FACULDADE DE MEDICINA
UNIVERSIDADE D
COIMBRA

MESTRADO INTEGRADO EM MEDICINA – TRABALHO FINAL

LUÍS HENRIQUE CERVEIRA DA CRUZ PINTO

Renoprotective Mechanisms of SGLT2 Inhibitors in Type 2 Diabetes Patients with Chronic Kidney Disease

ARTIGO DE REVISÃO NARRATIVA

ÁREA CIENTÍFICA DE ENDOCRINOLOGIA

Trabalho realizado sob a orientação de:

PROFESSORA DOUTORA MARIA LEONOR VIEGAS GOMES

DOUTORA DIANA FILIPA DA SILVA CATARINO

Abril/2022

RENOPROTECTIVE MECHANISMS OF SGLT2 INHIBITORS IN TYPE 2 DIABETES PATIENTS WITH CHRONIC KIDNEY DISEASE

MECANISMOS RENOPROTETORES DOS INIBIDORES DOS CO-TRANSPORTADORES RENAIS DE SÓDIO-GLUCOSE DO TIPO 2 EM DOENTES COM DIABETES MELLITUS TIPO 2 E DOENÇA RENAL CRÓNICA

Autor: Luís Henrique Cerveira da Cruz Pinto [a] – henriquecerveira@hotmail.com
Co-orientador: Dr.ª Diana Filipa da Silva Catarino, MD [a,b] – diana_catarino@hotmail.com
Orientador: Dr.ª Maria Leonor Viegas Gomes, PhD [a,b] – mgomes@fmed.uc.pt

(a) Faculdade de Medicina, Universidade de Coimbra, Portugal

(b) Serviço de Endocrinologia, Diabetes e Metabolismo, Centro Hospitalar e Universitário de Coimbra, Coimbra, Portugal

INDEX

ABSTRACT

***Background**:* Diabetes *mellitus* is a global epidemic estimated to affect more than 420 million people worldwide and predicted to impact about 630 million until 2045. Chronic kidney disease is a frequent complication of diabetes *mellitus*, especially the type 2, which is more common and associated with the patient's lifestyle. The sodium-glucose cotransporter 2 inhibitors (iSGLT2), approved as antidiabetic agents, improve the glycaemic control in patients with type 2 diabetes *mellitus* and chronic kidney disease whose estimated glomerular filtration rate is equal or more than 30 mL/min/1.73 m^2. When compared to other glucose-lowering agents, iSGLT2 showed a greater capacity to slow the progression of chronic kidney disease, indicating the existence of other mechanisms of action that allow them to exert renoprotection.

Objectives: This review aims to describe the potential renoprotective mechanisms of iSGLT2 proposed thus far.

Methods: Published articles were searched by applying the keywords in PubMed and Medline, from 2013 to 2022.

Discussion/Conclusion: The mechanisms that underlie the renoprotection achieved with iSGLT2 are not fully known. However, it is believed that the renal benefits of these agents derive from a combination of different endocrine, metabolic, haemodynamic and biochemical mechanisms, that may involve natriuresis, restoration of the renal tubuloglomerular feedback, reduction of blood pressure and other vascular effects, reduction of intrarenal hypoxia, anti-inflammatory and antifibrotic effects, among others.

Keywords: Type 2 Diabetes *Mellitus*; Chronic Kidney Disease; Inhibitors of SGLT2; Renoprotective Mechanisms

RESUMO

***Contexto**:* A diabetes *mellitus* é uma epidemia global, estimando-se que afete mais de 420 milhões de pessoas no mundo e prevendo-se que abarque cerca de 630 milhões até 2045. A doença renal crónica é uma complicação frequente da diabetes *mellitus*, especialmente da de tipo 2, a qual é mais comum e relacionada com o estilo de vida. Os inibidores dos co-transportadores renais de sódio-glucose do tipo 2 (iSGLT2), aprovados como agentes antidiabéticos, melhoram o controlo glicémico em doentes com diabetes *mellitus* tipo 2 e doença renal crónica cuja taxa de filtração glomerular estimada seja igual ou superior a 30 mL/min/1,73 m^2. Quando comparados aos restantes fármacos hipoglicemiantes, os iSGLT2 mostraram maior capacidade de retardar a progressão da doença renal crónica, apontando para a existência de outros mecanismos de ação que lhes permitam exercer renoproteção.

Objetivos: Esta revisão pretende descrever os potenciais mecanismos renoprotetores dos iSGLT2 até então propostos.

Métodos: Foi efetuada uma revisão da literatura publicada aplicando as palavras-chave na PubMed e Medline, desde 2013 até 2022.

Discussão/Conclusão: Os mecanismos que justificam a renoproteção por parte dos iSGLT2 não são completamente conhecidos. Ainda assim, acredita-se que os benefícios renais destes agentes resultem de um conjunto de diferentes mecanismos endócrinos, metabólicos, hemodinâmicos e bioquímicos, que podem envolver natriurese, restabelecimento do *feedback* tubuloglomerular renal, redução da pressão arterial e outros efeitos vasculares, redução da hipóxia intrarrenal, efeitos anti-inflamatórios e anti-fibróticos, entre outros.

Palavras-chave: Diabetes *Mellitus* Tipo 2; Doença Renal Crónica; Inibidores dos Co-transportadores Renais de Sódio-Glucose do Tipo 2; Mecanismos Renoprotetores

ABBREVIATIONS

iSGLT2: Inhibitors of sodium-glucose cotransporter 2
DM: Diabetes *mellitus*
T2DM: Type 2 diabetes *mellitus*
DKD: Diabetic kidney disease
CKD: Chronic kidney disease
eGFR: Estimated glomerular filtration rate
KDIGO: Kidney Disease: Improving Global Outcomes
WHO: World Health Organization
CVD: Cardiovascular disease
ESRD: End-stage renal disease
UACR: Urinary albumin-to-creatinine ratio
HbA1c: Glycated haemoglobin
RAAS: Renin-angiotensin-aldosterone system
ACEi: Angiotensin-converting enzyme inhibitors
ARB: Angiotensin receptor blockers
SGLTs: Sodium-glucose cotransporters
GLUTs: Facilitated glucose transporters
PRT: Proximal renal tubules
EMA: European Medicines Agency
FDA: Food and Drug Administration
CVOTs: Cardiovascular outcome trials
TmG: Maximum capacity of glucose transport
SNGFR: Single-nephron glomerular filtration rate
GFR: Glomerular filtration rate
TGF: Tubuloglomerular feedback
NHE2: Sodium-hydrogen exchanger-2 transporter
NHE3: Sodium-hydrogen exchanger-3 transporter
AMPK: Adenosine monophosphate-activated protein kinase
SIRT1: Sirtuin-1
mTORC1: Rapamycin-sensitive complex of mTOR
BCAA: Branched-chain aminoacids
NF-kB: Nuclear factor-kB
IL-6: Interleukin-6
MCP-1: Monocyte chemoattractant protein-1
TNFR1: Tumour necrosis factor receptor-1

TNF-α: Tumour necrosis factor alpha

MMP7: Matrix metalloproteinase 7

NLRP3: Nod-like receptor pyrin domain-containing protein 3

URAT1: Urate transporter-1

BP: Blood pressure

SNS: Sympathetic nervous system

FFA: Free fatty acids

ATP: Adenosine triphosphate

AQP2: Aquaporin-2 channels

NAFLD: Non-alcoholic fatty liver disease

NASH: Non-alcoholic steatohepatitis

EPO: Erythropoietin

HIF: Hypoxia-inducible factor

INTRODUCTION

Diabetes *mellitus* (DM) is a chronic metabolic disease responsible for serious multiorgan damage and one of the leading causes of death worldwide.(1, 2) Around 35% of patients with type 2 diabetes *mellitus* (T2DM), the most frequent type of diabetes, develop diabetic kidney disease (DKD) 15-25 years after the diagnosis.(3, 4) DKD is the leading cause of chronic kidney disease (CKD), associated with increased morbidity and mortality.(3, 5, 6) The association of metformin with one of the inhibitors of sodium-glucose cotransporter 2 (iSGLT2) is the first line of antihyperglycaemic treatment in many T2DM patients.(4, 7) In Europe, four iSGLT2 (canagliflozin, dapagliflozin, empagliflozin and ertugliflozin) are approved for clinical use in T2DM, when the estimated glomerular filtration rate (eGFR) is ≥30 mL/min/1.73 m^2, according to the KDIGO 2020 guideline.(7-9) Despite their primary glucose-lowering effect that rendered them such approval, iSGLT2 have shown cardiovascular benefits and the capacity to slow the progression of CKD and improve renal outcomes in T2DM.(10, 11) Their beneficial role in the kidney outcomes can be explained by their glucose-lowering effect, but also by a combination of glycaemic-independent mechanisms.(11-13)

The favourable results of recent clinical trials have prompted an extension of the iSGLT2 therapy to comprise T2DM patients with a low eGFR who were previously not eligible for this type of treatment.(4, 10, 14) In fact, iSGLT2 were shown to exert a great overall renal protection, while also significantly improving the cardiovascular outcomes.(11, 15) Given the high prevalence of CKD among T2DM patients, and the consequent increase of hospitalization rates, healthcare costs and premature mortality, as well as the decrease in quality of life, it is of major interest that the eligible patients are identified early and start therapy with iSGLT2 straightaway so that the CKD progression can be delayed.(4, 7, 15) Despite solid evidence for the beneficial role of iSGLT2, these drugs are still underprescribed among eligible patients, which appears to mainly result from clinical inertia and safety concerns in older patients.(16) Their recent introduction in the market and recent changes in clinical guidelines may also be a reason for underprescription.(17) Moreover, it seems that the decision to start iSGLT2 therapy is often deferred to endocrinologists.(16) Therefore, a better understanding of the therapeutic value of iSGLT2 in the management of CKD in T2DM patients, along with better awareness of the mechanisms that may underlie such benefits, must be promoted among all healthcare providers, especially primary care physicians, diabetologists, nephrologists and cardiologists.(16, 18, 19)

Our main goal is to analyse the published data on iSGLT2 therapy in patients with T2DM and CKD and review the main effects of these agents, along with the potential underlying mechanisms that might explain their renoprotective role. Here we focus on the impact of

iSGLT2 in the diabetic kidney. Other beneficial effects of iSGLT2, namely in the heart metabolism and cardiovascular system, are not part of this review's scope.

