



Inhibition of the Sodium–Proton Antiporter (Exchanger) is a Plausible Mechanism of Potential Benefit and Harm for Drugs Designed to Block Sodium Glucose Co-transporter 2

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Clinical trials of sodium glucose co-transporter 2 inhibitors (SGLT2i) in patients with type 2 diabetes and comorbid cardiovascular and kidney disease have shown reductions in major adverse cardiovascular events, heart failure hospitalizations, and attenuation of the progression of kidney disease. The magnitude of benefit appears to be greater than expected due to glycemic control, reduced blood pressure, and loss of adiposity. This impact is also independent from reduced renal function and lesser degrees of natriuresis and glycosuria. However, these agents have also been associated with limb amputation, Fournier's gangrene, diabetic ketoacidosis, metabolic bone disease, and increased hematopoiesis. A strong off-target effect of SGLT2i on the sodium–proton antiporter (exchanger) on the cell surface and intracellular organelles explains the wide-ranging effects of these agents. By slowing the restoration of pH within cells, SGLT2i activate secondary processes that mimic ischemic preconditioning in the heart and kidney and increased hematopoiesis in bone marrow which would explain salutary effects. Conversely, the inability to rapidly recover pH in ischemic peripheral tissues explains the progression of diabetic extremity ulcers, gangrene, propensity for metabolic bone disease, and diabetic ketoacidosis in patients who are predisposed. This paper will review the evidence for the strong off-target effect of SGLT2i on the sodium-proton exchanger and its potential effect on the organ systems and processes in which SGLT2i appear to have activity.

Keywords

SGLT-2 inhibitor; sodium-proton exchanger; sodium-hydrogen ex-

changer; empagliflozin; canagliflozin; dapagliflozin; ertugliflozin; sotagliflozin; cardiovascular disease; chronic kidney disease; adverse effects

1. Introduction

Patients with type 2 diabetes mellitus (T2DM) frequently develop cardiovascular and and/or kidney disease (WHO, 2016; Mozaffarian et al., 2016). The deleterious effects of T2DM have been described as microvascular (neuropathy, nephropathy, retinopathy) and macrovascular (coronary disease, stroke, peripheral arterial disease) (Ray et al., 2009). In recent decades, T2DM has been described as an independent risk factor for the two major phenotypes of heart failure (HF) - preserved and reduced ejection fraction (Fitchett et al., 2017; Lloyd-Jones et al., 2002; Owan et al., 2006). Further, it is associated with a four-fold increase in the rate of HF hospitalizations compared to those without T2DM (Zelniker et al., 2018). T2DM is also associated with non-healing lower extremity wounds such as foot ulcers, deep tissue osteomyelitis, metabolic bone disease, anemia, pancreatitis, and in advanced insulinopenic patients--diabetic ketoacidosis (DKA) (Armstrong et al., 2017; Gilbert and Pratley, 2015; Thomas et al., 2006).

The treatments for T2DM have been scrutinized for their independent link to organ system diseases above and beyond the risk of T2DM. Thiazolidinediones have been associated with worsening edema and increased risk for HF hospitalization, cardiovascular death, but not all-cause mortality (Kaul et al., 2010; Home et al., 2009; Lincoff et al., 2007). Oral sulfonylureas are mechanistically associated with hypoglycemia and the catecholamine surge that occurs with it provoking myocardial infarction (MI), stroke, and cardiovascular death (Douros et al., 2018; Powell et al., 2018).

Other safety events associated with particular agents are disputed with additional analyses over time such as pancreatitis (originally associated with glucagon-like peptide-1 agonists) and HF (originally associated with thiazolidinediones and dipeptidyl peptidase-4 inhibitors) (Li et al., 2014; Kaneko and Narukawa, 2017).

2. Cardiovascular safety assessed in clinical trials of antidiabetic agents

In 2008, the United States Food and Drug Administration (FDA) put forth guidelines for drug manufacturers to demonstrate that new anti-hyperglycemic medications should not increase the risk for MI, stroke, or cardiovascular death (McMurray et al., 2014). Specifically, the upper bound of the 95% confidence limit of the hazard ratio for major adverse cardiac events (nonfatal myocardial infarction, non-fatal stroke, or cardiovascular death) should not exceed 1.30 at the end of the trial nor > 1.80 at any interim analysis (Guidance, 2008). The shortcoming of this guidance is that it ignored the important connections between T2DM and the risk of HF, progression of kidney disease, and other organ system illnesses (Zannad et al., 2016). Additionally, while it leveraged the endpoints that were associated with hyperglycemia (MI, stroke, cardiovascular death) it failed to provide a framework that could adapt to the mechanism of action of the drug or consider important unique safety events for adjudication (e.g. extremity amputation, DKA, acute kidney injury). In the United States, there have been four FDA approved sodium glucose co-transporter inhibitors (SGLT2i) based on this guidance; their clinical implications have been well-demonstrated and are promising new tools for the care of T2DM patients with established cardiovascular disease, as evidenced by the recent 2018 American College of Cardiology Expert Consensus Decision (Writing Committee et al., 2018). However, there are concerning potential adverse effects – for example, a registry-based cohort study ($n = 34,426$) comparing outcomes between new users of SGLT2i and GLP1 receptor agonists found that SGLT2i were associated with increased risk of lower limb amputation and DKA compared to GLP1 receptor agonists (Ueda et al., 2018). In 2017 the European Medicines Agency issued a warning for this SGLT2i class limited at that time to canagliflozin, empagliflozin, and dapagliflozin concerning the risk of lower limb amputations (Diabetes patients, 2017). The array of clinical responses to this class of agents calls for an in-depth consideration of both target-mediated and off-target effects. Such an analysis of biologic mechanisms would account for positive outcomes such as a reduction in HF hospitalization, cardiovascular death, and attenuation of progression of diabetic kidney disease (Fig. 1), as well as adverse outcomes such as an increased risk of limb amputation, Fournier's gangrene, DKA, metabolic bone disease, and increased hematopoiesis.

