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Endothelial dysfunction as a potential contributor in diabetic nephropathy

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Abstract

The mechanisms that drive the development of diabetic nephropathy remain undetermined. Only 30–40% of patients with diabetes mellitus develop overt nephropathy, which suggests that other contributing factors besides the diabetic state are required for the progression of diabetic nephropathy. Endothelial dysfunction is associated with human diabetic nephropathy and retinopathy, and advanced diabetic glomerulopathy often exhibits thrombotic microangiopathy, including glomerular capillary microaneurysms and mesangiolysis, which are typical manifestations of endothelial dysfunction in the glomerulus. Likewise, diabetic mice with severe endothelial dysfunction owing to deficiency of endothelial nitric oxide synthase develop progressive nephropathy and retinopathy similar to the advanced lesions observed in humans with diabetes mellitus. Additionally, inhibitors of the renin–angiotensin system fail to be renoprotective in some individuals with diabetic nephropathy (due in part to aldosterone breakthrough) and in some mouse models of the disease. In this Review, we discuss the clinical and experimental evidence that supports a role for endothelial nitric oxide deficiency and subsequent endothelial dysfunction in the progression of diabetic nephropathy and retinopathy. If endothelial dysfunction is the key factor required for diabetic nephropathy, then agents that improve endothelial function or raise intraglomerular nitric oxide level could be beneficial in the treatment of diabetic nephropathy.

Introduction

Diabetic nephropathy and retinopathy are serious complications of diabetes mellitus, and can eventually progress to end-stage renal disease and blindness. Epidemiological studies,

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Competing interests
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Review criteria
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Author contributions
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however, show that only 30% of patients with diabetes develop overt nephropathy and that the incidence of diabetic nephropathy might be decreasing, particularly in patients with type 1 diabetes.[1,2] Histological evidence also shows that only a minority of patients with diabetic nephropathy exhibit advanced glomerular injury.[3,4] Therefore, it is important to note that not all patients with diabetes develop advanced stages of diabetic renal disease.

Similar observations have been noted in animal models of diabetes. Most diabetic animal models develop only early stages of diabetic nephropathy and not advanced lesions. For instance, glomerular hypertrophy, mesangial expansion, and thickening of the glomerular basement membrane—all of which are features of early diabetic nephropathy—can be induced in most animal models whereas advanced lesions (such as mesangiolysis, Kimmelstiel–Wilson nodules, and glomerular capillary microaneurysms) do not develop. Similarly, most animal models of diabetes only exhibit mild retinal vascular injury, which is characteristic of early features of diabetic retinopathy in humans. The observation that advanced diabetic lesions do not consistently develop in humans and animal models has led us to postulate that the development of these advanced lesions in diabetic nephropathy and retinopathy might require additional factors. This Review discusses the clinical and experimental evidence demonstrating that endothelial nitric oxide deficiency and subsequent endothelial dysfunction are key factors in the development of diabetic nephro-pathy and retinopathy. Therapies that could prove beneficial for the treatment of diabetic nephropathy and retinopathy are also discussed.

Diabetic nephropathy

Role of endothelial dysfunction

In 1989, Jensen *et al.*[5,6] first described that endothelial injury, as evidenced by an increase in plasma levels of von Willebrand factor, was present in patients with incipient type 1 diabetes. Importantly, endothelial function was more severely impaired in patients with overt nephropathy than in those with incipient nephropathy.[5,6] Other investigators later confirmed this association in both patients with type 1 diabetes and patients with type 2 diabetes.[7–9] Fioretto *et al.*[10] further documented that the increase in plasma levels of von Willebrand factor in patients with type 2 diabetes was positively correlated with glomerular injury whereas patients who did not exhibit endothelial dysfunction had intact renal morphology. This finding led to the hypothesis that the heterogeneous nature of glomerular injury observed in people with diabetes might be accounted for by the extent of endothelial injury in type 2 diabetes.[10] In addition, endothelial injury in diabetes is accompanied by structural abnormalities in the endothelium. For instance, an impairment of endothelial glycocalyx was found to be associated with increased permeability in the glomerular basement membrane, which might partially account for the microalbuminuria observed in patients with dia-betes.[11,12] loss of endothelial fenestrations is also another feature of early diabetes.[13,14] Importantly, such structural abnormalities of the endothelium were also accompanied by mesangial expansion and podocyte injury in patients with diabetes.[14]

Tubulointerstitial injury can also be observed in patients with type 1 or type 2 diabetes, but is more common in those with type 2 diabetes. Reports suggest that approximately 30% of patients with diabetes predominantly develop tubulointerstitial injury as opposed to glomerular disease.[15,16] Furthermore, the severity of the interstitial lesion is a determinant of renal disease progression in patients with type 2 diabetes.[17] Interestingly, tubulointerstitial injury develops independently of glom-erular injury in type 1 diabetes.[18] although the precise mechanism for diabetic tubulointerstitial injury remains unclear, some studies have suggested a role for endo-thelial dysfunction in this process.[19–21] Bangstad *et al.*[20] found an association between the ratio of asymmetric dimethylarginine: L-arginine (a marker of endothelial dysfunction) with tubulointerstitial injury in patients with type 1 diabetes.

likewise, Shibata *et al.*[19] suggested in 2009 that endothelial dysfunction could be responsible for the tubulointerstitial injury observed in rats with streptozotocin-induced diabetes. One mechanism by which endothelial dysfunction might induce tubulo-interstitial injury could be through plasma leaking from injured peritubular capillaries and eliciting an inflammatory tubulointerstitial response.[22]

Role of endothelial nitric oxide

Nitric oxide production is catalyzed by endothelial nitric oxide synthase (enOS) to maintain the integrity of endothelial cells. nitric oxide blocks endothelial cell activation and injury induced by cytokines (such as tumor necrosis factor), has antithrombogenic effects (for instance, by blocking the release of von Willebrand factor from Weibel–Palade bodies) and promotes vaso-dilation of the underlying vascular smooth muscle cells. Of note, one of the earliest features of endothelial dysfunction is a reduction in nitric oxide production in the endothelium.

