Cardiac effects of SGLT2 inhibitors: the sodium hypothesis

Edoardo Bertero¹,²*, Leticia Prates Roma¹, Pietro Ameri², and Christoph Maack¹*

¹Clinic for Internal Medicine III, University of the Saarland, Homburg 66421, Germany; and ²Cardiology Unit, IRCCS Policlinic Hospital San Martino & Department of Internal Medicine, University of Genova, Genova 16132, Italy

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Abstract

The effects of intense glycaemic control on macrovascular complications in patients with type 2 diabetes are incompletely resolved, and many glucose-lowering medications negatively affect cardiovascular outcomes. Recently, the EMPA-REG OUTCOME trial revealed that empagliflozin, an inhibitor of the sodium-glucose cotransporter 2 (SGLT2), substantially reduced the risk of hospitalization for heart failure, death from cardiovascular causes, and all-cause mortality in patients with type 2 diabetes mellitus at high cardiovascular risk. Although several mechanisms may explain this benefit, plasma volume contraction and a metabolic switch favouring cardiac ketone bodies oxidation have recently been proposed as the major drivers. Recent experimental work has prompted a novel and intriguing hypothesis, according to which empagliflozin may reduce intracellular sodium (Na⁺) load observed in failing cardiac myocytes by inhibiting the sarcolemmal Na⁺/H⁺ exchanger. Since elevated intracellular Na⁺ hampers mitochondrial Ca²⁺ handling and thereby, deteriorates energy supply and demand matching and the mitochondrial antioxidative defence systems, empagliflozin may positively affect cardiac function by restoring mitochondrial function, and redox state in the failing heart. Here, we review the current evidence for such a third mechanistic hypothesis, which may foster heart failure and diabetes research into a new direction which harbours several potential targets for therapeutic intervention.

Keywords

Empagliflozin • EMPA-REG OUTCOME • Diabetes • Heart failure • Sodium • Mitochondria

1. Introduction

Diabetes mellitus carries a high risk of heart failure (HF) and portends a dismal prognosis in patients with established HF. In particular, the risk of developing HF is nearly doubled in individuals with type 2 diabetes mellitus, and the relative risk increase is almost 9-fold for diabetic patients aged 45–54 years compared to non-diabetics.¹,² Furthermore, patients with both diabetes and HF have a nearly doubled mortality rate compared to non-diabetic HF patients³ and their median survival was reported to be 4 years.⁴ Although hyperglycaemia is a strong risk factor for microvascular complications associated with diabetes, the consequences of intensive glycaemic control on macrovascular complications are still debated. In fact, various glucose-lowering drugs increase the risk of hospitalization for HF,⁵,⁶ and hypoglycaemia may worsen cardiovascular outcome due to activation of inflammatory cytokines and neurohormonal imbalance.⁷ Therefore, clinical trials demonstrating the cardiovascular safety of antihyperglycaemic drugs have been advocated by both the US Food and Drug Administration and the European Medicines Agency.