2.1. The EMPA-REG OUTCOME trial

The Empagliflozin, Cardiovascular Outcomes, and Mortality in Type 2 Diabetes (EMPA-REG OUTCOME) trial was the first to show cardiovascular mortality reduction in T2DM patients with established cardiovascular disease treated with the SGLT2i empagliflozin. This trial randomized 7020 patients with T2DM, estimated glomerular filtration rate (eGFR) > 30 ml/min/1.73 m² and cardiovascular disease (CVD) to 10 mg or 25 mg of empagliflozin

daily or to placebo (Zinman et al., 2015). The primary composite endpoint of death from cardiovascular causes, nonfatal MI, or nonfatal stroke occurred in 10.5% in the pooled empagliflozin group vs. 12.1% in placebo (hazard ratio [HR] = 0.86, 95% confidence interval [CI] = 0.74-0.99, $p = 0.04$) in the 3.1 year follow up period. There were no significant differences in rates of MI or stroke, but there was a 38% lower risk of cardiovascular death and a 32% risk reduction for all-cause death (p -values < 0.001) with empagliflozin. The mortality effects were concordant with a reduction of HF hospitalization by 35% and HF death or hospitalization by 39% (p -values < 0.001), with these benefits seen in patients with (10%) and without (90%) documented HF at baseline (Writing Committee et al., 2018; Fitchett et al., 2016). The reduced adjudicated endpoint of HF hospitalization was supported by similar reductions of investigator-reported HF, and the introduction of loop diuretics as a proxy for HF symptoms. In the same trial, there was a 39% reduction in the relative hazard of incident or worsening nephropathy defined as either progression to macroalbuminuria (> 300 mg of albumin per gram of creatinine in spot urine), a doubling of the serum creatinine accompanied by an eGFR of ≤ 45 ml/min/1.73 m², the initiation of renal-replacement therapy, or death from renal disease (HR = 0.61, 95% CI = 0.53-0.71, $p < 0.001$). These findings appeared to be out of proportion to the 0.54% reduction in hemoglobin A1C, decrease (~ 4 mmHg) in systolic blood pressure, and decline (~ 2.0 kg) in body weight observed in the treatment group. Additionally, the cardiovascular outcomes were independent of baseline eGFR (Cherney et al., 2018).

2.2. The CANVAS program

The Canagliflozin Cardiovascular Assessment Study (CANVAS) Program included the CANVAS and CANVAS-Renal (CANVAS-R) study cohorts assembled into a randomized trial comparing canagliflozin 100 mg or 300 mg (100 mg with optional increase to 300 mg) to placebo control in 10,142 patients with T2DM and eGFR > 30 ml/min/1.73m² (Neal et al., 2013, 2017). Unlike EMPA-REG, CANVAS included only 56% of patients with established cardiovascular disease. Like EMPA-REG OUTCOME, the cardiovascular and renal benefits were evident with SGLT2i. The primary composite outcome of death from cardiovascular causes, nonfatal MI, or nonfatal stroke was lower with canagliflozin than with placebo, (HR = 0.86, 95% CI = 0.75-0.97, $p = 0.02$). HF hospitalization or cardiovascular death was reduced with canagliflozin compared with placebo (HR = 0.78, 95% CI = 0.67-0.91) as was fatal or hospitalized HF (HR = 0.70, 95% CI = 0.55-0.89) and hospitalized HF alone (HR = 0.67, 95% CI = 0.52-0.87) (Rådholm et al., 2018). Canagliflozin reduced the progression of albuminuria (HR = 0.73, 95% CI = 0.67-0.79) and the composite outcome of a sustained 40% eGFR reduction, the need for renal-replacement therapy, or death from renal causes (HR = 0.60, 95% CI = 0.47-0.77). Patients treated with canagliflozin had nearly a two-fold increased risk of limb amputation (6.3 vs. 3.4 participants per 1000 patient-years; HR = 1.97, 95% CI = 1.41-2.75); amputations were primarily of the toe or metatarsal (Diabetes patients, 2017). As in the EMPA-REG OUTCOME trial, the reductions in cardiorenal endpoints were out of proportion to changes in glycemic control, blood pressure, and body weight.

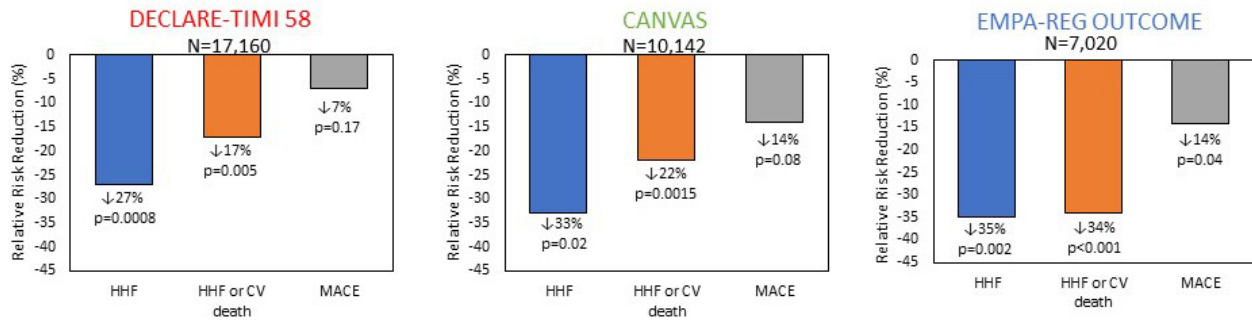


Figure 1. Cardiovascular outcomes from: the Dapagliflozin Effect on Cardiovascular Events (DECLARE-TIMI 58) trial, the Canagliflozin Cardiovascular Assessment Study (CANVAS), the Cardiovascular Outcome Event Trial in Type 2 Diabetes Mellitus Patients (EMPA-REG OUTCOME). Data reported as relative risk (RR) reduction percentage, p-value. RR reduction percentages calculated from hazard ratios for event rates per 1000 patient-years. HHF = heart failure hospitalization; CV = cardiovascular; MACE = major adverse cardiovascular event.

2.3. The DECLARE-TIMI 58 trial

Unlike EMPA-REG OUTCOME and CANVAS, the Dapagliflozin Effect on Cardiovascular Events (DECLARE-TIMI 58) trial recruited 17,160 T2DM patients (66% with cardiovascular disease) with eGFR > 60 ml/min/1.73 m². In the primary endpoint analyses, dapagliflozin 10 mg daily did not reduce major adverse cardiovascular events (MACE) (HR = 0.93, 95% CI = 0.84-1.03, $p = 0.17$), but did result in a lower rate of HF hospitalization or cardiovascular death (HR = 0.83, 95% CI = 0.73-0.95, $p = 0.005$) (Wiviott et al., 2018). The renal composite endpoint of sustained 40% eGFR reduction to < 60 ml/min/1.73 m², end stage renal disease, or death from renal causes was reduced with dapagliflozin (HR = 0.76, 95% CI = 0.67-0.87). DKA was more common with dapagliflozin than with placebo (0.3% vs. 0.1%, $p = 0.02$) and there were similar rates of amputation for dapagliflozin vs. placebo (1.4% vs. 1.3%, HR = 1.09, 95% CI = 0.84-1.4, $p = 0.53$). As in the EMPA-REG OUTCOME and CANVAS trials, the cardiorenal benefit was somewhat out of proportion to changes in glycemic control, blood pressure, and body weight.

