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REVIEW

Critical examination of mechanisms underlying the reduction in heart failure events with SGLT2 inhibitors: identification of a molecular link between their actions to stimulate erythrocytosis and to alleviate cellular stress

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Abstract

Sodium-glucose co-transporter 2 (SGLT2) inhibitors reduce the risk of serious heart failure events, even though SGLT2 is not expressed in the myocardium. This cardioprotective benefit is not related to an effect of these drugs to lower blood glucose, promote ketone body utilization or enhance natriuresis, but it is linked statistically with their action to increase haematocrit. SGLT2 inhibitors increase both erythropoietin and erythropoiesis, but the increase in red blood cell mass does not directly prevent heart failure events. Instead, erythrocytosis is a biomarker of a state of hypoxia mimicry, which is induced by SGLT2 inhibitors in manner akin to cobalt chloride. The primary mediators of the cellular response to states of energy depletion are sirtuin-1 and hypoxia-inducible factors (HIF-1α/HIF-2α). These master regulators promote the cellular adaptation to states of nutrient and oxygen deprivation, promoting mitochondrial capacity and minimizing the generation of oxidative stress. Activation of sirtuin-1 and HIF-1α/HIF-2α also stimulates autophagy, a lysosome-mediated degradative pathway that maintains cellular homoeostasis by removing dangerous constituents (particularly unhealthy mitochondria and peroxisomes), which are a major source of oxidative stress and cardiomyocyte dysfunction and demise. SGLT2 inhibitors can activate SIRT-1 and stimulate autophagy in the heart, and thereby, favourably influence the course of cardiomyopathy. Therefore, the linkage between erythrocytosis and the reduction in heart failure events with SGLT2 inhibitors may be related to a shared underlying molecular mechanism that is triggered by the action of these drugs to induce a perceived state of oxygen and nutrient deprivation.

Keywords SGLT2 inhibitors • Autophagy • Hypoxia-inducible factors • Sirtuin-1 • Cardioprotection

1. Introduction

In patients with Type 2 diabetes, sodium-glucose co-transporter 2 (SGLT2) inhibitors reduce the risk of serious heart failure events, specifically the risk of hospitalization for heart failure, and often cardiovascular death.[1] The benefit is most apparent in patients with a reduced ejection fraction.[2] Furthermore, dapagliflozin reduced the risk of cardiovascular death and hospitalizations for heart failure in patients with established systolic heart failure, including those without diabetes.[3] The mechanism of this cardioprotective benefit is unknown.

2. Mechanisms cannot explain SGLT2 inhibitor-mediated cardioprotection

It has been challenging to explain how SGLT2 inhibitors act to slow the evolution and progression of the cardiomyopathic process since SGLT2 is not present in the normal or failing heart.[4,5] SGLT2 is primarily expressed in the proximal renal tubule, where it mediates glucose reabsorption.[6] However, it is not clear how an action on the kidney to

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promote glycosuria might mitigate injurious processes in the myocardium and reduce the risk of serious heart failure events.

2.1 SGLT2 inhibitors do not produce cardioprotective effects by their action to lower glycated haemoglobin

Hyperglycaemia can produce deleterious effects on the heart,[7] but the magnitude of the blood glucose-lowering effect of SGLT2 inhibitors is modest, and in the large-scale trials with these drugs, the benefit on the reduction in heart failure hospitalizations was not related to the decline in glycated haemoglobin.[1,2] Drugs that exert greater antihyperglycaemic effects than SGLT2 inhibitors do not reduce the risk of serious heart failure events.[8] Furthermore, the favourable effect of SGLT2 inhibitors on heart failure is seen even in patients without Type 2 diabetes.[3] Hence, the antihyperglycaemic action of SGLT2 inhibitors cannot explain the ability of these drugs to reduce the evolution and progression of cardiomyopathy.

2.2 SGLT2 inhibitors do not produce cardioprotective effects as a result of their action to promote sodium excretion

SGLT2 inhibitors block sodium reabsorption in the proximal renal tubule, and some have hypothesized that the reduction in the risk of heart failure events is related to this natriuretic effect, leading to a decrease in intravascular or interstitial volume.[9,10] SGLT2 inhibitors can promote a short-term increase in urine volume in patients with acutely decompensated heart failure[11]; however, their effect on urinary sodium excretion and plasma volume in stable patients is modest and often transient.[12–15] Furthermore, SGLT2 inhibitors have not potentiated the effects of loop diuretics in clinical trials or cohort studies,[3,16] and they have not produced a meaningful immediate decline in circulating levels of natriuretic peptides,[3,17] as would be expected from a drug that acts primarily as a diuretic. Slowing of the rate of rise of natriuretic peptides seen during long-term treatment is likely the result of—rather than a contributing factor to—a favourable action of these drugs on left ventricular remodelling.[3,18] Moreover, SGLT2 inhibitors have not ameliorated symptoms of pulmonary or systemic congestion in clinical trials of patients with chronic heart failure.[17] Importantly, the use of SGLT2 inhibitors reduces the risk of cardiovascular death, whereas intensification of diuretic therapy has been associated with an increased mortality rate.[19]

