Received: 12 January 2022 | Revised: 4 March 2022 | Accepted: 30 March 2022

DOI: 10.1111/bph.15850

INVITED REVIEW

Amelioration of endothelial dysfunction by sodium glucose co-transporter 2 inhibitors: pieces of the puzzle explaining their cardiovascular protection

Xiaoling Li | Benedikt Preckel | Jeroen Hermanides | Markus W. Hollmann | Coert J. Zuurbier | Nina C. Weber

Department of Anesthesiology – Laboratory of Experimental Intensive Care and Anesthesiology (L.E.I.C.A.), Cardiovascular Science, Amsterdam University Medical Centers, location AMC, Amsterdam, The Netherlands

Correspondence
Nina C. Weber, Department of Anesthesiology – Laboratory of Experimental Intensive Care and Anesthesiology (L.E.I.C.A.), Cardiovascular Science, Amsterdam University Medical Centers, location AMC, Meibergdreef 11, 1105 AZ Amsterdam, The Netherlands.
Email: n.c.hauck@amsterdamumc.nl

Funding information
Chinese Scholarship Council (CSC), Grant/Award Number: 201906210054

Sodium glucose co-transporter 2 inhibitors (SGLT-2is) improve cardiovascular outcomes in both diabetic and non-diabetic patients. Preclinical studies suggest that SGLT-2is directly affect endothelial function in a glucose-independent manner. The effects of SGLT-2is include decreased oxidative stress and inflammatory reactions in endothelial cells. Furthermore, SGLT2is restore endothelium-related vasodilation and regulate angiogenesis. The favourable cardiovascular effects of SGLT-2is could be mediated via a number of pathways: (1) inhibition of the overactive sodium-hydrogen exchanger; (2) decreased expression of nicotinamide adenine dinucleotide phosphate oxidases; (3) alleviation of mitochondrial injury; (4) suppression of inflammation-related signalling pathways (e.g., by affecting NF-κB); (5) modulation of glycolysis; and (6) recovery of impaired NO bioavailability. This review focuses on the most recent progress and existing gaps in preclinical investigations concerning the direct effects of SGLT-2is on endothelial dysfunction and the mechanisms underlying such effects.

KEYWORDS
endothelial dysfunction, inflammation, reactive oxygen species, sodium glucose co-transporter 2 inhibitors, sodium-hydrogen exchanger

1 | INTRODUCTION

Patients with diabetes mellitus (DM) more frequently suffer from heart failure (HF), in particular heart failure with preserved ejection fraction (HFpEF), than individuals without DM (Seferovic et al., 2018). **Sodium glucose co-transporter 2** inhibitors (SGLT-2is), a novel class of glucose-lowering drugs, significantly reduce the risk of cardiovascular death and hospitalization in patients with existing HF, regardless of the presence of DM (Neal et al., 2017; Packer et al., 2020; Wiviott et al., 2019; Zinman et al., 2015). Treatment with **empagliflozin** also reduced the combined outcome of worsening HF, re-hospitalization for HF and death for HF in patients with acute HF (Damman et al., 2020). Recently, the EMPEROR-Preserved Phase III trial has established empagliflozin as the first potential therapy capable of improving cardiovascular outcome in patients suffering from HFpEF (Anker et al., 2021). The

Abbreviations: AGEs, advanced glycation end products; Ang II, angiotensin II; CM, cardiomyocyte; CMEC, cardiac microvascular endothelial cell; DM, diabetes mellitus; EC, endothelial cell; HAEC, human aortic endothelial cell; HCAEC, human coronary artery endothelial cell; HF, heart failure; HFpEF, heart failure with preserved ejection fraction; HIF-1α, hypoxia-induced factor-1α; HKII, hexokinase II; mtROS, mitochondrial reactive oxidative species; NCX, sodium-calcium exchanger; NHE, sodium-hydrogen exchanger; NOX, NAPDH oxidase; RAGE, receptor for advanced glycation end products; SFK, Src family of kinases; SGLT-2is, sodium glucose co-transporter 2 inhibitors; SMC, smooth muscle cell; VE-cadherin, vascular endothelial-cadherin.

pharmacological characteristics of the SGLT-2is discussed here are summarized in Table 1.

Until today, the exact mechanisms underlying these 'off-target' effects of SGLT-2is remain largely unknown. Earlier studies highlighted the direct cardiac effects of SGLT-2is (Kleinbongard et al., 2020; Packer, 2020), which are mediated by alleviation of oxidative stress, inflammation, apoptosis and Ca^{2+} overload of cardiomyocytes (CMs) (Trum et al., 2021; Uthman, Baartscheer, Schumacher, et al., 2018). The PROMIS-HFpEF trial prospectively demonstrated a high prevalence of coronary microvascular disorder and systemic endothelial dysfunction in patients with HF (Shah et al., 2018). Endothelial cells (ECs) form a monolayer over the inner surface of blood vessels (Kruger-Genge et al., 2019). In the adult human heart, ECs account for 12.2% of total cells within the arterial tissues and 7.8% within the ventricular regions (Litvinukova et al., 2020). Physiologically, ECs serve to maintain cardiovascular function by ensuring the production of endothelium-derived vasoactive factors, preventing monocyte adhesion and platelet aggregation, regulating the proliferation of smooth muscle cells (SMCs) as well as the contraction and relaxation of CMs (Monteiro et al., 2019). In patients with diabetes, hyperglycaemia impairs endothelial function and ultimately causes the development of macrovascular and microvascular complications (Shi & Vanhoutte, 2017). Thus, ECs might serve as a novel target to improve cardiac function of patients with HF.

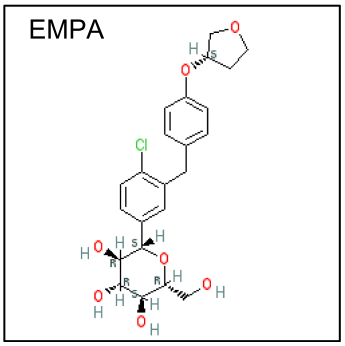
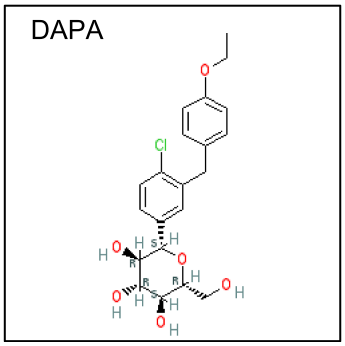
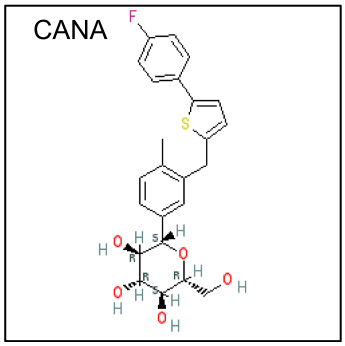
The SGLT-2is are known to directly ameliorate endothelial dysfunction in both euglycaemic and hyperglycaemic conditions (Alshnbari et al., 2020; Durante et al., 2021; Salvatore et al., 2021) and that empagliflozin mitigates endothelial and cardiac dysfunction in patients with HFpEF via reducing inflammatory-oxidative pathways (Kolijn et al., 2020). Our current review focuses on the potential role of improved endothelial function as a crucial contributor to the enhanced cardiac function in patients receiving SGLT-2is. We will review the current data and most recent progress in preclinical investigations concerning the direct effects of SGLT-2is on endothelial dysfunction, with the aim of improving the understanding of their marked cardiovascular effect in patients with HF.

2 | SGLT-2is INHIBIT REACTIVE OXYGEN SPECIES (ROS) PRODUCTION IN ECs

2.1 | Mechanisms underlying the increased ROS production in ECs

At moderate concentrations, ROS play an important role in maintaining the proliferation and survival of ECs, but excessive levels of ROS have detrimental effects on the vascular system (Forstermann et al., 2017). In patients with DM, hyperglycaemia accelerates protein glycation, giving rise to the advanced glycation end products (AGEs)

TABLE 1 Pharmacological characteristics of SGLT-2is

Structures (2D)			
Mechanism	• Reduce glucose re-absorption in kidney via inhibiting the activity of SGLT-2 in the proximal tubule, thereby lowering blood glucose in patients with diabetes		
Physiological actions	• Glycosuria • Weight loss • Blood pressure reduction		
Major benefits	• Decreased incidence of cardiovascular death • Lower risk of hospitalization for heart failure • Limited progression of chronic kidney disease		
Adverse events	• Enhanced incidence of genital mycotic infections • Development of euglycaemic diabetic ketoacidosis • Increased risk of fracture and amputation in lower limb (CANA only)		

Note: Structures of SGLT-2is are obtained from the IUPHAR/BPS Guide to PHARMACOLOGY (http://www.guidetopharmacology.org).
Abbreviations: CANA, canagliflozin; DAPA, dapagliflozin; EMPA, empagliflozin; SGLT-2, sodium glucose co-transporter 2.

(Kay et al., 2016). These AGEs bind with cell surface receptors for AGEs (**RAGE**) to activate downstream signalling pathways, such as those involving **ERK**, and subsequently increase ROS production (Yuan et al., 2019). Excessive ROS also trigger nuclear **poly ADP-ribose polymerase**, which inhibits activity of GADPH and activates the polyol pathway of glucose metabolism (Giri et al., 2018). This activated polyol pathway produces ROS, via depleting **$NADP^+$** and **glutathione**, as well as increasing the oxidation of NADPH, during the conversion of sorbitol to fructose. Inhibition of GADPH also accelerates the generation of diacylglycerol, subsequently activating **PKC** and stimulating NADPH oxidases (**NOX**s) to generate additional ROS (Yuan et al., 2019). Mitochondria are the central regulators for aerobic energy generation, and ROS are an essential by-product of this process. Diabetes and hyperglycaemia disrupt the mitochondrial respiratory chain and alter mitochondrial ultrastructure (e.g., mitochondrial fission and fusion), thereby increasing the ROS production within mitochondria (Brownlee, 2001; Forrester et al., 2018). Hyperglycaemia up-regulates both abundance and activity of the sodium-hydrogen exchangers (**NHE**) within ECs (Klug et al., 2021). Activated NHE promotes the influx of Na^+ and enhances intracellular Ca^{2+} levels, via triggering **sodium-calcium exchangers (NCX)** (Baartscheer et al., 2017). Increased intracellular Na^+ triggers the **NCX** and enhances Ca^{2+} influx into the cytosol. The increased cytosolic Ca^{2+} then stimulates the PKC–NOXs pathway, which further increases ROS production (Rastogi et al., 2016). Recently, Uthman et al. have directly shown the causal link between NHE activity and oxidative stress in ECs. The cytokine TNF-α enhanced NHE activity and intracellular Na^+, as well as ROS production, and the increased ROS generation was mitigated by cariporide, a potent inhibitor of NHE. The crucial role of the NHE/Na^+ axis in inflammation-related oxidative stress was further supported by the fact that sodium pump inhibitor **ouabain** increased intracellular Na^+ and ROS production in human ECs (Uthman et al., 2022).

Excessive ROS increase vascular tone and undermine cardiac inotropic function, contributing to cardiomyopathy (Ritchie & Abel, 2020). Oxidative stress causes uncoupling of the endothelial nitric oxide synthase (**eNOS**) and impairs **NO** production, the key endogenous vasodilator. ROS produced by NOXs also oxidize the **sarcoendoplasmic reticulum calcium transport ATPase (SERCA)** and limit the sensitivity of SMC to NO (Griendling et al., 2021). Besides, ROS induce vascular stiffness via up-regulating the expression of vasoactive factors, such as vascular endothelial growth factor (**VEGF**) and extracellular proteins such as **matrix metalloproteinases** (Griendling et al., 2021). Vascular remodelling elevates blood pressure and increases the intensity of cyclic stretch caused by cycles of vasoconstriction-dilation (Ohishi, 2018). Enhanced stretch might exacerbate oxidative stress via up-regulating expression of NOXs in ECs, further increasing ROS production within ECs (Li et al., 2021). Besides, oscillatory shear stress created by disturbed blood flow also induces oxidative stress in ECs via activating NOXs (Siu et al., 2016).

