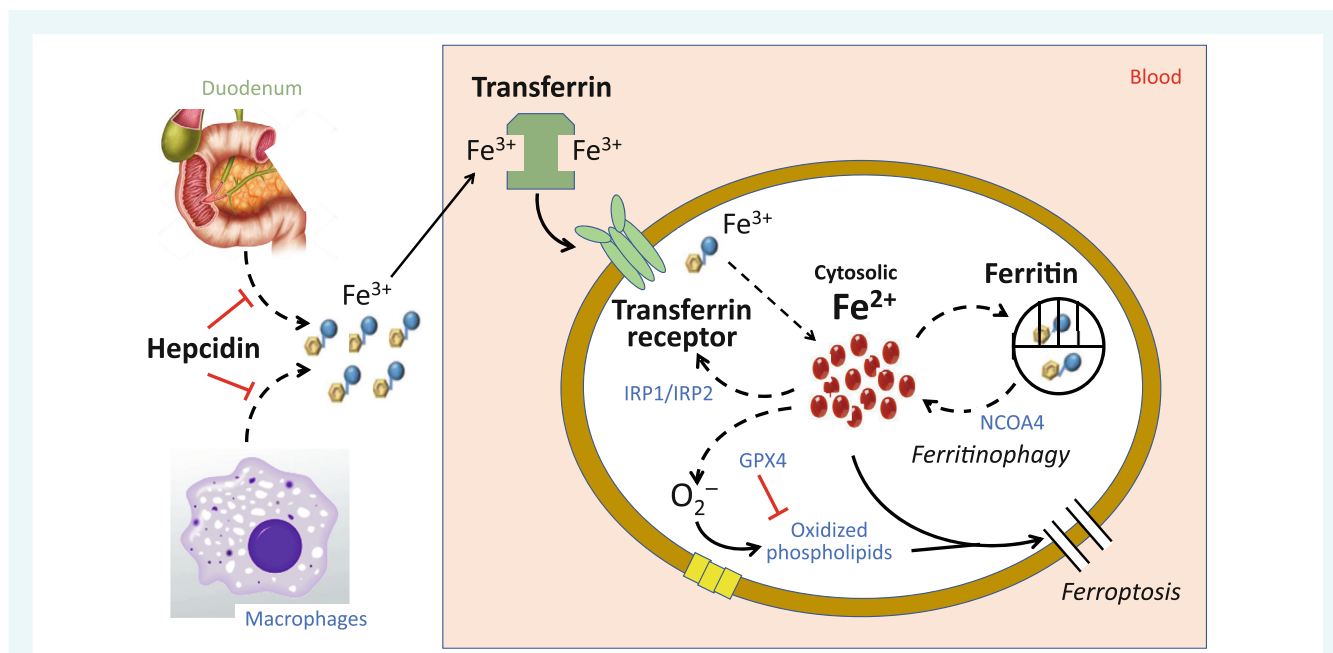
Figure 1 Mechanisms of iron homeostasis in erythroid precursors and cardiomyocytes. Hepcidin blocks the absorption of iron from the duodenum and the release of iron from macrophages and hepatocytes. Iron is bound to transferrin as ferric ion (Fe^{3+}) and is internalized when transferrin docks with the transferrin receptor (transferrin receptor protein 1). Iron is released into the cytosolic pool in its reactive form (Fe^{2+}), where it is available to be utilized by mitochondria in the synthesis of heme and iron−sulfur clusters. Cytosolic levels of Fe^{2+} are maintained in a tight range by the coordinated actions of iron regulatory proteins 1 and 2 (IRP1 and IRP2) and ferritin. Iron regulatory proteins stimulate TfR1 if cytosolic Fe^{2+} is low. Conversely, if Fe^{2+} increases, Fe^{2+} is sequestered as Fe^{3+} in a ferritin cage, which releases iron back into the cytosol by nuclear receptor coactivator 4 (NCOA4)-mediated ferritinophagy. If cytosolic level of highly reactive Fe^{2+} increases excessively, the resulting production of reactive oxygen species oxidizes membrane-bound phospholipids to promote ferroptosis; this oxidation and the resulting ferroptosis is prevented by glutathione peroxidase 4 (GPX4).