METHODS

We searched PubMed and Medline for published articles using the Medical Subjects Heading Terms (MESH) to create the following searching equation "(("Sodium-Glucose Transporter 2 Inhibitors"[Mesh]) AND "Renal Insufficiency, Chronic"[Mesh]) AND "Diabetes Mellitus, Type 2"[Mesh]". The search was restricted to the English language and all articles published from 2013 to March 11th 2022 were initially accepted. We obtained 197 references.

The inclusion criteria comprised clinical studies, observational studies, comparative studies, controlled clinical trials, meta-analysis, reviews, multicentre studies, randomized controlled trials, journal articles and practice guidelines. Commentary articles were also included when considered scientifically relevant. The Kidney Disease: Improving Global Outcomes (KDIGO) guidelines and diabetes reports from the World Health Organization (WHO) relevant to the scope of this review were similarly included.

Articles without full text available, focusing on different aspects of iSGLT2 than those intended for this review (e.g., cardiac metabolism), or outdated considering newer published studies were all excluded. The exclusion process was done through the screening of titles, abstracts and full text assessment.

By applying the aforementioned criteria, 41 references were selected as the final literary basis for this review.

THE KIDNEY IN TYPE 2 DIABETES MELLITUS

DM is a chronic metabolic disease in which the blood glucose levels are elevated, leading to serious multiorgan damage. According to the WHO, more than 420 million people currently live with type 1 or 2 DM worldwide, corresponding to 6% of the world's population,(1) and around 1.5 million deaths are directly linked to this disease each year, making it one of the world's leading causes of death and a global epidemic.(2) Its prevalence is predicted to increase by 48% until 2045, meaning that 629 million people are expected to develop diabetes.(3)

T2DM, the most common type of diabetes in adults, is characterised by resistance to insulin and/or insufficiency of its production.(2) This chronic metabolic condition is closely

associated with excessive body weight and physical inactivity, but also influenced by race, ethnicity and age.(1) CKD, cardiovascular disease (CVD) and heart failure are common complications that are interlinked and reduce the patient's quality of life by increasing morbidity and mortality.(20)

Diabetic Kidney Disease and Progression of Chronic Kidney Disease

DKD, also known as diabetic nephropathy, is the term used to define CKD as a complication of T2DM.(18) Along with diabetic retinopathy and neuropathy, DKD is one of the microvascular complications of T2DM and typically manifests 15-25 years after the initial diagnosis.(3) Its prevalence has stabilized due to advances in diabetes management,(4) but DKD remains responsible for 25-50% of all new cases of end-stage renal disease (ESRD), making it the leading cause of CKD requiring dialysis or kidney transplantation.(3, 21)

Despite having a multifactorial pathogenesis, with some unchangeable risk factors such as ethnic, familial and genetic predispositions, the patient's lifestyle holds a key role in the development of CKD, with hyperglycaemia, hypertension, dyslipidaemia, smoking and obesity typically present.(5)

The diagnosis of DKD is clinical and depends on the measurement of albuminuria and the eGFR.(22) It is established when the excretion of albumin in the urine is persistently high and/or the eGFR is sustainably low, after excluding other primary causes of kidney disease.(3, 21)

The levels of albuminuria are considered abnormal when ≥30 mg of albumin are found in a 24-hour urine sample or when the urinary albumin-to-creatinine ratio (UACR) is ≥30 mg/g.(18) Nonetheless, to define albuminuria at least two abnormal specimens of UACR within a period of 3 to 6 months are needed,(22) as these measurements can vary over time within individuals.(6)

Microalbuminuria (UACR ≥30 mg/g) is an early indicator of kidney damage(6, 14) that appears 5 years after the onset of the disease.(22) However, some patients reach ESRD without manifesting micro- or macroalbuminuria,(12) presenting with a low eGFR despite a relatively normal UACR.(6)

The eGFR, calculated through a validated formula, is considered abnormal when its value is ≤90 mL/min/1.73 m^2. An eGFR ≤60 mL/min/1.73 m^2 reflects a greater loss of kidney function and defines CKD.(6, 18) According to the levels of eGFR, five stages of CKD can be defined (table 1).(6, 7)

Table 1. Classification and prognostic risk of CKD according to eGFR and albuminuria.

			Albuminuria		
			Normal to mildly increased	Moderately increased	Severely increased
Stage	**eGFR (mL/min/1.73 m2)**	**Description**	<30 mg/g <3 mg/mmol	30-300 mg/g 3-30 mg/mmol	>300 mg/g >30 mg/mmol
1	≥90	Normal or high			
2	60-89	Mildly decreased			
3a	45-59	Mildly to moderately decreased			
3b	30-44	Moderately to severely decreased			
4	15-29	Severely decreased			
5	<15	Kidney failure			

eGFR: estimated glomerular filtration rate. Green: low risk. Yellow: moderately increased risk. Orange: high risk. Red: very high risk. Adapted from KDIGO 2020 guideline.

CKD consists of an abnormal renal structure and/or function present for more than 3 months and can be classified according to its cause, eGFR category and albuminuria category.(7) Since it is a frequent complication of T2DM that increases both the morbidity and mortality, it is of great importance that a tight glycaemic control is achieved and maintained to prevent its development or, if already established, slow its progression.(5, 6)

The progression of CKD increases the healthcare costs while decreasing the patient's quality of life, which is why early identification and pharmacological intervention are relevant to delay or prevent it.(23)

Management of Diabetic Kidney Disease

As mentioned, a rigorous hyperglycaemia control in diabetic patients is crucial to prevent and slow down the DKD progression. However, the management of DKD benefits from a multifaceted approach that goes beyond glycaemic control to include an efficient management of blood pressure, lipidic profile and lifestyle.(4, 22)

Current guidelines recommend a combination of metformin and one of the iSGLT2 as the first line of antihyperglycaemic treatment in patients with T2DM and CKD whose eGFR is ≥30 mL/min/1.73 m^2.(4, 7) Furthermore, the use of glycated haemoglobin (HbA1c) is recommended to monitor the glycaemic control.(7) Generally, the HbA1c goal is <7%, but the target should be individualized, ranging from <6.5% to <8.0% in patients with T2DM and CKD not receiving dialytic treatment.(3, 7)

iSGLT2 have a weak effect reducing HbA1c when compared to metformin, allowing a reduction of just 0.5-1.0%.(7, 19) Furthermore, their capacity to reduce HbA1c declines with the worsening of renal function, standing at 0.3-0.4% in CKD stage 3A (eGFR 45-59 mL/min/1.73 m^2), 0.2-0.3% in CKD stage 3B (eGFR 30-44 mL/min/1.73 m^2) and no reduction in CKD stage 4 (eGFR <30 mL/min/1.73 m^2).(10) Nevertheless, they have continuously shown

positive effects in reducing DKD progression and preventing CVD, motivating their use even when the glycaemic targets are achieved with metformin alone.(7, 24)

For the past 20 years, renin-angiotensin-aldosterone system (RAAS) inhibitors, including the angiotensin-converting enzyme inhibitors (ACEi) and angiotensin receptor blockers (ARB), have been the only agents shown to delay CKD progression in both diabetic and non-diabetic patients, while reducing the risk of kidney failure.(18, 23) However, these drugs are unable to reduce the risk of all-cause mortality. Recent clinical trials about the cardiovascular safety of iSGLT2 have shown a significantly lower risk of kidney function decline in comparison to placebo.(18) Moreover, the CREDENCE and DAPA-CKD trials have suggested that adding an iSGLT2 on top of RAAS inhibition can improve the cardiorenal outcomes in patients with and without T2DM, while decreasing the all-cause mortality.(18, 23) Under these premises, iSGLT2 are now recommended for renoprotection in T2DM patients with CKD,(7, 18) despite initially developed as glucose-lowering agents.(23)

GLUCOSE HOMEOSTASIS AND THE ROLE OF iSGLT2

The glucose homeostasis depends on a complex inter-organ interaction of different physiological processes, among which the gastrointestinal glucose absorption, glycogenolysis in the liver, hepatic and renal gluconeogenesis, and glucose reabsorption and excretion by the kidneys.(8)

The kidneys play a major role in the glucose metabolism and homeostasis, being responsible for 20-60% of glucose release into the systemic circulation and assuring a constant supply of glucose to the brain despite variations in its availability.(8) Moreover, the kidneys not only use glucose as an energetic substrate, but are also able to produce, filter and reabsorb it.(11)

In healthy individuals, the kidney filters approximately 180 litres of plasma per day, amounting to about 180 g of glucose found in the daily glomerular filtrate.(25, 26) This glucose quantity is almost entirely reabsorbed in the proximal renal tubules (PRT), otherwise it would represent a loss of 30% of the body's total caloric expenditure.(25)

Transport of Glucose in the Kidney: The SGLTs

Since the cellular membranes are impermeable to glucose,(19) this nutrient is transported by two types of carrier proteins: the sodium-glucose cotransporters (SGLTs) and the facilitated glucose transporters (GLUTs). SGLTs promote active glucose transport from the extracellular to the intracellular space against its concentration gradient while consuming

energy from sodium cotransport, whereas GLUTs passively transport glucose from the intracellular to the extracellular space following its gradient without energy consumption.(8, 26) The sodium cotransport implies adenosine triphosphate (ATP) consumption by the basolateral sodium-potassium pump of the epithelial tubular cells, which maintains a favourable sodium gradient that allows its entry into the cells along with glucose via SGLTs. Thus, glucose reabsorption starts actively in parallel to sodium reabsorption by SGLTs and ends passively with transport to the bloodstream by GLUTs (figure 1).(8, 19)

