Diabetic nephropathy: perspective on novel molecular mechanisms

Luigi Gnudi,[1] Richard JM Coward,[2] David A Long.[3]

[1]Cardiovascular Division, King's College London, SE1 9NH, UK;
[2]Academic Renal Unit, Dorothy Hodgkin Building, University of Bristol, Whitson Street, Bristol BS1 3NY, UK;
[3]Developmental Biology and Cancer Programme, Institute of Child Health, University College London, WC1N 1EH, UK.

Corresponding Authors:
Luigi Gnudi, e-mail : luigi.gnudi@kcl.ac.uk
David Long, PhD, e-mail : d.long@ucl.ac.uk

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Abstract

Diabetes is the major cause of end stage renal disease globally, and novel treatments are urgently needed. Current therapeutic approaches for diabetic nephropathy are focusing on the inhibition of the renin angiotensin aldosterone system, on glycaemic and lipid control, and life style changes. In this review we will highlight new molecular insights in our understanding of the initiation and progression of diabetic nephropathy including glomerular insulin resistance, dysregulation of cellular substrate utilisation, podocyte-endothelial communication and inhibition of tubular sodium coupled glucose reabsorption. We believe these mechanisms offer new therapeutic targets that can be exploited to develop important renoprotective treatments for diabetic nephropathy over the next decade.

INTRODUCTION

Diabetes mellitus is a metabolic disorder associated with chronic micro- and macrovascular complications. One of the most feared chronic microvascular complications is diabetic nephropathy (DN), currently the leading cause of end-stage renal disease (ESRD) in the Western world. Strikingly, 40-45% of patients with type-1 diabetes (T1D) develop DN and reach ESRD or die before its onset. Moreover, clinicians face a ~30% prevalence of patients with type-2 diabetes (T2D) and DN, with 45% of patients currently on dialysis having a primary diagnosis of diabetes, a population also at high risk of developing cardiovascular disease [1].

An early sign of DN is an increased amount of urinary protein, manifested by "albuminuria", which correlates with, and can predict, the progression of renal damage. Albuminuria arises from defects in the permeability of the glomerular filtration barrier consisting of glomerular endothelial cells (GECs) separated from specialized epithelia, called podocytes, by the glomerular basement membrane (GBM)[1]. Podocytes have extensive inter-digitating foot processes connected together by a slit diaphragm composed of proteins including nephrin and neph1, which interact with cytoplasmic adaptor and signalling proteins (PI3-Kinase, CD2AP, AKT, podocin). Nephrin is also linked with the podocyte actin cytoskeleton; the protein tyrosine kinase Fyn promotes nephrin phosphorylation which enhances its interaction with PI3-Kinase and PI3K-dependent phosphorylation of AKT and subsequently increases Rac1 activity, leading to modification of the actin cytoskeleton with maintenance of a normal podocyte anatomical structure and function [2, 3]. The structure and integrity of the glomerulus is also maintained by a complex local autocrine/paracrine network between the podocyte and the GECs

consisting of vascular growth factors and vasoactive peptides which is disrupted in DN [4]. The GECs are highly fenestrated with a unique ultrastructure lacking fenestrae diaphragms which facilitate water and small solutes permeability [5]. GECs are covered by a glycocalyx consisting mainly of proteoglycans which include core proteins such as syndecan and attached glycosaminoglycan side chains which appear to be important in regulating the permeability of the glomerulus [6].

Animal and human studies have established that the metabolic and haemodynamic changes that occur in diabetes lead to ultrastructural alterations of the glomerular filtration barrier, including podocyte foot process fusion and detachment, GBM thickening, a reduced endothelial cell glycocalyx, mesangial extracellular matrix accumulation and glomerulosclerosis **(Figure 1)**. These structural glomerular changes correlate with increasing albuminuria which has been proposed to be a marker of generalised systemic vascular dysfunction by the “Steno hypothesis” [7] and could represent a common pathogenetic mechanism for renal and extra-renal chronic vascular complications in diabetes [1].

Over the last 5-10 years our understanding of the molecular and cellular pathways by which diabetic kidney disease results in damage to the glomerular filtration barrier has increased. In this review, we will outline recent advances in glomerular insulin signalling, oxidative and endoplasmic reticulum (ER) stress and podocyte-endothelial communication that have revealed new exciting therapeutic directions for DN.

Insulin resistance as a mechanism for the predisposition of DN

Insulin is a metabolic hormone which not only regulates glucose and the metabolism of other substrates but also directly modulates the biology of specific cells in a variety of tissues. In both T1D and T2D patients, the ability of insulin to elicit cellular responses is impaired, a concept termed “cellular insulin resistance”, and is associated with DN [8]. Insulin resistance correlates with the development of microalbuminuria both in T1D and T2D patients and patients with T1D are more likely to have a strong family history of insulin resistance when compared with those without kidney disease [9]. Insulin resistance has been implicated in the development of glomerular hypertension and hyperfiltration [10], seen in the initial phase of diabetic kidney disease [11]. Furthermore, in both T1D and T2D patients, insulin resistance *per se* contributes to higher salt sensitivity, which closely associates with increases in blood pressure, albuminuria, and a decline in renal function [12, 13].