leads to cytosolic iron overload, ferroptosis and cardiomyopathy.[28] Conversely, if cytosolic Fe^{2+} declines to dysfunctional levels, ferritin is degraded, releasing iron from sequestration. The primary mechanism that mediates the degradation of ferritin is ferritinophagy, a lysosomal-dependent process that depends on nuclear receptor coactivator 4 (NCOA4), an autophagic cargo receptor that targets and directs ferritin for destruction (*Figure 1*).[29] If ferritinophagy is excessive, the resulting rise in cytosolic Fe^{2+} results in cardiomyocyte death and heart failure.[30] Conversely, if ferritinophagy is deficient, iron is not released from the ferritin nanocage in erythroid precursors, leading to anaemia, despite elevated tissue iron levels.[31] Excessive cellular iron uptake leads to enhanced degradation of NCOA4, suppression of ferritinophagy and augmented iron sequestration.[32]

Assessment of iron deficiency in patients without heart failure

In states of absolute iron deficiency due to poor dietary intake or gastrointestinal blood loss, a decrease in hepcidin leads to the enhanced duodenal absorption of iron and the augmented release of iron from macrophages and hepatocytes. The increase in circulating iron is prioritized for transport into heme-producing cells by

an increased expression of TfR1. At the same time, a decline in ferritin enables the release of iron into the cytosol from intracellular stores. Therefore, the combination of a decrease in hepcidin, an increase in TfR1 (or its secreted form, i.e. soluble transferrin receptor [sTfR]) and a decline in ferritin is typically seen when cytosolic Fe^{2+} is depleted due to absolute iron deficiency.[33–35] However, it should be noted that changes in these iron homeostasis proteins do not measure cytosolic Fe^{2+};[14,15] instead, they assess the cellular responses that are evoked during iron deficiency to restore cytosolic Fe^{2+}. In clinical practice, physicians have assumed that circulating levels of these proteins are closely correlated with their intracellular concentrations in heme-producing cells, although this assumption may not be valid. Nevertheless, the calculation of a ratio of sTfR to serum ferritin has been proposed as a superior diagnostic test for the identification of patients with iron deficiency,[34,36,37] based on the premise that the ratio reflects cellular efforts to enhance the entry of Fe^{2+} into cytosol from both extracellular and intracellular sources.

Historically, the diagnostic accuracy of blood tests used for the identification of iron-deficient states has been based on the evaluation of patients with anaemia due to absolute iron deficiency, typically established by the absence of iron stores in the bone marrow. However, the evaluation of iron staining in the bone marrow is challenging, since the aspirates are often inadequate

and the assessment is subjective.[38] Many patients with anaemia and no detectable bone marrow iron are not iron-deficient,[39,40] possibly because iron staining in bone marrow does not reflect iron stores in the liver, the most important storage depot for systemic iron. In light of these difficulties, serum ferritin has been considered to be the most accurate means of assessing systemic iron stores in patients without heart failure. In general, serum ferritin levels $<12-40\,\mu g/L$ are considered to be diagnostic of absolute iron deficiency, even in elderly patients with anaemia.[41,42] The requirement of an increase in sTfR (accompanying the low ferritin) further increases the likelihood that systemic iron stores are truly depleted.[43]

