Impact of sodium glucose cotransporter 2 (SGLT2) inhibitors on atherosclerosis: from pharmacology to pre-clinical and clinical therapeutics

Zhenghong Liu1, Xiaoxuan Ma1, Iqra Ilyas1, Xueying Zheng1, Sihui Luo1, Peter J. Little2,3, Danielle Kamato3, Amirhossein Sahebkar4,5, Weiming Wu6, Jianping Weng1,2* and Suowen Xu1,2*

1. Department of Endocrinology, The First Affiliated Hospital of USTC, Division of Life Sciences and Medicine, University of Science and Technology of China, Hefei, China.
2. Sunshine Coast Health Institute, University of the Sunshine Coast, Birtinya, QLD 4575, Australia.
3. School of Pharmacy, Pharmacy Australia Centre of Excellence, the University of Queensland, Woolloongabba, Queensland 4102, Australia.
4. Halal Research Center of IRI, FDA, Tehran, Iran.
5. Biotechnology Research Center, Pharmaceutical Technology Institute, Mashhad University of Medical Sciences, Mashhad, Iran.
6. Changshu Hospital Affiliated to Nanjing University of Chinese Medicine, Changshu, China.

*Corresponding author(s): Suowen Xu, PhD, E-mail: sxu1984@ustc.edu.cn; ORCID: 0000-0002-5488-5217; Jianping Weng, MD, PhD, E-mail: wengjp@ustc.edu.cn; ORCID: 0000-0002-7889-1697.

© The author(s). This is an open access article distributed under the terms of the Creative Commons Attribution License (https://creativecommons.org/licenses/by/4.0/). See http://ivyspring.com/terms for full terms and conditions.

Received: 2020.10.14; Accepted: 2021.01.17; Published: 2021.03.04

Abstract

Sodium-glucose cotransporter 2 inhibitors (SGLT2i) are new oral drugs for the therapy of patients with type 2 diabetes mellitus (T2DM). Research in the past decade has shown that drugs of the SGLT2i class, such as empagliflozin, canagliflozin, and dapagliflozin, have pleiotropic effects in preventing cardiovascular diseases beyond their favorable impact on hyperglycemia. Of clinical relevance, recent landmark cardiovascular outcome trials have demonstrated that SGLT2i reduce major adverse cardiovascular events, hospitalization for heart failure, and cardiovascular death in T2DM patients with/without cardiovascular diseases (including atherosclerotic cardiovascular diseases and various types of heart failure). The major pharmacological action of SGLT2i is through inhibiting glucose re-absorption in the kidney and thus promoting glucose excretion. Studies in experimental models of atherosclerosis have shown that SGLT2i ameliorate the progression of atherosclerosis by mechanisms including inhibition of vascular inflammation, reduction in oxidative stress, reversing endothelial dysfunction, reducing foam cell formation and preventing platelet activation. Here, we summarize the anti-atherosclerotic actions and mechanisms of action of SGLT2i, with an aim to emphasize the clinical utility of this class of agents in preventing the insidious cardiovascular complications accompanying diabetes.

Key words: SGLT2 inhibitors, diabetes, atherosclerosis, therapy, cardiovascular complications

Introduction

Atherosclerosis is the major potential pathology of most cardiovascular disease (CVD), including myocardial infarction (MI), heart failure (HF), stroke, and peripheral arterial disease [1]. CVDs are the leading cause of morbidity and mortality globally [1, 2]. In patients with diabetes, CVDs are the majority cause of premature mortality. Atherosclerosis is a slow-progressing inflammatory disease with a complex biochemical and cellular etiology characterized by the deposition of modified lipids in the arteries, the development of lipid-laden atherosclerotic plaques and ultimately the rupture of the plaque which precipitates the lethal clinical event being a heart attack or stroke [3, 4]. The conventional risk factors for atherosclerosis and its thrombotic complications include hypertension, obesity, smoking, dyslipidemia, depression, sedentary lifestyles and diabetes [1]. In particular, it is difficult to separate the effects of diabetes from those of other atherogenic factors. Patients with type 2 diabetes
mellitus (T2DM) have a higher risk of atherosclerosis and other complications compared with those without diabetes, and ~80 percent of mortality in individuals with T2DM is due to cardiovascular events [5-7].

Sodium-glucose cotransporter 2 inhibitors (SGLT2i) have been developed as hypoglycemic drugs that target SGLT2, the major glucose transporter in the kidney responsible for about 90 percent of glucose reabsorption from primary urine [8]. Recent evidence has suggested the use of SGLT2i as an adjunct to standard treatment to improve clinically relevant renal and cardiovascular outcomes in patients with T2DM [6, 9]. SGLT2i can reduce glycosylated hemoglobin, body weight, blood pressure, plasma volume, increase in erythrocyte mass, and improve cardiac energy metabolism, which imposes a positive influence on cardiovascular risk factors and outcomes [5, 10-12]. In light of the important clinical benefits of SGLT2i in improving cardiovascular outcomes, we provide a comprehensive and insightful overview of the pharmacological effects and underlying mechanisms of action of SGLT2i in CVD prevention, with a focus on mechanism addressing the accelerated atherosclerosis associated with diabetes.

The pharmacological basis of SGLT2i

The SGLT2 protein, encoded by SLC5A2, is a member of the sodium-glucose cotransporter family and it undertakes the function of transporting glucose from the renal tubule lumen to renal tubule epithelial cells [13]. SGLT2 is abundantly expressed in the anterior part of the proximal tubule [14, 15]. Mining of GTEx database indicated that SGLT2 and SGLT1 are expressed in the kidney and intestine, respectively (Figure S1).

Phlorizin, the first natural SGLT2i, was isolated from the root bark of apple trees in 1835. Due to its low water solubility and poor absorption in the gastrointestinal tract, it was not developed as an anti-hyperglycemic agent [16]. T-1095, a phlorizin derivative, overcomes some shortcomings of phlorizin, but could not go through clinical development [17]. Later, c-aryl glycosides derived from the basic structure of phlorizin were subsequently developed, such as dapagliflozin and canagliflozin [18, 19]. In addition to structural differences, they also have variable selectivity to SGLT1 and SGLT2 (Table 1) [20].

Table 1. Approved SGLT2 inhibitors in clinics

| SGLT2i   | Pubchem CID | Recommended starting dose (once daily) | Structure | selectivity (SGLT2:SGLT1) |
|----------|-------------|----------------------------------------|-----------|--------------------------|
| Empagliflozin | 11949646  | 10 mg                                  | ![Empagliflozin Structure](image) | ~ 2700:1                |
| Dapagliflozin  | 9887712   | 5 mg                                   | ![Dapagliflozin Structure](image) | ~ 1200:1                |
| Canagliflozin   | 24812758  | 100 mg                                 | ![Canagliflozin Structure](image) | ~ 414:1                 |
| Ipragliflozin    | 10453870  | 50 mg                                  | ![Ipragliflozin Structure](image) | ~ 860:1                 |
| Tofogliflozin    | 46908929  | 20 mg                                  | ![Tofogliflozin Structure](image) | ~ 3000:1                |
| Luseogliflozin   | 11988953  | 2.5 mg                                 | ![Luseogliflozin Structure](image) | ~ 1770:1                |
The expression of SGLT2 is up-regulated, and the urinary glucose excretion threshold is also higher in patients with hyperglycemia compared with healthy humans [21]. Inhibition of SGLT2 reduces glucose reabsorption, promotes urinary glucose excretion, and produces negative caloric balance, which leads to weight loss [22]. SGLT2i, including canagliflozin, dapagliflozin and empagliflozin, directly target SGLT2 instead of insulin secretion and insulin action as compared with other anti-hyperglycemic agents [13]. SGLT2i can thus be used on top of other oral glucose-lowering drugs and insulin to exert additive anti-hyperglycemic effects [14].

At present, there are four SGLT2i (empagliflozin, canagliflozin, dapagliflozin, ertugliflozin) approved by the US Food and Drug Administration (FDA) and the European Union [23, 24]. Some other drugs in the class like ipragliflozin, tofogliflozin and luseogliflozin are approved in Japan [25-27].