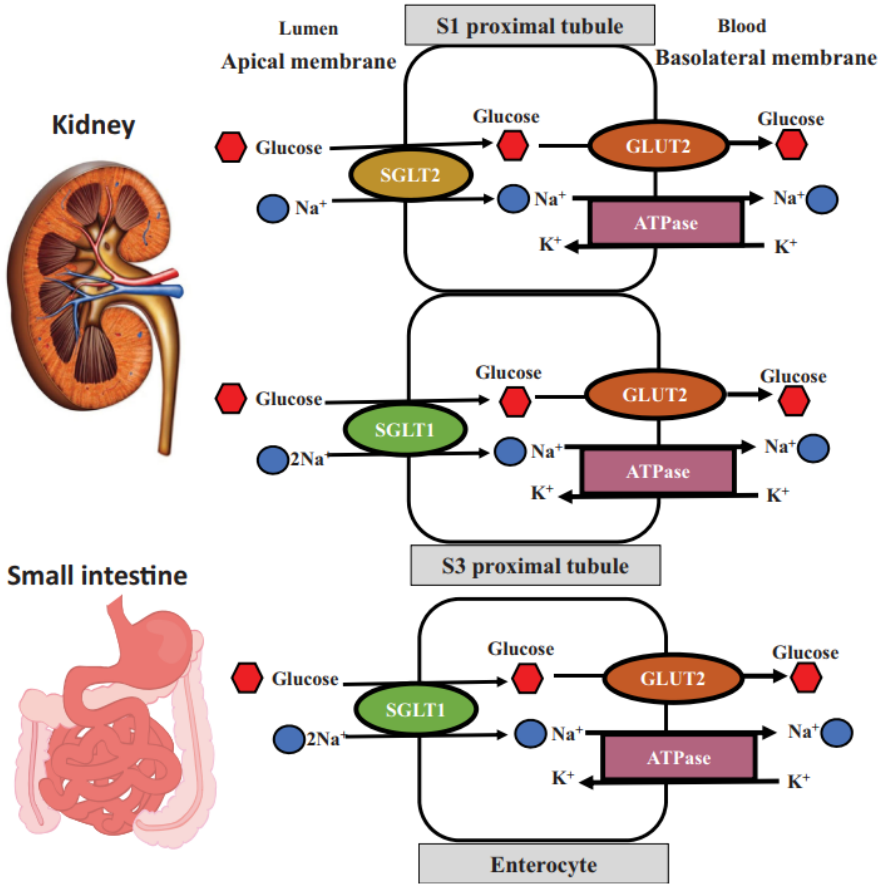

Figure 1: Glucose transport mechanisms in segments S1 and S2 of the renal tubules and in the enterocyte of the small intestine. Adapted from Brown E, et al.(8)

SGLT1 and SGLT2 are the two predominant and better studied isoforms of human SGLTs.(8, 26) SGLT1 is a low-capacity/high-affinity transporter with specificity for glucose and galactose, expressed in the small intestine's mucosa, heart and distal portion (S3) of the PRT, being responsible for 10% of glucose reabsorption. Meanwhile, SGLT2 is a high-capacity/low-affinity transporter specific for glucose, expressed almost exclusively and in higher concentration than SGLT1 in the kidney, mainly in the first segment (S1) of the PRT, being responsible for 90% of glucose reabsorption.(8, 19, 26)

SGLT2 Inhibitors

Phlorizin, a glycosuric compound discovered in the 19th century, was the first identified SGLT inhibitor, blocking both SGLT1 and SGLT2. This compound was studied in diabetes as a glucose-lowering agent but was not used as such since its oral absorption was poor, and diarrhoea would occur due to SGLT1 blockage in the gut. Nonetheless, phlorizin derivates with selectivity for the SGLT2 isoform were developed in the present century,(25) of which four are currently approved by the European Medicines Agency (EMA) and the USA Food and Drug Administration (FDA) for clinical use in T2DM (canagliflozin, dapagliflozin, empagliflozin and ertugliflozin), while sotagliflozin is approved only for T1DM.(8, 9)

After controversy with rosiglitazone, some concerns about the cardiovascular safety of glucose-lowering drugs arose, leading the FDA to demand proof of cardiovascular safety for new antidiabetic drugs in high-risk patients since 2008. Therefore, different cardiovascular outcome trials (CVOTs) were designed to evaluate cardiovascular outcomes in patients with T2DM.(19, 25, 27) Some of these trials also collected data on kidney outcomes as a secondary composite. Empagliflozin was evaluated in the EMPA-REG OUTCOME trial, canagliflozin in the CANVAS Program trial and dapagliflozin in the DECLARE-TIMI 58 trial.(25) These trials revealed that, apart from cardiovascular benefits, iSGLT2 were able to slow the progression of DKD and improve the kidney outcomes in T2DM patients with relatively preserved kidney function.(11) After such breakthrough revelations, the first dedicated renal outcome trial with canagliflozin (CREDENCE) was designed and eventually proved the cardiorenal benefits in adults with T2DM and CKD. Similar trials followed suit, such as the DAPA-CKD trial for dapagliflozin,(11, 28) and the EMPA-KIDNEY trial for empagliflozin.(4, 22) Ertugliflozin's efficacy and safety were more recently evaluated in its designated CVOT (VERTIS-CV).(20)

iSGLT2 were originally introduced as a new class of antidiabetics that would inhibit glucose reabsorption in the PRT, promoting glycosuria and decreasing hyperglycaemia.(29, 30) Thus, they were primarily approved for glycaemic control in T2DM patients, but only in those with preserved eGFR, as the later stages of CKD are characterised by attenuated glycosuria.(10) With the growing evidence that iSGLT2 can improve both the cardiovascular and the renal outcomes, as well as carrying out sodium-related effects that persist down to CKD stage 4, their use has been expanded far beyond their glucose-lowering effect. Initially-excluded patients with moderate CKD are now eligible for this treatment as prevention of cardiovascular complications, such as heart failure, and retardation of kidney function impairment.(10, 14, 19) The KDIGO 2020 guideline recommends the use of iSGLT2 in T2DM patients with CKD whose eGFR goes as low as 30 mL/min/1.73 m^2,(7) even though some of these drugs are now approved for CKD patients with an eGFR ≥25 mL/min/1.73 m^2.(18)

Glucose-lowering Effect of iSGLT2

Glycosuria depends on glycaemia and kidney function.(19) Plasmatic glucose concentrations exceeding a threshold of 160-180 mg/L correspond to an exceeded maximum capacity of glucose transport (TmG) of the PRT, leading to glycosuria.(26) In T2DM, the TmG is higher due to upregulation of SGLT2 and GLUT2 expression. Thus, the threshold for glycosuria and the renal glucose reabsorption increase, contributing to hyperglycaemia.(8, 26)

The primary rational for iSGLT2 prescription in T2DM was the inhibition of glucose reabsorption in the PRT, increasing its renal excretion to better control the glycaemia.(29, 30) This effect derives from two mechanisms: SGLT2 inhibition lowers the TmG and, consequently, the threshold for glycosuria, allowing an excretion of 60-80 g/day of glucose; and reduces glucotoxicity by decreasing the glycaemia, resulting in a better peripheral insulin sensitivity and amelioration of pancreatic beta-cell function.(8, 15) Furthermore, the glucose-lowering effect appears to be independent of insulin secretion and resistance, allowing iSGLT2 usage in all stages of T2DM, unless severe CKD is present.(15) In fact, renal impairment with progressively lower eGFR is accompanied by a progressive reduction of glucose excretion, hence reducing the glucose-lowering potency of iSGLT2.(8, 14)

Even though SGLT2 is normally responsible for 90% of glucose reabsorption, its inhibition merely allows an excretion of around 50% of the filtered glucose.(8, 28, 30) A compensatory increase in SGLT1-mediated glucose reabsorption to avoid hypoglycaemia explains this effect.(8) As a result, iSGLT2 are not the best at reducing HbA1c,(7, 19) yet their use is recommended to achieve better cardiorenal outcomes.(7, 24)

RENOPROTECTIVE MECHANISMS OF iSGLT2

Due to different metabolic and hemodynamic changes, T2DM is accompanied by renal structural changes that primarily affect the microcirculation.(19) The glomerular capillaries, basement membrane, podocytes and proximal tubular epithelium face mechanical stress attributable to changes in the glomerular arterioles, resulting in basal membrane thickening, mesangial expansion, renal hypertrophy and, ultimately, DKD-related nodular or diffuse glomerulosclerosis. Furthermore, reactive oxygen species and other inflammatory mediators are released, causing inflammation and fibrosis, reflecting in progressively lower eGFR and rising albuminuria.(19, 22) Nevertheless, despite a decline in functional nephrons, the single-nephron glomerular filtration rate (SNGFR) inversely increases as an adaptation to fewer nephrons, systemic arterial hypertension or higher metabolic demand, causing maladaptive glomerular hyperfiltration.(19, 31) Hyperglycaemia-induced glomerular hyperfiltration is

actually a fundamental mechanism for CKD development in diabetes.(18)

iSGLT2 are the antidiabetic class associated with the greatest overall renal protection.(15) Since the microvascular outcomes are improved with a better glycaemic control, their glucose-lowering effect can be considered an important mechanism behind their long-lasting benefit.(11) However, in patients with renal impairment, iSGLT2 only induce modest HbA1c reductions, yet they are still able to reduce the eGFR decline in T2DM patients with CKD stages 3 and 4, without additional safety concerns.(15, 32) In fact, the correlation between the cardiorenal benefits of iSGLT2 and their glucose-lowering effect is modest, suggesting that other glycaemic-independent pathways must be involved.(11, 13)

Although the exact renoprotective mechanisms are not entirely understood, different endocrine, metabolic, haemodynamic and biochemical pathways have been proposed.(11, 15, 33) There may be a combination of mechanisms at play since the clinical trials of other antidiabetic classes have not shown similar outcomes.(12) The proposed pleiotropic mechanisms involve natriuresis, restoration of tubuloglomerular feedback, blood pressure reduction and positive vascular effects, reduction of intrarenal hypoxia and tubular workload, increased autophagy, anti-inflammatory and antifibrotic properties.(11, 32, 33) Here we review these mechanisms.

