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Pathophysiology of diabetic nephropathy

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Abstract
Glomerular basement membrane thickening is common in patients with long-standing diabetes, with or without nephropathy. In the following period, there is a correlation between glomerular basement membrane thickness and fractional mesangial volumes and albuminuria. The mechanisms underlying the formation of albuminuria begin with expansion of the glomerular basement membrane (GBM) with deposition of type IV collagen and reduction of negatively charged heparin sulfate proteoglycan. One of the earliest cellular changes in diabetes is mesangial and tubular cell hypertrophy. Hyperglycemia causes hypertrophy in the kidney by stimulating growth factors such as insulin-like growth factor-1 (IGF-1), epidermal growth factor (EGF), platelet-derived growth factor (PDGF), VEGF, TGF-β and Ang II. However, antagonizing TGF-β in diabetic patients did not provide beneficial effects on renal function or proteinuria. Pathological features of diabetic nephropathy (DN) are mesangial expansion, nodular diabetic glomerulosclerosis (acellular Kimmelstiel-Wilson lesion), and diffuse glomerulosclerosis. Kidney structure is heterogeneous in diabetic patients; Only a subset of patients have typical diabetic glomerulopathy, whereas others have tubulointerstitial and vascular lesions that are much more advanced than glomerular lesions. There are features suggestive of normal or near-normal kidney structure and even glomerular ischemia. Treatment of diabetic animals with an ACE inhibitor attenuates p27 Kip1 and reduces renal hypertrophy.

Keywords: *Diabetic nephropathy, hyperglycemia, pathophysiology*

1. Introduction

Diabetic nephropathy is the leading cause of end-stage renal disease (ESRD) in the industrialized World.[1] Diabetic nephropathy is not only a significant burden on quality of life, but also predicts a poor prognosis despite advances in the medical treatment of renal and cardiovascular diseases.[2]

The pathophysiology of diabetic nephropathy is complex. Intrarenal hemodynamic abnormalities are thought to be the most important factors responsible for the initiation and progression of diabetic nephropathy.[3] Hyperglycemia is a necessary prerequisite, but genetic predisposition is also crucial for the development of diabetic nephropathy.[4] Genetic factors may directly affect the development of diabetic nephropathy or may be clustered with genes that affect cardiovascular disease.[1]

1.1. Pathology of kidney disease in diabetes

After the start of diabetes, kidney weight increases by an average of 15%. Early structural changes in the kidney are kidney enlargement involving both tubules and glomeruli.[5] This process includes predominantly hypertrophy and to a much lesser extent hyperplasia.[1] The kidney continues to increase in size until overt nephropathy occurs. Most diabetic patients have a sustained increase in glomerular volume and glomerular capillary lumen volume. These changes are accompanied by interstitium hypertrophy. Glomerular basement membrane thickening is common in patients with long-standing diabetes, whether or not they have nephropathy. In the following period, there is a correlation between glomerular basement membrane thickness and fractional mesangial volumes and albuminuria.[6]

The mechanisms underlying the occurrence of albuminuria begin with the expansion of the glomerular basement membrane (GBM) with accumulation of type IV collagen and reduction in negatively charged heparin sulfate proteoglycan. Expression of nephrin, a protein that controls permeability, is abnormally low in diabetic kidney disease.[7] Its transcription is suppressed by Angiotensin II (Ang II) and restored by renin-angiotensin system (RAS) blockers. Apoptosis of podocytes is triggered by several factors, including Ang II and TGF-β, and adhesion of podocytes to GBM is reduced by suppression of neuropilin-1 from enhanced glycation end products (AGEs). Podocyte loss also follows hyperglycemia-induced reactive oxygen species (ROS) generation, resulting in podocyte apoptosis or detachment. The generation of ROS in podocytes can be largely mediated by Nox4.[8,9] Migration of podocytes is also attenuated by reduction of neuropilin-1, thereby preventing surviving podocytes from covering bare areas of the GBM, which promotes the development of focal segmental glomerulosclerosis (FSGS).[6]