Diabetes is known to be associated with a reduction in nitric oxide bioavailability. The underlying mechanisms seem to be diverse, but include the effects of hyperglycemia,[23] advanced glycation end products,[24] uric acid,[25] asymmetric dimethylarginine[26] and oxi-dative stress[27] (Figure 1). Impairment of nitric oxide generation caused by polymorphisms in enOS is also thought to cause endothelial dysfunction and nitric oxide deficiency.[28] Over the past decade, several investigators have examined enOS polymorphisms in patients with diabetes. However, only some,[29–32] but not all studies,[33–36] have documented a positive association of specific eNOS polymorphisms with diabetic nephropathy. Hohenstein *et al.*[37] investigated eNOS expression in patients with type 2 diabetes using immunohistochemistry of renal biopsy samples and found that eNOS expression was increased in glom-eruli that had evidence of diabetic changes. Similarly, streptozotocin-induced diabetic rats exhibit an increase in eNOS expression in endothelial cells in the afferent arterioles and glomerulus.[38] Importantly, however, eNOS expression does not always correlate with eNOS activity. The generation of nitric oxide from L-arginine by eNOS requires the presence of cofactors that can be inactivated by oxidants, and when eNOS is ‘uncoupled’, superoxide as opposed to nitric oxide will be generated. Brodsky and colleagues[39] found that a high level of glucose induces uncoupling of eNOS, which causes a reduction in nitric oxide bioavailability and a concurrent increase in superoxide production.[39] Furthermore, Komers *et al.*[40] found that eNOS is present primarily in the uncoupled form and localizes to the cytosolic fraction of endothelial cells from diabetic rats. Since eNOS activation also requires its translocation into the plasma membrane in its coupled form,[40] it is likely that the increased level of eNOS in diabetes might be an inactivated form of the enzyme.

Experimental evidence

Streptozotocin-induced diabetes in mice

One of the current favored models for the study of diabetic nephropathy associated with type 1 diabetes is the model of streptozotocin-induced nephropathy in eNOS-knockout mice. However, numerous regimens for the induction of diabetes by streptozotocin are currently being used.[41–43] The animal Models of Diabetic Complications Consortium (AMDCC) recommends the injection of 50 mg/kg streptozotocin per day for 5 consecutive days to induce diabetes;[44] however, regimens that use 100 mg/kg streptozotocin per day for 2–3 days are also commonly used (and will be referred to as the ‘2-day method’ in this article).[45,46] Importantly, both methods are considered ‘low-dose streptozotocin’ compared with historical regimens.[41–43,47–49] Comparisons of the AMDCC-recommended regimen and the 2-day method in eNOS-knockout mice showed that although both induce diabetes effectively in mice, the 2-day protocol induced diabetes within 1 week while the AMDCC-

recommended regimen induced diabetes after 2 weeks. Moreover, the glomerular injury induced by the AMDCC-recommended regimen tended to be somewhat milder than that induced by the 2-day method (T. Nakagawa, unpublished work). although one might argue that the high doses of streptozotocin used by the 2-day method could cause tubulointerstitial injury owing to streptozotocin toxicity,[50] the AMDCC regimen also induced tubulointerstitial injury (T. Nakagawa, unpublished work). In addition, given our finding that insulin treatment substantially blocked tubulointerstitial and glomerular lesions,[49] it seems that tubulointerstitial injury occurred secondary to the diabetic state, and not from streptozotocin toxicity.

Diabetic nephropathy in eNos-knockout mice

The 2-day method has also been used to evaluate the role of eNOS in diabetic nephropathy. Consistent with previous findings, diabetic wild-type mice developed mild proteinuria but had preserved renal function. By contrast, diabetic eNOS-knockout mice developed clinical manifestations that resembled overt diabetic nephro pathy in humans. For example, hypertension, nephrotic albuminuria, and renal dysfunction were induced by administration of streptozotocin to eNOS-knockout mice.[49] Diabetic eNOS-knockout mice also exhibited higher mortality from progressive renal disease than other groups, including nondiabetic wild-type mice, diabetic wild-type mice and nondiabetic eNOS-knockout mice.[49] This finding is important because life expectancy is reduced in patients who have type 1 diabetes with advanced nephropathy[51] and mortality corre lates with the severity of renal injury in patients with diabetes.[52] These data indicate that a lack of endothelial nitric oxide could be a key factor in the development of advanced diabetic nephropathy.