Within the kidney many different cell types are insulin sensitive and express functional insulin receptors [14-17]. Furthermore, transgenic mouse models have revealed that inducing insulin resistance in different nephron compartments results in a variety of unfavourable renal phenotypes. In the glomerulus, approximately a decade ago it was discovered that human podocytes respond to insulin [14], and express the hallmark components of insulin sensitive cells including the insulin receptor, and key glucose transporters including GLUT4 and GLUT1. To elucidate the biological significance of insulin signalling in these cells, podocyte specific insulin receptor knockout mice were generated [18]. These animals developed albuminuria and a number of features of DN, including increased matrix production, glomerulosclerosis, and GBM thickening, but all in normoglycaemic conditions, suggesting that insulin resistance of this cell *per se* may be an important driver in glomerular diseases.

Insulin signaling is important in other parts of the nephron. Deletion of the insulin receptor In tubular epithelial cells widespread led to reduced natriuresis and hypertension [16]. Recent studies have begun to dissect out the precise function of the insulin receptor in specific tubular segments. These experiments revealed that loss of the insulin receptor in proximal tubules results in gluconeogenesis [19] while deletion in collecting ducts increased natriuresis and lowered blood pressure [20].

Diabetes provides an ideal environment consisting of increased adiposity, hyperglycaemia, and inflammation which are all important players in promoting podocyte insulin resistance and glomerular dysfunction [17](**Table 1**). A recent study has identified SMAD3 within the inflammation/ fibrosis pathway as an important modulator of podocyte insulin sensitivity in a model of obesity related DN [21]. In this work, mice fed a high fat diet exhibited an increase in kidney and podocyte SMAD3 expression levels which resulted in a severely fibrotic kidney; in these conditions SMAD3 knockout animals were protected from kidney damage and fibrosis. In parallel, fatty acid palmitate induces a SMAD3-mediated podocyte insulin resistance paralleled by mitochondrial dysfunction *in vitro*. These responses were exaggerated when animals became albuminuric, and could be rescued by SMAD3 blockade and restoration of podocyte insulin signalling [21]. Other studies have demonstrated that both Nucleotide-binding oligomerization domain containing protein 2 (NOD2)[22] and Toll-like receptor (TLR)[23] mediated-inflammation have an adverse effect on podocyte survival, insulin action, and glomerular permeability to protein. Decreased circulating adiponectin [24, 25], increased free fatty acids (FFA) levels [26], and defects in insulin action promote glomerular cells and podocyte dysfunction, and

albuminuria [27, 28]. Epigenetic mechanisms may also be important in determining insulin resistance [29]. This concept has not been studied in great detail to-date, but Kumar and colleagues have shown that insulin resistance induced by palmitate in human urinary podocyte cell lines is associated with an increase in histone H3K36me2 and reduced H3K27me3 on the promoter region of FOXO1, a regulator of gluconeogenic genes. This effect was long-lasting and persisted even after the normalisation of palmitate levels [30].

Glomerular insulin resistance, endoplasmic reticulum (ER) stress and autophagy in diabetic glomerulopathy

There are many consequences of insulin resistance within glomeruli, which are likely to contribute to the progression of DN. One key mechanism is changes to the mitochondria and the closely connected ER [31]. Mitochondrial metabolic overload results in increased cellular oxidative stress and ER-stress which leads to the activation of unfolded protein response (UPR)[32]. UPR is a positive cellular response that in its early phase either refolds accumulated unfolded proteins, or degrades unfolded protein by the ubiquitin-proteasome pathway. Misfolded proteins are detected by the ER membrane stress sensors protein kinase RNA-like ER kinase (PERK), inositol-requiring protein 1α (IRE1α) and activating transcription factor 6α (ATF6α) and its activator X-box binding protein-1 (XBP-1), which, in turn, activates several signalling events and trigger a compensatory response to prevent further accumulation of misfolded protein. However, when the unfolded protein and cellular damage exceeds a threshold, chronic and unresolved stress results in a change from an adaptive to pro-apoptotic responses [32].

There is some evidence that glucose/oxidative stress-mediated ER stress plays a role in chronic vascular complications in DN [33]. Hyperglycemia, or increased glycation of proteins have been shown to mediate apoptosis partly through increases in ER stress in cultured murine podocytes [34, 35]. Activation of the UPR has also been observed in mouse glomerular mesangial cells exposed to glucose and glucosamine [36], and in kidneys from diabetic rats administered streptozotocin for 16 weeks [37]. Microarray analysis of human biopsies from patients with established DN showed that UPR genes were upregulated proportionally to the severity of diabetic renal lesions [38]. Finally, recent experimental evidence has demonstrated that pharmacological inhibition of ER stress and stabilization of the UPR is beneficial in diabetic glomerulopathy [39].

