

1 **Diabetic nephropathy: perspective on novel molecular mechanisms**

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27 **Abstract**

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

38

39 **INTRODUCTION**

40 Diabetes mellitus is a metabolic disorder associated with chronic micro- and
41 macrovascular complications. One of the most feared chronic microvascular
42 complications is diabetic nephropathy (DN), currently the leading cause of end-stage
43 renal disease (ESRD) in the Western world. Strikingly, 40-45% of patients with type-
44 1 diabetes (T1D) develop DN and reach ESRD or die before its onset. Moreover,
45 clinicians face a ~30% prevalence of patients with type-2 diabetes (T2D) and DN,
46 with 45% of patients currently on dialysis having a primary diagnosis of diabetes, a
47 population also at high risk of developing cardiovascular disease [1].

48

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

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

70

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

80

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

86

87

88 **Insulin resistance as a mechanism for the predisposition of DN**

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

101

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

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

120

121

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

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

146

147

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

150 There are many consequences of insulin resistance within glomeruli, which are likely
151 to contribute to the progression of DN. One key mechanism is changes to the
152 mitochondria and the closely connected ER [31]. Mitochondrial metabolic overload
153 results in increased cellular oxidative stress and ER-stress which leads to the
154 activation of unfolded protein response (UPR)[32]. UPR is a positive cellular
155 response that in its early phase either refolds accumulated unfolded proteins, or
156 degrades unfolded protein by the ubiquitin-proteasome pathway. Misfolded proteins
157 are detected by the ER membrane stress sensors protein kinase RNA-like ER kinase
158 (PERK), inositol-requiring protein 1 α (IRE1 α) and activating transcription factor 6 α
159 (ATF6 α) and its activator X-box binding protein-1 (XBP-1), which, in turn, activates
160 several signalling events and trigger a compensatory response to prevent further
161 accumulation of misfolded protein. However, when the unfolded protein and cellular
162 damage exceeds a threshold, chronic and unresolved stress results in a change
163 from an adaptive to pro-apoptotic responses [32].

164

165 There is some evidence that glucose/oxidative stress-mediated ER stress plays a
166 role in chronic vascular complications in DN [33]. Hyperglycemia, or increased
167 glycation of proteins have been shown to mediate apoptosis partly through increases
168 in ER stress in cultured murine podocytes [34, 35]. Activation of the UPR has also
169 been observed in mouse glomerular mesangial cells exposed to glucose and
170 glucosamine [36], and in kidneys from diabetic rats administered streptozotocin for
171 16 weeks [37]. Microarray analysis of human biopsies from patients with established
172 DN showed that UPR genes were upregulated proportionally to the severity of
173 diabetic renal lesions [38]. Finally, recent experimental evidence has demonstrated
174 that pharmacological inhibition of ER stress and stabilization of the UPR is beneficial
175 in diabetic glomerulopathy [39].

176

177 Two studies have used transgenic mice to link podocyte insulin resistance with
178 mitochondrial function and ER stress. Ising and colleagues generated a mouse
179 model of podocyte mitochondrial dysfunction by specifically knocking out a key
180 molecule in this cell involved in mitochondrial fusion called prohibitin-2 [40]. This
181 caused a severe phenotype including glomerulosclerosis, renal failure and death at
182 approximately a month of age. They then went on to inhibit both the insulin receptor
183 and IGF-1 receptor (IGF1R) contemporaneously with podocyte-specific knockdown
184 of prohibitin-2. Inhibiting the insulin receptor alone, or in combination with the IGF1R
185 was partially protective and resulted in a significantly longer life span of the mice
186 [40]. This suggests that insulin resistance could reflect a “protective” resetting of
187 cellular substrate utilisation to shield from excess substrate flow to mitochondria with
188 “impaired” respiratory capabilities. In another study, Madhusudhan *et al.* have

