Diabetes Ther
https://doi.org/10.1007/s13300-020-00885-z

REVIEW

Effect of Sodium-Glucose Cotransporter-2 Inhibitors on Endothelial Function: A Systematic Review of Preclinical Studies

Afnan S. Alshnbari · Sophie A. Millar · Saoirse E. O'Sullivan · Iskandar Idris

Received: June 8, 2020

ABSTRACT

Introduction: While the beneficial effects of sodium-glucose cotransporter-2 (SGLT-2) inhibitors on cardiovascular and renal outcomes are recognized, their direct effects on endothelial function remain unclear. We, therefore, undertook a systematic review to evaluate the current literature in this area.

Methods: Electronic databases (PubMed, EMBASE, and Medline) were systematically searched using PRISMA guidelines for studies involving the in vitro, in vivo, or ex vivo administration of SGLT-2 inhibitors to animals, vascular tissue, or vascular endothelial cells.

Results: Of 144 retrieved publications, 24 experimental studies met the inclusion criteria. Reporting of possible sources of bias were poor, making the overall risk of bias difficult to assess. Within the 24 studies, the SGLT-2 inhibitors canagliflozin, ipragliflozin, empagliflozin, dapagliflozin, tofogliflozin, and luseogliflozin were assessed as interventions. Animal model studies (n = 17) demonstrated that all SGLT-2 inhibitors prevented endothelial dysfunction and enhanced endothelium-dependent vasorelaxation in diabetic and non-diabetic models. In vitro studies (n = 9) using human endothelial cells indicated a direct anti-inflammatory effect of dapagliflozin (1–100 nM) and canagliflozin, (10 μM), while empagliflozin (1 and 10 μM) improved viability of hyperglycemic cells. Potential mechanisms of action of the SGLT-2 inhibitors include a reduction in oxidative stress, modulation of adhesion molecules and reductions in pro-inflammatory cytokines.

Conclusions: Preclinical studies indicate that SGLT-2 inhibitors attenuate vascular dysfunction in preclinical models via a combination of mechanisms that appear to act independently of glucose-lowering benefits.

Keywords: Endothelial; Sodium-glucose co-transporter 2 inhibitors; Systematic review; Vascular

Digital Features To view digital features for this article go to: https://doi.org/10.6084/m9.figshare.12646328.

Electronic Supplementary Material The online version of this article (https://doi.org/10.1007/s13300-020-00885-z) contains supplementary material, which is available to authorized users.

A. S. Alshnbari · S. A. Millar · S. E. O'Sullivan · I. Idris (✉)
Vascular Research Group, Division of Medical Sciences and Graduate Entry Medicine, School of Medicine, University of Nottingham, Nottingham, UK
e-mail: iskandar.idris@nottingham.ac.uk

Published online: 27 July 2020

Key Summary Points

Sodium-glucose cotransporter-2 (SGLT2) inhibitors function through a novel mechanism of reducing renal tubular glucose reabsorption by inhibiting target SGLT2 receptors present in the renal tubule.

All studies which have reported experimental effects of SGLT-2 inhibitors suggest that this class of drug may exert 'off-target' cardiovascular benefits by modulating vascular endothelial cell activation and improving endothelial cell dysfunction, a critical early step in atherogenesis.

Chronic and acute treatment with dapagliflozin led to a significant endothelial-dependent vasorelaxation in the aorta of diabetic mice, which some studies suggest may be due to a direct effect on vascular cells.

The ex vivo and in vitro studies reviewed here support a possible class effect of SGLT-2 inhibitors on the regulation of endothelial function.

Anti-inflammatory effects of SGLT-2 inhibitors have been observed in diabetic nephropathy models, via a suppression of the advanced glycation endproducts (AGEs)-receptor pathway, as well as in in vitro studies, thereby implicating anti-inflammatory effects that are independent of glucose-lowering.

Systemic administration of SGLT-2 inhibitors markedly reduced expression of pro-inflammatory adhesion markers and cytokines in diabetic rodent models.

Arguably, the evidence from the experimental studies reported in this review points towards SGLT-2 inhibitors exerting additional benefits beyond their primary receptor targets in the renal tubule as well as acting independently of glucose control.

INTRODUCTION

Diabetes is a major public health problem [1] with an increasing economic burden worldwide and a strong link to cardiovascular disease [2]. The insulin resistance associated with type 2 diabetes (T2D) contributes to hyperglycemia, dyslipidemia, and hypertension, all of which significantly increase cardiovascular risk [3]. Such cardiovascular risk factors are associated with impaired endothelial function [4], a significant contributing factor to the pathogenesis of atherosclerotic vascular disease in patients with T2D [5–7]. Possible mechanisms of hyperglycemia-induced endothelial damage include insulin resistance and inflammation [8]. Nitric oxide (NO) [produced by endothelial NO synthase (eNOS)] exerts cardio-protective effects by inducing the relaxation of smooth muscle cells, thereby preventing several cascades of events, including migration of leukocytes into arteries, platelet adhesion, and smooth muscle cell proliferation [4, 9]. In diabetes, however, reduced eNOS activity and/or elevated reactive oxygen species (ROS) production reduces NO bioavailability and increases the production of harmful endothelial-derived vasoactive mediators such as endothelin-1 and ROS, leading to the progression of atherosclerosis and hypertension [4].

In recent years landmark clinical studies have reported the beneficial effects of the antidiabetic agents glucagon-like peptide-1 (GLP-1) receptor agonists and sodium-glucose co-transporter-2 (SGLT-2) inhibitors in reducing the risk of cardiovascular mortality in patients with T2D [10–12]. The findings of a recent network meta-analysis revealed that although both classes of antidiabetic drugs demonstrated cardiovascular benefits, SGLT-2 inhibitors were superior in reducing cardiovascular events compared to GLP-1 receptor agonists which was more beneficial in reducing the risk of nonfatal stroke [13].

SGLT-2 expression occurs in both the small intestine and kidney. These transporters act by modulating glucose reabsorption in the proximal renal tubules [14, 15]. Inhibition of SGLT-2 in kidneys facilitates excess urinary glucose excretion and lowers circulating blood glucose

levels in patients with diabetes. Although SGLT-2 expression has not been reported in cardiac and vascular tissues, recent clinical evidence has shown considerable reductions in blood pressure and improvement in endothelial function associated with their use. However, it is unclear if these effects are just due to the indirect glucose-lowering effect of these antidiabetic drugs [16, 17]. There is accumulating evidence on the effect of SGLT-2 inhibitors and their underlying mechanisms in animal and in vitro models of endothelial dysfunction [10]. Collectively, these indicate that SGLT-2 inhibitors are likely to have a direct influence on the cardiovascular system.

Consequently, a number of large clinical studies have been conducted to investigate endothelial function in patients treated with SGLT-2 inhibitors. The DEFENCE study found a significant improvement in endothelial function in patients with T2D receiving dapagliflozin 5 mg/day as an add-on therapy to metformin 750 mg/day [18]. Similarly, T2D patients with ischemic heart disease showed a significant reduction in surrogate markers for endothelial function following 12 weeks of dapagliflozin monotherapy [19]. An investigation by Solini et al. suggested that dapagliflozin might have a protective cardioprotective effect in preserving vasodilating capacity [20]. Similarly, Lunder et al. showed that empagliflozin 25 mg/day as add-on therapy to metformin therapy 2000 mg/day significantly reduced arterial stiffness in patients with type 1 diabetes [21]. Furthermore, the findings from a recent systematic review found that only SGLT-2 inhibitors significantly improved endothelial function, as assessed by flow-mediated dilation, in comparison to other classes of antidiabetic agents [22].