Changes in iron homeostasis proteins in patients with heart failure

In heart failure, the assessment of iron deficiency is complicated by the fact that such patients often have a systemic inflammatory state,[44] which enhances the production of hepcidin in the liver (*Figure 2*).[45] Circulating levels of hepcidin are increased in patients with heart failure[9,46,47] in proportion to the activation of proinflammatory pathways,[48–51] and the increase retards the entry of iron from the gastrointestinal tract and from macrophages, leading to a state of functional iron deficiency (akin to the anaemia of chronic disease[49,52,53]), which is resistant to oral iron supplementation, but can be responsive to treatment with intravenous iron.[54] Patients with heart failure can suffer from either absolute iron depletion or the functional iron deficiency of chronic inflammation, or both.[8,18,44,46–56] The relative importance of the two mechanisms in clinical practice has been difficult to discern from the available studies, most of which were small and evaluated highly selected patients. Furthermore, it is possible that the mechanisms leading to iron deficiency may shift as heart failure advances.[46,47]

In addition to their effects of hepcidin, activation of oxidative stress and proinflammatory pathways in heart failure can promote the synthesis of ferritin, independent of the level of cytosolic iron.[57,58] Additionally, the suppression of sirtuin-1 (SIRT1) in heart failure[59] can impair autophagic flux, thus limiting the ability of ferritinophagy to replete cytosolic Fe^{2+} levels.[60] As a result, the expected relationship between cytosolic Fe^{2+} and ferritin levels is disrupted, leading to ferritin levels that are disproportionately higher than the level of cytosolic Fe^{2+}, and undermining their ability to identify patients with iron depletion. Accordingly, the threshold for serum ferritin used for the diagnosis of iron-deficiency anaemia is shifted to a substantially higher level in patients with heart failure (to $300-600\,ng/ml$), and it is coupled with the requirement for a transferrin saturation $< 20\%$. However, the adequacy of this criterion adjustment for serum ferritin has been questioned.[7,61] In one study, patients with heart failure and an absolute iron deficiency
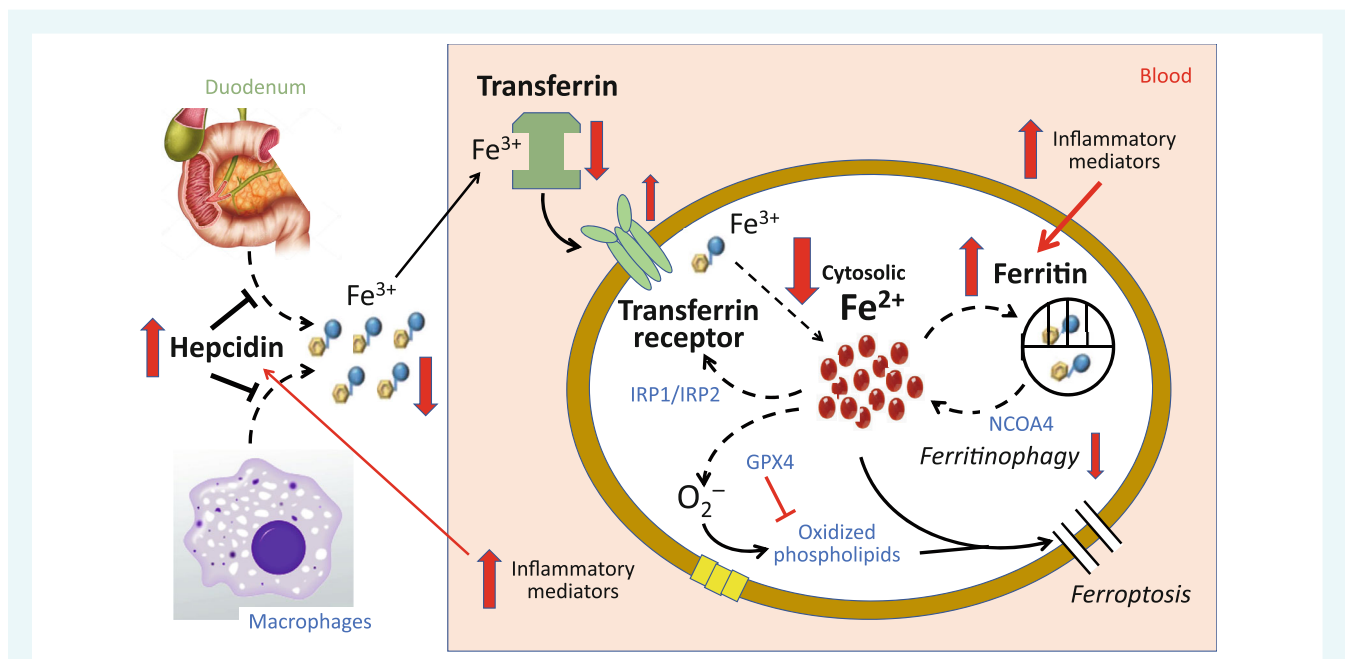

