



Sodium–glucose cotransporter inhibitors and kidney fibrosis: review of the current evidence and related mechanisms

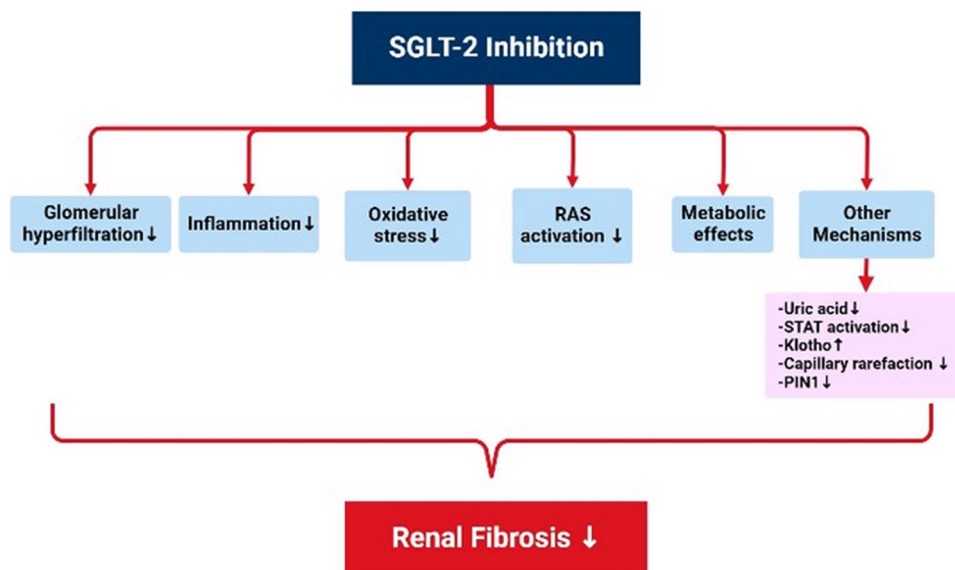
Baris Afsar¹ · Rengin Elsurur Afsar¹

Received: 7 September 2022 / Revised: 9 December 2022 / Accepted: 11 December 2022 / Published online: 19 December 2022
© The Author(s) under exclusive licence to Maj Institute of Pharmacology Polish Academy of Sciences 2022

Abstract

Sodium–glucose cotransporter inhibitors (SGLT2i) are a new class of anti-diabetic drugs that have beneficial cardiovascular and renal effects. These drugs decrease proximal tubular glucose reabsorption and decrease blood glucose levels as a main anti-diabetic action. Furthermore, SGLT2i decreases glomerular hyperfiltration by a tubuloglomerular feedback mechanism. However, the renal benefits of these agents are independent of glucose-lowering and hemodynamic factors, and SGLT2i also impacts the kidney structure including kidney fibrosis. Renal fibrosis is a common pathway and pathological marker of virtually every type of chronic kidney disease (CKD), and amelioration of renal fibrosis is of utmost importance to reduce the progression of CKD. Recent studies have shown that SGLT2i impact many cellular processes including inflammation, hypoxia, oxidative stress, metabolic functions, and renin–angiotensin system (RAS) which all are related with kidney fibrosis. Indeed, most but not all studies showed that renal fibrosis was ameliorated by SGLT2i through the reduction of inflammation, hypoxia, oxidative stress, and RAS activation. In addition, less known effects on SGLT2i on klotho expression, capillary rarefaction, signal transducer and activator of transcription signaling and peptidylprolyl cis/trans isomerase (Pin1) levels may partly explain the anti-fibrotic effects of SGLT2i in kidneys. It is important to remember that some studies have not shown any beneficial effects of SGLT2i on kidney fibrosis. Given this background, in the current review, we have summarized the studies and pathophysiologic aspects of SGL2 inhibition on renal fibrosis in various CKD models and tried to explain the potential reasons for contrasting findings.

Graphical abstract



Extended author information available on the last page of the article

Keywords Canagliflozin · Chronic kidney disease · Dapagliflozin · Empagliflozin · Fibrosis · Luseogliflozin · Sodium–glucose inhibitors

Abbreviations

AGE	Advanced glycation end-products
AKI	Acute kidney injury
α -SMA	Alpha-smooth muscle actin
CKD	Chronic kidney disease
CTGF	Connective tissue growth factor
DKD	Diabetic kidney disease
DM	Diabetes mellitus
EMT	Epithelial-to-mesenchymal transition
EPO	Erythropoietin
FN	Fibronectin
GFR	Glomerular filtration rate
HIF	Hypoxia-inducible factor
IR	Ischemia–reperfusion
MMP	Matrix metalloproteinase
MCP-1	Monocyte chemoattractant protein-1
mTOR	Mammalian target of rapamycin
PDGFB	Platelet-derived growth factor subunit B
PHD	Prolyl hydroxylase domain
ROS	Reactive oxygen species
STAT	Signal transducer and activator of transcription
SGLT2i	Sodium–glucose cotransporter inhibitors
TIMP	Tissue inhibitor of metalloproteinase
TGF- β	Transforming growth factor β
TCA	Tricarboxylic acid
TIF	Tubulointerstitial fibrosis
T2D	Type 2 diabetes
UNx	Unilateral nephrectomy
UUO	Unilateral ureteric obstruction

Introduction

Sodium–glucose cotransporter inhibitors (SGLT2i) are a new class of anti-diabetic drugs. Randomized clinical studies have shown that SGLT2i has cardiovascular and renal protective effects [1–4]. Although the main anti-diabetic action of SGLT2i is to decrease glucose reabsorption in proximal tubules, the pleiotropic actions of SGLT2i are critical for cardio-renal protection. Indeed, studies showed that the beneficial effects of SGLT2i were independent of blood glucose reduction and HbA1c [5, 6]. These drugs are also effective in patients with heart failure (HF) both with reduced ejection [2, 7] fraction and preserved ejection fraction [8]. Besides, SGLT2i improve estimated glomerular filtration rate (eGFR), kidney failure, and mortality independent of albuminuria. Thus, SGLT2i are recommended in patients

with type 2 diabetes and chronic kidney disease (CKD) [1, 9, 10]. Accordingly, the current KDIGO and ADA guidelines revised the threshold for initiation of SGLT2i therapy as eGFR > 20 mL/min/1.73 m² instead of eGFR > 30 mL/min/1.73 m² [11].

In diabetic state, there is increased proximal tubule absorption of glucose and sodium by SGLT2. This leads to a decrease in the luminal sodium chloride concentration, which is sensed by macula densa via the tubule glomerular feedback (TGF) mechanism, leading to dilatation of the renal afferent arteriole and glomerular hyperfiltration [12]. Experimental studies in diabetic rats demonstrated that SGLT2i reduced glomerular capillary pressure through TGF [13]. It is important to note that glomerular hyperfiltration is not unique to diabetic kidney disease (DKD), but occurs as a compensatory mechanism (increased single nephron GFR) in all progressive renal diseases. Although this initial compensation maintains the total GFR constant, the long-term consequences of mechanical stress induced by increased GFR are detrimental. High single nephron GFR damages the glomerular filtration barrier causing glomerulosclerosis and proteinuria, and eventually resulting in loss of remaining nephrons and kidney fibrosis [14–16]. Accordingly, the reno-protective mechanisms of SGLT2i is not unique to DKD. Indeed, recent clinical trials have shown the reno-protective effect of SGLT2i in non-diabetic kidney disease [9, 17]. Apart from these functional changes, SGLT2i-induced favorable renal structural changes, which have not been fully elucidated yet, might explain long-term effects of these agents [4]. Regardless of the cause, renal fibrosis is a common pathway and pathological marker of virtually every type of CKD [18]. Recent preclinical (Table 1) and clinical (Table 2) studies have shown that SGLT2i might modify kidney fibrosis by a variety of mechanisms. Given this background, in the current review, we have summarized the studies regarding the impact of SGLT2i on renal fibrosis in various CKD models.

Mechanisms of kidney fibrosis influenced by sodium–glucose cotransporter inhibition

Hypoxia-inducible factor, sodium–glucose cotransporter inhibition, and kidney fibrosis

As well known, hypoxia is a driver of tubulointerstitial fibrosis [19, 20]. Decreased hypoxia-inducible factor (HIF)

Table 1 Preclinical studies specifically summarizing the impact of sodium–glucose cotransporter inhibitors on kidney fibrotic markers and kidney fibrosis

References	Methods	Renal fibrosis related findings
[109]	Male T2DN rats divided to Group 1: Vehicle Group 2: LUSE (10 mg/kg/day) Group 3: Lisinopril (10 mg/kg/day) Group 4: LUSE (10 mg/kg/day) + Lisinopril (10 mg/kg/day) Group 5: Insulin Tx. were given for 3 months MT stain	Glomerulosclerosis, renal fibrosis, tubular necrosis, protein casts increased in Group 1 and decreased in Group 2. Insulin has no effect on these parameters Lisinopril reduced glomerular injury and protein casts but had no effect on renal fibrosis In Group 4 (combination), glomerular injury, renal fibrosis, and the formation of protein casts decreased more than in Group 2 and Group 3
[105]	Akita mice and WT controls were divided as (I): WT (II): WT + EMPA (300 mg/kg) of for 15 weeks (III): Type 1 diabetic Akita (IV): Type 1 diabetic Akita + EMPA (300 mg/kg) for 15 weeks PSR stain for renal collagen content In vivo and in vitro study For in vivo study, four groups of mice were used (Group 1): Control non-diabetic (db/m) (Group 2): Diabetic (db/db) (Group 3): db/db + DAPA (0.1 mg/kg/d by gavage for 12 weeks) (Group 4): db/db + DAPA (1 mg/kg/d by gavage for 12 weeks) For in vitro experiments PTECs used PAM and type 4 collagen staining used	EMPA decreased glomerular hyperfiltration and urinary albumin/creatinine ratio without affecting TGF- β and collagen expression Interstitial fibrosis higher in Group 2 versus Group 1 Interstitial fibrosis lower in Group 4 versus Group 2 DAPA decreased the expression of TGF- β and Type 4 collagen staining and which is increased in db/db mice
[106]	Male Enos knockout mice on a C57BL/6 background divided as (I): Control: citrate buffer injections (II): Control + EMPA (10 mg/kg/d) (III): Diabetic: Induced by STZ injections (55 mg/kg daily for 5 days) (IV): Diabetic + EMPA (10 mg/kg/d) (V): Diabetic + Telmisartan (3 mg/kg) EMPA and telmisartan: between 13 and 32 weeks MT, SR, and PAS staining Immunohistochemistry for FN and toll-like receptor 2	Significant glomerulosclerosis, tubular atrophy and TIF in Groups III and IV versus Groups 1 and 2 and 5 Telmisartan but not EMPA reduced glomerular FN deposition, glomerulosclerotic index, tubular atrophy, and TIF in diabetic mice MCP-1, FN and TGF- β transcriptions increased in Groups III and IV (not influenced by EMPA) In Group V, MCP-1 and FN transcriptions similar but TGF- β transcription was increased versus Group I

Table 1 (continued)

References	Methods	Renal fibrosis related findings
[110]	Diabetic male Dahl S rats (induced injection of 50 mg/kg, i.v. STZ) divided as Group 1: Vehicle control Group 2: LUSE (10 mg/kg/d) Group 3: Lisinopril (20 mg/kg) Group 4: LUSE (10 mg/kg/d) + Lisinopril (20 mg/kg) Group 5: Insulin MT staining	In vehicle diabetic rats, focal glomerulosclerosis, renal interstitial fibrosis, and tubular necrosis were observed Glomerular injury, outer medullary fibrosis, and tubular necrosis decreased more in Group 4 (combination therapy) versus Group 2 and 3 Insulin reduced glomerular injury and outer medullary fibrosis but it did not prevent tubular necrosis
[47]	Male DM Akita mice and non-DM C57BL/6 mice divided as (I): Control C57BL/6 mice (pure water for 12 weeks) (II): Control diabetic Akita mice (pure water for 12 weeks) (III): Diabetic Akita mice receiving DAPA (1.0 mg/kg/d per day) by gavage for 12 weeks (IV) Diabetic Akita mice receiving titrated doses of Insulin glargine (10–20 unit/kg/d per day) sc for 12 weeks PAS and PSR staining Renal expression of type IV collagen assessed	Interstitial fibrosis and type IV collagen expression was more severe in the Akita mice versus C57BL/6 mice Interstitial fibrosis and type IV collagen expression suppressed by both with DAPA and insulin but more with DAPA versus insulin
[104]	Sprague–Dawley rats undergo subtotal (5/6) nephrectomy (SNX) or sham surgery 1 week after SNX or sham randomized to receive DAPA (0.5 mg/kg), twice/day or vehicle Glomerular sclerosis was graded subjectively on a scale of 0–4 (Grade 0: normal Grade 4: Sclerotic area 75–100%) MT stain	Interstitial fibrosis, glomerulosclerosis, TGF- β 1, and type IV collagen expressions increased by SNX after 12 weeks' post-surgery which was unaffected by DAPA DAPA did not attenuate glomerulosclerosis, TGF- β 1, and type IV collagen expressions
[86]	Six groups of mice Group 1: Non-diabetic (db/m) vehicle Group 2: db/m + EMPA (10 mg/kg/day) Group 3: Diabetic (db/db) vehicle Group 4: db/db + EMPA (10 mg/kg/day) Group 5: db/db + metformin (250 mg/kg/day) Group 6: db/db + EMPA (mg/kg/d) + metformin (250 mg/kg/day) Vehicle or treatments performed by single daily oral gavage for 10 weeks MT, SR staining	Collagen I expression is increased with DAPA versus vehicle Collagen accumulation in cortical/outer medullary regions increased in all db/db mice versus db/m by MT but not by SR staining EMPA + metformin tended to reduce MT staining positivity and significantly decreased SR staining with no difference in FN staining EMPA decreased expression of TGF- β 1 but not collagen IV α and CTGF EMPA + metformin decreased expression of TGF- β 1 and collagen V α but not CTGF

Table 1 (continued)