Histological manifestations of diabetic nephropathy in diabetic eNOS-knockout mice also mimic those of human diabetic nephropathy. Importantly, this mouse model develops not only the early manifestations of diabetic nephropathy, such as mesangial expansion and thickening of the glomerular basement membrane, but also advanced lesions including mesangiolysis, Kimmelstiel–Wilson-like nodules, arteriolar hyalinosis, and tubulointerstitial disease.[49] In addition, abnormal angiogenesis is now recognized as a manifestation of human diabetic nephropathy;[53] the vessels that result from abnormal angiogenesis likely have a pathological role as antiangiogenic treatment has been shown to prevent proteinuria and renal hypertrophy in experimental models of diabetic nephropathy.[54–56] abnormal extraglomerular vessels are observed in wild-type mice with diabetic renal disease,[55,57] but the increase in capillary number is more marked in diabetic eNOS-knockout mice.[49] The mechanism for the development of abnormal angiogenesis might involve the effects of vascular endothelial growth factor (VEGF). In particular, the effects of VEGF on endothelial cells and macrophages can be enhanced by eNOS deficiency, which could contribute to the excessive proliferation of endothelial cells and abnormal angiogenesis observed in experimental models of diabetic nephropathy.[58,59]

Another histological manifestation of human diabetic nephropathy is tubulointerstitial injury. Diabetic mice that lack eNOS also develop tubulointerstitial injury that is more severe than that observed in diabetic wild-type mice.[21] Insulin treatment can prevent this tubulo interstitial injury,21 which indicates a role for hyperglycemia in mediating this type of injury. By contrast, lowering blood pressure does not prevent tubulo interstitial injury in diabetic eNOS-knockout mice.[21] These studies suggest that the diabetic state, coupled with the presence of eNOS deficiency or subsequent endo thelial dysfunction,[60] might have a key role in inducing tubulointerstitial injury in individuals with diabetes and that this phenomenon is independent of blood pressure.[21,49]

Diabetic retinopathy in eNos-knockout mice

Similar to diabetic nephropathy, most animal models of diabetes do not develop several features of retinal injury that are occasionally observed in humans with diabetic retinopathy. In fact, most models of diabetic mice develop only early stages of diabetic retinopathy: increased capillary permeability, breakdown of the blood retinal barrier, thickening of the capillary basement membrane, the appearance of pericyte ghosts, and the formation of acellular capillaries.[61,62] However, these manifestations are mild and do not completely replicate human diabetic retinopathy. In particular, upregulation of glial fibrillary acidic protein expression is associated with Müller cell activation in individuals with early diabetic retino pathy[63–65] while expression of glial fibrillary acidic protein is not dramatically different in streptozotocin-induced diabetic mice compared with wild-type mice[62] and is not increased in the *Ins2*^{Akita} mouse model of diabetes.[61] By contrast, diabetic eNOS-knockout mice display features of retinopathy that have not been observed in wild-type mice with streptozotocin-induced diabetes. These features resemble most of the major vascular hallmarks of human diabetic retino pathy, including increased thickness of the retinal capillary basement membrane, increased retinal vessel leakage, gliosis, and an increased number of acellular retinal capillaries (Figure 2).[66] These findings support the hypothesis that deficiency of endothelial nitric oxide contributes to endothelial dysfunction, which in turn has a critical role in the pathogenesis of diabetic retinopathy.[67]

Mechanisms of accelerated nephropathy

Several potential mechanisms exist through which a lack of endothelial nitric oxide could accelerate the development of diabetic nephropathy (Figure 3). First, endothe-lial dysfunction is known to alter renal autoregulation. Endothelial nitric oxide is produced by endothelial cells in afferent arterioles and the glomerulus, and to a lesser extent in efferent arterioles, and a lack of nitric oxide causes arteriolar disease.[68] under physio logical conditions, endothelial nitric oxide regulates intraglomerular pressure.[69,70] In settings in which production of nitric oxide is inhibited, however, an increase in systolic blood pressure coupled with altered arteriolar autoregulation results in the increased transmission of pressure to the glomerulus, which results in glomerular hypertension.[71] as such, an increase in glomerular pressure owing to endothelial nitric oxide deficiency might partially account for the development of mesangiolysis that is observed in human diabetic nephropathy. Consistent with this hypothesis, we have reported that lowering blood pressure dramatically prevented the progression of glomerular injury in diabetic eNOS-knockout mice.[21]

A second mechanism through which deficiency of endothelial nitric oxide could accelerate the development of diabetic nephropathy is by augmenting the response of many types of cells, including endothelial cells and macro phages, to VEGF. The diabetic state is known to cause an upregulation of VEGF expression, which induces new vessel formation in the kidney and retina,[53,72] stimulates renal hypertrophy[73] and causes protein uria in experimental models of diabetic nephro-pathy. [74,75] although diabetes is associated with a reduction in nitric oxide bioavailability (Figure 1), *in vitro* studies from our research group demonstrated that nitric oxide deficiency enhanced endothelial proliferation as well as macrophage chemotaxis in response to VEGF.[59,76] Similarly, Bussolati *et al.*[77] used a matrigel angiogenesis assay to demonstrate that nitric oxide inhibited VEGF-induced capillary growth and tube formation. We also documented that the effects of VEGF were enhanced in diabetic eNOS-knockout mice, as evidenced by increased macrophage infiltration and abnormal angiogenesis compared with diabetic wild-type mice.[59,76] although VEGF is deleterious in this model, VEGF is known to exert beneficial effects in many chronic and acute kidney diseases.[78,79] Such benefits could perhaps occur through nitric-oxide-dependent pathways, in which VEGF is able to induce the expression of endothelial nitric oxide to protect the kidney. By contrast, because eNOS is not available in this model,

VEGF could engage nitric-oxide-independent pathways and cause excessive endothelial proliferation or macrophage infiltration that might account for some of the deleterious effects of VEGF on the endothelium.[58]