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

198

199 Autophagy, regulated by the mammalian target of rapamycin complex 1 (mTORC1)
200 is, with the UPR, essential to maintain cellular homeostasis and in the context of ER
201 stress contributes towards the elimination of toxic and damaged cellular components
202 [42]. Haploinsufficiency of mTORC1 in podocytes or administration of rapamycin (a
203 mTORC1 inhibitor) resulting in activation of autophagy [43], has been shown to
204 prevent progressive DN [44, 45]. In contrast, mTORC1 activation in podocytes,
205 resulting in inhibition of autophagy, leads to accelerated DN [46]. Loss of insulin
206 sensitivity in cultured podocytes results in suppression of autophagy and addition of
207 rapamycin in these cells attenuates insulin resistance [47].

208

209

210 **Insulin resistance, the glomerular cell cytoskeleton and other mechanisms**

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

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

222

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

237

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

245

246

247 **Reactive oxygen species and diabetic nephropathy**

248 Over the last decade, an attractive unifying hypothesis has been put forward to
249 explain diabetic microvascular complications; specifically it was postulated that an
250 excess in cellular substrate availability leads to an increase in reactive oxygen
251 species (ROS) which in turn drives vascular complications in DN [62]. However, this
252 unifying hypothesis has been challenged by the negative results of antioxidant-based
253 clinical trials [63], and a new theory of “mitochondrial hormesis” has been proposed
254 [64], whereby the increased mitochondrial superoxide production is considered an
255 indicator of healthy mitochondria and physiologic oxidative phosphorylation.

256

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

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

266

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

278

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

286

287 AMP-activated protein kinase (AMPK) is a stress-activated kinase that is activated in
288 response to depleting ATP to preserve cell survival under conditions of reduced
289 substrate utilisation. AMPK activation has been involved in mitochondrial biogenesis
290 by leading to increased mitochondrial substrate utilisation and ATP generation, in
291 parallel with stimulation of antioxidant gene expression to ensure an optimal redox
292 balance [70]. Reduced AMPK, as seen in the diabetic kidney of both rodents and
293 humans [65], is associated with reduced catabolic activity (mitochondrial function)
294 [65], and reduced AMPK-mediated inhibition of NADPH oxidase (Nox2) resulting in
295 increased ROS production [71, 72]. Taken together, these results suggest that, in the
296 diabetic kidney, upregulation of AMPK could be therapeutically beneficial in DN [73]
297 to regulate nutrient utilisation and mitochondrial function towards maintenance of an
298 optimum redox balance.

299

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

303

304

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

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

312 setting of diabetes. There is clear evidence that the GECs glycocalyx is lost both
313 systemically and within the diabetic glomerulus, and that this contributes to both
314 cardiovascular and renal complications [6]. Mechanistically there are a number of
315 pathways which led to loss of the glomerular glycocalyx including hyperglycaemia
316 [75], and ROS [76].

317

318 During the early phases of diabetes an increase in VEGFA causes glycocalyx
319 shedding from the GECs. Furthermore, the inhibitory isoform of VEGFA called
320 VEGF-A_{165b} also plays a role in maintaining the GECs glycocalyx in diabetes. Oltean
321 et al. [77] have shown that in diabetic patients with progressive nephropathy, the
322 renal expression of VEGF-A_{165b} is lost. They went on to develop a number of murine
323 models of DN and have shown that genetic overexpression or pharmacological
324 administration of VEGF-A_{165b} to the mouse, acting through VEGF receptor 2 in the
325 GECs, restores damaged glomerular endothelial glycocalyx and improves renal
326 function. VEGF-A_{165b} also improved the permeability of isolated human diabetic
327 glomeruli suggesting the response is conserved across murine and human species
328 [77].