Natriuresis and Restoration of Tubuloglomerular Feedback

The SGLTs reabsorb sodium alongside glucose. The sodium transport follows an electrochemical gradient, from a higher concentration in the glomerular filtrate to a lower concentration in the cytoplasm of tubular cells, maintained by the basolateral sodium-potassium ATPase of epithelial tubular cells.(19) SGLT1 reabsorbs two sodium ions per glucose, using roughly twice the energy required by SGLT2, which only transports one sodium.(19, 25) Thus, the presence of SGLT2 in the early PRT allows an efficient use of energy.(25) Though SGLT2 regulates only 5% of the renal sodium reabsorption during normoglycaemia, in chronic hyperglycaemia that number reaches 14% given the increase in SGLT2 expression and tubular reabsorptive capacity. In this way, iSGLT2 not only promote glycosuria, but also natriuresis.(34)

In healthy individuals, the distal tubular sodium concentration influences the glomerular filtration rate (GFR) through a minute-by-minute autoregulatory feedback system called tubuloglomerular feedback (TGF).(10, 30) The macula densa is a group of cells at the end of the cortical thick ascending limb, where they form the juxtaglomerular apparatus. These cells sense changes in tubular fluid composition, via pathways involving the apical Na-K-2Cl cotransporter and sodium-hydrogen exchanger-2 transporter (NHE2), and regulate the renal blood flow and GFR through TGF and renin release.(9) Decreasing glomerular filtration lowers

the sodium delivery to the macula densa, whose cells sense the variation in sodium concentration and activate adenosine-related mechanisms that ultimately promote afferent arteriolar vasodilation, thereby increasing glomerular filtration. Therefore, the TGF maintains a constant GFR and renal perfusion despite changes in blood pressure and volume, crucial in conditions of volume depletion and hypotension.(10)

The transport of sodium in the PRT is largely mediated by the apical sodium-hydrogen exchanger-3 transporter (NHE3), responsible for 30% of sodium reabsorption and 70% of filtered sodium bicarbonate. Studies in mice showed co-localization and positive interference between SGLT2 and NHE3. In addition, SGLT2 inhibition suppressed the activity of NHE3, and NHE3 knock-out reduced SGLT2 expression and the natriuretic effect of iSGLT2. These findings suggest that the suppressive effect of iSGLT2 on NHE3 might contribute to their diuretic properties.(9, 11, 25) Not much is known regarding this arrangement, but some theories propose advantageous ties to acid-base balance in normal physiology, since empagliflozin inhibited NHE3 activity while lowering the urinary pH and bicarbonate excretion.(11, 25) Notably, ten different types of sodium-hydrogen exchangers (NHE) are known in humans and these antiporters exchange sodium for protons to restore pH when acid accumulates intracellularly. They are also found in the macula densa (NHE2) and thick ascending limb of Henle (NHE4). NHE2 plays an important role in salt-sensing and renin control by the macula densa, along with Na-K-2Cl cotransporters.(9)

Under the hyperglycaemic conditions of DM, a higher expression and activity of SGLT2 and a full recruitment of SGLT1 increase the tubular reabsorption of sodium. Consequently, the distal sodium delivery to the macula densa at the juxtaglomerular apparatus is reduced and inappropriately sensed as a reduction of effective circulating volume.(9, 11, 30) TGF is then activated given the lack of vasoconstrictive molecules acting on the afferent arteriole.(9) The reduced sodium transport to the macula densa causes a decline of ATP breakdown and adenosine production. Because adenosine is a strong vasoconstrictor, its reduction promotes vasodilation of the afferent glomerular arteriole, raising the intraglomerular capillary hydrostatic pressure and finally causing hyperfiltration.(5, 9)

Glomerular hyperfiltration is defined as an eGFR greater than two standard deviations than normal (between 120-140 mL/min/1.73 m^2).(22) Apart from the tubular mechanism above described, another pathophysiological mechanism is behind the DM hyperfiltration, consisting of glomerular haemodynamic abnormalities due to neurohormonal activation.(5) RAAS upregulation in DM boosts the angiotensin II levels, resulting in efferent arteriole vasoconstriction and aldosterone release. Simultaneously, the afferent arteriolar resistance declines on account of elevated circulating vasodilators, namely the atrial natriuretic peptide and nitric oxide, and insulin resistance or deficiency. This alternative pathway contributes to glomerular pressure increase and hyperfiltration, with subsequent glomerulosclerosis.(22)

Hyperfiltration and glomerular hypertension are common in early stages of DKD and are important pathophysiological factors for the initiation and progression of all proteinuric nephropathies, independently of their aetiology.(5, 12) Glomerular hyperfiltration leads to glomerular and tubulointerstitial fibrosis.(30) In fact, the kidneys grow large at the onset of DM with eGFR increment, and the individuals with bigger eGFR increases are at higher risk of kidney disease.(25) Nonetheless, the prevalence of glomerular hyperfiltration in T2DM patients stands at 6-23%, lower than in T1DM patients, possibly because of pre-existing comorbidities associated with renovascular diseases, such as hypertension, dyslipidaemia and obesity.(22)

By blocking SGLT2-mediated sodium reabsorption, iSGLT2 cause natriuresis and restore the distal sodium delivery to the macula densa, enhancing the TGF and reversing afferent arteriole vasodilation via adenosine-related mechanisms that promote vasoconstriction.(11, 14, 29, 35) In fact, around 10 g of extra sodium reach the macula densa daily as a direct result of SGLT2 inhibition.(12) Sodium reabsorption at the macula densa is an energy-dependent process wherein adenosine triphosphate is converted to adenosine, which binds to the adenosine-1 receptor at the afferent arteriole and induces vasoconstriction,(10) resulting in intraglomerular pressure decrease.(30, 35) The iSGLT2-induced glomerular pressure decrease manifests clinically as an acute eGFR drop of 4-6 mL/min/1.73 m^2 in the first weeks of treatment. Fortunately, this drop is not only reversible with discontinuation of iSGLT2 therapy, but also followed by a stabilization of long-term renal function decline with prolonged treatment.(10, 11, 14, 35) Moreover, the drop might account for the albuminuria-lowering effect of iSGLT2.(10, 14)

As the increased angiotensin II levels in DM further augment hyperfiltration by inducing efferent vasoconstriction,(18) vasodilation of the efferent arteriole may also underlie the iSGLT2-mediated hyperfiltration reduction.(14) The above-mentioned preglomerular haemodynamic effects of iSGLT2 were observed in T1DM models without certainty of holding true in patients with T2DM. This hypothesis was evaluated in early 2020 in a randomized clinical trial with dapagliflozin in T2DM patients. Dapagliflozin was found to reduce the intraglomerular pressure without increasing the renovascular resistance, suggesting that, unlike the effect of empagliflozin in T1DM patients, the dapagliflozin-induced acute eGFR drop in T2DM patients may be due to vasodilation of the renal efferent arteriole. Thus, despite the common eGFR drop in T1DM and T2DM, different underlying haemodynamic changes may occur. Though, the reason for such differences may be the different characteristics of the two studied cohorts regarding age, glycaemic control, diabetes duration, blood pressure, renal function and baseline haemodynamic status, as well as concomitant use of other drugs like insulin, ACEi and ARB.(9, 11) Additional investigation is required to better understand these differences.

Changes in Albuminuria

DKD starts as a glomerular disease characterised by increased glomerular pressure and hyperfiltration, clinically manifested as albuminuria.(22, 30) The supraphysiological GFR is accompanied by barotrauma that leads to ultrastructural changes, including glomerular hypertrophy and glomerulosclerosis, followed by renal failure. Similarly, the tubular region experiences structural changes such as tubular atrophy, interstitial fibrosis and peritubular capillary rarefaction, also correlated with kidney function decline.(21, 36) In fact, around 40% of T2DM patients show advanced tubulointerstitial lesions (thickening and reduplication of basement membrane, atrophy, fibrosis and chronic inflammation) despite only very mild glomerular changes.(5)

Microalbuminuria is an early manifestation of CKD in DM seen in around 25% of patients after 10 years of the onset of DM, progressing to macroalbuminuria at a rate of around 3% per year.(12) It is seen as an early marker of renal impairment and confirmatory evidence of DKD.(6, 14, 22) Though, progression to macroalbuminuria (defined as UACR ≥300 mg/g or ≥300 mg of albumin per 24-hours) is not absolute, with possible regression to normoalbuminuria.(3, 22) Albeit older studies suggesting that 80% of microalbuminuria cases could progress to macroalbuminuria in a period of 6-14 years, the improved management of glycaemia and cardiovascular risk factors has been linked with regression to normoalbuminuria in T1DM. The same effect was not observed in T2DM, in which studies showed an overall 50% prevalence of macroalbuminuria during a follow-up of 20 years, although such studies are now historical and new interventions became available since, like RAAS inhibition, better glycaemic control and multifactorial interventions.(22) Regardless, the screening of albuminuria allows identification of early CKD cases (microalbuminuria with preserved eGFR) so that disease-modifying therapies can be promptly initiated.(22)

Albuminuria is associated with a faster kidney function decline,(18) and adverse clinical outcomes, healthcare costs and disease burden all rise with its worsening. Thus, an increased UACR is now an important predictor of risk for CKD progression, cardiovascular events and mortality.(23) The early and sustained reduction in albuminuria seen during iSGLT2 therapy reflects their renoprotective effect, associating with a reduced risk of renal and cardiovascular outcomes in people with T2DM and CKD. Indeed, reductions in albuminuria might explain 50% of iSGLT2 treatment effects.(37) Accordingly, the assessment of albuminuria is vital for risk stratification and DKD follow-up, constituting an important prognostic factor for progression to ESRD.(3) Current guidelines recommend monitoring for CKD progression by assessing both eGFR and albuminuria at least annually.(18)

Nevertheless, advanced structural changes may be present long before clinically evident albuminuria, as T2DM may exhibit renal impairment with or without albuminuria.(3)

The prevalence of albuminuria in DKD has decreased over the last 20 years, marking a phenotype change in its clinical presentation.(18) Renal failure without significant preceding proteinuria is becoming a predominant form of DKD in which a low eGFR without increased UACR is registered, possibly arising from ischaemic vascular disease/non-glomerular injury or resulting from RAAS inhibition that consequently masks albuminuria.(6, 12, 21) Indeed, it is long known that RAAS blockade reduces the doubling rate of creatinine, albuminuria and progression to nephropathy, ESRD and death.(22) ACEi and ARB are able to delay the progression of proteinuric DKD to ESRD, hence being recommended for DKD with micro- or macroalbuminuria.(12)

From a haemodynamic perspective, ACEi and iSGLT2 show similar hyperfiltration reductions in T1DM. Studies adding iSGLT2 to RAAS inhibitors revealed further reduction in albuminuria by 30-40%, suggesting a clinically relevant additive reduction of intraglomerular pressure as both approaches can be combined in risk patients to maximize risk reduction.(30, 34) RAAS blockers bestow renoprotection by reducing intraglomerular pressure and proteinuria, and a similar effect is seen with iSGLT2 without direct RAAS interference.(12, 37)

In addition to their effect on eGFR, iSGLT2 were shown to reduce albuminuria.(5) Dapagliflozin promptly reduced the UACR after one week of administration in T2DM patients with CKD that had micro- or macroalbuminuria at baseline, an effect maintained over time when compared to placebo, independently of changes in blood pressure, HbA1c and eGFR.(5, 28) Similar findings were reported with canagliflozin, with bigger UACR decreases achieved with higher doses of canagliflozin and significantly less patients transiting to a higher UACR category compared to placebo.(5, 28) The early sustained reduction in albuminuria and retardation of kidney function decline with canagliflozin were independent of its glycaemic effects.(37) Likewise, the proteinuria-lowering effect has been demonstrated with empagliflozin in patients with micro- or macroalbuminuria and relatively preserved renal function, or with renal impairment despite lower HbA1c impact, suggesting a glucose-independent antiproteinuric role of iSGLT2 due to intrarenal haemodynamic changes.(34) Overall, different studies consistently demonstrated early and sustained UACR reductions with iSGLT2, supporting their renoprotective effects.(5) In individuals with T2DM and CKD, iSGLT2 are associated with a significant 24% reduction in albuminuria apparently mediated by decreases in blood pressure, intraglomerular pressure and hyperfiltration,(29) correlating with long-term beneficial kidney and cardiovascular outcomes.(37)