Figure 2 Mechanisms of functional iron deficiency in patients with heart failure. Red arrows indicate the shifts in iron homeostasis in patients with heart failure. Heart failure is often accompanied by increased systemic inflammation, which activates hepcidin, thus impairing the absorption of iron from the duodenum and the release of iron from macrophages and hepatocytes and leading to a decline in iron levels in circulating blood. Inflammation and oxidative stress also promote the synthesis of ferritin and suppress ferritinophagy, thus impairing the release of intracellular iron stores into the cytosol and leading to the depletion of bioreactive cytosolic Fe^{2+}. Transferrin receptor protein 1 may be down-regulated in the failing heart, but it is upregulated when cytosolic levels of Fe^{2+} are threatened. GPX4, glutathione peroxidase 4; IRP1 and IRP2, iron regulatory proteins 1 and 2; NCOA4, nuclear receptor coactivator 4.

often had normal serum levels of ferritin,[55] and in other reports, myocardial (but not serum) levels of ferritin reflected the presence of cardiac iron deficiency in experimental and clinical heart failure.[20,62] Furthermore, inflammation and nutritional status can influence transferrin levels, thus complicating the interpretation of transferrin saturation in heart failure.[63–66]

Given the limitations of both hepcidin and ferritin in states of chronic inflammation, the measurement of sTfR has been advocated as an additional diagnostic tool. Serum levels of sTfR are increased in both patients with an absolute or functional iron deficiency,[43,67] since the receptor is upregulated in response to a decrease in cytosolic iron, regardless of cause. However, the failing heart often shows decreased expression of TfR1,[68,69] perhaps the result of neurohormonal activation,[70,71] which may contribute to an intracellular iron deficiency in cardiomyocytes. However, it is possible that this down-regulation of TfR1 is modified into an upregulation of TfR1 when myocardial iron stores are depleted.[72,73]

The conventional definition of iron deficiency in patients with heart failure – based on the combination of serum ferritin and transferrin saturation – does not distinguish between absolute and functional iron deficiency. From the perspective of the failing heart or endangered erythropoiesis, the distinction may be unimportant, since both absolute and functional iron deficiency would be expected to deprive erythroid precursors and the myocardium of the iron required to maintain haemoglobin synthesis and ATP production. Patients who fulfill criteria for either absolute or functional iron deficiency have greater impairment in cardiac ATP production than those who are iron-replete.[74] It has been assumed that iron deficiency in the heart would be reflected in a depletion of myocardial iron stores, which can be assessed by myocardial biopsy or by non-invasive imaging.[75] Myocardial iron is characteristically reduced in heart failure,[19,20] often more so in patients with accompanying anaemia. However, myocardial staining for iron does not reflect the disordered regulation of bioreactive cytosolic Fe^{2+} that is caused by changes in iron handling proteins in the failing heart.[18] In fact, an iron deficiency state identified by low serum or myocardial ferritin can be accompanied by normal myocardial iron stores.[7,20] Instead, cardiac iron deficiency may be most reliably identified by an increase in the myocardial expression of TfR1 and a decrease in myocardial ferritin, rather than semi-quantitative assessments of myocardial iron content.[18,20,62,72] The ratio of TfR1 to ferritin in cardiomyocytes would reflect cellular efforts to enhance the entry of ferrous iron into cytosol from both extracellular and intracellular sources.