**Effects of SGLT2 inhibitors in CVD: clinical evidence**

Many clinical studies have consistently shown that SGLT2i have multiple cardioprotective functions which manifest as reduced CVD (Table 2). The landmark EMPA-REG OUTCOME study was the first to offer convincing evidence that anti-diabetic drugs can reduce the occurrence of cardiovascular events. The trial randomly selected 7,020 diabetic patients with CVD. 3-point MACE (major adverse cardiovascular events, including death from cardiovascular causes, nonfatal MI, and nonfatal stroke), the primary outcome, was reduced by 14%. Hospitalization for heart failure (HHF) was reduced by 35% [9]. However, the incidence of MI or stroke was not significant. The primary outcome is largely due to the reduction in death from cardiovascular causes [9]. Subsequent experiments on renal effects of empagliflozin treatment also demonstrated that, compared with placebo, empagliflozin treatment group showed a slower progression of renal disease [28]. Moreover, at 2020 at the European Society of Cardiology (ESC) annual meeting, the results of the EMPEROR-Reduced study, which extended the benefits of SGLT2i to patients with more advanced and severe chronic HF. The combined risk of HHF or cardiovascular death in patients receiving empagliflozin was 25% lower than placebo. In addition, empagliflozin-treated patients had a lower risk of serious renal outcomes [29, 30]. In secondary analysis of the EMPEROR-Reduced trial indicated that patients treated with empagliflozin had improvement in health status [31]. The efficacy and safety of empagliflozin in patients was not influenced by basal therapy with a neprilysin inhibitor [32]. Combined treatment with both drugs may produce additional benefits [32].

**Table 2. Completed clinical trials of SGLT2i in patients with T2DM, CVD or both**

| Drugs          | Trials       | Patients                                      | Median follow-up | Outcomes                  | References |
|----------------|--------------|-----------------------------------------------|------------------|---------------------------|------------|
| Empagliflozin  | EMPA-REG     | 7,020 T2DM patients with CVD                  | 3.1 years        | 0.86 (0.74-0.99) *        | [9]        |
|                | EMPEROR-Reduced | 3,600 patients with HF and reduced ejection fraction (540%) | 16 months        | --                        | [30]       |
| Canagliflozin  | CANVAS       | 10,142 T2DM patients with CVD or CV risk factors. | 2.4 years        | 0.86 (0.75-0.97) *        | [33]       |
|                | CREDENCE     | 4,401 T2DM patients with CKD                  | 2.6 years        | 0.80 (0.67-0.95) *        | [35]       |
| Dapagliflozin  | DECLARE-TIMI 58 | 17,160 T2DM patients with ASCVD or CV risk factors. | 4.2 years        | 0.93 (0.84-1.03)          | [36]       |
|                | DAPA-HF      | 4,744 patients with HF and reduced ejection fraction. | 18.2 months      | 0.82 (0.69-0.98)          | [38]       |
| Ertugliflozin  | VERTIS-CV    | 8,246 T2DM patients with ASCVD                 | 3.5 years        | 0.97 (0.85-1.11)          | [40]       |

ASCVD: atherosclerotic cardiovascular diseases; CKD: chronic kidney disease; CV: cardiovascular; CVD: cardiovascular diseases; HF: heart failure; HHF: hospitalization for heart failure; MACE: major adverse cardiovascular events; T2DM: type 2 diabetes mellitus; *** statistically significant difference.
safety outcomes and showed that canagliflozin treatment reduced 3-point MACE and the stand-alone endpoint of HHF, as well as the risk of the primary outcome (end-stage kidney disease, doubling of serum creatinine, renal or cardiovascular death). This study also supported the concept that drugs of the SGLT2i class have clinical efficacy regardless of patients’ HbA1c levels [34, 35].

The DECLARE-TIMI 58 trial indicated that treatment with dapagliflozin did not impact the 3-point MACE but significantly reduced the risk of HHF. Dapagliflozin also reduced the composite renal endpoint by 24 % [36, 37]. Another trial, DAPA-HF, has shown that among patients with HF and reduced ejection fraction, patients receiving dapagliflozin had a lower risk of exacerbating HF or cardiovascular death than patients receiving placebo, regardless of whether they have diabetes or not [38]. A post hoc analysis indicated that SGLT2i acted on background therapies of HF and reduced ejection fraction in a mechanistically-independent and complementary manner [39].

The VERTIS-CV study included 8,246 T2DM patients with confirmed disorder in coronary artery, cerebral and/or peripheral arterial system. The incidences of 3-point MACE in the ertugliflozin and placebo groups were similar, showing no significant difference, but ertugliflozin significantly reduced the risk of HHF [40]. Patients who used SGLT2i had a lower risk of ischemic heart disease than those who did not use SGLT2i. The decrease in systolic blood pressure caused by SGLT2i was partially responsible for the results observed [41].

Despite the above-mentioned clinical trials showing the reduction in cardiovascular events in the SGLT2i treated groups (compared to placebo), only empagliflozin and canagliflozin had protective effects on 3-point MACEs [42]. The favorable clinical outcomes are hypothesized to be mainly driven by reduction of the rate of HHF. However, some large multi-national observational studies in patients with T2DM and cardiovascular risk suggested beneficial effects of SGLT2i also directed to MI and stroke which are events most closely associated with atherosclerosis and its clinical sequelae [43, 44].

In contrast, another analysis found that glucose-lowering drugs including SGLT2i significantly reduced the risk of atherosclerotic events but had no significant effect on the risk of HF, indicating the need for further clinical and basic studies in this exciting new area of the therapeutics of diabetes and its CVD consequences [45].

A meta-analysis of three SGLT2i related clinical trials found that the reduction in 3-point MACE was not large, and this effect was limited to patients with established ASCVD [46]. Also, the UTOPIA trial investigated the effects of tofogliflozin in T2DM patients without apparent CVD and indicated that tofogliflozin treatment did not delay the progression of atherosclerosis by monitoring carotid intima-media thickness but lowered arterial stiffness by evaluating the changes in brachial-ankle pulse wave velocity [47-49]. This might be due to limited sample size and study duration [47]. Therefore, increasing the sample size and research duration may provide some clues for whether or not these drugs have an influence on the progression of atherosclerosis.

Effects of SGLT2 inhibitors on atherosclerosis: experimental evidence

Based on the notable cardiovascular benefits conferred by SGLT2i, research interest has been focused on the study of the anti-atherosclerotic effects of SGLT2i in suitable experimental models and several SGLT2i have been shown to ameliorate atherosclerosis in ApoE−/− mice, Ldlr−/− mice and rabbits (Table 3).

In the ApoE−/− mouse model, canagliflozin alleviated atherosclerosis by reducing the expression of monocyte chemoattractant protein-1 (MCP-1) and vascular cell adhesion molecule-1 (VCAM-1), accompanied by decreased levels of total cholesterol, triglyceride and glucose, and it also decreased heart rate, plaque size, and increased plaque stability [50]. Canagliflozin also suppressed lipid synthesis and interleukin (IL)-1β levels in ApoE−/− mice [51]. Similarly, luseogliflozin treatment inhibited the expression of intercellular cell adhesion molecule-1 (ICAM-1), IL-1β, IL-6, and tumor necrosis factor-α (TNF-α) [52]. Luseogliflozin treatment reduced macrophage accumulation in perivascular adipose tissue and reduced neointimal hyperplasia [53]. Ipragliflozin exerted similar actions (suppressed macrophage accumulation, reduced fibrosis and adipoocyte death) [54]. Empagliflozin reduced the levels of CD68, MCP-1, ICAM-1, TNF-α and nicotinamide adenine dinucleotide phosphate (NADPH) oxidase subunits and thereby ameliorated diabetes-induced endothelial dysfunction [55]. Moreover, several studies have indicated that empagliflozin increased tissue inhibitor of metalloproteinase (TIMP)/matrix metalloproteinase-2 (MMP-2) ratio and increased collagen content of developing plaques, rendering the plaques more stable [50, 56]. After empagliflozin treatment, the atherosclerotic plaque area was smaller, and the inflammatory cell infiltration in adipose tissue was reduced [57]. A further study indicated that empagliflozin reduced angiotensin II-induced neovessel formation and macrophage infiltration in
the abdominal aortic aneurysm lesions in ApoE−/− mice [58]. In addition, empagliflozin treatment also exerted atheroprotection by inhibiting the renin-angiotensin-aldosterone system and sympathetic activity [59]. In hyperglycemic STZ-diabetic mice, empagliflozin also reduced atherosclerotic plaques [60]. Another study used ApoE−/− mice as a model of non-atherogenic diet and disease and found that empagliflozin treatment inhibited the development of aortic atherosclerosis and increased ketone body levels [61]. Moreover, dapagliflozin treatment attenuated atherosclerosis, reduced macrophage infiltration, and enhanced plaque stability [62, 63]. Similar results were obtained after ipragliflozin treatment [62]. However, a study in ApoE−/− Irs2−/− mice indicated that dapagliflozin did not protect against the development of atherosclerosis in insulin-resistant mice under hypercholesterolemic conditions [64].