2.4. The CVD-REAL and CVD-REAL 2 studies

EMPA-REG OUTCOME, CANVAS, and DECLARE-TIMI 58 provided evidence of the efficacy and safety of currently approved SGLT2i in the randomized controlled trial setting. However, each trial had stringent inclusion and exclusion criteria, affecting the “real-world” applicability of these drugs (Kosiborod et al., 2017). Therefore, in support of these trials, the investigators in the Comparative Effectiveness of Cardiovascular Outcomes in New Users of Sodium-Glucose Cotransporter-2 Inhibitors (CVD-REAL) study sought to evaluate the efficacy but not safety of SGLT2i in reducing cardiovascular death and HF hospitalization in patients with T2DM in a large, multinational, observational study. Patients on SGLT2i were compared to patients on other glucose-lowering drugs. Data from 309,056 patients were collected from the United States (US), Norway, Denmark, Sweden, Germany and the United Kingdom (UK). Thirteen percent of patients had established ASCVD. There was an overall lower risk of HF hospitalization (HR = 0.61, 95% CI = 0.51-0.73, $p < 0.001$); death (HR = 0.49, 95% CI = 0.41-0.57, $p < 0.001$); and composite HF hospitalization or death (HR = 0.54, 95% CI = 0.48-0.60, $p < 0.001$) in patients treated with SGLT2i compared to other glucose-lowering agents.

There were geographic differences noted among the utilization of specific SGLT2i (92% dapagliflozin in Europe and 76% canagliflozin in the US); however, there was no significant absolute or relative difference in outcomes which suggests a class effect of SGLT2i in lowering cardiovascular risk.

In a subsequent study, the CVD-REAL 2 study, the investigators evaluated a broad range of cardiovascular outcomes in patients on SGLT2i compared with other antidiabetic agents. This study included over 400,000 patients from South Korea, Japan, Singapore, Israel, Australia and Canada; 27% had established cardiovascular disease (Kosiborod et al., 2018). Pooled analyses showed that the use of SGLT2i was associated with a lower risk of all cause death (HR = 0.51, 95% CI = 0.37-0.70, $p < 0.001$), HF hospitalization (HR = 0.64, 95% CI = 0.50-0.82, $p < 0.001$), composite death or HF hospitalization (HR = 0.60, 95% CI = 0.47-0.76, $p < 0.001$), MI (HR = 0.81, 95% CI = 0.74-0.88, $p < 0.001$), and stroke (HR = 0.68, 95% CI = 0.55-0.84, $p < 0.001$). These results were similar regardless of country and patient subgroup.

3. Common mechanisms of cardiorenal benefit

Any potential mechanism of SGLT2i benefiting the heart and the kidneys should consider the simultaneous impact on known and accepted risk factors for disease progression. For the outcome of HF and cardiovascular death, there are three principles of disease progression: 1) pressure overload, 2) volume overload, and 3) cardiomyopathy as it relates to structure, function, or resilience of the myocardium in response to disease. For diabetic nephropathy there are two mechanistic principles of treatment: 1) glycemic control and 2) blood pressure control.

3.1. Improvements in glycemic control

SGLT2 is located almost exclusively in the proximal renal tubule, while SGLT1 transporters are also located in the intestines, heart, and skeletal muscles (Heerspink et al., 2016). Glucose reabsorption in the kidneys occurs in a sodium-dependent manner via SGLT transporters, with most reabsorption occurring at the proximal convoluted tubule through SGLT2 and a smaller portion

in the distal segment of the proximal tubule by SGLT1 (Nauck, 2014). Once the maximum glucose transport capacity has been reached, excess glucose is excreted into the urine. SGLT2i lead to increased urinary glucose excretion by blocking SGLT2-mediated glucose reabsorption which is dependent upon the number of functioning nephrons and by proxy, the eGFR. SGLT2i as monotherapy and in combination with oral medications in T2DM as well as injectable therapies consistently improves glycemic control to a modest degree. Because SGLT2i increase glucose excretion in the urine, causing increased calorie loss and thus body weight reduction, there is less need for therapies that may have no benefit on cardiovascular and renal risk such as oral hypoglycemic agents and insulin, respectively. If we consider in context the pharmacologic inhibition of SGLT2 channels taken as a sole mechanism, there is no parallel antidiabetic agent that reduces hemoglobin A1c by ~0.5% and results in ~20-40% reductions in cardiovascular and renal events.

3.2. Blood pressure control

SGLT2i treatment is associated with reductions in systolic and diastolic blood pressure. Unlike vasodilators, this change in blood pressure is not associated with an increase in heart rate, suggesting that the sympathetic nervous system is not activated and that plasma volume is not persistently contracted (Baker et al., 2014; Cherney et al., 2014a). Reduction in blood pressure is likely multifactorial and related to natriuresis, non-fluid weight loss, as well as direct vascular effects (Inzucchi et al., 2015). The blood pressure reduction of ~4 mmHg is significant enough to partly influence both heart and kidney outcomes. This is supported by the observation that HF was the most responsive endpoint to SGLT2i, which is consistent with blood-pressure lowering trials (Brouwer et al., 2018).

3.3. Sodium and fluid loss

SGLT2i induce osmotic diuresis and natriuresis through the decreased reabsorption of glucose and sodium, resulting in decreased extracellular volume, possible reduction of vascular wall stress, improved cardiac function and potentially reduced congestion (Cherney et al., 2014b). An initial reduction in plasma volume may also lead to reduced myocardial stretch and natriuretic peptide levels, as well as improving symptom and functional capacity (Rajasekeran et al., 2016). SGLT2i have been shown to reduce the sodium content in skin which correlates with left ventricular mass (Schmieder et al., 2016; Schneider et al., 2017). In clinical practice, there may be a reduction in the need for thiazide and loop diuretics, and as a result, less diuretic-induced activation of the renin-angiotensin system, hypokalemia, and metabolic alkalosis. Unlike thiazide diuretics, SGLT2i induce natriuresis as well as osmotic diuresis via glucosuria which may decline over time with lower daily plasma glucose levels and compensatory renal mechanisms for sodium reabsorption. The increase in sodium excretion with SGLT2i may be long-lasting having an effect on blood pressure reduction in sustained and synergistic with other blood pressure lowering medication, perhaps due to whole body sodium content (Heise et al., 2016). While thiazides act on the distal convoluted tubule and are associated with hypokalemia, increased serum uric acid, and impaired glucose tolerance, SGLT2i act on the proximal

renal tubule and are associated with reductions in serum uric acid and no changes in serum potassium (Scheen, 2016). In summary, the diuretic-like properties of SGLT2i appear to account for the blood pressure reductions seen in T2DM; however, these effects do not appear to be substantial enough to account for the cardio-renal benefits of SGLT2i in clinical trials.