In this setting, the Empagliflozin, Cardiovascular Outcomes, and Mortality in Type 2 Diabetes (EMPA-REG OUTCOME) trial, in which the sodium-glucose cotransporter (SGLT)2 inhibitor empagliflozin (EMPA) was added on top of standard therapy in patients with type 2 diabetes and established cardiovascular disease, reported substantial risk reductions of hospitalization for HF (35% relative risk reduction), death from cardiovascular causes (38%), and all-cause mortality (32%). Of note, the cumulative incidence curves of EMPA and placebo separated already 3 months after randomization, and this was independent of atherothrombotic events (stroke and myocardial infarction).⁸ Furthermore, EMPA effects on HF hospitalization, cardiovascular death and all-cause mortality were consistent in patients with or without HF at baseline.⁹ Interestingly, the effect of a different SGLT2-inhibitor, canagliflozin, on death from cardiovascular causes (13%) as reported by the recent CANVAS Program, was less pronounced than the effect of EMPA in EMPA-REG OUTCOME despite similar reductions of HbA1c, body weight, or blood pressure, suggesting that the mechanisms of benefit in EMPA-REG may not be limited to mere SGLT2 inhibition.¹⁰
The pathophysiological mechanisms underlying the remarkable and—at least to this extent—unexpected results of the HF outcome in the EMPA-REG OUTCOME trial are unresolved. SGLT2 inhibition affects visceral adiposity, glycaemic control, hyperinsulinaemia, blood pressure, arterial stiffness, and albuminuria, but these effects have been generally considered insufficient to account for the precocity and magnitude of the outcome improvement observed with EMPA treatment. Although this latter most likely results from an interplay of beneficial effects, two mechanisms have been proposed as major drivers: the diuretic effect associated with SGLT2 inhibition with subsequent hemodynamic unloading of the left ventricle, and a shift in cardiac metabolism secondary to increased ketogenesis, coined the ‘diuretic’ and ‘thrifty substrate’ hypotheses, respectively. Here, we critically review experimental and clinical evidence supporting these two hypotheses and propose an additional possible mechanism through which EMPA may ameliorate HF, i.e. by directly restoring cardiac ion homeostasis in failing cardiac myocytes.

2. The diuretic hypothesis

The SGLTs are a family of proteins responsible for glucose translocation in a variety of tissues. The most extensively studied members of this family are SGLT1, which is expressed in small intestine, kidneys, liver, lungs, and heart capillaries in mice and in human cardiac myocytes, and SGLT2, primarily found in the kidney, but also expressed by pancreatic alpha cells. In the kidney, SGLT2 is located on the apical membrane of the epithelium of the first part of the proximal tubule, where it mediates reabsorption of 80–90% of filtered glucose, whereas the remaining 10–20% of glucose is taken up by SGLT1 in the distal segments of the proximal tubule. Both SGLT2 and SGLT1 cotransport Na+ together with glucose; hence, their activity is tightly coupled to the extrusion of Na+ by the Na+/K+ ATPase on the basolateral membrane, which generates a Na+ gradient between the tubular lumen and the cell and thus produces the driving force for glucose cotransport. The maximal glucose reabsorptive capacity of the healthy kidney is 375 mg/min, and therefore, a plasma glucose concentration of 180 mg/dl or more is required to produce glucosuria. In patients with type 2 diabetes, this threshold is remarkably increased, possibly as a consequence of increased SGLT2 activity. However, controversial results exist concerning SGLT2 expression in type 2 diabetes: whereas a first study reported an increase in SGLT2 expression and activity in exfoliated tubular cells isolated from the urine of diabetic individuals, two independent groups recently observed a reduction in SGLT2 expression in renal samples obtained from patients with type 2 diabetes, suggesting that SGLT1 may play a compensatory role.

SGLT2 inhibition primarily results in reduced reabsorption of glucose and Na+ in the proximal tubule. SGLT2 inhibitors are radically different from other glucose-lowering strategies as they directly affect the total glucose pool, which is completely independent of insulin. Furthermore, because of their unique mechanism of action, SGLT2 inhibitors do not increase the risk of hypoglycaemia, and therefore, can be combined with other glucose-lowering medications. Both natriuresis and the osmotic effect of glucosuria contribute to the diuretic effect of SGLT2 inhibitors with subsequent plasma volume contraction. Although natriuresis induced by SGLT2 inhibitors is transient, the sustained increase in hematocrit and the decrease in systolic (5–6 mmHg) and diastolic (1–2 mmHg) blood pressure observed in the EMPA-REG OUTCOME trial may be the result of a persistent volume depletion. Due to the outcome improvement in the EMPA-REG OUTCOME trial and its independence of thrombotic events, the EMPA diuretic effect is thought to be a major driver of its cardioprotective activity.

