Glucose and Blood Pressure-Dependent Pathways–The Progression of Diabetic Kidney Disease

Devang M. Patel 1,*, Madhura Bose 1 and Mark E. Cooper 1,2,*

1 Department of Diabetes, Monash University Central, Clinical School, Melbourne, VIC 3004, Australia; madhura.bose@monash.edu
2 Department of Endocrinology and Diabetes, The Alfred Hospital, Melbourne, VIC 3004, Australia
* Correspondence: devang.patel@monash.edu (D.M.P.); mark.cooper@monash.edu (M.E.C.)

Received: 20 February 2020; Accepted: 17 March 2020; Published: 23 March 2020

Abstract: The major clinical associations with the progression of diabetic kidney disease (DKD) are glycemic control and systemic hypertension. Recent studies have continued to emphasize vasoactive hormone pathways including aldosterone and endothelin which suggest a key role for vasoconstrictor pathways in promoting renal damage in diabetes. The role of glucose per se remains difficult to define in DKD but appears to involve key intermediates including reactive oxygen species (ROS) and dicarbonyls such as methylglyoxal which activate intracellular pathways to promote fibrosis and inflammation in the kidney. Recent studies have identified a novel molecular interaction between hemodynamic and metabolic pathways which could lead to new treatments for DKD. This should lead to a further improvement in the outlook of DKD building on positive results from RAAS blockade and more recently newer classes of glucose-lowering agents such as SGLT2 inhibitors and GLP1 receptor agonists.

Keywords: diabetic kidney disease; diabetic nephropathy; diabetic complications; vasoactive pathways

1. Introduction

Diabetes mellitus, commonly referred to as diabetes, is clinically characterized by hyperglycemia due to insulin insufficiency arising from a lack of insulin production or insulin insensitivity [1]. Diabetes is associated with numerous complications. These complications are wide-ranging and are mainly caused because of chronic elevation of blood glucose levels. Elevated blood glucose causes damage to small blood vessels and arteries, known as “microvascular disease” and “macrovascular disease” respectively. Underlying vascular injury causing organ and tissue damage results in diabetic complications. These complications include neuropathy (neural damage), retinopathy (eye disease) and nephropathy (kidney disease) [2]. In this review, we will primarily focus on diabetic nephropathy.

Diabetic nephropathy, more commonly known as diabetic kidney disease (DKD), remains a major cause of morbidity and mortality in both type 1 diabetes (T1D) and type 2 diabetes (T2D). Diabetic subjects have an elevated risk of DKD, with features of DKD developing in approximately half of all patients with T2D and one-third with type 1 diabetes. DKD is clinically defined by the presence of impaired renal function and/or elevated urinary albumin excretion and is the main cause of the end-stage renal disease (ESRD) in developed and developing countries. DKD in some parts of the world represents over 50% of patients requiring dialysis and/or transplantation. It is evident that hemodynamic and metabolic pathways interact to promote the development of DKD [3], as reflected by the clinical associations of systemic blood pressure and glycemic control with DKD. This review
will highlight some of the molecular mechanisms and key pathways associated with susceptibility and progression of DKD.

2. Vasoactive Pathways

2.1. RAAS Pathway

2.1.1. Renin-Angiotensin-Aldosterone System (RAAS)

The RAAS pathway is one of the best-studied vasoactive pathways (Figure 1). Historically, the RAAS was thought to be a major regulator of blood pressure, water, and electrolyte homeostasis [4,5]. However, over the last few decades, a role for the RAAS in regulating cell growth and differentiation, extracellular matrix (ECM) metabolism, and inflammation in chronic diseases has been reported [6–9]. Clinical trials of angiotensin-converting enzyme (ACE) inhibitors and angiotensin II (Ang II) receptor blockers (ARBs) supported the experimental findings and were shown to retard the progression of renal disease [10,11]. These studies established that the RAAS is a major player in the development and progression of DKD.

The canonical RAAS pathway begins with the production of angiotensinogen. First, renin converts angiotensinogen to angiotensin I (Ang I). Next, angiotensin-convertingenzyme ACE, also known as ACE-1, converts Ang I into angiotensin II (Ang II). The liver is a major source of circulating Ang I in mammals, but the expression of angiotensinogen has also been reported in other organs including the diabetic kidney [12,13] and the heart [14]. Ang II continues to be regarded as the primary effector molecule of the RAAS. Renin, aldosterone, and prorenin, the classical components of the RAAS, are now known to play major roles in DKD, making them attractive therapeutic targets (Figure 1). Important biological roles have also been discovered for other components of the RAAS, including metabolites of Angiotensin II, Ang (1-7) and Ang (1-9) and enzymes involved in Ang II synthesis and degradation, ACE2 and nephrilysin [15]. These make up the alternative vasodilator RAAS pathway and are discussed later in further detail.

Angiotensin II has several rapid effects, including constriction of the vascular tree, increased aldosterone secretion, the release of antidiuretic hormone, and increased myocardial contractility. In the glomerulus, Ang II increases intraglomerular pressure and induces alteration of glomerular basement permeability, promoting an increase in proteinuria [16]. Ang II also generates oxidative stress through NADPH (Nicotinamide Adenine Dinucleotide Phosphate) oxidase and acts as a promoter of inflammation and fibrosis, via activation of proinflammatory and prosclerotic cytokines [17]. Ang II primarily acts through two specific G-protein-coupled receptors, the angiotensin type 1 (AT1) and angiotensin type 2 (AT2) receptors. Both of these receptors are widely expressed and remain expressed at sites of diabetic complications [18]. The AT1, receptor subtype, is responsible for most of the physiological and pathological effects of Ang II. Interaction of Ang II and the AT1 receptor results in coupling of G proteins and generation of adenylyl cyclase, inducing many downstream pathways such as phospholipase C/inositol triphosphate/diacylglycerol/Ca2+ pathway, MAP kinases, tyrosine kinases and phosphatases, JAK signaling, NADPH oxidase, STAT pathways, and RhoA/Rho kinase [19]. In AT1 KO mice with induced diabetes, less renal injury develops. This is consistent with the pivotal role that the AT1 receptor has been observed to play in mediating diabetes-related renal injury [20]. AT1 receptor antagonists have demonstrated kidney protection in diabetic complications [17]. AT1 receptor inhibition reduces the production of fibrotic cytokines and ECM accumulation, improves renal albumin permeability via regulating cytokines such as vascular endothelial growth factor (VEGF), and alters podocyte structure and function via altered nephrin activity [21].

AT1 and AT2 receptors share only 34% sequence homology but both have a similar binding affinity for Ang II [22]. AT2 receptor expression is much lower than AT1 and may act as a functional antagonist to AT1 receptors by attenuating ROS-mediated damage generated by AT1 receptor action [23]. The role of the AT2 receptor in diabetic complications is not well defined, and contradictory results have been published. AT2 undergoes a complex tissue-specific regulation that may influence vascular
development and repair [24]. A study using an AT2 antagonist as well as experiments in AT2 KO mice suggested that suppression of AT2 in diabetes leads to reduced macrovascular diseases [25] although renal effects remain controversial. Previous studies have also suggested that AT2 activation is linked to antiproliferative and anti-inflammatory effects, and apoptosis [26–29].

Aldosterone is released from the adrenal glands in response to various angiotensins including Ang II and III and in response to changes in serum potassium concentrations. Within the kidney, aldosterone acts as a hormone to stimulate reabsorption of sodium ions and water and release potassium ions into the urine for excretion. The downstream effects of aldosterone are propagated via activation of the mineralocorticoid receptor (MR). Aldosterone thus promotes water and salt retention to increase blood volume, which ultimately results in increased blood pressure [30]. In response to declining blood pressure, the adrenal gland stimulates aldosterone release and increases sodium reabsorption. An increase in sodium alters the extracellular osmolarity, which produces a complementary rise in systemic blood pressure. After long-term ACE inhibition or ARBs, many patients show an increase in aldosterone levels and this may be accompanied by ongoing renal damage [31,32]. In experimental models, a MR blocker reduces albuminuria, glomerulosclerosis, renal macrophage infiltration, renal monocyte chemoattractant protein 1 (MCP-1) synthesis, and expression of MCP-1’s upstream transcription factor NF-κB[33]. Although aldosterone blockade may be a potential therapeutic target in DKD, caution needs to be taken as MR blockers are associated with hyperkalemia [34]. Nevertheless, the combination therapy of aldosterone blockade with ACE inhibition orARB may provide additional renoprotection in patients with DKD. This approach has been rekindled with the advent of new MR blockers with less hyperkalemia [35].

2.1.2. Vasodilatory Arm of RAAS

ACE2, Ang-(1-7) and Ang-(1-9) are key components of the noncanonical RAAS pathway [15,36]. Like ACE, ACE2 belongs to the family of zinc metalloproteases. ACE2 is a membrane-bound enzyme that is also found in a soluble form in the plasma as well as in tissues such as the heart, liver, kidney, brain, and blood vessels [37]. ACE2 gene expression is high in the kidney and low in the heart, aorta, lung, and retina. ACE and ACE2 share 40% amino acid sequence homology but have different substrate specificities [38]. ACE2 is a mono-carboxypeptidase with a single active site and a greater (~400-fold) affinity for Ang II than Ang I [39,40]. Since ACE2 degrades Ang II to Ang (1-7), it has been postulated that ACE2 opposes ACE to regulate the balance between Ang II and Ang (1-7) [41]. Experimental studies report that the expression level of ACE2 in the diabetic kidney varies depending on the stage of the disease. In the streptozotocin-induced diabetic rat, ACE2 expression was shown to be decreased in the proximal tubules, while glomerular expression was increased [42]. In the db/db mouse model, ACE2 mRNA and protein expression were shown to be up-regulated in the renal cortex in early diabetes [43,44]. Based on these data it was hypothesized that in the early stages of DKD, ACE2 is up-regulated as a protective mechanism against the increase in ACE-dependent Ang II formation and subsequent development of DKD. As a result of prolonged hyperglycemia and consequent activation of proinflammatory and profibrotic pathways, ACE2 expression becomes down-regulated and this deficiency in the vasodilatory arm of the RAAS may contribute to disease progression. This hypothesis was supported by the study from Ye and colleagues, which showed that mice infused with an ACE2 antagonist developed albuminuria and glomerulosclerosis [44].

Ang-(1-7) can be produced from Ang I by different endopeptidases, by catabolism of Ang (1-9) by ACE, and from Ang II by ACE2 [45,46]. Ang (1-7), acts through the MasR receptor [47] and is a potent vasodilator shown to have antihypertensive, anti-inflammatory, and antiproliferative properties [46,48,49]. These effects suggest that Ang (1-7) acts as a counter regulator to AT1 receptor-mediated effects of Ang II. Ang-(1-7) can activate different effectors such as cyclooxygenase-2 or COX-2, forkhead box protein O1 (FOXO1), and vasodilator mediators such as prostanoids and NO (nitric oxide). An elegant study by Benter and colleagues [50] suggested that treatment with Ang-(1-7) and/or the Ang-(1–7) receptor MasR agonist AVE-0991 reduces albuminuria and abrogates
the diabetes-induced abnormal vascular responsiveness to norepinephrine, endothelin-1, and Ang II. These observations suggest that Ang-(1-7) might be a renoprotective agent in diabetes. Ang-(1-7) also showed MasR-mediated cardioprotective effects via MAPK, phosphoinositide 3-kinase (PI3K)/Akt, and NADPH oxidase signaling pathways [51,52].

Ang-(1-9) was first detected in the early 1970s but was initially viewed to be biologically inactive and act indirectly by competing with Ang I for the active site of ACE [40,53,54]. The role of Ang (1-9) in DKD is not well understood, and the generation of Ang (1-9) from Ang II may only be relevant in the context of elevated Ang II [55]. However, there is increasing evidence to show that Ang-(1-9) has cardiovascular effects in vivo and in vitro via AT2 receptor activation [56–58]. It was recently shown that inhibition of the Rho-associated and coiled-coil-containing protein kinase signaling pathway increases ACE2 activity and plasma Ang-(1-9) levels [59]. Overexpression of various genes linked to vascular remodeling was normalized and mRNA eNOS (endothelial nitric oxide synthases) levels increased, revealing a novel role for Ang-(1-9) in vascular protection. Similarly, Ang-(1-9) infusion improved vasorelaxation and NO levels in spontaneously hypertensive stroke prone (SHRSP) rats. The same study also found that Ang-(1-9) increases NADPH oxidase 4 expression [60]. Nevertheless, further studies are needed to evaluate the significance of Ang (1-9) in the diabetic kidney.

2.1.3. The RAAS Inhibition as a Therapeutic Target

The negative impact of AngII in DKD has been well documented and it is well accepted that ACE inhibitors (ACEi) and ARBs prevent or delay the progression of DKD in both type 1 and 2 diabetes [10,11,61,62]. The specific role of ACEi was first studied in type 1 diabetic subjects [62] and currently, it is a recommended therapy for the patient with type 1 diabetes, normal GFR but with an early stage of kidney damage and microalbuminuria [63].

The ALLHAT (Antihypertensive Lipid-Lowering Treatment to Prevent Heart Attack Trial) [64] and ADVANCE (Action in Diabetes and Vascular Disease: Preterax and Diamicron MR Controlled Evaluation) [65] trials have reported data on renal endpoints, including “hard” outcomes. In ALLHAT, an ACEi (lisinopril) was not better than a diuretic (chlorthalidone), or a calcium channel blocker (amlodipine) on the renal composite outcomes. The absence of a significant difference in ESRD between lisinopril and either chlorthalidone or amlodipine was confirmed after an extended follow-up. However, the data obtained in ALLHAT have limited significance due to the absence of any data on albuminuria and blood pressure control was different among treatment groups [64,66]. The composite renal outcome, in the ADVANCE trial, was significantly lowering the perindopril group than in the placebo group, with the results mainly explained by the positive effect on the new-onset of microalbuminuria, whereas the impact on doubling serum creatinine or ESRD was not significant [65,67]. In MICRO-HOPE (Microalbuminuria Cardiovascular and Renal Outcomes–Heart Outcomes Prevention Evaluation), DIABYCAR, and the PERSUADE studies in T2D patients, no significant differences in the changes of serum creatinine or in renal outcomes were reported when comparing an ACEi with placebo [68–70]. However, in MICRO-HOPE [68] and DIABYCAR [69], the rate of new-onset of persistent macroalbuminuria was significantly lower and the rate of regression from macro to micro or from micro to normal albuminuria was numerically higher in the ACEi group compared with the placebo group. Several meta-analyses (without differentiating between type 1 and type 2 diabetes) support the use of ACEi in diabetic patients with diabetic nephropathy, especially in the presence of significant albuminuria [71–73].

Evidence of the renoprotective role of ARB in T2D patients is more robust than for ACEi. The Irbesartan Diabetic Nephropathy Trial (IDNT) [11] and Reduction of Endpoints in NIDDM with the AII Antagonist Losartan (RENAAL) [10] trials included a majority of hypertensive patients with already advanced chronic kidney diseases (CKD) [10,11]. The IDNT compared irbesartan versus placebo versus amlodipine [11]. RENAAL compared losartan with placebo [10]. In both studies, the effect on lowering albuminuria was significantly better in the ARB groups and the risk of the renal composite outcome was significantly lower with the ARB compared with the placebo.
The superiority of ARBs compared to ACEIs has been debated for a long time. In a direct comparison in randomized clinical trials in T2D patients with albuminuria, a similar effect on albuminuria and GFR decline was observed with telmisartan and enalapril [74]. The large ONTARGET trial, although not specifically dedicated to T2D patients, did not show any superiority in terms of renal endpoints or “hard” endpoints of telmisartan versus ramipril [75]. The pooled analysis also do not support any clear superiority of ARB over ACEIs in T2D patients [71,72,76–78].

Because of two different modes of blocking the RAAS (inhibiting the conversion of Ang I to Ang II versus blocking the activation of the angiotensin type 1 (AT1) receptor subtype) and the residual risk of nephropathy progression on either treatment alone, combination treatments with ACEi and ARB were explored in order to achieve better RAAS blockade. This intervention showed promising results regarding blood pressure reduction and proteinuria [79,80], but one of the larger trials, Ongoing Telmisartan Alone and in Combination with Ramipril Global Endpoint Trial (ONTARGET), was terminated because of a lack of long-term benefits and a greater risk of acute renal failure and hyperkalemia, as compared to monotherapy with either agent [81]. Data have emerged from clinical trials demonstrating that the use of “supratherapeutic doses” (doses greater than those approved for lowering blood pressure), compared with standard doses, has favorable safety, tolerability, and efficacy in reducing proteinuria in both diabetic and nondiabetic patients with chronic kidney disease. Supratherapeutic dosing with one agent may be a valuable approach for optimizing RAAS blockade and providing renoprotection [82] rather than combination approaches using different RAAS blockers.

To achieve superior RAAS blockade, the use of a direct inhibitor of the upstream enzyme, renin was explored. The addition of the renin inhibitor aliskiren to the ARB losartan in type 2 diabetic subjects with nephropathy in the AVOID study led to an enhanced reduction in proteinuria despite minimal differences in blood pressure between groups [83]. However, the larger subsequent ALTITUDE (Aliskiren Trial in Type 2 Diabetes Using Cardiovascular and Renal Disease Endpoints) trial was terminated early because of a lack of benefit and potential harm with a signal toward an increase in a combined cardiovascular endpoint when compared to monotherapy with an ACEi or ARB [84] and a clear increase in hyperkalemia and hypotension.

Another method of intensifying RAAS blockade involves the addition of a mineralocorticoid receptor antagonist (MRA) to ACEi or ARB, which has been shown to reduce albuminuria as well as inflammation and fibrosis in the kidney [85,86]. However, because of adverse side effects, this has limited its clinical utility especially in patients with impaired kidney function. The novel non-steroidal MRA finerenone appears to have a lower hyperkalemia risk despite having higher MR selectivity, potentially because of less renal accumulation than the older MRAs [87,88]. In patients with mild to moderate CKD and heart failure, finerenone reduced albuminuria and deterioration of renal function with a lower risk of hyperkalemia when compared to spironolactone [87]. In patients with type 2 diabetes, macroalbuminuria, and reduced kidney function addition of finerenone to ACEi or ARB treatment dose-dependently reduced albuminuria with only a 2–3% incidence of hyperkalemia [35]. Two-Phase 3 clinical trials (FIDELIO-DKD and FIGARO-DKD) are in progress to evaluate the effect of finerenone on nephropathy and cardiovascular disease in type 2 diabetic patients.