2.3 SGLT2 inhibitors do not produce cardioprotective effects by increasing the delivery of fuel or oxygen to the myocardium

In contrast to other antihyperglycaemic drugs, SGLT2 inhibitors promote ketogenesis through a combined action to enhance both gluconeogenesis and fatty acid oxidation.[20–22] Some have proposed that the resulting increase in circulating ketone bodies provides an efficient fuel for the failing heart.[23,24] However, ketonaemia is an established feature of patients with heart failure[25] and the failing myocardium already utilizes ketone bodies as a preferred fuel[26,27]—in the absence of a SGLT2 inhibitor.[26,27] Although the infusion of beta-hydroxybutyrate produces short-term increases in cardiac contractility and heart rate,[28] SGLT2 inhibitors do not produce these hemodynamic changes in clinical trials. Furthermore, SGLT2 inhibitors have not consistently enhanced ketone body consumption by the myocardium under experimental conditions.[21,29–32] Importantly, the increases in ATP production and cardiac efficiency seen following SGLT2 inhibition are not related to enhanced ketone body metabolism.[30,31]

An intriguing feature of SGLT2 inhibitors in clinical trials has been their action to increase the synthesis of erythropoietin and thereby increase red blood cell mass.[33] Theoretically, if the failing heart were hypoxic, erythrocytosis could increase oxygen delivery to the stressed myocardium, which might be particularly important in patients with coronary artery disease. However, there is no evidence for insufficient oxygen availability in hearts under states of hemodynamic stress.[34] Furthermore, in a large-scale trial, the magnitude of the benefits of SGLT2 inhibitors in heart failure did not depend on whether patients had underlying ischaemic heart disease; patients with an ischaemic aetiology did not benefit preferentially from SGLT2 inhibition.[3] Finally, when erythrocytosis is produced by erythropoietin-mimetic agents, the increase in red blood cell mass does not lead to favourable effects on cardiovascular death or heart failure hospitalizations in patients with chronic heart failure, even though the haematocrit was subnormal at the start of treatment.[35]

3. Erythrocytosis provides a statistical and molecular clue to the mechanism leading to the reduction in heart failure events with SGLT2 inhibitors

Although the failing heart does not benefit from the increase in oxygen delivery produced by an expanded red blood cell mass, the erythrocytosis seen with SGLT2 inhibitors may nevertheless be an indicator of the molecular events that underlie their cardioprotective benefits. When statistical mediation analyses are carried out in the large-scale trials with SGLT2 inhibitors, the increase in red blood cell counts emerges as the single most important variable to predict the decreased risk of cardiovascular death and heart failure hospitalizations produced by SGLT2 inhibitors.[36,37] This finding suggests that erythrocytosis is closely associated with the molecular pathways involved in cardioprotection, i.e. the enhanced erythropoiesis and the reduction in serious heart failure events are linked statistically not because the expanded red blood cell mass improves cardiac oxygenation, but because erythrocytosis and the cardioprotective effects have a shared underlying molecular mechanism.

4. Why do SGLT2 inhibitors stimulate erythropoietin? The concordant and counterbalancing effects of hypoxia-inducible factors and sirtuin-1

The primary regulators of erythropoietin synthesis are hypoxia-inducible factors (HIFs) that transactivate the gene for erythropoietin. Two inducible isoforms—HIF-1α and HIF-2α—are up-regulated by hypoxia or by drugs that mimic hypoxia under normoxic conditions (e.g. cobalt chloride).[38,39] HIF-1α and HIF-2α appear to activate the same downstream genes, but they do so differently in different tissues and under different conditions. Typically, HIF-1α promotes the transcription of genes encoding metabolic enzymes, transporters, and mitochondrial proteins that

decrease oxygen utilization,[40,41] whereas HIF-2α is the isoform that is the primary stimulus for erythropoietin synthesis.[39] Importantly, HIFs respond not only to oxygen deprivation but also to changes in caloric balance and to levels of glycaemia.[42,43] Both isoforms act to lower blood glucose, and in turn, glucose deprivation suppresses both HIF-1α and HIF-2α.[43,44] It is therefore noteworthy that HIF-2α mediates adaptation to hypoglycaemia,[45] whereas HIF-1α primarily activates genes typically triggered by hypoxia[41,46]—except for the synthesis of erythropoietin.