3. The ‘thrifty substrate’ hypothesis

The human heart derives 95% of its energy from mitochondrial oxidative phosphorylation, which under normal conditions is fuelled by fatty acids (FAs) (70%) and glucose (30%). The metabolic flexibility of the heart, i.e. its ability to switch among different substrates as energy sources, is crucial to maintain a constant ATP production in response to variations of workload and substrate availability. For instance, increased workload or β-adrenergic stimulation lead to a shift towards glucose oxidation, whereas FAs are the preferred substrates during prolonged fasting.

In the diabetic heart, the relative contribution of FAs to oxidative metabolism is dramatically increased (Figure 1). This metabolic switch is mainly a consequence of peripheral insulin resistance, resulting in dysregulated lipolysis and increased delivery of FAs to the heart. In fact, myocardial FA uptake mainly depends on FA concentrations in the blood, and their oxidation in cardiac myocytes inhibits glucose uptake through the Randle cycle. In addition, a more long-term switch towards FA oxidation is induced by activation of peroxisome proliferator-activated receptor alpha (PPAR-α), a nuclear transcription factor which enhances the expression of several key genes involved in FA metabolism. As a result, the diabetic heart is locked in a metabolically inflexible state, characterized by an almost complete reliance on FAs to fuel ATP production. On one hand, FA oxidation yields the highest amount of energy per mole (298 kcal/mol of 2-carbon unit for palmitate) compared to glucose (223.6 kcal/mol) and β-hydroxybutyrate oxidation (243.6 kcal/mol), and thus enhancing FA oxidation increases the delivery of reducing equivalents (NADH and FADH₂) to the electron transport chain. On the other hand, in the diabetic heart, the relative contribution of FAs to oxidative metabolism is dramatically increased (Figure 1). This metabolic switch is mainly a consequence of peripheral insulin resistance, resulting in dysregulated lipolysis and increased delivery of FAs to the heart. In fact, myocardial FA uptake mainly depends on FA concentrations in the blood, and their oxidation in cardiac myocytes inhibits glucose uptake through the Randle cycle. In addition, a more long-term switch towards FA oxidation is induced by activation of peroxisome proliferator-activated receptor alpha (PPAR-α), a nuclear transcription factor which enhances the expression of several key genes involved in FA metabolism. As a result, the diabetic heart is locked in a metabolically inflexible state, characterized by an almost complete reliance on FAs to fuel ATP production. On one hand, FA oxidation yields the highest amount of energy per mole (298 kcal/mol of 2-carbon unit for palmitate) compared to glucose (223.6 kcal/mol) and β-hydroxybutyrate oxidation (243.6 kcal/mol), and thus enhancing FA oxidation increases the delivery of reducing equivalents (NADH and FADH₂) to the electron transport chain. On the other
Figure 1 Increased fatty acid oxidation in the diabetic heart and the ‘thrift substrate’ hypothesis. In the diabetic heart (blue field), the relative contribution of fatty acids to oxidative metabolism is dramatically increased. Compared to glucose oxidation, fatty acid oxidation is less energetically efficient and leads to myocardial accumulation of triacylglycerols (TAG), increased production of reactive oxygen species, mitochondrial uncoupling and altered cellular ATP translocation from the mitochondria to the cytosol. Expression of uncoupling proteins 2 and 3 (UCP 2/3) is correlated to plasma fatty acid concentrations and may contribute to decreased energetic efficiency by producing a proton leak across the inner mitochondrial membrane (IMM). According to the ‘thrift substrate’ hypothesis (yellow field), the hyperketonemic state associated with Empagliflozin (EMPA) treatment may provide cardiac myocytes with a more energetically efficient substrate, i.e. β-hydroxybutyrate (BHOB), and shift cardiac metabolism away from fatty acid intermediates denoted as lipotoxicity, and may contribute to cardiac dysfunction.