This systematic review was therefore undertaken to evaluate the effect of SGLT-2 inhibitors on endothelial function in preclinical models. The evidence from animal and in vitro studies will enhance our understanding of the underlying molecular mechanisms of SGLT-2 inhibitors on endothelial dysfunction and in turn lead to the development of novel approaches to improved management of T2D patients, especially those with high risk of cardiovascular events.

METHODOLOGY

Database Search

This systematic review was performed following the Preferred Reporting Items for Systematic Review (PRISMA) guidelines [23]. Both the PubMed and EMBASE (including MEDLINE) electronic databases were searched from inception to May 2020 for publications reporting in vivo, ex vivo, or in vitro evaluation of the effect of SGLT-2 inhibitors on endothelial function using the following keywords: "SGLT-2", "sodium-glucose co-transporter inhibitor," "dapagliflozin," "canagliflozin," "empagliflozin," "ibragliflozin," "luseogliflozin," "endothelium," "vascular," "endothelial," "endothelia," "experimental study," "in vitro," "in vivo," "animal," "mice," "cell," and "ex-vivo." The searches were carried out by two independent researchers. In addition, the references of relevant articles were manually searched for any additional relevant articles.

This article is based on previously conducted studies and does not contain any studies with human participants or animals performed by any of the authors.

Study Selection and Data Extraction

Eligible studies were included if they were published in English, involved cell culture or animal models, assessed endothelial function evaluated by any method, and used SLGT-2 inhibitors as the intervention at any dose or duration. Studies were excluded if they were not in English, involved human clinical trials, did not use SGLT-2 inhibitors, used other antidiabetic medications or combined therapies, or if the study design was non-experimental. Two independent reviewers performed the extraction of relevant data.

Quality and Risk of Bias Assessment

Each eligible study was assessed for quality and risk of bias by two independent reviewers. For methodological quality assessment, data on randomization method, study duration, random sequence generation, allocation concealment, and blinding as well as other risks of bias information relating to the reporting and attrition bias from each study were extracted. Assessment of the risk of bias was completed using the SYRCLE tool [24] for the in vivo experiments. The internal validity of each study was assessed by performance bias, detection bias, and selection bias. The external validity of the animal population, drug interventions, and outcomes were assessed. Since the included studies did not report pre-defined primary and secondary outcomes, the reporting bias domain (selective outcome reporting) was not used in the assessment of the risk of bias. The scoring was as follows: low risk of bias (reported) was denoted as 'L'; high risk of bias (not reported), as 'H'; unclear or unknown risk of bias, as '?'. Since the experimental reporting on the methodology for animal models was generally considered to be relatively poor [25], other bias domains of reporting of randomization and blinding were included. Calculation of sample size and temperature control reporting were also included as other risks of bias domains. These items were scored as 'yes' if reported, and 'no' if not reported. Due to the substantial heterogeneity of the studies in terms of design and interventions, as well as limitations in the methodology used, a meta-analysis was not possible, and we present here only a qualitative systematic review.

RESULTS

Search Findings

A flow chart of the identification of eligible studies is shown in Fig. 1. A total of 144 articles were identified through searches of the PubMed, EMBASE, and MEDLINE electronic databases. After removal of duplications, 82 articles remained. A further 43 articles were excluded because they did not meet the inclusion criteria. Therefore, we evaluated the full text of 39 potentially relevant studies, of which 24 eligible experimental studies were selected for the final analysis, all of which met the inclusion criteria.

Study Characteristics

The characteristics of the included studies are summarized in Tables 1 and 2. Findings on the SGLT-2 inhibitors canagliflozin, ipragliflozin, empagliflozin, dapagliflozin, tofogliflozin, and luseogliflozin were reported.

Animal Models

A total of 18 studies reported in vivo data (13 in mice and four in rats, and one in rabbit; Table 1). Twelve studies used diabetic models, and four studies were performed in atherogenic models or an obese model. The SGLT-2 inhibitors used were empagliflozin (1–30 mg/kg/day), dapagliflozin (0.1–7 mg/kg/day), ipragliflozin (0.1–3 mg/kg/day), canagliflozin (2 studies, 1–30 mg/kg/day), tofogliflozin, and luseogliflozin (1–10 mg/kg/day). The duration of treatments ranged from 4 to 12 weeks. The rodents were aged between 5 weeks and 11 months at the beginning of the studies. All intervention administrations were oral gavage except one which was via intragastric route.

Three of the ex vivo studies included isolated pulmonary arteries from the thoracic aorta, one used the abdominal aorta, and one study used both the pulmonary and coronary arteries. Three of the ex vivo studies used dapagliflozin (1.0 nM–10 μM), four used empagliflozin (dose not reported), and two studies used canagliflozin (100 pmol/l–100 μmol/l).

In Vitro Studies

The in vitro studies (n = 9; Table 2) were performed in human umbilical vein endothelial cells (HUVECs), human aortic endothelial cells (HAECs), murine endothelial cells, or porcine coronary artery endothelial cells. The drug interventions included empagliflozin (6 studies; 0.1–10 μM, 24 h, 3 days or 30 min), dapagliflozin (3 studies; 1–100 nM, 3 days or 24 h) and