Another critical component of the cellular response to hypoxia in cardiomyocytes is up-regulation of sirtuin-1 (SIRT1),[42–44] a redox-sensitive nicotinamide adenine dinucleotide-dependent enzyme that deacetylates target proteins, many of which are responsible for the maintenance of blood glucose and cellular homoeostasis. As in the case for HIFs, the activity of SIRT1 is exquisitely responsive to nutrient deprivation as well as hypoxia[47,48]; a primary function of SIRT1 is to maintain blood glucose levels during states of hypoglycaemic stress.[49] Additionally, SIRT1 acts to mute cellular stress and exerts protective effects in a broad range of organs (including the heart),[50–52] explaining why SIRT1 may be the mechanistic link between caloric restriction and organismal longevity.[53]

4.1 Counterbalancing effects of HIF-1α and SIRT1

Interestingly, the actions of HIFs and SIRT1 oppose and counterbalance each other in many ways. With respect to glucose and lipid homoeostasis, HIF-1α switches cells from oxidative to glycolytic metabolism (thus allowing for the maintenance of ATP levels under hypoxic conditions), and HIF-2α acts to inhibit gluconeogenesis.[43,44] These effects—together with an action to inhibit fatty acid oxidation—explain why both isoforms act to lower blood glucose and to suppress ketone body formation.[54–57] However, SIRT1 signalling counteracts these effects on glucose and lipid metabolism. SIRT1 promotes glycogenolysis, gluconeogenesis, and fatty acid oxidation,[58] while inhibiting glycolysis,[59] and SIRT1 activates the rate-limiting step for ketone body production.[60] As a result of these actions, SIRT1 supports blood glucose and promotes ketogenesis, thus directly opposing the metabolic actions of HIF-1α.

At the same time, as part of its action to mute oxygen consumption, HIF-1α impairs the biogenesis of mitochondria,[61] whereas SIRT1 promotes the formation of healthy mitochondria and regulates mitochondrial quality control by facilitating the disposal of dysfunctional or damaged organelles.[62] HIF-1α and SIRT1 also appear to exert counterbalancing effects on inflammation. SIRT1 interacts directly with NF-κB to suppress its activation, thus suppressing myocardial and vascular inflammation.[63,64] In contrast, increased HIF-1α signalling promotes M1 polarization and enhances proinflammatory pathways in macrophages,[65–68] and NF-κB promotes the up-regulation of HIF-1α.[68,69]

4.2 Concordant and mutually reinforcing effects of HIF-2α and SIRT1

Interestingly, HIF-2α—the primary isoform responsible for erythropoiesis—has actions on inflammation that align with those of SIRT1 but are opposite to those of HIF-1α, i.e. HIF-2α mutes the inflammatory response that underlies insulin resistance in obesity.[70] Importantly, the oppositional effects of HIF-1α and HIF-2α in macrophages are paralleled by their counterbalancing actions on inflammatory processes in the heart. Cardiac activation of HIF-1α promotes inflammation,[71,72] whereas increased expression of HIF-2α in cardiomyocytes preserves mitochondrial integrity and prevents injury in experimental ischaemia.[73,74] Activation of HIF-2α (when produced by inhibitors of its degradation) attenuates experimental cardiac inflammation, fibrosis, and adverse remodelling.[75–78]

The parallelism between many (but not all) of the actions of SIRT1 and those of HIF-2α is intriguing, since SIRT1 increases the expression of HIF-2α, and in turn, HIF-2α (and erythropoietin, the protein product of HIF-2α transactivation) augments SIRT1 signalling in cardiomyocytes and other cell types.[79–82] The mutually stimulating components of the SIRT1/HIF-2α/erythropoietin axis mitigate the response to and the adverse consequences of disease states that promote cardiomyocyte stress and injury.

5. Coordinated actions of HIFs and SIRT1 on autophagic clearance of organellar sources of oxidative stress

Although HIFs and SIRT1 have counterbalancing or concordant effects under varying conditions, these master regulators act in a coordinated manner to stimulate the cellular housekeeping process of autophagy. Autophagy is a lysosomally mediated degradative pathway that maintains cellular homoeostasis by removing dangerous constituents and recycling cellular components. States of nutrient and oxygen deprivation are the primary triggers of autophagy,[83] and the degradation of cellular elements provides a supply of nutrients that can maintain cellular ATP during low-energy states. Importantly, autophagy is also stimulated by oxidative stress,[84] and in response, the process of cellular self-digestion clears the cytoplasm of damaged organelles (especially mitochondria and peroxisomes) that are the major sources of reactive oxygen species and cellular dysfunction in cardiomyocytes.[85] Yet, the housekeeping functions of autophagy are critically depressed in the myocardium of subjects with type 2 diabetes or with heart failure and play an important in promoting cardiac injury and accelerating cardiac dysfunction.[86–88] Conversely, experimental induction of autophagy has favourable effects to minimize ischaemia-reperfusion injury, mitigate left ventricular remodelling and retard the evolution of cardiomyopathy related to diverse causes.[89–93]