hand, there is general agreement that such dominance of FA oxidation negatively affects cardiac efficiency: first, FA oxidation is energetically less efficient than glucose oxidation, since the ATP production/O₂ consumption ratio (P/O) slightly favours glucose (P/O = 2.53) over FAs (2.33). Furthermore, excessive FA oxidation has been linked to an excess formation of reactive oxygen species (ROS), altered cellular ATP translocation from mitochondria to the cytosol, increased mitochondrial uncoupling, and energy expenditure due to futile cycling of FA intermediates. Finally, despite increased oxidation, FA delivery to the heart exceeds the demand of substrates for ATP production, giving rise to myocardial accumulation of lipids. The disruption of normal cellular signalling associated with lipid accumulation may result in apoptosis of cardiac myocytes, a process denoted as lipotoxicity, and may contribute to cardiac dysfunction.

The ‘thrift substrate’ or ‘fuel’ hypothesis was proposed independently by Ferrannini et al. and Mudaliar, who hypothesized that the removal of large amounts of glucose from the body and the subsequent reduction of the insulin/glucagon ratio associated with EMPA treatment may boost lipid mobilization and oxidation in the liver, hence stimulating ketogenesis (Figure 1). The resulting metabolic condition, characterized by a mildly hyperketonemic state akin to prolonged fasting, leads to avid myocardial uptake of β-hydroxybutyrate, the main ketone body, which competes with FA oxidation. This substrate shift is deemed to be cardioprotective because of the increased metabolic efficiency of ketone bodies oxidation, which may act as a ‘superfuel’ for the heart. In fact, the P/O ratio favours ketone bodies (P/O = 2.50) over FAs (2.33) and, although the P/O ratios of glucose and ketone bodies (2.53) do not differ significantly, β-hydroxybutyrate has a higher inherent energy compared to pyruvate because it is more reduced than the latter. The hemoconcentration resulting from SGLT2 inhibition may act synergistically with the metabolic substrate shift by enhancing O₂ delivery. Furthermore, the use of ketone bodies for ATP generation may be beneficial also by counteracting the detrimental effects of a disproportional reliance on FA oxidation (Figure 1). Finally, β-hydroxybutyrate has recently been linked to in vitro antioxidative and antiarrhythmic properties. To substantiate this hypothesis, an increase in both lipolysis and ketogenesis following SGLT2 inhibition has been observed in both animal models and human studies. Remarkably, the heart is the highest consumer of ketone bodies per unit mass and the ability of the heart to extract and oxidize ketone bodies is not impaired in HF patients. In fact, mitochondrial proteomics and metabolomic profiling performed in a mouse model of HF suggested that ketone bodies may represent a significant source of energy in the failing heart.

However, as pointed out in an insightful comment by Lopaschuk and Verma, several caveats exist concerning the ‘thrift substrate’ hypothesis. First, the mechanism underlying the increase in β-hydroxybutyrate levels in EMPA-treated patients is not resolved, nor is the time course of the hyperketonemic state associated with this drug. In fact, it cannot be ruled out that EMPA increases β-hydroxybutyrate by inhibiting its oxidation in the heart and skeletal muscle. Furthermore, there is no indisputable evidence that the switch toward ketone bodies oxidation is in fact beneficial to the failing heart. The causal relationship between the enhancement of myocardial metabolic efficiency and improved cardiac outcomes relies on the paradigm according to which the failing heart is an ‘engine out of fuel’, which has recently been challenged: genetically modified mice with whole-body creatine deficiency displayed unaltered contractility at baseline and normal recovery after myocardial infarction, suggesting that the metabolic efficiency required for maximal exercise.
capacity is not essential for the chronic stress response in the heart, and therefore, boosting myocardial metabolism in the failing heart with a 'superfuel' for the sake of just increasing ATP would not automatically translate into an improvement in cardiac function.48,49 Finally, even provided that this metabolic switch was cardioprotective, the increased metabolic efficiency should be beneficial in the setting of both HF and ischemia, but this is not consistent with the EMPA-REG OUTCOME results, where these two endpoints were differentially affected.8,12 Therefore, studies addressing time course, magnitude and correlation to cardiovascular outcomes of SGLT2-induced hyperketonemia are warranted to further interrogate the 'thirsty substrate' hypothesis. Remarkably, addressing these questions will also contribute to clarify whether enhancing myocardial metabolic efficiency is a viable therapeutic strategy in the setting of HF.