3.4. Weight Loss

SGLT2i are associated with modest (1.8 kg) short term but sustained reductions in body weight (Vasilakou et al., 2013). This occurs rapidly initially with diuresis and then is gradually consistent with daily caloric losses of glucose in urine until a plateau is reached; the weight reduction is sustained over time. Outside of bariatric surgery trials, which yield large reductions in body weight, clinical trials attempting to reduce body weight and improve cardiovascular outcomes have been disappointing. A greater than 10% loss of body weight with medication has been associated with reductions in cardiovascular deaths and adverse cardiovascular outcomes in patients with diabetes over the long term (Look AHEAD Research Group, 2016). However, the modest ~2 kg (< 5%) reduction in body weight is unlikely to completely account for the large reductions in HF, cardiovascular death, and/or progression of chronic kidney disease (CKD) observed in SGLT2i trials.

4. The sodium-proton antiporter (exchanger) channels

There are 10 described sodium-proton antiporters or sodium-hydrogen exchangers (NHE) in mammalian cells (Table 1, Fig. 2) (Kilić et al., 2014). These anti-porters exchange sodium for protons and work to restore pH in the setting of intracellular accumulation of acid. Additionally, there are NHE antiporters on the sarcolemmal membrane in cardiomyocytes which work as critical regulators of sodium within the sarcolemma at the level of intercalated discs and T-tubules (Fliegel et al., 1995). Increased intracellular concentration of sodium has been demonstrated to have deleterious effects on cardiomyocytes, altering the efficiency of calcium and hydrogen exchange (Pogwizd et al., 2003; Cingolani and Ennis, 2007). This increased intracellular sodium tendency has been well demonstrated in myocardial hypertrophy and HF states, both acute and chronic. Isoforms of NHE and tissue types are shown in Table 1. The NHE family has homology in structure to the SGLT family in terms of a funnel shape for sodium transport (Brouwer et al., 2018). It is well established that reductions in intracellular and tissue pH occur in the setting of systemic acidosis, hypoxia, and/or tissue ischemia due to impaired blood flow. The rapid restoration of intracellular pH is a normal function of all cells, and when this process is impaired, it results in both gene activation and repression governing a variety of metabolic processes in cells known as ischemic preconditioning. In short, provided the exposure to ischemia is sufficiently brief, ischemic preconditioning improves the hardiness of cells to the subsequent bout of ischemia; NHE upregulation occurs ischemic preconditioning as well as in many disease states. These changes may modulate responses to infarction, apoptosis, fibrosis, and have been important for drug development (Madonna and De, 2013). In the setting of chronic disease, NHE is upregulated and related to maladaptive processes including myocardial hypertrophy, sodium re-

Table 1. Subtypes of sodium-proton antiporters otherwise known as sodium hydrogen exchangers. These antiporter channels on the cell surface and surface of some organelles function by exchanging one proton out of the cell and one sodium into the cell, restoring intracellular pH.

NHE Subtype	Chromosome	Tissue/Cell Type	Comments	Inhibitor
NHE1 (Wakabayashi et al., 2013)	1	Cardiomyocyte, fibroblast, erythroblast, vascular endothelium	Upregulated in ischemic heart disease, heart failure, mediates hypertrophy	SGLT2i, Cariporide, Zoniporide, Remiporide, Amiloride
NHE2 (Rivarola et al., 2017)	2	Renal collecting duct	Co-dependent on aquaporin channels	Amiloride
NHE3 (Pogwizd et al., 2003)	5	Renal tubular epithelium Intestinal epithelium	Co-dependent on angiotensin II	SGLT2i? Tenapanor
NHE4 (Bourgeois et al., 2010)	2	Renal thick ascending limb of Henle, distal tubule	Ammonia efflux into urine	
NHE5 (Diering and Numata, 2014)	16	Neurons	Regulates endosome pH	
NHE6 (Xu et al., 2018)	X	Mitochondria, neurons, skeletal muscle, cardiomyocyte	Mutation results in Christian-Son Syndrome	
NHE7 (Lin et al., 2005)	X	Intracellular Golgi apparatus	Involved in lithium uptake	
NHE8 (Wang et al., 2015)	20	Retina, intestinal epithelium, testes	Responsible for mucosal integrity	
NHE9 (Beydoun et al., 2017)	3	Brain microvasculature	Upregulated in glioblastoma	
NHE10 (Lee et al., 2008)	4	Bone osteoclasts		

absorption, intracellular sodium accumulation, and fluid retention (Donowitz et al., 2013).

5. Drugs that inhibit the sodium-proton antiporter (exchanger)

The known and developed pharmacologic inhibitors of NHE include cariporide, zoniporide, rimaporide, eniporide, and the clinically utilized diuretic, amiloride. Cariporide (an NHE1 inhibitor) was developed as an adjunct to cardiac revascularization with the intent of preventing intracellular calcium overload by NHE1 inhibition. In the Guard During Ischemia Against Necrosis (GUARDIAN) trial, 11,590 patients who were hospitalized for an acute coronary syndrome or undergoing high-risk percutaneous coronary intervention or coronary artery bypass grafting (CABG) were randomized to one of three doses of cariporide (20, 80, or 120 mg), or placebo, administered as a 60-minute infusion every 8 hours for two to seven days (Chaitman, 2003). At day 36, patients treated with cariporide 120 mg demonstrated a nonsignificant 10% risk reduction in the primary endpoint of death or MI compared with placebo ($p = 0.12$). At this dose, patients undergoing CABG experienced a 25% risk reduction in death or MI ($p = 0.03$), which was sustained through six months ($p = 0.033$). The improvement resulted primarily from a 32% risk reduction in nonfatal MI ($p = 0.007$). Cardiovascular benefit was associated with the degree to which creatine kinase isoenzyme excursions

were attenuated by cariporide. This very short-term exposure to a strong NHE inhibitor suggested cardiovascular benefit, but unlike the SGLT2i trials, there was not a long period of exposure and there was a limited opportunity to characterize other changes such as blood pressure or changes in renal function/albuminuria. In contrast, the phase 2 placebo-controlled Evaluation of the Safety and Cardioprotective Effects of Eniporide in Acute Myocardial Infarction (ESCAMI) trial failed to show reductions in cumulative cardiac enzyme release when administered close to thrombolysis or percutaneous coronary intervention in acute myocardial infarction (Zeymer et al., 2001). An interpretation of GAURDIAN and ESCAMI pointed out that NHE1 inhibition would need to be pharmacologically present before the ischemic insult and additionally, there would need to be prompt reperfusion in order to have benefit of this mechanism of action (Avkiran and Marber, 2002). These principles would explain the disparate results of these trials and would also support the concept that long-term daily NHE1 inhibition would have a rationale as a myocardial protectant for bouts of ischemia.