329

330 VEGFA signalling is only one component of a complex system of molecular cross
331 talk between the podocyte and glycocalyx. New insights have revealed that
332 molecules produced by the endothelium can signal to the podocyte and then back to
333 the glomerular glycocalyx. Using transgenic murine models and conditionally
334 immortalised murine podocytes and GECs, Garsen et al have shown that endothelin-
335 1 (ET-1), an endothelial derived vasoconstrictor, is released by the GECs in diabetic
336 conditions and leads to shedding of the glycocalyx [78]. This is prevented by deleting

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

341

342

343 **SGLT2 and kidney disease**

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

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

361 renoprotection via upregulation of ACE2 and angiotensin 1-7/ 1-9 [86] which retains a
362 vasodilatory, anti-proliferative, anti-inflammatory and anti-oxidative stress effect [87].
363 Inhibition of SGLT2 increased expression and activity in diabetes [85] could lead to
364 restriction of chronic excess in substrates (glucose) availability, known to result in
365 AMPK inhibition [88], and promote a favourable renal outcome. Studies will have to
366 dissect whether inhibition of SGLT2 could result in AMPK activation and secondary
367 renoprotection.
368 In parallel to these “renal mechanisms”, natriuresis and plasma volume contraction
369 paralleled by blood pressure reduction has been also been proposed as a “systemic”
370 renoprotective mechanisms for SGLT2 inhibitors [89](**Figure 4**).

371

372 **Concluding Remarks and Future Perspectives**

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

385

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398

399

400 **TABLE 1**

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

406

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]

407

408 **Figure legends:**

409

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

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

416

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

419 Acute (early phase) exposure of cells to elevated glucose levels results in
420 upregulation of glucose oxidation with pyruvate-mediated stimulation of the
421 tricarboxylic acid (TCA) cycle with increased production of electron donors (NADH,
422 FADH₂) that, via the electron transport chain, will results in an excess generation of
423 superoxide (O₂⁻). Cells chronically exposed to elevated glucose levels (late phase)
424 will result in reduction in the availability of acetyl-CoA (secondary to inhibition of
425 pyruvate dehydrogenase activity) for the mitochondria resulting in reduced electron
426 transport chain activity, a fall in mitochondrial ATP production, less mitochondria
427 superoxide production and cellular dysfunction.

428

429 **Figure 3: Glomerular cell cross-talk and glycocalyx.**

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

433 (via the podocyte) can signal to the glomerular glycocalyx. Recently identified key
434 molecules such as VEGF-A_{165b}, VEGF-C, and angiopoietin-1 (ANGPT1) confer a
435 beneficial effect (green arrows) towards glycocalyx maintenance. Conversely VEGF-
436 A₁₆₅ and Endothelin (secreted by GECs and signals to the Endothelin-1 receptor in
437 the podocyte causing this cell to release heparanase, which then acts on the
438 glomerular glycocalyx to cleave heparin sulphate) promote shedding of the GECs
439 glycocalyx (red arrows).

440

441 **Figure 4: Proposed SGLT2 inhibition-mediated renoprotective mechanisms.**

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

448

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658 **OUTSTANDING QUESTIONS BOX**