Anti-inflammatory and Antifibrotic Effects

Diabetes includes an inflammatory component related with its main complications and evidenced by high plasmatic concentrations of proinflammatory cytokines thought to contribute

to progress of nephropathy.(9) Studies have shown that proximal tubular cells grown under high-glucose conditions secrete more inflammatory molecules and profibrotic cytokines, with *in vivo* activation of inflammatory pathways, macrophage recruitment and further tubular damage and interstitial fibrosis.(5)

Adenosine monophosphate-activated protein kinase (AMPK) and sirtuin-1 (SIRT1) are two enzymes that lower oxidative stress and inflammation through a coordinated activation of autophagy, a process that allows degradation of damaged mitochondria and peroxisomes.(9, 11) Therefore, these enzymes are vital for the homeostasis of podocytes, tubular cells, mesangial and glomerular endothelial cells. Furthermore, their antioxidant effect is widened by lowering the intracellular sodium content as their activation leads to NHE and epithelial sodium channels down-regulation. This is relevant because hypernatraemia promotes inflammatory cytokines and inhibits the anti-inflammatory function of innate and adaptative immune cells. In DKD, there is an impairment of AMPK and SIRT1 signalling that may contribute to development and progression of nephropathy.(9)

iSGLT2 stimulate autophagy by inducing both AMPK and SIRT1, thereby reducing cellular stress, and glomerular and tubular injury. In experimental models of DKD, AMPK activation preserved podocytes, renal tubular structure, and glomerular function, while SIRT1 overexpression improved the glomerular and tubulointerstitial injury. In brief, renoprotection with iSGLT2 may be explained by anti-inflammatory effects, evidenced by the balance between inflammatory and anti-inflammatory cytokines, and increase in autophagy.(9)

The activity of the rapamycin-sensitive complex of mTOR (mTORC1) modulates fibrogenesis by activating a pro-fibrotic programme that involves increased collagen gene expression and modulation of aminoacid transport. This complex integrates signals from nutrients like glucose and branched-chain aminoacids (BCAA) to control protein synthesis, cell size, proliferation and autophagy. Overactivation of mTORC1 in PRT cells has been reported in diabetes (most likely due to nutrient overload provoked by hyperglycaemia, metabolic syndrome and obesity), inducing tubular dysfunction, peritubular fibrosis, albuminuria and kidney disfunction, hence playing a key role in the pathophysiology of DKD. iSGLT2 decreased mTORC1 activity and the workload of PRT cells by inhibiting glucose transport through cells and BCAA uptake, which prevented fibrogenesis and development of DKD. Off note, these findings were reported in *Akita* mice (T1DM model) but, since mTORC1 was similarly activated in *db/db* mice (T2DM model), they might be extrapolated to T2DM in humans.(21)

Additionally, reductions in inflammatory factors implicated in the pathogenesis of DKD have been observed under iSGLT2 therapy. In experimental models of diabetes, markers of inflammation and fibrosis, including the nuclear factor-kB (NF-kB), interleukin-6 (IL-6) and monocyte chemoattractant protein-1 (MCP-1; also known as CCL2), were all decreased after iSGLT2 administration.(11, 33) Similarly, in T2DM patients, iSGLT2 promoted reductions in

urinary IL-6 and MCP-1, as well as serum tumour necrosis factor receptor-1 (TNFR1) and IL-6.(11)

The tumour necrosis factor alpha (TNF-α) is linked to progression of DKD, since its binding to its receptor TNFR1 in the podocytes leads to cytokine production and inflammation. This receptor is released in the blood circulation after enzymatic cleavage, allowing the prediction of ESRD risk through measurement of circulating TNFR1 in both T1DM and T2DM. Canagliflozin was shown to reduce TNFR1 levels in T2DM patients, independently associated with a slower decline of kidney function.(15, 33)

Hyperglycaemia triggers the podocytes, mesangial cells and tubular cells to release IL-6, which participates in local and systemic subclinical inflammation. Canagliflozin was proven to reduce this inflammatory mediator.(15, 33)

The matrix metalloproteinases are associated with kidney fibrosis by participating in the collagen metabolism. Elevated levels of matrix metalloproteinase 7 (MMP7) were reported in DKD patients with positive correlation to renal function.(33) MMP7 was targeted by canagliflozin in T2DM.(15, 19, 33)

Furthermore, decreases in the serum levels of leptin and fibronectin 1 were similarly observed with iSGLT2 therapy, whereas adiponectin levels increased.(15)

The nod-like receptor pyrin domain-containing protein 3 (NLRP3) inflammasome is a multimeric protein complex involved in inflammatory processes via production of interleukins 1b and 18. In animal models of DKD, a iSGLT2-induced increase in ketone bodies inhibited the NLPR3 inflammasome and, consequently, the two interleukins.(11, 19)

The anti-inflammatory activity of iSGLT2 results from multiple underlying mechanisms, of which intraglomerular pressure decline with attenuation in shear stress and glomerular wall tension, decrease in renal hypoxia, weight loss, reduction of adipose tissue inflammation, decrease of uricaemia, reduction of intrarenal angiotensinogen production, changes in energy substrate utilization and/or delivery to the heart and kidneys, and attenuation of oxidative stress.(10, 15, 19) In brief, local and systemic effects of iSGLT2 on inflammation and fibrosis may be renoprotective and particularly useful in T2DM patients with tubulointerstitial lesions.(5, 15)

Impact on Uric Acid Metabolism

The breakdown of dietary and endogenous purines produces uric acid, of which two thirds are excreted in the urine. Uric acid is almost entirely found in the form of urate anion at neutral physiological pH, but it can crystalize when in high concentrations at low pH, leading to nephrolithiasis and gout.(13)

Chronic hyperuricaemia has been linked to renal inflammation and increased risk of

development and progression of tubulointerstitial fibrosis and CKD, especially in diabetes, as well as hypertension and CVD.(11, 13, 15) Moreover, hyperuricaemia promotes insulin resistance by inhibiting insulin signalling pathways. Uric acid is, thus, seen as a modifiable risk factor for CKD progression in T2DM.(13)

The uric acid concentrations in T2DM are generally raised in comparison to non-diabetic individuals, contributing to the metabolic syndrome seen in this form of diabetes, even though they are often still within the normal range.(13)

iSGLT2 were shown to increase the uric acid excretion in T2DM rather than reducing its endogenous production, and consistently lowered the circulating uric acid levels, while improving the cardiovascular and renal outcomes.(11, 13, 15) Such effects were recorded within days and persisted in the long-term. Notably, similar significant uric acid reductions were seen with different iSGLT2 tested against control groups, indicating a class effect in the treatment of T2DM without substantial differences between agents and doses.(13, 15) Additionally, iSGLT2 appear to be the only glucose-lowering agents capable of reducing uric acid concentrations in T2DM.(13)

In the kidney, urate is freely filtered but almost all the filtered urate is reabsorbed in the first segment (S1) of the PRT, mostly via the urate transporter-1 (URAT1) and the facilitative hexose/urate transporter GLUT9b. The reabsorbed urate is then transported through the basolateral membrane of proximal cells via GLUT9a and secreted mainly in the second segment (S2) of the PRT and other distal parts of the nephron via the organic anion transporters OAT1 and OAT3.(13)

Albeit agents such as probenecid, losartan and lesinurad promoting uricosuria by inhibiting URAT1, it was initially believed that iSGLT2 would not affect this transporter, but rather exert uricosuric effects by supressing GLUT9b activity, given the less amounts of glucose to compete with urate for GLUT9b.(13) However, it was later proposed that the uricosuric effect of iSGLT2 also depends on URAT-1.(11, 19) Regardless of the underlying mechanisms, iSGLT2 are known to have an uricosuric effect that, along their glucose-lowering effect, may protect against adverse cardiovascular events and DKD progression, and assist the management of hyperuricaemia in diabetes.(10, 13)

Blood Pressure Lowering and Other Cardiovascular Effects

More than 70% of T2DM patients have an elevated systolic blood pressure (BP), importantly associated with cardiovascular and cerebrovascular events, as well as initiation and development of DKD,(10) since hypertension is a risk factor for progressive renal function loss, so much so that BP lowering has been proven to be renoprotective.(11)

Under iSGLT2 therapy, there were consistent BP reductions in T2DM patients, whether they had hypertension or not.(15) The systolic and diastolic BP faced reductions of approximately 4 mmHg and 2 mmHg, respectively.(11, 15) Notably, this BP-lowering effect seems to be accentuated in T2DM patients with prior hypertension and already taking antihypertensive drugs, in whom a mean reduction of 6 mmHg in the systolic BP was recorded.(34) Similarly, an average reduction of 2.6 mmHg in the nocturnal systolic BP has also been registered.(15)

The BP-lowering effect of iSGLT2 is most likely multifactorial.(10, 11) The already described iSGLT2-induced natriuresis leads to osmotic diuresis and contraction in plasma volume, the most likely mechanism behind the BP decrease.(8, 10, 11, 34) Supportive evidence of haemoconcentration includes the reported 7% reduction in plasma volume at 12 weeks of treatment alongside modest BP lowering of 5 mmHg, in addition to increased circulating and urinary RAAS hormones, and increased serum albumin and haematocrit. Albeit the comparable diuretic effects of iSGLT2 with thiazide diuretics, the plasma volume contraction seems to persist with iSGLT2 whereas a return to baseline by week 8 is expected with thiazides.(10) While thiazides induce a modest heart rate increase, the plasma volume contraction induced by iSGLT2 is not associated with reflex tachycardia, suggesting a possible blunting of the sympathetic nervous system (SNS) activity that may contribute to renoprotection.(10, 11) Dapagliflozin reduced the intrarenal tyrosine hydroxylase and norepinephrine in both obese mice and neurogenic hypertensive mice models, implying fewer SNS innervation and activity. The diabetic hyperactive sympathetic response was reduced by empagliflozin, which similarly supressed the renal sympathetic baroreflex. Thus, the cardiorenal effects of iSGLT2 may be related to sympathoinhibition.(11)

A recent 2021 study in T2DM patients with preserved kidney function demonstrated a significant decrease in the 24-hour BP with dapagliflozin, devoid of valuable changes in sodium excretion, suggesting that other natriuretic-independent mechanisms must justify the BP-lowering effect of iSGLT2.(11) iSGLT2 may ameliorate some vascular abnormalities related to diabetes by reducing the arterial stiffness, vascular resistance and endothelial dysfunction.(10, 11) Recent experimental and clinical studies have proposed a potential role of iSGLT2 in restoring the integrity of the glycocalyx, a gel-like structure that covers the endothelium found impaired in T2DM, possibly contributing to BP reduction.(11)

The BP-lowering capacity of iSGLT2 is maintained regardless of baseline kidney function. While the glucose-lowering efficacy and the clinical consequences of glucosuria (reductions in HbA1c and body weight) depend on kidney function and are attenuated in patients with eGFR <45 mL/min/1.73 m^2, the natriuresis-related effects, such as BP lowering, are maintained in T2DM patients with CKD.(10, 15) Moreover, the BP reduction is observed even in patients treated with other antihypertensive drugs, raising the possibility of synergistic

effects with RAAS blockers and other BP-lowering agents.(11, 15)

iSGLT2 were shown to reduce the risk of CKD progression by 45%, but this was not the only important positive renal outcome, as the risk of cardiovascular death or hospitalization due to heart failure was also reduced by 23%.(29) This new class of drugs deploys important cardiovascular effects apart from the mentioned BP-lowering effect, including improvement of ventricular loading conditions, reshaping of cardiac metabolism and bioenergetics, ventricular remodelling, direct cardioprotective and possible antiarrhythmic effects.(19) Nonetheless, such cardiovascular effects and underlying mechanisms go beyond the scope of this review.