References	Methods	Renal fibrosis related findings
[84]	<p>Long-Evans Tokushima Otsuka (LETO) rats and Otsuka Long-Evans Tokushima Fatty (OLETF) divided as</p> <p>Group 1: Non-diabetic LETO group (received saline)</p> <p>Group 2: Diabetic OLETF rats (received saline)</p> <p>Group 3: Diabetic OLETF rats received DAPA (1.0 mg/kg)</p> <p>Group 4: Diabetic OLETF rats received voglibose (0.6 mg/kg) (an alpha glucose inhibitor)</p> <p>Tx. duration was 12 weeks</p> <p>MT and PAS staining</p> <p>C57BL/6 N mice divided as</p> <p>Group 1: Control</p> <p>Group 2: Oxalate Induced CKD (50 μmoles/g for 7 days) + hydroxy ethyl cellulose (HEC) (Vehicle)</p> <p>Group 3: Oxalate Induced CKD (50 μmoles/g for 7 days) + EMPA (10 mg/kg for 7 days) (dissolved in HEC and administered by oral gavage)</p> <p>Group 4: Oxalate induced CKD (50 μmoles/g for 14 days) + hydroxy ethyl cellulose (HEC) (Vehicle)</p> <p>Group 5: Oxalate induced CKD (50 μmoles/g for 14 days) + EMPA 10 mg/kg (dissolved in HEC and administered by oral gavage for 14 days)</p> <p>PAS, silver, α-SMA and collagen 1 stain performed</p> <p>Four groups of mice with UNx</p> <p>Group 1: Non-diabetic mice (db/db)</p> <p>Group 2: Diabetic mice (db/db) at 18 weeks of age</p> <p>Group 3: db/db mice at 22 weeks of age</p> <p>Group 4: db/db mice + DAPA (1 mg/kg/d) between 4 weeks. From 18 to 22 weeks</p> <p>PAS staining</p>	<p>Mesangial widening, TIF, and total collagen content and Type IV collagen expression were increased in Group 2 but attenuated in Group 4 and more in Group 3</p> <p>EMPA did not affect interstitial fibrosis markers silver, α-SMA and collagen 1, and mRNA levels of FN-1, collagen IAI, fibroblast-specific protein-1, and TGF-β2 on days 7 and 14</p> <p>Mesangial expansion higher in db/db versus db/m</p> <p>Not meaningful tubulointerstitial injury in db/db mice (data not shown)</p> <p>In Group 2, renal TGF-β1, FN mRNA levels increased at 18 weeks, and further at 22 weeks, and a1(IV) collagen were increased only at 22 weeks of age versus Group 1</p> <p>DAPA tx. in Group IV reduced renal TGF-β1, FN, and collagen a1 mRNA expression to below week 18 values (normal levels)</p> <p>LUSE tx. decreased renal interstitial fibrosis at week 4 post-IR compared to vehicle</p> <p>Increased TGF-β1 expression in vehicle-treated IR group was suppressed by LUSE</p>
[102]		
[44]		
[98]	<p>Male C57BL/6 J mice were performed unilateral renal IR injury to AKI</p> <p>LUSE or its vehicle, carboxymethyl cellulose (0.1%), was administered 6 h after reperfusion (<i>po</i> 30 mg/kg/d) and then daily until day 7</p> <p>SR staining</p>	

Table 1 (continued)

References	Methods	Renal fibrosis related findings
[50]	<p>Five groups of Male Wistar rats</p> <p>Group 1: Control</p> <p>Group 2: UUO without treatment</p> <p>Group 3: Prophyllactic EMPA (10 mg/kg/d/po) before UUO</p> <p>Group 4: Immediate EMPA (10 mg/kg/d/po) after UUO</p> <p>Group 5: Delayed EMPA 10 mg/kg/d/po (received distilled water 1 week after UUO then empagliflozin for 2 weeks)</p>	<p>TGF-β1, FN levels and CTGF, α-SMA expressions increased in Group 2 versus Group 1 and significantly decreased in Groups 3 and 4 equally</p> <p>Interstitial fibrosis and cystic dilatation increased in Group 2 but not in Group 3</p> <p>Cystic dilatation, tubular necrosis, and inflammation was minimal and no fibrosis observed in Group 4. These changes were moderate in Group 5 including moderate fibrosis</p>
[111]	<p>H&E</p> <p>Male Wistar rats divided as</p> <p>Group 1: Received the sodium citrate buffer alone (Non-diabetic control)</p> <p>Group 2: Diabetic controls [DM induced in intraperitoneal injection of nicotinamide (230 mg/kg), 15 min prior to the injection of STZ (65 mg/kg, <i>ip</i>)]</p> <p>Group 3: Diabetic rats + DAPA (2 mg/kg/day)</p> <p>Group 4: Diabetic rats + irbesartan 30 mg/kg/day</p> <p>Group 5: Diabetic rats + DAPA (2 mg/kg/day) + irbesartan 30 mg/kg/day</p>	<p>Interstitial fibrosis and tubular atrophy increased in Group 2</p> <p>TGF-β1 elevated in Group 2 decreased in the same magnitude in Groups 3 and 4 and more in Group 5</p>
[112]	<p>Male wistar rats were divided as</p> <p>Group 1: Control</p> <p>Group 2: High fat diet (HFD)</p> <p>Group 3: HFD + DAPA (1 mg/kg/d) for 4 weeks</p> <p>Group 4: HFD + metformin (30 mg/kg/d) for 4 weeks</p>	<p>TGF-β1, Col IV expressions and TIF higher in Group 2 versus Group 1</p> <p>In HFD mice, DAPA or metformin decreased renal TGF-β1, collagen IV expressions, and TIF in the same magnitude</p>
[113]	<p>MT staining</p> <p>Male C57BL/6 (normal) and KK/Ay type 2 diabetic mice were divided into</p> <p>Group 1: Normal mice</p> <p>Group 2: Non-Nx, diabetic mice</p> <p>Nx diabetic mice which further divided into six</p> <p>(I) vehicle</p> <p>(II) IPRA (0.1 mg/kg)</p> <p>(III) IPRA (0.3 mg/kg)</p> <p>(IV) IPRA (1 mg/kg)</p> <p>(V) IPRA (3 mg/kg)</p> <p>(VI) losartan (30 mg/kg) for 4 weeks</p>	<p>Interstitial fibrosis occurs as an increased order in non-Nx DM mice and uni-Nx DM mice compared to normal mice</p> <p>In mice tx with IPRA (1 and 3 mg/kg) and losartan showed less interstitial fibrosis compared to uni-Nx DM mice</p>
	H&E, PAS staining	

Table 1 (continued)

References	Methods	Renal fibrosis related findings
[114]	<p>C57BL/6 J mouse were divided as</p> <p>Group 1: Low-fat diet (LF) (10 kcal % fat)</p> <p>Group 2: LF + DAPA (5 mg/kg/d for 26 weeks)</p> <p>Group 3: Western diet (WD) (42% milkfat, 34% sucrose, and 0.2% cholesterol)</p> <p>Group 4: WD + DAPA (5 mg/kg/d for 26 weeks)</p> <p>H&E, PSR stain</p>	<p>Collagen IV and FN accumulated more in the tubular interstitium in Group 3 versus Group 1 and 2 and attenuated by DAPA in Group 4 (less collagen and FN accumulation in Group 4 vs. Group 3)</p>
[115]	<p>Wistar rats divided into six, and treated for 35 consecutive days</p> <p>Group 1: Chow diet (Control)</p> <p>Group 2: Adenine 0.25% w/w</p> <p>Group 3: CANA (10 mg/kg/day po)</p> <p>Group 4: Adenine 0.25% w/w + CANA (10 mg/kg/day po)</p> <p>Group 5: CANA (25 mg/kg/d, po)</p> <p>Group 6: adenine 0.25% w/w + CANA (25 mg/kg/day po.)</p> <p>H&E and SR staining</p>	<p>In Group 2, significant acute tubular injury (in $61.4 \pm 4.8\%$ of examined tissue areas, score 3) and significant interstitial fibrosis ($32 \pm 5.4\%$, score 2) observed</p> <p>In Group 3, acute tubular injury ($33.7 \pm 5.4\%$, score 2) and interstitial fibrosis ($15.2 \pm 3.7\%$, score 1) were less</p> <p>In Group 6, acute tubular injury ($31.3 \pm 3.6\%$, score 2) and interstitial fibrosis ($17.3 \pm 3.4\%$, score 1) were similar as in Group 3</p>
[28]	<p>db/m mice (Mice on a C57BLKs/J background divided into three)</p> <p>(I) Non-diabetic (db/m) mice</p> <p>(II) Diabetic db/db (Lepr^{db/db}) mice</p> <p>(III) Diabetic db/db mice + LUSE (0.01% in chow; 15 mg/kg body weight/day) for 8 weeks</p> <p>HRPTECs used for in vitro studies</p> <p>PAS and PSR staining</p>	<p>LUSE decreased FN immunostaining in the kidneys of db/db mice</p> <p>LUSE decreased tubulointerstitial injury but did not attenuate glomerular sclerosis in diabetic mice</p>
[66]	<p>Type 2 diabetes induced in C57BL/6 J mice by injecting nicotinamide (120 mg/kg) and streptozotocin (100 mg/kg), by <i>ip</i> injection</p> <p>After 1 week, treated mice divided into three groups</p> <p>(Group 1): Control mice fed with normal diet</p> <p>(Group 2): Mice fed a high sucrose diet (HSD) forming (NA/STZ/Suc mice)</p> <p>(Group 3) NA/STZ/Suc mice + CANA mixed, at 0.03% (w/w) for 4 weeks</p> <p>Four groups of mice</p> <p>(i) Non-diabetic control</p> <p>(ii): Non-diabetic + CANA (10 mg/kg/day for 5 days)</p> <p>(iii) Diabetic mice (type 2 DM induced in New Zealand obese mice DM with a high-fat diet (30% fat)</p> <p>(iv): Diabetic mice + CANA (10 mg/kg/day for 5 days)</p> <p>PAS, MT staining</p>	<p>Collagen I A1 A2, CTGF, TGF-β expressions increased in Group 2 but normalized by CANA in Group 3 except TGF-β</p> <p>CANA reduced kidney fibrosis associated with decreased Pin 1 which is associated with fibrosis and inflammation</p> <p>Tubular fibrosis in DM mice (3.4 ± 0.9-fold) was suppressed by CANA (0.9 ± 0.3-fold)</p> <p>Mesangial expansion was not different among groups</p>
[56]		

Table 1 (continued)

References	Methods	Renal fibrosis related findings
[116]	DM was induced Male CD-1 mice by <i>ip</i> STZ injection (40 mg/kg) then divided as (i): DM mice + vehicle (ii): DM mice + losartan (10 mg/kg/d) (iii): DM mice + DAPA (1 mg/kg/d) (iv): DM mice + DAPA (5 mg/kg/d) H&E, PAS, MT, PSR staining Sprague–Dawley rats were divided as (I): Control (II): Control + EMPA (10 mg/kg) (III): Ang II (200 ng/kg/min), osmotic minipumps, <i>sc</i> (IV): Ang II + EMPA (10 mg/kg) After 2 weeks, TIF, Type I and IV collagen expression was analyzed PSR staining	Mesangial expansion and mesangial matrix hyperplasia occurred in diabetic mice but improved by DAPA and losartan TIF and macrophage infiltration in diabetic mice were only reduced by DAPA but not by losartan Ang II increased TIF, type I and type IV collagen expressions compared to control EMPA reduced TIF, and type I and IV collagen expression in Ang II-treated rats by reduction of inflammatory infiltrates
[55]	HK-2 cells incubated with (I): 25 mM D-glucose (high glucose) (II): L-glucose (L-glucose 20 mM + D-glucose 5.6 mM) (control) (III): Mannitol (mannitol, 20 mM + D-glucose, 5.6 mM) (osmolar control) (IV): EMPA at 0.5 μ M in dimethyl sulfoxide for 15 min prior to HG addition (25 mM for 15 min Epithelial (E-cadherin), and mesenchymal markers (FN, α -SMA and vimentin) are shown by immunoblotting Long-Evans rats divided as Group 1 (Control): Only received standard chow for 2 weeks Group 2: Chow diet for first week and then CANA at 5 mg/kg daily with chow diet for the second week Group 3: Chow diet + <i>sc</i> isoprenaline (ISO) at 50 mg/kg twice a week for the first week to induce oxidative stress Group 4: ISO (in the first week) + CANA 2nd the second week (same doses of ISO and CANA as above) H&E, PSR staining	High glucose suppressed E-cadherin expression, but increased expression of FN, α -SMA and vimentin EMPA restored E-cadherin expression, and suppressed HG-induced FN, α -SMA and vimentin suggesting inhibition of EMT Control and CANA animals maintain normal histology ISO tx. increased inflammatory cell infiltration and necrotic changes Group 1 and 2 rats showed normal collagen distribution and alignments, indicating an absence of fibrosis Excessive collagen deposition and fibrosis in ISO-treated rats was reduced upon CANA tx
[118]	Long-Evans rats divided as Group 1 (Control): Only received standard chow for 2 weeks Group 2: Chow diet for first week and then CANA at 5 mg/kg daily with chow diet for the second week Group 3: Chow diet + <i>sc</i> isoprenaline (ISO) at 50 mg/kg twice a week for the first week to induce oxidative stress Group 4: ISO (in the first week) + CANA 2nd the second week (same doses of ISO and CANA as above) H&E, PSR staining	Control and CANA animals maintain normal histology ISO tx. increased inflammatory cell infiltration and necrotic changes Group 1 and 2 rats showed normal collagen distribution and alignments, indicating an absence of fibrosis Excessive collagen deposition and fibrosis in ISO-treated rats was reduced upon CANA tx

Table 1 (continued)