One of the earliest cellular changes in diabetes is mesangial and tubular cell hypertrophy. Experimentally, avoiding hyperglycemia prevents kidney hypertrophy. Hyperglycemia causes hypertrophy in the kidney by stimulating growth factors including insulin like growth factor-1 (IGF-1), epidermal growth factor (EGF), platelet-derived growth factor (PDGF), VEGF, TGF-β and Ang II. Hyperglycemia also induces thrombospondin, a potent activator of latent TGF-β. However, antagonizing

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TGF-β in diabetic patients did not provide beneficial effects on kidney function or proteinuria.[10]

The pathological features of diabetic nephropathy (DN) are mesangial enlargement, nodular diabetic glomerulosclerosis (cellless Kimmelstiel-Wilson lesion) and diffuse glomerulosclerosis. Mesangiolysis probably plays an important role in cell loss and nodule formation. Increasing evidence suggests that local NO deficiency contributes to these histological lesions, particularly nodule formation.6 Nodular glomerular intercapillary lesions in advanced diabetic kidney disease are well-circumscribed eosinophilic and periodic acid-Schiff-positive masses in the central regions of peripheral glomerular lobules. When non-acellular, nodules contain pycnotic nuclei. It has been suggested that the nodules result from microaneurysmal dilatation of the associated capillaries, followed by mesangilysis and laminar organization of mesangial debris by lysis of the center of the lobule. Foamy cells usually surround the nodules. It is reported in 10% to 50% of biopsy specimens.[6]

Diffuse glomerular lesions are more common than nodular lesions. They consist of an increase in the mesangial matrix extending to contain the capillary lobes. In contrast to nodular lesions, which have little functional significance, the degree of diffuse glomerulosclerosis, and especially mesangial matrix enlargement, is associated with worsening kidney function.[11] In more severe disease, capillary wall thickening and mesangial enlargement lead to capillary constriction and hyalinization with periglomerular fibrosis. Podocytes are involved in the early stages of the course of diabetic kidney disease and an increase in foot spur width is observed with only slight increases in albuminuria. Longitudinal studies have shown a reduction in podocyte count, which is closely associated with proteinuria.[12,13]

Tubulointerstitial damage ultimately determines the rate of attrition of kidney function. In vitro studies demonstrate the pathogenic role of various diabetic substrates in promoting tubule hypertrophy, extracellular matrix (ECM) production and inflammation through multiple complex pathways leading to a chronic inflammatory infiltrate involving immune cells and macrophages.[7] Chemokines and their receptors, particularly monocyte chemotactic protein-1 (MCP-1/CCL2), RANTES/CCL5, IL-6, and tumor necrosis factor (TNF) receptors and adhesion molecules (e.g. ICAM-1), contribute to persistent inflammation that triggers a profibrotic cascade in the kidney.[7,14] Glomerular cells, TECs, macrophages/lymphocytes, and fibroblasts/myofibroblasts all lead to matrix deposition along the glomerular and tubular basement membranes and within the interstitium. Myofibroblasts support fibrosis progression in diabetic kidney disease by facilitating deposition of interstitial ECM. TNF receptors appear to be a potent biomarker for progressive kidney disease in diabetes. Th17 immune cells and their cytokine IL-17A are implicated in diabetes-mediated kidney injury and may be a promising therapeutic target.[15]

Arteriolar lesions are prominent in diabetes. The hyaline material progressively replaces the entire wall structure and contains both afferent and efferent vessels, which is highly specific for diabetes.[16]

Immunohistological examination is usually negative, but sometimes linear immunoglobulin G (IgG) can be observed in GBM. Tubulointerstitial fibrosis and tubular atrophy may be the best pathological correlations for the decline of GFR. Tubulointerstitial fibrosis and renal arteriolosclerosis are common.[6]

In fact, the kidney structure in diabetic patients is heterogeneous; only a subset of patients have typical diabetic glomerulopathy, while others have tubulointerstitial and vascular lesions that are much more advanced than glomerular lesions. There are features suggestive of normal or near-normal kidney structure and even glomerular ischemia.[16]