Metabolic Effects of iSGLT2

Around 80% of T2DM patients are obese or overweight. Common antidiabetic therapies cause weight gain and oral antidiabetics are unable to stop progressive beta-cell failure, with a quarter of patients eventually requiring insulin. iSGLT2 revolutionized the treatment of T2DM by permitting glycaemic control while limiting weight gain and beta-cell failure.(12) In fact, iSGLT2 promote a 2-3 kg weight loss within the first six months of therapy, attenuated afterwards possibly because of compensatory hyperphagia manifested by hunger for sugar-rich foods.(8, 19) However, the enhanced food intake is insufficient to fully compensate the continuous iSGLT2-mediated urinary fuel loss of glucose, otherwise no weight loss would be recorded.(29)

To counter the continuous caloric loss, physiological adaptative responses take place, specifically an increase of endogenous glucose production,(11) stimulated by decreased plasmatic insulin levels and increased plasmatic glucagon levels that follow iSGLT2 administration.(11, 28)

In the pancreas, iSGLT2 trigger glucagon secretion by alpha-cells,(12) with subsequent increase in plasmatic glucagon levels and glucagon-to-insulin ratio.(28, 29) Glucagon promotes the breakdown of glucose, generating pyruvate, and stimulates a transamination cascade of carbonic acids (pyruvate to alanine, oxaloacetate to aspartate and alfa-ketoglutarate to glutamate) that promotes protein catabolism for gluconeogenesis, paralleled by increased urea osmolyte generation and subsequent osmolyte-driven water conservation. iSGLT2 seem to shift the hepatic metabolism to preferentially produce ketones over glucose from gluconeogenesis, given that glucose production from pyruvate is energy demanding.(29)

Weight loss with iSGLT2 is mainly explained by glycosuric caloric loss, natriuresis and aquaresis, but also by changes in substrate utilization, including a fuel switch from glucose oxidation to increased lipolysis, fat oxidation and ketogenesis.(8) Glycogen and fat energy stores in the liver and skeletal muscle are depleted during iSGLT2 therapy, along with muscular

nitrogen reservoirs, as evidenced by reduction of the lean body mass. Under iSGLT2 regimens, diet- and muscle-derived energy and nitrogen substrates are used to counterbalance the urinary glucose and water loss. Simultaneously, there seems to be an increase in BCAA (valine, leucine and isoleucine) transfer from the muscle to the heart and kidney, where BCAA transaminases catabolize them into free fatty acids (FFA) and ketone bodies. BCAA constitute a special fuel that specifically supplies energy to the heart and kidneys, but not the liver, which lacks the BCAA transaminases needed for their catabolism.(29)

During iSGLT2 treatment stimulation of ketogenesis and lipolysis occurs, raising the circulating ketone bodies,(11, 29) particularly beta-hydroxybutyrate.(15, 28) In the setting of T2DM, glucose and FFA are inefficient energy sources for the heart and the kidneys, whereas beta-hydroxybutyrate constitutes a more energy-efficient fuel.(11, 15) Thus, the iSGLT2-induced metabolic shift towards energy-efficient ketone bodies may be cardiorenoprotective, improving the myocardial and renal efficiency and function.(15)

The heart benefits from ketone bodies usage because they allow the production of ATP in a more oxygen-efficient manner than FFA.(29) In normal conditions, 70% of the heart's energy expenditure derives from FFA oxidation.(12) However, during the persistent iSGLT2-induced hyperketonaemia, and according to the "thrifty substrate" hypothesis, beta-hydroxybutyrate is freely taken by the heart and oxidized in favour of FFA, because more ATP can be produced per molecule of oxygen invested, translating into a more energy-efficient cardiac workload and improvement of myocardial energetics.(12, 25, 28, 29) A similar mechanism may assist the diabetic kidneys by reducing regional hypoxia,(25) and ketone bodies may also reduce proximal tubular cell and podocyte damage by inhibiting the hyperactive mTORC1.(11) Furthermore, the increased ketogenesis suppresses the NLRP3 inflammasome activity, resulting in anti-inflammatory effects.(19)

iSGLT2 promote a sustained urinary energy loss in the form of glucose, causing initial water loss driven by osmotic diuresis, with consequent predisposition for dehydration. As a result, a physiological adaptation process that combines fuel generation with efficient water conservation ensues. iSGLT2 appears to shift the renal metabolism from pyruvate oxidation (ATP production using glucose via oxidative phosphorylation) to phosphoenolpyruvate generation (*de novo* glucose generation and storage using pyruvate). Some *in vitro* studies suggested that iSGLT2 administration augments the expression of key enzymes for gluconeogenesis, such as phosphoenolpyruvate carboxykinase (PEPCK) and glucose-6-phosphatase (G6Pase). This paradoxical gluconeogenesis is glucagon-driven and appears to be a kidney-specific effect of iSGLT2, as the expression of such enzymes was only recorded in the renal cortex, with no evidence in the liver. Furthermore, glycogen accumulation also took place in the kidney. A reduction in renal glycogen content would be expected, but instead its levels surge, suggesting that the kidney might reduce the use of glucose to utilize other sources

of energy, such as FFA and/or aminoacids.(29)

In mammals, the urea metabolism is crucial for renal water conservation. Urea-driven concentration of urine limits the osmotic diuresis during states of high sodium and chlorine excretion, preventing dehydration. Dapagliflozin increased the expression of the urea transporter UT-A1 in the renal medulla of diabetic and non-diabetic mice, with increased concentration of osmolytes at the interstitium and compensatory urea-driven water reabsorption taking place in the collecting duct. Water conservation is also achieved through vasopressin, a hormone that stimulates free-water reabsorption in the renal medulla via aquaporin-2 channels (AQP2). Dapagliflozin was shown to increase vasopressin release in humans and recent studies showed that vasopressin mediates the activation of AQP2 and facilitates UT-A1-driven urea transport. These findings suggest that iSGLT2 trigger vasopressin-mediated water conservation in the renal medulla. Furthermore, vasopressin exerts synergistic effects with glucagon to promote the breakdown of hepatic glycogen, while increasing the hepatic ureagenesis. Therefore, after the initial urine volume increase within the first 72 hours of iSGLT2 treatment, the diuresis returns to baseline levels and remains normal in the following weeks, despite persistently high solute excretion.(29)

The urinary loss of glucose under iSGLT2 therapy not only improves glycaemia, but additionally reduces the daily calorie balance and exploits the hepatic and muscular glycogen reservoirs, while increasing gluconeogenesis and improving the insulin sensitivity.(29) The caloric loss induced by these drugs leads to a reduction in adipose tissue and organ-specific fat content in the heart and liver, supressing systemic and local inflammation with predicted cardiorenal benefits.(10, 19)

In brief, hepatorenal water-conserving mechanisms are activated by iSGLT2 as compensation for the urinary glucose loss (through glycogen breakdown and *de novo* glucose synthesis from aminoacids), but also to provide urea needed to limit the osmotic diuresis that these drugs induce.(29)

Importantly, the increase in hepatic ketogenesis and circulating ketone bodies implies a risk of euglycaemic ketoacidosis,(12) particularly in patients with restricted carbohydrate intake (ketogenic diet), insulin deficiency or history of alcoholism.(18) However, this possible side-effect can be prevented through adequate hydration.(29)

In the liver, iSGLT2 also showed beneficial effects, especially by reducing steatosis and steatohepatitis.(10) Non-alcoholic fatty liver disease (NAFLD) represents a spectrum of liver diseases characterised by steatosis, with an estimated worldwide prevalence of 25%.(19, 36) Within the spectrum, a subgroup called non-alcoholic steatohepatitis (NASH) combines steatosis with hepatocyte injury and inflammation, with or without fibrosis.(36) NAFLD results from excessive organ-specific adiposity, commonly observed in patients with obesity, metabolic syndrome and T2DM,(10, 19) and increases the risk of cardiovascular events and

overall worse prognosis. The treatment strategies are limited, but iSGLT2 may favourably modulate NAFLD/NASH given their capacity to reduce adiposity.(19, 36) Indeed, iSGLT2 are the only glucose-lowering agents capable of reducing the levels of NAFLD markers, such as serum alanine aminotransferase, body weight and fatty liver index, in T2DM patients with NAFLD.(10, 36) Histological evidence of NAFLD improvement in T2DM patients receiving iSGLT2 was also reported.(10) Clinically, these benefits manifested by improved liver tests.(36)

In brief, the metabolic changes induced by iSGLT2, promoting water conservation and lower energy expenditure, are thought to improve the lifespan of the liver, heart and kidneys.(29)

Renal Oxygenation and Erythropoiesis

The renal tissue oxygenation depends on the balance between oxygen delivery, related to renal perfusion, and oxygen demand, related to ATP production necessary for tubular reabsorption. The “chronic hypoxia hypothesis” defends that chronic hypoxia, resulting from a disbalance between oxygen delivery and demand, is an important pathophysiological pathway driving CKD, regardless of its aetiology. This imbalance results from capillary damage that reduces glomerular and post-glomerular blood flow, enhanced in patients with DM.(9)