Nitric oxide is also known to negatively regulate the release of von Willebrand factor and P-selectin by inhibiting exocytosis of Weibel–Palade bodies in endothelial cells;[80] lack of eNOS results in the accelerated release of Weibel–Palade bodies in the mouse kidney.[81] This finding might account for the occasional observation of fibrin deposits in the glomeruli of patients with diabetic nephropathy.[82]

Therapeutic effects of RAS inhibition

Multiple vascular complications are present in 20% of patients with diabetes.[83] In particular, observations that diabetic retinopathy and nephropathy are closely associated with one another[84,85] have fostered the assumption that treatment of one complication could be also beneficial to the other. Yet, only a limited number of large-scale clinical trials have attempted to systematically address the therapeutic efficacy of simultaneously treating both diabetic retinopathy and nephropathy. Of note is the Diabetes Control and Complications Trial, which examined whether intensive control of blood glucose can reduce retinopathy and nephropathy in patients with type 1 diabetes.[86] another example is the UK Prospective Diabetes Study, which examined the longitudinal effects of blood glucose control or lowering of blood pressure on diabetic complications in patients with type 2 diabetes. Importantly, these studies demonstrated that treatments were effective in the prevention of both nephro pathy and retinopathy in their early stages regardless of diabetes type.[87]

Several large clinical trials have reported that inhibition of the renin–angiotensin system (RAS) slows the progression of renal disease in diabetes; however, the effect on diabetic retinopathy was not examined in these studies.[88–90] In turn, clinical studies from 2000 and 2007, respectively, examined the effects of RAS inhibitors on both diabetic nephropathy and retino pathy and have documented that the beneficial effect of RAS inhibition was observed only in diabetic nephro pathy and not in diabetic retinopathy.[91,92] For instance, the MICRO-HOPE study[91] documented that ramipril might have some beneficial effects on diabetic nephro pathy, but not on diabetic retinopathy. Similarly, the ADVANCE study showed that perindopril, when combined with indapamide, reduced the risk of new onset of micro-albuminuria, but had no effect on retino pathy in patients with type 2 diabetes.[92] By contrast, a 2009 study performed by Mauer *et al.*[93] documented an opposite outcome. The study included patients with type 1 diabetes and preclinical glomerulo pathy, and examined the effects of RAS-inhibitor treatment (over 5 years) on renal morphology and retino pathy.[93] Interestingly, in contrast to the results of the aforementioned studies,[91,92] this study documented that RAS inhibition blocked the progression of diabetic retino pathy, but not glomerulo pathy.[93] likewise, the DIRECT study reported that candesartan prevented retinopathy to some degree,[94,95] but did not prevent the development of microalbuminuria in normo-tensive patients with either type 1 or 2 diabetes.[96] Given these contradictory findings, controversy still exists as to whether RAS inhibitors are useful for the treatment of both diabetic nephropathy and retinopathy.

RaS blockade is considered a key treatment for diabetic nephropathy based on evidence that this approach could provide additional protective effects beyond that of lowering blood pressure.[90,97] However, lowering blood pressure in patients with diabetes seems to be difficult.[98] Target blood-pressure levels can only be achieved in 33% of patients with diabetes and overt nephropathy even with maximum doses of angiotensin-converting-enzyme (ACE) inhibitors or angiotensin-receptor blockers.[99] One potential reason for the difficulty in controlling blood pressure in patients with diabetes is that clinicians tend to

manage blood pressure less aggressively in patients with diabetes than in those without,[98] possibly owing to concerns about adverse effects, such as hyperkalemia or transient reductions in renal function. another potential explanation could be the development of 'aldosterone breakthrough'. This phenomenon occurs in approximately 40% of patients with diabetes who are treated with drugs that block the RaS.[100,101] Mehdi *et al.*[102] reported in 2009 that the addition of an aldosterone-receptor blocker to the treatment regimen of patients with diabetes who failed to respond to a maximum dose of an ACE inhibitor substantially reduced albuminuria.[102] However, the important question of why a sizable portion of patients with diabetic nephropathy exhibit a reduced response to RAS inhibitors and develop aldosterone breakthrough remains unanswered.

RAS inhibition and endothelial dysfunction

Brachial artery reactivity and flow-mediated vasodilatation, both of which are often used to evaluate endothelial function in clinical studies, are reduced in patients with diabetes.[103–105] In particular, african americans exhibit impaired brachial artery vasodilatation[106] and respond poorly to RAS blockade,[107–109] which suggests that the poor response of patients with diabetes to RAS inhibitors may be associated with endothelial dysfunction. Schalkwijk and colleagues[105] reported that ACE-inhibitor treatment for 5 weeks did not improve flow-mediated vasodilatation in patients with type 1 diabetes, but that this treatment effectively reduced blood pressure. Furthermore, Jawa *et al.*[110] compared the effects of ACE-inhibitor treatment in African Americans with type 2 diabetes who had persistent microalbuminuria or in whom microalbuminuria had resolved, and found that the poor response of patients with persistent micro-albuminuria to ACE-inhibitor therapy was associated with severely impaired vascular reactivity.