References	Methods	Renal fibrosis related findings
[25]	<p>Wistar rats divided as</p> <p>Group 1: Control</p> <p>Group 2: DM (ip injection 65 mg/kg body weight STZ)</p> <p>Group 3: DM+DAPA (1 mg/kg/d for 6 weeks)</p> <p>Group 4: DAPA (1 mg/kg/d 6 weeks) + losartan 20 mg/kg/day for last 3 weeks</p>	<p>DM induced collagen type 3 formation and MMP-9 mediated degradation of collagen type III and collagen type IV fragment formation and fibrosis which are decreased by DAPA</p> <p>Renal TGF-β1 PDGFB, CTGF, α-SMA mRNA expressions were increased in DM and decreased by DAPA except TGF-β1</p> <p>Combination DAPA + losartan had no synergistic effect in any of the observed parameters</p> <p>Renal FN gene expression decreased by 50% in DAPA-treated rats The DAPA + losartan combination had no additional effect on the decrement of FN accumulation</p>
[119]	<p>Collagen type III formation and collagen type IV fragments were measured in urinary samples</p> <p>MT and PRS staining</p> <p>Renal IR injury created in Sglt2-deficient (Sglt2^{-/-}) mice and WT C57BL/6 J mice</p> <p>Tubular injury scored as 0 (no tubular injury) to 3 (50% of tubules injured)</p>	<p>IR induced cortical and medullary tubular damage, cast formation, brush border loss, and immune cell infiltration and were similar between Sglt2^{-/-} and WT mice</p> <p>Renal mRNA expression of CC-chemokine ligand TGF-β1, and COL1A1 in same in Sglt2^{-/-} and WT mice</p> <p>Capillary rarefaction induced by IR and CD31 (a marker of endothelial cells) did not differ between Sglt2^{-/-} and WT mice</p> <p>High glucose increased expression of collagen IV, FN, TGF-β1 and α-SMA</p> <p>EMPA decreased expression of TGF-β1, and accumulation of FN collagen IV, α-SMA in dose-dependent manner</p>
[27]	<p>In vitro study using HK-2 cells</p> <p>Cells were cultured in the medium containing either 5.5 mM (normal glucose) or 25 mM glucose (high glucose) with or without EMPA (50 nM, 100 nM, 500 nM) for 72 h</p>	<p>Both low dose (0.003) and high (0.1) dose IPRA decreased tubular dilatation, crystal deposition which occur in adenine induced CKD with no statistical difference between low and high doses</p>
[120]	<p>C57BL mice divided as</p> <p>(I): Normal (received normal diet)</p> <p>(II): CKD [induced by 0.25% w/w adenine containing diet (no treatment)]</p> <p>(III): CKD + IPRA (0.03 mg/kg/d)</p> <p>(IV): CKD + IPRA (0.1 mg/kg/day)</p> <p>IPRA was orally administered to CKD mice for 4 weeks, concomitantly with adenine diet</p>	<p>Diabetic mice showed aberrant glycolysis [hexokinase 2 induction, and pyruvate kinase isozyme M2 dimer formation, increased fructose-6-phosphate, glucose-6-phosphate (G6P), and phosphofruktokinase] and decreased Sirt3 levels associated with EMT and kidney fibrosis</p>
[26]	<p>H&E, PAS, MT staining</p> <p>In vivo and in vitro study</p> <p>In vivo study: diabetic male CD-1 mice used (induced by intraperitoneal injections of STZ)</p> <p>In vitro study HK-2 cells were used</p> <p>EMPA (0.2, 1, or 5 mg/kg BW/d), CANA (30 mg/kg BW/d) were used for experiments</p> <p>SRS, PAS staining</p> <p>EMT were detected by double-positive labeling for E-cadherin and SMA or E-cadherin and vimentin</p>	<p>EMPA and CANA normalized these changes</p> <p>Smooth muscle protein 22α (SM22α), α-SMA, vimentin, E-cadherin expression is increased in diabetic mice and these changes are normalized by EMPA and CANA</p>

Table 1 (continued)

References	Methods	Renal fibrosis related findings
[121]	<p>Sprague–Dawley rats divided in to 4</p> <p>(I): Control</p> <p>(II): Control + EMPA (10 mg/kg/day)</p> <p>(III): Cyclosporine-A (CsA) nephropathy (CAN) (15 mg/kg/day, <i>ip</i>)</p> <p>(IV): CAN + EMPA (10 mg/kg/day)</p> <p>Cyclosporine and EMPA tx. started simultaneously for 4 weeks</p> <p>SRS and immunohistochemical evaluation of monocytes/macrophages), type I and type IV collagen expression</p>	<p>CsA increased glomerular fibrosis and TIF, renal inflammatory infiltrates type I and type IV collagen expression which all were decreased by EMPA</p>
[103]	<p>C57BL/6 J mice used in 2 models which used with 4 subgroups each</p> <p>Model 1</p> <p>(I): Sham</p> <p>(II): Placebo (UUO + vehicle)</p> <p>(III): UUO + EMPA (10–30 mg/kg/day)</p> <p>(IV): UUO + finerenone (3–10 mg/kg/day)</p> <p>Model 2</p> <p>(I): Sham</p> <p>(II): Placebo (Sub-Chronic IR Injury (sc/RI) + vehicle)</p> <p>(III): sc/RI mice + EMPA (10–30 mg/kg/d)</p> <p>(IV): sc/RI mice + finerenone (3–10 mg/kg/day)</p> <p>In both models</p> <p>EMPA and finerenone started 2 days before UUO or sc/RI</p> <p>Interstitial myofibroblast accumulation quantified via alpha-smooth muscle actin and interstitial collagen deposition via SR/Fast Green staining in both models</p>	<p>In UUO models, 11-fold increase in interstitial myofibroblast accumulation and sevenfold enhanced interstitial collagen fiber deposition compared to sham</p> <p>Finerenone 10 mg/kg but not EMPA reduced myofibroblast accumulation and collagen fiber deposition in UUO</p> <p>In UUO model, finerenone reduced the mRNA expression of αSMA and collagen types I, III, and IV but not</p> <p>CTGF and TGF-β. EMPA had no significant effect on the expression on these markers</p> <p>sc/RI mice showed an eightfold increase in interstitial myofibroblast accumulation and fourfold elevated interstitial collagen content as compared to sham</p> <p>Finerenone significantly decreased α-SMA and collagen deposition while EMPA only slightly reduced myofibroblast numbers and collagen deposition in sc/RI mice</p>
[122]	<p>Cardio-renal morbidity and mortality was studied in hypertensive and proteinuric renin-transgenic (mRen2)27 rats</p> <p>All animals in the study were initially placed on a diet with the captopril (300 mg/kg in food) from age 4–5 weeks then divided as</p> <p>(I): Control (continue to receive captopril only)</p>	<p>Group 2 rats developed increased cardiac and renal fibrosis and renal collagen Ia1 expression versus Group 1</p> <p>Higher doses finerenone and EMPA were protective for renal fibrosis compared to lower doses</p> <p>Cardio-renal fibrosis attenuated more in Group VII versus Group V and VI</p>

Table 1 (continued)

References	Methods	Renal fibrosis related findings
[46]	<p>(II): Placebo + Vehicle (10% EtOH, 40% Solutol, 50% water) + L-NAME (20 mg/L) at 8–9 weeks after washout period for 2 weeks and treated for up to 7 weeks</p> <p>(III): Finerenone 1 mg/kg/d in vehicle</p> <p>(IV): Finerenone 3 mg/kg/d in vehicle</p> <p>(V): EMPA 3 mg/kg/d in vehicle</p> <p>(VI): EMPA 10 mg/kg/d in vehicle</p> <p>(VII): Finerenone mg/kg/d + EMPA 3 mg/kg/d dissolved in vehicle</p> <p>Vehicle and dissolved medications begin at 8–9 weeks after washout of captopril continued for up to 7 weeks</p> <p>SR and Fast Green staining</p> <p>Male C57BL/6 J WT mice received</p> <p>(I): Standard diet (control)</p> <p>(II): HFD (High fat diet) (524 kcal/100 g, 60% energy as fat) for 20 weeks' vehicle (for the last 6 weeks synchronously with HFD)</p> <p>(III): HFD + IPRA (1 mg/kg/day) (for the last 6 weeks synchronously with HFD)</p> <p>SRS</p> <p>In vitro study using HRPTECs</p> <p>Cells were exposed to high D-glucose and TGF-β1 and DAPA was administered at 0.1, 1, or 10 nM</p> <p>C57BL/6 J mice divided as</p> <p>(I): Sham</p> <p>(II): UNx</p> <p>(III): UNx + high salt diet (4%)</p> <p>(IV): UNx + high salt diet (4%) + DOCA (60 days-release pellet (50–150 mg)</p> <p>EMPA (30 mg/kg/d) started 3 days before UNx via drinking water</p> <p>MT staining</p> <p>DM was induced in male Sprague–Dawley was divided as</p> <p>(I): Non-diabetic</p> <p>(II): Diabetic ice (by single <i>ip</i> injection (35 mg/kg) STZ) + saline</p> <p>(III): Diabetic mice + metformin (300 mg/kg/day, <i>po.</i>)</p> <p>(IV): Diabetic mice + Ursolic acid (50 mg/kg/day, <i>po.</i>)</p> <p>(V): Diabetic mice + EMPA (10 mg/kg/day, <i>po.</i>)</p> <p>(VI): Diabetic mice + Ursolic acid (50 mg/kg/d) + EMPA (10 mg/kg/d)</p> <p>H&E staining</p>	<p>Renal collagen Iα1 expression was decreased in same magnitude (no difference in intervention groups) in all finerenone, EMPA and combination groups compared to placebo</p> <p>COL1A2, COL6A3, and TGF-β1 mRNA expressions increased in HFD fed mice versus controls</p> <p>IPRA decreased COL1A2 and TGF-β expressions</p> <p>Fibrosis increased in HFD compared to SD in renal cortex</p> <p>IPRA decreased fibrotic area in renal cortex</p> <p>Combination of D-glucose and TGF-β1 increased CTGF2 mRNA which are decreased by DAPA 1 or 10 nM</p> <p>DAPA decreased ERK and Smad3 phosphorylation which are downstream mediators of TGF-β</p> <p>EMPA did not affect the level of fibrosis in UNx/DOCA/salt mice</p> <p>EMPA did not alter mRNA expression of FN, α-SMA and COL1A1</p> <p>In diabetic rats, TIMP, α-SMA, collagen IV, FN mRNA expressions increased and MMP-9 mRNA expression decreased</p> <p>DM increased P38MAPK a mediator of TGF-β1 signaling which reduced by EMPA</p> <p>TGF-β1 and Smad3 were activated and the expression of Smad7 was downregulated in the kidneys of DN rats EMPA reversed these changes</p>
[123]		
[108]		
[42]		

Table 1 (continued)

References	Methods	Renal fibrosis related findings
[40]	Male C57BL/6 J mice divided as (I): sham + vehicle (II): IR + vehicle (III): IR + DAPA (1 mg/kg/d by oral gavage from 1 week after reperfusion and until 6 weeks (total 5 weeks) PTECs used for in vitro studies H&E, PAS, MT, SRS used Total collagen content was expressed as micrograms per milligram of total protein Sprague Dawley rats with LSD (0.05% Sodium) divided as (I): Vehicle (olive oil, 0.3 mL, <i>sc.</i> , 3 weeks) (II): DM (induced by tacrolimus (1.5 mg/kg, <i>sc.</i> , 3 weeks) (III): Tacrolimus induced DM + EMPA (10 mg/kg by oral gavage for 3 weeks (IV): Tacrolimus induced DM + Gemigliptin (a DDP4i, 20 mg/kg by oral gavage, for 3 weeks) (V): Tacrolimus induced DM + EMPA (10 mg/kg) + Gemigliptin (20 mg/kg by oral gavage, for 3 weeks) TIF assessed by trichrome-stained tissue sections Male Wistar rats divided to four (I): Sham + Placebo (II): 5/6Nx + HSD (2%) + placebo (III): 5/6Nx + HSD + Telmisartan (5 mg/kg/day, qd) (IV): 5/6Nx + HSD + Empagliflozin (0.6 mg/kg/day, bid) SRS and collagen I immunostaining	TIF increased in the IR group compared with the sham group and DAPA tended to reverse these changes IR induced damage and depletion of mitochondria in proximal tubular cells were markedly relieved by DAPA Tacrolimus increased α -SAM and TGF- β 1 induced protein (β -igh3) expression versus vehicle EMPA or Gemigliptin independently decreased α -SAM and β -igh3 expression but more prominent in the EMPA + Gemigliptin than either tx. Alone Tacrolimus increased TIF score compared to vehicle (12.1 ± 1.7 vs. $0 \pm 0\%$) Both EMPA and Gemigliptin decrease TIF score independently 4.9 ± 1.2 for EMPA 4.5 ± 0.6 for Gemigliptin) EMPA and Gemigliptin in Combination decreased TIF score more prominently (1.9 ± 0.4) 5/6 Nx increased renal expression of collagen 1 and renal fibrosis Telmisartan and EMPA similarly decreased renal collagen 1 expression
[124]		
[107]		

AKI, acute kidney injury; α -SMA, alpha-smooth muscle actin; CKD, chronic kidney disease; CTGF, connective tissue growth factor; DAPA, dapagliflozin; EMPA, empagliflozin; Enos, endothelial nitric oxide synthase; EMT, epithelial-to-mesenchymal transition; FN, fibronectin; H&E, hematoxylin and eosin; HK-2, human kidney proximal tubule cell lines; HRPTECs, human renal proximal tubular epithelial cells; IR, ischemia–reperfusion; LSD, low sodium diet; LUSE, luseogliflozin; MT, Masson's trichrome; MMP, matrix metalloproteinase; MAPK, mitogen-activated protein kinase; MCP-1, monocyte chemoattractant protein-1; Nx, nephrectomized; PAM, periodic acid-methenamine silver; PAS, periodic acid Schiff; PSR, Picrosirius red; PDGFB, platelet-derived growth factor subunit B; PTC, proximal tubular cells; PTEC, proximal tubular epithelial cells; SR, Sirius red; STZ, streptozotocin; TIMP, tissue inhibitor of metalloproteinase; TGF- β , transforming growth factor β ; Tx, treatment; TIF, tubulointerstitial fibrosis; T2D, type 2 diabetes; UNX, unilateral nephrectomy; UUU, unilateral ureteric obstruction; WT, wild type

Table 2 Preclinical and clinical studies (Mixed) specifically summarizing the impact of sodium–glucose co-transporter inhibitors on kidney fibrotic markers and kidney fibrosis