As previously discussed, under the hyperglycaemic conditions of DM there is a higher SGLT2 expression, increasing glucose and sodium reabsorption.(11) Consequently, more ATP is consumed by the sodium-potassium pump, increasing the ATP demand and leading the PRT mitochondria to consume more oxygen.(9, 38) However, the hyperglycaemia-induced microvascular damage lowers the oxygen delivery capacity, causing hypoxia.(9, 11) Additionally, insulin resistance in T2DM is associated with decreased ATP production. In this sense, DM is characterised by increased renal oxygen demand with ATP deficit associated with reduced oxygen delivery, disturbing the balance that assures a proper kidney oxygenation.(9)

The intrarenal hypoxia experienced by diabetic kidneys is ameliorated by iSGLT2, which seem to reduce the renal oxygen demand and attenuate the microvascular changes that condition the renal oxygen supply. The iSGLT2-induced improvement of renal oxygenation may account for their renoprotective effects.(9, 33)

Anaemia is a common complication of CKD. When compared to non-diabetic patients with CKD, DKD patients are more likely to have low erythropoietin and vitamin D, manifesting anaemia earlier. Moreover, the levels of serum hepcidin, a hormone crucial in iron regulation, are elevated in T2DM, resulting in iron deficiency and anaemia, as well as worsening of insulin resistance.(22) An early iSGLT2 effect is the elevation of the haemoglobin levels, which persist

elevated throughout the administration.(39) Similarly, a modest 2-4% haematocrit increase has been recorded consistently for all four iSGLT2 approved in Europe, regardless of kidney function, except for CKD stage 4 in which iSGLT2 failed to significantly increase the haematocrit.(38) The decrease in plasma volume and consequent haemoconcentration partially explain such effects. However, iSGLT2-induced plasma contraction is limited to the first days of therapy and both the haemoglobin and haematocrit remain elevated afterwards, suggesting that other mechanisms take place.(38, 39) Indeed, iSGLT2 are thought to affect erythropoiesis.(39)

Renal interstitial fibroblasts that reside near the PRT produce erythropoietin (EPO) through a process regulated by the hypoxia-inducible factor (HIF). The increased oxygen demand in diabetes leads to tubulointerstitial hypoxia with damage to the PRT epithelial cells, triggering the transformation of EPO-producing fibroblasts into myofibroblasts uncapable of producing EPO but able to produce fibrogenic molecules, thus contributing to CKD and decreasing EPO levels in T2DM.(38, 39)

iSGLT2 reduce the ATP demand of the basolateral sodium-potassium pump in the tubular cells, given that less glucose is reabsorbed, and this may reduce the proximal tubular workload and improve the hypoxic conditions around the PRT, allowing the reversion of myofibroblasts into EPO-producing fibroblasts.(19, 38, 39) Dapagliflozin, canagliflozin and empagliflozin were all shown to increase the serum EPO levels within 2-4 weeks of treatment. Moreover, in patients taking dapagliflozin, the reticulocyte count increased at the same time of EPO and was followed by elevation of haemoglobin and haematocrit, reflecting the possible augmentation of erythropoiesis by iSGLT2.(38, 39)

Importantly, iSGLT2 shift the sodium reabsorption to distal nephron segments, increasing the medullary oxygen demand and setting the medulla at risk of hypoxia.(11, 39) In this setting, stimulation of HIF-2a has been reported, which further promotes erythropoiesis.(11)

The capacity of iSGLT2 to enhance erythropoiesis could improve the renal oxygenation by raising the circulating oxygen-carrying capacity, possibly explaining the reduction of acute kidney injury reported with these agents.(11, 39)

FUTURE PERSPECTIVES

Recent studies sought to evaluate the cardiorenal benefits of iSGLT2 in non-diabetic patients with heart failure and/or renal impairment, introducing a new era in which these drugs are used despite the presence of diabetes.(27)

The CREDENCE trial first proved the renoprotective effect of canagliflozin in T2DM

patients with DKD, confirming a marked reduction in the risk of CKD progression.(18, 40) Later, the DAPA-CKD trial confirmed a similar effect with dapagliflozin in patients with and without T2DM, and revealed a reduction of the all-cause mortality risk for the first time when added to RAAS inhibition.(18, 23) By evaluating primary kidney outcomes in both diabetic and non-diabetic patients, this trial confirmed that the renoprotective effects of iSGLT2 extend to the broader population with CKD, independently of T2DM.(31, 40) RAAS inhibitors have been the only available agents capable of reducing CKD progression until now, yet not reducing the all-cause mortality risk.(18, 40) However, the DAPA-CKD trial findings suggest an important role of iSGLT2 in the future treatment and management of CKD as a whole, unrestricted to T2DM.(23) The EMPA-KIDNEY trial is expected to consolidate these findings by accessing the efficacy of empagliflozin in patients with evidence of CKD, with or without DM and with or without albuminuria, presenting with an eGFR as low as 20 mL/min/1.73 m^2.(4, 22)

Given that an advanced age is one of the main barriers to initiate iSGLT2 treatment, owing to bigger safety concerns and difficulty distinguishing whether CKD is due to ageing or diabetes, the mentioned trials suggest that dapagliflozin can reduce the risk of kidney function deterioration and death even if changes in eGFR are age-related.(16)

DISCUSSION

T2DM induces upregulation of SGLT2 in the kidneys, which, in combination with an increased TmG, leads to enhanced glucose and sodium reabsorption in the PRT, contributing to hyperglycaemia.(8, 18, 26) This can be reversed by iSGLT2 that promote glycosuria, thus lowering the plasmatic glucose levels.(41) iSGLT2 were first introduced as glucose-lowering agents for T2DM, but restricted to relatively preserved kidney function, which limits such effect.(10) However, the growing evidence that iSGLT2 can improve cardiovascular and renal outcomes, independently of their glucose-lowering effect, has changed the paradigm of treatment with these agents.(18, 40) It is now believed that iSGLT2 exert renoprotection through a combination of different mechanisms.(12, 31)

Since SGLT2 reabsorbs sodium along glucose,(19) in T2DM more sodium is reabsorbed and less sodium chloride is delivered to the macula densa, causing preglomerular vasodilation and hyperfiltration, worsened by concomitant increase of angiotensin II levels.(18) The subsequent glomerular barotrauma, along pro-inflammatory and profibrotic changes, leads to tubulointerstitial damage.(18, 30) Fortunately, iSGLT2 promote natriuresis, possibly by supressing NHE3. The sodium delivery to the macula densa is restored, activating the TGF and promoting afferent vasoconstriction via adenosine-related mechanisms, thus contradicting

hyperfiltration.(31) Consequently to intraglomerular pressure reduction, acute eGFR drops are seen with iSGLT2 administration, but these are reversible and followed by long-term stabilization of kidney function,(11, 35) hence the recommendation by the KDIGO guideline to continue iSGLT2 until initiation of kidney replacement therapy even if the eGFR subsequently declines below 30 mL/min/1.73 m^2.(7)

The glomerular hypertension and hyperfiltration in diabetes causes barotrauma and ultrastructural changes in the kidney(21) that clinically manifest as albuminuria.(22, 36) Albuminuria is, thus, seen as an early marker of renal damage and clinical evidence of DKD,(6, 14) despite not being present in all patients with DKD.(18) Early and sustained reductions of albuminuria with iSGLT2 reflect their renoprotective action.(37) iSGLT2 reduce the intraglomerular pressure and contradict hyperfiltration, halting structural changes that would result in kidney function deterioration.(29) The screening of albuminuria allows early detection of DKD cases, crucial for early initiation of disease-modifying therapies that can improve the renal outcomes.(22) Moreover, albuminuria is also an important prognostic factor for CKD progression.(3) In this sense, the KDIGO guidelines recommend monitoring for CKD progression by accessing not just the eGFR, but also albuminuria at least annually, with great importance given that CKD is frequently underdiagnosed in T2DM patients due to low patient and physician awareness,(18) and that an accurate CKD diagnosis is the first step to overcome the therapeutic inertia towards the use of iSGLT2.(16)

Diabetes constitutes an inflammatory state that ultimately results in fibrosis and incites the progression of nephropathy.(5, 9) iSGLT2 exert local and systemic anti-inflammatory and anti-fibrotic effects through multiple mechanisms,(5, 15) evidenced by a balance between inflammatory and anti-inflammatory cytokines and increased autophagy.(9) These effects add to their renoprotective role.

One of the mechanisms underlying such anti-inflammatory properties of iSGLT2 might be the reduction in uric acid levels achieved during therapy.(10, 13) Uric acid, usually elevated in T2DM, is a modifiable risk factor for the progression of DKD,(13) as it plays a part in renal inflammation and tubulointerstitial fibrosis when chronically elevated.(11, 13) iSGLT2 are the only glucose-lowering agents that can lower uricaemia in T2DM, and are believed to do so by increasing its renal excretion through inhibition of URAT1 and GLUT9b.(13) This achievement may protect against adverse cardiovascular events and DKD progression.(10, 13)

The osmotic diuresis that follows iSGLT2-induced natriuresis leads to contraction in plasma volume, explaining their BP-lowering effect,(8, 10, 34) albeit only partially as other natriuretic-independent mechanisms complement this effect,(11) such as reduction of arterial stiffness, vascular resistance and endothelial dysfunction.(10, 11) The capacity of iSGLT2 to reduce BP is of great importance since a significative amount of T2DM patients are hypertensive(10) and hypertension contributes to renal failure and poor cardiovascular

outcomes.(11)

While most antidiabetics cause weight gain, iSGLT2 have the opposite effect within the first six months of therapy, important given that around 80% of T2DM patients are either overweight or obese.(12) The weight loss results not only from their glycosuric effect, but also from changes in substrate utilization that include a fuel switch from glucose oxidation to increased lipolysis and ketogenesis.(8) iSGLT2 induce metabolic changes that promote water conservation and reduce energy expenditure, with benefits for the kidneys, heart and liver.(29) Despite the changes in intrarenal haemodynamics (reduction of intraglomerular hyperfiltration), changes in the renal energy metabolism may also underlie the renoprotective effects of iSGLT2, namely the improved ATP utilization in the renal medulla.(29)

Certainly, the higher expression of SGLT2 in diabetes, and consequent increase of glucose and sodium reabsorption, leads to higher ATP consumption by the sodium-potassium pump in the PRT, increasing the renal oxygen demand.(9, 11) Simultaneously, the diabetes-related microvascular damage reduces the oxygen supply. As a result, the kidney experiences chronic hypoxia that drives it to fail.(9) iSGLT2 improve the intrarenal hypoxia, adding to their arsenal of renoprotective effects, probably by reducing the renal oxygen demand and exerting positive effects on the microvasculature,(9, 33) yet stimulation of erythropoiesis may also ensure the improvement of renal oxygenation by these agents.(11, 39)