In light of the influence of inflammation on circulating levels of conventionally-measured iron homeostatic proteins in heart failure, the available evidence suggests that the iron deficiency state in most patients with heart failure is functional, rather than absolute; a similar judgment has been reached by other investigators.[76] This conclusion is supported by observations that (i) hepcidin and ferritin are increased in patients with heart failure until the disease becomes advanced;[9,47] and (ii) iron-deficient patients with heart failure show clinical benefits in response to intravenously (but not orally) administered iron supplements,[77–80] presumably related to hepcidin-mediated inhibition of duodenal iron absorption. A similar discordance in the responsiveness to intravenous vs oral iron therapy has been observed when patients with chronic kidney disease and functional iron deficiency are treated with erythropoietin-stimulating agents.[81,82]

Effect of sodium–glucose cotransporter 2 inhibitors on iron homeostasis in patients with heart failure

Clinical studies of patients receiving SGLT2 inhibitors have shown that treatment with these drugs produces meaningful decreases in hepcidin, transferrin saturation and ferritin, while increasing sTfR (*Figure 3*).[2,10,68–70] This pattern has been noted consistently in placebo-controlled trials of patients who receive these drugs for diabetes or heart failure.

Changes in iron homeostasis biomarkers in placebo-controlled trials

In a placebo-controlled trial of 52 patients with type 2 diabetes studied after 12 weeks of treatment with dapagliflozin, Ghanim et al.[83] reported a 24% decrease in hepcidin (together with a 70% increase in erythroferrone, a hepcidin suppressor), a 22% decrease in transferrin saturation and a 32% decline in ferritin (all measured in plasma), while noting a 59% increase in TfR1 in mononuclear cells. In a placebo-controlled trial of 44 patients with type 2 diabetes studied after 12 weeks with empagliflozin, Thiele et al.[84] reported a 29% decrease in transferrin saturation and a 33% decline in ferritin in circulating blood. In a placebo-controlled trial of >2200 patients with heart failure and a reduced ejection fraction studied after 12 months of treatment with dapagliflozin, Docherty et al.[9] noted meaningful decreases in hepcidin and ferritin (with modest decreases in transferrin saturation) together with increases in sTfR. In all studies, the effects of SGLT2 inhibition were significantly different from placebo.

