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STATE OF THE ART REVIEW
Diabetes and metabolic disorders

Mechanisms of enhanced renal and hepatic erythropoietin synthesis by sodium–glucose cotransporter 2 inhibitors

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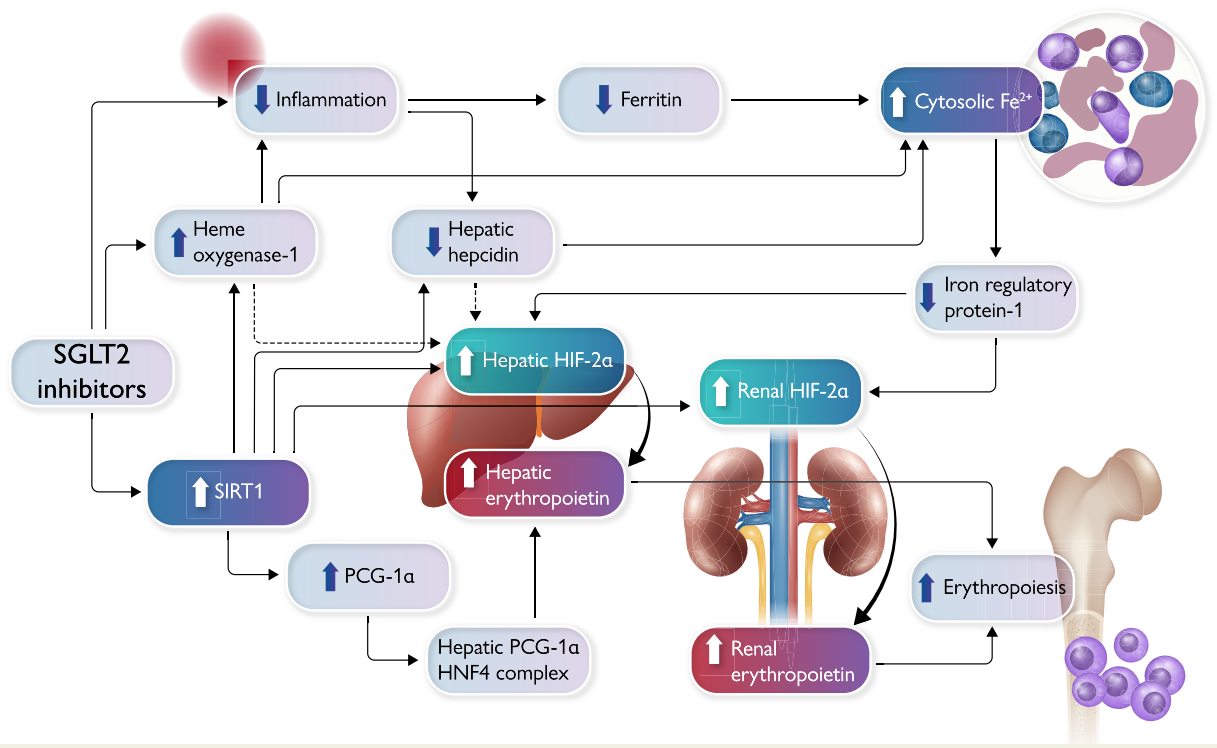
Graphical Abstract



Proposed mechanisms by which SGLT2-induced non-hypoxia-related cellular signaling might trigger erythropoietin synthesis by the kidney or liver. SGLT2 inhibitors activate nutrient deprivation cellular signaling, thus muting cellular stress and proinflammatory pathways. Up-regulation of sirtuin-1 (SIRT1) and heme oxygenase-1 may underlie these effects. The resulting reduction in inflammation-sensitive expression of hepcidin and ferritin increases the availability of bioreactive ferrous iron, which can stimulate expression of hypoxia-inducible factor-2α (HIF-2α) in the liver and kidney. Hypoxia-inducible factor-2α expression can be directly enhanced by up-regulation of SIRT1 and potentially by changes in hepcidin and heme oxygenase-1. In parallel with these events, SIRT1 activation may promote the hepatic formation of a PGC-1α–HNF4 complex, which can bind to the promoter region of the erythropoietin gene in a manner similar to HIF-2α, thus enhancing the transcription of erythropoietin in the liver. Dotted lines show effects demonstrated in tissues other than the liver or kidney, and not fully evaluated in hepatic or renal cells. HNF4, hepatocyte nuclear

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factor 4; PGC-1α, peroxisome proliferator-activated receptor-γ coactivator-1α; SGLT2, sodium–glucose cotransporter 2; HIF-2α, hypoxia-inducible factor-2α; SIRT1, sirtuin-1.