2.2. Endothelins

The endothelin family consists of three endothelins: endothelin-1 (ET-1), endothelin-2 (ET-2), and endothelin-3 (ET-3). Endothelin-1, a 21 amino acid peptide, is considered the most potent, vasoconstrictor in the kidney with proinflammatory, mitogenic, and profibrotic properties [89–92]. ET-1 exerts its renal actions mainly through the two endothelin receptor subtypes, ETA and ETB [90,93,94]. In the kidney, ET-1 and its receptors are widely expressed in every cell type [94,95]. ET-1 appears to have a vital role in modulating renal function under normal physiological conditions and is an important mediator of pathophysiological conditions such as DKD. ETA and ETB receptor antagonists prevent the development of hypertension, renal vasoconstriction, inflammation, proteinuria, glomerulosclerosis,
and tubulointerstitial fibrosis in experimental models of DKD [96–100]. The vasoconstrictive actions of endothelin-1 are primarily mediated via ETA receptors [93]. The ETB receptor is known to be involved in endothelin-1 clearance [101]. In various animal models, elevated expression of endothelin-1 has been observed in the diabetic kidney [98,101,102]. Experimental models of diabetic nephropathy have also shown increases in ETA receptor expression [102,103]. Under high glucose conditions in vitro, together with Ang II, increases in ET-1 mRNA and protein levels were observed [104,105]. Further, increased endothelin-1 increased ECM proteins via MAPK and PKC activation, and was associated with an increased concentration of ROS [104,106–108]. These findings support the view that renoprotection in the setting of diabetes may occur by antagonizing its actions. Indeed, Heerspink et al. recently showed that the ET antagonist, Atrasentan reduced the risk of renal events in patients with diabetes and CKD. These findings from the SONAR trial suggest a role for selective endothelin receptor antagonists in protecting renal function in patients with T2D at high risk of developing end-stage kidney disease [109]. These findings, build on a large number of studies with this [110] and other endothelin antagonists such as avosentan which have also demonstrated renoprotection [111].

2.3. Urotensin II

Urotensin II was first discovered nearly 40 years ago [112] and is considered the most potent vasoconstrictor identified to date [113]. However, its vasoconstrictive properties are restricted to certain regional vascular beds. Urotensin II is found primarily in the central nervous system but is also expressed at low levels in the kidney, spleen, prostate, thymus, adrenal glands, and small intestine and is also detected in blood and urine. It plays an important role in the pathogenesis of acute and chronic diseases, in stressful and adaptive reactions of the body, and in the development of pathological conditions such as renal failure and DKD [114].

Urotensin II has also been implicated in pancreatic beta-cell dysfunction and in diabetic retinopathy. Marked elevated levels of urotensin II and urotensin II receptor, also known as GPR14 expression have been observed in renal biopsies of patients with DKD [115–117]. This finding was further supported in animal experiments suggesting urotensin II/GPR14 as a mediator of renal fibrosis [118]. The molecular mechanism by which urotensin II promotes DKD is not well understood. Urotensin II causes ER stress and promotes the production of ECM in kidney tubular epithelial cells from diabetic mice, which leads to kidney fibrosis in diabetic nephropathy [116]. Increasing urotensin II expression in the kidney is associated with a significant increase in the synthesis of the profibrotic factors transforming growth factor-β1 (TGFβ1), fibronectin, and type IV collagen. Stimulation of the urotensin II receptor can also trigger vasodilation via an endothelium-dependent nitric oxide-mediated effect [119,120]. The role of urotensin II in kidney damage was confirmed in a clinical trial using the urotensin II receptor antagonist palosuran, which significantly reduced albumin excretion in diabetic patients with macrolebminuria [121]. Totsune et al. [122] found that patients with type II diabetes and renal dysfunction demonstrated significant increases in urinary urotensin II, in proportion to their level of renal dysfunction. Elevated urinary and circulating urotensin II levels in diabetic patients with renal impairment have also suggested a possible role for urotensin II in the mediation of progressive renal disease [122]. A study by Tian et al. [118] demonstrated increased transcription of urotensin II and the urotensin II receptor in the kidneys of animals with experimental diabetes and further reported AngII and TGFβ1 as possible mediators of the increase in urotensin II gene transcription (Figure 1).
3. Metabolic Pathways

3.1. Mitochondria and Reactive Oxygen Species (ROS)

The overproduction of ROS bridges the gap between altered metabolic pathways in the kidneys and disrupted renal hemodynamics known to be associated with DKD [123]. These pathways ultimately lead to inflammation, fibrosis, and endothelial dysfunction. Mitochondria produce energy in the form of adenosine triphosphate (ATP). This occurs via the electron transport chain, a series of proteins that work to create an electrochemical gradient using electrons sourced from fuels such as glucose, powered by hydrogen ion (H+) pumps, with energy harnessed to synthesize ATP and form water. When this process is dysregulated, such as in hyperglycemic states where glucose is in excess, it can result in excessive production of superoxide (O2−) and other ROS [2]. While ROS have a role in intracellular signaling, their accumulation can lead to oxidative stress, damage to critical cellular components (particularly protein and DNA), and cell death. Excessive ROS in the context of renal disease leads to renal fibrosis and a decline in renal function [124]. The damaging effect of ROS is thought to be mediated by activation of a number of pathways including PKC, NF-Kappa-B, hexosamine, and formation of advanced glycosylation end products (AGE). Physiological counter-regulatory mechanisms to protect against damage from ROS, in the form of antioxidants and repair enzymes have been explored. Antioxidants including coenzyme Q10 (ubiquinone), resveratrol, and ascorbic acid have been trialed in animal models of DKD with some evidence of therapeutic benefit [125–127] (Figure 2). Idebenone has preferential mitochondrial uptake by organs such as neurons, kidney, and cardiac tissues. Indeed, this compound is used in human respiratory chain diseases such as Friedreich ataxia where the mitochondrial generation of ATP appears to be preserved [128]. Similarly, mitoquinone has been trialed in mouse models of diabetes and showed improvement in glomerular function and albuminuria, potentially by promoting the destruction of defective mitochondria [128]. However, mitochondrial toxicity is a major concern in mitoquinone’s clinical utility [129]. Clinical trials using mitoquinone in CKD are underway and results are yet to be reported.
3.2. NADPH Oxidase (NOX)

NADPH oxidase (NOX) was originally discovered in neutrophils, where it produces vast quantities of \( O_2^- \) by electron transport to augment host-pathogen defenses [2]. In nonphagocytic cells, the ROS generated by NOX are postulated to act as second messengers. Activation of NOX can be triggered by several receptor-mediated pathways, such as receptors of AGEs, ANG II, and other cytokines and hormones [130,131]. Dysregulation of NOX activity is another source of oxidative stress contributing to the pathogenesis of DKD. NOX is also found in other cell types, where its physiological role is poorly understood but may involve signaling in the regulation of vascular tone and angiogenesis [124]. NOX-4 is a cytosolic and mitochondrial protein [132]. The potent NOX subunit NOX-4 is seen of increased levels in renal cells in animal models of DKD when compared to non-diabetic models [132]. Several studies suggest that upregulated NOX-4 is the primary source of ROS in the kidney, contributing to renal fibrosis and hence DKD [133].

In animal models, the deletion of NOX-4 and administration of a NOX-4/NOX-1 inhibitor GKT 137831 (Figure 2) have been shown to be renoprotective [134], but clinical trials in type 2 diabetic subjects did not show encouraging data albeit the trial duration was only for 3 months. Other NOX family members have also been explored. The NOX-1 isoform is considered to play a key role in atherosclerosis but a role in renal disease has not been confirmed [135]. Transgenic expression of NOX-5 in rodents, specifically in podocytes and mesangial cells, have demonstrated a likely pathogenic role for this isoform [136,137]. With NOX-2 playing a central role in immune defense it is critical that any NOX inhibitor is specific for the other isoforms without inhibiting NOX-2.

3.3. Nitric Oxide Synthase

Nitric oxide (NO) is a short-lived gas with biological and regulatory properties, produced via nitric oxide synthases (NOS). NO is a common free radical with a role in cellular signaling which is produced by numerous cell populations in mammals. Nitric oxide synthase (NOS) produces NO from NADPH, L-arginine, and oxygen. One of the major roles of NO is vascular dilatation following its release from endothelial cells. Indeed, NO is one of the most powerful vasodilators and is generally thought to be vasoprotective in the context of diabetes [138].

In diabetes, NOS is uncoupled following L-arginine depletion, where it produces superoxide instead of NO. This causes superoxide to accumulate at sites of diabetic complications [139]. Indeed, the administration of L-arginine to db/db mice prevents cardiac fibrosis [140]. There is, however, some controversy as to the contribution of NOS uncoupling to diabetic complications. Early in disease development, NO production within tissues is thought to increase [141] as a result of changes in NOS activity [139]. It has been postulated that the therapeutic blockade of this pathway could be beneficial at this stage of diabetes [142].

By contrast, most studies performed later in the progression of diabetes suggest that functional decline in complication-prone organs is seen in concert with a state of progressive NO deficiency [143]. These changes in NO production are attributed to multiple mechanisms such as glucose and AGE quenching, as well as inhibition and/or posttranslational modification of NOS. Indeed, several studies support this view, with chronic NO inhibition having been identified to have no effects [144] or detrimental outcomes for renal disease as a consequence of diabetes [145]. These complex temporal changes in NO production seen during the evolution of diabetic complications make it difficult to determine the clinical applicability of NOS activity inhibition, given that a deficiency in NO production seems to be an equally important pathological contributor to diabetic complications including DKD.

3.4. Dicarbonyl Synthesis

In diabetes, hyperglycemia leads to an increase in the accumulation of dicarbonyls in plasma and in cells because of increased formation or to decreased activity of the detoxifying system, or both. Increased dicarbonyl concentrations interfere with cellular homeostasis and this is referred to
as dicarbonyl stress [146]. Dicarbonyl stress has been identified as a major contributing factor to the progression of diabetic complications. Methylglyoxal (MGO), glyoxal (GO), and 3-deoxyglucosone (3-DG) are the main dicarbonyls that are present in human plasma and cells [147]. Among these, MGO is the most reactive and abundant dicarbonyl present in the body and thus has gained the most attention. MGO is associated with hyperglycemia in diabetes, diabetic vascular complications, hypertension, dyslipidemia, and obesity [148].

MGO is mainly formed by the nonenzymatic degradation of the triose phosphates, glyceraldehyde-3-phosphate (G3P) and dihydroxyacetone-phosphate (DHAP), as a byproduct of glucose metabolism [149]. Under physiological conditions formation of MGO constitutes only 0.1% of the glucotriose flux [150]. MGO is highly reactive and only 1% of MGO exists in the free unhydrated, monohydrated or dehydrated form. MGO primarily reacts with the arginine residues of proteins [146]. Furthermore, MGO can react with genomic DNA which can lead to genomic instability [151].

Plasma levels of MGO are associated with the prevalence of CKD in diabetes [152–154]. Higher plasma MGO levels are associated with an increased risk for lower eGFR and increased albuminuria in both T1D and T2D [155–157]. In addition to impaired detoxification, low eGFR may contribute to higher plasma concentrations of MGO in patients with diabetic nephropathy because of metabolic stress in tissues which may enhance the formation of MGO and/or inhibit its metabolism [158].

Glyoxalase 1 (Glo1) plays an important role in maintaining MGO levels with impairment of the rate of MGO detoxification by Glo1 potentially determining susceptibility to diabetic nephropathy. The knockdown of Glo1 increases MG-H1 residues in proteins of renal glomeruli and tubules, accompanied by the development of albuminuria and mesangial expansion [159]. Overexpression of Glo1 in glomerular mesangial cells decreases glucose-induced expression of mitochondrial oxidative phosphorylation complexes I, II, and III, indicating that MGO plays a role in high glucose-induced oxidative stress in mesangial cells [160]. Glo1 overexpression in diabetic rats also decreases markers for nephropathy, such as urinary KIM-1. Diabetes-induced loss of podocytes in the glomerulus, one of the early hallmarks of diabetic nephropathy [161], is also attenuated by overexpression of Glo1 [162]. Therefore, MGO may contribute to albuminuria through the accelerated loss of podocytes. Similar results were obtained using kidneys from nondiabetic Glo1 knockdown mice, indicating that increased MGO alone is sufficient to cause kidney dysfunction [163].

A clinical study in type 2 diabetic patients reported a positive association of serum MGO levels with the urinary albumin/creatinine ratio (ACR) at baseline while changes in the estimated glomerular filtration rate were inversely associated with MGO during follow-up [157]. Similarly, levels of urinary and plasma MGO levels correlated with basement membrane thickness in two cohorts of patients while MGO levels in red blood cells were higher in progressors vs. non-progressors of diabetic nephropathy. This link between MGO and renal disease was confirmed in another study, which reported the correlation of plasma MGO levels with serum creatinine and ACR in type 2 diabetic patients [152].

Similar to MG-H1, the level of the 3-DG derived AGE, 3-DG-H1, are elevated in experimental diabetes in the renal glomeruli [164]. Plasma levels of 3-DG also correlate with glomerular basement membrane thickness [154]. Kusunoki et al. reported elevated serum levels of 3-DG in diabetic patients with normoalbuminuria and further elevations in those patients with microalbuminuria and overt proteinuria [165]. Compared to 3-DG and MGO-derived AGEs, the glyoxal-derived AGE G-H1 is present at rather low levels, except in plasma [164,166,167]. Finally, the glyoxal-derived DNA product, GdG was elevated in the plasma of diabetic patients which suggests that glyoxal in contrast to methylglyoxal is more relevant for the glycation of DNA rather than for protein glycation.

MGO as a Therapeutic Target

Reducing the accumulation of MGO can provide new therapeutic opportunities for minimizing the pathophysiological conditions associated with MGO stress. Approaches to limit MGO stress are based on (1) the direct quenching of MGO, (2) the prevention or reduction of the formation of MGO, and (3) compounds inducing the expression of the MGO degrading enzyme, Glo1.
Aminoguanidine is effective in animal models of diabetes in lowering AGE formation possibly by quenching MGO and preventing diabetic complications including DKD [168,169] as well as retinopathy [170] and nephropathy [171]. Unfortunately, the use of aminoguanidine in two large clinical trials in individuals with T1D [172] and T2D [173] showed disappointing results and the trial was terminated. Interestingly, creatine shows structural analogy to aminoguanidine, and it has been shown that creatine is a scavenger for MGO under physiological conditions [174]. The dietary intake of creatine may thus provide a natural mechanism for the trapping of MGO. Experimental studies have shown that another drug that may quench MGO, alagebrium reduces large artery stiffness, left ventricular mass, diastolic stiffness of the heart, atherosclerosis, and diabetic nephropathy [175]. Clinical trials on alagebrium have shown mixed results. Two uncontrolled studies in hypertensive individuals have reported an improved aortic augmentation index and brachial artery flow-mediated dilation after alagebrium treatment [176,177]. Although the results from experimental studies were promising, several trials were prematurely discontinued because of financial reasons.

The natural vitamin B6 analog pyridoxamine has been described as an anti-glycating agent, which operates, at least partly, via the scavenging of MGO [178]. Clinical studies have shown variable data in phase II clinical trials. Pyridoxamine not only inhibited the formation of AGEs but also improved kidney function in T2D with overt nephropathy [179], whereas another trial found no beneficial effect of pyridoxamine on the reduction of serum creatinine levels [180].

Metformin is the most widely prescribed oral glucose-lowering agents for T2D, mainly due to the suppression of hepatic gluconeogenesis and increasing cellular uptake of glucose [181,182]. Metformin, similar in structure to the MGO scavenger aminoguanidine, is also able to trap MGO [183]. Indeed, it has been shown that metformin treatment reduces systemic plasma MGO levels [184,185], accompanied by increased levels of a metformin-MGO imidazolinone compound [186]. However, metformin has low MGO scavenging ability which suggests that scavenging of MGO might not be the primary effect by which metformin reduces systemic MGO concentrations [146] and this drug has not been shown to be renoprotective independent of its glucose lowering effect. However, one cannot exclude a potential renoprotective effect albeit only previously reported in an experimental study of a non-diabetic model of CKD [187].

Another treatment strategy for reducing MGO is the use of Glo1 inducers. Polyphenols are known to upregulate Glo1 expression [188–190]. Flavonoids are the largest group of polyphenols. It was reported, in a randomized, double-blind, placebo-controlled, crossover trial with pure flavonoids in healthy (pre)hypertensive men and women, that the polyphenol quercetin but not epicatechin decreased plasma MGO concentrations [191]. However, the effect could not be explained by an increase in the expression of Glo1, and therefore, the MGO-reducing capacity of quercetin is likely to be due to the scavenging activity of this drug [192].

Phenethyl isothiocyanate and sulforaphane [193] are compounds found in cruciferous vegetables that are known to activate nuclear factor erythroid 2-related factor 2 (Nrf2) [194]. Nrf2 plays an important role in protection against oxidative damage and the induction of antioxidant enzymes [195]. Activators of Nrf2 may be responsible for an increase in Glo1 mRNA, protein, and activity [193] levels, thus leading to a decrease in MGO levels [196]. Relatively low physiological concentrations of sulforaphane led to an increase in Glo1 expression [197] in lymphocytes. Studies found that trans-resveratrol (tRES) and hesperetin (HESP) coformulation is a potent inducer of Glo1 [198]. In a clinical trial, the tRES-HESP combination produced a 22% increase in the Glo1 activity of peripheral blood mononuclear cells together with a 37% decrease in plasma MGO [199]. Nevertheless, the renal effects of these various approaches to increase Glo1 have not been clearly described.

3.5. Advanced Glycation and RAGE

Advanced glycation of free amino groups on proteins and amino acids is a nonenzymatic post-translational modification, which begins with covalent attachment of heterogeneous sugar moieties. This reaction is influenced by many factors including intracellular glucose concentrations,
pH, and time. There is a large body of evidence to show that advanced glycation may modulate insulin secretion [200] and signaling [201,202], stabilize ECM proteins via cross-linking and modify collagens including type IV collagen, a basement membrane glycoprotein [203–205].