Depletion of **NO** is a crucial mediator of ROS-related cardiac dysfunction (Shah et al., 2021). EC-derived NO triggers **soluble guanylate cyclase (sGC)** to generate **cGMP** and activate PKG in adjacent CMs. Stimulation of PKG then leads to the phosphorylation of troponin I and reduction of myofilament Ca^{2+} sensitivity, thus enhancing myocardial relaxation in both isolated CMs and whole hearts (Feil et al., 2021; Król & Kepinska, 2021). Activation of the NO/sGC/cGMP/PKG signalling pathway also maintains phosphorylation of **titin** within CMs and prevents the development of cardiac hypertrophy (Shah et al., 2021). Moreover, oxidative stress leads to increased secretion of pro-inflammatory cytokines and chemokines from ECs, as well as up-regulated expression of adhesion molecules and enhanced monocyte-endothelial attachment (Yuan et al., 2019). Intensified ROS production activates the **Src family of kinases** (**SFK**) to phosphorylate vascular endothelial-cadherin (VE-cadherin), leading to VE-cadherin internalization and disruption of adherens junctions. Activated SFK also promotes the transformation from G actin to F actin to generate stress fibres under the cellular membrane, increasing the intracellular tension (Zhang et al., 2017). ROS are also involved in the endothelial dysfunction induced by stretch and oscillatory shear stress through intracellular cascades, such as **p38 MAPK**, ERK, **JNK** and the transcription factor NF-κB (Lehoux, 2006). The pivotal role of EC-derived ROS in development of cardiovascular disease is summarized in Figure 1.

2.2 | Anti-oxidative effect of SGLT-2is

Mounting evidence reveals a class effect of ROS inhibition for SGLT-2is. In vivo studies showed that **ipragliflozin** decreased urinary 8-hydroxy-2′-deoxyguanosine (a marker for DNA oxidative injury) in hyperglycaemic mice (Salim et al., 2016), and **dapagliflozin** attenuated elevated vascular ROS generation in aortic atherosclerotic tissues of diabetic mice (Leng et al., 2016). However, it might be considered that these observed ROS inhibitory effects of SGLT-2is were mediated by the decreased blood glucose in mice with diabetes.

Live cell imaging suggested that SGLT-2is directly inhibited inflammation-stimulated ROS production within human ECs from both venous and arterial vessels (Uthman et al., 2019). Two other studies showed that **empagliflozin** reduced ROS production within cardiac microvascular endothelial cells (CMECs) exposed to pro-inflammatory cytokines and uraemic acid (Juni et al., 2019, 2021). By preventing ROS accumulation within ECs, empagliflozin restored NO bioavailability in co-cultured CMs, indicating that the ROS inhibitory capacity of SGLT-2is contributes to the improvement of contraction and relaxation of adjacent CMs through an endothelial-NO pathway (Juni et al., 2019, 2021). Cellular senescence is another characteristic of diabetes-related endothelial dysfunction, which is promoted by hyperglycaemia-induced oxidative stress. Previous studies suggested that empagliflozin (0.1–100 nM) efficiently prevented senescence of porcine ECs exposed to high glucose, probably by inhibiting NOX- and **cyclooxygenase**-mediated ROS generation (Khemais-Benkhiat et al., 2020). Accordingly, the NOX inhibitor VAS-2870 and empagliflozin abolished the increased endothelial senescence induced by high glucose and **angiotensin II (Ang II)** (Khemais-Benkhiat et al., 2020; Park et al., 2021). A more recent study reported a novel

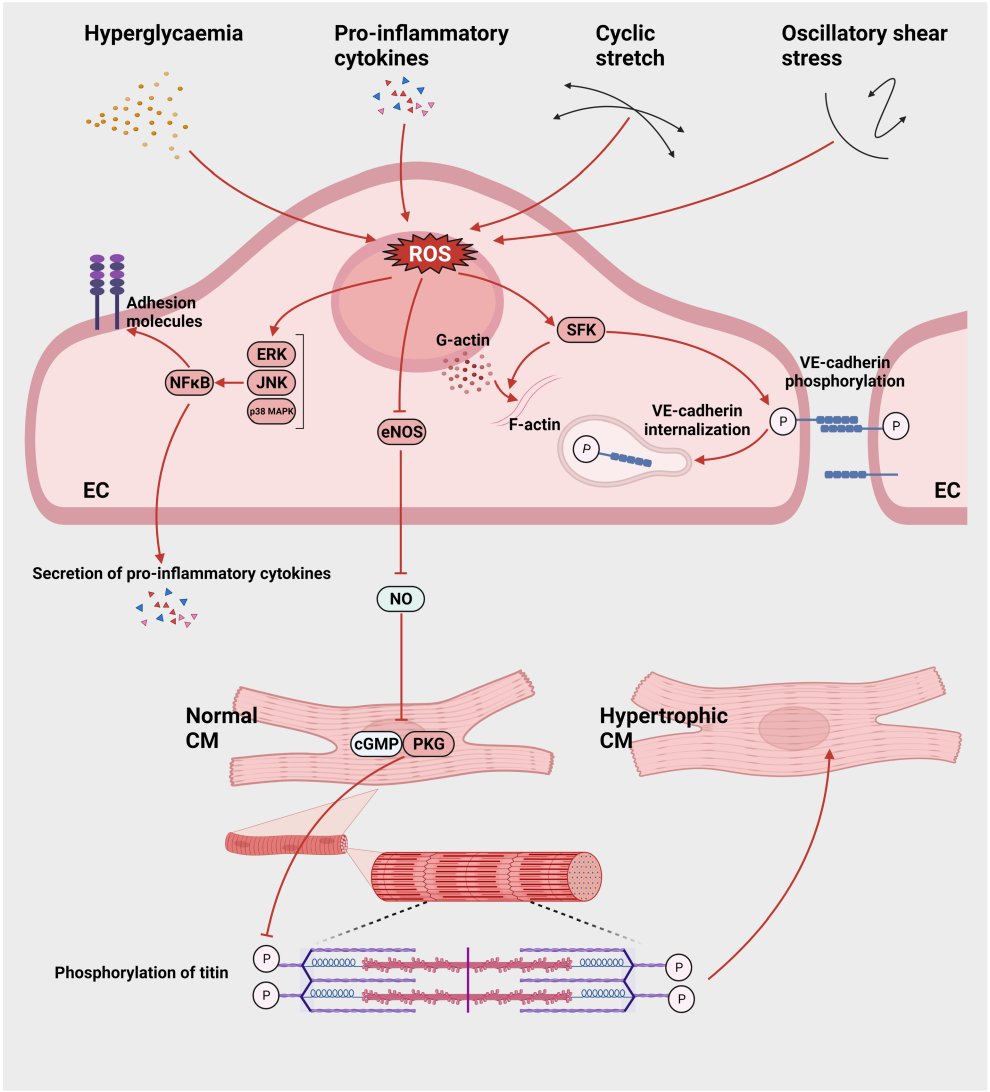


FIGURE 1 The pivotal role of reactive oxygen species (ROS) in endothelial dysfunction and CM hypertrophy. In patients with diabetes, hyperglycaemia along with inflammatory reaction, enhanced cyclic stretch and oscillatory shear stress, increases production of ROS in endothelial cells (ECs). ROS trigger vascular inflammation via activating multiple downstream pathways, including the kinases ERK, JNK and p38 MAPK, as well as by increasing the expression of NF-κB. Excessive ROS induce uncoupling of eNOS and loss of NO bioavailability of endothelial cells. The latter results in the inactivation of the PKG–cGMP signalling and hypo-phosphorylation of titin in adjacent cardiomyocytes (CM), thereby promoting CM hypertrophy. Increased ROS production promotes the formation of F-actin stress fibre and vascular endothelial (VE)-cadherin internalization via activating Src family kinases (SFK), leading to the disruption of adherens junction (created with Biorender.com)

ROS inhibitory effect of SGLT-2is in human coronary artery endothelial cells (HCAECs) undergoing enhanced cyclic stretch, suggesting that SGLT-2is might also alleviate oxidative stress caused by mechanical forces (Li et al., 2021). This study firstly showed that SGLT-2is prevented the loss of VE-cadherin and alleviated barrier dysfunction in HCAECs undergoing enhanced stretch, which was mediated by their ROS inhibitory effect (Li et al., 2021).

2.3 | Potential mechanisms underlying ROS inhibition by SGLT-2is

Several factors contribute to the anti-oxidative effect of SGLT-2is (summarized in Figure 2). SGLT-2is reversed the up-regulation of NOXs and inhibited oxidative stress in the macrovascular and microvascular systems (Ganbaatar et al., 2020; Kuno et al., 2020). In diabetic mice, empagliflozin reduced **NOX2** expression at mRNA levels, in aortic endothelium (Ganbaatar et al., 2020). Correspondingly, empagliflozin also suppressed the increase of NOX2 and **NOX4** in renal tissue of rats with acute kidney injury (Kuno et al., 2020). In vitro, empagliflozin exerted an inhibitory capacity similar to that of **GKT136901**, a specific inhibitor for NOX1 and NOX4, on ROS generation in HCAECs undergoing enhanced stretch. Combination of empagliflozin and GKT136901 did not further reduce the stretch-induced ROS production, suggesting that the anti-oxidative effect of empagliflozin is mediated via NOXs (Li et al., 2021). Furthermore, empagliflozin prevented hyperglycaemia-induced mitochondrial disruption, thereby attenuating the overproduction of cytosolic ROS and mitochondrial ROS (mtROS) in ECs isolated from mice and humans (Juni et al., 2021; Zhou et al., 2018). This mechanism is further supported by the fact that induction of mitochondrial fission abolished the inhibitory effects of empagliflozin on mtROS in mice CMECs (Zhou et al., 2018).

Another potential mechanism that might explain the antioxidant effects of SGLT2is is the direct inhibition of the NHE by SGLT2is, first discovered in CMs (Baartscheer et al., 2017; Uthman, Baartscheer, Bleijlevens, et al., 2018). A recent study from our laboratory showed that 10-μM cariporide blocked the increase of