Hypoxia-inducible factors and SIRT1 are important stimuli for the enhancement of autophagic flux; in doing so, their activation plays a protective role in preserving cellular health and viability and preventing maladaptive remodelling following cardiac injury.[94–98] Enhanced activity of HIF-1α and SIRT1 in cardiomyocytes promotes the autophagic clearance of damaged mitochondria, a process known as mitophagy.[97,98] Chronic hypoxia and ischaemic preconditioning in the myocardium stimulate HIF-1α and autophagy in parallel, and mitophagy is (in part) HIF-1α-dependent.[97,99] Additionally, the activation of SIRT1 stimulates HIF-2α,[79,80] which leads to enhanced clearance of damaged or dysfunctional peroxisomes, a process known as pexography.[100,101] Both SIRT1 and HIF-1α also act to directly preserve organellar function and integrity.[62,102] Mitochondria and peroxisomes are the major sources of reactive oxygen species and the injurious effects of oxidative stress in cardiomyocytes. Thus, the coordinated activation of HIFs and SIRT1—the key elements of the cellular response to oxygen and nutrient deprivation—is poised to ameliorate the organellar stress and cellular dysfunction seen in a broad range of myocardial disorders.

6. Role of HIF and SIRT1 signalling in the cardiac adaptation to stress

During embryonic development, HIF-1α protects the embryo from intrauterine hypoxia, but at the time of birth, normoxia suppresses HIFs as the heart switches from anaerobic to aerobic metabolism. The expression of HIFs remains low as long as the heart remains healthy, but if oxygen tension in the adult heart declines, HIFs are activated to facilitate oxygen delivery.[103] These transcription factors not only enhance the synthesis of erythropoietin but they also induce angiogenesis, and the development of a nurturing micro-circulation can increase oxygenation of hypertrophied or ischaemic myocardium. For these reasons, activation of HIF-1α and HIF-2α has been shown to ameliorate ischaemia-reperfusion injury in the heart,[76,104–107] whereas suppression of HIF-1α causes decompensation of experimental pressure overload or ischaemic states, which may be related to inadequate vascularization.[108] These observations demonstrate that activation of HIF-1α can have cardioprotective effects, at least in the short-term when oxygen tension is rapidly lowered, as in states of acute ischaemia.

However, several investigations have raised doubts about the cardioprotective benefits of HIF-1α when its activation is marked and sustained for long periods of time. HIF-1α maintains myocardial energy levels during hypoxia by shifting from oxidative to glycolytic metabolism; however, such an effect might limit cardiac performance, since glycolysis represents an inefficient mechanism of consuming oxygen in order to generate ATP.[109] Moreover, HIF-1α acts to impair mitochondrial biogenesis, thereby further limiting the generation of ATP needed to maintain cardiac performance.[108] Finally, HIF-1α (when activated by non-hypoxic stimuli) may play an important role in promoting inflammation in cardiomyocytes.[71,72] As a result of these mechanisms, marked, sustained, and isolated activation of HIF-1α leads to an impairment of cardiac performance. Specifically, prolonged pharmacological inhibition of the prolyl hydroxylases that is responsible for HIF-1α degradation (which stabilize HIF-1α at high levels) leads to worsening of cardiac function.[110] Similarly, marked activation produced by genetic overexpression of HIF-1α results in cardiomyopathy.[111]

6.1 Modulating influence of SIRT1 on the induction and actions of HIF-1α and HIF-2α during hypoxia or treatment with hypoxia-mimetic agents

However, experimental conditions that cause such extreme and isolated activation of HIF-1α may not be clinically relevant. During hypoxia or treatment with hypoxia-mimetic agents (e.g. cobalt chloride), the induction of HIFs is accompanied by coactivation of SIRT1 in the heart, liver, and endothelium.[82,112,113] The effects of SIRT1 on HIF-1α vary with the cell type,[114–117] but SIRT1 agonism inhibits HIF-1α in cardiomyocytes,[118] and SIRT1 opposes many of the actions of HIF-1α. SIRT1 counteracts the inhibitory effects of HIF-1α on oxidative metabolism, gluconeogenesis, and ketogenesis and on mitochondrial biogenesis and ATP production[57–61]; additionally, SIRT1 mutes activation of the inflammasome, thus negating the proinflammatory actions of HIF-1α.[63–69] SIRT1 also activates HIF-2α, further offsetting HIF-1α-triggered inflammatory pathways.[70–74]