Persistent hyperglycemia and oxidative stress accelerate the formation of AGEs [206]. In diabetes, not only do long-lived proteins become more heavily modified, but short-lived proteins are also altered by advanced glycation. In addition, glycolytic metabolites of glucose such as glyoxal and products of the Kreb’s citric acid cycle are much more efficient initiators of intracellular advanced glycation than glucose per se. AGE pathways are as heterogeneous as their products and occur as a result of complex biochemical reactions involving the formation of Amadori products, the pentose phosphate pathway glyceraldehyde-3-phosphate, and formation of the reactive carbonyl methylglyoxal, a dicarbonyl that was described earlier to play a role in diabetic complications [207].

The consequences of the modification of proteins by advanced glycation are numerous. Extracellular generation of AGEs has effects on matrix-matrix, cell-cell, or matrix-cell interactions. This has been shown under pathological conditions to excessively crosslink the matrix resulting in stiffening [208–210]. This may occur as a consequence of intracellular AGE modification of ECM proteins, altering their secretory properties and folding. AGEs can also interfere with cellular homeostasis via interaction with cellular receptors. There are many AGE receptors [211–214], but the role of the receptor for advanced glycation end products (RAGE) is the most widely studied in diabetic complications. RAGE is a pattern recognition receptor that binds to multiple ligands such as AGE modified proteins, HMGBl [215], S100 calgranulins [216], and β-amyloid [216]. RAGE appears to have a major role in immune and inflammatory responses [217,218]. The ligation of AGEs to RAGE also results in NAD(P)H oxidase [219] and mitochondrial [220] dependent ROS generation. In diabetes, AGEs can induce the production of chemokines such as MCP-1 [221–223], profibrotic cytokines, and growth factors including TGFβ1 [224–226] and connective tissue growth factor (CTGF), and the angiogenic growth factor VEGF [205].

The RAGE gene can produce a number of protein splice variants [227–229] but membrane-bound and circulating RAGE are the most common RAGE products [229]. Circulating soluble RAGE can also be produced via cleavage of membrane-bound RAGE [230]. The capacity of soluble RAGE, a so-called decoy receptor, to compete for ligands appears to play an important role in the development and progression of diabetic complications. In diabetic individuals with complications, studies now conclusively show that increases in soluble RAGE are predictive of both cardiovascular events [231–234] and all-cause mortality [234,235].

Glycosylated hemoglobin level (HbA1c) is widely used as a marker of glycemic control and predictor of diabetic complications, albeit HbA1c is an earlier rather than an advanced glycation product. Moreover, studies in both T1D and T2D conclusively show that elevation in HbA1c is one of the most useful prognostic indicators for risk in individuals with diabetes. Therefore, it is not totally surprising that elevations in circulating concentrations of RAGE ligands including AGEs [236] and HMGB1 [237] are predictive of macrovascular complications in diabetes. In addition, the urinary AGE concentration can act as a biomarker of DKD given that the ultimate fate of most AGE-modified proteins and peptides is getting excreted via kidney excretion [238–240].

In animal studies, B complex vitamins pyridoxamine (B6) and thiamine (B1) appeared to show evidence for reducing AGEs in preclinical studies, but have failed to show any major impact on DKD in clinical trials [241,242]. Alagebrum, which appears to have multiple actions including breaking crosslinks to dismantle AGEs as well as quenching MGO, has shown promising renal effects but when trialed in combination with an ACEi it did not confer additional renoprotection [243]. Further another AGE inhibitor, OPB-9195 [244] also can delay experimental diabetic nephropathy. Overexpression of glyoxalase-1 described earlier in this report is responsible for the removal of the AGE precursor MGO and this leads to a decrease in the tissue accumulation of AGEs [245,246]. A small-molecule RAGE inhibitor azeliragon has been trialed as a treatment in humans with Alzheimer’s
disease as AGEs interact with beta-amyloid in the formation of plaques; however, it was unexpectedly found to accelerate cognitive decline [247]. This failure of a RAGE inhibitor resulted in the closure of that drug discovery program and ultimately was not tested in DKD. Administration of soluble RAGE or RAGE-neutralizing antibodies [248] in rodent models of diabetes have also shown protection against complications [219,220,249–251]. Although the reduction in AGEs or targeting RAGE remain promising approaches, because of the adverse effect in some cases possibly as a result of an intrinsic role for RAGE in innate and adaptive immunity [252–254] more careful pharmacological targeting of this pathway is required (Figure 2).

**Figure 2.** Role of glucose in promoting dicarbonyl and oxidative stress and reducing NO availability in order to promote renal injury [125,126,128,134,168,178,183,242,243,246,247].

### 4. Hemodynamic and Metabolic Pathway Interactions

As outlined previously, metabolic and hemodynamic pathways interact to promote DKD [3]. The underlying molecular mechanisms are not fully explained. There are often common mediators of injury as a result of the activation of either pathway. This includes ROS, signaling pathways such as protein kinase C (PKC) and activation of both profibrotic and proinflammatory pathways. Furthermore, there appears to be direct interactions between the two pathways. For example, Thomas et al. identified the effects of Ang II infusion in generating AGE whereas AGE infusion promoted expression of various components of the RAAS [255]. In another study, Fukami et al. demonstrated that AGEs could activate autocrine Ang II signaling in mesangial cells [256]. Recently a potential molecular mechanism linking the AT1 receptor to a key mediator of biological effects of AGEs, RAGE was identified. It was shown that RAGE transactivation mediates Ang II-induced inflammation. This occurs via the formation of a heteromeric complex of the AT1 receptor with RAGE [257]. The relevance of these receptor interactions remains to be fully elucidated within the kidney including in the setting of diabetes.

### 5. New Targets for Renoprotection

#### 5.1. Sodium-Glucose Cotransporter 2 (SGLT2) Inhibitors

High-capacity, low-affinity SGLT2 transporters in the proximal tubules of the kidney are responsible for approximately 97% of the reabsorption of filtered glucose, thus minimizing glycosuria under normoglycemic conditions [258]. Sodium-glucose co-transporter-2 inhibitors are a unique class of anti-diabetic agents that have beneficial effects on blood pressure and body weight [259–262]. Under hyperglycemic conditions, the expression of SGLT2 in the proximal tubules is upregulated,
thus increasing the threshold for glycosuria in diabetic subjects [263]. Pharmacological inhibition of SGLT2 (SGLT2i) reduces the capacity of the renal tubules to reabsorb glucose by at least 50%, thereby increasing glycosuria and lowering blood glucose levels [264]. The blood glucose-lowering efficacy of SGLT2i has been confirmed in both placebo-controlled and active comparator studies, and the additional benefits of weight loss, blood pressure reduction, and negligible risk of hypoglycemia have made SGLT2i popular as second-line therapy after metformin in T2D [258].

Experimental data with SGLT2i demonstrated reductions in intraglomerular pressure, proteinuria and histological manifestations of glomerular and tubular damage, even in the absence of blood pressure reduction [265,266]. In clinical trials, the initiation of SGLT2i treatment causes an acute, reversible decrease in eGFR [267,268]. Substantial reductions in cardiovascular morbidity and mortality, as well as hospitalizations for heart failure in cardiovascular outcome trials (CVOTs), are exciting additional benefits to SGLT2i therapy [269,270]. Subsequent long-term trials have reported sustained reductions in albuminuria and preservation of eGFR with these agents [267,271].

Remarkable benefits on the development and progression of nephropathy have not been entirely understood often in the context of marginal reductions in glycemia and blood pressure as achieved in some of the CVOTs [268,272]. It has been hypothesized that the upregulation and activation of SGLT2 in diabetes results in an increased proximal tubular reabsorption of sodium via the sodium-glucose cotransporter. The reduced sodium concentration at the macula densa level activates tubuloglomerular feedback (TGF) leading to increased intraglomerular pressure and hyperfiltration. Pharmacological SGLT2i reverses these pathophysiological changes, causing less sodium reabsorption in the proximal tubule, thus reducing sodium and fluid retention as well as systemic blood pressure with the increase in sodium and glucose concentration at the macula densa triggering adenosine release, which is a paracrine mediator of TGF downregulation [272,273]. Adenosine enhances arteriolar tone, resulting in reduced intraglomerular pressure, reduction in albuminuria, and amelioration of hyperfiltration. Increased hydrostatic pressure in Bowman’s capsule, because of the increased osmotic concentration of sodium and glucose, further enhances the effect of SGLT2i.

SGLT2i causes a diminished consumption of adenosine and oxygen in the proximal tubule, and thus reduces susceptibility to acute kidney injury (AKI) [268] as was seen in the EMPA-REG clinical trial [271]. Animal experiments have shown that SGLT2i downregulates inflammatory markers, oxidative stress and fibrosis [272]. A meta-analysis has suggested that SGLT2i have moderate benefits on atherosclerotic major adverse cardiovascular events but these benefits appear to be confined to patients with established atherosclerotic cardiovascular disease. However, SGLT2i have robust benefits in reducing hospitalization for heart failure and progression of renal disease regardless of existing atherosclerotic cardiovascular disease or a history of heart failure [274]. A primary renal trial, the Canagliflozin and Renal Endpoints in Diabetes with Established Nephropathy Clinical Evaluation (CREDENCE) study, was stopped prematurely because of positive renal outcomes [275]. The recently published results showed that in patients with T2D and kidney disease, the risk of kidney failure and cardiovascular events was lower in the canagliflozin than in the placebo group.

5.2. Incretin-Related Therapies

Incretin-related therapies include dipeptidyl peptidase-4 inhibitors (DPP-4 inhibitors) and GLP-1 RAs (glucagon-like peptide type 1 receptor agonists). The GLP-1 peptide is a gastrointestinal hormone that acts as an incretin enhancing insulin secretion and has a pleiotropic effect on glucose metabolism. The incretin-based agents, GLP-1 RAs and DPP-4 inhibitors are novel antidiabetic drugs widely used as second-line therapy after metformin for the control of hyperglycemia in type 2 diabetic patients. GLP-1 RAs directly stimulate the GLP-1 receptor and DPP-4 inhibitors act by inhibiting the enzyme involved in the degradation of GLP-1, thereby increasing its serum concentration. Both drug classes exert their antihyperglycemic effect by stimulation of insulin secretion and suppression of glucagon secretion. In various rodent studies, incretin-based therapies decrease the activity of biomarkers of inflammation and fibrosis, urinary markers of oxidative stress, and glomerular leukocyte infiltration [276,277].
5.2.1. GLP-1 Receptor Agonists

Currently, there are numerous GLP-1 analogues available. In the kidney, GLP-1 RA treatment induces a proximal tubular natriuresis through inhibition of sodium reabsorption by the sodium-hydrogen exchanger-3 \[278\]. Subsequently, GLP-1 RA treatment significantly increases the fractional excretion of sodium, to some extent resembling the effects seen with SGLT-2 inhibition \[279,280\]. Interestingly, GLP-1 RA agents do not seem to influence tubuloglomerular feedback and do not affect renal blood flow or GFR which was seen with SGLT-2i \[281,282\].

Without affecting renal hemodynamics, GLP-1 RA agents reduce albuminuria and renal morphological changes in animal models of diabetic nephropathy \[283\]. GLP-1RAs have also been shown to reduce inflammation, macrophage infiltration, oxidative stress, and the accumulation of type IV collagen in the kidney \[278,284\]. The SCALE diabetes trial, a randomized clinical trial designed to study the benefit of liraglutide on weight reduction, noted that the drug caused a dose-dependent reduction in albuminuria \[285\]. In the LEADER and SUSTAIN-6 trials, treatment with liraglutide or semaglutide was associated with a significant reduction in secondary renal endpoints, driven by a reduction in the progression to macroalbuminuria but no apparent effect on harder endpoints related to renal function such as preventing end-stage renal failure \[286,287\]. Similarly, the ELIXA (Evaluation of Lixisenatide in Acute Coronary Syndrome) trial showed a significant reduction in both new-onset macroalbuminuria and progression of existing macroalbuminuria, but no significant effect on eGFR or the risk of doubling of serum creatinine \[288\]. In the EXSCEL trial (Exenatide Study of Cardiovascular Event Lowering Trial), a reduction of new-onset macroalbuminuria was also reported in patients treated with once-weekly exenatide compared to placebo, without significant changes in microalbuminuria and ESRD \[290\].

Integrated data from nine phase 2 and 3 trials for the GLP-1 RA dulaglutide showed reductions in albuminuria with dulaglutide compared to placebo or insulin glargine, although eGFR did not differ between treatment groups in these studies \[291\]. The multi-center, AWARD-7 trial, comparing 1-year treatment with two doses of dulaglutide to insulin glargine in type 2 diabetic patients with moderate to severe renal impairment, has shown a reduced decline in eGFR over the study period in both dulaglutide groups compared to the glargine group \[292\]. Positive results with respect to hard renal endpoints were reported in the REWIND study, which compared treatment with dulaglutide 1.5 mg once weekly to placebo in type 2 diabetic patients with established cardiovascular disease or at high cardiovascular risk.

In conclusion, most studies involving GLP-1 analogues show a favorable effect on albuminuria, although none of these trials studied renal endpoints as primary outcomes. Large randomized controlled trial targeting GLP-1 effects on pre-specified primary renal outcomes are required to enhance our understanding regarding the effects of this drug class on DKD as is now planned in the FLOW study (NCT03819153) with semaglutide.

5.2.2. DPP-4 Inhibitors

In the kidney, DPP-4 inhibition causes a distal natriuresis but does not generally significantly influence renal hemodynamics \[293,294\]. However, there are some preclinical studies where effects on eGFR have been reported with DPP-4 inhibitors. It has been postulated that this to be related to effects on stromal cell-derived factor 1 (SDF-1) another molecule that is modulated by DPP-4 \[295\]. Experimental studies with different DPP-4 inhibitors have shown reduced albuminuria and improvement in renal morphological damage in various models of nephropathy \[296,297\]. Although not fully characterized reductions in oxidative stress and in the formation of AGEs are believed to be possible molecular mechanisms for DPP-4 inhibitors affording renoprotection \[298\]. Among the available DPP-4 inhibitors, (linagliptin, saxagliptin, alogliptin, and sitagliptin) linagliptin has been the most extensively analyzed with regards to DKD since this is the only DPP-4 inhibitor with minimal renal metabolism.
In a pooled analysis of phase 3 trials in adults with T2D and albuminuria, 6-months of treatment with the DPP-4 inhibitor linagliptin reduced albuminuria by 28% compared to placebo [299]. Moreover, in a pooled analysis of 13 trials, a 16% reduction in composite adverse renal events was observed [300]. However, in a randomized controlled trial, MARLINA-T2D, linagliptin treatment did not significantly reduce albuminuria compared to placebo [301]. Recently, the large CVOT known as CARMELINA reported neutral outcomes for the secondary renal endpoint (ESRD, renal death or 40% reduction in eGFR) but the reduction in albuminuria was confirmed [302]. In the other large (TECOS and SAVOR-TIMI 53) CVOTs with DPP-4 inhibitors, sitagliptin and saxagliptin reduced albuminuria significantly compared to placebo [303,304]. The EXAMINE trial did not find any difference in eGFR or incidence of initiation of dialysis with the use of alogliptin compared with placebo [305]. Harder renal endpoints did not show a significant difference between treatment groups in the SAVOR-TIMI 53 trial [303], whereas eGFR was significantly lower in participants assigned to sitagliptin in the TECOS trial [304]. Thus, in summary, DPP-4 inhibitors appear to have a beneficial effect on albuminuria but if this relates to primarily being glucose lowering agents remains controversial.

Epidemiological data have indicated a link between anemia and progression of CKD in diabetes, but reduced hemoglobin is pathogenic or just reflects CKD is unknown [306]. Anemia is covariably associated with CKD and may be more severe for the same level of renal dysfunction in DKD [307]. Finally, the TREATS study used EPu to increase Hb in subjects with DKD but showed no benefit and indeed was associated with increased stroke [308].

6. Future Directions

Over the last two decades, there is an increasing body of data defining the molecular mechanisms responsible for the development and progression of DKD. With improved approaches to study the kidney such as single-cell sequencing, renal organoids, more sophisticated genomic and epigenomic approaches, and newer renal imaging techniques, more knowledge should become available on cell-specific changes in the diabetic kidney and molecular mechanisms of action by renoprotective drugs. With major progress over the last few years in reducing albuminuria and retarding a decline in GFR with some of the newer anti-diabetic agents, it is anticipated that the outlook for DKD will improve. With other new therapies mostly in the preclinical or early clinical phase, it is hoped that further breakthrough treatments will be identified either as a replacement or more likely as an adjunct treatment in diabetic subjects with or at risk of DKD.