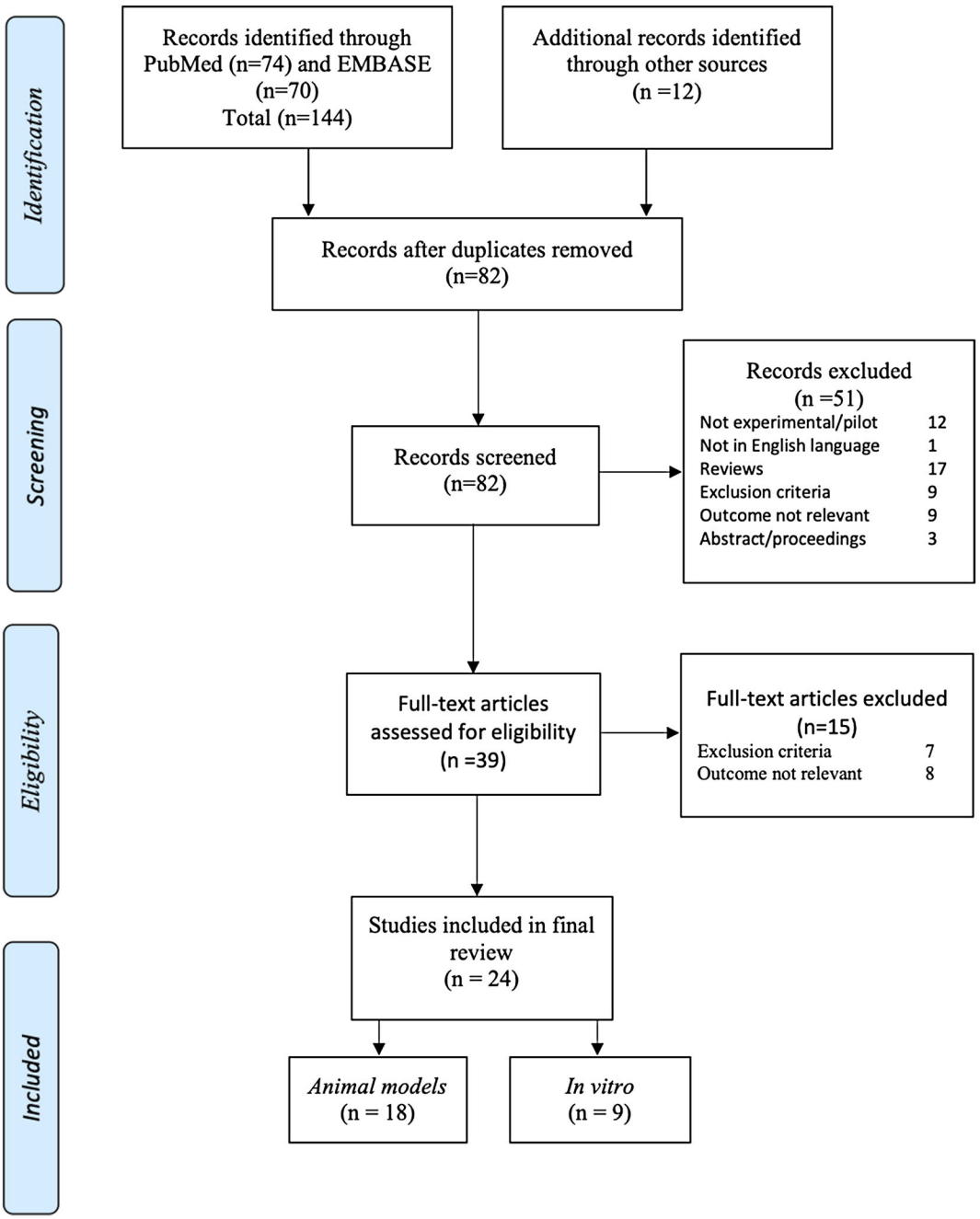

Fig. 1 Flow chart of the identification of eligible studies

canagliflozin (2 studies; 10 μM for 15 or 30 min). Before SGLT-2 inhibitors were added to the cell culture, the cells were stimulated with agents, such as tumor necrosis factor-α (TNFα), palmitate, high glucose, interleukin-1β (IL-1β), or acetylcholine, for various durations ranging from 1 to 24 h to induce damage to the cells/tissue so that any protective effects of the SGLT-2 inhibitors could be assessed.

Table 1 The effect of sodium-glucose co-transporter-2 inhibitors on endothelial function in animal models

Animal species	Animal model	Glyemic condition	Drug (dose)/route	Major finding	References
ApoE$^{-/-}$ mice	T1D	Hyperglycemic	Empagliflozin (20 mg/kg/day)/12 weeks/P.O	↓ MCP-1, VCAM-1, NADPH oxidase, NOX2, and p22phox mRNA expression in the atherosclerotic aorta	[29]
				↓ MCP-1, VCAM-1 mRNA and macrophage accumulation expressions in atherosclerotic lesions in the aortic root	
				↓ MCP-1, VCAM-1, CD68, NOX2, and p22phox RNA expression in the abdominal aorta	
				↓ PGE2 and TXB2 plasma level	
				↓ MCP-1, ICAM-1, VCAM-1 mRNA, CD68, p47phox, and p22phox expression in the PVAT	
				↓ Impairment of vascular endothelium-dependent relaxation in thoracic aortas in response of acetylcholine	
Dahl salt-sensitive rats	Hypertension	Euglycemic	Dapagliflozin (0.1 mg/kg/day)/6 weeks/P.O	↓ VCAM-1, E-selectin and eNOS protein expression	[54]
				↓ NF-κB, MCP1 and IL-6 protein expression	
ApoE$^{-/-}$ mice	Obesity	Euglycemic	Empagliflozin (10 mg/kg/day)/10 weeks/P.O	↓ Vcam-1 and MCP-1 mRNA	[55]
				Marginally ↓ Timp-1 and Timp-2 expression level in the aortic root (locally in the atherosclerotic lesion)	
Rtas	Healthy aortic ring	Euglycemic	Canagliflozin (10 μM)	↑ Endothelium-dependent vasodilation	[36]

Table 1 continued

Animal species	Animal model	Glyemic condition	Drug (dose)/route	Major finding	References
ApoE$^{-/-}$ mice	Obesity	Euglycemic	Canagliflozin (10 mg/kg/day)/5 weeks/P.O	↓ Vcam-1 and MCP-1 mRNA levels in the aortic root	[56]
db/db mice	T2D	Hyperglycemic	Canagliflozin (10 mg/kg/day)/5 weeks/P.O	↓ Impairment of vascular endothelium-dependent relaxation in thoracic aortas	[32]
C57Bl/6 J mice	–	Euglycemic	Dapagliflozin (1.0 μM)	↓ Impairment of vascular endothelium-dependent relaxation in thoracic aortas	[26]
ApoE$^{-/-}$ mice	Adult/obesity	Euglycemic	Dapagliflozin (1.0 mg/kg/day)/4 weeks/P.O	↓ Impairment of vascular endothelium-dependent relaxation in thoracic aortas	[26]
ApoE$^{-/-}$ mice	Aged/obesity	Euglycemic	Dapagliflozin (1.0 mg/kg/day)/4 weeks/P.O	↓ Impairment of vascular endothelium-dependent relaxation in thoracic aortas ↓ NFκB activation ↓ P-IκBα protein expression ↓ ICAM-1 and F4/80 protein expression	[26]
C57BLKS/J-leprdb/Leprdb mice	T2D	Hyperglycemic	Dapagliflozin (60 mg/kg diet; 0.006%)/8 weeks	↓ Impairment of vascular endothelium- dependent relaxation in thoracic aortas ↓ MCP-1, IL-1β, IL-17, IL-10, CCL5 and IL-6 circulating markers	[27]
White rabbits	Aortic smooth muscle	Euglycemic	Dapagliflozin 10, 30, 100, 300, and 1000 μM/30 min to 1 h	↑ Vasodilation in a concentration-dependent manner Activation of Kv channels and PKG, and was independent of other K^+ channels, Ca^{2+} channels, intracellular Ca^{2+}, and the endothelium	[34]