As dyslipidemia is an independent risk factor for atherosclerosis [65], it is also important to study how glycemic control affects the development of atherosclerosis in the presence of hyperlipidemia. Effective glycemic control with dapagliflozin not only reduced atherosclerosis, but also ameliorated plasma lipoprotein profiles in Ldlr−/− mice [66]. The benefits of dapagliflozin on atherosclerosis have also been demonstrated in experimental animals other than mice. For example, in a rabbit model of atherosclerosis, dapagliflozin was found to exhibit anti-atherosclerotic effects by modulating inflammatory responses (decreased expression of TNF-α, IL-1β, and IL-6) and macrophage polarization (toward M2 macrophages) under non-diabetic conditions [67].

### Table 3. Atheroprotective effects and mechanisms of SGLT2i in rodents

| Drugs        | Animal model                                                                 | Treatment dose and duration | Observations and mechanisms                                                                 | References |
|--------------|-------------------------------------------------------------------------------|-----------------------------|------------------------------------------------------------------------------------------------|------------|
| Empagliflozin| ApoE−/− mice with HFD containing 0.2% cholesterol                             | 10 mg/kg/day for 10 weeks via oral gavage | atherosclerosis↓, total cholesterol, fasting glucose↓, heart rate diastolic, blood pressure↓ | [56]       |
| Empagliflozin| ApoE−/− mice with STZ-induced diabetes and western type diet                 | 20 mg/kg/day for 12 or 8 weeks via oral gavage | atherosclerosis↓, endothelial dysfunction↓, plasma triglyceride↓, CD68, MCP-1↓, NADPH oxidase subunits↓, vasoconstrictive eicosanoids↓, prostaglandin E2, thromboxane B2↓ | [55]       |
| Empagliflozin| ApoE−/− mice with western type diet containing cholesterol                    | 1 mg/kg or 3 mg/kg for 10 weeks via oral gavage | atherosclerosis↓, TNF-α, IL-6, MCP-1, CD68↓, serum amyloid A↓, urinary microalbumin↓ | [57]       |
| Empagliflozin| Ang II-infused ApoE−/− mice                                                  | 1 mg/kg or 3 mg/kg for 4 weeks via oral gavage | abdominal aortic aneurysm↓, elastin degradation, neovessel formation, macrophage infiltration↓, CCL-2, CCL-5, VEGF↓, MMP-2, MMP-9↓, p38 MAPK, NF-κB↓ | [58]       |
| Empagliflozin| ApoE−/− mice with HFD                                                        | 30 mg/kg/day for 8 weeks via oral gavage | atherosclerosis↓, endogenous ketone body↓, mTORC1↓ | [61]       |
| Empagliflozin| STZ-diabetic mice with injections of LDLR and SRBI antisense oligonucleotides and high -cholesterol diet (HCD) for 16 weeks | 35 mg/kg/day for 3 weeks via drinking water | atherosclerosis↓, lipid↓, CD68↓ | [60]       |
| Empagliflozin| ApoE−/− mice with western type diet containing 0.2 % cholesterol             | 10 mg/kg/day for 5 weeks via drinking water | atherosclerosis↓, triglyceride, total cholesterol, LDL↓, the renin-angiotensin-aldosterone system and sympathetic activity↓, body weight↓ | [59]       |
| Empagliflozin| ApoE−/− mice with HFD and STZ-induced diabetes                               | 1.0 mg/kg/day for 12 weeks via oral gavage | atherosclerosis↓, macrophage infiltration↓, smooth muscle cell proliferation↓, fasting glucose↓, cholesterol crystals↓, IL-1β, IL-18, NLRP3, ROS↓, ROS-NLRP3-caspase-1 pathway | [62]       |
| Dapagliflozin| ApoE−/− mice with a high-fat, high-sodium diet                               | 3 mg/kg/day for 6 weeks via drinking water | No-effect on circulating inflammatory cells or cytokine level, no protection against atherosclerosis. | [64]       |
| Dapagliflozin| Ldlr−/− mice with STZ-induced diabetes and 0.15% cholesterol               | 25 mg/kg for 4 weeks via drinking water | atherosclerosis↓, plasma glucose, total cholesterol, triglycerides↓, lipoprotein clearance↓, HSPG and bile acid pathways↓ | [66]       |
| Dapagliflozin| Rabbit with 1% high-cholesterol diet and balloon injury in aorta             | 1 mg/kg for 8 weeks via drinking water | atherosclerosis↓, macrophage infiltration↓, TNF-α, IL-1β, IL-6, M2 macrophages↑ | [67]       |
| Canagliflozin| ApoE−/− mice with HFD containing 0.2% cholesterol                            | 10 mg/kg/day for 5 weeks via oral | atherosclerosis↓, total cholesterol, triglycerides↓, VCAM-1, MCP-1↓, TIMP-1↓, MMP-2↑ | [50]       |
| Canagliflozin| ApoE−/− mice with HFD containing 0.2% cholesterol                            | 30 mg/kg/day for 4 weeks via drinking water | energy expenditure↑, adiposity↓, liver lipid synthesis↓, IL-1β↓ | [51]       |
| Ipragliflozin| wild-type mice with Western-type diet                                        | 10 mg/kg/day for 10 weeks via drinking water | macropathologies accumulation, fibrosis, and adipocyte death↑ | [54]       |
| Dapagliflozin or Ipragliflozin | ApoE−/− mice with STZ-induced diabetes and atherogenic diet | 1.0 mg/kg/day for 4 weeks via drinking water | atherosclerosis↓, macrophage infiltration↓, foam cell formation, Hba1c↑, acat1↓, acat2↓ | [63]       |
| Luseogliflozin| ApoE−/− mice with NA- and STZ-induced diet                                   | 4 mg/kg/day for 4 weeks via drinking water | atherosclerosis↓, TNFα, INFα, IL-1, IL-6↓, ICAM-1, PECAM-1, MMP-9, MMP-2↓ | [52]       |
| Luseogliflozin| Wild-type mice fed with low-fat diet or HFD                                  | 18 mg/kg/day for 25 days via drinking water | adipocyte sizes↓, accumulation of macropathologies expressing PDGF-B, adiponectin gene expression↑ | [53]       |

ABCA1: ATP-binding cassette transporter A1; ACAT1: acetyl-coenzyme A acetyltransferase 1; CCL: chemokine (C-C motif) ligand; Hba1c: glycosylated hemoglobin; HFD: high-fat diet, HSPG: heparan sulfate proteoglycans; ICAM-1: intercellular cell adhesion molecule-1; LDLR: low-density lipoprotein receptor; IL-1β: interleukin-1β; IL-6: interleukin-6; Irs2: interleukin-18; MAPK: mitogen-activated protein kinase; MCP-1: monocyte chemoattractant protein-1; MMP-2: matrix metalloproteinase; NADPH: nicotinamide adenine dinucleotide phosphate; NF-κB: nuclear factor-κB; NLRP3: nucleotide-binding domain-like receptor protein 3; PDGF-B: platelet-derived growth factor-B; PECAM-1: platelet endothelial cell adhesion molecule-1; ROS: reactive oxygen species; SRBI: scavenger receptor B1; TIMP: tissue inhibitor of metalloproteinase; TNF-α: tumor necrosis factor-α; VCAM-1: vascular cell adhesion molecule-1; VEGF: vascular endothelial growth factor; VSMCs: vascular smooth muscle cells.
Mechanisms of action of SGLT2 inhibitors

The main mechanism for SGLT2i to exert hypoglycemic effects is to increase the excretion of glucose in urine [10, 68]. However, in diabetic patients, the mechanism of the inhibitory effect of SGLT2i on atherosclerosis, which is the cause of cardiovascular events, remains unclear. The focus of clinical trials is to study the impact of SGLT2i on cardiovascular events, deaths and safety outcomes, but the research on their mechanism is mainly based on preclinical studies. In the past decade, various targets and signaling pathways mediating SGLT2i’s cardioprotective actions have been revealed. The potential molecular targets and beneficial effects of SGLT2i on atherosclerosis are discussed as below (Figure 1 and Figure 2).