Abstract

Sodium–glucose cotransporter 2 (SGLT2) inhibitors reduce the risk of major heart failure events, an action that is statistically linked to enhanced erythropoiesis, suggesting that stimulation of erythropoietin and cardioprotection are related to a shared mechanism. Four hypotheses have been proposed to explain how these drugs increase erythropoietin production: (i) renal cortical reoxygenation with rejuvenation of erythropoietin-producing cells; (ii) counterregulatory distal sodium reabsorption leading to increased tubular workload and oxygen consumption, and thus, to localized hypoxia; (iii) increased iron mobilization as a stimulus of hypoxia-inducible factor-2α (HIF-2α)-mediated erythropoietin synthesis; and (iv) direct HIF-2α activation and enhanced erythropoietin gene transcription due to increased sirtuin-1 (SIRT1) signaling. The first two hypotheses assume that the source of increased erythropoietin is the interstitial fibroblast-like cells in the deep renal cortex. However, SGLT2 inhibitors do not alter regional tissue oxygen tension in the non-diabetic kidney, and renal erythropoietin synthesis is markedly impaired in patients with anemia due to chronic kidney disease, and yet, SGLT2 inhibitors produce an unattenuated erythrocytic response in these patients. This observation raises the possibility that the liver contributes to the production of erythropoietin during SGLT2 inhibition. Hypoxia-inducible factor-2α and erythropoietin are coexpressed not only in the kidney but also in hepatocytes; the liver is a major site of production when erythropoietin stimulation is maintained for prolonged periods. The ability of SGLT2 inhibitors to improve iron mobilization by derepressing hepcidin and ferritin would be expected to increase cytosolic ferrous iron, which might stimulate HIF-2α expression in both the kidney and liver through the action of iron regulatory protein 1. Alternatively, the established ability of SGLT2 inhibitors to enhance SIRT1 might be the mechanism of enhanced erythropoietin production with these drugs. In hepatic cell lines, SIRT1 can directly activate HIF-2α by deacetylation, and additionally, through an effect of SIRT in the liver, peroxisome proliferator-activated receptor-γ coactivator-1α binds to hepatic nuclear factor 4 to promote transcription of the erythropoietin gene and synthesis of erythropoietin. Since SIRT1 up-regulation exerts direct cytoprotective effects on the heart and stimulates erythropoietin, it is well-positioned to represent the shared mechanism that links erythropoiesis to cardioprotection during SGLT2 inhibition.

Keywords SGLT2 inhibitors • Erythropoietin • Erythropoiesis

Sodium–glucose cotransporter 2 (SGLT2) inhibitors reduce the risk of cardiovascular death and hospitalizations for heart failure in patients with type 2 diabetes, chronic kidney, or chronic heart failure with a reduced or preserved ejection fraction.[1,2] This benefit appears to be related to a direct action of these drugs on cardiomyocytes to enhance nutrient deprivation signaling and promote autophagic flux, thus reducing oxidative (and other cellular) stress and improving cellular survival in diverse injuries, including those induced by hyperglycemia, ischemia, pressure overload and cardiotoxic agents.[3,4] Sodium–glucose cotransporter 2 inhibitors exert cardioprotective effects in isolated cardiomyocytes (which do not express SGLT2) and *ex vivo* hearts independent of changes in environmental glucose, ketone bodies, or oxygen or neurohormonal influences as well as in animals in which SGLT2 has been knocked out.[5] Abrogation of the effects of SGLT2 inhibitors on autophagic pathways and interference with their signaling through sirtuins and adenosine monophosphate-activated protein kinase (AMPK) abolishes the actions of these drugs to reduce cellular stress and improve cellular viability.[3] Proteomic analyses have shown that SGLT2 inhibition leads to up-regulation of proteins that have an established role in promoting autophagy and reducing oxidative stress and cell death.[6]