4. The sodium hypothesis

Since the SGLT2 cotransports glucose with sodium (Na$^+$), SGLT2 inhibition induces not only glycosuria, but also natriuresis. Therefore, another—and potentially less anticipated—effect of SGLT2 inhibition is that tissue and cellular Na$^+$ homeostasis may be affected by the drug. In cardiac myocytes, Na$^+$ plays a key role for excitation-contraction coupling and mitochondrial redox regulation.50,51 As mentioned above, the heart mainly relies on mitochondrial oxidative metabolism to produce energy in the form of ATP. In particular, the reducing equivalents (NADH and FADH$_2$) produced by the Krebs cycle donate their electrons to complexes I and II of the electron transport chain, whose redox reactions are coupled with the translocation of H$^+$ to the mitochondrial intermembrane space (Figure 2). The proton motive force generated by H$^+$ translocation drives ADP phosphorylation at the F$_{1}$/F$_{0}$-ATP synthase, regenerating ATP. In response to an increase in energy requirements, the rise in ADP stimulates ATP production at the F$_{1}$/F$_{0}$-ATP synthase, thereby oxidizing NADH to NAD$^+$; simultaneously, β-adrenergic stimulation increases cytosolic Ca$^{2+}$ transients, which increases mitochondrial Ca$^{2+}$ uptake via the mitochondrial Ca$^{2+}$ uniporter (MCU). In mitochondria, Ca$^{2+}$ then activates Krebs cycle dehydrogenases to regenerate NADH. Thus, physiological workloads act on mitochondrial respiration and Krebs cycle in a coordinate fashion to preserve constant ratios of ATP/ADP and NADH/NAD$^+$, a process referred to as ‘parallel activation’.52 Furthermore, NADH and the Krebs cycle products malate and isocitrate are crucial to regenerate NADPH, which plays a pivotal role in oxidative defence by maintaining reduced glutathione, thioredoxin, and glutaredoxin pools. Therefore, mitochondrial Ca$^{2+}$ uptake is not only crucial for matching energy supply to demand, but also for preserving mitochondrial antioxidant capacity.53

Ca$^{2+}$ handling in cardiac myocytes is tightly intertwined with Na$^+$ handling via the activity of the sarcosomal and mitochondrial Na$^+$/Ca$^{2+}$ exchangers (NCX and NCLX, respectively). Under physiological conditions, the cardiac NCX almost exclusively functions in the forward mode, i.e. extruding Ca$^{2+}$ to the extracellular space, but its activity can be reversed in the early phase of the action potential depending on the membrane potential and the Na$^+$ and Ca$^{2+}$ transmembrane gradients.54 The NCLX is primarily responsible for Ca$^{2+}$ extrusion from mitochondria, but its kinetics are slower compared to Ca$^{2+}$ uptake via the MCU. This kinetic difference accounts for the aforementioned mitochondrial Ca$^{2+}$ accumulation following an increase in the rate and amplitude of cytosolic Ca$^{2+}$ transients, eventually boosting the Krebs cycle.