Author Contributions: D.M.P., M.B., and M.E.C. all contributed to writing, reviewing, and editing this manuscript. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Acknowledgments: We thank Birnbaum, M.D. for his reading and feedback on the manuscript.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Mathis, D.; Vence, L.; Benoist, C. Beta-Cell death during progression to diabetes. Nature 2001, 414, 792–798. [CrossRef] [PubMed]
2. Forbes, J.M.; Cooper, M.E. Mechanisms of diabetic complications. Physiol. Rev. 2013, 93, 137–188. [CrossRef] [PubMed]
3. Cooper, M.E. Interaction of metabolic and haemodynamic factors in mediating experimental diabetic nephropathy. Diabetologia 2001, 44, 1957–1972. [CrossRef] [PubMed]
4. Weir, M.R.; Dzau, V.J. The renin-angiotensin-aldosterone system: A specific target for hypertension management. Am. J. Hypertens. 1999, 12, 2058–2135. [CrossRef]
5. Navar, L.G.; Imig, J.D.; Zou, L.; Wang, C.T. Intrarenal production of angiotensin II. Semin. Nephrol. 1997, 17, 412–422.
6. Yoo, T.H.; Li, J.J.; Kim, J.J.; Jung, D.S.; Kwak, S.J.; Ryu, D.R.; Choi, H.Y.; Kim, J.S.; Kim, H.J.; Han, S.H.; et al. Activation of the renin-angiotensin system within podocytes in diabetes. *Kidney Int.* 2007, 71, 1019–1027. [CrossRef]

7. Huang, Y.; Noble, N.A.; Zhang, J.; Xu, C.; Border, W.A. Renin-stimulated TGF-beta1 expression is regulated by a mitogen-activated protein kinase in mesangial cells. *Kidney Int.* 2007, 72, 45–52. [CrossRef]

8. Nicholas, S.B.; Mauer, M.; Basgen, J.M.; Aguiniga, E.; Chon, Y. Effect of angiotensin II on glomerular structure in streptozotocin-induced diabetic rats. *Am. J. Nephrol.* 2004, 24, 549–556. [CrossRef]

9. Tone, A.; Shikata, K.; Ogawa, D.; Sasaki, S.; Nagase, R.; Sasaki, M.; Yozai, K.; Usui, H.K.; Okada, S.; Wada, J.; et al. Changes of gene expression profiles in macrophages stimulated by angiotensin II–angiotensin II induces MCP-2 through AT1-receptor. *J. Renin Angiotensin Aldosterone Syst.* 2007, 8, 45–50. [CrossRef]

10. Brenner, B.M.; Cooper, M.E.; de Zeeuw, D.; Keane, W.F.; Parving, H.H.; Remuzzi, G.; Snapinn, S.M.; Zhang, Z.; Shahnifar, S.; et al. Effects of losartan on renal and cardiovascular outcomes in patients with type 2 diabetes and nephropathy. *N. Engl. J. Med.* 2001, 345, 861–869. [CrossRef]

11. Lewis, E.J.; Hunsicker, L.G.; Clarke, W.R.; Berl, T.; Pohl, M.A.; Lewis, J.B.; Ritz, E.; Atkins, R.C.; Rohde, R.; Raz, I.; et al. Renoprotective effect of the angiotensin-receptor antagonist irbesartan in patients with nephropathy due to type 2 diabetes. *N. Engl. J. Med.* 2001, 345, 851–860. [CrossRef] [PubMed]

12. Ingelfinger, J.R.; Zuo, W.M.; Fon, E.A.; Ellison, K.E.; Dzau, V.J. In situ hybridization evidence for angiotensinogen messenger RNA in the rat proximal tubule. An hypothesis for the intrarenal renin angiotensin system. *J. Clin. Investig.* 1990, 85, 417–423. [CrossRef] [PubMed]

13. Terada, Y.; Tomita, K.; Nonoguchi, H.; Marumo, F. PCR localization of angiotensin II receptor and angiotensinogen mRNAs in rat kidney. *Kidney Int.* 1993, 43, 1251–1259. [CrossRef] [PubMed]

14. Feng, B.; Chen, S.; Chiu, J.; George, B.; Chakrabarti, S. Regulation of cardiomyocyte hypertrophy in diabetes at the transcriptional level. *Am. J. Physiol. Endocrinol. Metab.* 2008, 294, E1119–E1126. [CrossRef] [PubMed]

15. Maric, C. Vasoactive hormones and the diabetic kidney. *Sci World J.* 2008, 8, 470–485. [CrossRef] [PubMed]

16. Ruggenenti, P.; Cravedi, P.; Remuzzi, G. The RAAS in the pathogenesis and treatment of diabetic nephropathy. *Nat. Rev. Nephrol.* 2010, 6, 319–330. [CrossRef] [PubMed]

17. Warren, A.M.; Knudsen, S.T.; Cooper, M.E. Diabetic nephropathy: An insight into molecular mechanisms and emerging therapies. *Expert Opin. Ther. Targets* 2019, 23, 579–591. [CrossRef]

18. Kaschina, E.; Unger, T. Angiotensin AT1/AT2 receptors: Regulation, signalling and function. *Blood Press.* 2003, 12, 70–88. [CrossRef]

19. Higuchi, S.; Ohtsu, H.; Suzuki, H.; Shirai, H.; Frank, G.D.; Eguchi, S. Angiotensin II signal transduction through the AT1 receptor: Novel insights into mechanisms and pathophysiology. *Clin. Sci (Lond.)* 2007, 112, 417–428. [CrossRef]

20. Wichi, R.B.; Farah, V.; Chen, Y.; Irigoyen, M.C.; Morris, M. Deficiency in angiotensin AT1a receptors prevents diabetes-induced hypertension. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 2007, 292, R1184–R1189. [CrossRef]

21. Langham, R.G.; Kelly, D.J.; Cox, A.J.; Thomson, N.M.; Holtheofer, H.; Zaoui, P.; Pinel, N.; Cordonnier, D.J.; Gilbert, R.E. Proteinuria and the expression of the podocyte slit diaphragm protein, nephrin, in diabetic nephropathy due to type 2 diabetes and nephropathy. *Blood Press.* 2007, 53, 497–504. [CrossRef] [PubMed]

22. De Gasparo, M.; Husain, A.; Alexander, W.; Cath, K.J.; Chiu, A.T.; Drew, M.; Goodfriend, T.; Harding, J.W.; Inagami, T.; Timmermans, P.B. Proposed update of angiotensin receptor nomenclature. *Hypertension* 1995, 25, 924–927. [CrossRef] [PubMed]

23. Sourris, K.C.; Morley, A.L.; Koitka, A.; Samuel, P.; Coughlan, M.T.; Penfold, S.A.; Thomas, M.C.; Bierhaus, A.; Nawroth, P.P.; Yamamoto, H.; et al. Receptor for AGEs (RAGE) blockade may exert its renoprotective effects in patients with diabetic nephropathy via induction of the angiotensin II type 2 (AT2) receptor. *Diabetologia* 2010, 53, 2442–2451. [CrossRef] [PubMed]

24. Faria-Costa, G.; Leite-Moreira, A.; Henriques-Coelho, T. Cardiovascular effects of the angiotensin type 2 receptor. *Rev. Port. Cardiol.* 2014, 33, 439–449. [CrossRef]

25. Koitka, A.; Cao, Z.; Koh, P.; Watson, A.M.; Sourris, K.C.; Loufrani, L.; Soro-Paavonen, A.; Walther, T.; Woolard, K.J.; Jandeleit-Dahm, K.A.; et al. Angiotensin II subtype 2 receptor blockade and deficiency attenuate the development of atherosclerosis in an apolipoprotein E-deficient mouse model of diabetes. *Diabetologia* 2010, 53, 584–592. [CrossRef]
Brassard, P.; Amiri, F.; Thibault, G.; Schiffrin, E.L. Role of angiotensin type 1 and angiotensin type-2 receptors in the expression of vascular integrins in angiotensin II-infused rats. *Hypertension* **2006**, *47*, 122–127. [CrossRef]

Dandapat, A.; Hu, C.P.; Chen, J.; Liu, Y.; Khan, J.A.; Remee, F.; Carey, R.M.; Hermonat, P.L.; Mehta, J.L. Over-expression of angiotensin II type 2 receptor (agtr2) decreases collagen accumulation in atherosclerotic plaque. *Biochem. Biophys. Res. Commun.* **2008**, *366*, 871–877. [CrossRef]

Hu, C.; Dandapat, A.; Chen, J.; Liu, Y.; Hermonat, P.L.; Carey, R.M.; Mehta, J.L. Over-expression of angiotensin II type 2 receptor (agtr2) reduces arterogenesis and modulates LOX-1, endothelial nitric oxide synthase and heme-oxygenase-1 expression. *Atherosclerosis* **2008**, *199*, 288–294. [CrossRef]

Savoia, C.; Ebrahimian, T.; He, Y.; Gratton, J.P.; Schiffrin, E.L.; Touyz, R.M. Angiotensin II/AT2 receptor-induced vasodilation in stroke-prone spontaneously hypertensive rats involves nitric oxide and cGMP-dependent protein kinase. *J. Hypertens.* **2006**, *24*, 2417–2422. [CrossRef]

Briet, M.; Schiffrin, T.E. The role of aldosterone in the metabolic syndrome. *Curr. Hypertens. Rep.* **2011**, *13*, 163–172. [CrossRef]

Sato, A.; Saruta, T. Aldosterone breakthrough during angiotensin-converting enzyme inhibitor therapy. *Am. J. Hypertens.* **2003**, *16*, 781–788. [CrossRef]

Schjoedt, K.J.; Rossing, K.; Juhl, T.R.; Boomsma, F.; Tarnow, L.; Rossing, P.; Parving, H.H. Beneficial impact of spironolactone on nephrotic range albuminuria in diabetic nephropathy. *Kidney Int.* **2006**, *70*, 536–542. [CrossRef] [PubMed]

Han, S.Y.; Kim, C.H.; Kim, H.S.; Jee, Y.H.; Song, H.K.; Lee, M.H.; Han, K.H.; Kim, H.K.; Kang, Y.S.; Han, J.Y.; et al. Spironolactone prevents diabetic nephropathy through an anti-inflammatory mechanism in type 2 diabetic rats. *J. Am. Soc. Nephrol.* **2006**, *17*, 1362–1372. [CrossRef] [PubMed]

Epstein, M.; Williams, G.H.; Weinberger, M.; Lewin, A.; Krause, S.; Mukherjee, R.; Patni, R.; Beckerman, B. Selective aldosterone blockade with eplerenone reduces albuminuria in patients with type 2 diabetes. *Clin. J. Am. Soc. Nephrol.* **2006**, *1*, 940–951. [CrossRef] [PubMed]

Bakris, G.L.; Agarwal, R.; Chan, J.C.; Cooper, M.E.; Gansevoort, R.T.; Haller, H.; Remuzzi, G.; Rossing, P.; Schmieder, R.E.; Nowack, C.; et al. Effect of Finerenone on Albuminuria in Patients With Diabetic Nephropathy: A Randomized Clinical Trial. *JAMA* **2015**, *314*, 884–894. [CrossRef]

Mendoza-Torres, E.; Oyarzun, A.; Mondaca-Ruff, D.; Azocar, A.; Castro, P.F.; Jalil, J.E.; Chiong, M.; Lavandero, S.; Ocaraanza, M.P. ACE2 and vasoactive peptides: Novel players in cardiovascular/renal remodeling and hypertension. *Ther. Adv. Cardiovasc. Dis.* **2015**, *9*, 217–237. [CrossRef] [PubMed]

Ocaraanza, M.P.; Michea, L.; Chiong, M.; Lagos, C.F.; Lavandero, S.; Jalil, J.E. Recent insights and therapeutic perspectives of angiotensin-(1-9) in the cardiovascular system. *Clin. Sci. (Lond.)* **2014**, *127*, 549–557. [CrossRef] [PubMed]

Chamsi-Pasha, M.A.; Shao, Z.; Tang, W.H. Angiotensin-converting enzyme 2 as a therapeutic target for heart failure. *Curr. Heart Fail. Rep.* **2014**, *11*, 58–63. [CrossRef]

Tipnis, S.R.; Hooper, N.M.; Hyde, R.; Karrahn, E.; Christie, G.; Turner, A.J. A human homolog of angiotensin-converting enzyme. Cloning and functional expression as a captopril-insensitive carboxypeptidase. *J. Biol. Chem.* **2000**, *275*, 33238–33243. [CrossRef]

Rice, G.I.; Thomas, D.A.; Grant, P.J.; Turner, A.J.; Hooper, N.M. Evaluation of angiotensin-converting enzyme (ACE), its homologue ACE2 and neprilysin in angiotensin peptide metabolism. *Biochem. J.* **2004**, *383*, 45–51. [CrossRef]

Hamming, I.; Cooper, M.E.; Haagmans, B.L.; Hooper, N.M.; Korstanje, R.; Osterhaus, A.D.; Timens, W.; Turner, A.J.; Navis, G.; van Goor, H. The emerging role of ACE2 in physiology and disease. *J. Pathol.* **2007**, *212*, 1–11. [CrossRef]

Tikellis, C.; Johnston, C.I.; Forbes, J.M.; Burns, W.C.; Burrell, L.M.; Risvanis, J.; Cooper, M.E. Characterization of renal angiotensin-converting enzyme 2 in diabetic nephropathy. *Hypertension* **2003**, *41*, 392–397. [CrossRef] [PubMed]

Wysocki, J.; Ye, M.; Soler, M.J.; Gurley, S.B.; Xiao, H.D.; Bernstein, K.E.; Coffman, T.M.; Chen, S.; Battie, D. ACE and ACE2 activity in diabetic mice. *Diabetes* **2006**, *55*, 2132–2139. [CrossRef] [PubMed]

Ye, M.; Wysocki, J.; William, J.; Soler, M.J.; Cokic, I.; Battie, D. Glomerular localization and expression of Angiotensin-converting enzyme 2 and Angiotensin-converting enzyme: Implications for albuminuria in diabetes. *J. Am. Soc. Nephrol.* **2006**, *17*, 3067–3075. [CrossRef]
45. Ferrario, C.M. Angiotensin-(1-7) and antihypertensive mechanisms. *J. Nephrol.* 1998, 11, 278–283. [PubMed]
46. Trask, A.J.; Ferrario, C.M. Angiotensin-(1-7): Pharmacology and new perspectives in cardiovascular treatments. *Cardiovasc. Drug Rev.* 2007, 25, 162–174. [CrossRef]
47. Santos, R.A.; Simoes e Silva, A.C.; Maric, C.; Silva, D.M.; Machado, R.P.; de Buhr, I.; Heringer-Walther, S.; Pinheiro, S.V.; Lopes, M.T.; Bader, M.; et al. Angiotensin-(1-7) is an endogenous ligand for the G protein-coupled receptor Mas. *Proc. Natl. Acad. Sci. USA* 2003, 100, 8258–8263. [CrossRef]
48. Burns, K.D. The emerging role of angiotensin-converting enzyme-2 in the kidney. *Curr. Opin. Nephrol. Hypertens.* 2007, 16, 116–121. [CrossRef]
49. Ferrario, C.M.; Iyer, S.N. Angiotensin-(1-7): A bioactive fragment of the renin-angiotensin system. *Physiol. Heart Circ. Physiol.* 2006, 290, H684–H691. [CrossRef]
50. Benter, I.F.; Yousif, M.H.; Anim, J.T.; Cojocel, C.; Diz, D.I. Angiotensin-(1-7) prevents development of severe hypertension and end-organ damage in spontaneously hypertensive rats treated with L-NAME. *Am. J. Physiol. Heart Circ. Physiol.* 2006, 290, H684–H691. [CrossRef]
51. Nemoto, W.; Ogata, Y.; Nakagawasai, O.; Yaoita, F.; Tan-No, K. Angiotensin (1-7) prevents angiotensin II-induced nociceptive behaviour via inhibition of p38 MAPK phosphorylation mediated through spinal Mas receptors in mice. *Eur. J. Pain* 2014, 18, 1471–1479. [CrossRef]
52. Zheng, J.; Li, G.; Chen, S.; Bihl, J.; Buck, J.; Zhu, Y.; Xia, H.; Lazariguies, E.; Chen, Y.; Olson, J.E. Activation of the ACE2/Ang-(1-7)/Mas pathway reduces oxygen-glucose deprivation-induced tissue swelling, ROS production, and cell death in mouse brain with angiotensin II overproduction. *Neuroscience* 2014, 273, 39–51. [CrossRef]
53. Drummer, O.H.; Kourtis, S.; Johnson, H. Effect of chronic enalapril treatment on enzymes responsible for the catabolism of angiotensin I and formation of angiotensin II. *Biochem. Pharmacol.* 1990, 39, 513–518. [CrossRef]
54. Donoghue, M.; Hsieh, F.; Baronas, E.; Godbout, K.; Gosselin, M.; Stagliano, N.; Donovan, M.; Woolf, B.; Robison, K.; Jeyaseelan, R.; et al. A novel angiotensin-converting enzyme-related carboxypeptidase (ACE2) converts angiotensin I to angiotensin 1-9. *Circ. Res.* 2000, 87, E1–E9. [CrossRef]
55. Ocaranza, M.P.; Godoy, I.; Jalil, J.E.; Varas, M.; Collantes, P.; Pinto, M.; Roman, M.; Ramirez, C.; Copaja, M.; Diaz-Araya, G.; et al. Enalapril attenuates downregulation of Angiotensin-converting enzyme 2 in the late phase of ventricular dysfunction in myocardial infarcted rat. *Hypertension* 2006, 48, 572–578. [CrossRef]
56. Ocaranza, M.P.; Lavandero, S.; Jalil, J.E.; Moya, J.; Pinto, M.; Novoa, U.; Apablaza, F.; Gonzalez, L.; Hernandez, C.; Varas, M.; et al. Angiotensin-(1-9) regulates cardiac hypertrophy in vivo and in vitro. *J. Hypertens.* 2010, 28, 1054–1064. [CrossRef]
57. Flores-Munoz, M.; Godinho, B.M.; Almalik, A.; Nicklin, S.A. Adenoviral delivery of angiotensin-(1-7) or angiotensin-(1-9) inhibits cardiomyocyte hypertrophy via the mas or angiotensin type 2 receptor. *PLoS ONE* 2012, 7, e54556. [CrossRef]
58. Cha, S.A.; Park, B.M.; Gao, S.; Kim, S.H. Stimulation of ANP by angiotensin-(1-9) via the angiotensin type 2 receptor. *Life Sci.* 2013, 93, 934–940. [CrossRef] [PubMed]
59. Ocaranza, M.P.; Rivera, P.; Novoa, U.; Pinto, M.; Gonzalez, L.; Chiong, M.; Lavandero, S.; Jalil, J.E. Rho kinase inhibition activates the homologous angiotensin-converting enzyme-angiotensin-(1-9) axis in experimental hypertension. *J. Hypertens.* 2011, 29, 706–715. [CrossRef] [PubMed]
60. Flores-Munoz, M.; Work, L.M.; Douglas, K.; Denby, L.; Dominiczak, A.F.; Graham, D.; Nicklin, S.A. Angiotensin-(1-9) attenuates cardiac fibrosis in the stroke-prone spontaneously hypertensive rat via the angiotensin type 2 receptor. *Hypertension* 2012, 59, 300–307. [CrossRef] [PubMed]
61. Parving, H.H.; Lehnert, H.; Brochner-Mortensen, J.; Gomis, R.; Andersen, S.; Arner, P. The effect of irbesartan on the development of diabetic nephropathy in patients with type 2 diabetes. *N. Engl. J. Med.* 2001, 345, 870–878. [CrossRef] [PubMed]
62. Lewis, E.J.; Hunsicker, L.G.; Bain, R.P.; Rohde, R.D. The effect of angiotensin-converting enzyme inhibition on diabetic nephropathy. *The Collaborative Study Group*. *N. Engl. J. Med.* 1993, 329, 1456–1462. [CrossRef] [PubMed]
63. American Diabetes Association. Microvascular Complications and Foot Care: Standards of Medical Care in Diabetes-2018. *Diabetes Care* 2018, 41, S105–S118. [CrossRef] [PubMed]
64. Rahman, M.; Pressel, S.; Davis, B.R.; Nwachuku, C.; Wright, J.T., Jr; Whelton, P.K.; Barzilay, J.; Batuman, V.; Eckfeldt, J.H.; Farber, M.; et al. Renal outcomes in high-risk hypertensive patients treated with an angiotensin-converting enzyme inhibitor or a calcium channel blocker vs a diuretic: A report from the Antihypertensive and Lipid-Lowering Treatment to Prevent Heart Attack Trial (ALLHAT). Arch. Intern. Med. 2005, 165, 936–946. [CrossRef]

65. Patel, A.; Group, A.C.; MacMahon, S.; Chalmers, J.; Neal, B.; Woodward, M.; Billot, L.; Harrap, S.; Poulter, N.; Marre, M.; et al. Effects of a fixed combination of perindopril and indapamide on macrovascular and microvascular outcomes in patients with type 2 diabetes mellitus (the ADVANCE trial): A randomised controlled trial. Lancet 2007, 370, 829–840. [CrossRef]

66. Rahman, M.; Ford, C.E.; Cutler, J.A.; Davis, B.R.; Piller, L.B.; Whelton, P.K.; Wright, J.T., Jr; Barzilay, J.I.; Brown, C.D.; Colon, P.J., Sr.; et al. Long-term renal and cardiovascular outcomes in Antihypertensive and Lipid-Lowering Treatment to Prevent Heart Attack Trial (ALLHAT) participants by baseline estimated GFR. Clin. J. Am. Soc. Nephrol. 2012, 7, 989–1002. [CrossRef]

67. Thomopoulos, C.; Parati, G.; Zanchetti, A. Effects of blood-pressure-lowering treatment on outcome incidence in hypertension: 10–Should blood pressure management differ in hypertensive patients with and without diabetes mellitus? Overview and meta-analyses of randomized trials. J. Hypertens. 2017, 35, 922–944. [CrossRef]

68. Heart Outcomes Prevention Evaluation Study Investigators. Effects of ramipril on cardiovascular and microvascular outcomes in people with diabetes mellitus: Results of the HOPE study and MICRO-HOPE substudy. Lancet 2000, 355, 253–259.