Regardless of these counterbalancing effects on metabolism and inflammation, the coactivation of SIRT1 with HIFs promotes autophagy, and thus, acts to remove potentially dangerous constituents, thereby reducing oxidative and endoplasmic reticular stress and attenuating the resulting inflammatory response that leads to cellular dysfunction and death. Therefore, coordinated stimulation of HIFs and SIRT1 not only promotes erythropoiesis but it also mediates meaningful cardioprotective effects, thereby explaining the finding of a statistical linkage between erythrocytosis and the reduction in serious heart failure events.[36,37] Not surprisingly, the activities of HIF-2α and SIRT1 are reduced in various forms of cardiomyopathy. Suppression of these master regulators accelerates cardiac injury, and their induction ameliorates cardiomyocyte loss and dysfunction.[74,119–124]

7. Effects of SGLT2 inhibitors on HIFs, SIRT1, and autophagy and their link to cardioprotection

Because SGLT2 acts as a sensor of energy overabundance, there is an inverse relationship between SGLT2 and the activity of enzymes and transcription factors that are triggered by nutrient and oxygen deprivation. States of low-oxygen tension cause simultaneous down-regulation of SGLT2 but up-regulation of HIF-1α,[125] whereas high levels of renal tubular glucose promote the expression of SGLT2 but reduce the expression of SIRT1.[126]

7.1 Effect of SGLT2 inhibitors on SIRT1 and HIFs

Given these inverse relationships, it is not surprising that pharmacological inhibition of SGLT2 leads to activation of HIFs and SIRT1 (*Figure 1*). SGLT2 inhibitors have been shown to stimulate SIRT1 in a broad range of tissues, including the heart, kidney, and liver.[20,126–129] SIRT1-mediated activation of HIF-2α in the kidney can promote erythropoietin production in the interstitial fibroblasts that are the primary site of synthesis[79,130]; prolonged increases in HIF-2α activity enhances the growth of these specialized cells.[131] Additionally, activation of HIF-2α in the liver (also related to SIRT1 signalling) can contribute importantly to the production of erythropoietin.[132] The likelihood of SIRT1-mediated hepatic synthesis undermines the assumption that erythropoietin synthesis provoked by SGLT2 inhibitors is strictly limited to the renal medulla. Although these drugs have been hypothesized to provoke medullary hypoxia as a consequence of an action to increase oxygen consumption in distal tubular sites,[133,134] this response remains controversial. Furthermore, the isoform induced by SGLT2 inhibitors in the outer renal medulla in models of hypoxia/ischaemia appears to be HIF-1α, which is not the primary driver of erythropoietin synthesis.[135]

SGLT2 inhibitors have been reported to up-regulate HIF-1α in non-diabetic renal injury,[135,136] but down-regulate the isoform in the diabetic kidney[136]; yet, the effect of these drugs on HIF-1α in the heart has not yet been examined. If the activation of HIF-1α by SGLT2 inhibitors is confined to renal tissues, these drugs might not cause increases in cardiac HIF-1α that could excite inflammatory changes in the myocardium. Alternatively, if SGLT2 inhibitors were to be shown to enhance the activation of HIF-1α in the heart, any potential that HIF-1α would produce deleterious effects in cardiomyocytes might be negated by concomitant activation of SIRT1. In fact, it seems likely that, during treatment with SGLT2 inhibitors, the action of these drugs to promote SIRT1 signalling dominate and supersede any effects that might be related to the activation of HIF-1α. SGLT2 inhibition augments gluconeogenesis, fatty acid oxidation, and ketogenesis,[20,21,29,137,138] but these effects are explicable only by the actions of SIRT1,[58–60] and cannot be ascribed to HIF-1α.[54–57]