Sodium–glucose cotransporter 2 inhibitor-mediated stimulation of erythropoiesis

In light of these observations, it is noteworthy that SGLT2 inhibitors exert a consistent effect to increase hemoglobin and hematocrit in randomized controlled trials. Although originally ascribed to an effect to produce hemoconcentration as a result of natriuresis and plasma volume contraction, the effect of SGLT2 inhibitors to increase urinary sodium excretion and reduce plasma volume is transient,[7,8] and changes in body weight produced by these drugs are generally related to the urinary loss of calories.[9] Instead, increases in hemoglobin seen during SGLT2 inhibition are related to an enhanced production of erythropoietin and reticulocytosis and an expansion of red blood cell mass.[10–12] A marked increase in erythropoietin occurs rapidly following initiation of treatment, and then partially subsides during long-term therapy,[11–13] as a new set point for the equilibrium between erythropoietin and a higher hemoglobin is established.[14] If this feedback mechanism were not to occur, SGLT2 inhibitors would produce severe polycythemia.

Does the erythrocytosis produced by SGLT2 inhibitors contribute to their ability to reduce major heart failure events? Some have proposed that the erythrocytosis produced by these drugs might increase the delivery of oxygen to the heart, but there is no experimental or clinical evidence to support this hypothesis, either in patients with or without anemia. Other therapeutic approaches that increase hemoglobin in anemic patients (e.g. erythropoiesis-stimulating agents or prolyl hydroxylase inhibitors) do not reduce (and may increase) the risk of cardiovascular events, when given to patients with underlying heart or kidney disease.[15,16] Although statistical mediation analyses have identified short-term increases in hemoglobin as a correlate of the benefit of SGLT2 inhibitors to reduce heart failure events,[17–19] mediation analyses do not demonstrate that erythrocytosis causes cardioprotection. Instead, they indicate that erythrocytosis and the heart failure benefits produced by these drugs are related to a shared mechanism. Therefore, exploration of the mechanisms that are responsible for the increased erythropoietin may provide important insights as to the identity of a common pathway for the increases in hemoglobin and the decreased risk of heart failure events.

Regulation of erythropoietin production in the kidney and liver

During fetal development, the major site of erythropoietin production is the liver,[19,20] whereas in adults, erythropoietin is largely synthesized by peritubular fibroblast-like type-1 interstitial cells, located primarily in the deep renal cortex;[21–23] at both sites, erythropoietin production is exquisitely sensitive to tissue oxygen tension. A major difference between erythropoietin production in the kidney and liver is that hepatocytes can increase synthesis at a cellular level, whereas in the kidney, increased erythropoietin synthesis is achieved by proliferation of the peritubular fibroblast-like type-1 interstitial cells, each producing a fixed quantity of erythropoietin messenger RNA.[20]

In both the kidney and the liver, the major stimulus to the production of erythropoietin is hypoxia-inducible factor-2α (HIF-2α), which is expressed in the same cells that manufacture erythropoietin.[24,25] Coexpression of HIF-2α links erythropoietin synthesis to oxygen levels, since HIF-2α is degraded by prolyl hydroxylases that are stabilized by ambient levels of oxygen.[26] In addition, hepatocyte nuclear factor 4 (HNF4), which is also oxygen-sensitive, promotes transcription of the erythropoietin gene and may maintain tissue specificity of erythropoietin expression.[27,28]

With the onset of anemia and in the absence of kidney disease, the proliferation of interstitial cells extends the production of erythropoietin throughout the entire renal cortex.[22] However, during the evolution and progression of chronic kidney disease, the interstitial cells are transformed into myofibroblasts that can no longer synthesize erythropoietin, but they can drive the development of renal fibrosis.[29–31] Hepatic production of erythropoietin contributes importantly to systemic levels, if renal sources are impaired or if its synthesis is markedly stimulated, genetically or pharmacologically, for long periods.[19,32,33] During prolonged stimulated erythropoiesis in animals with normal renal function[32] or during prolyl hydroxylase inhibition for the treatment of anemia in patients with chronic kidney disease, the liver emerges as the primary site of erythropoietin production.[33–36] It is not known if the heightened synthesis of erythropoietin during SGLT2 inhibition is primarily renal or hepatic, especially during long-term therapy. However, it is noteworthy that the magnitude of erythrocytosis during SGLT2 inhibition is not attenuated in patients with estimated glomerular filtration rates <45 mL/min/1.73 m^2 or in patients who have anemia of chronic kidney disease.[37,38] Since the renal synthesis of erythropoietin in these patients is severely compromised,[29–31] it seems likely that the liver contributes importantly to the synthesis of erythropoietin seen during SGLT2 inhibition.