Table 1 continued

Animal species	Animal model	Glyemic condition	Drug (dose)/route	Major finding	References
C57Bl/6J mice	T1D	Hyperglycemic	Empagliflozin (10 mg/kg/day)/ 20 weeks/P.O	↓ Impairment of vascular endothelium-dependent relaxation in thoracic aortas ↓ ICAM1 and VCAM1 protein level upregulation	[30]
ApoE$^{-/-}$ mice	Atherosclerosis	Euglycemic	Empagliflozin (3 mg/kg/day)/8 weeks/P.O	↓ TNF-α, IL-6, MCP-1, and hsCRP circulating levels	[33]
(ZDF)rats	T2D	Hyperglycemic	Empagliflozin (10 mg/kg/day, 30 mg/kg/day)/6 weeks/P.O	↓ Impairment of vascular endothelium-dependent relaxation in thoracic aortas ↓ vascular oxidative stress	[57]
KK/Ay mice	T2D	Hyperglycemic	Ipragliflozin and dapagliflozin: (0.1–1 mg/kg/day) Tofogliflozin, canagliflozin, empagliflozin, and luseogliflozin: (1–10 mg/kg/day)/4 weeks/P.O	Improved IL-1β, IL-6, MCP-1, and TNF-α, ICAM-1, VCAM-1, and E-selectin circulation level	[47]
ApoE$^{-/-}$ mice	T1DM/obesity	Hyperglycemic	Dapagliflozin (1 mg/kg/day)/12 weeks/via intragastrical route	↓ NLRP3, IL-1β, and IL-18 serum level attenuation of vascular ROS production ↓ ROS formation and NLRP3, IL-1β, and IL-18 protein expression in aortic tissue	[39]
C57Bl/6 J mice	T1D	Hyperglycemic	Ipragliflozin (3 mg/kg/day)/3 weeks/P.O	↓ ICAM-1, VCAM-1, and MCP-1 RNA and protein expression ↑ impaired Akt & eNOS^{Ser1177}phosphorylation ↓ 8-OHdG ↓ Impairment of vascular endothelium- dependent relaxation in thoracic aortas	[28]
C57Bl/6J mice	T1D	Hyperglycemic	Canagliflozin, (30 mg/kg/day)/4 weeks/P.O	↓ Impairment of coronary vasodilation in the diabetic group only	[35]

Table 1 continued

Animal species	Animal model	Glyemic condition	Drug (dose)/route	Major finding	References
C57Bl/6J mice	Pulmonary arteries and coronary arteries	Hyperglycemic	Canaglflozin 100 pmol-1 nmol/l	↓ Vascular tone in pulmonary arteries only	[35]
			10 and 100 μmol/l	↑ Coronary vasodilation (SNP-induced)	
db/db mice	Diabetes/obesity	Hyperglycemic	0.03% empagliflozin/diet/10 weeks	↓ Impairment of vascular endothelium- dependent relaxation in thoracic aortas	[58]
				↓ elevated aortic superoxide	
Wistar rats	T1D	Hyperglycemic	Empagliflozin (30 or 10 mg/kg/day)/8 weeks/P.O	↓ Impairment of vascular endothelium- dependent relaxation in thoracic aortas	[31]

Kv Voltage-gated potassium channels, *PKG* protein kinase G, *Akt* protein kinase B, *ApoE*$^{-/-}$ *mice* apolipoprotein E (Apoe) knockout, *CCL5* chemokine ligand 5, *CD68* cluster of differentiation 68, *eNOS* endothelial nitric oxide synthase, *hsCRP* high-sensitivity C-reactive protein, *ICAM-1* intercellular adhesion molecule-1, *IL-17/10* interleukin-17/-10, *MCP-1* monocyte chemoattractant protein-1, *Mmp-2/-9* matrix metalloproteinases-2/-9, *NF-κB* nuclear factor kappa B, *NLRP3* NLR family pyrin domain containing 3, *NOX2* NADPH oxidase 2, *8-OHdG* 8-Oxo-2′-deoxyguanosine, *P-IκBα* phosphorylated- inhibitor of nuclear factor kappa B, *PGE_2* prostaglandin E_2, *PVAT* perivascular adipose tissue, *ROS* reactive oxygen species, *SNP* sodium nitroprusside, *T1D/T2D* type 1/type 2 diabetes, *Timp-2* tissue inhibitor of metalloproteinases 2, *TNF-α* tumor necrosis factor alpha, *TXB_2* thromboxane B2, *VCAM-1* vascular cell adhesion molecule-1, *P.O* oral gavage

Experimental Outcomes of SGLT-2 Inhibitors

The Effect of SGLT-2 Inhibitors on Endothelial Function

It has been shown in both diabetic and nondiabetic models that SGLT-2 inhibitors improve endothelial function in euglycemic and hyperglycemic conditions, as summarized in Table 1. The vascular response was examined in different studies to investigate the effects of SGLT-2 inhibitors on endothelial dysfunction and the proposed mechanism by which they reduce oxidative stress, namely, the formation of advanced glycation endproducts (AGEs) and their receptors (RAGEs), which is involved in AGE/RAGE signalling and inflammation.

The Direct Effect of SGLT-2 Inhibitors on Endothelial Cells

The direct involvement of SGLT-2 inhibitors in different cell lines was assessed, and the results are summarized in Table 2.

Qualitative and Risk of Bias Assessment

The overall and individual scores for the qualitative and risk of bias assessment of in vivo studies are summarized in the ESM (ESM Table S1; ESM Figure S1). Most of the included studies did not report sufficient information for the assessment of the risk of bias.

Although randomization was reported in six studies (50%), no detail was reported. Consequently, random allocation, housing, and assessment of outcome risk of bias were mostly unclear. Four studies (33%) reported blinding during the experimentation, three of which

Table 2 The direct effect of sodium-glucose co-transporter-2 inhibitors on endothelial function in cell culture

Cell lines	Drug (dose)	Stimulant (dose)	Major finding	References
HCAECs	Empagliflozin and dapagliflozin (1 μM)/2 h	TNFα (10 ng/ml)/4–24 h	↓ ROS level in TNFα-stimulated cells	[59]
HUVECs	Empagliflozin and dapagliflozin (1 μM)/2 h	TNFα (10 ng/ml)/4–24 h	↑ NO bioavailability in TNFα-stimulated cells	[59]
Porcine coronary artery endothelial cells	Empagliflozin (1–100 nmol/l)/30 min	HG (25 mmol/l) or H_2O_2 (100 μmol/l)/24 h	↓ SA-β-gal in HG-treated cells ↓ p21 and p16 expression level in HG-treated cells ↓ eNOS and VCAM-1 mRNA and protein expression level in HG-treated cells ↑ mRNA SGLT-1 and SGLT-2 expression in H_2O_2- and HG-treated ECs	[37]
HAAECs	Empagliflozin (50 μM)/24 h	Statically cultured or subjected to a steady wall shear stress of 10 dyne/cm	↓ Roundness of the cells under static conditions ↓ TNFα-associated HAAEC-NB4 cell adhesion under static and flow conditions ↓ NB4-HAAEC adhesion under static and perfused conditions ↑ HS intensity level under static and flow culture conditions in heparinase III-treated cells	[60]
HUVECs	Canagliflozin (0–50 μM), empagliflozin (0–50 μM), dapagliflozin (0–50 μM)/3 days		↓ DNA synthesis in a dose-dependent manner by dapagliflozin ↓ Proliferation in a dose-dependent manner by three SGLT-2 inhibitors Canagliflozin disrupts cell cycle progression, ↓ cyclin A expression and the phosphorylation of retinoblastoma protein	[61]
Murine endothelial cells	Empagliflozin (25 nM-10 μM)/24 h	HG (25 mM)/24 h	↓ Src-kinase, EGF receptor-kinase, protein kinase-C and Rho-kinase ↑ PAR2-mediated vasodilation in tissues cultured under hyperglycaemic conditions	[62]