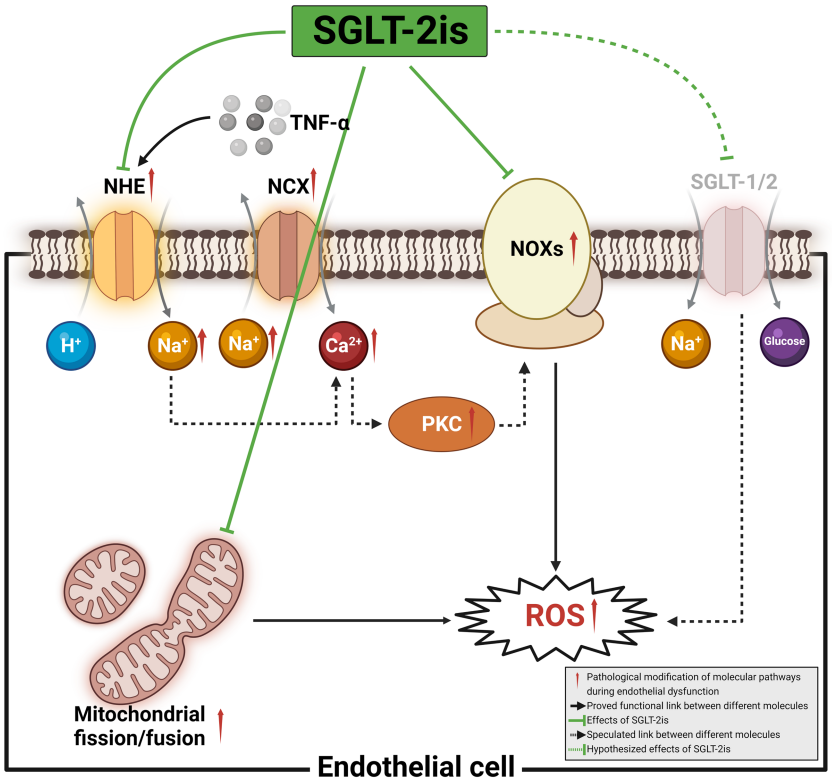


FIGURE 2 Mechanisms underlying the inhibitory effects of SGLT-2is on ROS levels. SGLT-2is inhibit ROS production of endothelial cells, independent of glucose. This anti-oxidative effect could be mediated via inhibition of NOXs, sodium-hydrogen exchanger (NHE) and mitochondrial fission/fusion. Recent studies show that TNF-α activates NHE to increase the intracellular Na^+ concentration, which triggers sodium-calcium exchange, via NCX, and enhances intracellular Ca^{2+} concentration. The increased cytosolic Ca^{2+} then stimulates the PKC-NOX pathway and promotes ROS generation. Whether the Na^+/Ca^{2+}/PKC/NOXs axis is involved in the ROS inhibitory effect of SGLT-2is still requires direct proof. The potential effect of SGLT-1/2 is also open for discussion because the presence of SGLT-2 in endothelial cells is still controversial (created with Biorender.com)

oxidative stress in HCAECs undergoing enhanced cyclic stretch, and this effect of cariporide on ROS production was not further enhanced when combined with empagliflozin. These data indirectly suggest the involvement of NHE in the anti-oxidative capacity of empagliflozin in ECs (Li et al., 2021). In 2021, Uthman et al. provided direct proof that empagliflozin inhibited ROS production in ECs via NHE inhibition, as empagliflozin treatment lowered the NHE activity and Na^+ concentration in human ECs triggered by TNF-α (measured with SNARF-AM and SBFI-AM fluorescence probes, respectively) and also mitigated the increased ROS production. The combination of empagliflozin and cariporide did not demonstrate additional ROS reduction in cells, showing that empagliflozin reduced TNF-α-induced ROS production via NHE inhibition (Uthman et al., 2022).

However, there is still ongoing discussion regarding the role of NHE in the inhibitory effect on ROS of SGLT-2is. In support of our finding, Cappetta et al. (2020) previously reported NHE inhibition by dapagliflozin in 'non-stimulated' HUVECs. In contrast, using cardiac microvascular ECs exposed to uraemic serum, Juni et al. (2021) recently observed a stronger ROS inhibitory capacity of 1-μM empagliflozin when compared with 10-μM cariporide (63% vs. 38%), indicating that part of the anti-oxidative effect of empagliflozin might be unrelated to NHE inhibition. Chung et al. (2020) reported a neutral effect of empagliflozin (1–30 μM) on NHE activity within isolated rat CMs, which is in contrast to the studies of Baartscheer et al. (2017), Uthman, Baartscheer, Bleijlevens, et al. (2018) and Zuurbier et al. (2021) showing that 1-μM empagliflozin inhibited the NHE activity in both isolated cardiac myocytes and isolated intact hearts of different rodents (mice and rabbits). Differences in the methods used might explain the differences between these studies.

The involvement of **SGLT-1/2** in the anti-oxidative effect of SGLT-2is has been recently discussed. Recent studies showed that high glucose and Ang II increased the expression of **SGLT-1** and -2 in porcine ECs and that empagliflozin showed an inhibitory effect on the induced SGLT-1/2 expression (Khemais-Benkhiat et al., 2020; Park et al., 2021). At 24 h, **sotagliflozin** (a dual inhibitor for SGLT-1 and -2) and empagliflozin abolished the Ang II-induced ROS production. Reduction of extracellular glucose and Na^+ concentrations significantly inhibited the pro-oxidant reaction to Ang II, indicating the crucial role of SGLT-1 and -2 in a glucose- and sodium-dependent ROS production (Park et al., 2021). Intriguingly, the sustained oxidative stress triggered by Ang II could also be alleviated by inhibition of NHE, NCX and NOXs, further supporting the functional link between the NHE/Na^+/Ca^{2+} pathway and ROS production by NOXs within ECs (Park et al., 2021).

However, expression of SGLT-2 in ECs is still a matter of debate, especially in the case of human cells. Mancini et al. (2018) showed the absence of SGLT-2 at mRNA level in HUVECs, corresponding with the most recent study of Juni et al. (2021) using human CMECs. In contrast, using Western blot, Behnammanesh et al. (2019) detected the presence of SGLT-2 in human ECs. Uthman et al. (2019) also reported a potential existence of SGLT-2 in human ECs at protein level with a commercially available antibody. But this signal for SGLT-2 protein persisted after the target gene had been silenced at mRNA level, and the qPCR revealed no existence of SGLT-2 (Uthman et al., 2019).

3 | SGLT-2is INHIBIT THE INFLAMMATORY RESPONSE OF ECs TO DIFFERENT STIMULI

3.1 | Increased vascular inflammation in ECs

During hyperglycaemia, binding of AGEs to RAGE elevates adhesion molecule expression, production of cytokines and growth factors via the activation of ERK, JNK and **PI3K** pathways. AGEs directly stimulate monocytes to produce inflammatory mediators such as the ILs and TNF-α, and thereby further enhance local inflammation of the vascular wall (Jin et al., 2018). Moreover, excessive production of ROS induces vascular inflammation in ECs via up-regulating the adhesion molecules **ICAM-1** and **VCAM-1** (Daiber et al., 2020). The oxidative stress triggers cytokine secretion mainly in an 'inflammasome-dependent' manner (Bai et al., 2020). Briefly, ROS activate NF-κB and up-regulate the expression level of the **NLRP3** inflammasome, as well as pro-**IL-1β**. Then, NLRP3 inflammasome is activated through assembling NLRP3, **caspase-1** and apoptosis-associated speck-like protein containing a CARD (caspase activation and recruitment domain), forming a complex for the final production of active caspase-1, IL-1β and **IL-18**. This process is also accelerated by ROS (Ferrucci & Fabbri, 2018; Toldo et al., 2021). Intriguingly, pro-inflammatory mediators released during inflammation, such as TNF-α can induce ROS production, thus promoting the 'vicious cycle' of oxidative stress and inflammation (Yuan et al., 2019).

3.2 | Anti-inflammatory capacity of SGLT-2is

A recent clinical trial revealed that 24-week treatment with empagliflozin significantly reduced serum ICAM-1 level and prevented leukocyte–endothelium interactions in patients with DM (Canet et al., 2021). Empagliflozin inhibited macrophage accumulation, as well the expression of the monocyte chemokine **CCL2** and ICAM-1 in the aortic arch of diabetic mice (Ganbaatar et al., 2020), and dapagliflozin attenuated the high-salt diet-induced up-regulation of VCAM-1 in euglycaemic rats and lowered NF-κB expression within rat ECs (Cappetta et al., 2020). Another study showed that empagliflozin limited Ang II-induced abdominal aortic aneurysm in ApoE knockout mice, partly through inhibiting activation of p38 MAPK and NF-κB in aortas, as well reducing macrophage infiltration within lesions (Ortega et al., 2019).

Consistent with these findings, in vitro studies revealed that dapagliflozin attenuated the increased ICAM-1 and VCAM-1 secretion of HUVECs exposed to hyperglycaemia or TNF-α for 24 h (Gaspari et al., 2018) and that empagliflozin inhibited the TNF-α triggered leukocyte adhesion towards human ECs cultured under flow (Cooper et al., 2019). However, the anti-inflammatory effect in Cooper et al.'s (2019) study was achieved by very with high concentration of empagliflozin (50 μM). Uthman et al. (2019) reported a neutral effect of empagliflozin (1 μM) on TNF-α-induced adhesion molecules expression in static human ECs. In the latter study, ICAM-1 and VCAM-1 were measured 4 h after TNF-α stimulation with flow cytometry, instead of using the supernatant of cell culture as in the study of Gaspari et al. (2018) (Uthman et al., 2019). The dose of SGLT-2is also differed in these two studies (1 μM vs. 1–5 nM, respectively). Yet, lower doses of dapagliflozin (1 and 10 nM) did not inhibit expression of adhesion molecules either in the study of Uthman et al. (2019). Additionally, static cells are not directly comparable with cells cultured under flow. Static cells develop a thinner glycocalyx layer than dynamically activated ECs, and the latter model is considered more physiologically relevant because in situ ECs are constantly exposed to mechanical forces generated by blood flow (Chistiakov et al., 2017; Haymet et al., 2021).

Uthman et al. (2020) reported a compound-specific effect of canagliflozin in inhibiting **IL-6** secretion by HCAECs exposed to lipopolysaccharide (LPS). In this study, only canagliflozin activated **AMPK**, while empagliflozin or dapagliflozin did not alter AMPK phosphorylation in HCAECs (Uthman et al., 2020). The different effects on AMPK might partly explain the diverse effects of the three SGLT-2is on vascular inflammation. Moreover, the decrease in IL-6 induced by canagliflozin was significantly diminished after knockdown of glycolytic hexokinase II (HKII), revealing a novel anti-inflammatory mechanism of canagliflozin, via inhibiting glycolysis (Uthman et al., 2020). In contrast, Abdollahi et al. recently showed that dapagliflozin attenuated the LPS-induced cytokine secretion (IL-6 and IL-8) from HUVECs. This anti-inflammatory effect of dapagliflozin was mediated via inhibition of **toll-like receptor 4** overexpression and NF-κB activation in ECs exposed to 20 $ng \cdot ml^{-1}$ LPS for 24 h, under normal (5.5 mM) and high (25 mM) glucose conditions. Another major finding from this study is that dapagliflozin converted the transition of macrophages from pro-inflammatory M1 phenotype to the anti-inflammatory M2 phenotype, which serves as a crucial therapeutic target during the progression of cardiovascular disease (Abdollahi et al., 2022). Intriguingly, in the former study, HCAECs were exposed to higher concentration of LPS (1 $\mu g \cdot ml^{-1}$) for a shorter period (3 h), and differences in exposures to LPS might partly explain the divergent results concerning IL reduction by dapagliflozin.

4 | SGLT-2is RESTORE IMPAIRED NO BIOAVAILABILITY

4.1 | Mechanisms of reduced NO bioavailability in ECs

Patients with HF show an increased vascular tone in both macro- and micro-vessels, mainly because of blunted NO bioavailability (Forstermann et al., 2017). Physiologically, NO production in ECs is initiated by phosphorylation of eNOS via the PI3K/protein kinase B (**Akt**) pathway. Hyperglycaemia compromises NO generation by inhibiting the expression of eNOS as well as suppressing the phosphorylation of the active site of eNOS (e.g., Ser1177) (Forstermann et al., 2017; Meza et al., 2019). In addition, excessive ROS in ECs consume NO, which can be prevented by anti-oxidative agents

(Meza et al., 2019). Next to the above-mentioned mechanisms, mechanical forces play a crucial role in modulating NO production within ECs. When exposed to unidirectional high shear stress (12–15 dyne·cm^{-2}), the glycocalyx transduces mechanical stimulation to intracellular compartments and triggers diverse downstream pathways, such as PI3K/Akt/eNOS, thus promoting the release of NO (Chistiakov et al., 2017). Increased blood glucose levels degrade the endothelial glycocalyx of diabetic mice (Zuurbier et al., 2005), suggesting a potential interaction between hyperglycaemia and the glycocalyx in eNOS-dependent NO production.