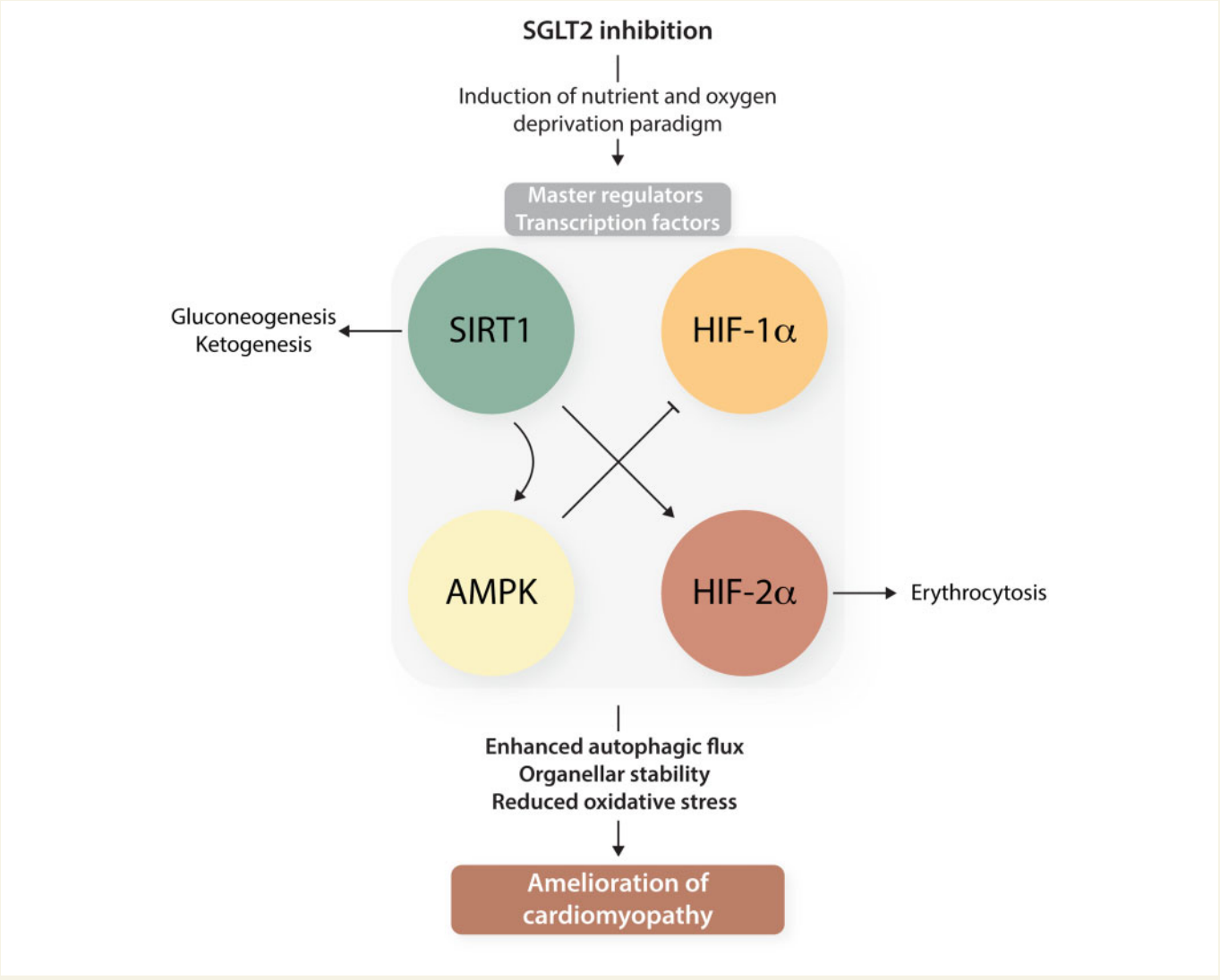

Figure 1 SGLT2 inhibitors may exert cardioprotective effects by inducing a nutrient- and oxygen-deprivation transcriptional paradigm. SGLT2, sodium-glucose co-transporter 2; SIRT1, sirtuin-1; HIF-1α, hypoxia-inducible factor isoform 1α; HIF-2α, hypoxia-inducible factor isoform 2α; AMPK, 5′-adenosine monophosphate-activated protein kinase.

Additionally, SGLT2 inhibitors modify macrophage polarization to promote an anti-inflammatory phenotype,[139,140] and they mute inflammation in the myocardium.[141] These actions are consistent with the activation of SIRT1, and they cannot be explained by HIF-1α.[63–68] Interestingly, anti-inflammatory changes in macrophage polarization are also seen with other hypoxia-mimetics (i.e. cobalt), but interestingly, these stimulate SIRT1 and HIF-2α in addition to HIF-1α.[82,142] Finally, SGLT2 inhibitors promote mitochondrial biogenesis,[21] an effect that reflects the actions of SIRT1 rather than those of HIF-1α, because the latter suppresses the formation of these organelles.[61,62]

7.2 Effect of SGLT2 inhibitors on other transcription factors and master regulators that can influence autophagic flux

It is possible that SGLT2 inhibitors may promote autophagy by mechanisms in addition to enhanced signalling through SIRT1 and HIFs. Activation of the Akt/mTOR pathway suppresses autophagic flux, and SGLT2 inhibitors may inhibit Akt/mTOR,[143] thereby enhancing autophagy.[144] Furthermore, SGLT2 inhibitors activate another energy deprivation sensor, adenosine monophosphate-activated protein kinase (AMPK),[29,141,143,145–148] which also acts to stimulate autophagy. The activity of both Akt/mTOR and AMPK in cardiomyocytes is sensitive to hypoxia.[149,150] It is, therefore, noteworthy that other hypoxia-mimetics (e.g. cobalt chloride) promote autophagy, an effect is accompanied by simultaneously inhibition of AKT/mTOR and activation of AMPK.[151]

There is considerable interdependence between SIRT1 and AMPK; these two enzymes can often mutually activate each other, and they share many common downstream targets[152]; in contrast, the effects of HIF-1α to promote autophagy are not mediated by AMPK.[153] Nevertheless, it seems unlikely that AMPK plays a dominant role in mediating the effects of most members of the SGLT2 inhibitor class of drugs since AMPK acts to suppress gluconeogenesis, ketogenesis, and erythrocytosis[154–156]; this profile of effects is opposite to that produced by most SGLT2 inhibitors.