Why do sodium–glucose cotransporter 2 inhibitors stimulate the production of erythropoietin?

Four hypotheses have been proposed to explain how these drugs might increase erythropoietin production: (i) renal cortical reoxygenation with rejuvenation of interstitial cells; (ii) counterregulatory distal sodium reabsorption leading to increased tubular workload and renal deep cortical and medullary hypoxia; (iii) increased iron mobilization as an inducer of HIF-2α-mediated erythropoietin synthesis; and (iv) HIF-2α activation and increased erythropoietin gene transcription due to increased nutrient deprivation signaling. This article reviews the merits and limitations of these four hypotheses (*Table 1*).

Sodium–glucose cotransporter 2 inhibitor-induced renal cortical reoxygenation with rejuvenation of hypoxic interstitial fibroblast-like cells is the stimulus to erythropoietin

Sano and Goto[39] proposed that increased glucose reabsorption places a metabolic burden on the proximal renal tubules, causing tubulointerstitial hypoxia. This hypoxic injury was hypothesized to cause the specialized interstitial fibroblast-like cells in the renal cortex to undergo transformation into dysfunctional myofibroblasts, which would be incapable of erythropoietin production, but promote renal fibrosis.[29,30] Hypothetically, inhibition of glucose reabsorption by SGLT2 inhibitors would alleviate the metabolic demands on the proximal tubules and reduce oxygen consumption, thus improving oxygenation in the renal cortex and potentially allowing dysfunctional fibroblasts to revert to a phenotype that which would be capable of erythropoietin synthesis (*Figure 1*).[40]

Yet, the available evidence does not support the 'renal cortical reoxygenation' hypothesis. Inhibition of glucose reabsorption by SGLT2 inhibitors has been reported to improve oxygenation in the renal cortex,[41] but this study used qualitative staining methods, and its findings have not been confirmed by quantitative methods (i.e. microelectrodes or magnetic resonance imaging).[42,43] Furthermore, the reversibility of interstitial fibroblast-like cell dysfunction is uncertain; stimulation of erythropoietin production in experimental renal fibrosis is localized to non-injured nephron segments, without evidence of reversion of differentiated myofibroblasts.[31] Importantly, acetazolamide improves oxygenation of the superficial renal cortex due to inhibition of proximal tubular sodium reabsorption,[44] but the drug does not promote erythropoiesis. Finally, any increase in oxygen tension in the deep renal cortex following SGLT2 inhibition would be expected to suppress (not activate) HIF-2α signaling and the synthesis of erythropoietin.[24]

Sodium–glucose cotransporter 2 inhibitor-mediated induction of renal hypoxia at the corticomedullary junction is the stimulus to the interstitial fibroblast-like cells that produce erythropoietin

The action of SGLT2 inhibitors to block sodium reabsorption in the proximal renal tubule leads to increased delivery of sodium to more distal portions of the nephron, where it is absorbed by counterregulatory mechanisms that are activated to limit the magnitude of natriuresis. These mechanisms include the reabsorption of sodium and solutes in the S3 segment of the proximal tubule, activation of Na,K,2Cl− cotransporter in the loop of Henle, and enhanced activity of the apical Na–Cl cotransporter in the distal convoluted tubule.[5,45,46] Increased sodium reabsorption in the S3 segment and loop of Henle would be expected to increase oxygen consumption in the deep cortex and outer medulla, respectively. Modeling studies have predicted that acute and chronic SGLT2 inhibition might be particularly likely to increase oxygen consumption and predispose to tissue hypoxia in the S3 segment,[47] potentially in close proximity to the specialized interstitial fibroblast-like cells in the deep cortex, especially if oxygen diffuses poorly through the renal parenchyma.[48] Experimental studies have noted that SGLT2 inhibition reduced oxygen tension in the deep cortex and outer medulla in diabetic rats,[41] changes that were accompanied by increased renal erythropoietin mRNA levels and reticulocytosis. Interestingly, inhibition of both SGLT1 and SGLT2 delivered additional solutes to segments beyond S3, possibly leading to hypoxia in the inner medulla (*Figure 1*).[49]

Table 1 Potential mechanisms mediating enhanced erythropoietin production during SGLT2 inhibition