4.2 | Restoration of NO by SGLT-2is

In vivo studies showed that SGLT-2is restored endothelium-dependent vasodilation in both hyperglycaemic and euglycaemic animals (Salim et al., 2016; Sayour et al., 2019). Additionally, in vitro studies showed that empagliflozin and dapagliflozin reversed the loss of NO in human ECs exposed to TNF-α (Juni et al., 2021; Uthman et al., 2019). However, as the phosphorylation of eNOS at Ser1177 in isolated ECs was not affected by empagliflozin, the rescued NO bioavailability in isolated human ECs was most likely mediated by the ROS inhibitory capacity of SGLT-2is (Juni et al., 2021; Uthman et al., 2019). In contrast, empagliflozin promoted eNOS phosphorylation via activating AMPK in ECs isolated from mice (Zhou et al., 2018), which could be partly explained by species difference. Moreover, the studies of Uthman et al. (2019) and Juni et al. (2021) were performed with static ECs. A further improvement in cellular studies could be the incorporation of dynamically activated ECs.

5 | SGLT-2is REVERSE THE DISTURBED ANGIOGENESIS OF ECs

5.1 | Pathological modulation of angiogenesis

Angiogenesis is defined as the formation of new capillaries from established vessels. Physiologically, this process is limited to the embryonic and post-natal stage and rarely found within healthy adults, apart from female reproductive organs (Eelen et al., 2020). Pathological alterations in angiogenesis are highly diverse in different organs/tissues. For example, excessive angiogenesis occurs in diabetic retinopathy/nephropathy, while the angiogenetic capacity is significantly impaired in cardiac and cerebral tissue of patients with DM (Okonkwo & DiPietro, 2017). Growth factors, such as the VEGFs, play key roles in promoting vascular growth and remodelling, and they can be markedly up-regulated by hyperglycaemia, ROS and pro-inflammatory mediators such as TNF-α, but mostly by the lack of oxygen in respective organs. Local hypoxic conditions activate hypoxia-induced factor (HIF)-1α and increase the expression of VEGFs (Apte et al., 2019). Conditional knockout of HIF-1α attenuated VEGF overproduction and prevented the development of diabetic retinopathy within mice, suggesting a crucial role of the HIF-1α/VEGFs pathway in pathological angiogenesis (Lin et al., 2011). Vasodilators, such as NO, are temporarily increased during pregnancy and may promote placental angiogenesis, but whether this effect persists in adult tissue is unclear (Umapathy et al., 2020).

5.2 | The effect of SGLT-2is on angiogenesis

The effect of SGLT-2is on angiogenesis is still controversial. Earlier, Zhou et al. (2018) had demonstrated that empagliflozin promoted angiogenesis of CMECs in diabetic mice, thus improving myocardial microcirculatory perfusion and cardiac function. This study showed that empagliflozin preserved angiogenetic capacity of isolated CMECs through stabilization of F-actin. The effect of empagliflozin was nearly abolished when it was combined with FCCP, a mitochondrial fission activator, to promote mtROS generation (Zhou et al., 2018). Excessive ROS arrested the cell cycle transition from G0/G1 to S and interrupted CMECs proliferation, which was re-established by empagliflozin and mdivi1 (an inhibitor for mitochondrial fission) (Zhou et al., 2018). Taken together, prevention of mitochondrial fission and subsequent oxidative stress by empagliflozin was involved in its pro-angiogenetic effects on CMECs. Correspondingly, a recent study from Nikolaou et al. (2021) showed that chronic administration (6 weeks) of empagliflozin reduced myocardial infarct size after ischaemia–reperfusion in non-diabetic mice, and this cardioprotective effect of empagliflozin could be partly explained by the improved survival of CMECs. An in vitro study with human ECs exposed to hypoxia/reoxygenation stress also showed that empagliflozin increased cellular viability via activating the **STAT3** pathway (Nikolaou et al., 2021). Using human aortic endothelial cells (HAECs), another study revealed that the autophagy is also involved in the pro-angiogenic effect of empagliflozin. The anti-leukaemia agent ponatinib induces vasculotoxicity via mitochondrial damage, while the autophagy-mediated removal of injured mitochondria represents a cardiovascular protective mechanism against the toxic effects of ponatinib. HAECs exposed to ponatinib showed decreases in autophagy marker expression (LC3-I/II), tube formation and cell viability, which were reversed by empagliflozin (Madonna et al., 2021).

In contrast, Behnammanesh et al. (2019) reported a robust anti-proliferative and anti-migration effect of canagliflozin in HUVECs and HAECs. In clinically relevant dosages, canagliflozin (5 and 10 μM) inhibited the proliferation of HUVECs by reducing the expression of cyclin A, as well as by reducing phosphorylation of the retinoblastoma protein, while empagliflozin and dapagliflozin barely influenced the proliferative capacity of ECs, using their physiological doses (1–2 μM) (Behnammanesh et al., 2019; Devineni et al., 2016; Tomlinson et al., 2017). The anti-proliferative effect of canagliflozin could also be beneficial, as it suppressed the increased proliferation and tubular formation of HUVECs during co-culture with Huh7 and HepG2 (hepatocyte-derived carcinoma cell lines), as well the enhanced production of angiogenic cytokines, such as IL-8, thus inhibiting the growth of liver cancer (Kaji et al., 2018). More recently, another study showed that canagliflozin had a dose-dependent inhibitory effect on the expression

TABLE 2 Major findings of the direct endothelial effects of SGLT-2is

References	Cells/tissues	Drug (dosage)	Stimulant (dosage)	Major findings
Abdollahi et al., 2022	HUVECs	DAPA (0.05 or 0.5 μM)/24 h	Lipopolysaccharide (20 ng·ml^{-1})/24 h, under normal (5.5 mM) or high (25 mM) glucose	IL-6 and IL-8 ↓ Toll-like receptor 4 ↓ NF-κB ↓
Behnammanesh et al., 2019	HUVECs HAECs Mice aortic ECs	CANA (0–50 μM)/1–3 days	…	Angiogenesis ↓ Cell proliferation ↓ Cyclin A and retinoblastoma phosphorylation ↓
Cappetta et al., 2020	HUVECs	DAPA (1 μM)	…	NHE activity ↓
	Rat vasculature	DAPA (0.1 mg·kg^{-1}·day^{-1})/6 weeks	High-salt (8% NaCl)/11 weeks	VCAM-1 ↓ NF-κB ↓
Cooper et al., 2019	HAECs	EMPA (50 μM)/24 h	TNF-α (10 μg·ml^{-1})/24 h Wall shear stress (10 dyne·cm^{-2})/24 h	Leukocyte–endothelium adhesion ↓ Glycocalyx integrity ↑
Ganbaatar et al., 2020	Abdominal aorta of Apo E$^{-/-}$ mice	EMPA (20 mg·kg^{-1}·day^{-1})/8–12 weeks	STZ (75 mg·kg^{-1})	NOX2 ↓ Macrophage accumulation ↓ Monocyte chemokine CCL2 ↓ ICAM-1 ↓
Gaspari et al., 2018	HUVECs	DAPA (1 nM to 1 mM)/24 h	TNF-α (10 ng·ml^{-1})/24 h Hyperglycaemia (10–30 mM)/24 h	ICAM-1 ↓ VCAM-1 ↓ NF-κB ↓
	Aortic sections of Apo E$^{-/-}$ mice	DAPA (1 mg·kg^{-1}·day^{-1})/4 weeks	High-fat diet (22% fat + 0.15% cholesterol)/20 weeks	ICAM-1 ↓
Juni et al., 2019	Human CMECs	EMPA (1 μM)/6 h	TNF-α (10 ng·ml^{-1})/6 h	Cytoplasmatic ROS ↓ Mitochondrial ROS ↓ NO bioavailability ↑
Juni et al., 2021	Human CMECs	EMPA (1 μM)/6 h	Uraemic serum (15%)/6 h	Cytoplasmatic ROS ↓ Mitochondrial ROS ↓ Mitochondrial fragmentation ↓ NO bioavailability ↑
Kaji et al., 2018	HUVECs	CANA (10 μM)/12 h	Co-culture with Huh7 and HepG2/24 h	HUVEC proliferation ↓ Tubular formation ↓ IL-8 ↓ Angiogenin ↓ Metalloproteinase-1 ↓
Khemais-Benkhiat et al., 2020	Porcine coronary artery ECs	EMPA (0.1–100 nM)/30 min to 96 h	High glucose (25 mmol·L^{-1})/48 or 96 h	Senescence-associated-beta-galactosidase ↓ p-21 and p-16 protein ↓ ROS ↓ NOX ↓ eNOS and NO ↑ Glucose uptake ↓ VCAM-1 ↓

TABLE 2 (Continued)

References	Cells/tissues	Drug (dosage)	Stimulant (dosage)	Major findings
	Porcine coronary segment	EMPA (100 nM)/30 min	High glucose (25 mmol·L^{-1})/24 h	SGLT-1 and -2 ↓ eNOS ↑ VCAM-1 ↓
Leng et al., 2016	Aortic sections of Apo E$^{-/-}$ mice	DAPA (1.0 mg·kg^{-1}·day^{-1})/12 weeks	STZ (130 mg·kg^{-1}) + high-fat diet (15% fat + 0.15% cholesterol)/4 weeks	ROS ↓ NLRP3 inflammasome ↓
Li et al., 2021	HCAECs	EMPA (1 μM)/24 h DAPA (1 μM)/24 h CANA (3 μM)/24 h	Cyclic stretch (1 Hz, 10%)/24 h	NHE-1 and NOX-mediated ROS ↓ Cell permeability ↓ VE-cadherin ↑
Luo et al., 2021	HUVECs	CANA (10 and 20 μM)/24 h	Co-culture with HepG2/24 h	Angiogenesis ↓ VEGF-A protein ↓
Madonna et al., 2021	HAECs	EMPA (100 and 300 nM)/0–48 h DAPA (100 nM)/0–48 h	PON (1.7 nM)/0–48 h	Tube formation ↑ Cell viability ↑ Autophagy ↑ Senescence ↑
Mancini et al., 2018	HUVECs HAECs	CANA (10 μM)/6 and 24 h	IL-1β (10 ng·ml^{-1})/6 and 24 h	IL-6 ↓ Monocyte chemokine CCL2 ↓ Monocyte adhesion ↓ AMPK activity ↑
Nikolaou et al., 2021	Mice CMECs	EMPA (10 mg·kg^{-1}·day^{-1})/6 weeks	Ischaemia (30 min)/reperfusion (2 h)	VEGF ↑ Superoxide dismutase 2 ↑ Cell survival ↑
	Human CMECs	EMPA (500 nM)/24 h	Hypoxia (3 h)/reoxygenation (1 h)	Cell viability ↑ ROS ↓
Park et al., 2021	Porcine coronary artery ECs	EMPA or sotagliflozin (100 nM)/30 min to 24 h	Ang II (100 nM)	ROS ↓ SGLT-1 and -2 ↓ Senescence-associated-β-galactosidase ↓ eNOS and NO ↑
	Porcine coronary artery ECs	EMPA or sotagliflozin (100 nM)/48 h	Microparticles from patients with coronary artery disease (10 nM)/48 h	SGLT-1 and -2 ↓ eNOS and NO ↑ VCAM-1 ↓
Ortega et al., 2019	Aortic aneurysm sections of Apo E$^{-/-}$ mice	EMPA (3 mg·kg^{-1}·day^{-1})/28 days	Ang II (1000 ng·kg^{-1}·day^{-1})/28 days	Macrophage infiltration ↓ p38 MAPK ↓ NF-κB ↓ VEGF ↓
	HAECs	EMPA (3 μM)/24 h	Ang II (1 μM)/24 h	Leukocyte–endothelium interactions ↓ ICAM-1 ↓ VCAM-1 ↓
Salim et al., 2016	Mice abdominal aorta	Ipragliflozin (3 mg·kg^{-1}·day^{-1})/3 weeks	STZ (150 mg·kg^{-1})	Akt phosphorylation ↑ eNOS phosphorylation ↑

(Continues)

TABLE 2 (Continued)