References	Methods	Renal fibrosis related findings
[43]	<p>Clinical and experimental study</p> <p>Clinical part: Kidney sections from human subjects with diabetic nephropathy</p> <p>Experimental part</p> <p>Group 1: Non-diabetic (db/m) mice (control)</p> <p>Group 2: Diabetic mice (db/db)</p> <p>Group 3: db/db + SGLT2i (JNJ- 39,933,673) at a concentration of 0.07 g/kg of diet</p> <p>PAS staining</p> <p>In vivo part</p> <p>Male C57/BL6 mice divided into</p> <p>Group 1: Non-diabetic control</p> <p>Group 2: Type 1 diabetic mice (induced by injecting 130 STZ <i>ip</i>) received saline for 8 weeks</p> <p>Group 3: Diabetic mice received saline for 12 weeks</p> <p>Group 4: Diabetic mice received saline for 16 weeks</p> <p>Group 5: Diabetic mice received DAPA (10 mg/kg/d) for 16 weeks</p>	<p>SGLT2 mRNA increased in kidney biopsies obtained from human subjects with diabetic nephropathy</p> <p>Extracellular matrix accumulation in Group 2 was prevented by SGLT2 inhibition in Group 3 as determined by FN, type IV collagen and fibrillary collagen accumulation</p> <p>In human biopsies, STAT1 TGF-β1, collagen IV and FN expressions and TIF increased in DN patients</p> <p>Diabetic mice showed increased STAT1/TGF-β1 signaling, collagen and FN expression, tubular atrophy, extracellular matrix accumulation</p> <p>TIF was higher compared to non-diabetic control and these changes increased as going from Group 2 to Group 4</p> <p>DAPA in Group 5 decreased STAT1/TGF-β1 signaling and TIF, collagen and fibronectin expressions</p> <p>In HK-2 cells, high glucose decreased expression of the epithelial marker E-cadherin and increased levels of α-SMA which were reversed by DAPA in a dose-dependent manner</p> <p>DAPA decreased STAT-1 activation in HK-2 cells associated with decreased HK-2 cells</p> <p>In Ang II incubated HK-2 cells SGLT2 Mrna expression increased in a concentration-dependent manner but losartan abolished this effect</p> <p>In mice, Ang II infusion evoked mesangial expansion, glomerulosclerosis TIF, FN-1 mRNA expression and these changes were attenuated by CANA</p>
[85]	<p>In vitro part: HK-2 cells used</p> <p><i>Clinical part</i>: Kidney biopsies from patients diagnosed with class III or IV DN and non-diabetic adjacent normal tissues from patients with renal cell carcinoma</p> <p>H&E, PAS, PSR, and MT staining</p> <p>Cell culture study: HK-2 cells incubated with Ang II (10^{-7} mol/L), with and without losartan (10^{-6} mol/L)</p> <p>Animal Study: WT mice (C57BL/6 background) divided as</p> <p>(I): No AngII infusion + water</p> <p>(II): No AngII infusion + CANA</p> <p>(III): AngII infusion</p> <p>(IV): AngII infusion + CANA (after 1 weeks of Ang II infusion (15 mg/kg/day in drinking water)</p> <p>AngII infusion by osmotic minipumps for 4 weeks</p> <p>Human study: Human kidney biopsies from Patients with proteinuric kidney disease without DM and secondary diseases (lupus, amyloid)</p>	<p>Human biopsy: SGLT2 mRNA negatively correlated with TIF</p>

SRS

Table 2 (continued)

References	Methods	Renal fibrosis related findings
[64]	<p>Clinical and experimental (in vitro and in vivo) study</p> <p>Clinical part: Postoperative tissue adjacent to the kidney carcinoma were collected from 36 patients with stage III DN (DN group; n = 18) and non-DN (NC group; n = 18)</p> <p>In vitro part: HK-2 cells incubated in high glucose and high glucose + EMPA</p> <p>In vivo part: c57 mice were divided</p> <p>(I): Control</p> <p>(II): Diabetic: induced by continuous <i>ip</i> injection of STZ (70 mg/kg/d) for 2 days, followed by high-fat feeding for 4 weeks</p> <p>(III): Diabetic + EMPA (10 mg/kg/d) administered 2 weeks after HFD for 8 weeks</p> <p>(IV): Diabetic + EMPA (10 mg/kg/d) + Compound C (10 mg/kg/d) for 8 weeks</p> <p>H&E, MT, PAS, and Oil Red O staining</p>	<p>Renal interstitial and perivascular fibrosis higher in section of DM patients compared to non-diabetic patients</p> <p>EMPA reduced lipid deposition in HK-2 cells</p> <p>Among four mouse groups kidney fibrosis, renal tubular atrophy and lipid deposition was increased in DM rats and EMPA reduced these detrimental findings</p> <p>Renal interstitial fibrosis was increased with EMPA + compound C compared to EMPA</p>

AngII, Angiotensin II; CANA, canagliflozin; DAPA, dapagliflozin; DN, diabetic nephropathy; EMPA, empagliflozin; HK-2, Hhuman kidney proximal tubule cell lines; FN, fibronectin; H&E, hematoxylin and eosin IP, intraperitoneal; MT, Masson's trichrome; PAS, periodic acid Schiff; PSR, Picro Sirius red; STAT, signal transducer and activator of transcription; SRS, Sirius red staining; SMA, smooth muscle actin; SGLT2i, sodium–glucose cotransporter inhibitors; STZ, streptozotocin; TGF- β , TIF, tubulointerstitial fibrosis; transforming growth factor β ; WT, wild type

function by SGLT2i may be one of the protective mechanisms for kidney fibrosis. Hypoxia may induce renal fibrosis by activating the HIF-1 α /human epididymis protein 4 (HE4)/nuclear factor kappa-light-chain-enhancer of activated B cells (NF- κ B) pathway [21]. Previously, it was suggested that there was a positive association between circulating HE4 and renal fibrosis [22, 23]. Huang et al. showed that dapagliflozin reduced HIF-1 α , HE4, and NF- κ B expressions along with decreased histologic kidney damage in contrast-induced acute kidney injury (AKI) model [24]. Dapagliflozin also decreased hypoxia-induced connective tissue growth factor (CTGF) and platelet-derived growth factor (PDGF) production, suggesting that its anti-fibrotic effects might be in direct connection with diminished hypoxia [25]. In diabetic mice HIF-1 α activation causes epithelial-to-mesenchymal transition (EMT), leading to tubulointerstitial injury and empagliflozin decreased renal fibrosis and EMT along with decreased HIF-1 α [26]. In human proximal kidney 2 (HK-2) cells, HIF-1 α expression is inhibited by SGLT2i during hypoxia [27, 28]. As SGLT2i decrease glucose uptake by renal proximal tubular cells (RPTCs), they reduce oxygen consumption and decrease expression, and increase proteasomal degradation of HIF1 α in RPTCs [29]. However, there are also contrasting findings. Although the above studies showed that high HIF-1 α in tubular epithelial cells contributes to renal fibrosis, HIF-1 α stabilization by prolyl hydroxylase domain (PHD) inhibitors has shown favorable effects in ischemic kidney injury [30]. In streptozotocin (STZ)-induced diabetic mice, HIF-1 α deficiency was related to more severe renal fibrosis and glomerular hypertrophy [31]. The reasons for these different findings are not exactly known, but the amount and duration of HIF activation may be an explanation. Indeed, it was suggested that the protective effect of HIF on the kidney occurs in AKI, but long-term permanent activation may be detrimental resulting in chronic inflammation, fibrosis, and extracellular matrix accumulation [32]. For example, after a 30 min period of ischemia, there is severe AKI in mice which exhibits a significant delay in the HIF response, compared to mice with mild AKI which elicits fast HIF response. In the group with a delayed response; HIF-1 α and HIF-2 α activation were inappropriately sustained with severe ischemia–reperfusion injury (IRI) leading to maladaptive renal repair and acute kidney injury (AKI) and that activation remained upregulated 4 weeks after injury. In contrast, HIF response in the mild AKI group, i.e., adaptive repair group, returned to normal levels well before 4 weeks [33]. Thus, it is probable that although acute short-term physiological HIF activation may be beneficial, persistent HIF activation may be detrimental in long term. In fact, there appears to be a time-dependent transition in the pattern of HIF activation from renal tubular epithelia and capillary endothelia to renal interstitial fibroblasts during chronic maladaptive renal repair resulting in renal

fibrosis [34]. Thus, the role of HIF activation in fibrogenesis may be context specific (e.g., IRI), duration specific (acute vs. chronic) or amount specific (high vs. low dose HIF). Furthermore, as SGLT2i shift tubular glucose and sodium absorption from the proximal tubule to the outer medulla, where SGLT1 partially compensates for SGLT2 blockade, cortical oxygen tension is expected to increase at the expense of lower medullary oxygen levels. This will, in turn, increase HIF signaling in the medulla, which stimulates erythropoietin (EPO) release and increases hematocrit [35]. In this regard, SGLT2 inhibition may be a double-edged sword to medullary oxygenation, and whether final outcome of medullary hypoxia is adverse or favorable to overall kidney function is unknown [32]. Furthermore, the impact of SGLT2 inhibition during AKI and CKD may operate via different mechanisms (Fig. 1). Thus, it is not hundred percent clear whether HIF-1 α has a detrimental or protective effect in the context of CKD, and future research is needed to obtain a deeper understanding of HIF in kidney fibrosis.

Inflammation, sodium–glucose cotransporter inhibition, and kidney fibrosis

In chronic kidney disease, there is increased pro-inflammatory markers and decreased anti-inflammatory markers favoring a chronic inflammatory state [36]. SGLT2i have many anti-inflammatory effects in kidney (Fig. 2). NLR family pyrin domain containing 3 (NLRP3) is one of the most important inflammatory mediator overactivated in CKD [37] and associated with kidney fibrosis [38]. Benetti et al. showed that empagliflozin decreased NLRP3 activation in the kidney and liver induced by a high-fat–high-sugar diet [39]. In IRI-induced fibrosis model, dapagliflozin prevented activation of NLRP3 inflammasome and increased anti-inflammatory metabolite itaconate and protected kidney against fibrosis [40]. It was suggested that β -hydroxybutyrate—a type of ketone body—inhibits NLRP3 activation [41]. As SGLT2i promote the elevation of ketone bodies, this may be another mechanism to explain the reno-protective role of SGLT2i. In rats with experimentally induced DM, increased tumor necrosis factor- α (TNF- α), interleukin-1 β (IL-1 β), and interleukin-6 (IL-6) levels were counterbalanced by empagliflozin [42]. Wang et al. showed that SGLT2i decrease macrophage infiltration, p65 subunit of NF- κ B toll-like receptor 4 (TLR4), monocyte chemoattractant protein 1 (MCP-1) and osteopontin in diabetic db/db mice [43]; which are in compliance with the findings of Tang et al. [44]. In diabetic mice, dapagliflozin inhibited pro-inflammatory macrophage (M1 type) infiltration, CD11c (a marker of M1 type macrophages), and CD14 expressions in the renal cortex with no effect on CD206 (specific for the anti-inflammatory (M2) subtype of macrophages). Both in the glomeruli and interstitial space, immunoperoxidase staining

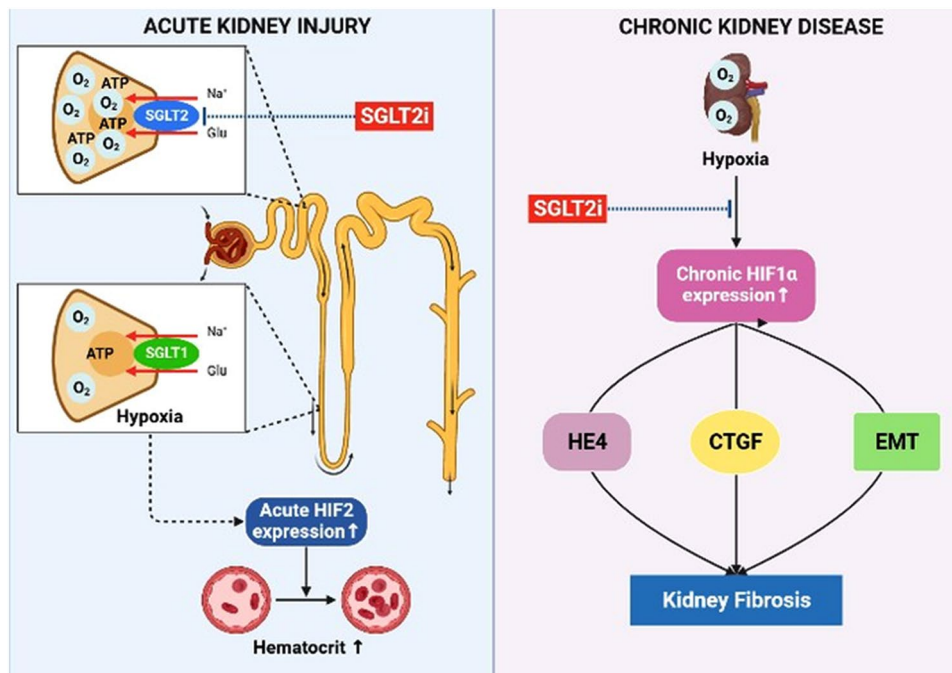


Fig. 1 The inhibition of sodium–glucose cotransport 2 during acute kidney injury (AKI) and chronic kidney disease may activate different mechanisms. During AKI, sodium–glucose cotransport 2 inhibitors (SGLT2i) impair proximal sodium and glucose reabsorption from the proximal tubule which results in decreased ATP consumption and increased oxygen tension in proximal tubular cells. This in turn shifts sodium glucose reabsorption to distal parts of the proximal tubule close to the medulla which SGLT1 plays a major role in reabsorption. The increased need for reabsorption in distal parts necessitates more energy consumption which results in a decrease in ATP and oxygen tension in the medulla. This in turn decreased hypoxia-inducible fac-

tor-2 alpha (HIF-2 α) degradation resulting in increased EPO secretion and increased hematocrit levels. The net balance between hypoxia and increased hematocrit will determine whether the final outcome of medullary hypoxia is adverse or beneficial to overall kidney function. In chronic kidney disease, there is persistent hypoxia leading to chronic HIF-1 α activation. Chronic HIF-1 activation induces kidney fibrosis by a variety of mechanisms including increased human epididymis protein 4 (HE4), connective tissue growth factor (CTGF), and epithelial-to-mesenchymal transition (EMT). SGLT2 inhibition during CKD decreases tissue hypoxia and kidney fibrosis may inhibit HE4, CTGF, and EMT downstream

showed increased F4/80, a marker for M1 macrophages, which was reduced by dapagliflozin [45]. In high-fed mice, ipragliflozin treatment suppressed the ratio of M1-type (CD11c + CD206- cells) to M2-type tissue macrophages (CD11c-CD206 + cells) in peri-renal fat tissue [46]. In Akita mice, dapagliflozin decreased macrophage infiltration into glomeruli and interstitium, and decreased transforming growth factor β (TGF- β), C-C motif chemokine ligand 2 (CCL2), osteopontin, and intercellular adhesion molecule 1 (ICAM-1) expressions [47]. In streptozotocin-induced diabetic rats, empagliflozin reduced receptor for advanced glycation end-products (RAGE) gene and protein expressions, M1 macrophage infiltration (F4/80), and expression of pro-fibrotic markers such as MCP-1, ICAM-1, TGF- β , and CTGF, which are AGE-RAGE signaling [48]. In renal proximal tubular cells exposed to high glucose, dapagliflozin inhibited high mobility group box 1 (HMGB1)/RAGE/NF κ B signaling pathway, leading to attenuation inflammatory (downregulation of ICAM-1, MCP-1, and NF- κ B mRNA levels) and fibrosis (decreased fibronectin and collagen I mRNA) markers [49]. Abbas et al. demonstrated that

empagliflozin decreased NF- κ B activation in the unilateral ureteric obstruction (UUO) model of nephropathy [50], which plays an important role in renal fibrosis and fibroblast activation [51]. Furthermore, empagliflozin decreased TLR4 expression, which is involved in NF- κ B-mediated inflammation [52]. In a clinical study using the transcriptomics approach, Heerspink et al. demonstrated that compared with glimepiride, canagliflozin (300 mg/day) decreased plasma levels of TNF receptor 1 and IL-6 during 2 years of follow-up [53].