Heart failure is associated with an impairment in both Ca$^{2+}$ and Na$^+$ handling within cardiac myocytes (Figure 2). In particular, failing cardiac myocytes display a decreased amplitude and velocity of cytosolic Ca$^{2+}$ transients and increased diastolic cytosolic Ca$^{2+}$ ([Ca$^{2+}$]$_i$) and Na$^+$ concentrations ([Na$^+$]$_i$). The predominant defect in Ca$^{2+}$ handling is the reduced Ca$^{2+}$ load of the sarcoplasmic reticulum (SR), which results from decreased Ca$^{2+}$ uptake by the sarco/endoplasmic reticulum Ca$^{2+}$-ATPase (SERCA) and Ca$^{2+}$ leak from ryanodine receptors.55,56 Furthermore, expression and activity of the NCX are increased,58,59 partly compensating the reduction in SERCA activity, but likely contributing to the depletion of SR Ca$^{2+}$ load by exporting more Ca$^{2+}$ to the extracellular space. In addition, because of the privileged communication between SR and mitochondria, a reduced Ca$^{2+}$ release from the SR reduces mitochondrial Ca$^{2+}$ uptake and steady-state mitochondrial Ca$^{2+}$ ([Ca$^{2+}$]$_{i,m}$).60

An important discovery in the early to mid 2000’s was that [Na$^+$]$_i$ is increased in failing cardiac myocytes, mainly as a consequence of (i) increased Na$^+$ influx via the late Na$^+$ current,61 (ii) increased activity of the sarcolemmal Na$^+$/H$^+$ exchanger (NHE),61 and/or (iii) a reduction in Na$^+$ /K$^+$ ATPase activity.62 Moreover, (iv) SGLT1 expression in the heart is upregulated both in animal models of type 2 diabetes and in cardiac tissue harvested from patients with diabetic cardiomyopathy, and its activity contributes to the increase in [Na$^+$]$_i$.63,64 In principle, intracellular Na$^+$ overload may have positive effects on cytosolic Ca$^{2+}$ handling, as it prevents NCX from extruding Ca$^{2+}$ during diastole and stimulates the reverse-mode function of this exchanger during the action potential, thereby promoting more trans-sarcoclemmal Ca$^{2+}$ influx with enhanced SR Ca$^{2+}$ load and amplitude of cytosolic Ca$^{2+}$ transients.61

Metabolically, however, an increase in [Na$^+$]$_i$, may have adverse effects. As described above, mitochondrial Ca$^{2+}$ uptake plays a key role for mitochondrial energy supply-and-demand matching and to keep the antioxidative capacity in a reduced state to prevent oxidative stress. Since Ca$^{2+}$ is exported out of mitochondria into the cytosol by a Na$^+$/Ca$^{2+}$ exchanger (i.e. the NCLX), an increase in cytosolic Na$^+$ increases the driving force for mitochondrial Ca$^{2+}$ extrusion. In fact, we observed that in isolated cardiac myocytes from guinea pigs with or without HF, elevation of [Na$^+$]$_i$ hampers mitochondrial Ca$^{2+}$ accumulation during physiological workload transitions. The reduction in [Ca$^{2+}$]$_{i,m}$ impairs Ca$^{2+}$-induced stimulation of Krebs cycle dehydrogenases, hindering regeneration of NADH and NADPH.60 While NADH is required for ATP production, NADPH is a key mediator of the mitochondrial antioxidative defence, since superoxide generated by the electron transport chain is rapidly dismutated to hydrogen peroxide (H$_2$O$_2$), which in turn is eliminated by enzymes that are continuously regenerated by NADPH, i.e. glutathione peroxidase, peroxiredoxin, and glutaredoxin. Thus, elevated [Na$^+$]$_i$, ultimately increases mitochondrial ROS emission,53 which may further aggravate intracellular Na$^+$ overload by activating the late sodium Na$^+$ current.51 Furthermore, oxidative stress increases the vulnerability of the heart to neurohormonal hyperactivation and arrhythmias,65 and ROS are also involved in signalling pathways promoting hypertrophy, fibrosis and cell death.66

On this basis, counteracting intracellular Na$^+$ overload and restoring Na$^+$ handling in failing cardiac myocytes was observed to improve mitochondrial energetics and oxidative defence, and this approach is considered a promising therapeutic strategy for HF. In a guinea pig model of HF induced by pressure overload and chronic sympathetic stimulation, increasing [Ca$^{2+}$]$_i$, by inhibiting the NCLX prevented oxidation of the NAD(P)H pools and decreased mitochondrial ROS emission. On a whole heart level, NCLX inhibition blunted cardiac hypertrophic
remodelling, preserved contractile function and decreased the incidence of ventricular arrhythmias.  