References	Cells/tissues	Drug (dosage)	Stimulant (dosage)	Major findings
				Monocyte chemokine CCL2 ↓ ICAM-1 ↓ VCAM-1 ↓
Uthman et al., 2019	HCAECs HUVECs	EMPA (1 μM)/4–24 h DAPA (1 μM)/4–24 h	TNF-α (10 ng·ml^{-1})/4–24 h	ROS ↓ NO bioavailability ↑
Uthman et al., 2020	HCAECs	CANA (10 μM)/16 h	Lipopolysaccharide (1 μg·ml^{-1})/3 h	IL-6 ↓ ERK1/2 phosphorylation ↓ AMPK phosphorylation ↑ HKII ↓
Uthman et al., 2022	HCAECs HUVECs	EMPA (1 μM)/6 h	TNF-α (10 ng·ml^{-1})/6 h	NHE activity ↓ Intracellular Na^+ ↓ ROS ↓
Zhou et al., 2018	Mice CMECs	EMPA (10 mg·kg^{-1}·day^{-1})/20 weeks	STZ (50 mg·kg^{-1})/5 days	Angiogenesis ↑ Migration ↑ Wound healing ↑ Cytoplasmatic ROS ↓ Mitochondrial ROS ↓ Mitochondrial fission and fusion ↓ Senescence ↓

Abbreviations: Akt, protein kinase B; AMPK, AMP-activated protein kinase; Ang II, angiotensin II; CANA, canagliflozin; CMEC, cardiac microvascular endothelial cell; DAPA, dapagliflozin; EMPA, empagliflozin; eNOS, endothelial nitric oxide synthase; HAEC, human aortic endothelial cell; HCAEC, human coronary artery endothelial cell; HKII, hexokinase II; HUVEC, human umbilical vein endothelial cell; ICAM-1, intracellular adhesion molecule 1; NHE, sodium-hydrogen exchanger; NLRP3, NOD-like receptor pyrin domain containing 3; NOX, nicotinamide adenine dinucleotide phosphate oxidase; PON, ponatinib; ROS, reactive oxygen species; SGLT, sodium glucose co-transporter; STZ, streptozotocin; VCAM-1, vascular cell adhesion molecule 1; VE-cadherin, vascular endothelial cadherin.

of VEGF-A and angiogenesis of HUVECs co-cultured with HepG2 cells (Luo et al., 2021).

Several factors might explain the observed opposing effects of canagliflozin and empagliflozin on angiogenesis. Firstly, these studies were performed with ECs under different 'stress' conditions. Cells in the empagliflozin studies were activated with pathological stimuli (diabetes, hypoxia/reoxygenation and ponatinib) that impaired their cellular viability and angiogenesis capacity (Madonna et al., 2021; Nikolaou et al., 2021; Zhou et al., 2018), while the anti-angiogenic effect of canagliflozin was reported in 'non-stimulated' cells or ECs co-cultured with cancer cells (Behnammanesh et al., 2019; Kaji et al., 2018; Luo et al., 2021). The potential involvement of SGLT-1 is also of importance, considering that the anti-proliferating effect seems to be compound-specific for canagliflozin (Behnammanesh et al., 2019). Compared with empagliflozin and dapagliflozin, canagliflozin was relatively less selective for SGLT-2 over SGLT-1, and the latter is highly expressed in human ECs (Ohgaki et al., 2016). However, in a HepG2 and ECs co-culture model, canagliflozin reduced glucose uptake and growth of liver cancer cells via inhibiting SGLT-2, rather than SGLT-1 (Kaji et al., 2018). Moreover, the effect of SGLT-2is on ECs proliferation and migration might vary among different organs and tissues, and more research is required to explain these existing differences.

6 | SUMMARY AND PERSPECTIVE

In summary, inhibitors of SGLT-2 demonstrate prominent cardiovascular protective effects in patients with existing HF, with and without diabetes (Anker et al., 2021; Packer et al., 2020). Whereas earlier studies focused on the direct effects of SGLT-2is on CMs, recent work has also emphasized ECs as a promising therapeutic target of SGLT-2is (Durante et al., 2021; Salvatore et al., 2021). Preclinical studies show that SGLT-2is exert favourable effects against endothelial dysfunction, including inhibiting oxidative stress and inflammatory reactions, restoring NO bioavailability and modulating angiogenesis (the major findings of published work are listed in Table 2). This property might contribute to the cardiovascular benefits observed in patients receiving SGLT-2is (Figure 3).

However, there is still much debate about the mechanisms underlying these observed endothelial effects and whether the SGLT-2 transporter is involved. To our knowledge, SGLT-2 is not expressed in human ECs (Juni et al., 2019; Mancini et al., 2018; Uthman et al., 2019), and the protective effects of empagliflozin, dapagliflozin and canagliflozin are therefore most likely mediated via 'off-target' activities, for example, NHE inhibition and glycolysis modulation (Baartscheer et al., 2017; Cappetta et al., 2020; Li et al., 2021; Uthman et al., 2022). However, some studies using porcine cells reported redox-sensitive up-regulation of SGLT-1/2 in ECs, an effect that was attenuated by empagliflozin (Khemais-Benkhiat et al., 2020; Park et al., 2021). Studies with SGLT-2 knockout animals would be able to further explore the potential role of SGLT-2 inhibition in the endothelial effects of these drugs. Besides, species differences can only partly explain these discrepancies, and in vivo studies using rats and mice are vulnerable to the influence of their biokinetic parameters, making it difficult to extrapolate the results to humans (Saeidnia et al., 2015). Moreover, the relatively limited studies in human ECs are of high diversity in origin (micro- or macro-circulation) and developmental stage (adult or postnatal) of the cells (Alshnbari et al., 2020). Further studies with CMECs are required, considering the high prevalence and clinical relevance of coronary microvascular dysfunction in patients with HF (Shah et al., 2018). Remarkably, most of these in vitro studies have been performed in static cells.

Additionally, it is noticeable that the cardiovascular protection of SGLT-2is might be partly mediated by their capacity to switch substrates. SGLT-2is administration facilitates the shift of substrate utilization from carbohydrate to fat and increases ketone generation. This metabolic conversion might optimize cardiac efficiency and reduce oxygen consumption in endangered myocardium

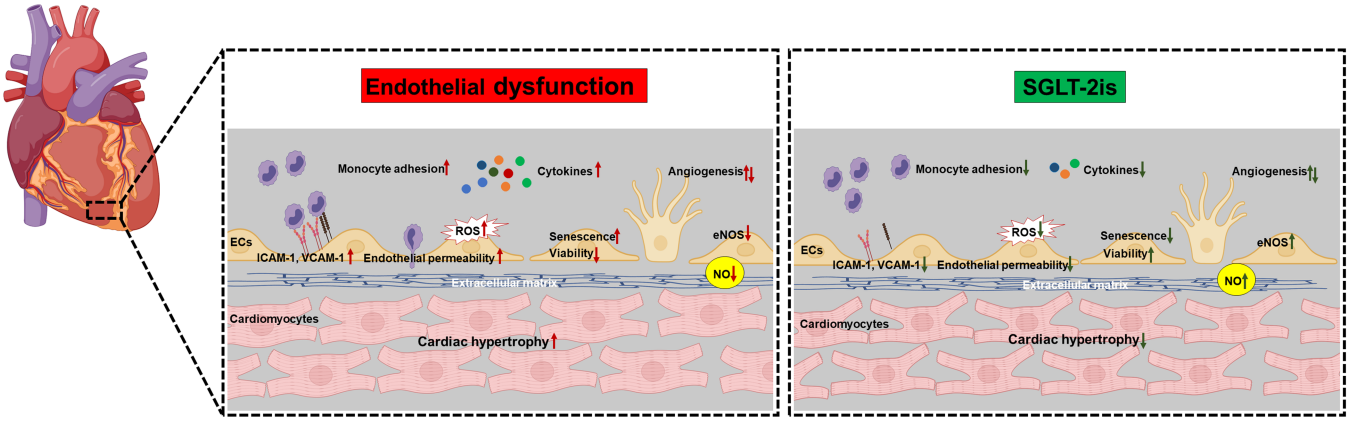


FIGURE 3 Direct endothelial protection by SGLT-2is. SGLT-2is have favourable effects on endothelial cells (ECs), including (1) inhibition of ROS production, (2) prevention of the inflammatory reaction, (3) restoration of NO bioavailability, (4) modulation of angiogenesis and senescence and (5) improvement of cellular viability. The anti-inflammatory effect of SGLT-2is include the down-regulation of VCAM-1 and ICAM-1, the reduction in cytokine secretion and prevention of monocyte–endothelium adhesion. SGLT-2is could also restore NO bioavailability in endothelial cells, via improving phosphorylation of eNOS and scavenging ROS (created with Biorender.com)

(Ferrannini et al., 2016). Thus, it is possible that SGLT-2is ameliorate diabetic microvascular complications via inducing low-grade ketonaemia. These benefits might be mediated via improved fuel energetics, alleviated hypoxia and via the anti-inflammatory/oxidative effects of ketone bodies. However, further studies are needed to explore the potential involvement of ketone generation in the cardiovascular benefits of SGLT-2is (Mudaliar et al., 2021).

Taken together, given that endothelial dysfunction is a crucial promoter for the development of HF and that SGLT-2is exert promising protective effects on ECs, SGLT-2is show great potential in the treatment of cardiovascular disease. Investigations regarding the direct effects of SGLT-2is on the endothelium are needed for a better understanding of the mechanisms underlying these beneficial cardiovascular effects.

6.1 | Nomenclature of targets and ligands

Key protein targets and ligands in this article are hyperlinked to corresponding entries in http://www.guidetopharmacology.org and are permanently archived in the Concise Guide to PHARMACOLOGY 2021/22 (Alexander, Fabbro et al., 2021a, b; Alexander, Kelly et al., 2021a, b; Alexander, Mathie, et al., 2021).

ACKNOWLEDGEMENT

XL is supported by the Chinese Scholarship Council (CSC) (No. 201906210054).

CONFLICT OF INTEREST

No conflict of interest to be disclosed.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article because no new data were created or analysed in this study.

ORCID

Nina C. Weber https://orcid.org/0000-0003-1111-9042

REFERENCES

Abdollahi, E., Keyhanfar, F., Delbandi, A. A., Falak, R., Hajimiresmaiel, S. J., & Shafiei, M. (2022). Dapagliflozin exerts anti-inflammatory effects via inhibition of LPS-induced TLR-4 overexpression and NF-κB activation in human endothelial cells and differentiated macrophages. *European Journal of Pharmacology*, *918*, 174715. https://doi.org/10.1016/j.ejphar.2021.174715

Alexander, S. P., Fabbro, D., Kelly, E., Mathie, A., Peters, J. A., Veale, E. L., Armstrong, J. F., Faccenda, E., Harding, S. D., Pawson, A. J., Southan, C., Davies, J. A., Beuve, A., Brouckaert, P., Bryant, C., Burnett, J. C., Farndale, R. W., Friebe, A., Garthwaite, J., … Waldman, S. A. (2021a). THE CONCISE GUIDE TO PHARMACOLOGY 2021/22: Catalytic receptors. *British Journal of Pharmacology*, *178*(Suppl 1), S264–s312. https://doi.org/10.1111/bph.15541

Alexander, S. P., Fabbro, D., Kelly, E., Mathie, A., Peters, J. A., Veale, E. L., Armstrong, J. F., Faccenda, E., Harding, S. D., Pawson, A. J., Southan, C., Davies, J. A., Boison, D., Burns, K. E., Dessauer, C., Gertsch, J., Helsby, N. A., Izzo, A. A., Koesling, D., … Wong, S. S. (2021b). THE CONCISE GUIDE TO PHARMACOLOGY 2021/22: Enzymes. *British Journal of Pharmacology*, *178*(S1), S313–S411. https://doi.org/10.1111/bph.15542