Table 2 continued

Cell lines	Drug (dose)	Stimulant (dose)	Major finding	References
HUVECs	Canagliflozin (10 μM)/30 min	IL-1β (10 ng/ml) for 6 h	↓ IL-6 and MCP-1 protein and MRNA expression	[45]
HAECs	Canagliflozin (10 μM)/15 min	IL-1β (5 ng/ml) for 4 h	↓ IL-6 and MCP-1 protein and mRNA expression	[45]
HUVECs	Dapagliflozin (1.0–5.0 nM)/24 h Dapagliflozin (100 nM)/24 h	TNFα (10 ng/ml) or HG (10–30 mM) for 24 h	↓ ICAM-1 & VCAM-1 protein levels ↑ PAI-1 protein ↓ ICAM-1, PAI-1 mRNA and protein expression in hyperglycemia-treated cells	[26]
HUVECs	Empagliflozin (0.1–100 μM)	VEGF (10 ng/ml) for 1 h	Neutral effect of the drug on endothelial cell proliferation	[35]
HUVECs	Empagliflozin (1 or 10 μM)/6 days	Ach (1 μM)/30 min after cultured under HG (30 mM)	↑ viability of hyperglycemic endothelial cells	[57]

EGF Epidermal growth factor, H_2O_2 hydrogen peroxide, *HAAECs* human abdominal 2a aortic endothelial cells, *HAECs* human aortic endothelial cells, *HCAECs* human coronary artery endothelial cells, *HG* high glucose, *HUVECs* human umbilical vein endothelial cell, *NO* nitric oxide, *PAI-1* plasminogen, *PAR-2* proteinase activated receptor 2, *SGLT-2* sodium-glucose co-transporter-2

reported blinding of outcome assessor and one reported blinding of the examiners to the allocation of animals. Therefore, risk of bias related to blinding of the examiner and assessor as well as allocation concealment was assessed as unclear. Measures to reduce random housing (performance bias) were reported in two studies (16%), with a further two studies not reporting random housing (high risk of bias) and the remaining eight studies unclear. Only in five of the studies (42%) were the baseline characteristics between the control and intervention group reported. Since the reporting was quite poor for animal experiments, most of the possible bias sources were scored as unclear risk (ESM Table S1). Four studies (33%) reported details of dropouts to score risk of attrition bias as low level. None of the studies reported sample size calculation to explain and justify the number of animals used per group.

DISCUSSION

The aim of this systematic review was to assess existing evidence on the protective effects of SGLT-2 inhibitors on endothelial function in preclinical models. All of the studies published to date which have reported on the experimental effects of SGLT-2 inhibitors suggest that this class of drug may exert cardiovascular benefits by modulating vascular endothelial cell activation and improving endothelial cell dysfunction, a critical early step in atherogenesis.

These beneficial effects are likely due to (1) glucose-lowering effects, thereby preventing downstream glucotoxicity, such as AGE formation, AGE/RAGE signalling, reduction of oxidative stress, and inflammation and impairment of vascular function, and (2) direct effects on vascular endothelial cells (as summarized in Fig. 2).

Chronic and acute treatment with dapagliflozin led to a significant endothelial-dependent vasorelaxation in the aorta of diabetic mice [26, 27]. These data are consistent with results from studies that used ipragliflozin [28], empagliflozin [29–31], and canagliflozin [32, 33] in diabetic mice. Likewise, the findings from ex vivo studies indicated that various SGLT-2 inhibitors could induce direct vasorelaxation [26, 34–36]. This beneficial effect on endothelial cell function points to several potential mechanisms of vasodilation, including modulation of adhesion molecules, attenuation of inflammation, activation of eNOS phosphorylation, potassium/calcium channel mediation independent of the endothelium;,decreased cardiac macrophage infiltration, and reduced oxidative stress. This beneficial effect could be due to the sustained reduction in plasma glucose concentration, which was observed even during shorter treatment periods, suggesting the reliability and effectiveness of glucose-lowering therapy with SGLT-2 inhibitors. However, given that the $ApoE^{-/-}$ mice used in the study by Gaspari et al. [26] were nondiabetic and did not exhibit a change in glucose metabolism induced by dapagliflozin, it remains uncertain whether this class of drug exerts a direct effect on blood vessels [26]. A recent study found a significant increase in sodium-glucose transporter-1 (SGLT1) and SGLT-2 expression in high glucose-treated porcine endothelial cells [37]. Thus, it is possible that the direct effect of SGLT-2 inhibitors, via inhibition of SGLT-2 in the vascular wall, contributes to improvement in endothelial function.

The ex vivo and in vitro studies reviewed support a possible class effect of the SGLT-2 inhibitors on the regulation of endothelial function that includes the reduction of inflammatory cytokine secretion and hyperglycemia-mediated protein expression, as well as improved viability of hyperglycemic endothelial cells. Overall, the beneficial effects of SGLT-2 inhibitors on endothelial dysfunction appear to be consistent across all of the outcomes measured, such as vascular function and oxidative stress. None of the studies investigated reported any adverse effects related to treatment with SGLT-2 inhibitors. Oxidative stress is known to lead to cell and tissue damage via the production of ROS, such as active oxygen and free radicals, with oxidative stress markers, including thiobarbituric acid reactive substances (TBARS), reported to be significantly elevated in people with T2D with non-alcoholic fatty liver disease [38]. In vitro experiments have demonstrated attenuation of protein kinase B (Akt) and eNOS phosphorylation in HUVECs treated with methylglyoxal, the precursor of AGEs, indicating that this effect seems to be at least partially attributable to the improvement in eNOS function in a hyperglycemic state [28]. It has been argued that this anti-oxidant effect is most likely due to the inhibition of NADPH oxidase activity and a decreased serum level of the AGE precursor methylglyoxal [31, 39]. However, the glucose-lowering effect of SGLT-2 inhibitors in diabetic mice significantly decreased oxidative stress, as determined by urinary excretion of 8-oxo-2′-deoxyguanosine and in cardiac and vascular tissue, whole blood, aorta, and the heart [28].

Inflammation has been implicated in vascular dysfunction in diabetes [40]. As a result, several studies have examined the ideal approach to prevent and manage this inflammatory response [41, 42]. Anti-inflammatory effects of SGLT-2 inhibitors were previously observed in diabetic nephropathy models, and subsequently proposed to occur via a suppression of the RAGE pathways [43]. This, in turn, has led to many further potential pathways being proposed, ranging from direct effects on the expression/release of pro-inflammatory mediators or indirect effects via oxidative balance, hyperglycemia-induced cytokine production, immune system function, and/or obesity-related inflammation [44]. Systemic administration of the SGLT-2 inhibitors empagliflozin, ipragliflozin, or luseogliflozin led to markedly reduced expression of pro-inflammatory adhesion markers and cytokines, such as IL-6, monocyte chemoattractant protein-1 (MCP-1), and intercellular adhesion molecule-1 (ICAM-1) in diabetic rodent models [28, 45–47]. Canagliflozin administration inhibited inflammation in metabolic tissue of mice fed a high-fat diet. In keeping with these previous observations, an in vitro study showed that dapagliflozin