Improving endothelial dysfunction

Endothelial dysfunction is an initial key event of atherosclerosis and an important contributor to vascular diseases [69, 70]. Substantial evidence showed that SGLT2i ameliorate endothelial dysfunction and improve endothelium-dependent vasodilation. Dapagliflozin regulated glycemic indices, which could improve flow-mediated vasodilation, arterial stiffness and endothelial function in patients with T2DM [71-73].

Several preclinical studies have demonstrated that endothelial dysfunction can be prevented by SGLT2i in different experimental models. Empagliflozin prevented the increased expression of atherothrombotic markers and improved endothelial function in ZSF1 rats that have metabolic syndrome and associated insulin resistance [74]. In this context, empagliflozin treatment decreased aortic stiffness and suppressed endothelial dysfunction by promoting glycosuria in a mouse model of T2DM [75]. Furthermore, empagliflozin attenuated high glucose-induced endothelial senescence and dysfunction by inhibiting the local angiotensin system [76]. Similarly, dapagliflozin reduced arterial stiffness and endothelial dysfunction in diabetic mice and enhanced diastolic function in a non-diabetic model [77, 78]. SGLT2i reversed endothelial activation and endothelial nitric oxide synthases (eNOS) deficit under diabetic conditions [78]. These results are
consistent with those of Gaspari et al. [79], who found that dapagliflozin treatment attenuated vascular endothelial cell activation and induced significant endothelium-independent vasorelaxation in ApoE<sup>−/−</sup> mice. Importantly, Tahara et al. [80] conducted a comparative experiment to compare the effects of six SGLT2i (luseogliflozin, ipragliflozin, tofogliflozin, empagliflozin, canagliflozin and dapagliflozin) on diabetes-related complications in T2DM mice and determined that all SGLT2i examined prevented the development of endothelial dysfunction suggesting this is a class effect for these agents although the commonality of reduced glycemia cannot be totally excluded.

**Improving vascular smooth muscle cell dysfunction**

Excessive proliferation and migration of vascular smooth muscle cells (VSMCs) as part of the development of the neointima play a crucial role in the pathogenesis of atherosclerosis [81, 82]. In this regard, VSMC growth and migration were significantly blunted in diabetic patients after canagliflozin treatment at clinically relevant doses [83]. Heme oxygenase-1 (HO-1) is a newly discovered target of canagliflozin. Treatment of VSMCs with canagliflozin stimulated HO-1 expression/activity [83].

Combination therapy with ipragliflozin and empagliflozin inhibited VSMC proliferation and the formation of neointima after vascular injury [84]. Furthermore, empagliflozin improved coronary microvascular function and contractile function [85]. Ipragliflozin also had the same actions (inhibiting the proliferation and migration of monocytes and VSMCs in vitro) [54].

**Attenuation of macrophage inflammation, foam cell formation, and M1 polarization**

Macrophage inflammation, foam cell formation, and M1 polarization are critical events in the development of atherosclerosis [86]. Empagliflozin ameliorated cardiac macrophage infiltration in db/db mice [87]. Similar findings were reported by Pennig et al. [60] using STZ-induced diabetic mice. Glucose-lowering effects conferred by empagliflozin alleviated the proliferation of plaque-resident macrophages and the atherosclerotic plaque size was significantly smaller [60]. In addition, mechanistic studies revealed that empagliflozin reduced the accumulation of M1 polarized macrophages, and redirected the macrophage phenotype toward an anti-inflammatory Figure 2. Potential cardiovascular actions of SGLT2i. SGLT2i have pleiotropic cardiovascular protective effects, such as: reduce weight, blood pressure, blood glucose, insulin resistance and glucotoxicity in patients, increases hemoconcentration, and inhibits oxidative stress and inflammation. The most direct effect of SGLT2i is inhibition of the reabsorption of glucose and a diuretic and natriuretic effect. In addition, SGLT2i also exerts other effects such as regulating ion channels, activating autophagy, inhibiting iron overload, attenuating activation of the NLRP3 inflammasome, and inhibiting the signaling of advanced glycation end products. The synergistic effects of these benefits may provide a therapeutic basis for the cardioprotective effects of SGLT2i.**
M2 phenotype, reduced obesity-related chronic inflammation, attenuated insulin resistance, and activated AMP-activated protein kinase (AMPK) [88, 89]. Similarly, canagliflozin directly inhibited the secretion of endothelial pro-inflammatory cytokine (MCP-1 and IL-6) through AMPK-dependent and -independent mechanisms [90]. AMPK activation increased ATP production and reduced ATP consumption [89]. The expression of lectin-like oxidized low-density lipoprotein receptor-1 (Lox-1) and acetyl-coenzyme A acetyltransferase 1 (ACAT1) genes was down-regulated in peritoneal macrophages isolated from diabetic mice receiving dapagliflozin, while the expression of ATP-binding cassette transporter A1 (ABCA1) was up-regulated [63].

In addition, macrophage infiltration into atherosclerotic lesions was reduced by dapagliflozin treatment [63]. In an infarction model in non-diabetic rats, dapagliflozin increased signal transducer and activator of transcription 3 (STAT3) activity, STAT3 nuclear translocation, and M2 macrophage infiltration. [91]. Similar results were reported in a rabbit model, in which dapagliflozin increased M2 macrophages and inhibited toll-like receptor 4/nuclear factor-kappa B signaling pathway which serve as master regulators of inflammatory responses in macrophages [67].

**Prevention of platelet activation**

Platelet adhesion, activation and aggregation in plaques are key events in atherothrombosis [92]. The reduction in blood glucose by dapagliflozin treatment normalized reticulated platelet levels [93]. Spigoni et al. [94] showed that empagliflozin and dapagliflozin reduced inflammation and oxidative stress and might reduce ADP-stimulated platelet activation. Empagliflozin reduced the plasma concentration of plasminogen activator inhibitor-1 in patients with T2DM, which inhibited the development of thrombotic diseases [95]. Therefore, plaque stabilization and inhibition of thrombosis are the potential mechanisms of SGLT2i-mediated cardiovascular protection [94].

**Attenuation of oxidative stress**

The development of atherosclerosis is closely related to oxidative stress. SGLT2i reduce oxidative stress in patients, experimental animals, and cultured cells. After SGLT2i treatment, NADPH oxidase subunits (NOX1, NOX2, NOX4, p22phox, and p47phox) were reduced [55, 96-98]. Surrogate parameters of oxidative stress, 3-nitrotyrosine- and hydroxynonenal-positive proteins, were almost normalized [99]. Moreover, parameters of pathological oxidative stress (hydrogen peroxide, 3-nitrotyrosine, lipid peroxide) were attenuated in cardiomyocytes [100] and urinary excretion of 8-hydroxydeoxyguanosine was reduced [97, 101]. Inhibition of oxidative stress restores the bioavailability of NO and explains the vasoprotective benefits of SGLT2i [102]. Kolijn et al. [100] conducted more in-depth mechanistic research and observed that empagliflozin improved endothelial vasorelaxation via reducing pro-inflammatory/pro-oxidative pathways and eNOS-dependent PKGIα (cyclic guanosine monophosphate-dependent protein kinase G Iα) oxidation. SGLT2i improved PAR2 (proteinase-activated receptor 2)-mediated NO5-dependent vasodilation, which is compromised by oxidative stress though an NAPDH oxidase/ROS-dependent signaling pathway [103].

**Reduced inflammation**

Compared with most current glucose-lowering agents, SGLT2i have actions in reducing tissue inflammation. Evidence in mouse models suggested that SGLT2i inhibited the expression of circulating inflammatory molecules (TNF-α, MCP-1, PECAM-1, VCAM-1, ICAM-1, IL-1β, and IL-6) associated with atherosclerosis [52, 56, 62, 77]. Also, human evidence indicated that canagliflozin might induce changes in TNFR1, IL-6, MMP7, serum leptin, adiponectin and fibronectin 1 [104, 105]. Empagliflozin reduced superoxide production in leukocytes and reduced hs-CRP in patients with T2DM [106]. SGLT2i have the capacity to inhibit inflammation and reverse the adverse factors of atherosclerosis.