7.3 The intriguing paradox of nutrient- and oxygen-deprivation signalling and autophagy stimulation in the absence of SGLT2 expression

How can SGLT2 inhibitors to promote SIRT1 signalling in the heart if SGLT2 is not expressed in the myocardium? SGLT2 inhibitors have profound effects in many organs that do not express SGLT2 or bind SGLT2 inhibitors,[4,5,157] including cardiac and skeletal muscle, liver, and adipose depots.[20,158–161] How does inhibition of SGLT2 influence the structure and function of these tissues? SGLT2 inhibition induces loss of calories in the urine, which (by reducing glucose levels in the tissues) triggers a fasting-like transcriptional paradigm in organs throughout the body.[143] Activation of SIRT1 is a cornerstone of the organismal response to starvation (since SIRT1 serves to maintain blood glucose),[49] and thus, glycosuria (and its effects on environmental glucose) activates SIRT1 systemically; this activation occurs in all tissues, regardless of whether they express SGLT2.[20] Additionally, SGLT2 may act as a central sensor for the energy state of the organism, and thus, suppression of its activity can stimulate SIRT1 even if there is no actual loss of calories in the urine.[125,126] By virtue of either or both mechanisms, nutrient-deprivation signalling provoked by SGLT2 inhibition can activate HIFs and AMPK (in addition to SIRT1) in diverse organs.

The concerted systemic activation of multiple master regulators involved in nutrient- and oxygen-deprivation signalling likely underlies the ability of empagliflozin, dapagliflozin, and canagliflozin to promote autophagic flux in many organs, including the heart,[145,162–164] both in diabetic and in non-diabetic rodent models. Such an action may explain why SGLT2 inhibitors act to reduce oxidative stress, normalize mitochondrial structure and function, and mute the activity of the NLRP3 inflammasome in the stressed myocardium.[21,141,162,165] Enhanced autophagy may also account for the effect of SGLT2 inhibitors to ameliorate ischaemia-reperfusion injury and post-infarction remodelling and to minimize micro-vascular dysfunction, hypertrophy and fibrosis, enhance contractile performance, and ameliorate the course of experimental cardiomyopathy.[21,92,93,158,159,165,166]

7.4 Influence of SIRT1/HIF-1α/HIF-2α signalling on sodium transporters and intracellular sodium concentration

It has recently been hypothesized that SGLT2 inhibitors may reduce the risk of serious heart failure events by an effect to inhibit ion channels or exchangers that mediate an increase in intracellular sodium, with its attendant adverse consequences on cardiomyocyte survival.[167,168] SGLT2 inhibitors decrease the cytosolic concentration of sodium, but they have not yet been shown to exert an inhibitory action on a specific sodium influx mechanism (i.e. the sodium-hydrogen exchanger).[169] It is therefore noteworthy that there exists an inverse relationship between SIRT1/HIF-1α/HIF-2α signalling and many transport mechanisms that mediate sodium influx into cells. SIRT1 overexpression leads to inhibition of the epithelial sodium channel,[170] and conversely, overexpression of this channel leads to suppression of SIRT1.[171] In parallel, activation of HIF-1α/HIF-2α by the hypoxia-mimetic cobalt leads to inhibition of NHE1,[172] which can also be suppressed by activation of AMPK.[148] Inhibition of Akt/mTOR signalling causes inhibition of NHE3,[173] an effect that is mediated by enhanced autophagy. Therefore, the action of SGLT2 inhibitors to promote nutrient-deprivation signalling may explain the ability of these drugs to lower sodium concentrations in cardiomyocytes, thus contributing to their cardioprotective effects.[168]

8. Effects of other antihyperglycaemic drugs on HIFs, SIRT1, and autophagy

Since autophagy is activated in response to nutrient deprivation, drugs that lower glucose may increase autophagic flux, especially in hyperglycaemic states. Transcriptionally, Type 2 diabetes presents itself as a state of nutrient overabundance, and autophagy is suppressed.[174] However, autophagic flux is increased in Type 1 diabetes, suggesting that it is the hyperinsulinaemia of Type 2 diabetes—rather than the hyperglycaemia—that causes down-regulation of autophagy under conditions of glucose intolerance.[159] It is therefore noteworthy that insulin itself suppresses autophagic flux, potentially by inhibiting SIRT1 or activating Akt/mTOR signalling.[175,176]