In a proteomics analysis of 72 patients with type 2 diabetes reported by Ferrannini et al.,[85] SGLT2 inhibition for 4 weeks led to increases in sTfR and decreases in ferritin and neogenin (a positive regulator of hepcidin), all with false discovery rates <1%. Interestingly, the effect on ferritin was exceptionally consistent, with a false discovery rate p-value of 6.7×10^{-8}. Of >3700 proteins examined, 43 were differentially-expressed as a result of SGLT2 inhibition, and of these, 5 were involved in iron metabolism. In a proteomics analysis of >1100 patients with heart failure reported by Zannad et al.,[2] SGLT2 inhibition for 12 weeks led to increases in TfR1 and erythropoietin, both with false discovery rates <1%. TfR1 was the protein that was most consistently changed by empagliflozin among >1200 proteins that were measured, with a false discovery rate p-value of 2.5×10^{-12}. Increases in erythropoietin with SGLT2 inhibitors have been reported in other placebo-controlled trials.[1]
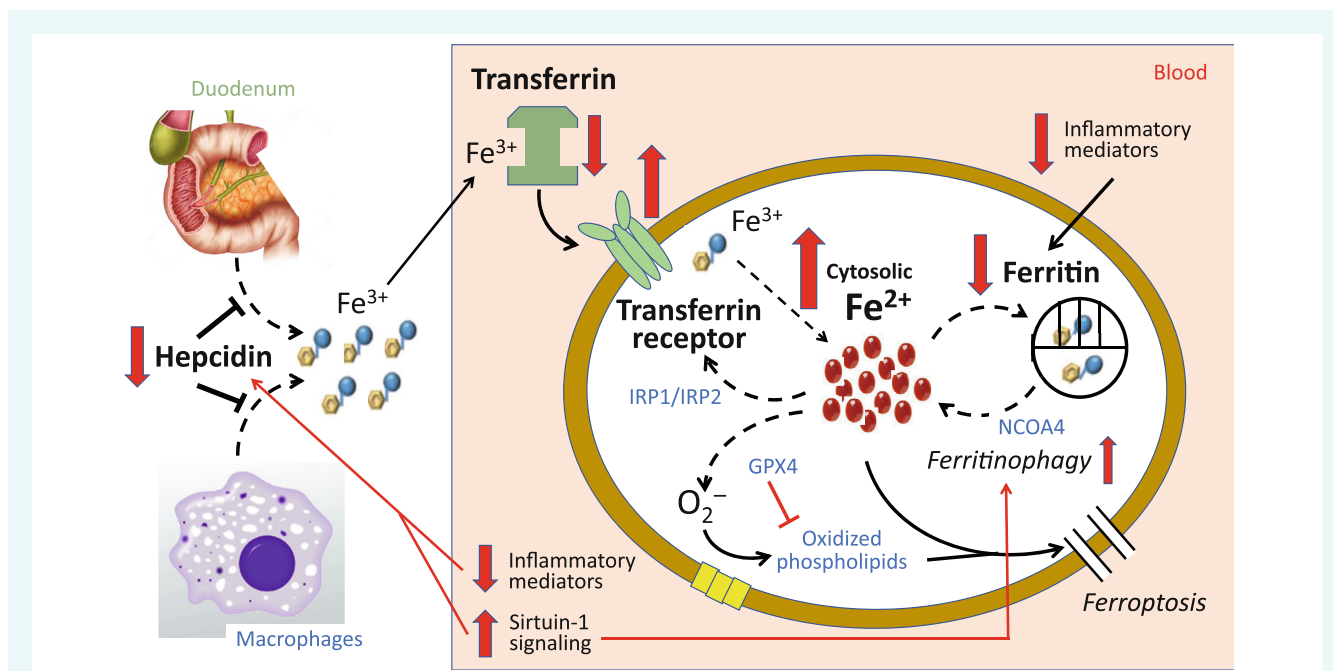
Figure 3 Alleviation of functional iron deficiency in patients with heart failure during treatment with sodium−glucose cotransporter 2 inhibitors (SGLT2). Red arrows indicate the shifts in iron homeostasis in patients with heart failure treated with SGLT2 inhibitors. Due to their action to upregulate sirtuin-1 and to suppress inflammatory mediators, SGLT2 inhibitors down-regulate hepcidin, thus enhancing the absorption of iron from the duodenum and the release of iron from macrophages and hepatocytes. Concomitant increased expression of transferrin receptor protein 1 − often measured as soluble transferrin receptor in circulating blood − augments the transit of iron into erythroid precursors and cardiomyocytes. Suppression of inflammation and upregulation of sirtuin-1 also mutes the synthesis of ferritin and promotes ferritinophagy; thus, SGLT2 inhibitors promote the release of Fe^{2+} into the cytosol from intracellular stores. The net effect of these pathways is to alleviate the deficit of bioreactive Fe^{2+}, thus enabling the synthesis of heme (in erythroid precursors) and ATP (in cardiomyocytes). GPX4, glutathione peroxidase 4; IRP1 and IRP2, iron regulatory proteins 1 and 2; NCOA4, nuclear receptor coactivator 4.