**Regulation of iron metabolism**

Iron metabolism occurs as a complex interplay between iron per se, inflammation and atherosclerosis [107]. Iron overload promotes the formation of highly reactive forms of oxygen free radicals, which accelerates atherosclerosis [108-111]. Serum ferritin is a reliable indicator of iron stores [110, 112]. High transferrin saturation signals iron overload [108]. Recent proteomic findings in plasma of T2DM demonstrated significant decrement in ferritin following empagliflozin treatment [113]. In addition, dapagliflozin treatment significantly reduced circulating hepcidin and ferritin concentrations [114]. Regulating iron metabolism might be one of the novel mechanisms of action of SGLT2i in cardiovascular protection but this area requires more investigation.

**Promoting autophagy**

Autophagy is related to the clearance of apoptotic macrophages from atherosclerotic plaques [115]. Blocking autophagy renders macrophages more susceptible to cell death and promotes necrosis in advanced atherosclerosis [115]. Canagliflozin
inhibited intracellular glucose metabolism and promoted autophagy that might be associated with inhibited 6-phosphofructo-2-kinase (PFK2) expression and increased AMPK phosphorylation [116]. Autophagy is closely related to AMPK and sirtuin-1 (SIRT1). Canagliflozin upregulated the expression of SIRT1 [117]. Similarly, empagliflozin treatment activated AMPK and enhanced cardiac autophagy [118]. Following MI in patients with diabetes, empagliflozin inhibited ROS and restored autophagy to normalize the size and number of mitochondria [119]. Empagliflozin treatment increased the level of mitochondrial SIRT3 and enhanced the activation of TLR9, thereby activating autophagy [120]. Therefore, enhancing autophagy might be a potential mechanism for SGLT2i to exert atheroprotective effects.

**Regulation of ion exchange channels**

K⁺ channels regulating depolarization/hyperpolarization are the main determinants of vascular tone. The voltage-dependent K⁺ (Kv) channels could be the target of dapagliflozin. The vasodilatory effect of dapagliflozin occurred through direct activation of protein kinase G and subsequent activation of Kv channels [121].

Na⁺/H⁺ exchanger 1 (NHE1) in endothelial cells might be another target of SGLT2i. Dapagliflozin inhibited the activity of NHE1 in endothelial cells to reverse endothelial activation [78]. Empagliflozin treatment directly inhibited NHE1 mediated Na⁺ influx, thereby reducing myocardial cytoplasmic Na⁺, regardless of SGLT2 activity [122]. However, the latest research proves that empagliflozin treatment did not inhibit cardiac NHE1 activity [123]. It remains unclear whether SGLT2i affect the progression of atherosclerosis through targeting ion channels.

**Increasing ketone bodies**

An important feature of diabetic patients treated with SGLT2i is the increase of circulating ketone bodies [124]. Ferrannini et al. [125-127] indicated that increased β-hydroxybutyrate (BHB) promote ketone bodies as metabolic substrates and result in improved energy metabolism of the heart. In addition to the involvement in energy metabolism, other protective effects have been proposed for ketone bodies. For example, preclinical findings demonstrate that BHB has a strong anti-inflammatory effect. Empagliflozin has been reported to significantly increase the abundance of serum BHB leading to inhibition of NLRP3 and reduction of IL-1β levels [128]. The importance of ketone bodies as an adjuster of the benefits of SGLT2i in atherosclerosis remain uncertain.

**Reduced body weight**

Inhibition of glucose reabsorption leads to calorie loss, accompanied by weight loss [129]. Several meta-analyses of clinical trials in patients with T2DM have suggested that body weight was significantly reduced following SGLT2i treatment [130, 131]. SGLT2i convert glucose metabolism into fatty acids and ketones, and enhance fat utilization that are favorable factors which confer anti-atherosclerotic effects.

**Regulation of diuresis, natriuresis, homoconcentration and blood pressure**

SGLT2i have natriuretic and diuretic effects [124]. Induction of diuresis and natriuresis by SGLT2i decrease plasma volume and contribute to systolic and diastolic blood pressure control [132, 133]. Hypertension is a contributing factor to atherosclerosis and its thrombotic complications [1]. Reductions in blood pressure were greater with empagliflozin compared with placebo [134]. Natriuresis also activates the tubuloglomerular feedback response [135]. The synergistic effect of these several mechanisms may provide an indirect but useful basis for the anti-atherosclerotic effects of SGLT2i.

**Lowering the level of uric acid**

SGLT2i treatment resulted in lower circulating levels of uric acid [136-138]. Uric acid is considered an activator of oxidative stress and inflammation, which induces activation of the NLRP3 inflammasome [124, 139]. Lowering uric acid might be an indirect mechanism of SGLT2i to improve atherosclerosis, and its deeper mechanism remains to be evaluated.

**Inhibition of NLRP3 inflammasome**

Nucleotide-binding domain-like receptor protein 3 (NLRP3) inflammasome plays a vital role in inflammation and immunity [140]. The activation of NLRP3 inflammasome and the subsequent release of IL-1β and IL-18 contribute to the pathogenesis of atherosclerosis and HF [141-143]. Current research on the effect of SGLT2i on NLRP3 inflammasome is focused on diabetic nephropathy, steatohepatitis, cardiomyopathy and atherosclerosis.

Empagliflozin attenuated the activation of NLRP3 inflammasome in a Ca²⁺-dependent manner [144]. Kim et al. [128] demonstrated that empagliflozin significantly inhibited the activation of NLRP3 inflammasome by increasing serum BHB levels and reducing insulin levels in T2DM and CVD patients, regardless of glycemic control. Dapagliflozin treatment reduced the production of NLRP3 protein and ROS in aortic tissues, thereby partially reversing...
the formation of atherosclerosis [62]. Dapagliflozin also inhibited the activation of NLRP3 inflammasome by activating AMPK and mTORC2 [145, 146]. In conclusion, SGLT2i attenuates the activation of NLRP3 inflammasome, which might help explain its inhibitory effect on atherosclerosis.

**Reduction of advanced glycation end-products**

The binding of advanced glycation end-products (AGEs) to endothelial AGE receptors (RAGE) stimulates oxidative stress and expression of cytokines, chemokines, and adhesion molecules [147]. Methylglyoxal, a primary precursor of AGEs, decreased the phosphorylation of eNOSSer1177 and protein kinase B (Akt), which inhibited eNOS activity. SGLT2i decreased the levels of methylglyoxal, prevented AGE formation and AGE/RAGE signaling, and ameliorated decreased phosphorylation of eNOSSer1177 and Akt, thus conferring atheroprotective effects [96, 97, 101].

**Conclusions and perspectives**

As a new category of oral hypoglycemic agents, SGLT2i have a specific mechanism of action and target glucose removal which is distinct from other hypoglycemic agents. By increasing the excretion of urinary glucose, SGLT2i regulate glucose levels without an increased risk of hypoglycemic events. A recent observational study suggested that SGLT2i might be more effective than GLP-1RA in ameliorating cardiovascular outcomes of T2DM with comparable rate of adverse events [148]. In addition, SGLT2i significantly decreased the risk of HF or cardiovascular death independent of diabetes status in patients on background therapy for HF [39, 149].

The cardiovascular actions and anti-inflammatory effects of SGLT2i have been excellently reviewed elsewhere [11, 150-153]. Here, we provide a focused review of the protective effects of SGLT2i in different stages of atherosclerosis (the leading cause of CVD), illuminating the molecular targets of this category of drugs in atheroprotection. In patients with diabetes, SGLT2i show cardio-renal protection and have important clinical advantages but there are also some adverse reactions. The most commonly observed adverse effect is polyuria. Empagliflozin increased the risk of urogenital infections in women and men [9]. Another important safety concern, observed in the CANVAS trial, was amputations and fractures of the legs and feet in patients treated with canagliflozin compared with placebo [33]. However, a recent real-world study suggested that the risk of amputations in patients treated with SGLT2i was not higher compared with other anti-diabetic drugs [154]. Also, the application of SGLT2i for patients with type 1 diabetes should be considered with caution due to increased incidence of ketoacidosis and diarrhea [155]. Long-term systemic side effects of SGLT2i are warranted to be evaluated in large-scale randomized controlled trials.