The precise mechanisms for the reduced effectiveness of ACE inhibitors in patients with diabetes remain unclear; however, endothelial dysfunction presumably contributes to aldosterone breakthrough. In general, aldosterone is predominantly produced in the adrenal grand where its synthesis is regulated by nitric oxide. Indeed, a lack of endothelial nitric oxide accelerates aldosterone synthesis in the human adrenal grand.[111] In addition, nitric oxide deficiency enhances the vaso-constrictive effects of aldosterone in renal afferent arterioles,[112,113] which could contribute to renal disease progression. In support of this hypothesis, Ikeda *et al.*[114] have demonstrated that chronic inhibition of nitric oxide increased plasma concentrations of aldosterone, which mediated renal inflammation and fibrosis in rats. Conversely, nitric oxide inhibits the synthesis of aldo sterone in glomerulosa cells from bovine adrenal gland.[115] Thus, endothelial dysfunction could have a key role in inducing aldosterone breakthrough.

Over the past 2 years, the aldosterone–mineralocorticoid receptor system has been found to exist in the rat kidney and retina, and has been postulated to have a causal role in diabetic nephropathy and retinopathy. In the kidney, podocytes have been found to produce aldosterone, and this production is further enhanced in response to high levels of glucose to cause podocyte apoptosis in the diabetic rat kidney.[116] In support of these findings, Wilkinson-Berka *et al.*[117] demonstrated that blocking the mineralocorticoid receptor prevented retinal angiogenesis and attenuated the inflammatory response in a rat model of oxygen-induced retinopathy that has features of prematurity in humans. Hence, blocking the aldosterone system could be a novel therapeutic target in diabetic retinopathy and nephropathy.

RAS blockade in diabetic eNos-knockout mice

The response of diabetic eNOS-knockout mice to anti-hypertensive agents seems to be similar to that observed in patients with diabetes. We found that hydralazine markedly

lowered systemic blood pressure and prevented the progression of diabetic glomerular injury as well as retinopathy in this experimental mouse model.[21] By contrast, and in line with some of the findings from studies of ACE inhibition in humans with diabetes, the ACE inhibitor enalapril blocked retinopathy but not nephro pathy in diabetic eNOS-knockout mice (T. nakagawa, unpublished work). Blood pressure was only trans iently reduced by enalapril (10 mg/kg per day) in the first 2 weeks, with blood pressure levels later returning to levels similar to those in untreated diabetic eNOS-knockout mice after this time.[118] likewise, enalapril had only minor effects in reducing urinary albumin excretion in diabetic eNOS-knockout mice although the same treatments markedly reduced renal disease in diabetic wild-type mice.[118] Of note, enalapril had no effect in preventing mesangial expansion and reducing extracellular matrix deposition in diabetic eNOS-knockout mice. One could argue that the refractoriness of blood pressure and glomerular injury in diabetic eNOS-knockout mice to these ACE inhibitors could be due to the low dose used. However, support for this explanation is limited, since an excessive dose of enalapril (up to 50 mg/kg per day) also failed to lower blood pressure and had no effect on glomerular injury.[118] An intriguing finding is that ACE-inhibitor treatment blocked the forma tion of the acellular capillaries in the retina of diabetic eNOS-knockout mice despite having no effect on blood pressure (T. nakagawa, unpublished work). Hence, the precise mechanisms by which diabetic nephropathy and retinopathy develop could differ slightly, although eNOS deficiency or endothelial dysfunction might still contribute to the development of both lesions in an indivi dual with diabetes.

Of interest, the diabetic eNOS-knockout mice that received ACE-inhibitor therapy probably developed aldosterone breakthrough since the treatment did not suppress but, rather paradoxically, increased serum aldosterone concentration.[118] In support of this hypothesis, the aldosterone antagonist spironolactone substantially lowered blood pressure and reduced renal injury in this model of diabetic nephropathy.[118] The fact that diabetic wild-type mice did not show such paradoxical response to RAS inhibition supports the notion that endothelial dysfunction is involved in the pathogenesis of aldosterone breakthrough (Figure 4). A possible pathological role of the retinal aldosterone system in retinal vasculopathy has also been implicated;[119] however, its involvement in the pathogenesis of retinopathy in diabetic eNOS-knockout mice has not been studied.

Conclusions

Diabetic eNOS-knockout mice have several disease manifestations that resemble those of human diabetic nephropathy and retinopathy. In particular, this model displays advanced lesions that are rarely seen in other animal models of diabetes. Data from experiments using diabetic eNOS-knockout mice indicate that endothelial nitric oxide deficiency or endothelial dysfunction could contribute to advanced nephropathy and retinopathy in patients with diabetes. agents that stimulate endothelial function, such as statins and agonists of peroxi-some proliferator-activated receptors[120] might benefit patients with diabetes in part because of these actions. More importantly, we suggest that new therapies specifically aimed at increasing nitric oxide levels within the endothelium are developed to determine whether such agents provide additional benefit in treating diabetic nephropathy and retinopathy.