Oxidative stress, sodium–glucose cotransporter inhibition, and kidney fibrosis

Increased oxidative stress is one of the hallmarks of CKD. In a rat model of diabetic nephropathy, Wu et al. showed that the activities of superoxide dismutase (SOD), glutathione peroxidase (GSH) in the diabetic nephropathy group were decreased and the levels of malondialdehyde (MDA) and nitric oxide (NO) were increased compared to controls. However, after treatment with empagliflozin, the activities

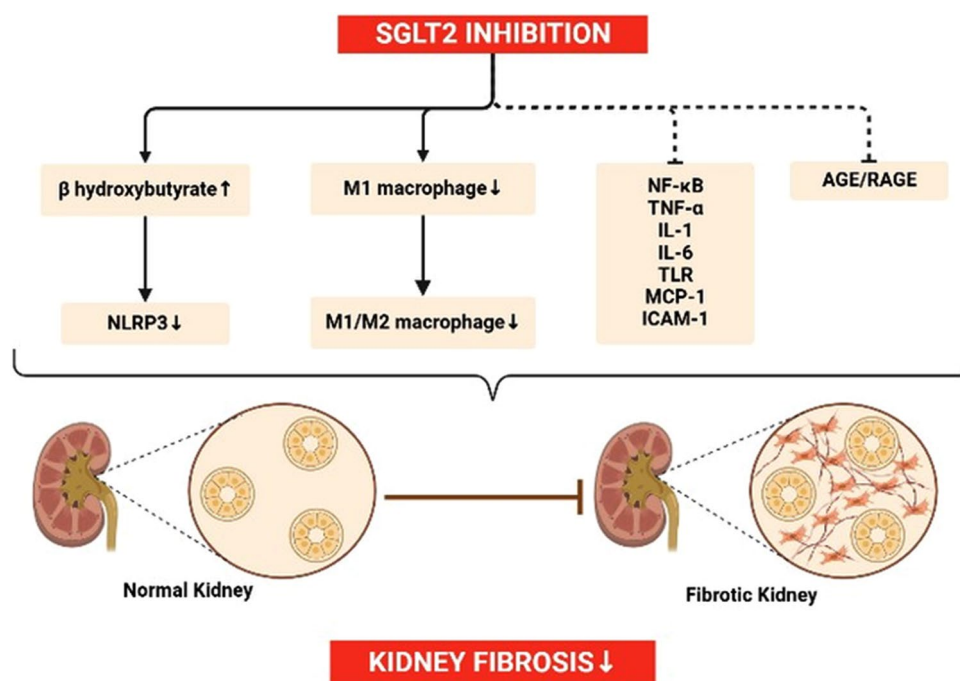


Fig. 2 Sodium–glucose cotransport 2 inhibitors (SGLT2i) exert many anti-inflammatory effects which ameliorate kidney fibrosis. They increase ketone body formation including β -hydroxybutyrate which inhibits the Nod-like receptor family and pyrin domain containing 3 (NLRP3) inflammasome. SGLT2i decreases macrophage infiltration to the kidney and decreases the pro-inflammatory (M1)/anti-inflammatory (M2) macrophage ratio. They decrease many inflammatory

pathways and molecules including nuclear factor kappa-light-chain-enhancer of activated B cells (NF- κ B), tumor necrosis factor- α (TNF- α), interleukin-1 β (IL-1 β), interleukin-6 (IL-6), toll-like receptors (TLRs), monocyte chemoattractant protein 1 (MCP-1), intercellular adhesion molecule 1 (ICAM-1), and advanced glycation end-products (AGE)–RAGE signaling

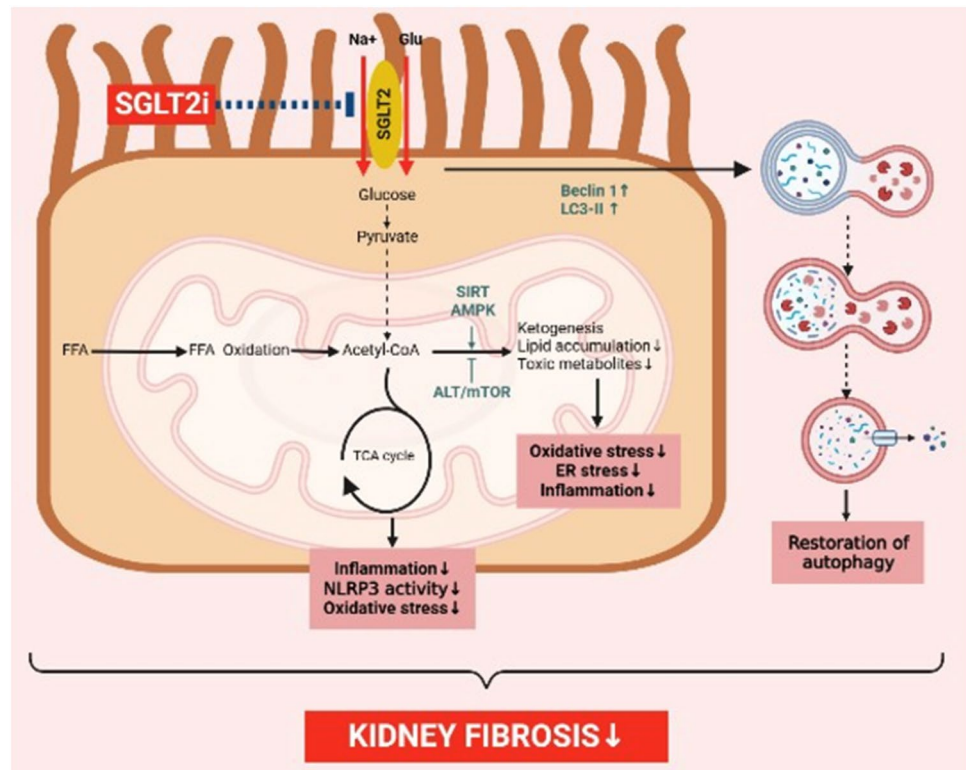
of SOD and GSH increased and the levels of MDA and NO decreased [42]. These findings are important since the increase in reactive oxygen species (ROS) triggers inflammation, endothelial dysfunction, increases expression of TGF- β 1, and induces renal fibrosis [54]. In a diabetic (db/db) mice model, dapagliflozin decreased oxidative stress and ROS production (both 0.1 mg/kg and 1 mg/kg doses) and decreased the number of apoptotic cells and the expressions of the pro-apoptotic factors, Caspase-12 and Bax [45]. Tang et al. showed that in db/db diabetic mice, dapagliflozin decreased Nox4, Nox2, and its regulatory subunit p47phox. Furthermore, thiobarbituric acid reactive substances (TBARS) levels, which show the level of lipid peroxidation, were also decreased by dapagliflozin [44]. In Akita mice, dapagliflozin decreased Nox4 both in vivo and in vitro along with decreased apoptosis [47]. In proximal tubular epithelial cells, high glucose increased superoxide and hydrogen peroxide generation, oxidative stress-dependent TRAF3IP2 upregulation, NF- κ B, and p38 MAPK activation, which was inhibited by empagliflozin [55]. In New Zealand obese mice with a high-fat diet, canagliflozin ameliorated hyperglycemia-associated augmentation of urinary 8-isoprostane levels [56]. Ipragliflozin and 30% calorie restriction almost normalized the oxidized glutathione levels in the renal cortex of

BTBR ob/ob diabetic mice as shown by mass spectrometry [57].

Metabolism, sodium–glucose cotransporter inhibition, and kidney fibrosis

SGLT2i has many metabolic effects, the most prominent one is shifting glucose to free fatty acid utilization (Fig. 3) [58]. Decreased toxic lipid metabolites in mesangial cells, podocytes, and proximal tubular reduce oxidative stress, endoplasmic reticulum stress, and pro-inflammatory processes [59]. SGLT2i increase ketone body formation via starvation-like response including activation of sirtuin1/activated protein kinase and adenosine monophosphate-activated protein kinase (AMPK) signaling and inhibition of protein kinase B (AKT)/mammalian target of rapamycin (mTOR) signaling [13, 60–62]. Increased lipid toxicity produces ROS, which induces inflammatory mediators and damages renal tubular epithelial cells. Furthermore, deficient lipophagy increases tubular lipid deposition and increased fibrosis in diabetic nephropathy [63]. Zhang et al. showed that empagliflozin decreased lipid accumulation in renal tubular cells which is associated with decreased fibrosis. Additionally, empagliflozin reduced lipid expression and showed reduced lipid

Fig. 3 Sodium–glucose cotransporter 2 inhibitors (SGLT2i) have many beneficial metabolic effects that retard kidney fibrosis. They block glucose entry to tubular cells and inhibit glycolysis. They simultaneously increase free fatty acid oxidation and ketone body formation resulting in decreased toxic lipid accumulation in proximal tubular cells resulting in decreased inflammation, and oxidative and endoplasmic reticulum (ER) stress. They regulate the tricarboxylic acid (TCA) cycle and decrease TCA cycle metabolites in renal proximal tubular cells. SGLT2i restore autophagy markers Beclin-1 and LC3-II and retard kidney fibrosis



droplet activity [64]. Thus, decreasing lipid toxicity may be another favorable mechanism of SGLT2i.

The impact of SGLT2i on the tricarboxylic acid (TCA) cycle is also worth mentioning. Ke et al. showed that TCA cycle metabolites were accumulated, NLRP3 inflammasome, mTOR/HIF-1 α signaling were increased in the IRI-induced fibrosis model in mice compared to sham, all of which are strikingly blocked by dapagliflozin. Authors suggest that TCA cycle metabolites accumulate due to decreased metabolic processes in the fibrotic kidney. However, dapagliflozin boosted TCA cycle derivative itaconate by decreasing isocitrate dehydrogenase 2 and increasing immune-responsive gene 1 and mitochondrial citrate carrier. RNA sequence data showed that dapagliflozin tended to restore decreased TCA cycle enzymes including α -ketoglutarate dehydrogenase, succinate dehydrogenase, fumarase, and malate dehydrogenase. Furthermore, itaconate prevented activation of the NLRP3 inflammasome and protected the kidney against fibrosis. These findings identify a novel mechanism coupling inflammation and metabolism for reno-protection of SGLT2 inhibition for kidney fibrosis and CKD. The accumulation of lipid droplets in renal tubules was markedly attenuated by dapagliflozin treatment. These changes were associated with a decrease in NLRP3 inflammasome activation [40]. Ding et al. demonstrated that STZ-induced CD1 mice showed aberrant glycolysis (indicated by hexokinase 2 induction, and pyruvate kinase isozyme M2 dimer formation,

increased fructose-6-phosphate, glucose-6-phosphate (G6P), and phosphofructokinase) and decreased Sirt3 levels associated with EMT and kidney fibrosis. Empagliflozin and canagliflozin normalized these changes. These findings are important since inhibition of glycolysis has been demonstrated to be a relevant strategy for inhibiting kidney fibrosis [65].

Activation of AMPK, which is an important energy sensor of the cell, is also activated by SGLT2 inhibition. Inoue et al. showed that canagliflozin activated AMPK in the kidney and reduced the expression of fibrotic and inflammatory markers such as collagen I, CTGF, F4/80, TGF- β , and MCP-1 [66]. Supporting these findings, empagliflozin treatment restored phosphorylated AMPK levels in renal proximal tubular cells and increased autophagy markers Beclin1 and LC3II by decreasing mTOR activation [67]. SGLT2i also exerts anti-fibrotic effects via post-translational modification. Post-translational O-GlcNAcylation, which induces O-linked N-acetylglucosamine (O-GlcNAc) is a known contributor to glucose toxicity in diabetes, and increased O-GlcNAcylation increases fibrotic signaling in kidneys [68]. Hodrea et al. showed that dapagliflozin reduced O-GlcNAcylation resulting in decreased fibrosis in streptozotocin-induced type 1 diabetes in adult male Wistar rats [25].

Last but not the least, SGLT2i may decrease endoplasmic reticulum (ER) stress by downregulating key ER stress markers such as phosphorylated eukaryotic initiation factor-2 α (peIF2 α), activating transcription factor 4 (ATF4), and C/

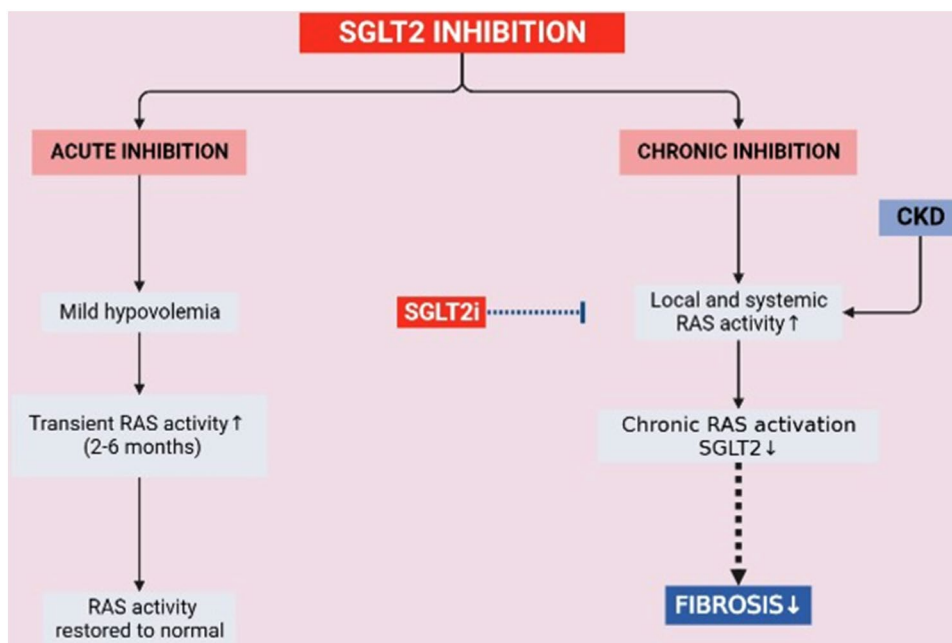
EBP homologous protein (CHOP; an ER-stress induced apoptotic marker), and the apoptotic marker Caspase 3 [69, 70], both of which are associated with kidney fibrosis [71].