Two studies have used transgenic mice to link podocyte insulin resistance with mitochondrial function and ER stress. Ising and colleagues generated a mouse model of podocyte mitochondrial dysfunction by specifically knocking out a key molecule in this cell involved in mitochondrial fusion called prohibitin-2 [40]. This caused a severe phenotype including glomerulosclerosis, renal failure and death at approximately a month of age. They then went on to inhibit both the insulin receptor and IGF-1 receptor (IGF1R) contemporaneously with podocyte-specific knockdown of prohibitin-2. Inhibiting the insulin receptor alone, or in combination with the IGF1R was partially protective and resulted in a significantly longer life span of the mice [40]. This suggests that insulin resistance could reflect a “protective” resetting of cellular substrate utilisation to shield from excess substrate flow to mitochondria with “impaired” respiratory capabilities. In another study, Madhusudhan *et al*. have

elegantly shown that under diabetic conditions ER adaptive mechanisms are impaired in the podocyte and that this is exacerbated when the cell is rendered more insulin resistant. Studying human and murine DN they discovered that nephropathy was associated with alterations in the UPR with impairment of the nuclear translocation of XBP-1. Genetic ablation of the transcription factor XBP-1 or activation of ATF6 (downstream of XBP-1) in the podocyte of diabetic mice aggravates DN. Of interest, mice with genetically impaired podocyte insulin signalling exhibited impaired UPR (XBP-1 activation) that was associated with more severe diabetic kidney disease when compared with diabetic controls [41].

Autophagy, regulated by the mammalian target of rapamycin complex 1 (mTORC1) is, with the UPR, essential to maintain cellular homeostasis and in the context of ER stress contributes towards the elimination of toxic and damaged cellular components [42]. Haploinsufficiency of mTORC1 in podocytes or administration of rapamycin (a mTORC1 inhibitor) resulting in activation of autophagy [43], has been shown to prevent progressive DN [44, 45]. In contrast, mTORC1 activation in podocytes, resulting in inhibition of autophagy, leads to accelerated DN [46]. Loss of insulin sensitivity in cultured podocytes results in suppression of autophagy and addition of rapamycin in these cells attenuates insulin resistance [47].

Insulin resistance, the glomerular cell cytoskeleton and other mechanisms

Experiments using podocyte cell lines have begun to reveal other downstream targets of insulin resistance which may play a role in DN. Addition of exogenous insulin to human podocytes in culture led to cytoskeletal rearrangement [18], a

process which has been pharmacologically targeted using small molecules as a novel therapy for DN [48]. Other studies [49] have identified the cytoskeleton protein septin-7 as playing an important role in the regulation of insulin-mediated translocation of GLUT4 vesicles to the plasma membrane and the control of podocyte glucose transport. Insulin may also modulate calcium signalling in podocytes which has been shown to be important in maintaining cytoskeletal dynamics by altering the expression of canonical transient receptor potential-6 channel-TRPC6 [50] and large-conductance Ca(2+)-activated K(+) channels [51].

Insulin stimulates the Phosphoinositide 3-kinase (PI3K) pathway and causes AKT activation. In normal physiology, insulin stimulation of podocytes results in AKT phosphorylation (activation), while, in insulin-resistant disease settings such as diabetes, a number of reports have shown an early loss of glomerular AKT phosphorylation whilst AKT signaling is maintained in the tubular compartment of the kidney [28]. AKT exists in three isoforms with AKT2 being located specifically in the podocyte within the kidney [52]. A loss of podocyte AKT2 activation is detrimental when there is chronic kidney disease associated with nephron loss [52]. AKT2 is the major isoform through which insulin signals [53]. It is currently not completely clear if the loss of renal AKT activation is detrimental in the setting of diabetes as a number of studies have shown an increase in AKT phosphorylation in the vasculature in experimental animal models of diabetes [54-58], and pharmacological inhibition of the AKT activation by AS101, may confer renoprotection in diabetes [59]. More work will have to be performed to dissect the exact role of AKT in diabetic kidney disease.

Insulin can also modulate the renin-angiotensin-aldosterone system, critical for regulating glomerular haemodynamics in DN, by increasing the expression and activity of angiotensin converting enzyme-2 (ACE2)[60]. Further work is required to identify other downstream targets of podocyte insulin signalling ideally using systems biology genomic and proteomic approaches. Candidate molecules altered by insulin signalling might include recently identified genes found to be associated with the early stages of albuminuria in in-bred strains of mice [61].

Reactive oxygen species and diabetic nephropathy

Over the last decade, an attractive unifying hypothesis has been put forward to explain diabetic microvascular complications; specifically it was postulated that an excess in cellular substrate availability leads to an increase in reactive oxygen species (ROS) which in turn drives vascular complications in DN [62]. However, this unifying hypothesis has been challenged by the negative results of antioxidant-based clinical trials [63], and a new theory of “mitochondrial hormesis” has been proposed [64], whereby the increased mitochondrial superoxide production is considered an indicator of healthy mitochondria and physiologic oxidative phosphorylation.

Recent research has found a reduction of superoxide in the kidneys of streptozotocin (STZ)-induced diabetic Akita-mice, as assessed by a combination of *in vivo* real-time transcutaneous fluorescence, confocal microscopy, and electron paramagnetic resonance analysis [65]. The authors of this study found that chronic exposure to high glucose levels (as occurs in diabetes) results in disrupted mitochondria, which was associated with a reduced respiration and a lowering in mitochondrial

superoxide. Interestingly, genetic or pharmacological correction of mitochondrial dysfunction by improving substrate utilisation was recently found to be renoprotective in a mouse model of tubulointerstitial fibrosis [66].