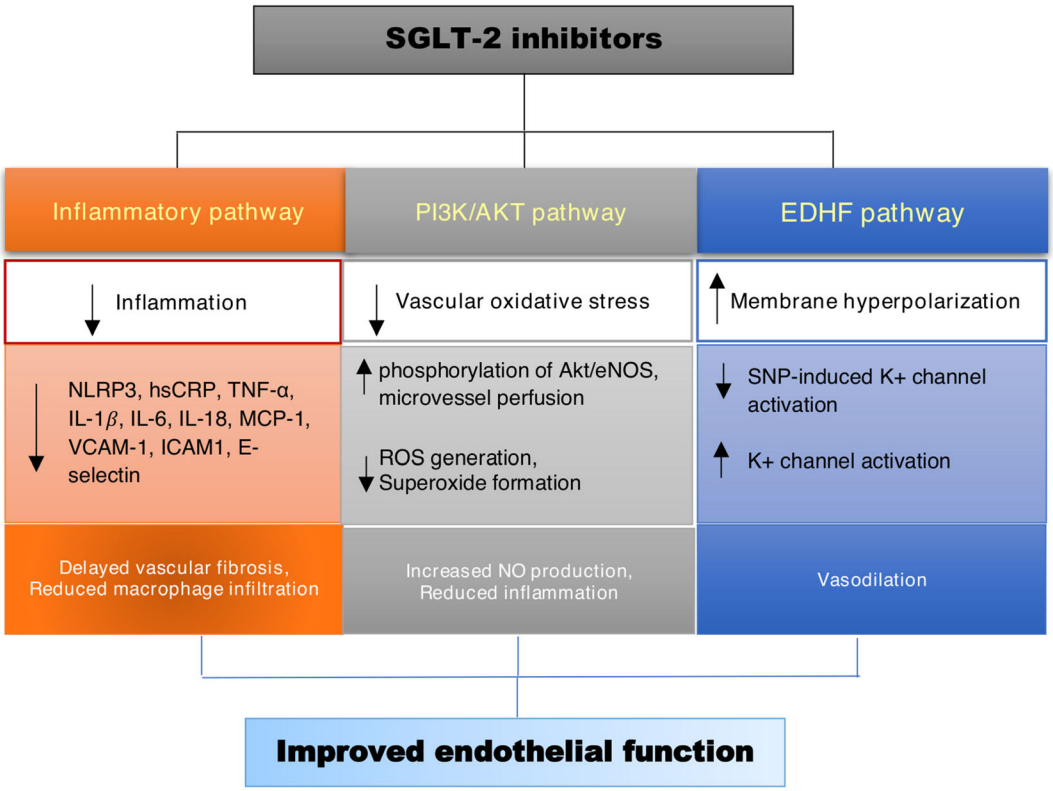

Fig. 2 Summary of potential mechanisms involved in the protective effects of sodium-glucose co-transporter-2 (*SGLT-2*) inhibitors on endothelial function. *Akt* Protein kinase B, *EDHF* endothelium-derived hyperpolarizing factor, *NLRP3* NLR family pyrin domain containing 3, *SNP* sodium nitroprusside; see footnotes to Tables 1 and 2 for other definitions

treatment inhibited TNFα induction of ICAM-1 and vascular cell adhesion molecule-1 (VCAM-1) protein expression in human endothelial cells [45]. Similarly, dapagliflozin inhibited IL-1β-stimulated MCP-1 and IL-6 secretion [26]. Taken together these in vivo and in vitro results strongly suggest direct underlying mechanisms of action of SGLT-2 inhibitors on endothelial function that are independent of beneficial glucose-lowering effects [26].

The doses of SLGT-2 inhibitors used in these studies were determined from the therapeutic dose required to reach a peak concentration, or in the case of in vitro studies, the concentration that reflected the maximum plasma concentration. Therefore, for example, canagliflozin, which reaches a peak concentration of about 7 μmol/l in human plasma, was employed at a therapeutically relevant concentration of 10 μmol/l, while dapagliflozin and empagliflozin, which produce peak concentrations of 1–2 μmol/l, were employed at doses of 1 μmol/l [45].

These observations from animal and cell model experiments are supported by clinical studies, as a reduction in biomarkers of cardiovascular inflammation (namely, highly sensitive C-reactive protein) was observed in diabetic patients treated with dapagliflozin [48]. The cardiovascular benefits observed in multiple clinical trials were seen to occur independently of glycemic or lipid control [49]. The underlying pathophysiological mechanisms of the improved cardiovascular outcomes observed was hypothesized to be due to the diuretic (and, therefore, anti-hypertensive) effects of SGLT-2 inhibitors [50]. In addition, the 'thrifty substrate' hypothesis proposes a shift in metabolism from glucose and lipids to ketone bodies as a direct effect of SGLT-2 inhibition [51].

Mudaliar et al. [49] argue that although diuretic and vascular endothelium effects could have an impact on cardiovascular improvements, such considerable benefits are highly unlikely in the short period of 3 months. Notably, the vascular dysfunction associated with T2D was initially thought to occur due to hyperglycemia, as a result of interactions between various pathways [52]. Arguably, the evidence from the experimental studies reported in this review point towards SGLT-2 inhibitors exerting additional benefits beyond glucose control.

LIMITATIONS

Due to insufficient or poor-quality methodological reporting of the included studies, as well as considerable heterogeneity, the observations reported should be interpreted with caution. Furthermore, although well-established tools exist for the assessment of methodology quality of human clinical trials, there are currently no validated or standardized grading scales available for the assessment of methodological quality in animal studies. Another limitation of our review may be the inclusion of only published studies, which may be a potential source of publication bias [53]; unpublished results or conference abstracts may have reported negative findings.

CONCLUSIONS

In this systematic review, we have demonstrated that experimental evidence suggests that SGLT-2 inhibitors are effective at attenuating vascular dysfunction in preclinical models, with complex underlying mechanisms that may act independently to hyperglycemia-related benefits. The evidence from these preclinical studies supports the demonstrated improved vascular functional outcomes of human clinical trials. Further studies are needed to clarify how these compounds are acting in the vasculature and to discover their target sites of action.

ACKNOWLEDGEMENTS

Funding. No funding was received for this study or publication of this article. The Rapid Service Fee was funded by the authors. PhD studentship was sponsored by the Saudi Arabia government scholarship scheme.

Authorship. All named authors meet the International Committee of Medical Journal Editors (ICMJE) criteria for authorship for this article, take responsibility for the integrity of the work as a whole, and have given their approval for this version to be published.

Disclosures. Afnan S. Alshnbari, Sophie A. Millar, and Saoirse E. O'Sullivan and Iskandar Idris have nothing to disclose.

Compliance with Ethics Guidelines. This article is based on previously conducted studies and does not contain any studies with human participants or animals performed by any of the authors.

REFERENCES

1. Chen L, Magliano DJ, Zimmet PZ. The worldwide epidemiology of type 2 diabetes mellitus–present and future perspectives. Nat Rev Endocrinol. 2011;8(4):228–36.

2. Ebong IA, Goff DC Jr, Rodriguez CJ, Chen H, Sibley CT, Bertoni AG. Association of lipids with incident heart failure among adults with and without diabetes mellitus: multiethnic study of atherosclerosis. Circ Heart Fail. 2013;6(3):371–8.