69. Marre, M.; Lievre, M.; Chatellier, G.; Mann, J.F.; Passa, P.; Menard, J.; Investigators, D.S. Effects of low dose ramipril on cardiovascular and renal outcomes in patients with type 2 diabetes and raised excretion of urinary albumin: Randomised, double blind, placebo controlled trial (the DIABHYCAR study). BMJ 2004, 328, 495. [CrossRef] [PubMed]

70. Daly, C.A.; Fox, K.M.; Remme, W.J.; Bertrand, M.E.; Ferrari, R.; Simoons, M.L.; Investigators, E. The effect of perindopril on cardiovascular morbidity and mortality in patients with diabetes in the EUROPA study: Results from the PERSUADE substudy. Eur. Heart J. 2005, 26, 1369–1378. [CrossRef]

71. Thomopoulos, C.; Parati, G.; Zanchetti, A. Effects of blood-pressure-lowering treatment on outcome incidence in hypertension: 10–Should blood pressure management differ in hypertensive patients with and without diabetes mellitus? Overview and meta-analyses of randomized trials. J. Hypertens. 2017, 35, 922–944. [CrossRef]

72. Catala-Lopez, F.; Macias Saint-Gerons, D.; Gonzalez-Bermejo, D.; Rosano, G.M.; Davis, B.R.; Ridao, M.; Zaragoza, A.; Montero-Corominas, D.; Tobias, A.; de la Fuente-Honrubia, C.; et al. Cardiovascular and Renal Outcomes of Renin-Angiotensin System Blockade in Adult Patients with Diabetes Mellitus: A Systematic Review with Network Meta-Analyses. PLoS Med. 2016, 13, e1001971. [CrossRef]

73. Vejakama, P.; Thakkinstian, A.; Lertrattananon, D.; Ingsathit, A.; Ngarmukos, C.; Attia, J. cardio-protective effects of renin-angiotensin system blockade and other antihypertensive drugs in patients with diabetes mellitus: Results of the ONTARGET study: A multicentre, randomised, double-blind, controlled trial. Diabetologia 2012, 55, 566–578. [CrossRef]

74. Barnett, A.H.; Bain, S.C.; Bouter, P.; Karlberg, B.; Madsbad, S.; Jervell, J.; Mustonen, J. Angiotensin-receptor blocker blockade versus converting-enzyme inhibition in type 2 diabetes and nephropathy. N. Engl. J. Med. 2004, 351, 1952–1961. [CrossRef]

75. Mann, J.F.; Schmieder, R.E.; McQueen, M.; Dyal, L.; Schumacher, H.; Pogue, J.; Wang, X.; Maggioni, A.; Budaj, A.; Chaitbirapan, S.; et al. Renal outcomes with telmisartan, ramipril, or both, in people at high vascular risk (the ONTARGET study): A multicentre, randomised, double-blind, controlled trial. Lancet 2008, 372, 547–553. [CrossRef]

76. Wu, H.Y.; Huang, J.W.; Lin, H.J.; Liao, W.C.; Peng, Y.S.; Hung, K.Y.; Wu, K.D.; Tu, Y.K.; Chien, K.L. Comparative effectiveness of renin-angiotensin system blockers and other antihypertensive drugs in patients with diabetes: Systematic review and bayesian network meta-analysis. BMJ 2013, 347, f6008. [CrossRef]

77. Kunz, R.; Friedrich, C.; Wolbers, M.; Mann, J.F. Meta-analysis: Effect of monotherapy and combination therapy with inhibitors of the renin angiotensin system on proteinuria in renal disease. Ann. Intern. Med. 2008, 148, 30–48. [CrossRef] [PubMed]

78. Strippoli, G.F.; Craig, M.; Deeks, J.J.; Schena, F.P.; Craig, J.C. Effects of angiotensin converting enzyme inhibitors and angiotensin II receptor antagonists on mortality and renal outcomes in diabetic nephropathy: Systematic review. BMJ 2004, 329, 828. [CrossRef] [PubMed]
79. Mogensen, C.E.; Neldam, S.; Tikkkanen, I.; Oren, S.; Viskoper, R.; Watts, R.W.; Cooper, M.E. Randomised controlled trial of dual blockade of renin-angiotensin system in patients with hypertension, microalbuminuria, and non-insulin dependent diabetes: The candesartan and lisinopril microalbuminuria (CALM) study. *BMJ* **2000**, *321*, 1440–1444. [CrossRef] [PubMed]

80. Andersen, N.H.; Poulsen, P.L.; Knudsen, S.T.; Poulsen, S.H.; Eiskjaer, H.; Hansen, K.W.; Helleberg, K.; Mogensen, C.E. Long-term dual blockade with candesartan and lisinopril in hypertensive patients with diabetes: The CALM II study. *Diabetes Care* **2005**, *28*, 273–277. [CrossRef]

81. Yusuf, S.; Teo, K.K.; Pogue, J.; Dyal, L.; Copland, I.; Schumacher, H.; Dagenais, G.; Sleight, P.; Anderson, C. Telmisartan, ramipril, or both in patients at high risk for vascular events. *N. Engl. J. Med.* **2008**, *358*, 1547–1559. [CrossRef] [PubMed]

82. Palmer, B.F. Supratherapeutic doses of angiotensin receptor blockers to decrease proteinuria in patients with chronic kidney disease. *Am. J. Nephrol.* **2008**, *28*, 381–390. [CrossRef] [PubMed]

83. Parving, H.H.; Persson, F.; Lewis, J.B.; Lewis, E.J.; Hollenberg, N.K. Aliskiren combined with losartan in type 2 diabetes and nephropathy. *N. Engl. J. Med.* **2008**, *358*, 2433–2446. [CrossRef] [PubMed]

84. Parving, H.H.; Brenner, B.M.; McMurray, J.J.; de Zeeuw, D.; Haffner, S.M.; Solomon, S.D.; Chaturvedi, N.; Persson, F.; Desai, A.S.; Nicolaiades, M.; et al. Cardiorenal end points in a trial of aliskiren for type 2 diabetes. *N. Engl. J. Med.* **2012**, *367*, 2204–2213. [CrossRef]

85. Chrysostomou, A.; Becker, G. Spironolactone in addition to ACE inhibition to reduce proteinuria in patients with chronic renal disease. *N. Engl. J. Med.* **2001**, *345*, 925–926. [CrossRef]

86. Ando, K.; Ohtsu, H.; Uchida, S.; Kaname, S.; Arakawa, Y.; Fujita, T.; Group, E.S. Anti-albuminuric effect of the aldosterone blocker eplerenone in non-hypertensive patients with albuminuria: A double-blind, randomised, placebo-controlled trial. *Lancet Diabetes Endocrinol.* **2014**, *2*, 944–953. [CrossRef]

87. Pitt, B.; Kober, L.; Ponikowski, P.; Gheorghiade, M.; Filippatos, G.; Krum, H.; Nowack, C.; Kolkhof, P.; Kim, S.Y.; Zannad, F. Safety and tolerability of the novel non-steroidal mineralocorticoid receptor antagonist BAY 94-8862 in patients with chronic heart failure and mild or moderate chronic kidney disease: A randomized, double-blind trial. *Eur. Heart J.* **2013**, *34*, 2453–2463. [CrossRef]

88. Kolkhof, P.; Borden, S.A. Molecular pharmacology of the mineralocorticoid receptor: Prospects for novel therapeutics. *Mol. Cell Endocrinol.* **2012**, *350*, 310–317. [CrossRef]

89. Sugimoto, K.; Fujimori, A.; Yuyama, H.; Tahara, A.; Fujimura, A. Renal protective effect of YM598, a selective endothelin type A receptor antagonist. *J. Cardiovasc. Pharmacol.* **2004**, *44*, S451–S454. [CrossRef]

90. Feldstein, C.; Romero, C. Role of endothelins in hypertension. *Am. J. Ther.* **2003**, *10*, 78–88. [CrossRef] [PubMed]

91. Neuhofer, W.; Kohan, D.E. The renal medullary endothelin system in control of sodium and water excretion and systemic blood pressure. *Curr. Opin. Nephrol. Hypertens.* **2006**, *15*, 34–40. [CrossRef]

92. Neuhofer, W.; Pittrow, D. Role of endothelin and endothelin receptor antagonists in renal disease. *Eur. J. Clin. Investig.* **2006**, *36*, 78–88. [CrossRef] [PubMed]

93. Sorokin, A.; Kohan, D.E. Physiology and pathology of endothelin-1 in renal mesangium. *Am. J. Physiol. Renal Physiol.* **2003**, *285*, F579–F589. [CrossRef] [PubMed]

94. Dean, R.; Zhuo, J.; Alcorn, D.; Casley, D.; Mendelsohn, F.A. Cellular localization of endothelin receptor subtypes in the rat kidney following in vitro labelling. *Clin. Exp. Pharmacol. Physiol.* **1996**, *23*, 524–531. [CrossRef]

95. Cosenzi, A.; Bernobich, E.; Trevisan, R.; Milutinovic, N.; Borri, A.; Bellini, G. Nephroprotective effect of bosentan in diabetic rats. *J. Cardiovasc. Pharmacol.* **2003**, *42*, 752–756. [CrossRef]

96. Kelly, D.J.; Skinner, S.L.; Gilbert, R.E.; Cox, A.J.; Cooper, M.E.; Wilkinson-Berka, J.L. Effects of endothelin or angiotensin II receptor blockade on diabetes in the transgenic (mRen-2)27 rat. *Kidney Int.* **2000**, *57*, 1882–1894. [CrossRef]

97. Ding, S.S.; Qiu, C.; Hess, P.; Xi, J.F.; Zheng, N.; Clozel, M. Chronic endothelin receptor blockade prevents both early hyperfiltration and late overt diabetic nephropathy in the rat. *J. Cardiovasc. Pharmacol.* **2003**, *42*, 48–54. [CrossRef]

98. Hocher, B.; Schwarz, A.; Reinbacher, D.; Jacobi, J.; Lun, A.; Priem, F.; Bauer, C.; Neumayer, H.H.; Raschack, M. Effects of endothelin receptor antagonists on the progression of diabetic nephropathy. *Nephron* **2001**, *87*, 161–169. [CrossRef]
100. Sasser, J.M.; Sullivan, J.C.; Hobbs, J.L.; Yamamoto, T.; Pollock, D.M.; Carmines, P.K.; Pollock, J.S. Endothelin A receptor blockade reduces diabetic renal injury via an anti-inflammatory mechanism. *J. Am. Soc. Nephrol.* 2007, 18, 143–154. [CrossRef]

101. Pfab, T.; Thone-Reineke, C.; Theilig, F.; Lange, I.; Witt, H.; Maser-Gluth, C.; Bader, M.; Stasch, J.P.; Ruiz, P.; Bachmann, S.; et al. Diabetic endothelin B receptor-deficient rats develop severe hypertension and progressive renal failure. *J. Am. Soc. Nephrol.* 2006, 17, 1082–1089. [CrossRef] [PubMed]

102. Hargrove, G.M.; Dufresne, J.; Whiteside, C.; Muruve, D.A.; Wong, N.C. Diabetes mellitus increases endothelin-1 gene transcription in rat kidney. *Kidney Int.* 2000, 58, 1534–1545. [CrossRef] [PubMed]

103. Khan, M.A.; Dashwood, M.R.; Mumtaz, F.H.; Thompson, C.S.; Mikhailidis, D.P.; Morgan, R.J. Upregulation of endothelin A receptor sites in the rabbit diabetic kidney: Potential relevance to the early pathogenesis of diabetic nephropathy. *Nephron* 1999, 83, 261–267. [CrossRef] [PubMed]

104. Glogowski, E.A.; Tsiani, E.; Zhou, X.; Fantus, I.G.; Whiteside, C. High glucose alters the response of mesangial cell protein kinase C isoforms to endothelin-1. *Kidney Int.* 1999, 55, 486–499. [CrossRef]

105. Kohno, M.; Horio, T.; Ikeda, M.; Yokokawa, K.; Fukui, T.; Yasunari, K.; Kurihara, N.; Takeda, T. Angiotensin II stimulates endothelin-1 secretion in cultured rat mesangial cells. *Kidney Int.* 1992, 42, 860–866. [CrossRef]

106. De Zeeuw, D.; Coll, B.; Anto, D.; Brennan, J.J.; Tang, H.; Houser, M.; Correa-Rotter, R.; Hou, F.F.; Kitzman, D.W.; Kohan, D.E. Ectopic expression of urotensin II and urotensin II receptor in human diabetic nephropathy. *Curr. Protein. Pept. Sci.* 2018, 21, 2218–2221. [CrossRef]

107. Heerspink, H.J.L.; Makino, H.; et al. The endothelin antagonist atrasentan lowers residual albuminuria in patients with type 2 diabetes and chronic kidney disease (SONAR): A double-blind, randomised, placebo-controlled trial. *Lancet* 2019, 393, 1937–1947. [CrossRef]

108. Hua, H.; Goldberg, H.J.; Fantus, I.G.; Whiteside, C.I. High glucose-enhanced mesangial cell extracellular signal-regulated protein kinase activation and alpha1(IV) collagen expression in response to endothelin-1: Role of specific protein kinase C isozymes. *Diabetes* 2001, 50, 2376–2383. [CrossRef]

109. Hughes, A.K.; Stricklett, P.K.; Padilla, E.; Kohan, D.E. Effect of reactive oxygen species on endothelin-1 production by human mesangial cells. *Kidney Int.* 1996, 49, 181–189. [CrossRef]

110. Heerspink, H.J.L.; Parving, H.H.; Andress, D.L.; Bakris, G.; Correa-Rotter, R.; Hou, F.F.; Kitzman, D.W.; Kohan, D.; Makino, H.; McMurray, J.J.V.; et al. Atrasentan and renal events in patients with type 2 diabetes and chronic kidney disease (SONAR): A double-blind, randomised, placebo-controlled trial. *Lancet* 2019, 393, 1937–1947. [CrossRef]

111. Mann, J.F.; Green, D.; Jamerson, K.; Ruliope, L.M.; Kuranoff, S.J.; Littke, T.; Viberti, G.; Group, A.S. Atrasentan for overt diabetic nephropathy. *J. Am. Soc. Nephrol.* 2010, 21, 527–535. [CrossRef]

112. Langham, R.G.; Kelly, D.J. Urotensin II and the kidney. *Curr. Opin. Nephrol. Hypertens.* 2013, 22, 107–112. [CrossRef]

113. Sugo, T.; Murakami, Y.; Shimomura, Y.; Harada, M.; Abe, M.; Ishibashi, Y.; Kitada, C.; Miyajima, N.; Suzuki, N.; Mori, M.; et al. Identification of urotensin II-related peptide as the urotensin II-immunoreactive molecule in the rat brain. *Biochem. Biophys. Res. Commun.* 2003, 310, 860–866. [CrossRef]

114. Svistunov, A.A.; Tarasov, V.V.; Shakhmardanova, S.A.; Sologova, S.S.; Bagatiriya, E.T.; Chubarev, V.N.; Galenko-Yaroshevsky, P.A.; Avila-Rodriguez, M.F.; Barreto, G.E.; Aliev, G. Urotensin II: Molecular Mechanisms of Biological Activity. *Curr. Protein. Pept. Sci.* 2018, 19, 924–934. [CrossRef] [PubMed]

115. Langham, R.G.; Kelly, D.J.; Gow, R.M.; Zhang, Y.; Dowling, J.K.; Thomson, N.M.; Gilbert, R.E. Increased expression of urotensin II and urotensin II receptor in human diabetic nephropathy. *Am. J. Kidney Dis.* 2004, 44, 826–831. [CrossRef]

116. Pang, X.X.; Bai, Q.; Wu, F.; Chen, G.J.; Zhang, A.H.; Tang, C.S. Urotensin II Induces ER Stress and EMT and Increase Extracellular Matrix Production in Renal Tubular Epithelial Cell in Early Diabetic Mice. *Kidney Blood Press. Res.* 2016, 41, 434–449. [CrossRef]

117. Chen, G.J.; Wu, F.; Pang, X.X.; Zhang, A.H.; Shi, J.B.; Lu, M.; Tang, C.S. Retraction statement: ‘Urotensin II inhibits autophagy in renal tubular epithelial cells and induces extracellular matrix production in early diabetic mice’ by Guan-Jong Chen, Fei Wu, Xin-Xin Pang, Ai-Hua Zhang, Jun-Bao Shi, Min Lu and Chao-Shu Tang. *J. Diabetes Investig.* 2017, 8, 629. [CrossRef]