Alexander, S. P., Kelly, E., Mathie, A., Peters, J. A., Veale, E. L., Armstrong, J. F., Faccenda, E., Harding, S. D., Pawson, A. J., Southan, C., Buneman, O. P., Cidlowski, J. A., Christopoulos, A., Davenport, A. P., Fabbro, D., Spedding, M., Striessnig, J., Davies, J. A., Ahlers-Dannen, K. E., … Zolghadri, Y. (2021a). THE CONCISE GUIDE TO PHARMACOLOGY 2021/22: Other Protein Targets. *British Journal of Pharmacology*, *178*(S1), S1–S26. https://doi.org/10.1111/bph.15537

Alexander, S. P., Kelly, E., Mathie, A., Peters, J. A., Veale, E. L., Armstrong, J. F., Faccenda, E., Harding, S. D., Pawson, A. J., Southan, C., Davies, J. A., Amarosi, L., Anderson, C. M. H., Beart, P. M., Broer, S., Dawson, P. A., Hagenbuch, B., Hammond, J. R., Inui, K. I., … Verri, T. (2021b). THE CONCISE GUIDE TO PHARMACOLOGY 2021/22: Transporters. *British Journal of Pharmacology*, *178*(Suppl 1), S412–s513. https://doi.org/10.1111/bph.15543

Alexander, S. P., Mathie, A., Peters, J. A., Veale, E. L., Striessnig, J., Kelly, E., Armstrong, J. F., Faccenda, E., Harding, S. D., Pawson, A. J., Southan, C., Davies, J. A., Aldrich, R. W., Attali, B., Baggetta, A. M., Becirovic, E., Biel, M., Bill, R. M., Catterall, W. A., … Zhu, M. (2021). THE CONCISE GUIDE TO PHARMACOLOGY 2021/22: Ion channels. *British Journal of Pharmacology*, *178*(Suppl 1), S157–s245. https://doi.org/10.1111/bph.15539

Alshnbari, A. S., Millar, S. A., O'Sullivan, S. E., & Idris, I. (2020). Effect of sodium-glucose cotransporter-2 inhibitors on endothelial function: A systematic review of preclinical studies. *Diabetes Ther*, *11*(9), 1947–1963. https://doi.org/10.1007/s13300-020-00885-z

Anker, S. D., Butler, J., Filippatos, G., Ferreira, J. P., Bocchi, E., Bohm, M., Brunner-La Rocca, H. P., Choi, D. J., Chopra, V., Chuquiure-Valenzuela, E., Giannetti, N., Gomez-Mesa, J. E., Janssens, S., Januzzi, J. L., Gonzalez-Juanatey, J. R., Merkely, B., Nicholls, S. J., Perrone, S. V., Piña, I. L., … EMPEROR-Preserved Trial Investigators. (2021). Empagliflozin in heart failure with a preserved ejection fraction. *The New England Journal of Medicine*, *385*, 1451–1461. https://doi.org/10.1056/NEJMoa2107038

Apte, R. S., Chen, D. S., & Ferrara, N. (2019). VEGF in signaling and disease: Beyond discovery and development. *Cell*, *176*(6), 1248–1264. https://doi.org/10.1016/j.cell.2019.01.021

Baartscheer, A., Schumacher, C. A., Wust, R. C., Fiolet, J. W., Stienen, G. J., Coronel, R., & Zuurbier, C. J. (2017). Empagliflozin decreases myocardial cytoplasmic Na^+ through inhibition of the cardiac Na^+/H^+ exchanger in rats and rabbits. *Diabetologia*, *60*(3), 568–573. https://doi.org/10.1007/s00125-016-4134-x

Bai, B., Yang, Y., Wang, Q., Li, M., Tian, C., Liu, Y., Aung, L. H. H., Li, P. F., Yu, T., & Chu, X. M. (2020). NLRP3 inflammasome in endothelial dysfunction. *Cell Death & Disease*, *11*(9), 776. https://doi.org/10.1038/s41419-020-02985-x

Behnammanesh, G., Durante, Z. E., Peyton, K. J., Martinez-Lemus, L. A., Brown, S. M., Bender, S. B., & Durante, W. (2019). Canagliflozin inhibits human endothelial cell proliferation and tube formation. *Frontiers in Pharmacology*, *10*, 362. https://doi.org/10.3389/fphar.2019.00362

Brownlee, M. (2001). Biochemistry and molecular cell biology of diabetic complications. *Nature*, *414*(6865), 813–820. https://doi.org/10.1038/414813a

Canet, F., Iannantuoni, F., Marañon, A. M., Díaz-Pozo, P., López-Domènech, S., Vezza, T., Navarro, B., Solá, E., Falcón, R., Bañuls, C., Morillas, C., Rocha, M., & Víctor, V. M. (2021). Does empagliflozin modulate leukocyte-endothelium interactions, oxidative stress, and inflammation in type 2 diabetes? *Antioxidants (Basel)*, *10*(8). https://doi.org/10.3390/antiox10081228

Cappetta, D., De Angelis, A., Ciuffreda, L. P., Coppini, R., Cozzolino, A., Micciche, A., Dell'Aversana, C., D'Amario, D., Cianflone, E.,

Scavone, C., Santini, L., Palandri, C., Naviglio, S., Crea, F., Rota, M., Altucci, L., Rossi, F., Capuano, A., Urbanek, K., & Berrino, L. (2020). Amelioration of diastolic dysfunction by dapagliflozin in a non-diabetic model involves coronary endothelium. *Pharmacological Research*, *157*, 104781. https://doi.org/10.1016/j.phrs.2020.104781

Chistiakov, D. A., Orekhov, A. N., & Bobryshev, Y. V. (2017). Effects of shear stress on endothelial cells: Go with the flow. *Acta Physiologica (Oxford, England)*, *219*(2), 382–408. https://doi.org/10.1111/apha.12725

Chung, Y. J., Park, K. C., Tokar, S., Eykyn, T. R., Fuller, W., Pavlovic, D., Swietach, P., & Shattock, M. J. (2020). Off-target effects of SGLT2 blockers: Empagliflozin does not inhibit Na^+/H^+ exchanger-1 or lower $[Na^+]_i$ in the heart. *Cardiovascular Research*, *117*, 2794–2806. https://doi.org/10.1093/cvr/cvaa323

Cooper, S., Teoh, H., Campeau, M. A., Verma, S., & Leask, R. L. (2019). Empagliflozin restores the integrity of the endothelial glycocalyx in vitro. *Molecular and Cellular Biochemistry*, *459*(1–2), 121–130. https://doi.org/10.1007/s11010-019-03555-2

Daiber, A., Steven, S., Vujacic-Mirski, K., Kalinovic, S., Oelze, M., Di Lisa, F., & Münzel, T. (2020). Regulation of vascular function and inflammation via cross talk of reactive oxygen and nitrogen species from mitochondria or NADPH oxidase-implications for diabetes progression. *International Journal of Molecular Sciences*, *21*(10). https://doi.org/10.3390/ijms21103405

Damman, K., Beusekamp, J. C., Boorsma, E. M., Swart, H. P., Smilde, T. D. J., Elvan, A., Eck, J. W. M., Heerspink, H. J. L., & Voors, A. A. (2020). Randomized, double-blind, placebo-controlled, multicentre pilot study on the effects of empagliflozin on clinical outcomes in patients with acute decompensated heart failure (EMPA-RESPONSE-AHF). *European Journal of Heart Failure*, *22*(4), 713–722. https://doi.org/10.1002/ejhf.1713

Devineni, D., Polidori, D., Curtin, C., Stieltjes, H., Tian, H., & Wajs, E. (2016). Single-dose pharmacokinetics and pharmacodynamics of canagliflozin, a selective inhibitor of sodium glucose cotransporter 2, in healthy Indian participants. *Clinical Therapeutics*, *38*(1), 89–98. e81. https://doi.org/10.1016/j.clinthera.2015.11.008

Durante, W., Behnammanesh, G., & Peyton, K. J. (2021). Effects of sodium-glucose co-transporter 2 inhibitors on vascular cell function and arterial remodeling. *International Journal of Molecular Sciences*, *22*(16). https://doi.org/10.3390/ijms22168786

Eelen, G., Treps, L., Li, X., & Carmeliet, P. (2020). Basic and therapeutic aspects of angiogenesis updated. *Circulation Research*, *127*(2), 310–329. https://doi.org/10.1161/circresaha.120.316851

Feil, R., Lehners, M., Stehle, D., & Feil, S. (2021). Visualising and understanding cGMP signals in the cardiovascular system. *British Journal of Pharmacology*. https://doi.org/10.1111/bph.15500

Ferrannini, E., Mark, M., & Mayoux, E. (2016). CV protection in the EMPA-REG OUTCOME trial: A "thrifty substrate" hypothesis. *Diabetes Care*, *39*(7), 1108–1114. https://doi.org/10.2337/dc16-0330

Ferrucci, L., & Fabbri, E. (2018). Inflammageing: Chronic inflammation in ageing, cardiovascular disease, and frailty. *Nature Reviews. Cardiology*, *15*(9), 505–522. https://doi.org/10.1038/s41569-018-0064-2

Forrester, S. J., Kikuchi, D. S., Hernandes, M. S., Xu, Q., & Griendling, K. K. (2018). Reactive oxygen species in metabolic and inflammatory signaling. *Circulation Research*, *122*(6), 877–902. https://doi.org/10.1161/CIRCRESAHA.117.311401

Forstermann, U., Xia, N., & Li, H. (2017). Roles of vascular oxidative stress and nitric oxide in the pathogenesis of atherosclerosis. *Circulation Research*, *120*(4), 713–735. https://doi.org/10.1161/CIRCRESAHA.116.309326

Ganbaatar, B., Fukuda, D., Shinohara, M., Yagi, S., Kusunose, K., Yamada, H., Soeki, T., Hirata, K. I., & Sata, M. (2020). Empagliflozin ameliorates endothelial dysfunction and suppresses atherogenesis in diabetic apolipoprotein E-deficient mice. *European Journal of Pharmacology*, *875*, 173040. https://doi.org/10.1016/j.ejphar.2020.173040

Gaspari, T., Spizzo, I., Liu, H., Hu, Y., Simpson, R. W., Widdop, R. E., & Dear, A. E. (2018). Dapagliflozin attenuates human vascular endothelial cell activation and induces vasorelaxation: A potential mechanism for inhibition of atherogenesis. *Diabetes & Vascular Disease Research*, *15*(1), 64–73. https://doi.org/10.1177/1479164117733626

Giri, B., Dey, S., Das, T., Sarkar, M., Banerjee, J., & Dash, S. K. (2018). Chronic hyperglycemia mediated physiological alteration and metabolic distortion leads to organ dysfunction, infection, cancer progression and other pathophysiological consequences: An update on glucose toxicity. *Biomedicine & Pharmacotherapy*, *107*, 306–328. https://doi.org/10.1016/j.biopha.2018.07.157

Griendling, K. K., Camargo, L. L., Rios, F. J., Alves-Lopes, R., Montezano, A. C., & Touyz, R. M. (2021). Oxidative stress and hypertension. *Circulation Research*, *128*(7), 993–1020. https://doi.org/10.1161/CIRCRESAHA.121.318063

Haymet, A. B., Bartnikowski, N., Wood, E. S., Vallely, M. P., McBride, A., Yacoub, S., Biering, S. B., Harris, E., Suen, J. Y., & Fraser, J. F. (2021). Studying the endothelial glycocalyx in vitro: What is missing? *Front Cardiovasc Med*, *8*, 647086. https://doi.org/10.3389/fcvm.2021.647086

Jin, X., Liu, L., Zhang, Y., Xiang, Y., Yin, G., Lu, Y., Shi, L., Dong, J., & Shen, C. (2018). Advanced glycation end products enhance murine monocyte proliferation in bone marrow and prime them into an inflammatory phenotype through MAPK signaling. *Journal Diabetes Research*, *2018*, 2527406–10. https://doi.org/10.1155/2018/2527406