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

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

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

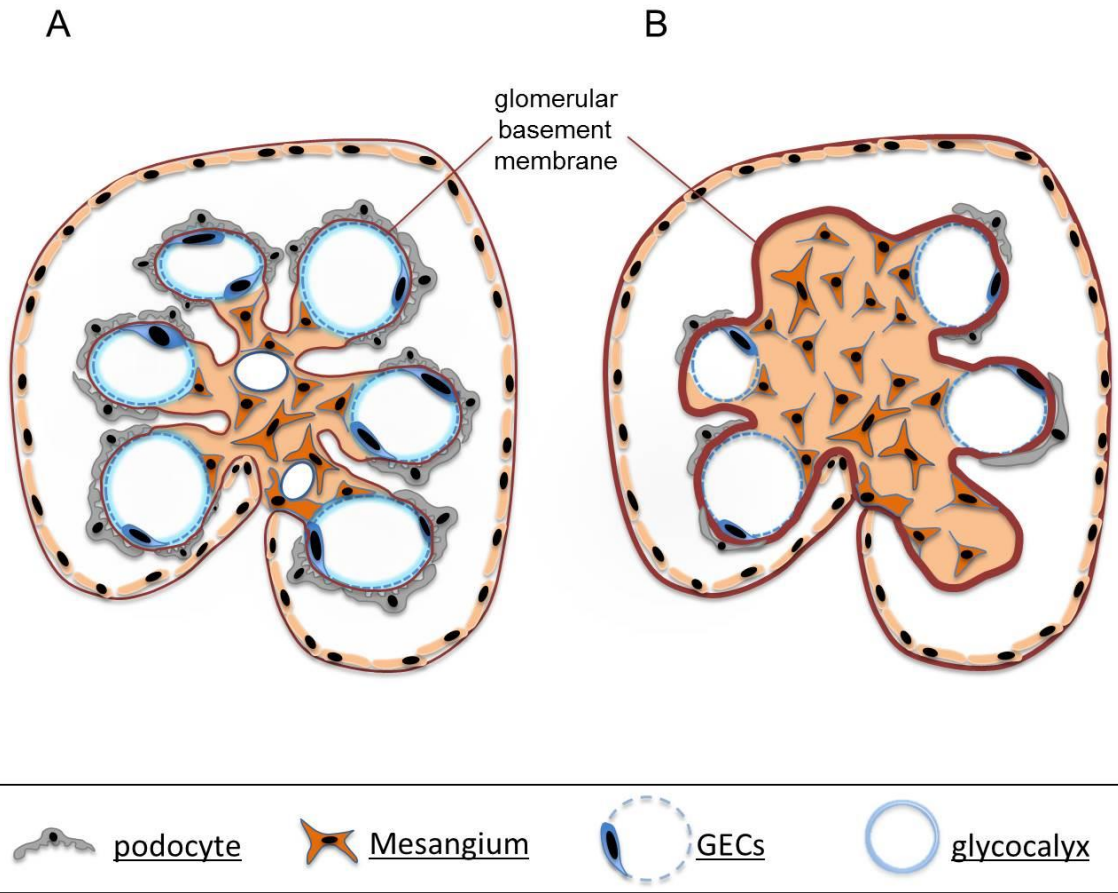
668 - Could targeting the glycocalyx be a new therapeutic approach for diabetic
669 nephropathy? What are the important molecular signals from the podocyte and
670 endothelium which regulate the glycocalyx?

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

675 **TRENDS BOX:**

- 676 - Insulin resistance is a key mechanism for diabetic glomerulopathy.
- 677 - Disruption in the molecular communication between glomerular podocytes
678 and endothelia is critical in the progression of diabetic nephropathy.
- 679 - Raised (but not too elevated) mitochondrial superoxide cellular levels in
680 parallel with healthy mitochondria are protective against progression of
681 diabetic kidney disease.
- 682 - A reduction in maximal mitochondrial respiration and reserve capacity could
683 represent an important driving force for kidney disease progression in
684 diabetes.
- 685 - Inhibition of SGLT2-mediated sodium-coupled glucose transport confers
686 renoprotection of similar magnitude of inhibitors of the renin-angiotensin-
687 aldosterone system.
- 688