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Key points

- Not all patients with diabetes mellitus develop advanced diabetic nephropathy
- Similar to some patients with diabetes, animal models of the disease usually develop only early manifestations of diabetic nephropathy (such as mesangial expansion) and nephropathy (such as pericyte ghost formation)
- Severe endothelial dysfunction might be required for the development of advanced diabetic nephropathy in humans as well as animals
- Not all patients with diabetic nephropathy benefit from treatment with inhibitors of the renin-angiotensin system (RAS)
- The effects of RAS inhibitors on diabetic nephropathy and retinopathy might differ
- Failure of RAS inhibitors to prevent the progression of diabetic nephropathy might be accounted for by endothelial dysfunction

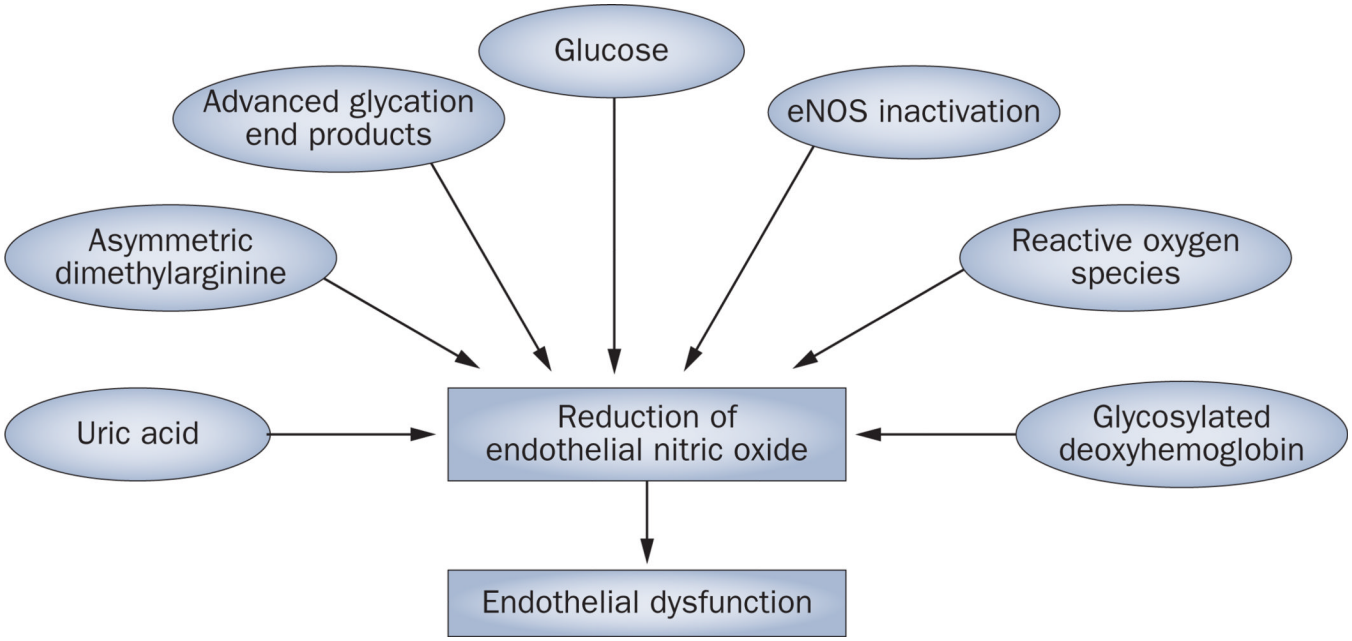

Figure 1.
Factors that contribute to the development of endothelial dysfunction in patients with diabetes. Factors including reactive oxygen species, eNOS inactivation and uric acid contribute to a reduction in the levels of nitric oxide in the endothelium, which in turn leads to endothelial dysfunction. Abbreviation: eNOS, endothelial nitric oxide synthase.