With regard to SGLT2 inhibition, a direct effect of EMPA on cardiac myocytes was initially considered unlikely because most studies could neither observe the expression of SGLT2 in rodent nor in human heart. Although one group reported low levels of SGLT2 mRNA expression in human cardiac samples, this observation was subsequently ascribed to technical issues, and a recent study found no SGLT2 protein expression in human hearts, neither in healthy subjects nor under pathologic conditions. However, Baartscheer et al. recently reported that EMPA reduced [Na⁺], and [Ca²⁺], in isolated ventricular myocytes. Interestingly, the EMPA effect was independent of the presence of glucose and largely abolished after incubation with the NHE inhibitor caripriodine, suggesting that EMPA inhibits the NHE. Finally, consistent with the effect of [Na⁺], on the activity of NCLX, EMPA also increased [Ca²⁺]. Although evidence of a direct EMPA action on ion handling is limited to in vitro experiments, Habibi et al. recently documented a recovery in diastolic dysfunction in diabetic db/db mice treated with EMPA for 5 weeks. In this study, cardiac ion handling was not directly evaluated, but a reduction in myocardial expression of the serum- and glucocorticoid-inducible kinase 1 (SGK1) was observed, whose activity is known to modulate NHE activity via Akt-dependent signaling, providing additional mechanistic through which EMPA may restore cardiac [Na⁺], in a more long-term fashion. Finally, as assessed by Na⁺ magnetic resonance imaging, diabetic patients treated with dapagliflozin display a decreased Na⁺ tissue content of the skin, which was shown to correlate with left ventricular mass in patients with chronic kidney disease.

Na⁺/H⁺ exchanger is upregulated in HF. This increase in NHE expression has been attributed to increased conversion of pyruvate to lactate, causing intracellular acidosis. Although this metabolic derangement has not been associated with diabetes, increased NHE activity was reported in several animal models of type 2 diabetes. In this setting, decreasing [Na⁺], by NHE inhibition with caripriodine was cardioprotective. In particular, in the db/db mouse model of ischemia/reperfusion, caripriodine reduced the rise in [Na⁺], at the end of ischemia and prevented ventricular tachycardia from degenerating into ventricular fibrillation during reperfusion, underscoring the role of elevated [Na⁺], in creating a favourable substrate for ventricular arrhythmias. In another work, based on the Goto–Kakizaki rat model of type 2 diabetes, cardiac myocytes displayed enhanced NHE activity resulting in intracellular Na⁺ overload. This animal model is notable in that rats do not develop hypertension, obesity or hyperlipidemia, thus eliminating the confounding effects associated with these conditions. In this setting, the increase in [Na⁺], was associated with the development of left ventricular hypertrophy, most likely via activation of the intracellular kinase Akt. Caripriodine treatment decreased both [Na⁺], and Akt activation and reversed the hypertrophic phenotype.

In light of these works, it is tempting to speculate that the positive results of the EMPA-REG OUTCOME trial may be—at least in part—related to direct effects of EMPA on cardiac ion homeostasis. By decreasing [Na⁺], and restoring mitochondrial Ca²⁺ handling, EMPA may ameliorate mitochondrial energetic mismatch and production of ROS, thus interrupting the vicious circle which underlies Na⁺ overload and oxidative stress in cardiomyocytes. This strategy has already proven effective in preserving myocardial contractile function in animal