Treatment of diabetic animals with an ACE inhibitor attenuates p27 Kip1 and reduces renal hypertrophy. Inactivation of p27 Kip1 in mice, which made diabetic by streptozotocin (STZ), results in less diabetic nephropathy.[17] Although cell cycle arrest is necessary, it may not be sufficient for the development of hypertrophy. Complementary signals that increase RNA and protein synthesis are needed for cell growth. In the early stages of diabetes, kidney cells and especially mesangial cells activate the mammalian target of rapamycin (mTOR).[18,19] mTORC1 mainly acts on two important downstream targets: eukaryotic translation initiation factor 4E-binding protein1 (4EBP1) and serine/threonine protein kinase p70S6 kinase 1 (S6K1). Phosphorylation of 4EBP1 leads to the release of eIF4E and ultimately initiates cap-dependent translation of mRNAs. On the other hand, S6K1 phosphorylates the 40S ribosomal protein S6 and plays a role in the regulation of cellular and organ hypertrophy.[20] In fact, inhibition of the mTOR pathway or knockdown of S6K1 by rapamycin reduces renal hypertrophy in rodent models of diabetes.[19,21] In addition, rapamycin treatment can reduce basement membrane thickening, mesangial matrix deposition, as well as renal inflammatory markers, resulting in significantly less albuminuria.[22]

1.2. Renin-angiotensin-aldosterone system

Angiotensinogen synthesized by the liver enters the circulation, where angiotensin I is formed by renin, a peptidase secreted from the juxtaglomerular apparatus (JGA) of the kidney. Angiotensin I is exposed to angiotensin-converting enzyme, where its terminal two amino acids are removed and converted to angiotensin II. Angiotensin II, the main effector molecule of RAS, then binds to its type 1 receptor (AT1R), causing vasoconstriction, sodium retention, thirst, and aldosterone secretion.[23]

Based on animal models of 5/6 nephrectomy and diabetic glomerulosclerosis, it is plausible that disruption of the RAAS cascade could result in renoprotection. In animal models, while causing hemodynamic damage through efferent arteriolar vasoconstriction, the use of ACE inhibitors and angiotensin receptor blockers (ARBs) effectively widens the efferent arteriole, leading to glomerular relaxation followed by reduction of glomerular hypertrophy and damage. RAAS blockade has also been shown to impair the inflammatory and fibrosing effects of various cytokines, including TGF-β, in systemic hypertension, which further reduces glomerular hypertension. Thus, the net effect of RAAS antagonism is renoprotective at multiple levels.[24]

Furthermore, the ability of tubule succinate to induce JGA renin secretion suggests that this phenomenon is likely an important determinant of JGA function in both physiological and pathophysiological settings. For example, high succinate has been detected in both plasma and urine in diabetic rats.[25]

1.3. Podocytes

Podocytes (or visceral epithelial cells) are specialized cells of neuroepithelial origin that surround the glomerular capillaries. Long projections of podocytes surround the capillaries, and between these projections is a slit diaphragm. It has been determined that there are many proteins in these foot protrusions that surround the capillaries. Nephrin is a protein that plays a functional role in the structure of the slit diaphragm.[26] There is growing evidence to suggest that nephrine may play an important role in glomerular filtration and the development of proteinuria. In diabetes, premature flattening and retraction of the foot ridges are associated with thickening of the glomerular basement membrane. Indeed, there is a decrease in the number of filtration slits in diabetic rats with a decrease in nephrin expression.[26,27]

In diabetic nephropathy, there is a marked reduction in the number of anionic groups such as sialic acid and heparan sulfate proteoglycans (HSPG) in the glomerular basement membrane, which has been associated with a loss of charge permeability selectivity. The loss of permeability selectivity is most likely due to changes in podocyte function and structure. Podocyte dysfunction plays an important role in the pathogenesis of various progressive nephropathies, including diabetic kidney disease.[13,28]

Albumin uptake has been demonstrated in human, rat and mouse podocytes in vivo and in mouse and human podocytes in vitro.[29] Accumulation of endocytous protein in podocytes is also demonstrated by podocyte vacuolization in proteinuric patients, and endocytic uptake of tracer proteins in podocytes has been demonstrated experimentally in vivo. The albumin-binding receptor megalin has also been identified in rat podocytes and more recently in human podocytes and provides a mechanism for endocytic uptake of albumin and other proteins.[30,31] It has been suggested that if the filtered proteins are not removed, they will clog the glomerular filter due to the podocyte cleft membrane. Such a theoretical occlusion of the slit diaphragm can be reduced by megalin, endocytosis of proteins with podocyte. It should be emphasized that the endocytic uptake of albumin in podocytes is minimal compared to that in the proximal tubule.[30]