Juni, R. P., Al-Shama, R., Kuster, D. W. D., van der Velden, J., Hamer, H. M., Vervloet, M. G., Eringa, E. C., Koolwijk, P., & van Hinsbergh, V. W. M. (2021). Empagliflozin restores chronic kidney disease-induced impairment of endothelial regulation of cardiomyocyte relaxation and contraction. *Kidney International*, *99*(5), 1088–1101. https://doi.org/10.1016/j.kint.2020.12.013

Juni, R. P., Kuster, D. W. D., Goebel, M., Helmes, M., Musters, R. J. P., van der Velden, J., Koolwijk, P., Paulus, W. J., & van Hinsbergh, V. W. M. (2019). Cardiac microvascular endothelial enhancement of cardiomyocyte function is impaired by inflammation and restored by empagliflozin. *JACC Basic Transl Sci*, *4*(5), 575–591. https://doi.org/10.1016/j.jacbts.2019.04.003

Kaji, K., Nishimura, N., Seki, K., Sato, S., Saikawa, S., Nakanishi, K., Furukawa, M., Kawaratani, H., Kitade, M., Moriya, K., Namisaki, T., & Yoshiji, H. (2018). Sodium glucose cotransporter 2 inhibitor canagliflozin attenuates liver cancer cell growth and angiogenic activity by inhibiting glucose uptake. *International Journal of Cancer*, *142*(8), 1712–1722. https://doi.org/10.1002/ijc.31193

Kay, A. M., Simpson, C. L., & Stewart, J. A. Jr. (2016). The role of AGE/-RAGE signaling in diabetes-mediated vascular calcification. *Journal Diabetes Research*, *2016*, 6809703. https://doi.org/10.1155/2016/6809703

Khemais-Benkhiat, S., Belcastro, E., Idris-Khodja, N., Park, S. H., Amoura, L., Abbas, M., Auger, C., Kessler, L., Mayoux, E., Toti, F., & Schini-Kerth, V. B. (2020). Angiotensin II-induced redox-sensitive SGLT1 and 2 expression promotes high glucose-induced endothelial cell senescence. *Journal of Cellular and Molecular Medicine*, *24*(3), 2109–2122. https://doi.org/10.1111/jcmm.14233

Kleinbongard, P., Bøtker, H. E., Ovize, M., Hausenloy, D. J., & Heusch, G. (2020). Co-morbidities and co-medications as confounders of cardioprotection—Does it matter in the clinical setting? *British Journal of Pharmacology*, *177*(23), 5252–5269. https://doi.org/10.1111/bph.14839

Klug, N. R., Chechneva, O. V., Hung, B. Y., & O'Donnell, M. E. (2021). High glucose-induced effects on Na^+-K^+-$2Cl^-$ cotransport and Na^+/H^+ exchange of blood-brain barrier endothelial cells: Involvement of SGK1, PKCβII, and SPAK/OSR1. *American Journal of Physiology. Cell Physiology*, *320*(4), C619–C634. https://doi.org/10.1152/ajpcell.00177.2019

Kolijn, D., Pabel, S., Tian, Y., Lódi, M., Herwig, M., Carrizzo, A., Zhazykbayeva, S., Kovács, Á., Fülöp, G. Á., Falcão-Pires, I., Reusch, P. H., Linthout, S. V., Papp, Z., van Heerebeek, L., Vecchione, C., Maier, L. S., Ciccarelli, M., Tschöpe, C., Mügge, A., … Hamdani, N. (2020). Empagliflozin improves endothelial and cardiomyocyte function in human heart failure with preserved ejection fraction via reduced pro-inflammatory-oxidative pathways and protein kinase Gα oxidation. *Cardiovascular Research*, *117*(2), 495–507. https://doi.org/10.1093/cvr/cvaa123

Król, M., & Kepinska, M. (2021). Human nitric oxide synthase—Its functions, polymorphisms, and inhibitors in the context of inflammation, diabetes and cardiovascular diseases. *International Journal of Molecular Sciences*, *22*(1), 56. https://doi.org/10.3390/ijms22010056

Kruger-Genge, A., Blocki, A., Franke, R. P., & Jung, F. (2019). Vascular endothelial cell biology: An update. *International Journal of Molecular Sciences*, *20*(18). https://doi.org/10.3390/ijms20184411

Kuno, A., Kimura, Y., Mizuno, M., Oshima, H., Sato, T., Moniwa, N., Tanaka, M., Yano, T., Tanno, M., Miki, T., & Miura, T. (2020). Empagliflozin attenuates acute kidney injury after myocardial infarction in diabetic rats. *Scientific Reports*, *10*(1), 7238. https://doi.org/10.1038/s41598-020-64380-y

Lehoux, S. (2006). Redox signalling in vascular responses to shear and stretch. *Cardiovascular Research*, *71*(2), 269–279. https://doi.org/10.1016/j.cardiores.2006.05.008

Leng, W., Ouyang, X., Lei, X., Wu, M., Chen, L., Wu, Q., Deng, W., & Liang, Z. (2016). The SGLT-2 inhibitor dapagliflozin has a therapeutic effect on atherosclerosis in diabetic ApoE$^{-/-}$ mice. *Mediators of Inflammation*, *2016*, 6305735. https://doi.org/10.1155/2016/6305735

Li, X., Römer, G., Kerindongo, R. P., Hermanides, J., Albrecht, M., Hollmann, M. W., Zuurbier, C. J., Preckel, B., & Weber, N. C. (2021). Sodium glucose co-transporter 2 inhibitors ameliorate endothelium barrier dysfunction induced by cyclic stretch through inhibition of reactive oxygen species. *International Journal of Molecular Sciences*, *22*(11), 6044. https://www.mdpi.com/1422-0067/22/11/6044

Lin, M., Chen, Y., Jin, J., Hu, Y., Zhou, K. K., Zhu, M., le, Y. Z., Ge, J., Johnson, R. S., & Ma, J. X. (2011). Ischaemia-induced retinal neovascularisation and diabetic retinopathy in mice with conditional knockout of hypoxia-inducible factor-1 in retinal Muller cells. *Diabetologia*, *54*(6), 1554–1566. https://doi.org/10.1007/s00125-011-2081-0

Litvinukova, M., Talavera-Lopez, C., Maatz, H., Reichart, D., Worth, C. L., Lindberg, E. L., Kanda, M., Polanski, K., Heinig, M., Lee, M., Nadelmann, E. R., Roberts, K., Tuck, L., Fasouli, E. S., DeLaughter, D. M., McDonough, B., Wakimoto, H., Gorham, J. M., Samari, S., … Teichmann, S. A. (2020). Cells of the adult human heart. *Nature*, *588*(7838), 466–472. https://doi.org/10.1038/s41586-020-2797-4

Luo, J., Sun, P., Zhang, X., Lin, G., Xin, Q., Niu, Y., Chen, Y., Xu, N., Zhang, Y., & Xie, W. (2021). Canagliflozin modulates hypoxia-induced metastasis, angiogenesis and glycolysis by decreasing HIF-1α protein synthesis via AKT/mTOR pathway. *International Journal of Molecular Sciences*, *22*(24). https://doi.org/10.3390/ijms222413336

Madonna, R., Barachini, S., Moscato, S., Ippolito, C., Mattii, L., Lenzi, C., Balistreri, C. R., Zucchi, R., & de Caterina, R. (2021). Sodium-glucose cotransporter type 2 inhibitors prevent ponatinib-induced endothelial senescence and disfunction: A potential rescue strategy. *Vascular Pharmacology*, *142*, 106949. https://doi.org/10.1016/j.vph.2021.106949

Mancini, S. J., Boyd, D., Katwan, O. J., Strembitska, A., Almabrouk, T. A., & Kennedy, S. (2018). Canagliflozin inhibits interleukin-1β-stimulated cytokine and chemokine secretion in vascular endothelial cells by AMP-activated protein kinase-dependent and -independent mechanisms. *Scientific Reports*, *8*(1), 5276. https://doi.org/10.1038/s41598-018-23420-4

Meza, C. A., La Favor, J. D., Kim, D. H., & Hickner, R. C. (2019). Endothelial dysfunction: Is there a hyperglycemia-induced imbalance of NOX and NOS? *International Journal of Molecular Sciences*, *20*(15). https://doi.org/10.3390/ijms20153775

Monteiro, J. P., Bennett, M., Rodor, J., Caudrillier, A., Ulitsky, I., & Baker, A. H. (2019). Endothelial function and dysfunction in the cardiovascular system: The long non-coding road. *Cardiovascular Research*, *115*(12), 1692–1704. https://doi.org/10.1093/cvr/cvz154

Mudaliar, S., Hupfeld, C., & Chao, D. L. (2021). SGLT2 inhibitor-induced low-grade ketonemia ameliorates retinal hypoxia in diabetic retinopathy—A novel hypothesis. *The Journal of Clinical Endocrinology and Metabolism*, *106*(5), 1235–1244. https://doi.org/10.1210/clinem/dgab050

Neal, B., Perkovic, V., Mahaffey, K. W., de Zeeuw, D., Fulcher, G., Erondu, N., Shaw, W., Law, G., Desai, M., & Matthews, D. R. (2017). Canagliflozin and cardiovascular and renal events in type 2 diabetes. *The New England Journal of Medicine*, *377*(7), 644–657. https://doi.org/10.1056/NEJMoa1611925

Nikolaou, P. E., Efentakis, P., Abu Qourah, F., Femminò, S., Makridakis, M., Kanaki, Z., Varela, A., Tsoumani, M., Davos, C. H., Dimitriou, C. A., Tasouli, A., Dimitriadis, G., Kostomitsopoulos, N., Zuurbier, C. J., Vlahou, A., Klinakis, A., Brizzi, M. F., Iliodromitis, E. K., & Andreadou, I. (2021). Chronic empagliflozin treatment reduces myocardial infarct size in nondiabetic mice through STAT-3-mediated protection on microvascular endothelial cells and reduction of oxidative stress. *Antioxidants Redox Signaling*, *34*(7), 551–571. https://doi.org/10.1089/ars.2019.7923

Ohgaki, R., Wei, L., Yamada, K., Hara, T., Kuriyama, C., Okuda, S., Ueta, K., Shiotani, M., Nagamori, S., & Kanai, Y. (2016). Interaction of the sodium/glucose cotransporter (SGLT) 2 inhibitor canagliflozin with SGLT1 and SGLT2. *The Journal of Pharmacology and Experimental Therapeutics*, *358*(1), 94–102. https://doi.org/10.1124/jpet.116.232025

Ohishi, M. (2018). Hypertension with diabetes mellitus: Physiology and pathology. *Hypertension Research*, *41*(6), 389–393. https://doi.org/10.1038/s41440-018-0034-4

Okonkwo, U. A., & DiPietro, L. A. (2017). Diabetes and wound angiogenesis. *International Journal of Molecular Sciences*, *18*(7). https://doi.org/10.3390/ijms18071419

Ortega, R., Collado, A., Selles, F., Gonzalez-Navarro, H., Sanz, M.-J., Real, J. T., & Piqueras, L. (2019). SGLT-2 (sodium-glucose cotransporter 2) inhibition reduces Ang II (angiotensin II)-induced dissecting abdominal aortic aneurysm in ApoE (apolipoprotein E) knockout mice. *Arteriosclerosis, Thrombosis, and Vascular Biology*, *39*(8), 1614–1628. https://doi.org/10.1161/ATVBAHA.119.312659

Packer, M. (2020). Molecular, cellular, and clinical evidence that sodium-glucose cotransporter 2 inhibitors act as neurohormonal antagonists when used for the treatment of chronic heart failure. *Journal of the American Heart Association*, *9*(16), e016270. https://doi.org/10.1161/jaha.120.016270

Packer, M., Anker, S. D., Butler, J., Filippatos, G., Pocock, S. J., Carson, P., Januzzi, J., Verma, S., Tsutsui, H., Brueckmann, M., Jamal, W., Kimura, K., Schnee, J., Zeller, C., Cotton, D., Bocchi, E., Böhm, M., Choi, D. J., Chopra, V., … Zannad, F. (2020). Cardiovascular and renal outcomes with empagliflozin in heart failure. *The New England Journal of Medicine*, *383*(15), 1413–1424. https://doi.org/10.1056/NEJMoa2022190