3. Tabit CE, Chung WB, Hamburg NM, Vita JA. Endothelial dysfunction in diabetes mellitus: molecular mechanisms and clinical implications. Rev Endocr Metab Disorders. 2010;11(1):61–74.

4. Versari D, Daghini E, Virdis A, Ghiadoni L, Taddei S. Endothelial dysfunction as a target for prevention of cardiovascular disease. Diabetes Care. 2009;32(Suppl 2):S314–21.

5. Creager MA, Luscher TF, Cosentino F, Beckman JA. Diabetes and vascular disease: pathophysiology, clinical consequences, and medical therapy: Part I. Circulation. 2003;108(12):1527–32.

6. Bakker W, Eringa EC, Sipkema P, van Hinsbergh VW. Endothelial dysfunction and diabetes: roles of hyperglycemia, impaired insulin signaling and obesity. Cell Tissue Res. 2009;335(1):165–89.

7. Kim JA, Montagnani M, Koh KK, Quon MJ. Reciprocal relationships between insulin resistance and endothelial dysfunction: molecular and pathophysiological mechanisms. Circulation. 2006;113(15):1888–904.

8. Calles-Escandon J, Cipolla M. Diabetes and endothelial dysfunction: a clinical perspective. Endocr Rev. 2001;22(1):36–52.

9. Taddei S, Ghiadoni L, Virdis A, Versari D, Salvetti A. Mechanisms of endothelial dysfunction: clinical significance and preventive non-pharmacological therapeutic strategies. Curr Pharm Des. 2003;9(29):2385–402.

10. Lim S, Eckel RH, Koh KK. Clinical implications of current cardiovascular outcome trials with sodium glucose cotransporter-2 (SGLT2) inhibitors. Atherosclerosis. 2018;272:33–40.

11. Marso SP, Daniels GH, Brown-Frandsen K, et al. Liraglutide and cardiovascular outcomes in type 2 diabetes. N Engl J Med. 2016;375(4):311–22.

12. Kristensen SL, Rørth R, Jhund PS, et al. Cardiovascular, mortality, and kidney outcomes with GLP-1 receptor agonists in patients with type 2 diabetes: a systematic review and meta-analysis of cardiovascular outcome trials. Lancet Diabetes Endocrinol. 2019;7(10):776–85.

13. Fei Y, Tsoi M-F, Cheung BMY. Cardiovascular outcomes in trials of new antidiabetic drug classes: a network meta-analysis. Cardiovasc Diabetol. 2019;18(1):112.

14. Vrhovac I, Eror DB, Klessen D, et al. Localizations of Na(+)-D-glucose cotransporters SGLT1 and SGLT2 in human kidney and of SGLT1 in human small intestine, liver, lung, and heart. Pflügers Arch. 2015;467(9):1881–988.

15. Imprialos KP, Sarafidis PA, Karagiannis AI. Sodium–glucose cotransporter-2 inhibitors and blood pressure decrease: a valuable effect of a novel antidiabetic class? J Hypertens. 2015;33(11):2185–97.

16. Chilton R, Tikkanen I, Cannon C, et al. Effects of empagliflozin on blood pressure and markers of arterial stiffness and vascular resistance in patients with type 2 diabetes. Diabetes Obes Metab. 2015;17(12):1180–93.

17. Ott C, Jumar A, Striepe K, et al. A randomised study of the impact of the SGLT2 inhibitor dapagliflozin on microvascular and macrovascular circulation. Cardiovasc Diabetol. 2017;16(1):26.

18. Shigiyama F, Kumashiro N, Miyagi M, et al. Effectiveness of dapagliflozin on vascular endothelial function and glycemic control in patients with early-stage type 2 diabetes mellitus: DEFENCE study. Cardiovasc Diabetol. 2017;16(1):84.

19. Zainordin NA, Hatta SFWM, Mohamed Shah FZ, et al. Effects of dapagliflozin on endothelial dysfunction in type 2 diabetes with established ischemic heart disease (EDIFIED). J Endocrine Soc. 2020;4(1):bvz017. https://doi.org/10.1210/jendso/bvz017.

20. Solini A, Giannini L, Seghieri M, et al. Dapagliflozin acutely improves endothelial dysfunction, reduces aortic stiffness and renal resistive index in type 2 diabetic patients: a pilot study. Cardiovasc Diabetol. 2017;16(1):138.

21. Lunder M, Janić M, Japelj M, Juretič A, Janež A, Šabovič M. Empagliflozin on top of metformin treatment improves arterial function in patients with type 1 diabetes mellitus. Cardiovasc Diabetol. 2018;17(1):153.

22. Batzias K, Antonopoulos AS, Oikonomou E, et al. Effects of newer antidiabetic drugs on endothelial function and arterial stiffness: a systematic review and meta-analysis. J Diabetes Res. 2018;2018:1232583. https://doi.org/10.1155/2018/1232583.

23. Moher D, Liberati A, Tetzlaff J, Altman DG. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. PLoS Med. 2009;6(7):e1000097.

24. Hooijmans CR, Rovers MM, de Vries RB, Leenaars M, Ritskes-Hoitinga M, Langendam MW. SYRCLE's risk of bias tool for animal studies. BMC Med Res Methodol. 2014;14(1):43.

25. Kilkenny C, Parsons N, Kadyszewski E, et al. Survey of the quality of experimental design, statistical analysis and reporting of research using animals. PLoS ONE. 2009;4(11):e7824.

26. Gaspari T, Spizzo I, Liu H, et al. Dapagliflozin attenuates human vascular endothelial cell activation and induces vasorelaxation: a potential mechanism for inhibition of atherogenesis. Diabetes Vasc Dis Res. 2018;15(1):64–73.

27. Lee DM, Battson ML, Jarrell DK, et al. SGLT2 inhibition via dapagliflozin improves generalized vascular dysfunction and alters the gut microbiota in type 2 diabetic mice. Cardiovasc Diabetol. 2018;17(1):62.

28. Salim HM, Fukuda D, Yagi S, Soeki T, Shimabukuro M, Sata M. Glycemic control with ipragliflozin, a novel selective SGLT2 inhibitor, ameliorated endothelial dysfunction in streptozotocin-induced diabetic mouse. Front Cardiovasc Med. 2016;3:43.

29. Ganbaatar B, Fukuda D, Shinohara M, et al. Empagliflozin ameliorates endothelial dysfunction and suppresses atherogenesis in diabetic apolipoprotein E-deficient mice. Eur J Pharmacol. 2020;875:173040.

30. Zhou H, Wang S, Zhu P, Hu S, Chen Y, Ren J. Empagliflozin rescues diabetic myocardial microvascular injury via AMPK-mediated inhibition of mitochondrial fission. Redox Biol. 2018;15:335–46.

31. Oelze M, Kroller-Schon S, Welschof P, et al. The sodium-glucose co-transporter 2 inhibitor empagliflozin improves diabetes-induced vascular dysfunction in the streptozotocin diabetes rat model by interfering with oxidative stress and glucotoxicity. PLoS ONE. 2014;9(11):e112394.

32. Aroor AR, Das NA, Carpenter AJ, et al. Glycemic control by the SGLT2 inhibitor empagliflozin decreases aortic stiffness, renal resistivity index and kidney injury. Cardiovasc Diabetol. 2018;17(1):108.