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**Figure 2** The sodium hypothesis. Failing cardiac myocytes display an elevated intracellular Na⁺ concentration as a result of (i) increased late Na⁺ current (Iₙ,ᵢ), (ii) increased activity of the sarcolemmal Na⁺/H⁺ exchanger (NHE), (iii) reduced Na⁺/K⁺ ATPase (NKA) activity and, in diabetic hearts, (iv) increased expression and activity of the Na⁺/glucose cotransporter 1 (SGLT1). Intracellular Na⁺ overload accelerates Ca²⁺ efflux from mitochondria via the mitochondrial Na⁺/Ca²⁺ exchanger (NCLX). The reduction in mitochondrial matrix Ca²⁺ concentration impairs Ca²⁺-induced stimulation of Krebs cycle dehydrogenases during workload transitions, thereby hindering regeneration of reducing equivalents which are required for matching energy supply to demand and preserving antioxidative capacity. Empagliflozin (EMPA) may have a positive impact on failing cardiac myocytes by correcting Na⁺ handling via NHE inhibition. ADP, adenosine diphosphate; ATP, adenosine triphosphate; ETC, electron transport chain; MCU, mitochondrial Ca²⁺ unipporter; NAD⁺/NADH, nicotinamide adenine dinucleotide oxidized/reduced; NCX, sarcolemmal Na⁺/Ca²⁺ exchanger; NKA, Na⁺/K⁺ ATPase; IMM, inner mitochondrial membrane; OMM, outer mitochondrial membrane; RyR, ryanodine receptor; SERCA, sarcoplasmic reticulum Ca²⁺ ATPase.
models of HF and diabetes, where increasing [Ca\(^{2+}\)]\(_{\text{m}}\) also reduced the incidence of ventricular arrhythmias and sudden cardiac death.\(^6\),\(^7\),\(^8\) This is consistent with the results of the EMPA-REG OUTCOME trial, in which the reduced incidence of sudden cardiac death in the EMPA-treated group (1.1% vs. 1.6%) contributed to the overall risk reduction of death for cardiovascular causes. A possible caveat concerning this hypothesis is that, as previously mentioned, in the EMPA-REG OUTCOME trial, HF and ischemia were affected to a different extent, whereas animal models predict that NHE inhibition should prove beneficial also in the setting of myocardial ischemia.\(^6\) Finally, although the available evidence discussed above does not support SGLT2 expression in the human heart,\(^15\),\(^16\) it cannot definitely be excluded that EMPA acts on cardiac cells, including non-cardiac myocytes with possible paracrine effects on cardiac myocytes, by other means than NHE inhibition.

### 5. Conclusions

The impressive outcome improvement reported by the EMPA-REG OUTCOME trial investigators is most likely the result of pleiotropic effects exerted by EMPA on the heart and kidneys, however, experimental and clinical evidence to support these mechanisms is currently largely lacking. Furthermore, the three hypotheses discussed above are not mutually exclusive, and thus beneficial effects on hemodynamics, metabolism, and cardiac ion handling may coexist and contribute to the overall benefit. The 'Na\(^{+}\) hypothesis' presented here posits the intriguing possibility that EMPA may directly counteract the intracellular Na\(^{+}\) overload observed in diabetic and failing cardiac myocytes, thereby possibly improving mitochondrial energetics and preventing oxidative stress. Until now, EMPA effects on cardiac ion homeostasis have been studied only in vitro, and whether the effects of EMPA in vivo also impact cardiac Na\(^{+}\) (Na\(^{+}\)), currently remains unresolved. Future experimental studies should be directed at addressing the effect of EMPA and other SGLT2 inhibitors on cardiac Na\(^{+}\) and Ca\(^{2+}\) handling in vivo. In particular, it would be of interest to evaluate the effects of EMPA treatment on mitochondrial redox state in animal models of type 2 diabetes and HF. Given the long-held concept that elevated cardiac myocyte Na\(^{+}\) levels may play an important pathophysiological role in the progression of the disease, the EMPA-REG data may spark renewed enthusiasm towards directly targeting cytosolic Na\(^{+}\) homeostasis in heart failure and possibly also diabetes.

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### Conflict of interest

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### References

6. Ingelheim. Conflict of interest:...