Renin–angiotensin system: sodium–glucose cotransporter inhibition and kidney fibrosis

SGLT2i may impact differently on the systemic renin–angiotensin system (RAS) depending on whether inhibition is acute or occurs in the context of CKD, when RAS is already activated (Fig. 4). Not only systemic RAS, but more importantly local RAS activates fibrotic pathways in the kidney [72]. Indeed, the anti-fibrotic actions of angiotensin II (AngII) type 1 receptor (AT1R) antagonism [73] and angiotensin-converting enzyme (ACE) inhibition [74] have been already demonstrated in murine models of UUO and progressive renal fibrosis. Furthermore, RAS inhibition has already been shown to reduce renal fibrosis in DKD [75–77]. On the other hand, AngII type 2 receptor (AT2R) agonism, which has opposite actions to classical AT1R receptor is protective against kidney fibrosis [78]. The pro-fibrotic action of RAS activation is suggested to be due to the exacerbation of renal inflammation [79, 80], induction of abnormal autophagy [81], increase in the synthesis of TGF- β [82], and increased collagen synthesis [83]. There is now clinical evidence suggesting that RAS activation is blunted when SGLT2i is used. In Otsuka Long-Evans Tokushima Fatty (OLETF) rats, 12 weeks of dapagliflozin reduced urinary AngII and angiotensinogen levels, and AT1R expression in a model of T2D with DKD [84]. In New Zealand obese mice with a high-fat diet, canagliflozin decreased the expression of renal angiotensinogen [56]. In non-diabetic patients with

nephrotic range proteinuria, SGLT2 is positively associated with angiotensinogen, renin, and ACE mRNA levels. The same study showed that in proximal HK-2 cells, acute but not chronic (4 week) AngII stimulated SGLT2 expression in a dose-dependent manner. Incubation of HK-2 cells with losartan (AT1R antagonist) abolished the effects of AngII, indicating that AngII stimulates SGLT2 expression via AT1R [85]. In contrast, Gallo et al. showed that both plasma and intra-renal renin activity is increased after 10 weeks of empagliflozin treatment in a diabetic model of db/db mice without change in intra-renal AngII levels [86]. In patients with type 2 diabetes, 12 weeks of dapagliflozin (10 mg/day), resulted in increased plasma renin activity and aldosterone levels, possibly due to reduced plasma volume [87]. The study by Tanaka et al. showed that canagliflozin did not change plasma aldosterone levels despite increased plasma renin activity and urine volume [88]. In OLETF rats, empagliflozin treatment was associated with an increase in urinary angiotensinogen excretion [89]. These contradictory findings may be of concern given the fact that in CKD and DKD, there is already activated RAS. However, the rise of renin activity seems to be transient. In a retrospective study performed in patients with type 2 diabetes, Sawamura et al. showed that there was no change in plasma renin activity and plasma aldosterone levels receiving SGLT2i for 2–6 months [90]. As SGLT2i decrease glucose load in proximal tubules and increase glucose load in distal tubules, the impact of SGLT2i on the RAS system may differ in proximal and distal tubules. The contradictory effects of SGLT2i on proximal (decreased RAS activation) and distal tubules (increased RAS activation) may compensate each other with resultant non-significant changes in intra-renal RAS [29]. However,

Fig. 4 The inhibition of SGLT2 may impact differently on the systemic renin–angiotensin system (RAS) depending on whether inhibition is acute or occurs in the context of CKD when RAS is already activated. When SGLT2i given, they cause mild hypovolemia which results in increased temporary renin–angiotensin system activation (RAS) which is normalized in the 2–6-month period. During chronic kidney disease (CKD), there is already local kidney RAS activation and SGLT2i in CKD decreases local kidney RAS activation resulting in kidney fibrosis



more studies are warranted to understand the systemic and intra-renal RAS in the context of SGLT2 inhibition.

Other potential mechanisms related sodium–glucose cotransporter inhibition and kidney fibrosis

The interaction with uric acid (UA) and SGLT2i is a concern for renal fibrosis. Increased UA levels were related to increased renal fibrosis [91]. SGLT2i reduce plasma UA by increased renal urate elimination by competition of extra glucose for the urate transporter GLUT9 and decreased UA levels [92, 93]. Uric acid excretion by SGLT2i was linked to urinary glucose excretion and is attenuated during the concomitant pharmacologic blockade of urate transporter 1 [94]. SGLT2i may repress tubulointerstitial fibrosis by influencing signal transducer and activator of transcription-1 (STAT-1) activity. STAT1 plays a role in high glucose-induced oxidative stress and expression of TGF- β 1, as well as the production of the extra-cellular matrix proteins collagen IV and fibronectin. Concurrently, high glucose activates STAT1 and TGF β and is involved in tubulointerstitial fibrosis. It was shown that dapagliflozin suppressed pro-fibrotic factors STAT1 and TGF- β 1 both in vivo (measured by mRNA in tubulointerstitium compartment of the renal biopsy cores) and in vitro (in cultured renal proximal cells) independent of glucose-lowering effect [95].

Decreased klotho expression has been suggested as a risk factor for the development of kidney fibrosis and hypertension [96]. Zhou et al. showed that klotho ameliorates kidney injury and fibrosis, and normalizes blood pressure by targeting the RAS [97]. In a model of UUO, Abbas et al. showed that prophylactic administration of empagliflozin increased klotho expression in both immediate and delayed treatment groups, which was decreased by UUO. Authors suggested that by increasing klotho, empagliflozin may be potentially protective against kidney fibrosis [50].

Renal fibrosis was associated with capillary rarefaction and the role of SGLT2i on capillary rarefaction deserves mention. Zhang et al. investigated the effect of luseogliflozin on capillary rarefaction in AKI induced by ischemia–reperfusion. They showed that IRI decreased CD31, an endothelial marker. Luseogliflozin treatment attenuated CD31-positive cell loss, suggesting a protective role for endothelial cells. The decline of CD31 during IR is also associated with increased hypoxia, and luseogliflozin treatment ameliorated hypoxia as determined by pimonidazole staining. NG2, an activated pericyte marker, was also decreased by luseogliflozin treatment suggesting that luseogliflozin ameliorated IRI-induced renal capillary rarefaction and detachment of pericytes from endothelial cells [98], both of which were implicated in the development of renal interstitial fibrosis [99]. Moreover, luseogliflozin decreased capillary

rarefaction and maintained the capillary network by increasing the vascular endothelial growth factor (VEGF)-A level [98].

Another potential anti-fibrotic action of canagliflozin may be due to the suppression peptidylprolyl cis/trans isomerase (Pin 1). Pin 1 previously has been shown to play a role in tubulointerstitial fibrosis [100]. Inoue et al. showed that in type 2 diabetic model mice, Pin1 expression increased along with fibrosis, but Pin1 protein levels were normalized by canagliflozin [66].

Perspectives and conclusion

As the above discussion suggests, most, but not all, studies showed that SGLT2i are potentially protective against kidney fibrosis, a common pathway for progressive CKD. However, there are also contrasting findings. Liu et al. showed that in patients with type 2 diabetes and stage 3 CKD, ertugliflozin (5 or 15 mg/d) did not reduce fibrosis markers, such as PDGF-AA and PDGF-AB/BB [101]. In oxalate-induced nephrocalcinosis, empagliflozin did not have any protective effect against renal fibrosis, and did not decrease α -smooth muscle actin and collagen 1 staining, and mRNA levels of fibronectin-1, collagen 1a1, fibroblast-specific protein-1, and TGF- β 2 expressions. Authors suggest that the reno-protective effects of SGLT2i in DK do not occur in non-diabetic oxalate-induced nephrocalcinosis, a form of progressive CKD driven by primary tubulointerstitial injury [102]. One potential explanation is that renal anti-fibrotic effects mediated by the SGLT2i empagliflozin are ambiguous, might depend on the presence of type 2 diabetes, and/or are indirect consequences of the primary metabolic/hemodynamic action in the long term [103]. Similarly, in 5/6 (subtotal) nephrectomized rats, dapagliflozin has no protective effect on glomerulosclerosis, tubulointerstitial fibrosis, or overexpression of the pro-fibrotic cytokine, TGF- β 1 [104]. Furthermore, Vallon et al. demonstrated that TGF- β and renal collagen expression, as determined by Sirius red binding, was not ameliorated in diabetic Akita mice [105]. In male C57BL/6 Enos knockout mice, empagliflozin did not decrease tubular atrophy, or tubulointerstitial fibrosis in streptozotocin-induced DM [106]. Why are the results conflicting? The exact answer is not known, but SGLT2i may impact many processes (HIF, inflammation, oxidative stress, metabolism, RAS). Besides, the impact of SGLT2 inhibition on some biological pathways (e.g., HIF, RAS) is not consistent and somewhat controversial. The type of experimental design, the duration of SGLT2 inhibition (acute vs. chronic), the time of administration (prophylactic, immediate or delayed), the dosage (low vs. high dose), and variable drug actions in different species may be responsible for these diverse findings. Unfortunately, the clinical data are scarce and most of

the studies are based on in vitro and in vivo experiments. The direct extrapolation of experimental findings to humans may be problematic and specific studies should be planned to investigate the anti-fibrotic effects of SGLT2i on kidney fibrosis in humans.

There is also controversy about whether the anti-fibrotic effects of SGLT2i are independent of TGF. Zeng et al. showed that the anti-fibrotic action of empagliflozin is independent of the TGF mechanism [107]. However, Tauber et al. demonstrated that empagliflozin did not have any protective effects against kidney fibrosis in the uninephrectomy/high salt/doca model, and since functional TGF mechanism is necessary to reduce chronic hyperfiltration and protect against glomerular damage, albuminuria, and fibrosis, authors suggested TGF may not be working properly in these animals [108].

Last but not the least, most of the results are from animal studies. Besides, assessment of kidney fibrosis necessitates renal biopsy, which might not be feasible just to determine whether SGLT2i decreased renal fibrosis. Thus, it is important to remember that the animal data may not be directly translated into clinical data. However, it is advisable that specific interest could be given to assess whether SGLT2i would decrease renal fibrosis at least in patients for whom renal biopsy was performed for other reasons.

In conclusion, SGLT2 inhibition seems to be a promising alternative to retard kidney fibrosis by influencing a variety of mechanisms. Although human data are scarce, most of the experimental studies showed favorable effects in ameliorating kidney fibrosis. As direct extrapolation of experimental animal data to humans may be problematic, studies are warranted to investigate whether SGLT2i are protective against kidney fibrosis, which is a final common pathway for all kinds of CKD progression.

Authors' contributions BA was the owner of the project, gathered data, wrote the manuscript. REA helped to write the manuscript, reviewed, and approved the final version.

Funding None declared.

Data availability The manuscript has no associated data.

Declarations

Conflict of interest All authors declare that they have no conflict of interest.

References

- Perkovic V, Jardine MJ, Neal B, Bompoint 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.
- McMurray JJV, Solomon SD, Inzucchi SE, Køber L, Kosiborod MN, Martinez FA, et al. Dapagliflozin in patients with heart failure and reduced ejection fraction. *N Engl J Med*. 2019;381:1995–2008.
- Zinman B, Wanner C, Lachin JM, Fitchett D, Bluhmki E, Hantel S, et al. Empagliflozin, cardiovascular outcomes, and mortality in type 2 diabetes. *N Engl J Med*. 2015;373:2117–28.
- Wanner C, Inzucchi SE, Lachin JM, Fitchett D, von Eynatten M, Mattheus M, et al. Empagliflozin and progression of kidney disease in type 2 diabetes. *N Engl J Med*. 2016;375:323–34.
- Cooper ME, Inzucchi SE, Zinman B, Hantel S, von Eynatten M, Wanner C, et al. Glucose control and the effect of empagliflozin on kidney outcomes in type 2 diabetes: an analysis from the EMPA-REG outcome trial. *Am J Kidney Dis*. 2019;74:713–5.
- Heerspink HJ, Desai M, Jardine M, Balis D, Meininger G, Perkovic V. Canagliflozin slows progression of renal function decline independently of glycemic effects. *J Am Soc Nephrol*. 2017;28:368–75.
- Packer M, Anker SD, Butler J, Filippatos G, Pocock SJ, Carson P, et al. Cardiovascular and renal outcomes with empagliflozin in heart failure. *N Engl J Med*. 2020;383:1413–24.
- Anker SD, Butler J, Filippatos G, Ferreira JP, Bocchi E, Böhm M, et al. Empagliflozin in heart failure with a preserved ejection fraction. *N Engl J Med*. 2021;385:1451–61.
- Heerspink HJL, Stefánsson BV, Correa-Rotter R, Chertow GM, Greene T, Hou FF, et al. Dapagliflozin in patients with chronic kidney disease. *N Engl J Med*. 2020;383:1436–46.
- Neuen BL, Young T, Heerspink HJL, Neal B, Perkovic V, Billot L, et al. SGLT2 inhibitors for the prevention of kidney failure in patients with type 2 diabetes: a systematic review and meta-analysis. *Lancet Diabetes Endocrinol*. 2019;7:845–54.
- de Boer IH, Khunti K, Sadusky T, Tuttle KR, Neumiller JJ, Rhee CM, et al. Diabetes management in chronic kidney disease: a consensus report by the American Diabetes Association (ADA) and kidney disease: improving global outcomes (KDIGO). *Kidney Int*. 2022;102:974–89.
- Vallon V, Thomson SC. The tubular hypothesis of nephron filtration and diabetic kidney disease. *Nat Rev Nephrol*. 2020;16:317–36.
- Vallon V, Verma S. Effects of SGLT2 inhibitors on kidney and cardiovascular function. *Annu Rev Physiol*. 2021;83:503–28.
- Brenner BM, Lawler EV, Mackenzie HS. The hyperfiltration theory: a paradigm shift in nephrology. *Kidney Int*. 1996;49:1774–7.
- Denic A, Mathew J, Lerman LO, Lieske JC, Larson JJ, Alexander MP, et al. Single-nephron glomerular filtration rate in healthy adults. *N Engl J Med*. 2017;376:2349–57.
- Fattah H, Layton A, Vallon V. How do kidneys adapt to a deficit or loss in nephron number? *Physiology (Bethesda)*. 2019;34:189–97.
- Cherney DZI, Dekkers CCJ, Barbour SJ, Catran D, Abdul Gafar AH, Greasley PJ, et al. Effects of the SGLT2 inhibitor dapagliflozin on proteinuria in non-diabetic patients with chronic kidney disease (DIAMOND): a randomised, double-blind, crossover trial. *Lancet Diabetes Endocrinol*. 2020;8:582–93.
- Yan H, Xu J, Xu Z, Yang B, Luo P, He Q. Defining therapeutic targets for renal fibrosis: exploiting the biology of pathogenesis. *Biomed Pharmacother*. 2021;143:112115.
- Fine LG, Bandyopadhyay D, Norman JT. Is there a common mechanism for the progression of different types of renal diseases other than proteinuria? Towards the unifying theme of chronic hypoxia. *Kidney Int Suppl*. 2000;75:S22–6.
- Tanaka T, Nangaku M. The role of hypoxia, increased oxygen consumption, and hypoxia-inducible factor-1 alpha in progression of chronic kidney disease. *Curr Opin Nephrol Hypertens*. 2010;19:43–50.