By deepened understanding of the mechanism of action of SGLT2i, the adverse reactions after drug treatments could be reduced. Results of recent clinical trials involving individuals without diabetes might repurpose this drug as “a drug for cardiorenal protection” [156]. Taken together, SGLT2i have broad therapeutic prospects, and their pharmacological mechanisms and precise molecular targets beyond SGLT2 inhibition and glycemic control need to be elucidated in future studies.

**Supplementary Material**

Supplementary figure.
http://www.thno.org/v11p4502s1.pdf

**Acknowledgements**

This study was supported by grants from National Natural Science Foundation of China [Grant No’s. 82070464 to SX, 81941022 to JW, 81530025 to JW], Program for Innovative Research Team of The First Affiliated Hospital of USTC (to JW), Strategic Priority Research Program of Chinese Academy of Sciences [Grant No. XDB38010100 to JW] and the National Key R&D Program of China [Grant No. 2017YFC1309603 to JW]. This work was also supported by Local Innovative and Research Teams Project of Guangdong Pearl River Talents Program [2017B011311], China International Medical Foundation (to XZ), Natural Science Foundation of Anhui Province [Grant No. 006223066002 to SL].

**Competing Interests**

The authors have declared that no competing interest exists.

**References**

1. Libby P, Buring JE, Badimon L, Hansson GK, Deanfield J, Bittencourt MS, et al. Atherosclerosis. Nat Rev Dis Primers. 2019; 5: 56. doi: 10.1038/s41572-019-0106-2.
2. Einarsen TR, Acs A, Ludwig C, Panton UH. Prevalence of cardiovascular disease in type 2 diabetes: a systematic literature review of scientific evidence from across the world in 2007-2017. Cardiovasc Diabetol. 2018; 17: 83. doi: 10.1186/s12933-018-0728-6.
3. Lusis AJ. Atherosclerosis. Nature. 2000; 407: 233-41.
4. Wang D, Yang Y, Lei Y, Tzvetkov NT, Liu X, Yeung AWK, et al. Targeting foam cell formation in atherosclerosis: therapeutic potential of natural products. Pharmacol Rev. 2019; 71: 596-670.
5. Abdul-Ghani M, Del Prato S, Chilton R, DeFronzo RA. SGLT2 inhibitors and cardiovascular risk: lessons learned from the EMPA-REG OUTCOME study. Diabetes Care. 2016; 39: 717-25.
6. Gore MO, McGuire DK, Lingvay I, Rosenstock J. Predicting cardiovascular risk in type 2 diabetes: the heterogeneity challenges. Curr Cardiol Rep. 2015; 17: 607. doi: 10.1007/s11886-015-0607-7.
7. Feng X, Sureda A, Jafari S, Menbari Z, Tewari D, Annunziata G, et al. Berberine in cardiovascular and metabolic diseases: from mechanisms to therapeutics. Theranostics. 2019; 9: 1923-51.

8. Scheen AJ. Pharmacodynamics, efficacy and safety of sodium-glucose cotransporter 2 (SGLT2) inhibitors for the treatment of type 2 diabetes mellitus. Drugs. 2015; 75: 33-59.

9. Zinnman B, Wanner C, Lachin JM, Fitchett D, Bluemink H, Hantel S, et al. Empagliflozin, cardiovascular outcomes, and mortality in type 2 diabetes. N Engl J Med. 2015; 373: 2117-28.

10. Nauck MA. Update on developments with SGLT2 inhibitors in the management of type 2 diabetes. Drug Des Devel Ther. 2014; 8: 1335-80.

11. Lopaschuk GD, Verma S. Mechanisms of cardiovascular benefits of sodium glucose co-transporter 2 (SGLT2) inhibitors: a state-of-the-art review. JACC Basic Transl Sci. 2020; 5: 632-44.

12. Lambers Heerspink HJ, de Zeeuw D, Wie L, Leslie B, List J. Dapagliflozin, a glucose-regulating drug with diuretic properties in subjects with type 2 diabetes. Diabetes Obes Metab. 2013; 15: 853-62.

13. Abdul-Ghani MA, Norton L, Defronzo RA. Role of sodium-glucose co-transporter type 2 (SGLT2) inhibitors for the treatment of type 2 diabetes mellitus. Advanced therapy. 2019; 11: 515-31.

14. Ferrannini E, Solini A. SGLT2 inhibition in diabetes mellitus: rationale and clinical prospects. Nat Rev Endocrinol. 2012; 8: 495-502.

15. Vallon V, Thomson SC. Targeting renal glucose reabsorption to treat hyperglycaemia: the pleiotropic effects of SGLT2 inhibition. Diabetologia. 2017; 60: 215-25.

16. Ehrenkranz JR, Lewis NG, Kahn CR, Roth J. Phlorizin: a review. Diabetes Metab Res Rev. 2005; 21: 51-8.

17. Oku A, Ueta K, Arakawa K, Ishihara T, Nawano M, Kuronuma Y, et al. T-1095, an inhibitor of renal Na+-glucose cotransporters, may provide a novel approach to treating diabetes. Diabetes. 1999; 48: 1794-800.

18. Weng W, Ellsworth BA, Nirschl AA, McCann PJ, Patel M, Girotra RN, et al. Discovery of dapagliflozin: a potent, selective renal sodium-dependent glucose co-transporter 2 (SGLT2) inhibitor for the treatment of type 2 diabetes. J Med Chem. 2008; 51: 1145-9.

19. Nomura S, Sakamaki S, Hongu M, Kawanishi E, Koga Y, Sakamoto T, et al. Discovery of canagliflozin, a novel C-glucoside with thiophene ring, as sodium-dependent glucose co-transporter 2 inhibitor for the treatment of type 2 diabetes mellitus. J Med Chem. 2010; 53: 6355-60.

20. Gremerl R, Thomas L, Eckhardt M, Himmelbach F, Sauer A, Sharp DE, et al. Empagliflozin, a novel selective sodium-glucose co-transporter 2 (SGLT2) inhibitor: characterisation and comparison with other SGLT2 inhibitors. Diabetes Obes Metab. 2012; 14: 83-90.

21. Rabizadeh S, Nakhjavani M, Esteghamati A. Cardiovascular and renal benefits of SGLT2 inhibitors: a narrative review. Int J Endocrinol Metab. 2019; 17: e84335. doi: 10.1186/s12933-020-01077-y.

22. Bolinder J, Ljunggren O, Kullberg J, Johansson L, Wilding J, Langkilde T, et al. Empagliflozin and health-related quality of life in patients with heart failure with reduced ejection fraction: the EMPEROR-Reduced trial. Eur Heart J. 2019; 41: 2379-92.

23. Ni L, Yuan C, Chen G, Zhang C, Wu X. SGLT2i: beyond the glucose-lowering effect. Cardiovasc Diabetol. 2020; 19: 98.

24. Wiviott SD, Raz I, Bonaca MP, Mosenzon O, Kato ET, Cahn A, et al. Dapagliflozin and cardiovascular outcomes in type 2 diabetes. N Engl J Med. 2019; 380: 347-57.

25. Kato ET, Silverman MG, Mosenzon O, Zelniker TA, Cahn A, Furtado RHM, et al. Effect of dapagliflozin on heart failure and mortality in type 2 diabetes mellitus. Circulation. 2019; 139: 2528-36.

26. Poole RM, Prossler JE. Tofogliflozin: first global approval. Drugs. 2014; 74: 73-80.

27. Markham A, Elkinson S. Luseogliflozin: first global approval. Drugs. 2021; 81: 667-77.

28. Wanner C, Inzucchi SE, Lachin JM, Fitchett D, von Eynatten M, Mattei S, et al. Empagliflozin and cardiovascular and renal events in type 2 diabetes and chronic kidney disease according to baseline HbA1c, including those with HbA1c <7%: results from the CREDENCE trial. Circulation. 2020; 141: 407-10.

29. Perkovic V, Jardine MJ, Neal B, Bompont S, Heerspink HJL, Charytan DM, et al. Canagliflozin and renal outcomes in type 2 diabetes and nephropathy. N Engl J Med. 2019; 380: 2295-306.

30. Packer M, Anker SD, Butler J, Filippatos G, Ferreira JP, Pocock SJ, et al. Cardiovascular and renal events in type 2 diabetes. N Engl J Med. 2017; 377: 644-57.

31. Cannon CP, Perkovic V, Agarwal R, Baldassarre J, Bakris G, Charytan DM, et al. Evaluating the effects of canagliflozin on cardiovascular and renal events in patients with type 2 diabetes mellitus and chronic kidney disease according to baseline HbA1c, including those with HbA1c <7%: results from the CREDENCE trial. Circulation. 2020; 141: 407-10.