Zoniporide is a parental NHE1 antagonist that in clinical models had a similar ischemic-protective effect as cariporide (Clements-Jewery et al., 2004). In a pig model of cardiac arrest, zoniporide prevented reductions in left ventricular myocardial distensibility during the interval of cardiac arrest and extracorporeal

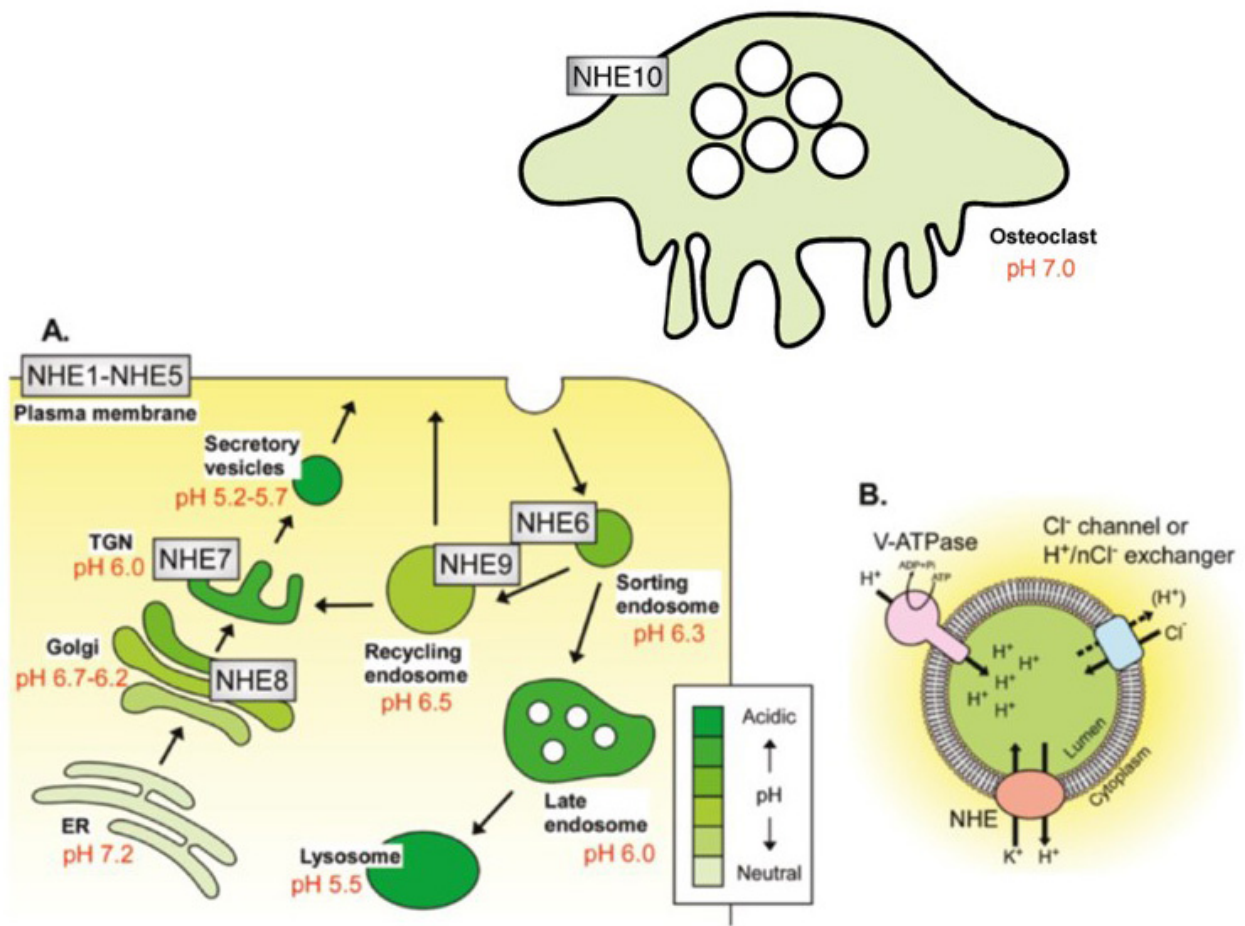


Figure 2. Sodium-hydrogen exchanger (NHE) functions and locations within cells. (A) Locations of NHEs 1-5 in the plasma membrane and NHEs 6 - 9 in the organelles of somatic cells, and NHE10 in osteoclastic cells. (B) Illustration of pH regulation in somatic organelles. TGN = trans-Golgi network; ER = endoplasmic reticulum. Adapted from Ohgaki et al. and Yuan et al. (Ohgaki et al., 2011; Yuan et al., 2018).

circulation. Additionally, zoniporide was resulted in greater effectiveness in termination and prevention of recurrent ventricular fibrillation. These energy effects are consistent with NHE-1 inhibition protecting mitochondrial bioenergetic function and limiting Ca²⁺ overload (Ayoub et al., 2010; Lamoureux et al., 2016). Rimeporide is an oral NHE1 inhibitor being tested in Duchenne's muscular dystrophy (Rimeporide in Patients with Duchenne Muscular Dystrophy [RIM4DMD] Trial) (Rimeporide, 2016). Amiloride was approved by the FDA in 1981 and works by inhibiting epithelial sodium channel in the distal nephron. It has also been shown to block the NHE3 antiporter on the apical surface of the proximal tubule cells in the nephron, abolishing more than 80% of the action of angiotensin II on the secretion of hydrogen ions in proximal tubule cells. Finally, spironolactone has been indirectly linked to NHE1 inhibition in models where desoxycorticosterone induced hypertension results in an upregulation in aortic NHE1 and spironolactone prevents this injury without a change in the transcription of NHE1 (Carreño et al., 2015).

6. SGLT2i pleiotropic inhibition of cardiorenal sodium-proton exchange

We recognize that SGLT2i inhibition of the family of NHE's is speculative and much remains to be proven in testable hypotheses. In preclinical models, empagliflozin has been demonstrated to have 80% of the NHE1 inhibitory effect of cariporide in both rat and rabbit ventricular myocytes (Baartscheer et al., 2017). These preclinical trials treated healthy cardiomyocytes with physiologically relevant concentrations 0.25-1 $\mu\text{mol/l}$ of empagliflozin, 5.5 mmol/l or 11 mmol/l glucose, 10 $\mu\text{mol/l}$ cariporide, or 20 mmol NH₄Cl (NH₄⁺) either alone or in combination. NHE1 inhibition was inferred by the delay in pH recovery after an acute acidic load via a pulse of NH₄⁺. In control conditions, pH quickly recovered to normal values after NH₄⁺ pulse. Recovery of pH was totally inhibited as expected by cariporide. In the presence of empagliflozin, the NHE flux was strongly reduced by approximately 80% of that with cariporide. This effect was independent of glucose and SGLT1 in cardiomyocytes. Isolated healthy mouse ventricular cardiomyocytes were treated with 1 $\mu\text{mol/l}$ empagliflozin,

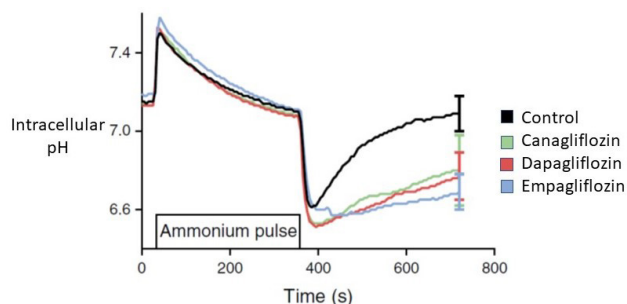
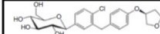
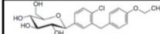
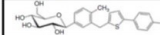
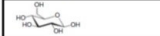


Figure 3. Sodium glucose co-transporter 2 inhibitors mediate sodium-hydrogen exchanger 1 inhibition and blunting of the restoration of pH of cardiomyocytes in response to an acid challenge. All p -values < 0.001. Adapted from Uthman et al. (Ayoub et al., 2010).