Mechanistic implications of changes in iron homeostasis during sodium−glucose cotransporter 2 inhibition

Increases in erythropoietin will not yield meaningful increases in red blood cell production if a deficiency in cytosolic levels of bioreactive Fe^{2+} impairs the synthesis of haemoglobin. It is therefore interesting that SGLT2 inhibition leads to changes in iron regulatory proteins that ensure that cytosolic levels of Fe^{2+} are supported (*Figure 3*). The decreases in hepcidin seen following SGLT2 inhibition should increase the gastrointestinal absorption of iron and the release of iron from macrophages and hepatocytes. SGLT2 inhibition produces a small decrease in transferrin saturation, presumably because iron is rapidly transported out of the bloodstream and into cells as a consequence of the upregulation of TfR1/sTfR. The augmented transport of iron into erythroid precursors and cardiomyocytes would increase cytosolic levels of bioreactive Fe^{2+}, as long as iron is not sequestered within the ferritin nanocage. It is therefore noteworthy that SGLT2 inhibitors produce a decrease in serum ferritin; if this decline reflects a decline in ferritin in erythroid precursors and cardiomyocytes, such an action would ensure an increase in cytosolic levels of Fe^{2+}.

How do SGLT2 inhibitors cause increases in sTfR/TfR1 and erythropoietin and decreases in hepcidin and ferritin? SGLT2 inhibitors promote a state of starvation mimicry, which is characterized by upregulation of nutrient deprivation signalling, especially SIRT1.[59] SIRT1 is responsible for the increased gluconeogenesis and ketogenesis that follows the loss of calories in the urine seen following inhibition of SGLT2.[86,87] Upregulation of SIRT1 increases signalling through hypoxia-inducible factor-2α (HIF-2α),[88] the primary positive regulator of erythropoietin synthesis. At the same time, activation of SIRT1 also leads to the suppression of hepcidin synthesis in both the liver and macrophages.[89] Upregulation of SIRT1 also increases the activation of PGC-1α, which can upregulate the expression of TfR1.[90] Finally, since the principal mechanism by which intracellular ferritin declines is related to ferritinophagy, it is noteworthy that SIRT1 can promote ferritinophagy either by a direct effect to promote autophagic flux[59,60] or through stimulation of HIF-2α-mediated increases in the expression of NCOA4,[91] thus promoting ferritinophagy. Additionally, the well-established action of SGLT2 inhibitors to mute inflammation in the heart and bone marrow[92−94] (also the result of enhanced SIRT1 signalling[93−95]) might contribute to the observed decline in both hepcidin and ferritin. Finally, increased erythropoietin activity upregulates TfR1 and reduces transferrin saturation, ferritin

and hepcidin.[96–98] Importantly, if cytosolic Fe^{2+} levels were to rise excessively, SIRT1 can prevent the deleterious consequences of iron overload by its action to suppress ferroptosis.[99,100]

Therefore, through their effect to enhance nutrient deprivation signalling and suppress inflammation, SGLT2 inhibitors act to increase in bioreactive cytosolic Fe^{2+} together with an increase in erythropoietin, thus stimulating erythroid precursors and haemoglobin synthesis to produce an increase in red blood cell mass. Similar changes in the heart would promote the synthesis of heme-containing proteins in iron-deficient cardiomyocytes, which are essential for the synthesis of ATP in the energy-starved failing heart. Upregulation of TfR1 also promotes autophagic clearance of damaged mitochondria, which is essential for both erythroid maturation and cardioprotection.[101] Accordingly, an increase in bioreactive cytosolic Fe^{2+} may explain the ability of SGLT2 inhibitors to simultaneously increase ATP production within the heart and to promote erythrocytosis in the bone marrow.[102] This parallelism may explain why erythrocytosis closely parallels and predicts the cardioprotective effect of these drugs. In fact, an increase in haemoglobin is the most important statistical mediator of the ability of empagliflozin, canagliflozin and ertugliflozin to reduce the risk of major heart failure events in large-scale cardiovascular outcome trials.[3–5]