1.4. Transforming growth factor-1

Excessive accumulation of extracellular protein production (ECM) in the kidney is thought to play an increasingly important role in the development and progression of diabetic nephropathy. It has been shown to play a key role in mediating the profibrotic effect of various pathological stimuli such as transforming growth factor-β (TGF-β), hyperglycemia, and angiotensin II; many of these functions are developed in the diabetic environment, leading to excessive ECM deposition in the diabetic kidney.[26] TGF-β stimulates the synthesis of the extracellular matrix, including type I collagen, type IV collagen, fibronectin and laminin.[1] TGF-β also upregulates another growth factor, connective tissue growth factor (CTGF), through various signaling pathways involving specific molecules known as Smads.[26]

Studies in animal models of both type 1 and type 2 diabetes further reveal TGF-β as an important mediator of diabetic kidney disease. TGF-β1 mRNA and protein levels are increased both glomerularly and tubularly in diabetic rats and mice.[32]

In almost all renal cell types (except podocytes) studied in tissue culture, high ambient glucose has been shown to increase the expression and bioactivity of TGF-β; these include proximal tubular epithelial cells, glomerular mesangial and endothelial cells, and interstitial fibroblasts. High glucose also increases TGF-β type II receptor in all kidney cells studied, including podocytes.[33,34]

Blocking the activity of the renal TGF-β system in diabetic animals provided evidence that the development of kidney disease is due to overactivity of this system in diabetes. Neutralizing monoclonal antibodies to TGF-β prevent glomerular hypertrophy and reduce the increase in TGF-β1, type IV collagen and fibronectin mRNAs in STZ-induced diabetes mellitus in mice. Furthermore, antibody treatment in the db/db mouse, a model of type 2 diabetes, prevents mesangial matrix expansion without affecting albuminuria. This last finding suggests that unlike hypertrophic and prosclerotic renal manifestations of diabetes, diabetic proteinuria is mediated by increased podocyte-derived VEGF instead of TGF-β.[35,36]

1.5. Oxidative stress and diabetic renal damage

Hyperglycemia causes vascular injury through complex overlapping pathways, including formation of enhanced glycation end products (AGE), activation of protein kinase C (PKC), and production of ROS. There is increasing evidence to suggest that ROS may play an important role in the initiation and progression of diabetic nephropathy.[23] The effects of antioxidant therapy have been demonstrated in animal studies, but convincing evidence for clinical efficacy is still lacking.

Excessive ROS production via Nicotinamide adenine dinucleotide phosphate (NADPH) oxidase (Nox) has played a role in the pathogenesis of diabetic nephropathy. Diabetes-induced translocation of PKC, particularly PKC-a, to renal membranes has been associated with increased NADPH-induced superoxide production and high concentrations of vascular endothelial growth factor (VEGF) in kidney, serum, and urine. reduced albuminuria and glomerulosclerosis, which increase with diabetes.[24] In both diabetic rodents and AGE-treated mesangial cells, increased VEGF as well as blockade of Nox or PKC-α reduced cytosolic superoxide formation and PKC activation. Moreover, renal extracellular matrix accumulation of fibronectin and collagen IV was reduced by the Nox inhibitor aposine. Therefore, inhibition of Nox is likely to provide a new therapeutic target for diabetic nephropathy.

Ethical approval

This study does not require approval from the Ethics Committee for Animal Experiments.

Conflict of interest

There are no conflicts of interest associated with this research publication, according to the authors.

Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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This situation does not exist.