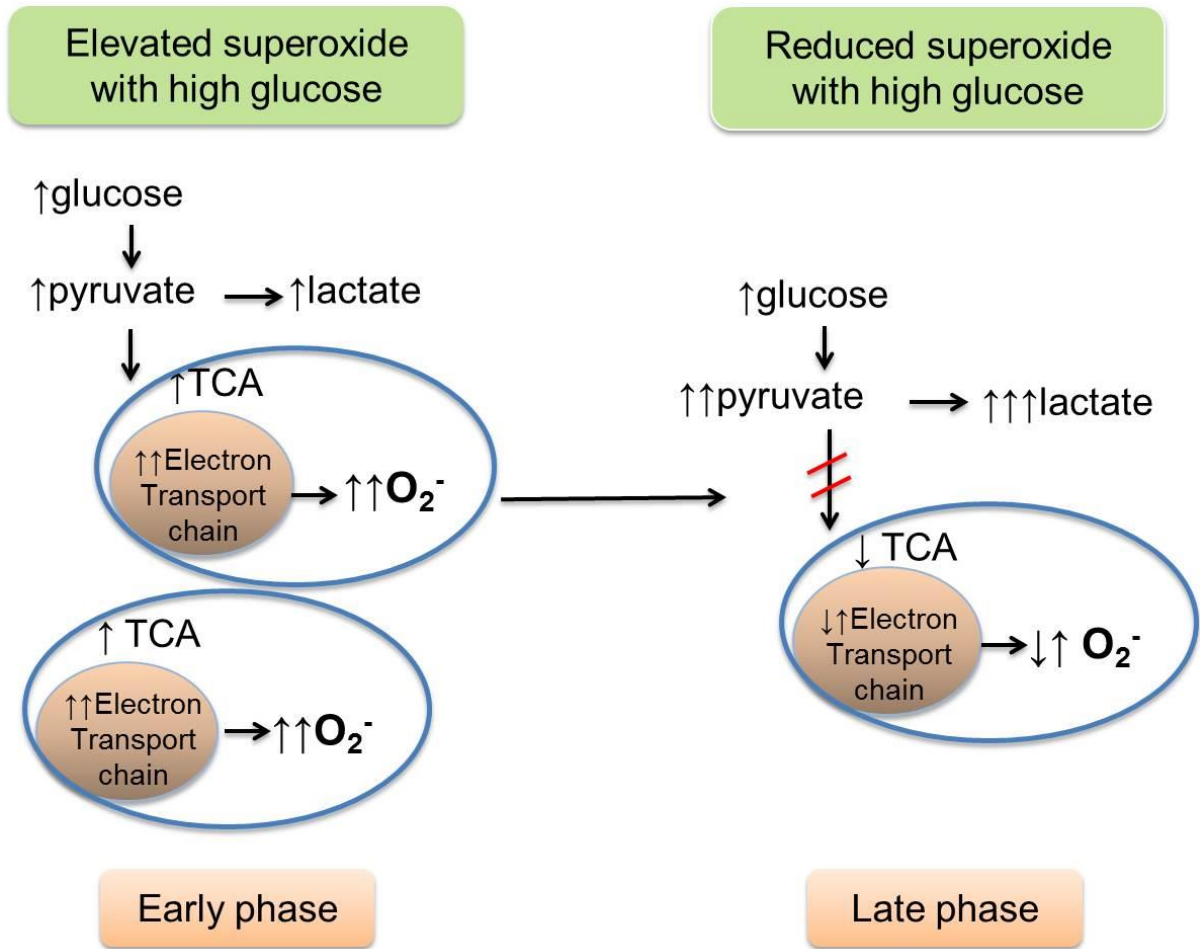
689 **Figure 1**



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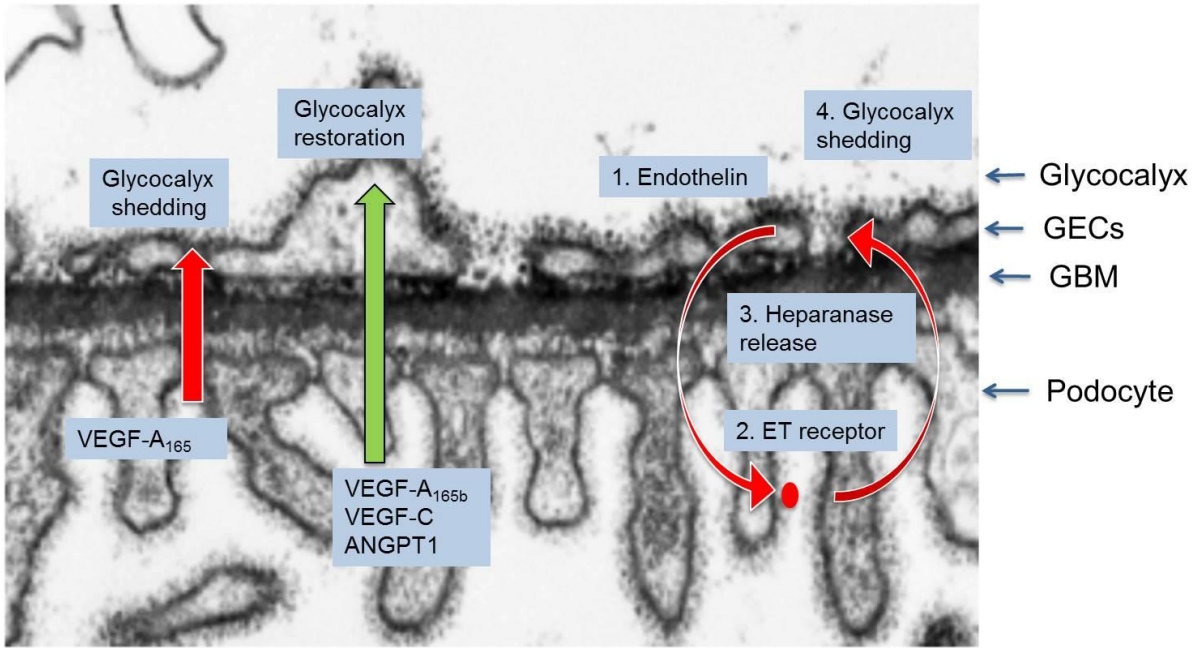
692 **Figure 2**



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