From experimental animal studies it appears that increased cytosolic superoxide and other non-mitochondrial sources of ROS generation play a prominent role in diabetic kidney disease and that strategies involving a more targeted (towards specific cellular compartments such as the cytosol) antioxidant approach, may be important to optimize renoprotection in diabetes [67]. Along these lines, human studies have shown that leukocytes obtained from patients with diabetes and DN (when compared with non-diabetics or patients with diabetes without DN) have a reduced maximal respiration and reserve capacity [68, 69] suggesting that chronic metabolic stress in the presence of a reduced mitochondrial function (being this primary or secondary) will manifest with low ATP-linked respiration, low reserve capacity and reduced mitochondrial ROS generation.

It could be speculated that metabolic stress could initially (early phase) promote an excess production of mitochondrial superoxide [62] that will lead, in a subsequent chronic phase (late phase), towards mitochondrial damage, progressive deterioration in bioenergetic cellular function, reduced ATP synthesis and cell death. Future work will address these questions, and need to evaluate whether cells are able to maintain adequate number of healthy mitochondria which can then burn sufficient substrates for energy production and maintain a “balanced” level of ROS (**Figure 2**).

AMP-activated protein kinase (AMPK) is a stress-activated kinase that is activated in response to depleting ATP to preserve cell survival under conditions of reduced substrate utilisation. AMPK activation has been involved in mitochondrial biogenesis by leading to increased mitochondrial substrate utilisation and ATP generation, in parallel with stimulation of antioxidant gene expression to ensure an optimal redox balance [70]. Reduced AMPK, as seen in the diabetic kidney of both rodents and humans [65], is associated with reduced catabolic activity (mitochondrial function) [65], and reduced AMPK-mediated inhibition of NADPH oxidase (Nox2) resulting in increased ROS production [71, 72]. Taken together, these results suggest that, in the diabetic kidney, upregulation of AMPK could be therapeutically beneficial in DN [73] to regulate nutrient utilisation and mitochondrial function towards maintenance of an optimum redox balance.

Overall, more work is required in the DN field to dissect between these opposing theories specifically by examining mitochondrial function and specific ROS moieties both *in vitro* and in tissues.

Vascular endothelial growth factor-A (VEGFA) and the glycocalyx

Recent studies have shown a connection between insulin resistance and the subsequent production of VEGFA in podocytes [74]. This finding is likely to be important in the setting of DN with many elegant studies using transgenic mice highlighting the importance of podocyte VEGFA levels in the progression of this condition [4]. A new aspect of VEGFA signalling in the glomerulus is potential cross talk between VEGFA secreted from podocytes and the GECs glycocalyx in the

setting of diabetes. There is clear evidence that the GECs glycocalyx is lost both systemically and within the diabetic glomerulus, and that this contributes to both cardiovascular and renal complications [6]. Mechanistically there are a number of pathways which led to loss of the glomerular glycocalyx including hyperglycaemia [75], and ROS [76].

During the early phases of diabetes an increase in VEGFA causes glycocalyx shedding from the GECs. Furthermore, the inhibitory isoform of VEGFA called VEGF-A_{165b} also plays a role in maintaining the GECs glycocalyx in diabetes. Oltean et al. [77] have shown that in diabetic patients with progressive nephropathy, the renal expression of VEGF-A_{165b} is lost. They went on to develop a number of murine models of DN and have shown that genetic overexpression or pharmacological administration of VEGF-A_{165b} to the mouse, acting through VEGF receptor 2 in the GECs, restores damaged glomerular endothelial glycocalyx and improves renal function. VEGF-A_{165b} also improved the permeability of isolated human diabetic glomeruli suggesting the response is conserved across murine and human species [77].

VEGFA signalling is only one component of a complex system of molecular cross talk between the podocyte and glycocalyx. New insights have revealed that molecules produced by the endothelium can signal to the podocyte and then back to the glomerular glycocalyx. Using transgenic murine models and conditionally immortalised murine podocytes and GECs, Garsen et al have shown that endothelin-1 (ET-1), an endothelial derived vasoconstrictor, is released by the GECs in diabetic conditions and leads to shedding of the glycocalyx [78]. This is prevented by deleting

the ET-1 receptor specifically in the podocyte. This is therapeutically intriguing as there are ET-1 receptor antagonists that have been shown to ameliorate early microalbuminuric diabetic kidney disease [79]. In the future, therapeutic approaches to maintain the GECs glycocalyx should be explored in more detail (**Figure 3**).

SGLT2 and kidney disease

Poor glycaemic control and hyperinsulinaemia (at least in the early phase of diabetes) lead to upregulation of SGLT2 expression and proximal tubular SGLT2-mediated sodium-glucose reabsorption [80], which in turns is believed to also contribute to higher blood pressure levels [81]. SGLT2 inhibitors have recently been developed as oral hypoglycaemic agents [82]. The SGLT2 antagonists block the sodium-coupled energy dependent glucose proximal tubular reabsorption resulting in improvement in diabetes control, weight loss and blood pressure lowering. Recent clinical trials have demonstrated a dramatic cardiovascular [83] and renoprotective [84] effect of the SGLT2 inhibitor empaglifozin.