1 $\mu\text{mol/l}$ dapagliflozin, 3 $\mu\text{mol/l}$ canagliflozin or control vehicle (note that these concentrations were based on the maximum plasma concentration of each drug at physiologic doses (Scheen et al., 2014)). As shown in Figure 3, all three compounds considerably slowed the restoration of intracellular pH after an NH_4^+ pulse. Additionally, in a model of NHE1 binding, all three SGLT2i were found to bind efficiently at the Na pocket of NHE1 (815 amino acids) as shown in Figure 4 (note glucose was used as a control as it binds weakly to the Na pocket at much lower affinities). The glucoside portion of the drug molecule bound toward the Na^+ binding site on the extracellular side of the channel and the aglycone portion of the drug molecule blocks the aperture. SGLT2 inhibition of NHE1 on cardiomyocytes reduced intracellular concentrations of sodium, subsequently reducing calcium overloading which is a common pathophysiologic mechanism of contractile failure (Uthman et al., 2018a). The reduction in intracellular sodium influx was significant and clearly associated with the introduction of the SGLT2i in the experimental model. Langendorff constant-flow perfused mouse hearts were treated with SGLT2i for 30 minutes; there was coronary dilation with a modest reduction in coronary perfusion pressure and no change in rate-pressure product or measures of myocardial energetics. It is also known that SGLT2i do not influence the cardiomyocyte action potential. At this time, the physiologic impact and direct cardiac tissue effect of SGLT2i have not been fully assessed, but we believe preclinical data suggest blunting of the recovery of pH may mimic ischemic preconditioning which is a set of actions that have protective effects on cell survival, function, and organ adaptation (Karmazyn et al., 2001). Finally, dapagliflozin has been demonstrated to attenuate the upregulation of NHE1 in response to injury due to lipoprotein polysaccharide in preclinical models using rat cardiac fibroblasts (Ye et al., 2018).

In the kidney, under physiologic concentrations of glucose, SGLT2-mediated glucose uptake has been shown to stimulate NHE3 (834 amino acids) which is involved in the secretion of ammonia and reabsorption of sodium bicarbonate (Pessoa et al., 2014; Layton et al., 2015). Under normal conditions, NHE3 is responsible for a considerable amount of sodium reabsorption in the proximal tubule (Fenton et al., 2017). NHE3 transcription is upregulated by angiotensin II which itself is activated in CKD and HF.

Molecular structure	Compound	Binding energy (kJ/mol)
	EMPA	-34.3 (-8.2 kcal/mol)
	DAPA	-32.2 (-7.7 kcal/mol)
	CANA	-37.2 (-8.9 kcal/mol)
	Glucose*	-24.3 (-5.8 kcal/mol)

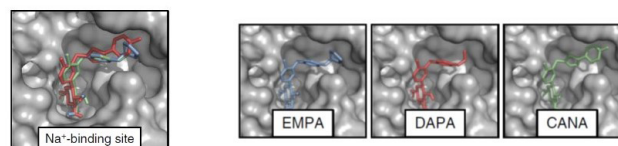


Figure 4. Molecular structure and binding energy of sodium glucose co-transporter 2 inhibitors on the sodium-hydrogen exchanger 1 channel. Glucose was used as a negative control. EMPA = empagliflozin; DAPA = dapagliflozin; CANA = canagliflozin. Adapted from Uthman et al. (Ayoub et al., 2010)

In a HF rat model, the upregulation of NHE3 likely contributed to attenuation of the natriuresis and volume expansion (Inoue et al., 2012). SGLT2i has resulted in decreased activity and function of NHE3, possibly contributing to natriuresis, reduction in blood pressure, and a tissue protective effect at the level of the renal tubule (Gallo et al., 2015). As with NHE1, glucose binds to NHE3, and while one would expect SGLT2i to bind directly to NHE3, at the time of this writing, we could find no report of binding models or attempted provocative studies of SGLT2i on NHE3 (Brouwer et al., 2018). NHE3 inhibition may work in concert with natriuresis and glycosuria to trigger tubuloglomerular feedback and reduce glomerular blood flow (de Albuquerque et al., 2018). By this mechanism, the hyperfiltration glomerular injury in T2DM may be attenuated.

7. SGLT2i and sodium-proton antiporter (exchanger) mediated effects in other organ systems

7.1. Hematopoietic system

SGLT2i result in increased hemoglobin and hematocrit levels (Zinman et al., 2016). Univariate analysis of potential mediators for empagliflozin's reduction in cardiovascular demonstrated that hematocrit (increase of 5%) – a surrogate for both hemoglobin concentration and plasma volume reduction – was associated with a 52% risk reduction cardiovascular mortality, however, the associations with both lower and higher hemoglobin concentrations are complex and this relationship may be spurious (Inzucchi, 2017; Brown et al., 2001; Lee et al., 2018). This increase in hemoglobin and hematocrit has been hypothesized to also coincide with the renal tubular benefit of SGLT2i (Sano et al., 2016). Erythroblasts and reticulocytes express NHE 1 which is reduced as the red cell matures (Liu et al., 2010). It has been demonstrated that NHE participates in the regulation of hematopoiesis (Zerbini et al., 1998). Hemoglobin provides better tissue oxygenation and is regulated through hypoxia-inducible factor 1-alpha expression

and subsequent erythropoietin secretion, factors that are influenced by SGLT2i inhibition of NHE1 (Chang et al., 2016). Note that while the rise in hematocrit was thought to increase stroke risk, a post-hoc analysis of EMPA-REG OUTCOME found no elevated risk and CVD-REAL observed a reduced risk (Zinman et al., 2017). Thus we believe the rise in hemoglobin and hematocrit with SGLT2i may not all be attributed to a diuretic effect and while not yet demonstrated with NHE1 inhibition, an increase in erythropoiesis should be considered. At this time we are not aware of studies evaluating SGLT2i on red cell mass or measured plasma volume, hence this is a current knowledge gap.