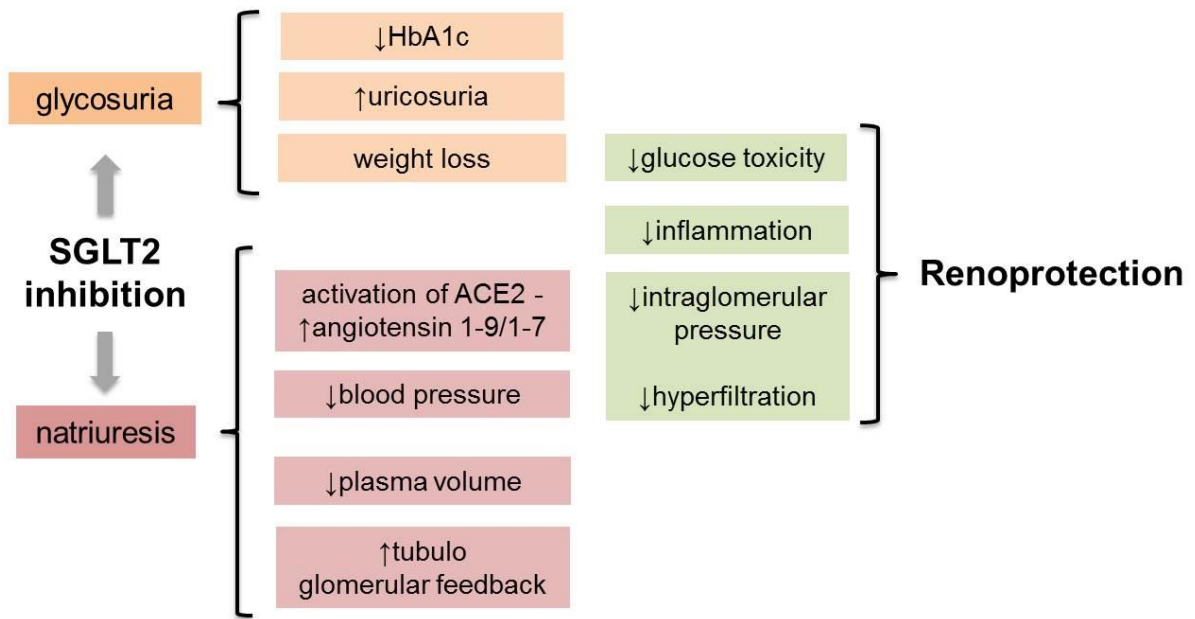
695 **Figure 3**



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698 **Figure 4**



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