Park, S. H., Belcastro, E., Hasan, H., Matsushita, K., Marchandot, B., Abbas, M., Toti, F., Auger, C., Jesel, L., Ohlmann, P., Morel, O., & Schini-Kerth, V. B. (2021). Angiotensin II-induced upregulation of SGLT1 and 2 contributes to human microparticle-stimulated endothelial senescence and dysfunction: Protective effect of gliflozins. *Cardiovascular Diabetology*, *20*(1), 65. https://doi.org/10.1186/s12933-021-01252-3

Rastogi, R., Geng, X., Li, F., & Ding, Y. (2016). NOX activation by subunit interaction and underlying mechanisms in disease. *Frontiers in Cellular Neuroscience*, *10*, 301. https://doi.org/10.3389/fncel.2016.00301

Ritchie, R. H., & Abel, E. D. (2020). Basic mechanisms of diabetic heart disease. *Circulation Research*, *126*(11), 1501–1525. https://doi.org/10.1161/CIRCRESAHA.120.315913

Saeidnia, S., Manayi, A., & Abdollahi, M. (2015). From in vitro experiments to in vivo and clinical studies; pros and cons. *Current Drug Discovery Technologies*, *12*(4), 218–224. https://doi.org/10.2174/1570163813666160114093140

Salim, H. M., Fukuda, D., Yagi, S., Soeki, T., Shimabukuro, M., & Sata, M. (2016). Glycemic control with ipragliflozin, a novel selective SGLT2 inhibitor, ameliorated endothelial dysfunction in streptozotocin-induced diabetic mouse. *Front Cardiovasc Med*, *3*, 43. https://doi.org/10.3389/fcvm.2016.00043

Salvatore, T., Caturano, A., Galiero, R., Di Martino, A., Albanese, G., Vetrano, E., Sardu, C., Marfella, R., Rinaldi, L., & Sasso, F. C. (2021). Cardiovascular benefits from gliflozins: Effects on endothelial function. *Biomedicine*, *9*(10). https://doi.org/10.3390/biomedicines9101356

Sayour, A. A., Korkmaz-Icoz, S., Loganathan, S., Ruppert, M., Sayour, V. N., Olah, A., Benke, K., Brune, M., Benkő, R., Horváth, E. M., Karck, M., Merkely, B., Radovits, T., & Szabó, G. (2019). Acute canagliflozin treatment protects against in vivo myocardial ischemia-reperfusion injury in non-diabetic male rats and enhances endothelium-dependent vasorelaxation. *Journal of Translational Medicine*, *17*(1), 127. https://doi.org/10.1186/s12967-019-1881-8

Seferovic, P. M., Petrie, M. C., Filippatos, G. S., Anker, S. D., Rosano, G., Bauersachs, J., Paulus, W. J., Komajda, M., Cosentino, F., de Boer, R. A., Farmakis, D., Doehner, W., Lambrinou, E., Lopatin, Y., Piepoli, M. F., Theodorakis, M. J., Wiggers, H., Lekakis, J., Mebazaa, A., … McMurray, J. J. V. (2018). Type 2 diabetes mellitus and heart failure: A position statement from the Heart Failure Association of the European Society of Cardiology. *European Journal of Heart Failure*, *20*(5), 853–872. https://doi.org/10.1002/ejhf.1170

Shah, A. K., Bhullar, S. K., Elimban, V., & Dhalla, N. S. (2021). Oxidative stress as a mechanism for functional alterations in cardiac hypertrophy and heart failure. *Antioxidants (Basel)*, *10*(6). https://doi.org/10.3390/antiox10060931

Shah, S. J., Lam, C. S. P., Svedlund, S., Saraste, A., Hage, C., Tan, R. S., Beussink-Nelson, L., Ljung Faxén, U., Fermer, M. L., Broberg, M. A., Gan, L. M., & Lund, L. H. (2018). Prevalence and correlates of coronary microvascular dysfunction in heart failure with preserved ejection fraction: PROMIS-HFpEF. *European Heart Journal*, *39*(37), 3439–3450. https://doi.org/10.1093/eurheartj/ehy531

Shi, Y., & Vanhoutte, P. M. (2017). Macro- and microvascular endothelial dysfunction in diabetes. *Journal of Diabetes*, *9*(5), 434–449. https://doi.org/10.1111/1753-0407.12521

Siu, K. L., Gao, L., & Cai, H. (2016). Differential roles of protein complexes NOX1-NOXO1 and NOX2-p47phox in mediating endothelial redox responses to oscillatory and unidirectional laminar shear stress. *The Journal of Biological Chemistry*, *291*(16), 8653–8662. https://doi.org/10.1074/jbc.M115.713149

Toldo, S., Mezzaroma, E., Buckley, L. F., Potere, N., Di Nisio, M., Biondi-Zoccai, G., Van Tassell, B. W., & Abbate, A. (2021). Targeting the NLRP3 inflammasome in cardiovascular diseases. *Pharmacology & Therapeutics*, *236*, 108053. https://doi.org/10.1016/j.pharmthera.2021.108053

Tomlinson, B., Hu, M., Zhang, Y., Chan, P., & Liu, Z. M. (2017). Evaluation of the pharmacokinetics, pharmacodynamics and clinical efficacy of empagliflozin for the treatment of type 2 diabetes. *Expert Opinion on Drug Metabolism & Toxicology*, *13*(2), 211–223. https://doi.org/10.1080/17425255.2017.1258401

Trum, M., Riechel, J., & Wagner, S. (2021). Cardioprotection by SGLT2 inhibitors—Does it all come down to Na^+? *International Journal of Molecular Sciences*, *22*(15). https://doi.org/10.3390/ijms22157976

Umapathy, A., Chamley, L. W., & James, J. L. (2020). Reconciling the distinct roles of angiogenic/anti-angiogenic factors in the placenta and maternal circulation of normal and pathological pregnancies. *Angiogenesis*, *23*(2), 105–117. https://doi.org/10.1007/s10456-019-09694-w

Uthman, L., Baartscheer, A., Bleijlevens, B., Schumacher, C. A., Fiolet, J. W. T., Koeman, A., Jancev, M., Hollmann, M. W., Weber, N. C., Coronel, R., & Zuurbier, C. J. (2018). Class effects of SGLT2 inhibitors in mouse cardiomyocytes and hearts: Inhibition of Na^+/H^+ exchanger, lowering of cytosolic Na^+ and vasodilation. *Diabetologia*, *61*(3), 722–726. https://doi.org/10.1007/s00125-017-4509-7

Uthman, L., Baartscheer, A., Schumacher, C. A., Fiolet, J. W. T., Kuschma, M. C., Hollmann, M. W., Coronel, R., Weber, N. C., & Zuurbier, C. J. (2018). Direct cardiac actions of sodium glucose cotransporter 2 inhibitors target pathogenic mechanisms underlying heart failure in diabetic patients. *Frontiers in Physiology*, *9*, 1575. https://doi.org/10.3389/fphys.2018.01575

Uthman, L., Homayr, A., Juni, R. P., Spin, E. L., Kerindongo, R., Boomsma, M., Hollmann, M. W., Preckel, B., Koolwijk, P., van Hinsbergh, V., Zuurbier, C. J., Albrecht, M., & Weber, N. C. (2019). Empagliflozin and dapagliflozin reduce ROS generation and restore NO bioavailability in tumor necrosis factor α-stimulated human coronary arterial endothelial cells. *Cellular Physiology and Biochemistry*, *53*(5), 865–886. https://doi.org/10.33594/000000178

Uthman, L., Kuschma, M., Romer, G., Boomsma, M., Kessler, J., Hermanides, J., Hollmann, M. W., Preckel, B., Zuurbier, C. J., & Weber, N. C. (2020). Novel anti-inflammatory effects of canagliflozin involving hexokinase II in lipopolysaccharide-stimulated human coronary artery endothelial cells. *Cardiovascular Drugs and Therapy*, *35*, 1083–1094. https://doi.org/10.1007/s10557-020-07083-w

Uthman, L., Li, X., Baartscheer, A., Schumacher, C. A., Baumgart, P., Hermanides, J., Preckel, B., Hollmann, M. W., Coronel, R., Zuurbier, C. J., & Weber, N. C. (2022). Empagliflozin reduces oxidative stress through inhibition of the novel inflammation/NHE/$[Na^+]$c/ROS-pathway in human endothelial cells. *Biomedicine & Pharmacotherapy*, *146*, 112515. https://doi.org/10.1016/j.biopha.2021.112515

Wiviott, S. D., Raz, I., Bonaca, M. P., Mosenzon, O., Kato, E. T., Cahn, A., Silverman, M. G., Zelniker, T. A., Kuder, J. F., Murphy, S. A., Bhatt, D. L., Leiter, L. A., McGuire, D. K., Wilding, J. P. H., Ruff, C. T., Gause-Nilsson, I. A. M., Fredriksson, M., Johansson, P. A., Langkilde, A. M., & Sabatine, M. S. (2019). Dapagliflozin and cardiovascular outcomes in type 2 diabetes. *The New England Journal of Medicine*, *380*(4), 347–357. https://doi.org/10.1056/NEJMoa1812389

Yuan, T., Yang, T., Chen, H., Fu, D., Hu, Y., Wang, J., Yuan, Q., Yu, H., Xu, W., & Xie, X. (2019). New insights into oxidative stress and inflammation during diabetes mellitus-accelerated atherosclerosis. *Redox Biology*, *20*, 247–260. https://doi.org/10.1016/j.redox.2018.09.025

Zhang, W. J., Li, P. X., Guo, X. H., & Huang, Q. B. (2017). Role of moesin, Src, and ROS in advanced glycation end product-induced vascular endothelial dysfunction. *Microcirculation*, *24*(3), e12358. https://doi.org/10.1111/micc.12358

Zhou, H., Wang, S., Zhu, P., Hu, S., Chen, Y., & Ren, J. (2018). Empagliflozin rescues diabetic myocardial microvascular injury via AMPK-mediated inhibition of mitochondrial fission. *Redox Biology*, *15*, 335–346. https://doi.org/10.1016/j.redox.2017.12.019

Zinman, B., Wanner, C., Lachin, J. M., Fitchett, D., Bluhmki, E., Hantel, S., Mattheus, M., Devins, T., Johansen, O. E., Woerle, H. J., Broedl, U. C., & Inzucchi, S. E. (2015). Empagliflozin, cardiovascular outcomes, and mortality in type 2 diabetes. *The New England Journal of Medicine*, *373*(22), 2117–2128. https://doi.org/10.1056/NEJMoa1504720

Zuurbier, C. J., Baartscheer, A., Schumacher, C. A., Fiolet, J. W. T., & Coronel, R. (2021). SGLT2 inhibitor empagliflozin inhibits the cardiac Na^+/H^+ exchanger 1: Persistent inhibition under various experimental conditions. *Cardiovascular Research*, *117*, 2699–2701. https://doi.org/10.1093/cvr/cvab129

Zuurbier, C. J., Demirci, C., Koeman, A., Vink, H., & Ince, C. (2005). Short-term hyperglycemia increases endothelial glycocalyx permeability and acutely decreases lineal density of capillaries with flowing red blood cells. *Journal of Applied Physiology (Bethesda, MD: 1985)*, *99*(4), 1471–1476. https://doi.org/10.1152/japplphysiol.00436.2005

How to cite this article: Li, X., Preckel, B., Hermanides, J., Hollmann, M. W., Zuurbier, C. J., & Weber, N. C. (2022). Amelioration of endothelial dysfunction by sodium glucose co-transporter 2 inhibitors: pieces of the puzzle explaining their cardiovascular protection. *British Journal of Pharmacology*, *179*(16), 4047–4062. https://doi.org/10.1111/bph.15850