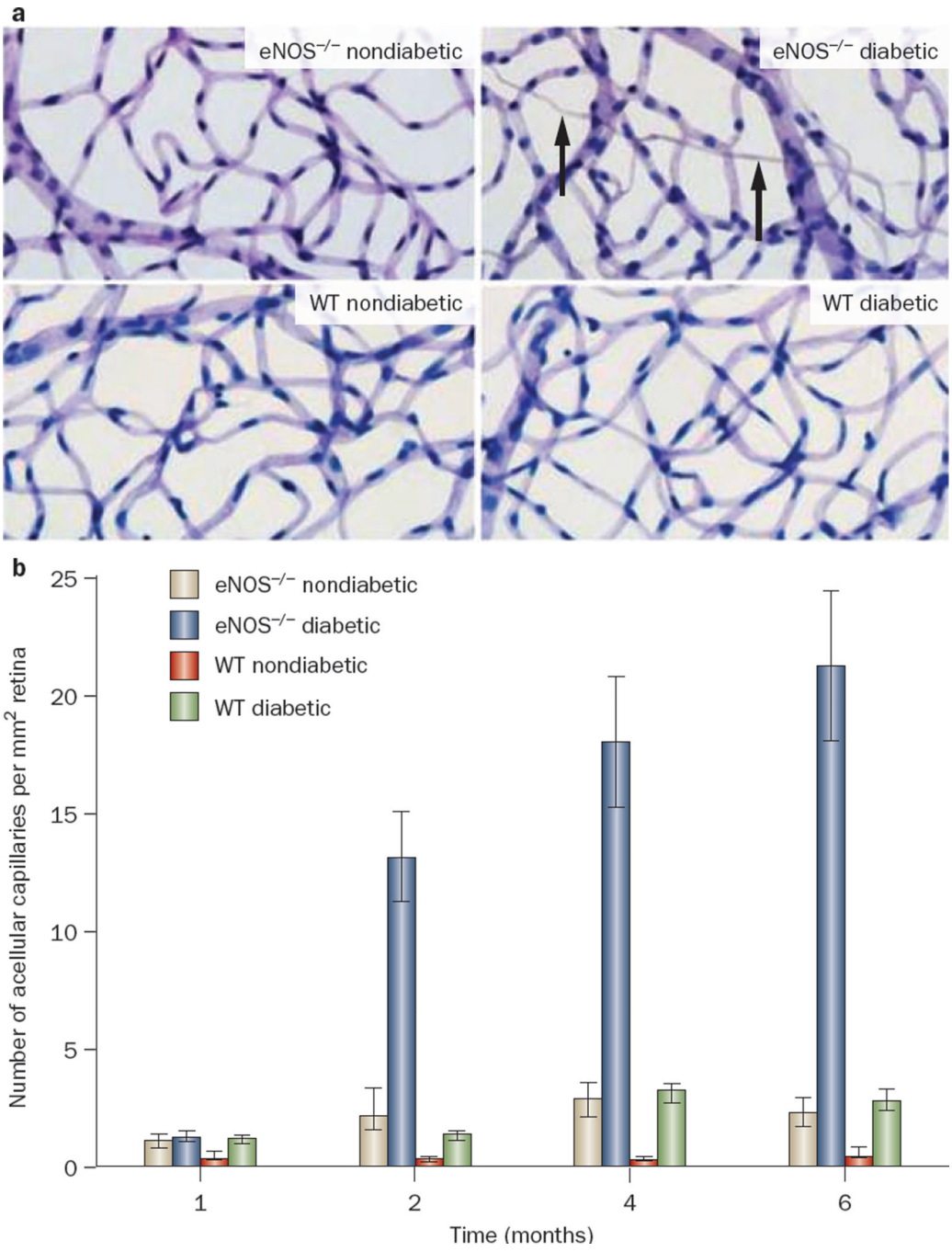

Figure 2.

Development of acellular capillaries in the retina of diabetic eNOS-knockout mice. **a** | Representative images of retinal vessels from nondiabetic wild-type (WT nondiabetic) and diabetic wild-type (WT diabetic) mice 6 months after onset of diabetes treatment, and nondiabetic eNOS-knockout (eNOS$^{-/-}$ nondiabetic) and diabetic eNOS-knockout (eNOS$^{-/-}$ diabetic) mice 4 months after onset of diabetes. The arrows indicate the presence of acellular capillaries in diabetic eNOS-knockout mice. **b** | Quantitative measurements of acellular capillaries in the various mice. Abbreviations: eNOS, endothelial nitric oxide synthase; W T,

wild-type C57BL/6 mice. Permission obtained from Investigative Ophthalmology & visual Science © Li, Q. *et al. Invest. Ophthalmol. Vis. Sci.* 51, 5240–5246 (2010).