118. Tian, L.; Li, C.; Qi, J.; Fu, P.; Yu, X.; Li, X.; Cai, L. Diabetes-induced upregulation of urotensin II and its receptor plays an important role in TGF-beta1-mediated renal fibrosis and dysfunction. *Am. J. Physiol. Endocrinol. Metab.* 2008, 295, E1234–E1242. [CrossRef] [PubMed]
119. Rodriguez-Moyano, M.; Diaz, I.; Dionisio, N.; Zhang, X.; Avila-Medina, J.; Calderon-Sanchez, E.; Trebak, M.; Rosado, J.A.; Ordonez, A.; Smani, T. Urotensin-II promotes vascular smooth muscle cell proliferation through store-operated calcium entry and EGFR transactivation. *Cardiovasc. Res.* **2013**, *100*, 297–306. [CrossRef]

120. Song, N.; Ding, W.; Chu, S.; Zhao, J.; Dong, X.; Di, B.; Tang, C. Urotensin II stimulates vascular endothelial growth factor secretion from adventitial fibroblasts in synergy with angiotensin II. *Circ. J.* **2012**, *76*, 1267–1273. [CrossRef]

121. Papadopoulos, P.; Bousette, N.; Giaid, A. Urotensin-II and cardiovascular remodeling. *Peptides* **2008**, *29*, 764–769. [CrossRef] [PubMed]

122. Totsune, K.; Takahashi, K.; Arihara, Z.; Sone, M.; Murakami, O.; Ito, S.; Kikuya, M.; Ohkubo, T.; Hashimoto, J.; Imai, Y. Elevated plasma levels of immunoreactive urotensin II and its increased urinary excretion in patients with Type 2 diabetes mellitus: Association with progress of diabetic nephropathy. *Peptides* **2004**, *25*, 1809–1814. [CrossRef] [PubMed]

123. Jha, J.C.; Banal, C.; Chow, B.S.; Cooper, M.E.; Jandeleit-Dahm, K. Diabetes and Kidney Disease: Role of Oxidative Stress. *Antioxid. Redox Signal.* **2016**, *25*, 657–684. [CrossRef] [PubMed]

124. Badal, S.S.; Danesh, F.R. New insights into molecular mechanisms of diabetic kidney disease. *Am. J. Kidney Dis.* **2014**, *63*, S63–S83. [CrossRef] [PubMed]

125. Sourris, K.C.; Harcourt, B.E.; Tang, P.H.; Morley, A.L.; Huynh, K.; Penfold, S.A.; Coughlan, M.T.; Cooper, M.E.; Nguyen, T.V.; Ritchie, R.H.; et al. Ubiquinone (coenzyme Q10) prevents renal mitochondrial dysfunction in an experimental model of type 2 diabetes. *Free Radic. Biol. Med.* **2012**, *52*, 716–723. [CrossRef] [PubMed]

126. Huang, S.S.; Ding, D.F.; Chen, S.; Dong, C.L.; Ye, X.L.; Yuan, Y.G.; Feng, Y.M.; You, N.; Xu, J.R.; Miao, H.; et al. Resveratrol protects podocytes against apoptosis via stimulation of autophagy in a mouse model of diabetic nephropathy. *Sci. Rep.* **2017**, *7*, 45692. [CrossRef]

127. Lee, E.Y.; Lee, M.Y.; Hong, S.W.; Chung, C.H.; Hong, S.Y. Blockade of oxidative stress by vitamin C ameliorates albuminuria and renal sclerosis in experimental diabetic rats. *Yonsei Med. J.* **2007**, *48*, 847–855. [CrossRef]

128. Hausse, A.O.; Aggoun, Y.; Bonnet, D.; Sidi, D.; Munnich, A.; Rotig, A.; Rustin, P. Idebenone and reduced cardiac hypertrophy in Friedreich’s ataxia. *Heart* **2002**, *87*, 346–349. [CrossRef]

129. Pokrzywinski, K.L.; Biel, T.G.; Kryndushkin, D.; Rao, V.A. Therapeutic Targeting of the Mitochondria Initiates Excessive Superoxide Production and Mitochondrial Depolarization Causing Decreased mtDNA Integrity. *PLoS ONE* **2016**, *11*, e0168283. [CrossRef] [PubMed]

130. Asaba, K.; Tojo, A.; Onozato, M.L.; Goto, A.; Quinn, M.T.; Fujita, T.; Wilcox, C.S. Effects of NADPH oxidase inhibitor in diabetic nephropathy. *Kidney Int.* **2005**, *67*, 1890–1898. [CrossRef]

131. Thallas-Bonke, V.; Thorpe, S.R.; Coughlan, M.T.; Fukami, K.; Yap, F.Y.; Sourris, K.C.; Penfold, S.A.; Bach, L.A.; Cooper, M.E.; Forbes, J.M. Inhibition of NADPH oxidase prevents advanced glycation end product-mediated damage in diabetic nephropathy through a protein kinase C-alpha-dependent pathway. *Diabetes* **2008**, *57*, 460–469. [CrossRef] [PubMed]

132. Block, K.; Gorin, Y.; Aboud, H.E. Subcellular localization of Nox4 and regulation in diabetes. *Proc. Natl. Acad. Sci. USA* **2009**, *106*, 14385–14390. [CrossRef] [PubMed]

133. Sedeek, M.; Callera, G.; Montezano, A.; Gutsol, A.; Heitz, F.; Szynidea, C.; Page, P.; Kennedy, C.R.; Burns, K.D.; Touyz, R.M.; et al. Critical role of Nox4-based NADPH oxidase in glucose-induced oxidative stress in the kidney: Implications in type 2 diabetic nephropathy. *Am. J. Physiol. Renal Physiol.* **2010**, *299*, F1348–F1358. [CrossRef] [PubMed]

134. Jha, J.C.; Gray, S.P.; Di Marco, E.; Okabe, J.; Szynidea, C.; Heitz, F.; Montezano, A.C.; de Haan, J.B.; Kouli, C.; El-Osta, A.; Andrews, K.L.; et al. NADPH oxidase 1 plays a key role in diabetes mellitus-accelerated atherosclerosis. *Circulation* **2013**, *127*, 1888–1902. [CrossRef] [PubMed]

135. Jha, J.C.; Banal, C.; Okabe, J.; Gray, S.P.; Hettige, T.; Chow, B.S.M.; Thallas-Bonke, V.; De Vos, L.; Holterman, C.E.; Coughlan, M.T.; et al. NADPH Oxidase Nox5 Accelerates Renal Injury in Diabetic Nephropathy. *Diabetes* **2017**, *66*, 2691–2703. [CrossRef] [PubMed]
137. Holterman, C.E.; Thibodeau, J.F.; Towaij, C.; Gutsol, A.; Montezano, A.C.; Parks, R.J.; Cooper, M.E.; Touyz, R.M.; Kennedy, C.R. Nephropathy and elevated BP in mice with podocyte-specific NADPH oxidase 5 expression. J. Am. Soc. Nephrol. 2014, 25, 784–797. [CrossRef]

138. Mariotto, S.; Menegazzi, M.; Suzuki, H. Biochemical aspects of nitric oxide. Curr. Pharm. Des. 2004, 10, 1627–1645. [CrossRef] [PubMed]

139. Satoh, M.; Fujimoto, S.; Haruna, Y.; Arakawa, S.; Horike, H.; Komai, N.; Sasaki, T.; Tsujioka, K.; Makino, H.; Kashihara, N. NAD(P)H oxidase and uncoupled nitric oxide synthase are major sources of glomerular superoxide in rats with experimental diabetic nephropathy. Am. J. Physiol. Renal Physiol. 2005, 288, F1144–F1152. [CrossRef]

140. Khaidar, A.; Marx, M.; Lubec, B.; Lubec, G. L-arginine reduces heart collagen accumulation in the diabetic db/db mouse. Circulation 1994, 90, 479–483. [CrossRef]

141. Komers, R.; Allen, T.J.; Cooper, M.E. Role of endothelium-derived nitric oxide in the pathogenesis of the renal hemodynamic changes of experimental diabetes. Diabetes 1994, 43, 1190–1197. [CrossRef]

142. Choi, K.C.; Lee, S.C.; Kim, S.W.; Kim, N.H.; Lee, J.U.; Kang, Y.J. Role of nitric oxide in the pathogenesis of diabetic nephropathy in streptozotocin-induced diabetic rats. Korean J. Intern. Med. 1999, 14, 32–41. [CrossRef] [PubMed]

143. Prabhakar, S.S. Role of nitric oxide in diabetic nephropathy. Semin. Nephrol. 2004, 24, 333–344. [CrossRef] [PubMed]

144. Soulis, T.; Cooper, M.E.; Sastra, S.; Thallas, V.; Panagiotopoulos, S.; Bjerrum, O.J.; Jerums, G. Relative contributions of advanced glycation and nitric oxide synthase inhibition to aminoguanidine-mediated renoprotection in diabetic rats. Diabetologia 1997, 40, 1141–1151. [CrossRef]

145. Kamijo, H.; Higuchi, M.; Hora, K. Chronic inhibition of nitric oxide production aggravates diabetic nephropathy in Otsuka Long-Evans Tokushima Fatty rats. Nephron. Physiol. 2006, 104, p12–p22. [CrossRef] [PubMed]

146. Schalkwijk, C.G.; Stehouwer, C.D.A. Methylglyoxal, a Highly Reactive Dicarbonyl Compound, in Diabetes, Its Vascular Complications, and Other Age-Related Diseases. Physiol. Rev. 2020, 100, 407–461. [CrossRef] [PubMed]

147. Nigro, C.; Leone, A.; Fiory, F.; Prevenzano, I.; Nicolo, A.; Mirra, P.; Beguinot, F.; Miele, C. Dicarbonyl Stress at the Crossroads of Healthy and Unhealthy Aging. Cells 2019, 8, 749. [CrossRef] [PubMed]

148. Brings, S.; Fleming, T.; Freichel, M.; Muckenthaler, M.U.; Herzig, S.; Nawroth, P.P. Dicarbonyls and Advanced Glycation End-Products in the Development of Diabetic Complications and Targets for Intervention. Int. J. Mol. Sci. 2017, 18, 984. [CrossRef]

149. Phillips, S.A.; Thornalley, P.J. The formation of methylglyoxal from triose phosphates. Investigation using a specific assay for methylglyoxal. Eur. J. Biochem. 1993, 212, 101–105. [CrossRef]

150. Thornalley, P.J. Modification of the glyoxalase system in human red blood cells by glucose in vitro. Biochem. J. 1988, 254, 751–755. [CrossRef]

151. Tamae, D.; Lim, P.; Wuenenschell, G.E.; Termini, J. Mutagenesis and repair induced by the DNA advanced glycation end product N2-1-(carboxyethyl)-2′-deoxyguanosine in human cells. Biochemistry 2011, 50, 2321–2329. [CrossRef] [PubMed]

152. Lu, J.; Randell, E.; Han, Y.; Adeli, K.; Krahn, J.; Meng, Q.H. Increased plasma methylglyoxal level, inflammation, and vascular endothelial dysfunction in diabetic nephropathy. Clin. Biochem. 2011, 44, 307–311. [CrossRef] [PubMed]

153. Nakayama, K.; Nakayama, M.; Iwabuchi, M.; Terawaki, H.; Sato, T.; Kohno, M.; Ito, S. Plasma alpha-oxoaldehyde levels in diabetic and non-diabetic chronic kidney disease patients. Am. J. Nephrol. 2008, 28, 871–878. [CrossRef]

154. Beisswenger, P.J.; Drummond, K.S.; Nelson, R.G.; Howell, S.K.; Szwergold, B.S.; Mauer, M. Susceptibility to diabetic nephropathy is related to dicarbonyl and oxidative stress. Diabetes 2005, 54, 3274–3281. [CrossRef] [PubMed]

155. Hanssen, N.M.J.; Scheijen, J.; Jorsal, A.; Parving, H.H.; Tarnow, L.; Rossing, P.; Stehouwer, C.D.A.; Schalkwijk, C.G. Higher Plasma Methylglyoxal Levels Are Associated With Incident Cardiovascular Disease in Individuals With Type 1 Diabetes: A 12-Year Follow-up Study. Diabetes 2017, 66, 2278–2283. [CrossRef] [PubMed]
156. Hanssen, N.M.J.; Westerink, J.; Scheijen, J.; van der Graaf, Y.; Stehouwer, C.D.A.; Schalkwijk, C.G.; Group, S.S. Higher Plasma Methylglyoxal Levels Are Associated With Incident Cardiovascular Disease and Mortality in Individuals With Type 2 Diabetes. *Diabetes Care* **2018**, *41*, 1689–1695. [CrossRef]

157. Jensen, T.M.; Vistisen, D.; Fleming, T.; Nawroth, P.P.; Rossing, P.; Jorgensen, M.E.; Lauritzen, T.; Sandbaek, A.; Witte, D.R. Methylglyoxal is associated with changes in kidney function among individuals with screen-detected Type 2 diabetes mellitus. *Diabet. Med.* **2016**, *33*, 1625–1631. [CrossRef]

158. Rabbani, N.; Sebekova, K.; Sebekova, K., Jr.; Heidland, A.; Thornalley, P.J. Accumulation of free adduct glycation, oxidation, and nitration products follows acute loss of renal function. *Kidney Int.* **2007**, *72*, 1113–1121. [CrossRef]

159. Giacco, F.; Du, X.; D’Agati, V.D.; Milne, R.; Sui, G.; Geoffrion, M.; Brownlee, M. Knockdown of glyoxalase 1 mimics diabetic nephropathy in nondiabetic mice. *Diabetes* **2014**, *63*, 291–299. [CrossRef]

160. Kim, K.M.; Kim, Y.S.; Jung, D.H.; Lee, J.; Kim, J.S. Increased glyoxalase I levels inhibit accumulation of oxidative stress and an advanced glycation end product in mouse mesangial cells cultured in high glucose. *Exp. Cell Res.* **2012**, *318*, 152–159. [CrossRef]

161. Pagtalunan, M.E.; Miller, P.L.; Jumping-Eagle, S.; Nelson, R.G.; Myers, B.D.; Rennke, H.G.; Coplon, N.S.; Sun, L.; Meyer, T.W. Podocyte loss and progressive glomerular injury in type II diabetes. *J. Clin. Investig.* **1997**, *99*, 342–348. [CrossRef] [PubMed]

162. Brouwers, O.; Niessen, P.M.; Miyata, T.; Ostergaard, J.A.; Flyvbjerg, A.; Peutz-Kootstra, C.J.; Sieber, J.; Mundel, P.H.; Brownlee, M.; Janssen, B.J.; et al. Glyoxalase-1 overexpression reduces endothelial dysfunction and attenuates early renal impairment in a rat model of diabetes. *Diabetologia* **2014**, *57*, 224–235. [CrossRef]

163. Queisser, M.A.; Yao, D.; Geisler, S.; Hammes, H.P.; Lochnit, G.; Schleicher, E.D.; Brownlee, M.; Preissner, K.T. Hyperglycemia impairs proteasome function by methylglyoxal. *Diabetes* **2010**, *59*, 670–678. [CrossRef]

164. Thornalley, P.J.; Battah, S.; Ahmed, N.; Karachalias, N.; Agalou, S.; Babaei-Jadidi, R.; Dawnay, A. Quantitative screening of advanced glycation endproducts in cellular and extracellular proteins by tandem mass spectrometry. *Biochem. J.* **2003**, *375*, 581–592. [CrossRef]

165. Kusunoki, H.; Miyata, S.; Ohara, T.; Liu, B.F.; Uriuhara, A.; Kojima, H.; Suzuki, K.; Miyazaki, H.; Yamashita, Y.; Inaba, K.; et al. Relation between serum 3-deoxyglucosone and development of diabetic microangiopathy. *Diabetes Care* **2003**, *26*, 1889–1894. [CrossRef] [PubMed]

166. Genuith, S.; Sun, W.; Cleary, P.; Gao, X.; Sell, D.R.; Lachin, J.; Group, D.E.R.; Monnier, V.M. Skin advanced glycation end products glucosepane and methylglyoxal hydroimidazolone are independently associated with long-term microvascular complication progression of type 1 diabetes. *Diabetes* **2015**, *64*, 266–278. [CrossRef] [PubMed]

167. Schmidt, R.; Bohme, D.; Singer, D.; Frolov, A. Specific tandem mass spectrometric detection of AGE-modified arginine residues in peptides. *J. Mass Spectrom.* **2015**, *50*, 613–624. [CrossRef] [PubMed]

168. Soulsis, T.; Cooper, M.E.; Vranes, D.; Bucala, R.; Jerums, G. Effects of aminoguanidine in preventing experimental diabetic nephropathy are related to the duration of treatment. *Kidney Int.* **1996**, *50*, 627–634. [CrossRef] [PubMed]

169. Soulsis-Liparota, T.; Cooper, M.; Papazoglou, D.; Clarke, B.; Jerums, G. Retardation by aminoguanidine of development of albuminuria, mesangial expansion, and tissue fluorescence in streptozocin-induced diabetic rat. *Diabetes* **1991**, *40*, 1328–1334. [CrossRef] [PubMed]

170. Hammes, H.P.; Martin, S.; Federlin, K.; Geisen, K.; Brownlee, M. Aminoguanidine treatment inhibits the development of experimental diabetic retinopathy. *Proc. Natl. Acad. Sci. USA* **1991**, *88*, 11555–11558. [CrossRef] [PubMed]

171. Kihara, M.; Schmelzer, J.D.; Poduslo, J.F.; Curran, G.L.; Nickander, K.K.; Low, P.A. Aminoguanidine effects on nerve blood flow, vascular permeability, electrophysiology, and oxygen free radicals. *Proc. Natl. Acad. Sci. USA* **1991**, *88*, 6107–6111. [CrossRef] [PubMed]

172. Bolton, W.K.; Cattran, D.C.; Williams, M.E.; Adler, S.G.; Appel, G.B.; Cartwright, K.; Foiles, P.G.; Freedman, B.I.; Raskin, P.; Ratner, R.E.; et al. Randomized trial of an inhibitor of formation of advanced glycation end products in diabetic nephropathy. *Am. J. Nephrol.* **2004**, *24*, 32–40. [CrossRef] [PubMed]

173. Freedman, B.I.; Wuerth, J.P.; Cartwright, K.; Bain, R.P.; Dippe, S.; Hershon, K.; Mooradian, A.D.; Spinowitz, B.S. Design and baseline characteristics for the aminoguanidine Clinical Trial in Overt Type 2 Diabetic Nephropathy (ACTION II). *Control. Clin. Trials* **1999**, *20*, 493–510. [CrossRef]
174. Lobner, J.; Degen, J.; Henle, T. Creatine is a scavenger for methylglyoxal under physiological conditions via formation of N-(4-methyl-5-oxo-1-imidazolin-2-yl)sarcosine (MG-HCr). *J. Agric. Food Chem.* 2015, 63, 2249–2256. [CrossRef] [PubMed]