Hypothesis	Proposed mechanism	Supporting observations	Limitations and concerns
Renal cortical reoxygenation with rejuvenation of erythropoietin-producing cells	SGLT2 inhibitors reduce sodium and glucose transport and oxygen consumption in the proximal tube, potentially alleviating hypoxia in the superficial cortex	Improved renal cortical oxygenation using qualitative staining methods	SGLT2 inhibitors do not influence cortical oxygen tension using quantitative methods. Acetazolamide inhibits proximal tubular function (akin to SGLT2 inhibitors), but does not induce erythrocytosis. Up-regulation of sodium reabsorption in distal convoluted tubule would be expected to aggravate (not alleviate) cortical hypoxia
Increased distal tubular workload, leading to hypoxia at the corticomedullary junction	Counterregulatory increased distal sodium reabsorption and workload at S3 segment, and loop of Henle increases oxygen consumption and causes hypoxia in the deep cortex and outer medulla, which stimulates interstitial fibroblast-like 1 cells	SGLT2 inhibition has been reported to reduce oxygen tension in deep cortex and outer medulla in diabetic mice	No evidence for renal hypoxia following SGLT2 inhibition in non-diabetic animals or humans. Drug-induced interference with distal sodium reabsorption does not influence erythrocytosis. SGLT2 inhibitors induce erythrocytic response in patients with marked impairment of renal interstitial fibroblast-like cell function
Increased iron mobilization, triggering enhanced HIF-2α signaling	SGLT2 inhibition acts to derepress hepcidin and ferritin (and increase heme oxygenase-1), thus promoting increased iron availability in the cytosol	IRP1 acts a sensor of cytosolic ferrous iron and can modulate expression of HIF-2α mRNA in the liver and kidney in an oxygen-independent manner	No study has evaluated the effect of SGLT2 inhibitors on IRP1 expression
SIRT1 up-regulation, triggering enhanced HIF-2α signaling or erythropoietin gene transcription	Up-regulation of SIRT1 by SGLT2 inhibition acts to stimulate the activity of both HIF-2α and erythropoietin in an oxygen-independent manner	Sirtuin-1 has been shown to mediate the cellular benefits of SGLT2 inhibition. SIRT1 can activate HIF-2α directly (or through heme oxygenase-1) or can promote transcription of erythropoietin gene through formation of a complex with PGC-1α and HNF4	No studies have evaluated whether SGLT2 inhibitors increase activity of HIF-2α and HNF4 in the liver or kidney

HNF4, hepatocyte nuclear factor 4; HIF-2α, hypoxia-inducible factor-2α; IRP1, iron regulatory protein 1; SGLT2, sodium glucose cotransporter 2; SIRT1, sirtuin-1.

Despite these observations, the available evidence does not provide substantial support to the 'renal corticomedullary hypoxia' hypothesis as a mechanism of increased erythropoietin production. In experimental and clinical studies of renal oxygenation, SGLT2 inhibition produced hypoxia in the deep cortex and outer medulla in diabetic subjects, but intriguingly, not in those without diabetes;[42] yet, patients without diabetes still manifest a robust erythrocytosis following SGLT2 inhibition.[50,51] Furthermore, acetazolamide blocks sodium reabsorption in the S2 segment[52,53] and increases sodium delivery to the S3 segment (with the potential for tubular hypoxia), and yet, the drug does not induce an increase in hematocrit. Increased sodium delivery and tubular workload at the loop of Henle leading to outer medullary hypoxia cannot explain the increase in erythropoiesis, since the increased tubular workload and oxygen consumption would be blocked by loop diuretics;[44] yet, patients with heart failure prescribed loop diuretics still manifest a strong erythropoietic response.[54] Moreover, although blockade of SGLT1 should alleviate the hypoxia in the S3 segment,[55] dual SGLT1 and SGLT2 inhibition does not produce a potentiated erythrocytosis, as compared with selective SGLT2 inhibitors.[56] Most importantly, SGLT2 inhibitors produce a robust increase in hematocrit in patients with stage 3b/4 chronic kidney disease and in patients with renal anemia,[37,38] even though these patients manifest a diminished distal tubular sodium delivery due to glomerular hypofiltration,[57] and they manifest a severe impairment of renal synthesis of erythropoietin,[29–31] rebutting the possibility that SGLT2 inhibitors enhance renal erythropoietin production as a result of increased corticomedullary tubular workload.