33. Han JH, Oh TJ, Lee G, et al. The beneficial effects of empagliflozin, an SGLT2 inhibitor, on atherosclerosis in ApoE (-/-) mice fed a western diet. Diabetologia. 2017;60(2):364–76.

34. Li H, Shin SE, Seo MS, et al. The anti-diabetic drug dapagliflozin induces vasodilation via activation of PKG and Kv channels. Life Sci. 2018;197:46–55.

35. Han Y, Cho YE, Ayon R, et al. SGLT inhibitors attenuate NO-dependent vascular relaxation in the pulmonary artery but not in the coronary artery. Am J Physiol Lung Cell Mol Physiol. 2015;309(9):L1027–36.

36. Sayour AA, Korkmaz-Icöz S, Loganathan S, et al. Acute canagliflozin treatment protects against in vivo myocardial ischemia–reperfusion injury in non-diabetic male rats and enhances endothelium-dependent vasorelaxation. J Transl Med. 2019;17(1):127.

37. Khemais-Benkhiat S, Belcastro E, Idris-Khodja N, et al. Angiotensin II-induced redox-sensitive SGLT1 and 2 expression promotes high glucose-induced endothelial cell senescence. J Cell Mol Med. 2020;24(3):2109–22.

38. Narasimhan S, Gokulakrishnan K, Sampathkumar R, et al. Oxidative stress is independently associated with non-alcoholic fatty liver disease (NAFLD) in subjects with and without type 2 diabetes. Clin Biochem. 2010;43(10):815–21.

39. Leng W, Ouyang X, Lei X, et al. The SGLT-2 inhibitor dapagliflozin has a therapeutic effect on atherosclerosis in diabetic ApoE(-/-) mice. Mediat Inflamm. 2016;2016:6305735.

40. Lastra G, Manrique C. Perivascular adipose tissue, inflammation and insulin resistance: link to vascular dysfunction and cardiovascular disease. Horm Mol Biol Clin Investig. 2015;22(1):19–26.

41. Donate-Correa J, Martín-Núñez E, Muros-de-Fuentes M, Mora-Fernández C, Navarro-González JF. Inflammatory cytokines in diabetic nephropathy. J Diabetes Res. 2015;2015:948417.

42. Yaribeygi H, Farrokhi FR, Rezaee R, Sahebkar A. Oxidative stress induces renal failure: a review of possible molecular pathways. J Cell Biochem. 2018;119(4):2990–8.

43. Ojima A, Matsui T, Nishino Y, Nakamura N, Yamagishi S. Empagliflozin, an inhibitor of sodium-glucose cotransporter 2 exerts anti-inflammatory and antifibrotic effects on experimental diabetic nephropathy partly by suppressing AGEs-receptor axis. Horm Metab Res. 2015;47(9):686–92.

44. Satirapoj B. Sodium-glucose cotransporter 2 inhibitors with renoprotective effects. Kidney Dis (Basel). 2017;3(1):24–322.

45. Mancini SJ, Boyd D, Katwan OJ, et al. Canagliflozin inhibits interleukin-1β-stimulated cytokine and

chemokine secretion in vascular endothelial cells by AMP-activated protein kinase-dependent and-independent mechanisms. Sci Rep. 2018;8(1):5276.

46. Han JH, Oh TJ, Lee G, et al. The beneficial effects of empagliflozin, an SGLT2 inhibitor, on atherosclerosis in ApoE −/− mice fed a western diet. Diabetologia. 2017;60(2):364–76.

47. Tahara A, Takasu T, Yokono M, Imamura M, Kurosaki E. Characterization and comparison of SGLT2 inhibitors: part 3. Effects on diabetic complications in type 2 diabetic mice. Eur J Pharmacol. 2017;809:163–71.

48. Ferrannini E, Ramos SJ, Salsali A, Tang W, List JF. Dapagliflozin monotherapy in type 2 diabetic patients with inadequate glycemic control by diet and exercise: a randomized, double-blind, placebo-controlled, phase 3 trial. Diabetes Care. 2010;33(10):2217–24.

49. Mudaliar S, Alloju S, Henry RR. Can a shift in fuel energetics explain the beneficial cardiorenal outcomes in the EMPA-REG OUTCOME study? Unifying hypothesis. Diabetes Care. 2016;39(7):1115–22.

50. McMurray J. EMPA-REG–the "diuretic hypothesis". J Diabetes Complicat. 2016;30(1):3–4.

51. Bertero E, Prates Roma L, Ameri P, Maack C. Cardiac effects of SGLT2 inhibitors: the sodium hypothesis. Cardiovasc Res. 2017;114(1):12–8.

52. Kitada M, Zhang Z, Mima A, King GL. Molecular mechanisms of diabetic vascular complications. J Diabetes Investig. 2010;1(3):77–89.

53. Dickersin K. The existence of publication bias and risk factors for its occurrence. JAMA. 1990;263(10):1385–9.

54. Cappetta D, De Angelis A, Ciuffreda LP, et al. Amelioration of diastolic dysfunction by dapagliflozin in a non-diabetic model involves coronary endothelium. Pharmacol Res. 2020;157:104781.

55. Dimitriadis GK, Nasiri-Ansari N, Agrogiannis G, et al. Empagliflozin improves primary haemodynamic parameters and attenuates the development of atherosclerosis in high fat diet fed APOE knockout mice. Mol Cell Endocrinol. 2019;494:110487.

56. Nasiri-Ansari N, Dimitriadis GK, Agrogiannis G, et al. Canagliflozin attenuates the progression of atherosclerosis and inflammation process in APOE knockout mice. Cardiovasc Diabetol. 2018;17(1):106.

57. Steven S, Oelze M, Hanf A, et al. The SGLT2 inhibitor empagliflozin improves the primary diabetic complications in ZDF rats. Redox Biol. 2017;13:370–85.

58. Lin B, Koibuchi N, Hasegawa Y, et al. Glycemic control with empagliflozin, a novel selective SGLT2 inhibitor, ameliorates cardiovascular injury and cognitive dysfunction in obese and type 2 diabetic mice. Cardiovasc Diabetol. 2014;13:148.

59. Uthman L, Homayr A, Juni RP, Set al. Empagliflozin and dapagliflozin reduce ROS generation and restore NO bioavailability in tumor necrosis factor α-stimulated human coronary arterial endothelial cells. Cell Physiol Biochem. 2019;53(5):865–86.

60. Cooper S, Teoh H, Campeau MA, Verma S, Leask RL. Empagliflozin restores the integrity of the endothelial glycocalyx in vitro. Mol Cell Biochem. 2019;459(1):121–30.

61. Behnammanesh G, Durante ZE, Peyton KJ, et al. Canagliflozin inhibits human endothelial cell proliferation and tube formation. Front Pharmacol. 2019;10(362). https://doi.org/10.3389/fphar.2019.00362.

62. El-Daly M, Pulakazhi Venu VK, Saifeddine M, et al. Hyperglycaemic impairment of PAR2-mediated vasodilation: prevention by inhibition of aortic endothelial sodium-glucose-co-transporter-2 and minimizing oxidative stress. Vascul Pharmacol. 2018;109:56–71.