175. Engelen, L.; Stehouwer, C.D.; Schalkwijk, C.G. Current therapeutic interventions in the glycation pathway: Evidence from clinical studies. *Diabetes Obes. Metab.* 2013, 15, 677–689. [CrossRef]

176. Little, W.C.; Zile, M.R.; Kitzman, D.W.; Hundley, W.G.; O’Brien, T.X.; Degroof, R.C. The effect of alagebrium chloride (ALT-711), a novel glucose cross-link breaker, in the treatment of elderly patients with diastolic heart failure. *J. Card. Fail.* 2005, 11, 191–195. [CrossRef]

177. Zieman, S.J.; Melenovsky, V.; Clattenburg, L.; Corretti, M.C.; Capriotti, A.; Gerstenblith, G.; Kass, D.A. Advanced glycation endproduct crosslink breaker (alagebrium) improves endothelial function in patients with isolated systolic hypertension. *J. Hypertens.* 2007, 25, 577–583. [CrossRef]

178. Voziyan, P.A.; Hudson, B.G. Pyridoxamine as a multifunctional pharmaceutical: Targeting pathogenic glycation and oxidative damage. *Cell Mol. Life Sci.* 2005, 62, 1671–1681. [CrossRef]

179. Williams, M.E.; Bolton, W.K.; Khalifah, R.G.; Degenhardt, T.P.; Schotzinger, R.J.; McGill, J.B. Effects of pyridoxamine in combined phase 2 studies of patients with type 1 and type 2 diabetes and overt nephropathy. *Am. J. Nephrol.* 2007, 27, 605–614. [CrossRef]

180. Lewis, E.J.; Greene, T.; Spitalewiz, S.; Blumenthal, S.; Berl, T.; Hunsicker, L.G.; Pohl, M.A.; Rohde, R.D.; Raz, I.; Yerushalmy, Y.; et al. Pyridoxin in type 2 diabetic nephropathy. *J. Am. Soc. Nephrol.* 2012, 23, 131–136. [CrossRef]

181. Giannarelli, R.; Aragona, M.; Coppelli, A.; Del Prato, S. Reducing insulin resistance with metformin: The evidence today. *Diabetes Metab.* 2003, 29, 6528–6535. [CrossRef]

182. Viollet, B.; Guigas, B.; Sanz Garcia, N.; Leclerc, J.; Foretz, M.; Andreelli, F. Cellular and molecular mechanisms of metformin: An overview. *Clin. Sci. (Lond.)* 2012, 122, 253–270. [CrossRef] [PubMed]

183. Ruggiero-Lopez, D.; Lecomte, M.; Moinet, G.; Patereau, G.; Lagarde, M.; Wiernsperger, N. Reaction of methylglyoxal with isolated systolic hypertension. *Am. J. Hypertens.* 2007, 20, 1671–1681. [CrossRef]

184. Beisswenger, P.J.; Howell, S.K.; Dargusch, R.; Ehren, J.L.; Okada, S.; Sharma, K.; Schubert, D. Fisetin lowers methylglyoxal concentrations of methylglyoxal in type 2 diabetes. *Exp. Clin. Endocrinol. Diabetes* 2014, 122, 316–319. [CrossRef]

185. Kender, Z.; Fleming, T.; Kopf, S.; Torzska, P.; Grolmusz, V.; Herzig, S.; Schleicher, E.; Racz, K.; Reismann, P.; Raz, I.; Yerushalmy, Y.; et al. Pyridoxin in type 2 diabetic nephropathy. *J. Am. Soc. Nephrol.* 2012, 23, 131–136. [CrossRef]

186. Stoica, R.; Avdeef, A.; Guimaraes, J.R.; DeBakker, R.; Michel, A.; Homberg, J.; Schmitz, W. Dicarbonyl reactions of pyridoxal phosphate. *J. Agric. Food Chem.* 2005, 53, 198–202. [CrossRef] [PubMed]

187. Christensen, M.; Jensen, J.B.; Jakobsen, S.; Jessen, N.; Kjeldsen, S.; Christensen, J.B.; Capriotti, A.; Gerstenblith, G.; Kass, D.A.; Melenovsky, V.; et al. Pyridoxin in type 2 diabetic nephropathy. *J. Am. Soc. Nephrol.* 2012, 23, 131–136. [CrossRef]

188. Cheng, A.S.; Cheng, Y.H.; Chiou, C.H.; Chang, T.L. Resveratrol upregulates Nrf2 expression to attenuate methylglyoxal-induced protein glycation and limits the complications of diabetes. *PLoS ONE* 2011, 6, e21226. [CrossRef]

189. Maher, P.; Dargusch, R.; Ehren, J.L.; Okada, S.; Sharma, K.; Schubert, D. Fisetin lowers methylglyoxal levels in type 2 diabetes. *Exp. Clin. Endocrinol. Diabetes* 2014, 122, 316–319. [CrossRef]

190. Yeh, W.J.; Hsia, S.M.; Lee, W.H.; Wu, C.H. Polyphenols with antiglycation activity and mechanisms of action: A review of recent findings. *J. Food Drug Anal.* 2017, 25, 84–92. [CrossRef]

191. Van den Eynde, M.D.G.; Geleijnse, J.M.; Scheijen, J.; Hanssen, N.M.J.; Dower, J.I.; Afman, L.A.; Stehouwer, C.D.A.; Hollman, P.C.H.; Schalkwijk, C.G. Quercetin, but Not Epicatechin, Decreases Plasma Concentrations of Methylglyoxal in Adults in a Randomized, Double-Blind, Placebo-Controlled, Crossover Trial with Pure Flavonoids. *J. Nutr.* 2018, 148, 1911–1916. [CrossRef] [PubMed]

192. Kender, Z.; Fleming, T.; Kopf, S.; Torzska, P.; Grolmusz, V.; Herzig, S.; Schleicher, E.; Racz, K.; Reismann, P.; Raz, I.; Yerushalmy, Y.; et al. Pyridoxin in type 2 diabetic nephropathy. *J. Am. Soc. Nephrol.* 2012, 23, 131–136. [CrossRef]

193. Xue, M.; Rabbani, N.; Momiji, H.; Imbasi, P.; Anwar, M.M.; Kitteringham, N.; Park, B.K.; Souma, T.; Moriguchi, T.; Yamamoto, M.; et al. Transcriptional control of glyoxalase 1 by Nrf2 provides a stress-responsive defence against dicarbonyl glycation. *Biochem. J.* 2012, 443, 213–222. [CrossRef] [PubMed]
194. Das, B.N.; Kim, Y.W.; Keum, Y.S. Mechanisms of Nrf2/Keap1-dependent phase II cytoprotective and detoxifying gene expression and potential cellular targets of chemopreventive isothiocyanates. *Oxid. Med. Cell. Longev.* 2013, 2013, 839409. [CrossRef]

195. Mann, G.E.; Niehueser-Saran, J.; Watson, A.; Gao, L.; Ishii, T.; de Winter, P.; Siow, R.C. Nrf2/ARE regulated antioxidant gene expression in endothelial and smooth muscle cells in oxidative stress: Implications for atherosclerosis and preeclampsia. *Sheng Li Xue Bao* 2007, 59, 117–127.

196. Hsu, W.H.; Lee, B.H.; Chang, Y.Y.; Hsu, Y.W.; Pan, T.M. A novel natural Nrf2 activator with PPARgamma-agonist (monacolin K) enhances the toxicity of methylglyoxal and hyperglycemia. *Toxicol. Appl. Pharmacol.* 2013, 272, 842–851. [CrossRef]

197. Alfarano, M.; Pastore, D.; Fogliano, V.; Schalkwijk, C.G.; Oliviero, T. The Ecto-5’-Nucleotidase (CD73) mediates the extracellular signalling of extracellular nucleotides: Implications for cardiovascular diseases and metabolic syndrome. *Antioxid. Redox Signal.* 2019, 30, 354–374. [CrossRef] [PubMed]

198. Reddy, M.A.; Li, S.L.; Sahar, S.; Kim, Y.S.; Xu, Z.G.; Lanting, L.; Natarajan, R. Key role of Src kinase in S100B-induced activation of the receptor for advanced glycation end products in vascular smooth muscle cells. *J. Biol. Chem.* 2006, 281, 13685–13693. [CrossRef] [PubMed]

199. Kass, D.A.; Shapiro, E.P.; Kawaguchi, M.; Capriotti, A.R.; Scuteri, A.; de Groof, R.C.; Lakatta, E.G. Improved arterial compliance by a novel advanced glycation end-product crosslink breaker. *Circulation* 2001, 104, 1464–1470. [CrossRef]

200. Thornalley, P.J. Glyoxalase 1 Modulation in Obesity and Diabetes. *Antioxid. Redox Signal.* 2019, 30, 354–374. [CrossRef] [PubMed]
212. Sourris, K.C.; Forbes, J.M. Interactions between advanced glycation end-products (AGE) and their receptors in the development and progression of diabetic nephropathy—Are these receptors valid therapeutic targets. *Curr. Drug Targets* 2009, 10, 42–50. [CrossRef]

213. Sundblad, V.; Croci, D.O.; Rabinovich, G.A. Regulated expression of galectin-3, a multifunctional glycan-binding protein, in haematopoietic and non-haematopoietic tissues. *Histol. Histopathol.* 2011, 26, 247–265. [CrossRef] [PubMed]

214. Yan, S.F.; Yan, S.D.; Ramasamy, R.; Schmidt, A.M. Tempering the wrath of RAGE: An emerging therapeutic strategy against diabetic complications, neurodegeneration, and inflammation. *Ann. Med.* 2009, 41, 408–422. [CrossRef] [PubMed]

215. Nogueira-Machado, J.A.; Volpe, C.M.; Veloso, C.A.; Chaves, M.M. HMGB1, TLR and RAGE: A functional tripod that leads to diabetic inflammation. *Expert. Opin. Targets* 2011, 15, 1023–1035. [CrossRef]

216. Heizmann, C.W.; Ackermann, G.E.; Galichet, A. Pathologies involving the S100 proteins and RAGE. *Subcell. Biochem.* 2007, 45, 93–138. [CrossRef]

217. Bierhaus, A.; Schiekofer, S.; Schwaninger, M.; Andressy, M.; Humpert, P.M.; Chen, J.; Hong, M.; Luther, T.; Henle, T.; Kloting, I.; et al. Diabetes-associated sustained activation of the transcription factor nuclear factor-kappaB. *Diabetes* 2001, 50, 2792–2808. [CrossRef]

218. Schmidt, A.M.; Hori, O.; Chen, J.X.; Li, J.F.; Crandall, J.; Zhang, J.; Cao, R.; Yan, S.D.; Brett, J.; Stern, D. Advanced glycation endproducts interacting with their endothelial receptor induce expression of vascular cell adhesion molecule-1 (VCAM-1) in cultured human endothelial cells and in mice. A potential mechanism for the accelerated vasculopathy of diabetes. *J. Clin. Investig.* 1995, 96, 1395–1403. [CrossRef]

219. Wendt, T.M.; Tanji, N.; Guo, J.; Kislinger, T.R.; Qu, W.; Lu, Y.; Bucciarelli, L.G.; Rong, L.L.; Moser, B.; Markowitz, G.S.; et al. RAGE drives the development of glomerulosclerosis and implicates podocyte activation in the pathogenesis of diabetic nephropathy. *Am. J. Pathol.* 2003, 162, 1123–1137. [CrossRef]

220. Coughlan, M.T.; Thorburn, D.R.; Penfold, S.A.; Laskowski, A.; Harcourt, B.E.; Sourris, K.C.; Tan, A.L.; Fukami, K.; Thallas-Bonke, V.; Nawroth, P.P.; et al. RAGE-induced cytosolic ROS promote mitochondrial superoxide generation in diabetes. *J. Am. Soc. Nephrol.* 2009, 20, 742–752. [CrossRef]

221. Inagaki, Y.; Yamagishi, S.; Okamoto, T.; Takeuchi, M.; Amano, S. Pigment epithelium-derived factor prevents advanced glycation end products-induced monocyte chemoattractant protein-1 production in microvascular endothelial cells by suppressing intracellular reactive oxygen species generation. *Diabetologia* 2003, 46, 284–287. [CrossRef]

222. Isoda, K.; Folco, E.; Marwali, M.R.; Ohsuzu, F.; Libby, P. Glycated LDL increases monocyte CC chemokine receptor 2 expression and monocyte chemoattractant protein-1-mediated chemotaxis. *Atherosclerosis* 2008, 198, 307–312. [CrossRef] [PubMed]

223. Yamagishi, S.; Inagaki, Y.; Okamoto, T.; Amano, S.; Koga, K.; Takeuchi, M.; Makita, Z. Advanced glycation end product-induced apoptosis and overexpression of vascular endothelial growth factor and monocyte chemoattractant protein-1 in human-cultured mesangial cells. *J. Biol. Chem.* 2002, 277, 20309–20315. [CrossRef] [PubMed]

224. Li, J.H.; Huang, X.R.; Zhu, H.J.; Oldfield, M.; Cooper, M.; Truong, L.D.; Johnson, R.J.; Lan, H.Y. Advanced glycation end products activate Smad signaling via TGF-beta-dependent and independent mechanisms: Implications for diabetic renal and vascular disease. *FASEB J.* 2004, 18, 176–178. [CrossRef]

225. Tsuchida, K.; Makita, Z.; Yamagishi, S.; Atsumi, T.; Miyoshi, H.; Obara, S.; Ishida, M.; Ishikawa, S.; Yasumura, K.; Koike, T. Suppression of transforming growth factor beta and vascular endothelial growth factor in diabetic nephropathy in rats by a novel advanced glycation end product inhibitor, OPB-9195. *Diabetes* 2009, 58, 2792–2808. [CrossRef] [PubMed]

226. Yamagishi, S.; Inagaki, Y.; Okamoto, T.; Amano, S.; Koga, K.; Takeuchi, M. Advanced glycation end products-induced monocyte chemoattractant protein-1 production in microvascular endothelial cells by suppressing intracellular reactive oxygen species generation. *Diabetologia* 2003, 46, 284–287. [CrossRef]

227. Isoda, K.; Folco, E.; Marwali, M.R.; Ohsuzu, F.; Libby, P. Glycated LDL increases monocyte CC chemokine receptor 2 expression and monocyte chemoattractant protein-1-mediated chemotaxis. *Atherosclerosis* 2008, 198, 307–312. [CrossRef] [PubMed]

228. Yamagishi, S.; Inagaki, Y.; Okamoto, T.; Amano, S.; Koga, K.; Takeuchi, M.; Makita, Z. Advanced glycation end product-induced apoptosis and overexpression of vascular endothelial growth factor and monocyte chemoattractant protein-1 in human-cultured mesangial cells. *J. Biol. Chem.* 2002, 277, 20309–20315. [CrossRef] [PubMed]

229. Li, J.H.; Huang, X.R.; Zhu, H.J.; Oldfield, M.; Cooper, M.; Truong, L.D.; Johnson, R.J.; Lan, H.Y. Advanced glycation end products activate Smad signaling via TGF-beta-dependent and independent mechanisms: Implications for diabetic renal and vascular disease. *FASEB J.* 2004, 18, 176–178. [CrossRef]

230. Tsuchida, K.; Makita, Z.; Yamagishi, S.; Atsumi, T.; Miyoshi, H.; Obara, S.; Ishida, M.; Ishikawa, S.; Yasumura, K.; Koike, T. Suppression of transforming growth factor beta and vascular endothelial growth factor in diabetic nephropathy in rats by a novel advanced glycation end product inhibitor, OPB-9195. *Diabetes* 2009, 58, 2792–2808. [CrossRef] [PubMed]

231. Yamagishi, S.; Inagaki, Y.; Okamoto, T.; Amano, S.; Koga, K.; Takeuchi, M. Advanced glycation end products inhibit de novo protein synthesis and induce TGF-beta overexpression in proximal tubular cells. *Kidney Int.* 2003, 63, 464–473. [CrossRef]

232. Hudson, B.I.; Carter, A.M.; Harja, E.; Kalea, A.Z.; Arriero, M.; Yang, H.; Grant, P.J.; Schmidt, A.M. Identification, classification, and expression of RAGE gene splice variants. *FASEB J.* 2008, 22, 1572–1580. [CrossRef] [PubMed]

233. Kalea, A.Z.; Reigner, N.; Yang, H.; Arriero, M.; Schmidt, A.M.; Hudson, B.I. Alternative splicing of the murine receptor for advanced glycation end-products (RAGE) gene. *FASEB J.* 2009, 23, 1766–1774. [CrossRef] [PubMed]
229. Yonekura, H.; Yamamoto, Y.; Sakurai, S.; Petrova, R.G.; Abedin, M.J.; Li, H.; Yasui, K.; Takeuchi, M.; Makita, Z.; Takasawa, S.; et al. Novel splice variants of the receptor for advanced glycation end-products expressed in human vascular endothelial cells and pericytes, and their putative roles in diabetes-induced vascular injury. *Biochim. J.* 2003, 370, 1097–1109. [CrossRef]

230. Zhang, L.; Bukulin, M.; Kojro, E.; Roth, A.; Metz, V.V.; Fahrenholz, F.; Nawroth, P.P.; Bierhaus, A.; Postina, R. Receptor for advanced glycation end products is subjected to protein ectodomain shedding by metalloproteinases. *J. Biol. Chem.* 2008, 283, 35507–35516. [CrossRef]

231. Colhoun, H.M.; Betteridge, D.J.; Durrington, P.; Hitman, G.; Neil, A.; Livingstone, S.; Charlton-Menys, V.; Zhang, L.; Bukulin, M.; Kojro, E.; Roth, A.; Metz, V.V.; Fahrenholz, F.; Nawroth, P.P.; Bierhaus, A.; Yonekura, H.; Yamamoto, Y.; Sakurai, S.; Petrova, R.G.; Abedin, M.J.; Li, H.; Yasui, K.; Takeuchi, M.; Makita, Z.; Ishikawa, S.; Yasumura, K.; Fujii, W.; Yanagisawa, K.; Kawata, T.; Koike, T.; Nakamura, S.; Makita, Z.; Ishikawa, S.; Yasumura, K.; Fujii, W.; Yanagisawa, K.; Kawata, T.; Koike, T. Prevention and reversal of diabetic nephropathy in spontaneous diabetic rats is prevented by OPB-9195, a novel inhibitor of advanced glycation. *Diabetes* 1997, 46, 895–899. [CrossRef] [PubMed]
245. Brouwers, O.; Niesen, P.M.; Ferreira, I.; Miyata, T.; Scheffer, P.G.; Teerlink, T.; Schrauwen, P.; Brownlee, M.; Stehouwer, C.D.; Schalkwijk, C.G. Overexpression of glyoxalase-I reduces hyperglycemia-induced levels of advanced glycation end products and oxidative stress in diabetic rats. *J. Biol. Chem.* **2011**, *286*, 1374–1380. [CrossRef]