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References

1. Chen S, Khoury C, Ziyadeh FN. Pathophysiology and pathogenesis of diabetic nephropathy. *Seldin and Giebisch's The Kidney: Physiology and Pathophysiology*. 2012:2605-2632. doi:10.1016/B978-0-12-381462-3.00078-1
2. Hemmelgarn BR, Manns BJ, Lloyd A, et al. Relation between kidney function, proteinuria, and adverse outcomes. *JAMA*. 2010;303(5):423-429. doi:10.1001/JAMA.2010.39
3. Li B, Yao J, Kawamura K, et al. Real-time observation of glomerular hemodynamic changes in diabetic rats: Effects of insulin and ARB. *Kidney Int*. 2004;66.
4. Freedman BI, Bostrom M, Daeihagh P, Bowden DW. Genetic factors in diabetic nephropathy. *Depth Review Clin J Am Soc Nephrol*. 2007;2:1306-1316. doi:10.2215/CJN.02560607
5. Fioretto P, Steffes MW, Brown DM, Mauer SM. An Overview of Renal Pathology in Insulin-dependent diabetes mellitus in relationship to altered glomerular hemodynamics. *American Journal of Kidney Diseases*. 1992;20(6):549-558. doi:10.1016/S0272-6386(12)70217-2
6. Tang S, Sharma K. *Pathogenesis, Clinical Manifestations, and Natural History of Diabetic Kidney Disease*. (Feehally J, Floege J, Tonelli M, Johnson RJ, eds.).; 2018.
7. Reidy K, Kang HM, Hostetter T, Susztak K. Molecular mechanisms of diabetic kidney disease. *J Clin Invest*. 2014;124(6):2333-2340. doi:10.1172/JCI72271
8. Sharma K, RamachandraRao S, Qiu G, et al. Adiponectin regulates albuminuria and podocyte function in mice. *J Clin Invest*. 2008;118(5):1645-1656. doi:10.1172/JCI32691
9. Jha JC, Thallas-Bonke V, Banal C, et al. Podocyte-specific Nox4 deletion affords renoprotection in a mouse model of diabetic nephropathy. Diabetologia. 2016;59(2):379-89. doi: 10.1007/s00125-015-3796-0
10. Voelker J, Berg PH, Sheetz M, et al. Anti-TGF-b1 antibody therapy in patients with diabetic nephropathy. *J Am Soc Nephrol. 2017;28(3):953-962. doi: 10.1681/ASN.2015111230*
11. Fioretto P, Steffes MW, Sutherland DER, Mauer M. Sequential renal biopsies in insulin-dependent diabetic patients: Structural factors associated with clinical progression. *Kidney Int*. 1995;48(6):1929-1935. doi:10.1038/KI.1995.493
12. Marshall SM. The podocyte: A major player in the development of diabetic nephropathy? *Hormone and Metabolic Research*. 2005;37(SUPPL. 1):9-16. doi:10.1055/S-2005-861397/ID/19/BIB
13. Wolf G, Chen S, Ziyadeh FN. Perspectives in diabetes from the periphery of the glomerular capillary wall toward the center of disease podocyte ınjury comes of age in diabetic nephropathy. Diabetes, 2005;54:1626-1634.
14. W Tang SC, Han Yiu W. Innate immunity in diabetic kidney disease. Nat Rev Nephrol, 2020:206-222. doi:10.1038/s41581-019-0234-4
15. Lavoz C, Sánchez Matus Y, Orejudo M, et al. Interleukin-17A blockade reduces albuminuria and kidney injury in an accelerated model of diabetic nephropathy. Kidney Int. 2019;95(6):1418-1432. doi:10.1016/j.kint.2018.12.031
16. Cohen Tervaert TW, Mooyaart AL, Amann K, et al. Pathologic classification of diabetic nephropathy. J Am Soc Nephrol. 2010;21(4):556-563. doi:10.1681/ASN.2010010010
17. Wolf G, Schroeder R, Zahner G, Stahl RAK, Shankland SJ. High glucose-induced hypertrophy of mesangial cells requires p27Kip1, an inhibitor of cyclin-dependent kinases. *Am J Pathol*. 2001;158(3):1091. doi:10.1016/S0002-9440(10)64056-4
18. Sataranatarajan K, Mariappan MM, Myung JL, et al. Regulation of elongation phase of mRNA translation in diabetic nephropathy: Amelioration by rapamycin. *Am J Pathol*. 2007;171(6):1733. doi:10.2353/AJPATH.2007.070412
19. Mori H, Inoki K, Masutani K, et al. The mTOR pathway is highly activated in diabetic nephropathy and rapamycin has a strong therapeutic potential. *Biochem Biophys Res Commun*. 2009;384(4):471-475. doi:10.1016/j.bbrc.2009.04.136
20. Choo AY, Blenis J. Cell Cycle Not all substrates are treated equally: Implications for mTOR, rapamycin-resistance, and cancer therapy. *Cell Cycle*. 2009;8(4):567-572. doi:10.4161/cc.8.4.7659
21. Chen JK, Chen J, Thomas G, Kozma SC, Harris RC. S6 kinase 1 knockout inhibits uninephrectomy- or diabetes-induced renal hypertrophy. *Am J Physiol Renal Physiol*. 2009;297(3):585-593. doi:10.1152/ajprenal.00186.2009
22. Yang Y, Wang J, Qin L, et al. Rapamycin prevents early steps of the development of diabetic nephropathy in rats. *Am J Nephrol*. 2007;27(5):495-502. doi:10.1159/000106782
23. Gilbert RE, et al. Vasoactive molecules and the kidney. 2016:325-353. Doi: 10.1016/B978-1-4160-6193-9.10012-0
24. Tangri N, Stevens LA, Griffith J, et al. A predictive model for progression of chronic kidney disease to kidney failure. *JAMA*. 2011;305(15):1553-1559. doi:10.1001/JAMA.2011.451
25. Vargas SL, Toma I, Jung JK, Meer EJ, Peti-Peterdi J. Activation of the succinate receptor GPR91 in macula densa cells causes renin release. *J Am Soc Nephrol*. 2009;20(5):1002. doi:10.1681/ASN.2008070740
26. Cao Z, Cooper ME. Pathogenesis of diabetic nephropathy. Journal of Diabetes Investigation, 2011;2:243-247. doi:10.1111/j.2040-1124.2011.00131.x