7.2. Vasculature

Empagliflozin has been shown to reduce arterial stiffness in patients with type I DM (Cherney et al., 2014a). The improved arterial compliance and smooth muscle relaxation mediated through NHE1 inhibition may be beneficial in patients at risk for HF and the progression of CKD. In patients with T2DM and hypertension, empagliflozin was associated with reductions in markers of arterial stiffness and vascular resistance (Chilton et al., 2015). Thus, the dual diuretic-like effect of SGLT2i combined with the vascular effects of NHE1 inhibition may explain the sustained reduction of blood pressure in SGLT2i trials across a range of eGFR at the lower ends which would normally not be as responsive to diuretics alone.

7.3. Cellular metabolism

The ketone genesis observed with SGLT2i is likely independent of NHE blockade (Soler et al., 1994). However the elevated level of beta hydroxybutyrate seen with SGLT2i results in a shift in metabolism from fatty acids and glucose – which are less energy efficient in the setting of T2DM – towards the more energy-efficient ketones (Ferrannini et al., 2016a). This in turn improves myocardial and renal metabolic efficiency while reducing oxygen consumption (Ferrannini et al., 2016b; Mudaliar et al., 2016). The ketone hypothesis is under study for cardiovascular and renal benefit and is potentially positioned to explore the proclivity for T2DM patients who are ketosis prone, to develop DKA while administered SGLT2i in the absence of hyperglycemia or insulinopenia (Lopaschuk and Verma, 2016). The NHE family of channels may be influenced by ketogenesis, for example, NHE1 appears to mediate the brain swelling that occurs in preclinical models of DKA (Rose et al., 2015).

7.4. Metabolic bone disease

Chronic metabolic acidosis is associated with activation of osteoclastic activity and suppression of osteoblasts. Likewise, a chronically impaired NHE mechanism in osteocytes is a tractable mechanism for metabolic bone disease that is akin to that of CKD (Blair et al., 2018). NHE1, NHE3, and NHE5 are all expressed in bone cells, although inhibition of these channels has not been performed to evaluate the effects on bone cells. In trials of dapagliflozin, there were higher rates of fracture with SGLT2i. In a dedicated bone mineral density study, dapagliflozin 10 mg compared to metformin had no impact on imaging or biochemical measures of bone health (Ljunggren et al., 2012). Canagliflozin in a dedicated bone mineral density study was associated with markers of bone resorption consistent with the activation of osteoclasts

potentially through NHE (Bilezikian et al., 2016). There are potentially kidney-bone metabolic effects to consider with SGLT2i including renal phosphate retention which has been shown over the short term to induce the secretion of fibroblast-growth factor-23 in health volunteers (Blau et al., 2018). Accordingly, there were numerically higher rates of fracture in EMPA-REG OUTCOME and CANVAS which did not meet statistical significance (FDA Briefing Document, 2016; Invokana and Invokamet, 2018; Watts et al., 2016).

7.5. Diabetic non-Healing ulcers, extremity amputation, Fournier's gangrene

Tissue hypoxia and acidosis is an accepted model for the progression of non-healing diabetic ulcers most commonly seen in the diabetic foot. The same factors contribute to arterial ulcers and critical limb ischemia as the occurs in those with and without T2DM. NHE1 is active in neuronal cells, fibroblasts, subcutaneous stromal cells, and on the vascular endothelium (Castañeda-Corral et al., 2012). In this setting, NHE1 is essential for the restoration of intracellular pH after bouts of ischemia. Thus, it is conceivable that NHE1 inhibition by SGLT2i is the mechanism by which these agents, most notably canagliflozin, have been associated with lower extremity amputations and rare cases of Fournier's gangrene, which may be an extension of the well-recognized genital mycotic infections associated with SGLT2i (Kumar et al., 2017). Because canagliflozin is used clinically at doses corresponding to highest tissue concentration in preclinical models, it is congruous that canagliflozin has had the strongest differential amputation risk among the SGLT2i (Ohgaki et al., 2016; Mudaliar et al., 2015).

8. Discussion

We have described in detail how a strong, off-target effect on NHE1 and NHE3 could explain the substantial clinical effects seen in terms of HF hospitalization, cardiovascular death, and attenuation of the progression of kidney disease reported in the EMPA-REG OUTCOME, CANVAS, and DECLARE TIMI-58 trials. A meta-analysis of the three trials found that the reduction in atherosclerotic events and cardiovascular death was dependent on patients having known atherosclerotic disease at the time of event (Zelniker et al., 2018). While there was a trend towards significance for patients with multiple risk factors, it was not statistically significant. However, reductions in HF hospitalization were consistently reduced across all patients. Additional retrospective studies have also observed these clinical findings (Clegg et al., 2019). Upregulation of NHE1 in the setting of known myocardial disease and pharmacologic inhibition of NHE1 with SGLT2i would also explain the effects reported on the rates of fatal ischemic stroke and MI; importantly, NHE1 inhibition as a mechanism is more likely to impact the survivability of these events and hence is part of the explanation of the greater reduction cardiovascular death than in the nonfatal events themselves. The biologic plausibility that SGLT2i are also NHE inhibitors is very strong. It is based on what is known about the molecular homology of SGLT and NHE channels, binding studies, and interventional physiologic experiments which all demonstrate a direct NHE inhibitory of three SGLT2i: empagliflozin, canagliflozin, and dapagliflozin (Uthman et al., 2018b).