The benefits of iSGLT2 in elderly patients are similar or greater than in younger patients, despite the higher chance of developing complications.(16) Physicians should individualize iSGLT2 treatment by accessing the benefits of reducing CKD progression and all-cause mortality risk against the potential risks of initial increased diuresis, diabetic ketoacidosis and urinary tract and genital mycotic infections, while considering underlying comorbidities and concomitant medications.(18)

CONCLUSION

iSGLT2 have revolutionized the treatment of T2DM far beyond the initial glucose-lowering goal, with growing evidence of their important renoprotective role. In the present century, multiple studies have demonstrated the improvement of renal outcomes in diabetic patients receiving iSGLT2 therapy, namely the reduction of CKD progression and death. A broader combination of effects and respective underlying mechanisms other than glycaemic control may explain the renoprotective role of iSGLT2. Such mechanisms are not yet fully understood, opening new doors for future clinical and scientific investigation. Nevertheless, the existing knowledge on these agents has already motivated an extension of their usage to include diabetic patients suffering from CKD, while previewing a promising role in the future

management of CKD as a whole, including those without diabetes

REFERENCES

1. WHO. The Global Diabetes Compact 2021 [Available from: https://cdn.who.int/media/docs/default-source/diabetes/gdc_need_to_know_web.pdf?sfvrsn=7a4af558_4&download=true.
2. WHO. Diabetes 2022 [Available from: https://www.who.int/health-topics/diabetes#tab=tab_1.
3. Kleinaki Z, Kapnisi S, Theodorelou-Charitou SA, Nikas IP, Paschou SA. Type 2 diabetes mellitus management in patients with chronic kidney disease: an update. Hormones (Athens). 2020;19(4):467-76.
4. Triozzi JL, Parker Gregg L, Virani SS, Navaneethan SD. Management of type 2 diabetes in chronic kidney disease. BMJ Open Diabetes Res Care. 2021;9(1).
5. Fioretto P, Zambon A, Rossato M, Busetto L, Vettor R. SGLT2 Inhibitors and the Diabetic Kidney. Diabetes Care. 2016;39 Suppl 2:S165-71.
6. Tong L, Adler S. Glycemic control of type 2 diabetes mellitus across stages of renal impairment: information for primary care providers. Postgrad Med. 2018;130(4):381-93.
7. KDIGO 2020 Clinical Practice Guideline for Diabetes Management in Chronic Kidney Disease. Kidney Int. 2020;98(4s):S1-s115.
8. Brown E, Wilding JPH, Alam U, Barber TM, Karalliedde J, Cuthbertson DJ. The expanding role of SGLT2 inhibitors beyond glucose-lowering to cardiorenal protection. Ann Med. 2021;53(1):2072-89.
9. Cianciolo G, De Pascalis A, Gasperoni L, Tondolo F, Zappulo F, Capelli I, et al. The Off-Target Effects, Electrolyte and Mineral Disorders of SGLT2i. Molecules. 2020;25(12).
10. Cherney DZ, Kanbay M, Lovshin JA. Renal physiology of glucose handling and therapeutic implications. Nephrol Dial Transplant. 2020;35(Suppl 1):i3-i12.
11. Sen T, Heerspink HJL. A kidney perspective on the mechanism of action of sodium glucose co-transporter 2 inhibitors. Cell Metab. 2021;33(4):732-9.
12. Sarafidis P, Ferro CJ, Morales E, Ortiz A, Malyszko J, Hojs R, et al. SGLT-2 inhibitors and GLP-1 receptor agonists for nephroprotection and cardioprotection in patients with diabetes mellitus and chronic kidney disease. A consensus statement by the EURECA-m and the DIABESITY working groups of the ERA-EDTA. Nephrol Dial Transplant. 2019;34(2):208-30.
13. Bailey CJ. Uric acid and the cardio-renal effects of SGLT2 inhibitors. Diabetes Obes Metab. 2019;21(6):1291-8.
14. Scheen AJ. Pharmacokinetic/Pharmacodynamic Properties and Clinical Use of SGLT2 Inhibitors in Non-Asian and Asian Patients with Type 2 Diabetes and Chronic Kidney Disease. Clin Pharmacokinet. 2020;59(8):981-94.
15. Scheen AJ. Sodium-glucose cotransporter type 2 inhibitors for the treatment of type 2 diabetes mellitus. Nat Rev Endocrinol. 2020;16(10):556-77.
16. Jeong SJ, Lee SE, Shin DH, Park IB, Lee HS, Kim KA. Barriers to initiating SGLT2 inhibitors in diabetic kidney disease: a real-world study. BMC Nephrol. 2021;22(1):177.
17. Bramlage P, Lanzinger S, Tittel SR, Hess E, Fahrner S, Heyer CHJ, et al. Guidelines adherence in the prevention and management of chronic kidney disease in patients with diabetes mellitus on the background of recent European recommendations - a registry-based analysis. BMC Nephrol. 2021;22(1):184.
18. Mende CW. Chronic Kidney Disease and SGLT2 Inhibitors: A Review of the Evolving Treatment Landscape. Adv Ther. 2022;39(1):148-64.
19. Zelniker TA, Braunwald E. Mechanisms of Cardiorenal Effects of Sodium-Glucose

Cotransporter 2 Inhibitors: JACC State-of-the-Art Review. J Am Coll Cardiol. 2020;75(4):422-34.
20. Khunti K. SGLT2 inhibitors in people with and without T2DM. Nat Rev Endocrinol. 2021;17(2):75-6.
21. Kogot-Levin A, Hinden L, Riahi Y, Israeli T, Tirosh B, Cerasi E, et al. Proximal Tubule mTORC1 Is a Central Player in the Pathophysiology of Diabetic Nephropathy and Its Correction by SGLT2 Inhibitors. Cell Rep. 2020;32(4):107954.
22. Stephens JW, Brown KE, Min T. Chronic kidney disease in type 2 diabetes: Implications for managing glycaemic control, cardiovascular and renal risk. Diabetes Obes Metab. 2020;22 Suppl 1:32-45.
23. Garcia Sanchez JJ, Thompson J, Scott DA, Evans R, Rao N, Sörstadius E, et al. Treatments for Chronic Kidney Disease: A Systematic Literature Review of Randomized Controlled Trials. Adv Ther. 2022;39(1):193-220.
24. de Boer IH, Caramori ML, Chan JCN, Heerspink HJL, Hurst C, Khunti K, et al. Executive summary of the 2020 KDIGO Diabetes Management in CKD Guideline: evidence-based advances in monitoring and treatment. Kidney Int. 2020;98(4):839-48.
25. Thomson SC, Vallon V. Renal Effects of Sodium-Glucose Co-Transporter Inhibitors. Am J Cardiol. 2019;124 Suppl 1(Suppl 1):S28-s35.
26. Vlotides G, Mertens PR. Sodium-glucose cotransport inhibitors: mechanisms, metabolic effects and implications for the treatment of diabetic patients with chronic kidney disease. Nephrol Dial Transplant. 2015;30(8):1272-6.
27. Jacob S, Krentz AJ, Deanfield J, Rydén L. Evolution of Type 2 Diabetes Management from a Glucocentric Approach to Cardio-Renal Risk Reduction: The New Paradigm of Care. Drugs. 2021;81(12):1373-9.
28. Milder TY, Stocker SL, Samocha-Bonet D, Day RO, Greenfield JR. Sodium-glucose cotransporter 2 inhibitors for type 2 diabetes-cardiovascular and renal benefits in patients with chronic kidney disease. Eur J Clin Pharmacol. 2019;75(11):1481-90.
29. Marton A, Kaneko T, Kovalik JP, Yasui A, Nishiyama A, Kitada K, et al. Organ protection by SGLT2 inhibitors: role of metabolic energy and water conservation. Nat Rev Nephrol. 2021;17(1):65-77.
30. Nelinson DS, Sosa JM, Chilton RJ. SGLT2 inhibitors: a narrative review of efficacy and safety. J Osteopath Med. 2021;121(2):229-39.
31. Patel AB, Mistry K, Verma A. DAPA-CKD: Significant Victory for CKD with or without Diabetes. Trends Endocrinol Metab. 2021;32(6):335-7.
32. Miyoshi H, Kameda H, Yamashita K, Nakamura A, Kurihara Y. Protective effect of sodium-glucose cotransporter 2 inhibitors in patients with rapid renal function decline, stage G3 or G4 chronic kidney disease and type 2 diabetes. J Diabetes Investig. 2019;10(6):1510-7.
33. 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(7):1154-66.
34. Rajasekeran H, Cherney DZ, Lovshin JA. Do effects of sodium-glucose cotransporter-2 inhibitors in patients with diabetes give insight into potential use in non-diabetic kidney disease? Curr Opin Nephrol Hypertens. 2017;26(5):358-67.
35. Oshima M, Jardine MJ, Agarwal R, Bakris G, Cannon CP, Charytan DM, et al. Insights from CREDENCE trial indicate an acute drop in estimated glomerular filtration rate during treatment with canagliflozin with implications for clinical practice. Kidney Int. 2021;99(4):999-1009.
36. Wanner C, Marx N. SGLT2 inhibitors: the future for treatment of type 2 diabetes mellitus and other chronic diseases. Diabetologia. 2018;61(10):2134-9.
37. Oshima M, Neuen BL, Li J, Perkovic V, Charytan DM, de Zeeuw D, et al. Early Change in Albuminuria with Canagliflozin Predicts Kidney and Cardiovascular Outcomes: A Post Hoc Analysis from the CREDENCE Trial. J Am Soc Nephrol. 2020;31(12):2925-36.
38. Sano M, Goto S. Possible Mechanism of Hematocrit Elevation by Sodium Glucose

Cotransporter 2 Inhibitors and Associated Beneficial Renal and Cardiovascular Effects. Circulation. 2019;139(17):1985-7.
39. Maruyama T, Takashima H, Oguma H, Nakamura Y, Ohno M, Utsunomiya K, et al. Canagliflozin Improves Erythropoiesis in Diabetes Patients with Anemia of Chronic Kidney Disease. Diabetes Technol Ther. 2019;21(12):713-20.
40. 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(15):1436-46.
41. Cruz JE, Ahuja T, Bridgeman MB. Renal and Cardiac Implications of Sodium Glucose Cotransporter 2 (SGLT2) Inhibitors: The State of the Science. Ann Pharmacother. 2018;52(12):1238-49.