Sodium–glucose cotransporter 2 inhibitor-mediated iron mobilization promotes hypoxia-inducible factor-2α signaling in the kidney and liver

Many of the disease states that are treated with SGLT2 inhibitors are characterized by anemia of chronic disease, a chronic inflammatory state that is characterized by increased levels of two major iron regulatory proteins—hepcidin and ferritin.[58,59] Increases in the synthesis of

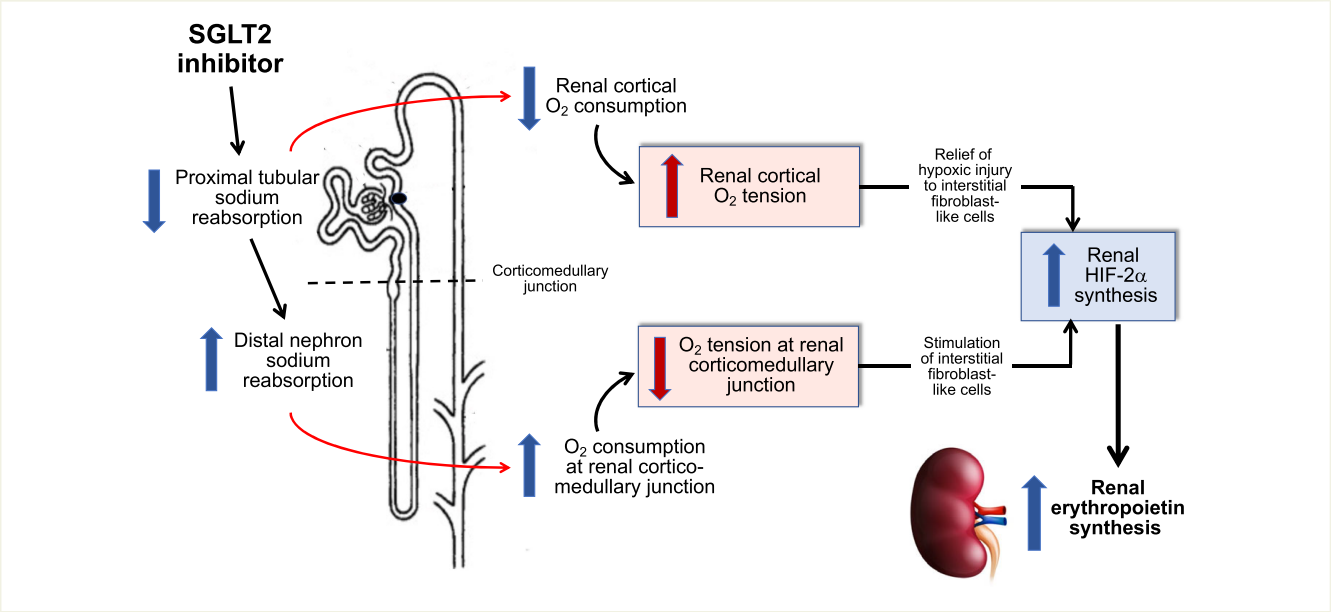

Figure 1 Proposed mechanisms by which changes in regional renal oxygenation by SGLT2 inhibitors might trigger erythropoietin synthesis by the kidney. The energy required for sodium transport in the renal tubules is the principal determinant of oxygen consumption, and enhanced sodium reuptake may predispose to localized hypoxia. Changes in oxygen tension to the interstitial fibroblast-like cells at the corticomedullary junction may influence viability of these cells or may decrease the activation of prolyl hydroxylases, thus enhancing the synthesis of hypoxia-inducible factor-2α (HIF-2α), the main driver of erythropoietin synthesis. SGLT2, sodium–glucose cotransporter 2.

hepcidin by the liver block the absorption of iron from the duodenum and the release of iron from the reticuloendothelial system.[60] Increases in ferritin in heme-producing cells result in the sequestration of ferrous iron in an intracellular nanocage, preventing its release into the cytosol.[61] Increases in hepcidin and ferritin are responsible for the development of a state of functional iron deficiency in patients with type 2 diabetes, chronic kidney disease, and chronic heart failure, in the face of adequate total body iron stores.[62–64]