21. Zhang L, Liu L, Bai M, Liu M, Wei L, Yang Z, et al. Hypoxia-induced HE4 in tubular epithelial cells promotes extracellular matrix accumulation and renal fibrosis via NF- κ B. *Faseb j*. 2020;34:2554–67.
22. LeBleu VS, Teng Y, O'Connell JT, Charytan D, Müller GA, Müller CA, et al. Identification of human epididymis protein-4 as a fibroblast-derived mediator of fibrosis. *Nat Med*. 2013;19:227–31.
23. Chen P, Yang Q, Li X, Qin Y. Potential association between elevated serum human epididymis protein 4 and renal fibrosis: a systemic review and meta-analysis. *Medicine (Baltimore)*. 2017;96:e7824.
24. Huang X, Guo X, Yan G, Zhang Y, Yao Y, Qiao Y, et al. Dapagliflozin attenuates contrast-induced acute kidney injury by regulating the HIF-1 α /HE4/NF- κ B pathway. *J Cardiovasc Pharmacol*. 2022;79:904–13.
25. Hodrea J, Balogh DB, Hosszu A, Lenart L, Besztercei B, Koszegi S, et al. Reduced O-GlcNAcylation and tubular hypoxia contribute to the antifibrotic effect of SGLT2 inhibitor dapagliflozin in the diabetic kidney. *Am J Physiol Renal Physiol*. 2020;318:F1017–29.
26. Li J, Liu H, Takagi S, Nitta K, Kitada M, Srivastava SP, et al. Renal protective effects of empagliflozin via inhibition of EMT and aberrant glycolysis in proximal tubules. *JCI Insight*. 2020;5:1.
27. Ndibalema AR, Kabuye D, Wen S, Li L, Li X, Fan Q. Empagliflozin protects against proximal renal tubular cell injury induced by high glucose via regulation of hypoxia-inducible factor 1- α . *Diabetes Metab Syndr Obes*. 2020;13:1953–67.
28. Bessho R, Takiyama Y, Takiyama T, Kitsunai H, Takeda Y, Sakagami H, et al. Hypoxia-inducible factor-1 α is the therapeutic target of the SGLT2 inhibitor for diabetic nephropathy. *Sci Rep*. 2019;9:14754.
29. Ravindran S, Munusamy S. Renoprotective mechanisms of sodium–glucose co-transporter 2 (SGLT2) inhibitors against the progression of diabetic kidney disease. *J Cell Physiol*. 2022;237:1182–205.
30. Nordquist L, Friederich-Persson M, Fasching A, Liss P, Shoji K, Nangaku M, et al. Activation of hypoxia-inducible factors prevents diabetic nephropathy. *J Am Soc Nephrol*. 2015;26:328–38.
31. Jiao Y, Jiang H, Lu H, Yang Y, Zhang Y, Zhang K, et al. Deficiency of hypoxia inducible factor-1 α promoted progression of diabetic nephropathy with hypertension. *Exp Ther Med*. 2018;16:3658–62.
32. Hesp AC, Schaub JA, Prasad PV, Vallon V, Laverman GD, Bjornstad P, et al. The role of renal hypoxia in the pathogenesis of diabetic kidney disease: a promising target for newer renoprotective agents including SGLT2 inhibitors? *Kidney Int*. 2020;98:579–89.
33. Xu ZH, Wang C, He YX, Mao XY, Zhang MZ, Hou YP, et al. Hypoxia-inducible factor protects against acute kidney injury via the Wnt/ β -catenin signaling pathway. *Am J Physiol Renal Physiol*. 2022;322:F611–24.
34. Chen B, Brem AS, Gong R. The Janus view: dual roles for hypoxia-inducible factor in renal repair after acute kidney injury. *Am J Physiol Renal Physiol*. 2022;323:F1–f3.
35. Kanbay M, Tapoi L, Ureche C, Tanriover C, Cevik E, Demiray A, et al. Effect of sodium–glucose cotransporter 2 inhibitors on hemoglobin and hematocrit levels in type 2 diabetes: a systematic review and meta-analysis. *Int Urol Nephrol*. 2022;54:827–41.
36. Afsar B, Afsar RE, Ertuglu LA, Covic A, Kanbay M. Nutrition, immunology, and kidney: looking beyond the horizons. *Curr Nutr Rep*. 2022;11:69–81.
37. Komada T, Muruve DA. The role of inflammasomes in kidney disease. *Nat Rev Nephrol*. 2019;15:501–20.
38. Zhang H, Wang Z. Effect and regulation of the NLRP3 inflammasome during renal fibrosis. *Front Cell Dev Biol*. 2019;7:379.
39. Benetti E, Mastrocola R, Vitarelli G, Cutrin JC, Nigro D, Chiazza F, et al. Empagliflozin protects against diet-induced NLRP-3 inflammasome activation and lipid accumulation. *J Pharmacol Exp Ther*. 2016;359:45–53.
40. Ke Q, Shi C, Lv Y, Wang L, Luo J, Jiang L, et al. SGLT2 inhibitor counteracts NLRP3 inflammasome via tubular metabolite itaconate in fibrosis kidney. *Faseb j*. 2022;36:e22078.
41. Youm YH, Nguyen KY, Grant RW, Goldberg EL, Bodogai M, Kim D, et al. The ketone metabolite β -hydroxybutyrate blocks NLRP3 inflammasome-mediated inflammatory disease. *Nat Med*. 2015;21:263–9.
42. Wu X, Li H, Wan Z, Wang R, Liu J, Liu Q, et al. The combination of ursolic acid and empagliflozin relieves diabetic nephropathy by reducing inflammation, oxidative stress and renal fibrosis. *Biomed Pharmacother*. 2021;144:112267.
43. Wang XX, Levi J, Luo Y, Myakala K, Herman-Edelstein M, Qiu L, et al. SGLT2 protein expression is increased in human diabetic nephropathy: SGLT2 protein inhibition decreases renal lipid accumulation, inflammation, and the development of nephropathy in diabetic mice. *J Biol Chem*. 2017;292:5335–48.
44. Tang L, Wu Y, Tian M, Sjöström CD, Johansson U, Peng XR, et al. Dapagliflozin slows the progression of the renal and liver fibrosis associated with type 2 diabetes. *Am J Physiol Endocrinol Metab*. 2017;313:E563–76.
45. Terami N, Ogawa D, Tachibana H, Hatanaka T, Wada J, Nakatsuka A, et al. Long-term treatment with the sodium glucose cotransporter 2 inhibitor, dapagliflozin, ameliorates glucose homeostasis and diabetic nephropathy in db/db mice. *PLoS ONE*. 2014;9:e100777.
46. Okuma H, Mori K, Nakamura S, Sekine T, Ogawa Y, Tsuchiya K. Ipragliflozin ameliorates diabetic nephropathy associated with perirenal adipose expansion in mice. *Int J Mol Sci*. 2021;22:1.
47. Hatanaka T, Ogawa D, Tachibana H, Eguchi J, Inoue T, Yamada H, et al. Inhibition of SGLT2 alleviates diabetic nephropathy by suppressing high glucose-induced oxidative stress in type 1 diabetic mice. *Pharmacol Res Perspect*. 2016;4:e00239.
48. 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;47:686–92.
49. Yao D, Wang S, Wang M, Lu W. Renoprotection of dapagliflozin in human renal proximal tubular cells via the inhibition of the high mobility group box 1-receptor for advanced glycation end products-nuclear factor- κ B signaling pathway. *Mol Med Rep*. 2018;18:3625–30.
50. Abbas NAT, El Salem A, Awad MM. Empagliflozin, SGLT(2) inhibitor, attenuates renal fibrosis in rats exposed to unilateral ureteric obstruction: potential role of klotho expression. *Naunyn Schmiedebergs Arch Pharmacol*. 2018;391:1347–60.
51. Huang J, Chen Z, Li J, Chen Q, Li J, Gong W, et al. Protein kinase CK2 α catalytic subunit ameliorates diabetic renal inflammatory fibrosis via NF- κ B signaling pathway. *Biochem Pharmacol*. 2017;132:102–17.
52. Panchapakesan U, Pegg K, Gross S, Komala MG, Mudaliar H, Forbes J, et al. Effects of SGLT2 inhibition in human kidney proximal tubular cells—Renoprotection in diabetic nephropathy? *PLoS ONE*. 2013;8:e54442.
53. Heerspink HJL, Perco P, Mulder S, Leierer J, Hansen MK, Heinzel A, et al. Canagliflozin reduces inflammation and fibrosis biomarkers: a potential mechanism of action for beneficial effects of SGLT2 inhibitors in diabetic kidney disease. *Diabetologia*. 2019;62:1154–66.
54. Stieger N, Worthmann K, Teng B, Engeli S, Das AM, Haller H, et al. Impact of high glucose and transforming growth