The mechanism by which SGLT2 inhibitors exert their renoprotective effects is currently unknown. One possibility is that the improvement in renal disease is secondary to activation of tubuloglomerular feedback, a prime mechanism that determines a reduction in glomerular capillary pressure [85]. A complementary mechanism may be that the inhibition of enhanced tubuli sodium-coupled glucose transport seen in diabetes would result in diminished tubulointerstitial injury and progression of DN [81]. The use of SGLT2 inhibitors in combination with inhibitors of the renin-angiotensin-aldosterone system in patients with diabetes may confer some

renoprotection via upregulation of ACE2 and angiotensin 1-7/ 1-9 [86] which retains a vasodilatory, anti-proliferative, anti-inflammatory and anti-oxidative stress effect [87]. Inhibition of SGLT2 increased expression and activity in diabetes [85] could lead to restriction of chronic excess in substrates (glucose) availability, known to result in AMPK inhibition [88], and promote a favourable renal outcome. Studies wll have to dissect whether inhibition of SGLT2 could result in AMPK activation and secondary renoprotection.

In parallel to these “renal mechanisms”, natriuresis and plasma volume contraction paralleled by blood pressure reduction has been also been proposed as a “systemic” renoprotective mechanisms for SGLT2 inhibitors [89](**Figure 4**).

Concluding Remarks and Future Perspectives

Glomerular cellular insulin resistance plays an important role in mitochondrial dysfunction-ER stress and the UPR, which contribute to glomerular cell dysfunction and progressive kidney disease. AMPK, with its important role in mitochondrial function, could represent a potential target for treatment in DN; more studies are required to assess the role of AMPK on podocyte biology and the regulation of the glomerular filtration barrier. A link between the tubular compartment and the glomerulus is evident in the pathophysiology of tubular SGLT2-mediated Na-coupled glucose reabsorption in diabetes, however it is not yet completely clear what are the mechanisms underlying these beneficial effects. Future studies will need to better dissect the cellular mechanisms underlying the proposed pathways outlined in this article, specifically focusing on the physiology of the nephron as a whole entity, and by identifying potentially targetable molecules for future treatment.

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TABLE 1

Diabetes results in inflammation, increased adiposity and chronic hyperglycaemia which drive podocyte insulin resistance resulting in disruption to podocytes and the glomerulus. (NOD2: Nucleotide-binding oligomerization domain containing protein 2, TLR: Toll-like receptor, SMAD: vertebrate homologues of *Sma* and *Mad*; FFAs: free fatty acids; SHP-1: Src homology-2 domain-containing phos- phatase-1)

Diabetes related phenotypes	Molecular mechanisms	Glomerular phenotype	REF #
Chronic inflammation	Increased NOD2	Increased pro-inflammatory responses and impaired insulin signaling	[22]
	Increased TLR	Podocyte inflammation and insulin resistance	[23]
	Increased SMAD	Mitochondrial dysfunction and insulin resistance in podocytes	[21]
Obesity	Decreased adiponectin	Albuminuria and increased oxidative stress in podocytes	[24]
	Increased FFAs	Inhibition of insulin-stimulated glucose uptake in human podocytes	[26]
Elevated blood sugar	Increased SHP-1	Podocyte insulin resistance and detachment	[27]
	Ubiquitination and degradation of components of insulin signaling pathway	Diabetic glomerulopathy and albuminuria	[28]

Figure legends:

Figure 1: Schematic structure of a normal and diabetic glomeruli.

(A) Schematic representation of a normal glomerular structure. (B) The major glomerular structural changes occurring in diabetic glomerulopathy. Note the extensive mesangial expansion, the thickening of the glomerular basement membrane (GBM), the detachment of podocyte, and the impairment in the glycocalyx and glomerular endothelial cells (GECs).

Figure 2: Hypothetical shift of superoxide level imbalance in diabetic complications.

Acute (early phase) exposure of cells to elevated glucose levels results in upregulation of glucose oxidation with pyruvate-mediated stimulation of the tricarboxylic acid (TCA) cycle with increased production of electron donors (NADH, $FADH_2$) that, via the electron transport chain, will results in an excess generation of superoxide (O_2^-). Cells chronically exposed to elevated glucose levels (late phase) will result in reduction in the availability of acetyl-CoA (secondary to inhibition of pyruvate dehydrogenase activity) for the mitochondria resulting in reduced electron transport chain activity, a fall in mitochondrial ATP production, less mitochondria superoxide production and cellular dysfunction.

Figure 3: Glomerular cell cross-talk and glycocalyx.