27. Bonnet F, Cooper ME, Kawachi H, Allen TJ, Boner G, Cao Z. Irbesartan normalises the deficiency in glomerular nephrin expression in a model of diabetes and hypertension. *Diabetologia*. 2001;44(7):874-877. doi:10.1007/S001250100546

28. Pavenstädt H, Kriz W, Kretzler M. Cell biology of the glomerular podocyte. *Physiol Rev*. 2003;83(1):253-307. doi:10.1152/PHYSREV.00020.2002

29. Eyre J, Ioannou K, Grubb BD, et al. Statin-sensitive endocytosis of albumin by glomerular podocytes. *Am J Physiol Renal Physiol*. 2007;292(2):674-681. doi:10.1152/AJPRENAL.00272.2006

30. EI Christensen RNHB. Renal filtration, transport, and metabolism of albumin and albuminuria, in Seldin and Giebisch's The Kidney. 2013.

31. Smithies O. Why the kidney glomerulus does not clog: A gel permeation/diffusion hypothesis of renal function. *Proc Natl Acad Sci U S A*. 2003;100(7):4108-4113. doi:10.1073/PNAS.0730776100

32. Sharma K, Ziyadeh FN. Renal hypertrophy is associated with upregulation of TGF-beta 1 gene expression in diabetic BB rat and NOD mouse. *Am J Physiol*. 1994;267(6 Pt 2):F1094-F1101. doi:10.1152/AJPRENAL.1994.267.6.F1094

33. Ziyadeh FN, Hoffman BB, Han DC, et al. Long-term prevention of renal insufficiency, excess matrix gene expression, and glomerular mesangial matrix expansion by treatment with monoclonal antitransforming growth factor-β antibody in db/db diabetic mice. *Proc Natl Acad Sci U S A*. 2000;97(14):8015-8020. doi:10.1073/PNAS.120055097

34. Ziyadeh FN. Mediators of diabetic renal disease: The case for TGF-as the major mediator. *J Am Soc Nephrol*. 2004;15 Suppl 1:S55-S57. doi:10.1097/01.ASN.0000093460.24823.5B

35. Chen S, Jim B, Ziyadeh FN. Podocyte-derived vascular endothelial growth factor mediates the stimulation of 3(IV) collagen production by transforming growth factor-1 in mouse podocytes. *Diabetes*. 2004;53(11):2939-2949. doi: 10.2337/diabetes.53.11.2939.

36. Flyvbjerg A, Dagnaes-Hansen F, Frieda B, Schrijvers F, Tilton R. Amelioration of long-term renal changes in obese type 2 diabetic mice by a neutralizing vascular endothelial growth factor antibody. *Diabetes*. 2002;51(10):3090-3094. doi: 10.2337/diabetes.51.10.3090.