- factor- β on bioenergetic profiles in podocytes. *Metabolism*. 2012;61:1073–86.
55. Das NA, Carpenter AJ, Belenchia A, Aroor AR, Noda M, Siebenlist U, et al. Empagliflozin reduces high glucose-induced oxidative stress and miR-21-dependent TRAF3IP2 induction and RECK suppression, and inhibits human renal proximal tubular epithelial cell migration and epithelial-to-mesenchymal transition. *Cell Signal*. 2020;68:109506.
 56. Woods TC, Satou R, Miyata K, Katsurada A, Dugas CM, Klingenberg NC, et al. Canagliflozin prevents intrarenal angiotensinogen augmentation and mitigates kidney injury and hypertension in mouse model of type 2 diabetes mellitus. *Am J Nephrol*. 2019;49:331–42.
 57. Tanaka S, Sugiura Y, Saito H, Sugahara M, Higashijima Y, Yamaguchi J, et al. Sodium–glucose cotransporter 2 inhibition normalizes glucose metabolism and suppresses oxidative stress in the kidneys of diabetic mice. *Kidney Int*. 2018;94:912–25.
 58. Ferrannini E, Muscelli E, Frascerra S, Baldi S, Mari A, Heise T, et al. Metabolic response to sodium–glucose cotransporter 2 inhibition in type 2 diabetic patients. *J Clin Invest*. 2014;124:499–508.
 59. DeFronzo RA, Reeves WB, Awad AS. Pathophysiology of diabetic kidney disease: impact of SGLT2 inhibitors. *Nat Rev Nephrol*. 2021;17:319–34.
 60. Packer M. SGLT2 inhibitors produce cardiorenal benefits by promoting adaptive cellular reprogramming to induce a state of fasting mimicry: a paradigm shift in understanding their mechanism of action. *Diabetes Care*. 2020;43:508–11.
 61. Tomita I, Kume S, Sugahara S, Osawa N, Yamahara K, Yasuda-Yamahara M, et al. SGLT2 inhibition mediates protection from diabetic kidney disease by promoting ketone body-induced mTORC1 inhibition. *Cell Metab*. 2020;32:404–19.e6.
 62. Hawley SA, Ford RJ, Smith BK, Gowans GJ, Mancini SJ, Pitt RD, et al. The Na⁺/glucose cotransporter inhibitor canagliflozin activates AMPK by inhibiting mitochondrial function and increasing cellular AMP levels. *Diabetes*. 2016;65:2784–94.
 63. Han Y, Xiong S, Zhao H, Yang S, Yang M, Zhu X, et al. Lipophagy deficiency exacerbates ectopic lipid accumulation and tubular cells injury in diabetic nephropathy. *Cell Death Dis*. 2021;12:1031.
 64. Zhang Z, Ni L, Zhang L, Zha D, Hu C, Zhang L, et al. Empagliflozin regulates the AdipoR1/p-AMPK/p-ACC pathway to alleviate lipid deposition in diabetic nephropathy. *Diabetes Metab Syndr Obes*. 2021;14:227–40.
 65. Ding H, Jiang L, Xu J, Bai F, Zhou Y, Yuan Q, et al. Inhibiting aerobic glycolysis suppresses renal interstitial fibroblast activation and renal fibrosis. *Am J Physiol Renal Physiol*. 2017;313:F561–75.
 66. Inoue MK, Matsunaga Y, Nakatsu Y, Yamamotoya T, Ueda K, Kushiyaama A, et al. Possible involvement of normalized Pin1 expression level and AMPK activation in the molecular mechanisms underlying renal protective effects of SGLT2 inhibitors in mice. *Diabetol Metab Syndr*. 2019;11:57.
 67. Lee YH, Kim SH, Kang JM, Heo JH, Kim DJ, Park SH, et al. Empagliflozin attenuates diabetic tubulopathy by improving mitochondrial fragmentation and autophagy. *Am J Physiol Renal Physiol*. 2019;317:F767–80.
 68. Goldberg H, Whiteside C, Fantus IG. O-linked β -N-acetylglucosamine supports p38 MAPK activation by high glucose in glomerular mesangial cells. *Am J Physiol Endocrinol Metab*. 2011;301:E713–26.
 69. Shibusawa R, Yamada E, Okada S, Nakajima Y, Bastie CC, Maeshima A, et al. Dapagliflozin rescues endoplasmic reticulum stress-mediated cell death. *Sci Rep*. 2019;9:9887.
 70. Afsar B, Hornum M, Afsar RE, Ertuglu LA, Ortiz A, Covic A, et al. Mitochondrion-driven nephroprotective mechanisms of novel glucose lowering medications. *Mitochondrion*. 2021;58:72–82.
 71. Xie Y, Jing E, Cai H, Zhong F, Xiao W, Gordon RE, et al. Reticulon-1A mediates diabetic kidney disease progression through endoplasmic reticulum–mitochondrial contacts in tubular epithelial cells. *Kidney Int*. 2022;102:293–306.
 72. Siragy HM, Carey RM. Role of the intrarenal renin-angiotensin-aldosterone system in chronic kidney disease. *Am J Nephrol*. 2010;31:541–50.
 73. Gross O, Schulze-Lohoff E, Koepke ML, Beirowski B, Addicks K, Bloch W, et al. Antifibrotic, nephroprotective potential of ACE inhibitor vs. AT1 antagonist in a murine model of renal fibrosis. *Nephrol Dial Transpl*. 2004;19:1716–23.
 74. Rubel B, Stock J, Ciner A, Hiller H, Giergert R, Müller GA, et al. Antifibrotic, nephroprotective effects of paricalcitol versus calcitriol on top of ACE-inhibitor therapy in the COL4A3 knockout mouse model for progressive renal fibrosis. *Nephrol Dial Transpl*. 2014;29:1012–9.
 75. Feldman DL, Jin L, Xuan H, Contrepas A, Zhou Y, Webb RL, et al. Effects of aliskiren on blood pressure, albuminuria, and (pro)renin receptor expression in diabetic TG(mRen-2)/27 rats. *Hypertension*. 2008;52:130–6.
 76. Han KH, Kang YS, Han SY, Jee YH, Lee MH, Han JY, et al. Spironolactone ameliorates renal injury and connective tissue growth factor expression in type II diabetic rats. *Kidney Int*. 2006;70:111–20.
 77. Koszegi S, Molnar A, Lenart L, Hodrea J, Balogh DB, Lakat T, et al. RAAS inhibitors directly reduce diabetes-induced renal fibrosis via growth factor inhibition. *J Physiol*. 2019;597:193–209.
 78. Morrissey JJ, Klahr S. Effect of AT2 receptor blockade on the pathogenesis of renal fibrosis. *Am J Physiol*. 1999;276:F39–45.
 79. Crowley SD, Rudemiller NP. Immunologic effects of the renin-angiotensin system. *J Am Soc Nephrol*. 2017;28:1350–61.
 80. Liu Z, Huang XR, Chen HY, Penninger JM, Lan HY. Loss of angiotensin-converting enzyme 2 enhances TGF- β /Smad-mediated renal fibrosis and NF- κ B-driven renal inflammation in a mouse model of obstructive nephropathy. *Lab Invest*. 2012;92:650–61.
 81. Mao N, Tan RZ, Wang SQ, Wei C, Shi XL, Fan JM, et al. Ginsenoside Rg1 inhibits angiotensin II-induced podocyte autophagy via AMPK/mTOR/PI3K pathway. *Cell Biol Int*. 2016;40:917–25.
 82. Lizakowski S, Tylicki L, Renke M, Rutkowski P, Heleniak Z, Sławińska-Morawska M, et al. Aliskiren and perindopril reduce the levels of transforming growth factor- β in patients with non-diabetic kidney disease. *Am J Hypertens*. 2012;25:636–9.
 83. Cuevas CA, Gonzalez AA, Inestrosa NC, Vio CP, Prieto MC. Angiotensin II increases fibronectin and collagen I through the β -catenin-dependent signaling in mouse collecting duct cells. *Am J Physiol Renal Physiol*. 2015;308:F358–65.
 84. Shin SJ, Chung S, Kim SJ, Lee EM, Yoo YH, Kim JW, et al. Effect of sodium–glucose co-transporter 2 inhibitor, dapagliflozin, on renal renin-angiotensin system in an animal model of type 2 diabetes. *PLoS ONE*. 2016;11:e0165703.
 85. Miyata KN, Lo CS, Zhao S, Liao MC, Pang Y, Chang SY, et al. Angiotensin II up-regulates sodium–glucose co-transporter 2 expression and SGLT2 inhibitor attenuates Ang II-induced hypertensive renal injury in mice. *Clin Sci (Lond)*. 2021;135:943–61.
 86. Gallo LA, Ward MS, Fotheringham AK, Zhuang A, Borg DJ, Flemming NB, et al. Once daily administration of the SGLT2 inhibitor, empagliflozin, attenuates markers of renal fibrosis without improving albuminuria in diabetic db/db mice. *Sci Rep*. 2016;6:26428.
 87. 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.
88. Tanaka H, Takano K, Iijima H, Kubo H, Maruyama N, Hashimoto T, et al. Factors affecting canagliflozin-induced transient urine volume increase in patients with type 2 diabetes mellitus. *Adv Ther*. 2017;34:436–51.
 89. Takeshige Y, Fujisawa Y, Rahman A, Kittikuluth W, Nakano D, Mori H, et al. A sodium–glucose co-transporter 2 inhibitor empagliflozin prevents abnormality of circadian rhythm of blood pressure in salt-treated obese rats. *Hypertens Res*. 2016;39:415–22.
 90. Sawamura T, Karashima S, Nagase S, Nambo H, Shimizu E, Higashitani T, et al. Effect of sodium–glucose cotransporter-2 inhibitors on aldosterone-to-renin ratio in diabetic patients with hypertension: a retrospective observational study. *BMC Endocr Disord*. 2020;20:177.
 91. Ryu ES, Kim MJ, Shin HS, Jang YH, Choi HS, Jo I, et al. Uric acid-induced phenotypic transition of renal tubular cells as a novel mechanism of chronic kidney disease. *Am J Physiol Renal Physiol*. 2013;304:F471–80.
 92. Bailey CJ. Uric acid and the cardio-renal effects of SGLT2 inhibitors. *Diabetes Obes Metab*. 2019;21:1291–8.
 93. Novikov A, Fu Y, Huang W, Freeman B, Patel R, van Ginkel C, et al. SGLT2 inhibition and renal urate excretion: role of luminal glucose, GLUT9, and URAT1. *Am J Physiol Renal Physiol*. 2019;316:F173–85.
 94. Suijk DLS, van Baar MJB, van Bommel EJM, Iqbal Z, Krebber MM, Vallon V, et al. SGLT2 inhibition and uric acid excretion in patients with type 2 diabetes and normal kidney function. *Clin J Am Soc Nephrol*. 2022;17:663–71.
 95. Huang F, Zhao Y, Wang Q, Hillebrands JL, van den Born J, Ji L, et al. Dapagliflozin attenuates renal tubulointerstitial fibrosis associated with type 1 diabetes by regulating STAT1/TGFβ1 signaling. *Front Endocrinol (Lausanne)*. 2019;10:441.
 96. Kanbay M, Demiray A, Afsar B, Covic A, Tapoi L, Ureche C, et al. Role of klotho in the development of essential hypertension. *Hypertension*. 2021;77:740–50.
 97. Zhou L, Mo H, Miao J, Zhou D, Tan RJ, Hou FF, et al. Klotho ameliorates kidney injury and fibrosis and normalizes blood pressure by targeting the renin-angiotensin system. *Am J Pathol*. 2015;185:3211–23.
 98. Zhang Y, Nakano D, Guan Y, Hitomi H, Uemura A, Masaki T, et al. A sodium–glucose cotransporter 2 inhibitor attenuates renal capillary injury and fibrosis by a vascular endothelial growth factor-dependent pathway after renal injury in mice. *Kidney Int*. 2018;94:524–35.
 99. Gomez IG, Roach AM, Nakagawa N, Amatucci A, Johnson BG, Dunn K, et al. TWEAK-Fn14 signaling activates myofibroblasts to drive progression of fibrotic kidney disease. *J Am Soc Nephrol*. 2016;27:3639–52.
 100. Shen ZJ, Hu J, Shiizaki K, Kuro-o M, Malter JS. Phosphate-induced renal fibrosis requires the prolyl isomerase pin1. *PLoS ONE*. 2016;11:e0150093.
 101. Liu H, Sridhar VS, Lovblom LE, Lytvyn Y, Burger D, Burns K, et al. Markers of kidney injury, inflammation, and fibrosis associated with ertugliflozin in patients with CKD and diabetes. *Kidney Int Rep*. 2021;6:2095–104.
 102. Ma Q, Steiger S, Anders HJ. Sodium glucose transporter-2 inhibition has no renoprotective effects on non-diabetic chronic kidney disease. *Physiol Rep*. 2017;5:1.
 103. Droebner K, Pavkovic M, Grundmann M, Hartmann E, Goea L, Nordlohne J, et al. Direct blood pressure-independent anti-fibrotic effects by the selective nonsteroidal mineralocorticoid receptor antagonist finerenone in progressive models of kidney fibrosis. *Am J Nephrol*. 2021;52:588–601.
 104. Zhang Y, Thai K, Kepecs DM, Gilbert RE. Sodium–glucose linked cotransporter-2 inhibition does not attenuate disease progression in the rat remnant kidney model of chronic kidney disease. *PLoS ONE*. 2016;11:e0144640.
 105. Vallon V, Gerasimova M, Rose MA, Masuda T, Satriano J, Mayoux E, et al. SGLT2 inhibitor empagliflozin reduces renal growth and albuminuria in proportion to hyperglycemia and prevents glomerular hyperfiltration in diabetic Akita mice. *Am J Physiol Renal Physiol*. 2014;306:F194–204.
 106. Gangadharan Komala M, Gross S, Mudaliar H, Huang C, Pegg K, Mather A, et al. Inhibition of kidney proximal tubular glucose reabsorption does not prevent against diabetic nephropathy in type 1 diabetic eNOS knockout mice. *PLoS ONE*. 2014;9:e108994.
 107. Zeng S, Delic D, Chu C, Xiong Y, Luo T, Chen X, et al. Antifibrotic effects of low dose SGLT2 inhibition with empagliflozin in comparison to Ang II receptor blockade with telmisartan in 5/6 nephrectomised rats on high salt diet. *Biomed Pharmacother*. 2022;146:112606.
 108. Tauber P, Sinha F, Berger RS, Gronwald W, Dettmer K, Kuhn M, et al. Empagliflozin reduces renal hyperfiltration in response to uninephrectomy, but is not nephroprotective in UNx/DOCA/Salt mouse models. *Front Pharmacol*. 2021;12:761855.
 109. Kojima N, Williams JM, Takahashi T, Miyata N, Roman RJ. Effects of a new SGLT2 inhibitor, luseogliflozin, on diabetic nephropathy in T2DN rats. *J Pharmacol Exp Ther*. 2013;345:464–72.
 110. Kojima N, Williams JM, Slaughter TN, Kato S, Takahashi T, Miyata N, et al. Renoprotective effects of combined SGLT2 and ACE inhibitor therapy in diabetic Dahl S rats. *Physiol Rep*. 2015;3:1.
 111. Abdel-Wahab AF, Bamagous GA, Al-Harizy RM, ElSawy NA, Shahzad N, Ibrahim IA, et al. Renal protective effect of SGLT2 inhibitor dapagliflozin alone and in combination with irbesartan in a rat model of diabetic nephropathy. *Biomed Pharmacother*. 2018;103:59–66.
 112. Jaikumkao K, Pongchaidecha A, Chueakula N, Thongnak LO, Wanchai K, Chatsudthipong V, et al. Dapagliflozin, a sodium–glucose co-transporter-2 inhibitor, slows the progression of renal complications through the suppression of renal inflammation, endoplasmic reticulum stress and apoptosis in prediabetic rats. *Diabetes Obes Metab*. 2018;20:2617–26.
 113. Tahara A, Takasu T. Prevention of progression of diabetic nephropathy by the SGLT2 inhibitor ipragliflozin in uninephrectomized type 2 diabetic mice. *Eur J Pharmacol*. 2018;830:68–75.
 114. Wang D, Luo Y, Wang X, Orlicky DJ, Myakala K, Yang P, et al. The sodium-glucose cotransporter 2 inhibitor dapagliflozin prevents renal and liver disease in western diet induced obesity mice. *Int J Mol Sci*. 2018;19:1.
 115. Ali BH, Al Salam S, Al Suleimani Y, Al Za'abi M, Abdelrahman AM, Ashique M, et al. Effects of the SGLT-2 inhibitor canagliflozin on adenine-induced chronic kidney disease in rats. *Cell Physiol Biochem*. 2019;52:27–39.
 116. Cai T, Ke Q, Fang Y, Wen P, Chen H, Yuan Q, et al. Sodium–glucose cotransporter 2 inhibition suppresses HIF-1α-mediated metabolic switch from lipid oxidation to glycolysis in kidney tubule cells of diabetic mice. *Cell Death Dis*. 2020;11:390.
 117. Castoldi G, Carletti R, Ippolito S, Colzani M, Barzaghi F, Stella A, et al. Renal anti-fibrotic effect of sodium glucose cotransporter 2 inhibition in angiotensin II-dependent hypertension. *Am J Nephrol*. 2020;51:119–29.
 118. Hasan R, Lasker S, Hasan A, Zerín F, Zamila M, Parvez F, et al. Canagliflozin ameliorates renal oxidative stress and inflammation by stimulating AMPK-Akt-eNOS pathway in the isoprenaline-induced oxidative stress model. *Sci Rep*. 2020;10:14659.
 119. Nespoux J, Patel R, Zhang H, Huang W, Freeman B, Sanders PW, et al. Gene knockout of the Na(+)-glucose cotransporter SGLT2

- in a murine model of acute kidney injury induced by ischemia-reperfusion. *Am J Physiol Renal Physiol.* 2020;318:F1100–12.
120. Yamato M, Kato N, Kakino A, Yamada KI, Inoguchi T. Low dose of sodium–glucose transporter 2 inhibitor ipragliflozin attenuated renal dysfunction and interstitial fibrosis in adenine-induced chronic kidney disease in mice without diabetes. *Metabol Open.* 2020;7:100049.
 121. Castoldi G, Carletti R, Ippolito S, Colzani M, Barzagli F, Stella A, et al. Sodium–glucose cotransporter 2 inhibition prevents renal fibrosis in cyclosporine nephropathy. *Acta Diabetol.* 2021;58:1059–70.
 122. Kolkhof P, Hartmann E, Freyberger A, Pavkovic M, Mathar I, Sandner P, et al. Effects of finerenone combined with empagliflozin in a model of hypertension-induced end-organ damage. *Am J Nephrol.* 2021;52:642–52.
 123. Pan X, Phanish MK, Baines DL, Dockrell MEC. High glucose-induced Smad3 linker phosphorylation and CCN2 expression are inhibited by dapagliflozin in a diabetic tubule epithelial cell model. *Biosci Rep.* 2021;41:1.
 124. Ko EJ, Shin YJ, Cui S, Lim SW, Chung BH, Yang CW. Effect of dual inhibition of DPP4 and SGLT2 on tacrolimus-induced diabetes mellitus and nephrotoxicity in a rat model. *Am J Transpl.* 2022;22:1537–49.

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Springer Nature or its licensor (e.g. a society or other partner) holds exclusive rights to this article under a publishing agreement with the author(s) or other rightsholder(s); author self-archiving of the accepted manuscript version of this article is solely governed by the terms of such publishing agreement and applicable law.

Authors and Affiliations

Baris Afsar¹  · Rengin Elsurer Afsar¹

✉ Baris Afsar
 afsarbrs@yahoo.com; barisafsar@sdu.edu.tr

¹ Department of Nephrology, School of Medicine, Suleyman Demirel University, Isparta, Turkey