Sodium–glucose cotransporter 2 inhibitors reduce serum hepcidin and ferritin in both type 2 diabetes and chronic heart failure, thus acting to potentially alleviate the functional iron deficiency.[65–67] In part, the changes in hepcidin might be related to stimulation of erythropoiesis, which can suppress hepcidin through an effect of erythroferrone that is released from proliferating erythroid precursors;[68,69] however, observations concerning an effect of SGLT2 inhibitors on erythroferrone are inconsistent,[10,65] and suppression of erythroferrone would not be expected to lower ferritin levels.[70] Therefore, it seems more likely that decreases in hepcidin and ferritin with SGLT2 inhibitors are related to their actions to mute inflammation (*Graphical Abstract*).[64] The anti-inflammatory effects of these drugs may be related to enhanced nutrient deprivation signaling, to activation of heme oxygenase-1, or by a direct effect on intracellular proinflammatory signaling pathways.[3,71,72]

Regardless of the mechanism, decreases in hepcidin and ferritin would lead to heightened release of iron from macrophage and intracellular storage sites, respectively, Furthermore, activation of heme oxygenase-1 by SGLT2 inhibitors promotes the degradation of heme, further increasing the release of iron into the cytosol.[71] The combined effect of these cellular events would improve iron mobilization into erythroid precursors (thereby facilitating hemoglobin production) and into cardiomyocytes (thereby facilitating ATP production).[4] This conceptual framework may explain why SGLT2 inhibitors promote myocardial iron repletion by cardiac magnetic resonance, although the method quantifies tissue iron content rather than cytosolic iron levels.[73] Alleviation of a cytosolic iron deficiency state by SGLT2 inhibitors may explain why patients with the most significant iron deficiency prior to treatment appear to experience the greatest benefit from these drugs with respect to the reduction in heart failure events.[67]

Interestingly, by enhancing the levels of cytosolic iron, SGLT2 inhibitors are poised to influence the expression of proteins that are responsive to iron availability and are linked directly to HIF-2α-mediated erythropoietin production. Drug-induced decreases in hepatic hepcidin promote up-regulation of HIF-2α in enterocytes and potentially the liver;[74,75] the mechanism involves enhancement of ferroportin and subsequent inhibition of the prolyl hydroxylases that degrade HIF-2α.[74–76] Up-regulation of heme oxygenase-1 may also contribute to enhance hypoxia-inducible factor signaling.[77,78] Perhaps most importantly, the primary mechanisms of intracellular iron sensing are the iron-regulatory proteins, IRP1 and IRP2, and SGLT2 inhibitor-mediated increases in cytosolic ion would be expected to suppress the expression of IRP1, thereby increasing the expression of HIF-2α mRNA in both the kidney and liver (*Graphical Abstract*).[79–81] Iron chelation suppresses cytosolic iron, and thus, HIF-2α transcriptional activity and protein expression.[82] Studies of the effect of SGLT2 inhibitors on IRP1 are needed to confirm this hypothesis.

Sodium–glucose cotransporter 2 inhibitor up-regulation of sirtuin-1 directly activates hypoxia-inducible factor-2α signaling and promotes erythropoietin gene transcription

Sodium–glucose cotransporter 2 inhibitors promote a state of starvation mimicry, which is characterized by glycosuria, gluconeogenesis, ketogenesis and shrinkage of adipose depots at a physiological level, and by up-regulation of nutrient deprivation signaling at a cellular level.[83]

When cells perceive that they are deprived of nutrients, they activate numerous master switches that prioritize cellular health and survival over cellular growth and replication.[84] These cellular switches act to promote cellular housekeeping through enhanced autophagic flux; to reduce oxidative and endoplasmic reticulum stress; to mute proinflammatory and profibrotic pathways; and to improve cellular homeostasis and viability.[85] The nutrient deprivation signals that are most relevant to the action of SGLT2 inhibitors are sirtuin-1 (SIRT1), sirtuin-3 (SIRT3), AMPK and peroxisome proliferator-activated receptor-γ coactivator-1α (PGC-1α).[3] The induction of glycosuria (and urinary caloric loss) stimulates the production of SIRT1 in the liver.[86]

Numerous experimental studies have demonstrated that SGLT2 inhibitors exert direct cardioprotective effects on the heart, which are accompanied by increased phosphorylation of AMPK and increased expression of SIRT1, SIRT3, and PGC-1α.[3,4] Inhibition or genetic silencing of AMPK, SIRT1, and SIRT3 abolishes the favorable effects of SGLT2 inhibitors to inhibit cellular stress and inflammation.[3] Sodium–glucose cotransporter 2 inhibitors act directly to up-regulate nutrient deprivation signaling even though SGLT2 is not expressed in the healthy or failing heart. Possible mechanisms include direct docking of these drugs with target proteins, an effect on other glucose transporters or an effect mediated through ketone bodies acting as signaling molecules.[3]