Consequently, it is difficult to interpret experimental studies that report an effect of antihyperglycaemic drugs on autophagy, since their net result may not only be mediated by an action of glucose-lowering to promote autophagy but by an effect of augmented insulin signalling or other actions to suppress autophagy. Glucagon-like peptide-1 (GLP-1) receptor agonists have been reported to increase autophagy by enhancing activation of AMPK/SIRT1,[177,178] but these drugs are not hypoxia-mimetics, and in fact, hypoxia and HIF-1α signalling act to reduce GLP-1 secretion.[179,180] Furthermore, GLP-1 receptor agonists promote the secretion of insulin and its suppressive effects on autophagy.[176] Analogously, dipeptidyl peptidase 4 inhibitors have been reported to promote autophagy,[181] but these drugs also enhance CXCR4 chemokine signalling,[182] which decreases autophagic flux.[183] Finally, although thiazolidinediones can stimulate AMPK in the heart,[184] their actions to promote insulin signalling and sodium influx can increase the risk of serious heart failure events.[185]

8.1 Effect of metformin on autophagy and its potential interaction with SGLT2 inhibitors

In contrast to antihyperglycaemic drugs that act by promoting the secretion or action of insulin, metformin reduces insulin secretion, and the drug enhances autophagy through its ability to function as an agonist of AMPK in diabetic and non-diabetic hearts under stress.[91,144,186,187] However, metformin does not appear to have meaningful effects to stimulate SIRT1 or HIFs. Metformin inhibits gluconeogenesis and ketosis[188] (which are activated by SIRT1 signalling[58] and starvation[189]), and the drug decreases the haematocrit[190]—presumably because its stimulatory action on AMPK suppresses HIF-1α—an effect opposite to that of SIRT1.[191] Therefore, despite its effect to activate AMPK, metformin does not evoke a state of fasting and hypoxia mimicry or lead to broad-based up-regulation of enzymes and transcription factors that involved in the response to nutrient and oxygen deprivation. Interestingly, there is little evidence that isolated AMPK activation with metformin has favourable effects on the development of heart failure in the clinical setting.[192] Therefore, the well-established effects of SGLT2 inhibitors to reduce serious heart failure events may be related to their ability to promote a wide-ranging enhancement of master regulators that are responsible for pattern of fasting and hypoxia mimicry.

Nevertheless, it is noteworthy that metformin and SGLT2 inhibitors share an ability to stimulate AMPK in the myocardium. The meaningfulness of this overlap is supported by the observation that the magnitude of the benefit of SGLT2 inhibitors on serious heart failure events in large-scale trials may be attenuated in patients receiving metformin. In the CANVAS trials, canagliflozin reduced the risk of cardiovascular death or hospitalization for heart failure by 36% in patients not receiving metformin, but by only 12% in those receiving metformin (interaction $P = 0.03$).[193] In the EMPA-REG OUTCOME trial, empagliflozin reduced the risk of cardiovascular death by 54% in patients not receiving metformin, but by 29% in those receiving metformin (interaction $P = 0.07$).[194] The benefit in metformin users was still meaningful in the trial that evaluated empagliflozin, but was not in the trial that studied canagliflozin. The greater metformin-related attenuation seen in the CANVAS trials may be related to the greater and more direct AMPK activation produced by canagliflozin than by empagliflozin.[146,147]

9. Synthesis and clinical implications

SGLT2 inhibitors produce erythrocytosis, which has led investigators to propose that these effects increase oxygen delivery to the failing heart. However, it seems more likely that erythrocytosis is a biomarker of an action of SGLT2 inhibitors to induce a hypoxia- and fasting-like transcriptional paradigm, which involves the activation of SIRT-1 and HIF-2α signalling. Transactivation of these downstream mediators not only promotes the synthesis of erythropoietin but it ameliorates cardiomyocyte inflammation and dysfunction, either directly or by stimulating the autophagic clearance of damaged organelles (mitochondria and peroxisomes). This conceptual framework can explain the strong statistical linkage between erythrocytosis and the reduction in serious heart failure events observed in large-scale randomized controlled trials with SGLT2 inhibitors.

Conflict of interest: Dr Packer has recently consulted for Abbvie, Actavis, Akcea, Amgen, AstraZeneca, Boehringer Ingelheim, Cardiorentis, Daiichi Sankyo, Johnson & Johnson, NovoNordisk, Pfizer, Sanofi, Synthetic Biologics, and Theravance.

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