Transmission electron microscopy image of the glomerular filtration barrier (podocyte glomerular basement membrane (GBM), glomerular endothelial cells (GEC), and glycocalyx) highlighting how molecules produced by the podocytes and endothelium

(via the podocyte) can signal to the glomerular glycocalyx. Recently identified key molecules such as VEGF-A_{165b}, VEGF-C, and angiopoietin-1 (ANGPT1) confer a beneficial effect (green arrows) towards glycocalyx maintenance. Conversely VEGF-A_{165} and Endothelin (secreted by GECs and signals to the Endothelin-1 receptor in the podocyte causing this cell to release heparanase, which then acts on the glomerular glycocalyx to cleave heparin sulphate) promote shedding of the GECs glycocalyx (red arrows).

Figure 4: Proposed SGLT2 inhibition-mediated renoprotective mechanisms.

SGLT2 inhibition blocks sodium-glucose coupled glucose reabsorption at the S1 S2 segment of the proximal tubule. The net result is loss of glucose and sodium (the latter especially in patients on renin angiotensin aldosterone blockade) in the urine, with secondary weight loss, improvement in glycaemic control, blood pressure fall, and plasma volume contraction. These effects confer cardiac and renal protection in patients with diabetes. (ACE2: angiotensin converting enzyme 2)

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OUTSTANDING QUESTIONS BOX

- What are the main pathway/s that link insulin action, mitochondrial function and UPR?

- What are the mitochondrial-driven mechanisms that predispose towards faster kidney disease progression in diabetes? Is there an alteration in the mitochondria driven UPR-mediated response towards cell survival or is it a primary alteration in UPR response?

- Does AMPK, with its important role as a regulator of nutrient utilisation and mitochondrial function, represent a real answer to diabetes mediated ER-stress mitochondria dysfunction, UPR, and, if so, what are the mechanisms?

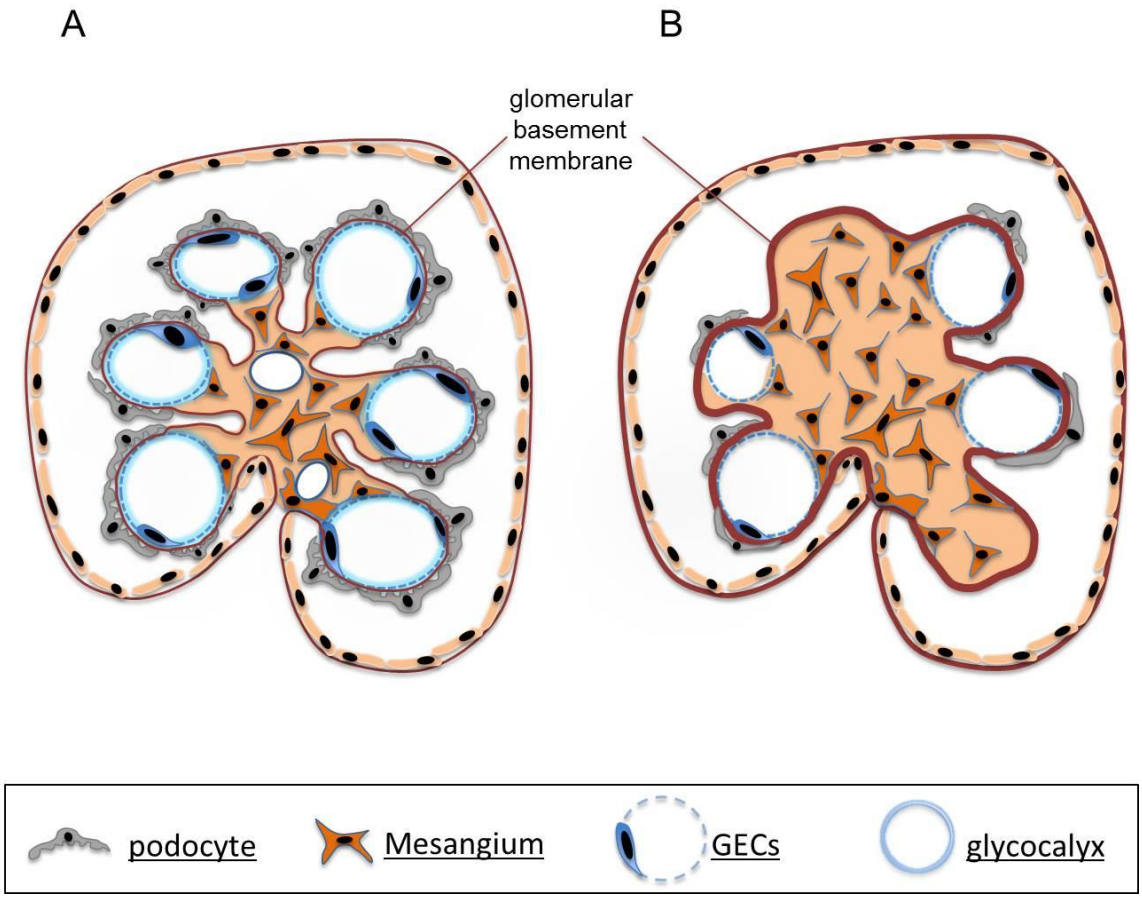
- Could targeting the glycocalyx be a new therapeutic approach for diabetic nephropathy? What are the important molecular signals from the podocyte and endothelium which regulate the glycocalyx?