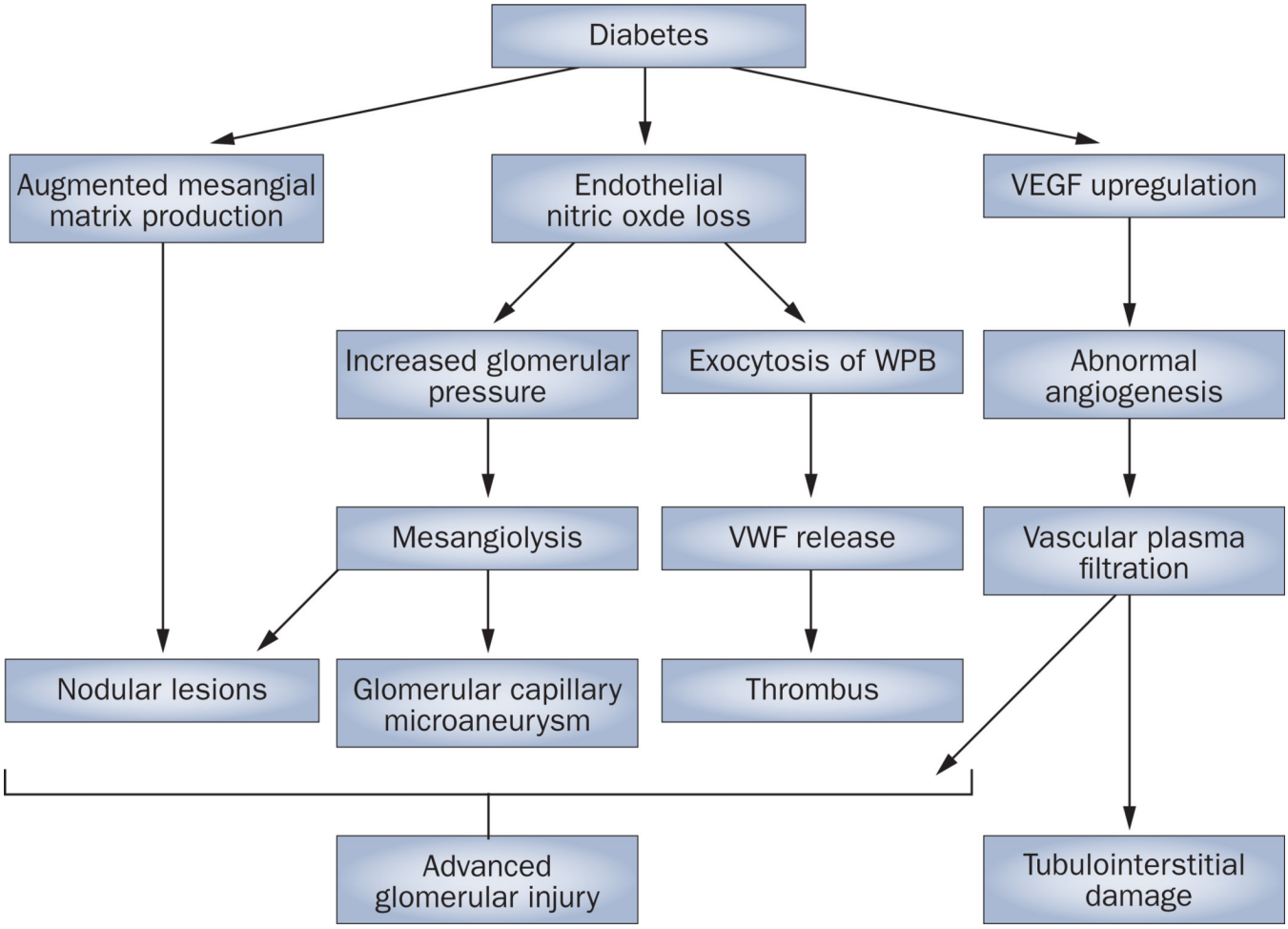

Figure 3.

Potential mechanisms by which endothelial nitric oxide deficiency causes advanced renal injury in diabetes. A number of mechanisms contribute to the development of diabetic nephropathy in patients with diabetes. Changes to the production of the mesangial matrix, loss of endothelial nitric oxide and upregulation of VEGF ultimately lead to advanced glomerular injury and tubulointerstitial damage. Abbreviations: VEGF, vascular endothelial growth factor; VWF, von Willebrand factor; WPB, Weibel–Palade bodies.

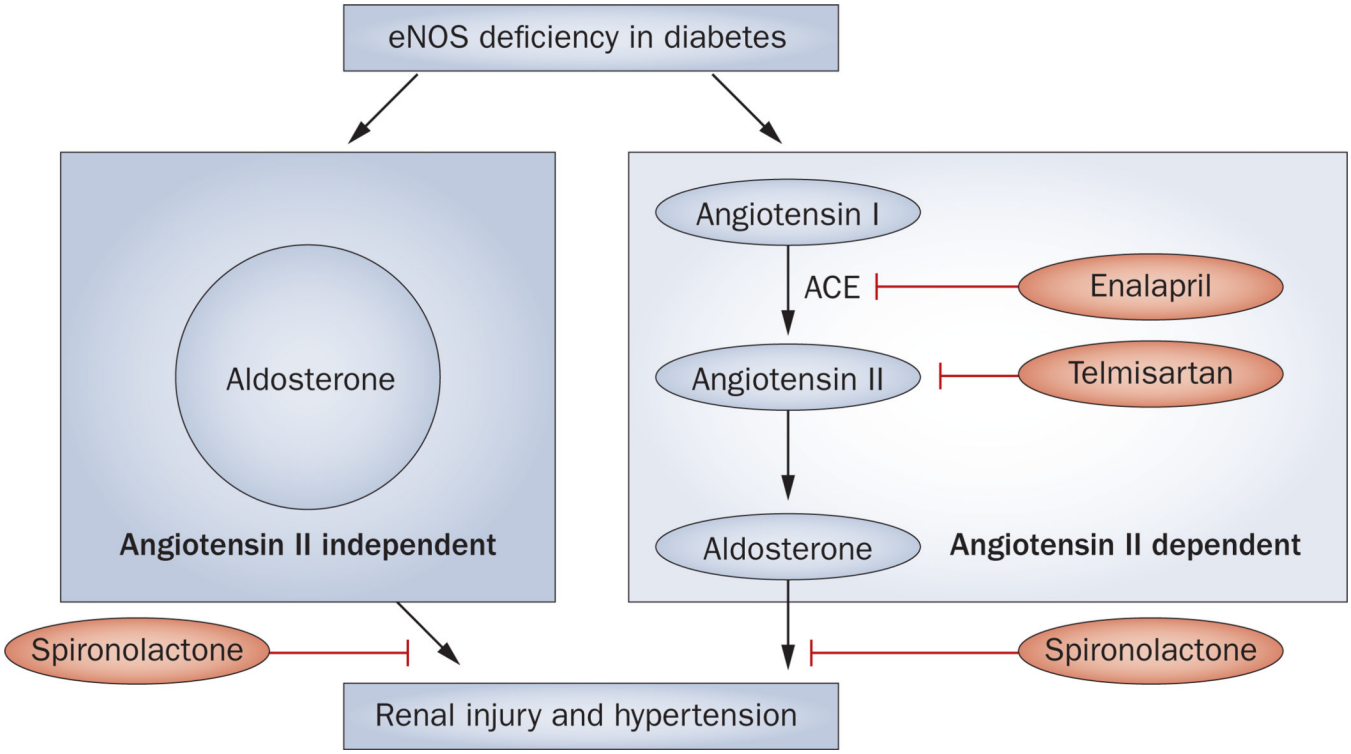

Figure 4.
Proposed mechanism by which aldosterone causes renal injury and hypertension in diabetes independent of angiotensin II. According to data from studies in eNOS-knockout mouse, eNOS deficiency in combination with diabetes leads to angiotensin-II-independent increases in aldosterone levels, which in turn contributes to renal injury and hypertension. The aldosterone antagonist spironolactone is able to prevent renal injury and hypertension by blocking angiotensin-II-independent and angiotensin-II-dependent aldosterone action while enalapril (an ACE inhibitor) and telmisartan (an angiotensin-receptor blocker) are able to prevent angiotensin-II-dependent renal injury and hypertension. Abbreviations: ACE, angiotensin-converting enzyme; eNOS, endothelial nitric oxide synthase.