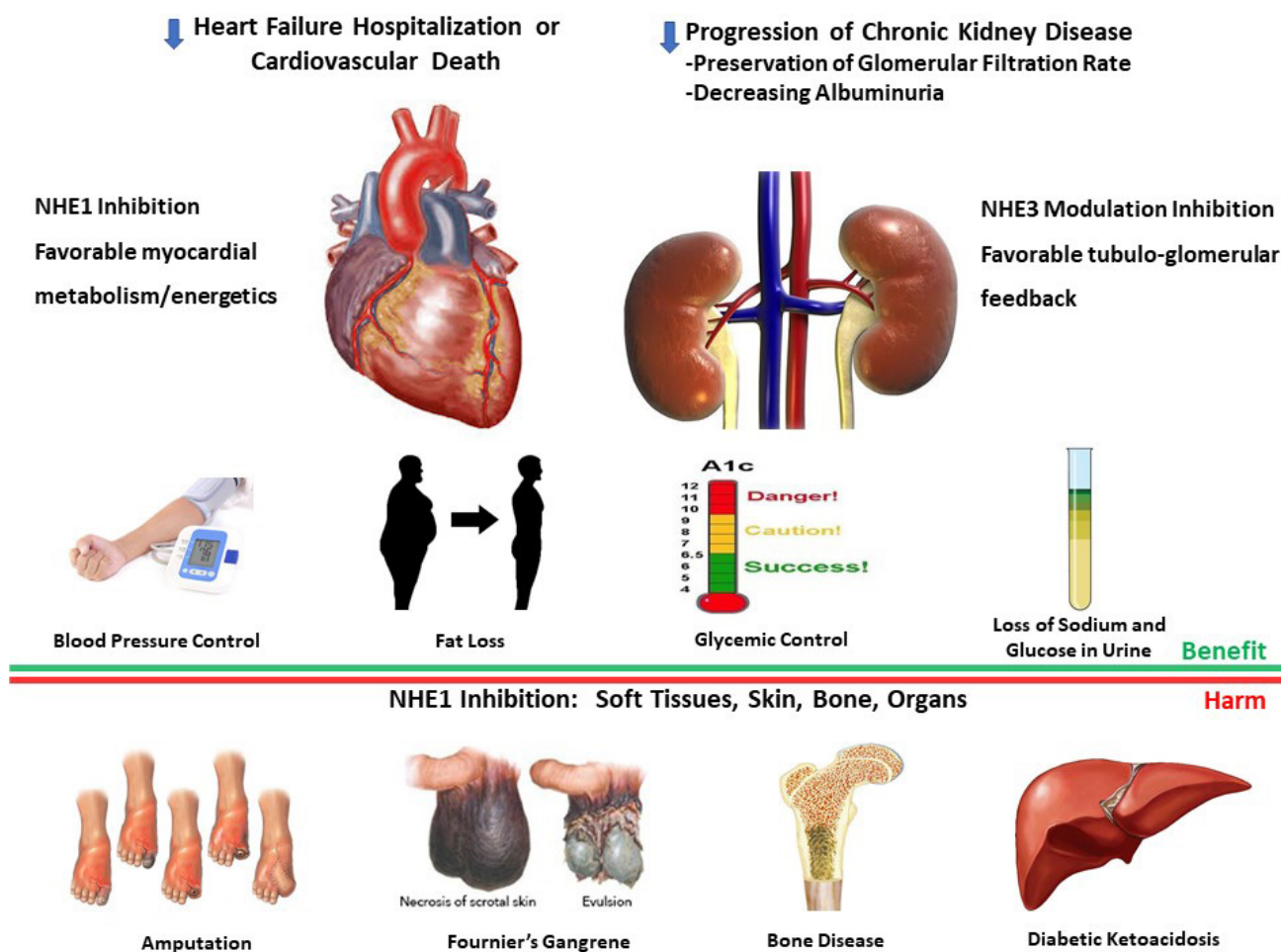


Figure 5. Inhibition of sodium-proton antiporters in the heart, kidney, bone, and peripheral tissues account for cardiorenal benefit as well as the known serious adverse effects of sodium glucose co-transporter 2 inhibitors. NHE = sodium-hydrogen exchanger.

Recent reviews have called attention to NHE1 and relationships to myocardial energetics as the most plausible mechanism explaining the favorable impact on both phenotypes in HF (Packer et al., 2017a,b; Verma et al., 2018; Chin et al., 2018). Mimicking the physiology of ischemic preconditioning and favorable upregulation of gene programs that result in improved myocardial performance and resistance to hypoxic injury at the cellular level would explain benefit in HF with preserved or reduced ejection fraction. Because the cardiovascular benefits of SGLT2i are independent from the degree of HbA1C reduction and baseline eGFR, it is very likely that pharmacologic ischemic preconditioning and/or more favorable myocardial energetics are the mechanisms of myocardial benefit as opposed to diuresis or theorized chronic volume contraction. In support of this idea, inhibition of hematoblast NHE1 and upregulation of erythropoiesis are concordant with the cardiovascular benefits of SGLT2i. The improvement of T2DM and CKD associated anemia could be viewed as salutary effects for the heart as well (McCullough et al., 2013).

Inhibition of renal NHE3 with SGLT2i may be a mechanism to explain further protection against the adverse impact of angiotensin II in the proximal tubules. As in the heart, SGLT2i inhi-

bition of NHE attenuates renal ischemia reperfusion injury (Chang et al., 2016). Additionally, the role of NHE3 inhibition in concert with glycosuria and natriuresis on tubulo-glomerular feedback resulting in an increase in afferent arteriolar tone and a reduction in glomerular blood flow are areas of active research to explain the renal impact of SGLT2i. This clinical effect appears to be a relatively immediate minor reduction in eGFR which is then sustained and protected from the time related loss of eGFR seen in T2DM. There is a reduction in the progression of albuminuria, but this appears to be a minor effect.

In our view, SGLT2i inhibition of NHE represents a plausible mechanistic pathway for the positive cardiorenal effects as well as the potential adverse effects of DKA, metabolic bone disease, amputation, and Fournier's gangrene (Fig. 5). Of these three, the amputation risk is most plausibly explained by NHE inhibition. The progression of diabetic foot ulcers, critical limb ischemia, deep tissue infection, and osteomyelitis are all related to tissue hypoxia and acidosis. If SGLT2i can slow the restoration of pH in the setting of ischemia or acid loading as well as inhibit the upregulation of NHE in the setting of infection, it is understandable how SGLT2i – despite improving glycemic control and exerting cardiorenal benefit

– could accelerate the risks of amputations in T2DM patients with foot ulcers/gangrene/critical limb ischemia. In one analysis from CANVAS, patients who suffered one or more amputations had the greatest relative risk reductions in cardiovascular endpoints. While a sub-analysis of the EMPA-REG OUTCOME trial did not find an increased amputation risk, the trial did not specifically report critical limb ischemia as an adverse outcome, while CANVAS did (Inzucchi et al., 2018). Thus, the differential amputation risk between the two studies may be partly attributable to a difference in definitions. NHE inhibition appears to be a logical explanation that could account for the amputation risk.

There are many ongoing large clinical trials with SGLT2i as a treatment for HF and CKD in those with T2DM and in some trials, even those without T2DM (Verma et al., 2018). The off-target NHE inhibitory effects of SGLT2i support the rationale for SGLT2i in patients without T2DM. If positive outcomes are observed in the absence of smaller reductions in HbA1C, body weight, and blood pressure, then inhibition of NHE as a global mechanism would be further supported. There is a great need for additional preclinical investment in SGLT2i and NHE inhibition as this off-target effect may be amenable to drug design changes to optimize this important cellular metabolic effect.

9. Summary

The class of SGLT2i agents as strong inhibitors of NHE1 will likely be shown to either moderate or inhibit NHE3 resulting in favorable changes at the cellular and tissue levels in the heart and kidney. NHE inhibition is the most plausible explanation for the cardiorenal benefit of these agents. In contrast, careful consideration of NHE inhibition brings to light the notion that this same mechanism likely explains the adverse effects of these agents described in human use including DKA, metabolic bone disease, amputations, and Fournier's gangrene.

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Conflict of Interest

The authors declare no competing interests.

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