- How does SGLT2 inhibition confer reno-protection? Is it about SGLT2-driven sodium and volume loss (systemic effect) or tubuloglomerular feedback and inflammation (intrarenal effect)? Are these mechanisms behind the renoprotective effects of these drugs?

TRENDS BOX:

- Insulin resistance is a key mechanism for diabetic glomerulopathy.
- Disruption in the molecular communication between glomerular podocytes and endothelia is critical in the progression of diabetic nephropathy.
- Raised (but not too elevated) mitochondrial superoxide cellular levels in parallel with healthy mitochondria are protective against progression of diabetic kidney disease.
- A reduction in maximal mitochondrial respiration and reserve capacity could represent an important driving force for kidney disease progression in diabetes.
- Inhibition of SGLT2-mediated sodium-coupled glucose transport confers renoprotection of similar magnitude of inhibitors of the renin-angiotensin-aldosterone system.

689 **Figure 1**



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692 **Figure 2**

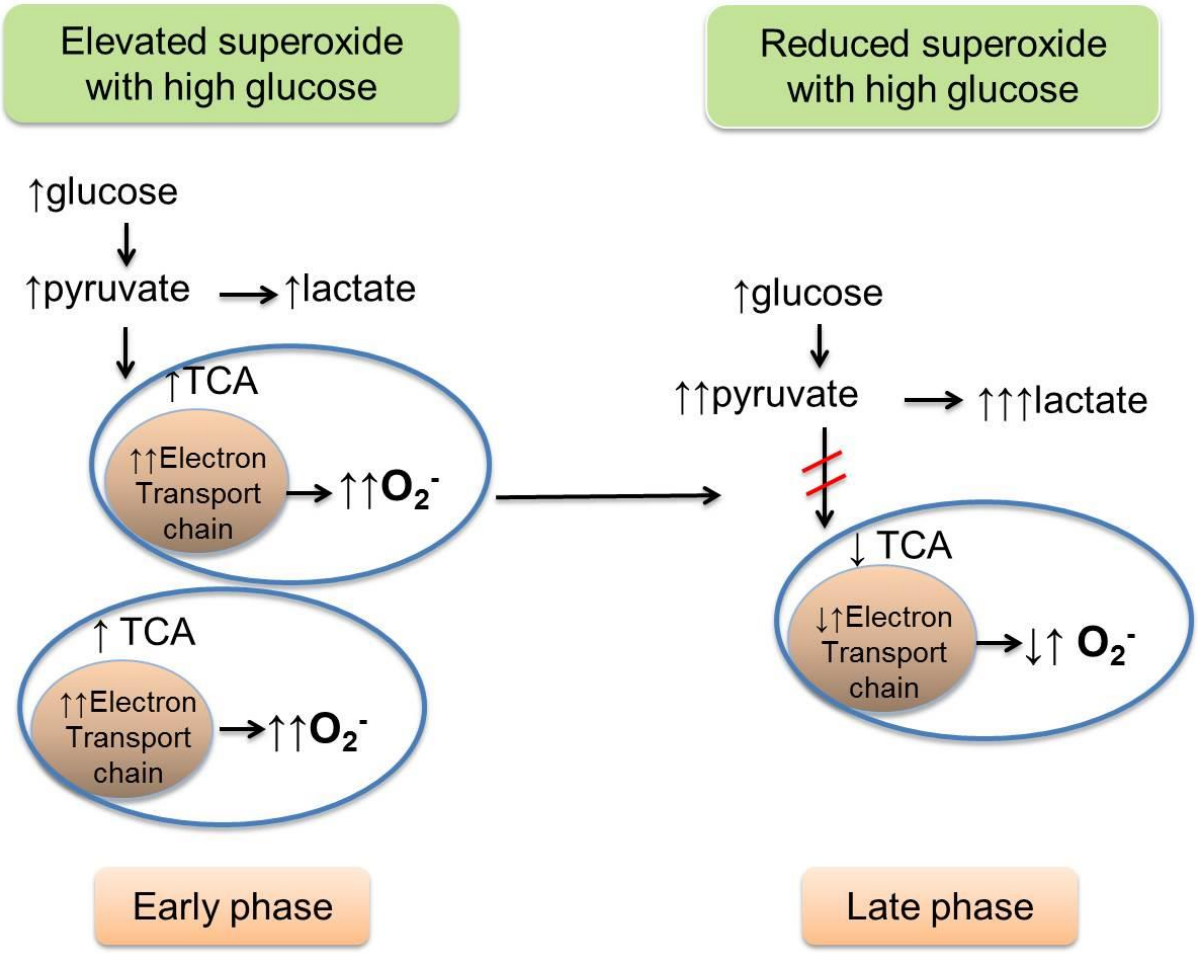

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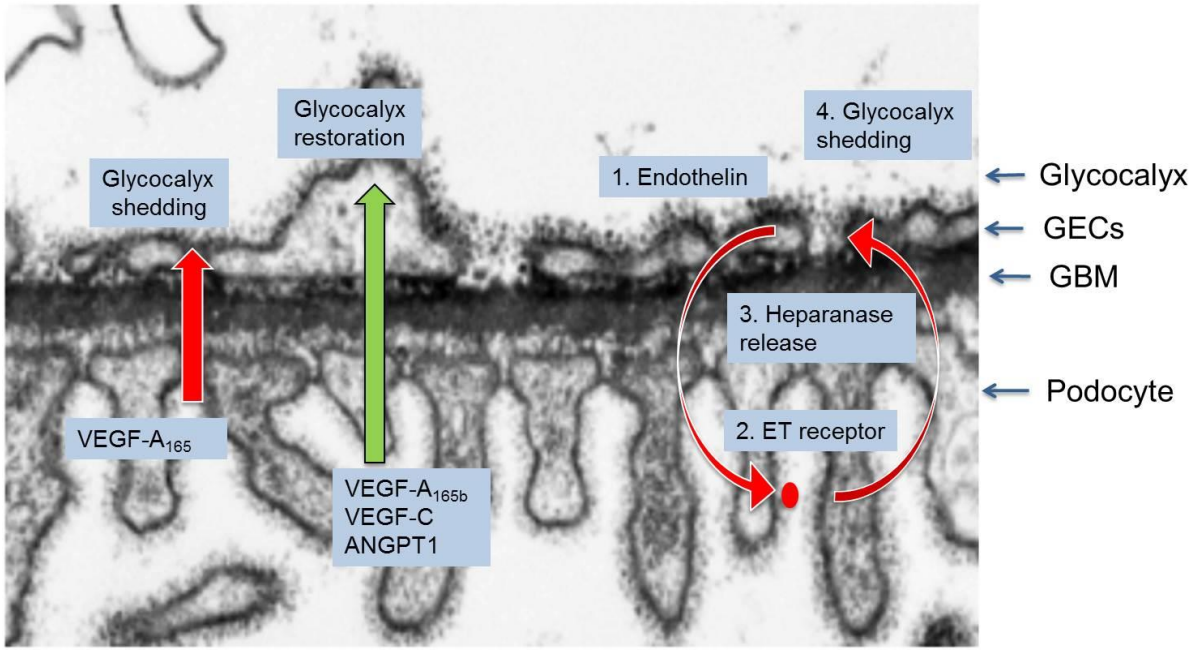
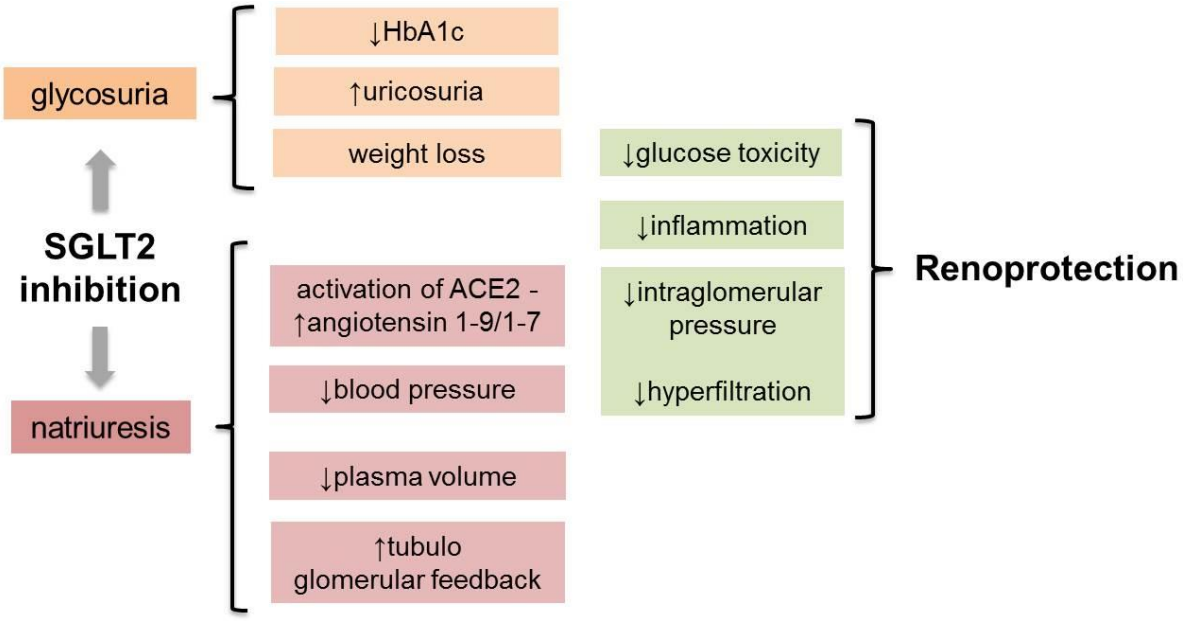
Figure 3


Glycocalyx restoration
Glycocalyx shedding
1. Endothelin
4. Glycocalyx shedding
Glycocalyx
GECs
GBM
3. Heparanase release
Podocyte
2. ET receptor
VEGF-A165
VEGF-A165b
VEGF-C
ANGPT1

698 **Figure 4**



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