To exert their effects to maintain cellular homeostasis, SIRT1 and AMPK act as cellular energy sensors. The activity of SIRT1 is dependent on the levels of NAD+ (the oxidized form of nicotinamide adenine dinucleotide), and thus, is activated during low-energy states that are characterized by high cellular levels of NAD+.[87] AMPK senses the ratio of AMP to ATP, and when supplies of ATP are limited, AMPK is phosphorylated, leading to activation of SIRT1.[88] Additionally, SIRT1 is up-regulated during states of glucose deprivation, and it acts to maintain blood glucose, primarily by interacting with PGC-1α in the liver to promote hepatic gluconeogenesis.[87,89,90] In furtherance of its function as an energy sensor, SIRT1 is also activated by hypoxia,[91] thereby providing a mechanistic link by which SIRT1 might function to promote the synthesis of erythropoietin.[92]

In fact, in an immortalized hepatic cell line, SIRT1 has been shown to directly activate HIF-2α by virtue of its action to deacetylate specific lysine residues.[93,94] Additionally, SIRT1 can promote the expression of heme oxygenase-1[95] and its downstream effects on hypoxia-inducible factor signaling,[77,78] and the anti-inflammatory actions of SIRT1 signaling can suppress hepcidin.[96] Most importantly, when SGLT2 inhibitors activate SIRT1 in the liver,[86] SIRT1 deacetylates PGC-1α to promote hepatic gluconeogenesis, a metabolic event that requires the formation of a complex between PGC-1α and HNF4.[97,98] Hepatocyte nuclear factor 4 functions as an oxygen sensor and binds to the promoter and 3′ enhancer region of the erythropoietin gene to stimulate erythropoietin transcription in hepatocytes[27,28,98,99] and may be the primary driver of hepatic erythropoietin synthesis during fetal development.[100] Therefore, as a result of up-regulation of hepatic SIRT1, SGLT2 inhibitors are well-positioned to promote the production of erythropoietin by the liver.

These observations, taken collectively, indicate that SGLT2 inhibitors—acting through several mechanisms mediated through SIRT1—might be able to directly activate HIF-2α and HNF4 and promote erythropoietin gene transcription, independent of hypoxia or the action of prolyl hydroxylases (*Graphical Abstract*). Additional studies are needed to confirm or refute this hypothesis. Although SGLT2 inhibitors have been reported to up-regulate HIF-2α in the heart;[72] their effect on the activity of HIF-2α and HNF4 in the liver and kidney has not yet been studied.

Summary and conclusions

Understanding the mechanisms of enhanced erythropoietin production may provide important insights into the pathways that mediate the cardioprotective effects of SGLT2 inhibitors. The available evidence does not support the hypothesis that drug-mediated changes in renal tubular workload and oxygen consumption are likely to trigger hypoxia-mediated increases in erythropoietin production by interstitial fibroblast-like 1 cells in the deep cortex and outer medulla of the kidney. Instead, it seems likely that the action of SGLT2 inhibitors to up-regulate signaling through SIRT1 may directly or indirectly up-regulate HIF-2α and/or HNF4 to promote transcription of the erythropoietin gene, particularly in the liver. Hepatic synthesis may be particularly important during long-term therapy and in patients with chronic kidney disease and anemia in whom interstitial fibroblast-like 1 cells are severely compromised, but who nevertheless show an unattenuated erythrocytic response to SGLT2 inhibitors. Since SIRT1 up-regulation exerts direct cardioprotective effects and stimulates erythropoietin, it may represent the shared mechanism that links erythropoiesis to the heart failure benefits of SGLT2 inhibition.

Data availability

No new data were generated or analysed in support of this research.

Conflict of interest

During the past three years, M.P. reports personal fees for consulting from Abbvie, Actavis, Amarin, Amgen, AstraZeneca, Boehringer Ingelheim, Caladrius, Casana, CSL Behring, Cytokinetics, Imara, Lilly, Moderna, Novartis, Reata, Relypsa, and Salamandra.

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