32. Perkovic V, Jardine MJ, Neal B, Bompont S, Heerspink HJL, Charytan DM, et al. Canagliflozin and renal outcomes in type 2 diabetes and nephropathy. N Engl J Med. 2019; 380: 2295-306.

33. Packer M, Butler J, Filippatos G, Ferreira JP, Pocock SJ, et al. Empagliflozin and health-related quality of life in patients with heart failure with reduced ejection fraction: the EMPEROR-Reduced trial. Eur Heart J. 2021. doi: 10.1093/eurheartj/ehaa1007.
51. Day EA, Ford RJ, Lu JH, Lu R, Lundberg L, Desjardins EM, et al. The SGLT2 inhibitor canagliflozin suppresses lipid synthesis and interleukin-1 beta in ApoE deficient mice. Biochem J 2020; 477: 2347-61.

52. Nakatsu Y, Kokubo H, Bumdelger B, Yoshizumi M, Yamamotoya T, Matsunaga Y, et al. The SGLT2 inhibitor luseogliflozin rapidly normalizes aortic mRNA levels of inflammation-related but not lipid-metabolism-related genes and suppresses atherosclerosis in diabetic ApoE KO mice. Int J Mol Sci 2017; 18: doi: 10.3390/ijms18081704.

53. Mori Y, Terasaki M, Hiromura M, Saito T, Kushima H, Koshibu M, et al. Luseogliflozin attenuates neointimal hyperplasia after wire injury in high-fat-diet-fed mice via inhibition of perivascular adipose tissue remodeling. Cardiovasc Diabetol. 2019; 18: 143: doi: 10.1186/s12933-019-0947-5.

54. Mori K, Tsachiya K, Nakamura S, Miyagi Y, Shiba K, Ogawa Y, et al. Lp-PLA2 and neointimal expansion inhibits cuff-induced vascular remodeling in mice. Cardiovasc Diabetol. 2019; 18: 83: doi: 10.1186/s12933-019-0886-1.

55. Ganbaatar B, Fukuda D, Shinohara M, Yagi S, Kusunose K, Yamada H, et al. Empagliflozin ameliorates endothelial dysfunction and suppresses arteriosclerosis in diabetic apolipoprotein E-deficient mice. Eur J Pharmacol. 2020; 175: 105404. doi: 10.1016/j.ejphar.2020.175404.

56. Dimitriadis GK, Nasiri-Ansari N, Agrogiannis G, Kostakis ID, Randeva HS, et al. Effects of dapagliflozin on endothelial function in type 2 diabetes with established ischemic heart disease (EDIFIED). J Endocr Soc. 2020; 4: bvo217. doi: 10.1210/jendos/bvo217.

57. Solini A, Giannini L, Seghieri M, Vitolo E, Taddei S, Ghiadoni L, et al. Dapagliflozin acutely improves endothelial function, reduces aortic stiffness and renal resistive index in type 2 diabetic patients: a pilot study. Cardiovasc Diabetol. 2017; 16: 138: doi: 10.1186/s12933-017-0621-8.

58. Park SH, Farooq MA, Gaertner S, Bruckert C, Qureshi AW, Lee HH, et al. Empagliflozin improved systolic blood pressure, endothelial dysfunction and heart remodeling in the metabolic syndrome ZSF1 rat. Cardiovasc Diabetol. 2020; 19: 19: doi: 10.1186/s12933-020-00997-7.

59. Aroor AR, Das NA, Carpenter AJ, Habibi J, Jia G, Ramirez-Perez FI, et al. Glycemic control by the SGLT2 inhibitor empagliflozin decreases aortic stiffness, renal resistivity index and kidney injury. Cardiovasc Diabetol. 2018; 17: 108: doi: 10.1186/s12933-018-0759-8.

60. Khamaisi-Benkhat S, Betancor E, Yrías-Khadja N, Park SH, Amoura L, Abbas M, et al. Angiotensin II-induced redox-sensitive SGLT2 and 1 expression promotes high glucose-induced endothelial cell senescence. J Cell Mol Med. 2020; 24: 2109-22.

61. Lee DM, Battson ML, Jarrell DK, Hou S, Ecton KE, Weir TL, et al. SGLT2 inhibition via dapagliflozin improves generalized vascular dysfunction and alters the gut microbiota in type 2 diabetic mice. Cardiovasc Diabetol. 2018; 17: 62: doi: 10.1186/s12933-018-0708-x.

62. Cappetta D, De Angelis A, Ciferri F, Coppi, R, Cozzolino A, Miecchi A, et al. Amelioration of diabetic dysfunction by dapagliflozin in a non-diabetic model involves coronary endothelium. Pharmacological Research. 2020; 157: doi: 10.1016/j.phrs.2020.104781.

63. Gaspari T, Spizzo I, Liu H, Hu Y, Simpson RW, Widdop RE, et al. Dapagliflozin attenuates human vascular endothelial cell activation and induces vasorelaxation: A potential mechanism for inhibition of atherosclerosis. Diab Vasc Dis Res. 2018; 15: 64-73.

64. Takahara 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.

65. Beckman JA, Creager MA, Libby P. Diabetes and atherosclerosis: epidemiology, pathophysiology, and management. JAMA. 2002; 287: 2570-81.

66. Creager MA, Luscher TF, Cosentino F, Beckman JA. Diabetes and vascular disease: pathophysiology, clinical consequences, and medical therapy: Part I. Circulation. 2003; 107: 1527-32.

67. Behnammanesh G, Durante GL, Khanna YP, Peyton KJ, Durante W. Inhibition by Empagliflozin Promotes Fat Utilization and Browning and Inhibition of Neointima Hyperplasia in a Mouse Model of Atherosclerosis. Atheroscler Rep. 2020; 22: 59. doi: 10.1007/s11883-020-00870-8.

68. Shrikrishnapalasuriy N, Shaikh A, Ruslan AM, Sharaf G, Udaiawar M, Price DE, et al. Dapagliflozin is associated with improved glycemic control and weight reduction at 44 months of follow-up in a secondary care diabetes clinic in the UK. Diabetes Metab Syndr. 2020; 14: 257-9.