Implications for understanding the pathogenesis and consequences of iron deficiency in heart failure

In patients with heart failure, the effect of SGLT2 inhibitors to increase TfR1 and decrease hepcidin and ferritin would be expected to increase the level of bioreactive cytosolic Fe^{2+} in cells involved in the production of heme and iron−sulfur clusters. This increase in cytosolic iron − coupled with an increase in erythropoietin − can explain the effect of SGLT2 inhibitors to promote erythrocytosis even in patients who are deemed to be iron-deficient before treatment.[9] In fact, SGLT2 inhibitors induced significant erythropoiesis even in iron-deficient patients who were *anaemic* prior to treatment[10]; that is, before SGLT2 inhibition, cytosolic levels of Fe^{2+} in these patients were apparently insufficient to support adequate red blood cell production. Patients who have heart failure and anaemia are often poorly responsive to erythropoietin-stimulating agents if they are iron-deficient despite the use of oral supplements.[103] The finding that SGLT2 inhibitors can rapidly correct anaemia without iron supplementation[10] indicates that iron deficiency in these patients (who had mild-to-moderate heart failure) was likely to be functional, rather than absolute, that is, it was related to inflammation-mediated increases in hepcidin and ferritin, which were reversed by treatment with SGLT2 inhibitors. Such a conclusion is consistent with previous studies.[7,46–54] Reports that patients with heart failure suffer from an absolute iron deficiency have been based on a small sample size,[55,56,63] the lack of confirmed bone marrow iron depletion,[53,55] or the evaluation of people with advanced disease.[47]

Importantly, an increase in bioreactive cytosolic Fe^{2+} is likely to be relevant in augmenting mitochondrial production of ATP in cardiomyocytes,[22,73] and thus, retarding the progression of heart failure. It is therefore noteworthy that the effect of SGLT2 inhibitors to reduce heart failure hospitalizations was particularly marked in patients who were most iron-deficient prior to therapy (i.e. serum ferritin <100 ng/ml)[9] and who might be expected to be particularly responsive to interventions that would increase cytosolic Fe^{2+}. Analogously, patients with heart failure who have decreased transferrin saturations (i.e. <20%) were most likely to experience an improvement in cardiac performance and a decrease in heart failure hospitalizations following intravenous iron supplementation,[77,78] although this finding remains uncertain.[79] Nevertheless, the parallelism of these observations suggests that a SIRT1-mediated improvement in iron homeostasis may mediate many of the benefits of SGLT2 inhibitors in heart failure. This hypothesis is consistent with the findings of both statistical mediation analyses and proteomics analyses of placebo-controlled trials, which have highlighted the effects of these drugs on erythropoiesis and its regulators as prominent features of their pharmacological profile and therapeutic benefits.[2–6,85]

It should be noted that SGLT2 inhibitors exert their parallel benefits on red blood cell production and heart failure events even though treatment with these drugs produces decreases in transferrin saturation and serum ferritin and increases in sTfR. This pattern of changes would typically lead physicians to conclude that patients were becoming more (rather than less) iron-deficient during treatment with these drugs. In fact, after 12 months of treatment, patients receiving dapagliflozin in the DAPA-HF trial were far more likely to fulfill diagnostic criteria of an iron-deficiency state than patients receiving placebo, even though patients treated with the SGLT2 inhibitor continued to demonstrate a robust erythrocytic response and the magnitude of erythrocytosis was not attenuated in those with a diagnosis of iron deficiency prior to treatment.[9] These intriguing observations raise important questions about our current reliance on the measurement of circulating levels of iron homeostatic proteins to reach conclusions about the level of bioreactive cytosolic Fe^{2+} within erythrocyte precursor cells or cardiomyocytes when patients are being treated with SGLT2 inhibitors.

Conflict of interest: none declared.

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