246. Shinohara, M.; Thornalley, P.J.; Giardino, I.; Beisswenger, P.; Thorpe, S.R.; Onorato, J.; Brownlee, M. Overexpression of glyoxalase-I in bovine endothelial cells inhibits intracellular advanced glycation endproduct formation and prevents hyperglycemia-induced increases in macromolecular endocytosis. *J. Clin. Investig.* **1998**, *101*, 1124–1147. [CrossRef]

247. Galasko, D.; Bell, J.; Mancuso, J.Y.; Kupiec, J.W.; Sabbagh, M.N.; van Dyck, C.; Thomas, R.G.; Aisen, P.S.; Alzheimer’s Disease Cooperative, S. Clinical trial of an inhibitor of RAGE-Abeta interactions in Alzheimer disease. *Neurology* **2014**, *82*, 1536–1542. [CrossRef]

248. Flyvbjerg, A.; Denner, L.; Schrijvers, B.F.; Tilton, R.G.; Mogensen, T.H.; Paludan, S.R.; Rasch, R. Long-term renal effects of a neutralizing RAGE antibody in obese type 2 diabetic mice. *Diabetes* **2004**, *53*, 166–172. [CrossRef]

249. Myint, K.M.; Yamamoto, Y.; Doi, T.; Kato, I.; Harashima, A.; Yonekura, H.; Watanabe, T.; Shinohara, H.; Takeuchi, M.; Tsuneyama, K.; et al. RAGE control of diabetic nephropathy in a mouse model: Effects of RAGE gene disruption and administration of low-molecular weight heparin. *Diabetes* **2006**, *55*, 2510–2522. [CrossRef]

250. Soro-Paavonen, A.; Watson, A.M.; Li, J.; Paavonen, K.; Koitka, A.; Calkin, A.C.; Barit, D.; Coughlan, M.T.; Drew, B.G.; Lancaster, G.I.; et al. Receptor for advanced glycation end products (RAGE) deficiency attenuates the development of atherosclerosis in diabetes. *Diabetes* **2008**, *57*, 2461–2469. [CrossRef]

251. Tan, A.L.; Sourris, K.C.; Harcourt, B.E.; Thallas-Bonke, V.; Penfold, S.; Andrikopoulos, S.; Thomas, M.C.; O’Brien, R.C.; Bierhaus, A.; Cooper, M.E.; et al. Disparate effects on renal and oxidative parameters following RAGE deletion, AGE accumulation inhibition, or dietary AGE control in experimental diabetic nephropathy. *Am. J. Physiol. Renal Physiol.* **2010**, *298*, F763–F770. [CrossRef] [PubMed]

252. Manfredi, A.A.; Capobianco, A.; Esposito, A.; De Cobelli, F.; Canu, T.; Monno, A.; Raucci, A.; Sanvito, F.; Doglioni, C.; Nawrot, P.P.; et al. Maturing dendritic cells depend on RAGE for in vivo homing to lymph nodes. *J. Immunol.* **2008**, *180*, 2270–2275. [CrossRef] [PubMed]

253. Moser, B.; Desai, D.D.; Downie, M.P.; Chen, Y.; Yan, S.F.; Herold, K.; Schmidt, A.M.; Clynnes, R. Receptor for advanced glycation end products expression on T cells contributes to antigen-specific cellular expansion in vivo. *J. Immunol.* **2007**, *179*, 8051–8058. [CrossRef] [PubMed]

254. Tian, J.; Avalos, A.M.; Mao, S.Y.; Chen, B.; Senthi, K.; Wu, H.; Parroche, P.; Drabic, S.; Golenbock, D.; Sirois, C.; et al. Toll-like receptor 9-dependent activation by DNA-containing immune complexes is mediated by HMGB1 and RAGE. *Nat. Immunol.* **2007**, *8*, 487–496. [CrossRef]

255. Forbes, J.M.; Thorpe, S.R.; Thallas-Bonke, V.; Pete, J.; Thomas, M.C.; Deemer, E.R.; Bassal, S.; El-Osta, A.; Long, D.M.; Panagiotopoulos, S.; et al. Modulation of soluble receptor for advanced glycation end products by angiotensin-converting enzyme-1 inhibition in diabetic nephropathy. *J. Am. Soc. Nephrol.* **2004**, *15*, 2263–2372. [CrossRef]

256. Fukami, K.; Ueda, S.; Yamagishi, S.; Kato, S.; Inagaki, Y.; Takeuchi, M.; Motomiya, Y.; Bucala, R.; Iida, S.; Tamaki, K.; et al. AGEs activate mesangial TGF-beta-Smad signaling via an angiotensin II type I receptor interaction. *Kidney Int.* **2004**, *66*, 2137–2147. [CrossRef]

257. Pickering, R.J.; Tikellis, C.; Rosado, C.J.; Tsonetes, D.; Dimitropoulos, A.; Smith, M.; Huet, O.; Seeber, R.M.; Abhayawardana, R.; Johnstone, E.K.; et al. Transactivation of RAGE mediates angiotensin-induced inflammation and atherogenesis. *J. Clin. Investig.* **2019**, *129*, 406–421. [CrossRef]

258. Vasilakou, D.; Karagiannis, T.; Athanasiadou, E.; Mainou, M.; Liakos, A.; Bekiari, E.; Sarigianni, M.; Matthews, D.R.; Tsapou, A. Sodium-glucose cotransporter 2 inhibitors for type 2 diabetes: A systematic review and meta-analysis. *Ann. Intern. Med.* **2013**, *159*, 262–274. [CrossRef]

259. Cherney, D.Z.; Perkins, B.A. Sodium-glucose cotransporter 2 inhibition in type 1 diabetes: Simultaneous glucose lowering and renal protection? *Can. J. Diabetes* **2014**, *38*, 356–363. [CrossRef]

260. Cherney, D.Z.; Perkins, B.A.; Soleymaniou, N.; Har, R.; Fagan, N.; Johansen, O.E.; Woerle, H.J.; von Eynatten, M.; Broedl, U.C. The effect of empagliflozin on arterial stiffness and heart rate variability in subjects with uncomplicated type 1 diabetes mellitus. *Cardiovasc. Diabetol.* **2014**, *13*, 28. [CrossRef]
261. Perkins, B.A.; Cherney, D.Z.; Partridge, H.; Soleymanlou, N.; Tschirhart, H.; Zinman, B.; Fagan, N.M.; Kaspers, S.; Woerle, H.J.; Broedl, U.C.; et al. Sodium-glucose cotransporter 2 inhibition and glycemic control in type 1 diabetes: Results of an 8-week open-label proof-of-concept trial. *Diabetes Care* **2014**, *37*, 1480–1483. [CrossRef] [PubMed]

262. Stanton, R.C. Sodium glucose transport 2 (SGLT2) inhibition decreases glomerular hyperfiltration: Is there a role for SGLT2 inhibitors in diabetic kidney disease? *Circulation* **2014**, *129*, 542–544. [CrossRef] [PubMed]

263. Liu, J.J.; Lee, T.; DeFronzo, R.A. Why Do SGLT2 inhibitors inhibit only 30–50% of renal glucose reabsorption in humans? *Diabetes* **2012**, *61*, 2199–2204. [CrossRef] [PubMed]

264. Rahmoune, H.; Thompson, P.W.; Ward, J.M.; Smith, C.D.; Hong, G.; Brown, J. Glucose transporters in human proximal tubular cells isolated from the urine of patients with non-insulin-dependent diabetes. *Diabetes* **2005**, *54*, 3427–3434. [CrossRef]

265. Gembardt, F.; Bartun, C.; Jarzewska, N.; Mayouxi, E.; Todorov, V.T.; Hohenstein, B.; Hugo, C. The SGLT2 inhibitor empagliflozin ameliorates early features of diabetic nephropathy in BTBR ob/ob type 2 diabetic mice with and without hypertension. *Am. J. Physiol. Renal Physiol.* **2014**, *307*, F317–F325. [CrossRef]

266. Terami, N.; Ogawa, D.; Tachibana, H.; Hatanaka, T.; Wada, J.; Nakatsuka, A.; Eguchi, J.; Horiguchi, C.S.; Nishii, N.; Yamada, H.; 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. [CrossRef]

267. Yale, J.F.; Bakris, G.; Cariou, B.; Yue, D.; David-Neto, E.; Xi, L.; Figueroa, K.; Wajs, E.; Usiskin, K.; Meininger, G. Efficacy and safety of canagliflozin in subjects with type 2 diabetes and chronic kidney disease. *Diabetes Obes. Metab.* **2015**, *15*, 463–473. [CrossRef]

268. Thomas, M.C.; Cherney, D.Z.I. The actions of SGLT2 inhibitors on metabolism, renal function and blood pressure. *Diabetologia* **2018**, *61*, 2098–2107. [CrossRef]

269. Zinman, B.; Wanner, C.; Lachin, J.M.; Fitchett, D.H.; Husain, M.; Cherney, D.Z. Sodium Glucose Cotransporter 2 Inhibitors in the Treatment of Diabetes Mellitus: Cardiovascular and Kidney Effects, Potential Mechanisms, and Clinical Applications. *Circulation* **2016**, *134*, 752–772. [CrossRef] [PubMed]

270. Rajasekeran, H.; Lytvin, Y.; Bozovic, A.; Lovshin, J.A.; Diamandis, E.; Cattran, D.; Husain, M.; Perkins, B.A.; Advani, A.; Reich, H.N.; et al. Urinary adenosine excretion in type 1 diabetes. *Am. J. Physiol. Renal Physiol.* **2017**, *313*, F184–F191. [CrossRef] [PubMed]

271. Heerspink, H.J.; Perkins, B.A.; Fitchett, D.H.; Husain, M.; Cherney, D.Z. Sodium Glucose Cotransporter 2 Inhibitors in the Treatment of Diabetes Mellitus: Cardiovascular and Kidney Effects, Potential Mechanisms, and Clinical Applications. *Circulation* **2016**, *134*, 752–772. [CrossRef] [PubMed]

272. Zelniker, T.A.; Viviotti, S.D.; Raz, I.; Im, K.; Goodrich, E.L.; Bonaca, M.P.; Mosenzon, O.; Kato, E.T.; Cahn, A.; Furtdo, R.H.M.; et al. SGLT2 inhibitors for primary and secondary prevention of cardiovascular and renal outcomes in type 2 diabetes: A systematic review and meta-analysis of cardiovascular outcome trials. *Lancet* **2019**, *393*, 31–39. [CrossRef]

273. Perkovic, V.; Jardine, M.J.; Neal, B.; Bompoint, S.; Heerspink, H.J.L.; Charytan, D.M.; Edwards, R.; Agarwal, R.; Bakris, G.; Bull, S.; et al. Canagliflozin and Renal Outcomes in Type 2 Diabetes and Nephropathy. *N. Engl. J. Med.* **2019**, *380*, 2295–2306. [CrossRef] [PubMed]

274. Park, C.W.; Kim, H.W.; Ko, S.H.; Lim, J.H.; Ryu, G.R.; Chung, H.W.; Han, S.W.; Shin, S.J.; Bang, B.K.; Breyer, M.D.; et al. Long-term treatment of glucagon-like peptide-1 analog exendin-4 ameliorates diabetic nephropathy through improving metabolic anomalies in db/db mice. *J. Am. Soc. Nephrol.* **2007**, *18*, 1227–1238. [CrossRef]

275. Liu, W.J.; Xie, S.H.; Liu, Y.N.; Kim, W.; Jin, H.Y.; Park, S.K.; Shao, Y.M.; Park, T.S. Dipeptidyl peptidase IV inhibitor attenuates kidney injury in streptozotocin-induced diabetic rats. *J. Pharmacol. Exp. Ther.* **2012**, *340*, 248–255. [CrossRef]

276. Thomas, M.C. The potential and pitfalls of GLP-1 receptor agonists for renal protection in type 2 diabetes. *Diabetes Metab.* **2017**, *43*, 2520–2527. [CrossRef]
295. Takashima, S.; Fujita, H.; Fujishima, H.; Shimizu, T.; Sato, T.; Morii, T.; Tsukiyama, K.; Narita, T.; Takahashi, T.; Drucker, D.J.; et al. Stromal cell-derived factor-1 is upregulated by dipeptidyl peptidase-4 inhibition and has protective roles in progressive diabetic nephropathy. *Kidney Int.* 2016, 90, 783–796. [CrossRef]

296. Alter, M.L.; Ott, I.M.; von Websky, K.; Tsupyrykov, O.; Sharkovska, Y.; Krause-Relle, K.; Raila, J.; Henze, A.; Klein, T.; Hocher, B. DPP-4 inhibition on top of angiotensin receptor blockade offers a new therapeutic approach for diabetic nephropathy. *Kidney Blood Press. Res.* 2012, 36, 119–130. [CrossRef]

297. Gangadharan Komala, M.; Gross, S.; Zaky, A.; Pollock, C.; Panchapakesan, U. Saxagliptin reduces renal tubulointerstitial inflammation, hypertrophy and fibrosis in diabetes. *Nephrology (Carlton)* 2016, 21, 423–431. [CrossRef]

298. Ishibashi, Y.; Matsui, T.; Maeda, S.; Higashimoto, Y.; Yamagishi, S. Advanced glycation end products evoke endothelial cell damage by stimulating soluble dipeptidyl peptidase-4 production and its interaction with mannose 6-phosphate/insulin-like growth factor II receptor. *Cardiovasc. Diabetol.* 2013, 12, 125. [CrossRef]

299. Groop, P.H.; Cooper, M.E.; Perkovic, V.; Emser, A.; Woerle, H.J.; von Eynatten, M. Linagliptin lowers albuminuria on top of recommended standard treatment in patients with type 2 diabetes and renal dysfunction. *Diabetes Care* 2013, 36, 3460–3468. [CrossRef]

300. Cooper, M.E.; Perkovic, V.; McGill, J.B.; Groop, P.H.; Wanner, C.; Rosenstock, J.; Hehnke, U.; Woerle, H.J.; von Eynatten, M. Kidney Disease End Points in a Pooled Analysis of Individual Patient-Level Data From a Large Clinical Trials Program of the Dipeptidyl Peptidase 4 Inhibitor Linagliptin in Type 2 Diabetes. *Am. J. Kidney Dis.* 2015, 66, 441–449. [CrossRef]

301. Groop, P.H.; Cooper, M.E.; Perkovic, V.; Hocher, B.; Kanasaki, K.; Haneda, M.; Schernthaner, G.; Sharma, K.; Stanton, R.C.; Toto, R.; et al. Linagliptin and its effects on hyperglycaemia and albuminuria in patients with type 2 diabetes and renal dysfunction: The randomized MARLINA-T2D trial. *Diabetes Obes. Metab.* 2017, 21, 1610–1619. [CrossRef][PubMed]

302. Rosenstock, J.; Perkovic, V.; Johansen, O.E.; Cooper, M.E.; Kahn, S.E.; Marx, N.; Alexander, J.H.; Pencina, M.; Toto, R.D.; Wanner, C.; et al. Effect of Linagliptin vs Placebo on Major Cardiovascular Events in Adults With Type 2 Diabetes and High Cardiovascular and Renal Risk: The CARMELINA Randomized Clinical Trial. *JAMA* 2019, 321, 69–79. [CrossRef][PubMed]

303. Cornel, J.H.; Bakris, G.L.; Stevens, S.R.; Alvarsson, M.; Bax, W.A.; Chuang, L.M.; Engel, S.S.; Lopes, R.D.; McGuire, D.K.; Rifflin, A.; et al. Effect of Sitagliptin on Kidney Function and Respective Cardiovascular Outcomes in Type 2 Diabetes: Outcomes From TECOS. *Diabetes Care* 2016, 39, 2304–2310. [CrossRef][PubMed]

304. Mosenzon, O.; Leibowitz, G.; Bhatt, D.L.; Cahn, A.; Hirshberg, B.; Wei, C.; Im, K.; Rozenberg, A.; Yanuv, I.; Stahre, C.; et al. Effect of Saxagliptin on Renal Outcomes in the SAVOR-TIMI 53 Trial. *Diabetes Care* 2017, 40, 69–76. [CrossRef]

305. White, W.B.; Cannon, C.P.; Heller, S.R.; Nissen, S.E.; Bergenstal, R.M.; Bakris, G.L.; Perez, A.T.; Fleck, P.R.; Mehta, C.R.; Kupfer, S.; et al. Alogliptin after acute coronary syndrome in patients with type 2 diabetes. *N. Engl. J. Med.* 2013, 369, 1327–1335. [CrossRef]

306. Vlagopoulos, P.T.; Tighiouart, H.; Weiner, D.E.; Griffith, J.; Pettitt, D.; Salem, D.N.; Levey, A.S.; Sarnak, M.J. Anemia as a risk factor for cardiovascular disease and all-cause mortality in diabetes: The impact of chronic kidney disease. *J. Am. Soc. Nephrol.* 2005, 16, 3403–3410. [CrossRef]

307. Okada, H.; Hasegawa, G.; Tanaka, M.; Osaka, T.; Shiotsu, Y.; Narumiya, H.; Inoue, M.; Nakano, K.; Nakamura, N.; Fukui, M. Association between Hemoglobin Concentration and the Progression or Development of Albuminuria in Diabetic Kidney Disease. *PLoS ONE* 2015, 10, e0129192. [CrossRef]

308. Pfeffer, M.A.; Burdmann, E.A.; Chen, C.Y.; Cooper, M.E.; de Zeeuw, D.; Eckardt, K.U.; Feyzi, J.M.; Ivanovich, P.; Kewalramani, R.; Levey, A.S.; et al. A trial of darbepoetin alfa in type 2 diabetes and chronic kidney disease. *N. Engl. J. Med.* 2009, 361, 2019–2032. [CrossRef]