69. Sita S, Tomasoni L, Atzeni F, Ambrosio G, Cordiano C, Catapano A, et al. From endothelial dysfunction to atherosclerosis. Autoimmun Rev. 2010; 9: 830-4.
Macrophages in Diet-induced Obese Mice. ElBioMedicine. 2017; 20: 137-49.
89. Koyani CN, Plastira I, Sourij H, Hallstrom S, Schmidt A, Rainer PP, et al. Empagliflozin protects heart from inflammation and energy depletion via AMPA activation. Pharmacol Res. 2020; 158: 104870. doi: 10.1016/j.phrs.2020.104870.
90. Mancini SJ, Boyd D, Katwain OJ, Strembitska A, Almabrouk TA, Barrett TJ, et al. Sodium-glucose co-transporter 2 inhibitors antagonize lipotoxicity in human myeloid angiogenic cells and ADP-dependent activation in human platelets: potential relevance to prevention of cardiovascular events. Cardiovasc Drugs. 2020; 39: 107703. doi: 10.1016/j.jcd.2020.107703.
91. Sakurai S, Jojima T, Iijima T, Tomaru T, Usui I, Aso Y. Empagliflozin attenuates the plasma concentration of plasminogen activator inhibitor-1 (PAI-1) in patients with type 2 diabetes: Association with improvement of fibrinolysis. J Diabetes Complications. 2020; 34: 107703. doi: 10.1016/j.jdiacomp.2020.107703.
92. Wu MD, Atkinson TM, Lindner JR. Platelets and von Willebrand factor oxidation. Cardiovasc Res. 2020. doi: 10.1093/cvr/cvaa123.
93. Kraaikan MJ, Lee MK, Al-Sharea A, Dragoljevic D, Barrett TJ, Montenont E, et al. Neutrophil-derived S100 calcium-binding proteins A8/A9 promote reticulated thrombocytosis and atherogenesis in diabetes. J Clin Invest. 2017; 127: 2133-47.
94. Spigoni V, Fantuzzi F, Carubbi C, Pozzi G, Masselli E, Gobbi G, et al. Sodium-glucose co-transporter 2 inhibitors antagonize lipotoxicity in human myeloid angiogenic cells and ADP-dependent activation in human platelets: potential relevance to prevention of cardiovascular events. Cardiovasc Drugs. 2020; 39: 107703. doi: 10.1016/j.jcd.2020.107703.
95. Sakurai S, Jojima T, Iijima T, Tomaru T, Usui I, Aso Y. Empagliflozin attenuates the plasma concentration of plasminogen activator inhibitor-1 (PAI-1) in patients with type 2 diabetes: Association with improvement of fibrinolysis. J Diabetes Complications. 2020; 34: 107703. doi: 10.1016/j.jdiacomp.2020.107703.
96. Wu MD, Atkinson TM, Lindner JR. Platelets and von Willebrand factor oxidation. Cardiovasc Res. 2020. doi: 10.1093/cvr/cvaa123.
97. Sayour AA, Korkmaz-Icouc S, Loganathan S, Ruppert M, Sayour VN, Olah A, 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: 127. doi: 10.1186/s12967-019-1881-8.
98. Steven S, Oelze M, Hanf A, Kroller-Schon S, Kashani F, Roohani S, et al. The SGLT2 inhibitor empagliflozin improves the primary diabetic complications in ZDF rats. Redox Biol. 2017; 13: 370-85.
99. Koolin D, Pabel S, Tian Y, Lodi M, Herwig M, Carrizzo A, et al. Empagliflozin improves endothelial and cardiomyocyte function in human heart failure with preserved ejection fraction via reduced pro-inflammatory-oxidative pathways and protein kinase Galpha. Biochem Pharmacol. 2018; 152: 45-59.
100. Kraakman MJ, Lee MK, Al-Sharea A, Dragoljevic D, Barrett TJ, Montenont E, et al. Neutrophil-derived S100 calcium-binding proteins A8/A9 promote reticulated thrombocytosis and atherogenesis in diabetes. J Clin Invest. 2017; 127: 2133-47.
101. Villa BB, Bicocchi P, Mulder S, Leiser U, Hansen MK, Heinzel A, et al. Empagliflozin reduces inflammation and fibrosis biomarkers: a potential mechanism for action of SGLT2 inhibitors in diabetic kidney disease. Diabetologia. 2019; 62: 1154-66.
127. Ferrannini E, Baldi S, Frascerra S, Astiarraga B, Heise T, Bizzotto R, et al. Shift to fatty substrate utilization in response to sodium-glucose cotransporter 2 inhibition in subjects without diabetes and patients with type 2 diabetes. Diabetes. 2016; 65: 1190-5.

128. Kim SR, Lee SG, Kim SH, Kim JH, Choi E, Cho W, et al. SGLT2 inhibition modulates NLRP3 inflammasome activity via ketones and insulin in diabetes with cardiovascular disease. Nat Commun. 2020; 11: 2127.

129. Rajev SP, Cuthbertson DJ, Wilding JPH. Energy balance and metabolic changes with sodium-glucose co-transporter 2 inhibition. Diabetes Obes Metab. 2016; 18: 125-34.

130. Storgaard H, Glud LL, Bennet C, Grundahl MF, Christensen MB, Knop FK, et al. Benefits and harms of sodium-glucose co-transporter 2 inhibitors in patients with type 2 diabetes: a systematic review and meta-analysis. PLoS One. 2016; 11: e0166125. doi: 10.1371/journal.pone.0166125.

131. Cai X, Yang W, Gao X, Chen Y, Zhou L, Zhang S, et al. The association between the dosage of SGLT2 inhibitor and weight reduction in type 2 diabetes patients: a meta-analysis. Obesity (Silver Spring). 2018; 26: 70-80.

132. Heerspink HJL, Perkins BA, Fitchett DH, Husain M, Cherney DZI. Sodium glucose cotransporter 2 inhibitors in the treatment of diabetes mellitus: cardiovascular and kidney effects. Potential Mechanisms, and Clinical Applications. Circulation. 2016; 134: 752-7.

133. Lytvyn Y, Bjornstad P, Udell JA, Lovshin JA, Cherney DZI. Sodium glucose cotransporter-2 inhibitor in heart failure: potential mechanisms, clinical applications, and summary of clinical trials. Circulation. 2017; 136: 1643-58.

134. Tikkanen L, Narko K, Zeller C, Grein A, Salsali A, Broedl UC, et al. Empagliflozin reduces blood pressure in patients with type 2 diabetes and hypertension. Diabetes Care. 2015; 38: 420-8.

135. Davies MJ, Trujillo A, Vijapurkar U, Damaraju CV, Meininger G. Effect of canagliflozin on serum uric acid in patients with type 2 diabetes mellitus. Diabetes Obes Metab. 2015; 17: 426-9.

136. Zhao Y, Xu L, Tian D, Xia P, Zheng H, Wang L, et al. Effects of sodium-glucose co-transporter 2 (SGLT2) inhibitors on serum uric acid level: A meta-analysis of randomized controlled trials. Diabetes Obes Metab. 2018; 20: 458-62.

137. Hao Z, Huang X, Shao H, Tian F. Effects of dapagliflozin on serum uric acid levels in hospitalized type 2 diabetic patients with inadequate glycemic control: a randomized controlled trial. Ther Clin Risk Manag. 2018; 14: 2407-13.

138. Braga TT, Formi MF, Correa-Costa M, Ramos RN, Barbuto JA, Branco P, et al. Soluble uric acid activates the NLRP3 inflammasome. Sci Rep. 2017; 7: 39884. doi: 10.1038/srep39884.

139. De Nardo D, Latz E. NLRP3 inflammasomes link inflammation and metabolic disease. Trends Immunol. 2011; 32: 373-9.

140. Kim SR, Lee SG, Kim SH, Kim JH, Choi E, Cho W, et al. SGLT2 inhibition modulates NLRP3 inflammasome activity via ketones and insulin in diabetes with cardiovascular disease. Nat Commun. 2020; 11: 2127.

141. Isermann B, Bierhaus A, Humpert PM, Rudofsky G, Chavakis T, Ritzel R, et al. [AGE-RAGE: a hypothesis or a mechanism?]. Herz. 2004; 29: 504-9.

142. Lytvyn Y, Bjornstad P, Udell JA, Lovshin JA, Cherney DZI. Sodium glucose cotransporter-2 inhibitor in heart failure: potential mechanisms, clinical applications, and summary of clinical trials. Circulation. 2017; 136: 1643-58.

143. Gupta H, Callaway JB, Ting JP. Inflammasomes: mechanism of action, role in disease, and therapeutics. Nat Med. 2015; 21: 677-87.

144. Byrne NJ, Matsumura N, Maayah ZH, Ferdauossi M, Takahara S, Darwesh AM, et al. Empagliflozin blunts worsening cardiac dysfunction associated with reduced NLRP3 (nucleotide-binding domain-like receptor protein 3) inflammasome activation in heart failure. Circulation: Heart Failure. 2020; 13. doi: 10.1161/CIRCHEARTFAILURE.119.006277.

145. Ye Y, Bajaj M, Yang HC, Perez-Polo JR, Birnbaum Y. SGLT-2 inhibition with dapagliflozin reduces the activation of the Nlrp3/ASC inflammasome and attenuates the development of diabetic cardiomyopathy in mice with type 2 diabetes. further augmentation of the effects with saxagliptin, a DPP4 inhibitor. Cardiovasc Drugs Ther. 2017; 31: 119-32.

146. Chen H, Tran D, Yang HC, Nylander S, Birbaum Y, Ye Y. Dapagliflozin and tacrolimus have additive effects on the attenuation of the activation of the NLRP3 inflammasome and the progression of diabetic cardiomyopathy: an AMPK-mTOR interplay. Cardiovasc Drugs Ther. 2020; 34: 443-61.

147. Isermann B, Birhaus A, Humpert PM, Rudofksy G, Chavakis T, Ritzel R, et al. [AGE-RAGE: a hypothesis or a mechanism?]. Herz. 2004; 29: 504-9.

148. Longato E, Di Camillo B, Sparacino G, Cubian L, Avogaro A, Fadini GP. Cardiovascular outcomes of type 2 diabetic patients treated with SGLT-2 inhibitors versus GLP-1 receptor agonists in real-life. BMJ Open Diabetes Res Care. 2020; 8. doi: 10.1136/bmjdrc-2020-001451