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Kidney growth, hypertrophy and the unifying mechanism of diabetic complications

Review Article

J. Satriano

Division of Nephrology-Hypertension, Department of Medicine, The Veterans Administration San Diego Healthcare System, University of California San Diego, San Diego, CA, USA and The Stein Institute for Research on Aging, University of California, San Diego, CA, USA

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Summary. Michael Brownlee has proposed a 'Unifying Mechanism' of hyperglycemia-induced damage in diabetes mellitus. At the crux of this hypothesis is the generation of reactive oxygen species (ROS), and their impact on glycolytic pathways.

Diabetes is the leading cause of chronic kidney failure. In the early phase of diabetes, prior to establishment of proteinuria or fibrosis, comes kidney growth and hyperfiltration. This early growth phase consists of an early period of hyperplasia followed by hypertrophy. Hypertrophy also contributes to cellular oxidative stress, and may precede the ROS perturbation of glycolytic pathways described in the Brownlee proposal. This increase in growth promotes hyperfiltration, and along with the hypertrophic phenotype appears required for hyperglycemia-induced cell damage and the progression of downstream diabetic complications. Here we will evaluate this growth phenomenon in the context of diabetes mellitus.

Keywords: Type I diabetes – Hyperfiltration – Tubuloglomerular feedback – Reactive oxygen species – Polyamines – Hypertrophy

Abbreviations: AGE, advanced glycation end products; Ang II, angiotensin II; APR, absolute proximal reabsorption; 4E-BP1, eIF-4E binding protein-1; BrdU, 5-bromodeoxyuridine; CKI, cyclin dependent kinase inhibitor; DAG, diacylglycerol; DFMO, difluoromethylornithine; ECM, extracellular matrix; EGF, epidermal growth factor; eIF-4E, eukaryotic initiation factor 4E; EMT, epithelial-to-mesenchymal transition; ERK1,2, extracellular signal-regulated protein kinases; ESRD, end-stage renal disease; FGF, fibroblast growth factor; FPR, fractional proximal reabsorption; GAPDH, glyceraldehyde-3 phosphate dehydrogenase; GFR, glomerular filtration rate; HGF, hepatocyte growth factor; IGF-1, insulin-like growth factor; MAPK, mitogen-activated protein kinases; MnSOD, manganese superoxide dismutase; mTOR, mammalian target of rapamycin; NF-κB, nuclear factor kappa B; ODC, ornithine decarboxylase; p27, $p27^{KIP1}$; p27+/+, $p27^{KIP1}$ wild type mice; p27−/−, $p27^{KIP1}$ deficient mice; PAI-1, plasminogen activator inhibitor-1; PCNA, proliferating cell nuclear antigen; PDGF, platelet-derived growth factor; PKC, protein kinase C; RAGE, receptor of advanced glycation end products; ROS, reactive oxygen species; STZ, streptozotocin; TGF-β, transforming growth factor-beta; TGF, tubuloglomerular feedback; UCP-1, uncoupling protein-1; VEGF, vascular endothelial growth factor

Introduction

Diabetes is one of the fastest growing pandemics affecting our world today. It has increased from an estimated 30 million people in 1985 to 194 million by 2003, and is expected to grow to approximately 350 million by 2025 (King et al., 1998). In the United States alone, one in three born in the year 2000 is projected to develop diabetes within their lifetime (Narayan et al., 2003). In 2007, diabetes will cause 3.5 million deaths worldwide.

In the past two decades the cases of end-stage renal disease (ESRD) in the United States have nearly quadrupled. A primary cause of ESRD is diabetic nephropathy. The increasing incidence of diabetes and ESRD constitute a large and growing component of the patient load globally. However, the mechanisms responsible for the onset and progression of diabetes have not been fully resolved.

Brownlee's "unifying mechanism" of diabetes mellitus

Subsets of cells, including the mesangial cells of the glomerulus, are particularly subject to the tissue damaging effects of hyperglycemia. This is attributed to the inability to effectively down-regulate glucose transporters in a hyperglycemic environment of diabetes (Haneda et al., 2003).

At least four pathways are associated with glucose mediated cellular damage. The first mechanism discovered in 1966 is the increased flux through the polyol pathway (Fig. 1) (Gabbay et al., 1966). Glucose is reduced to

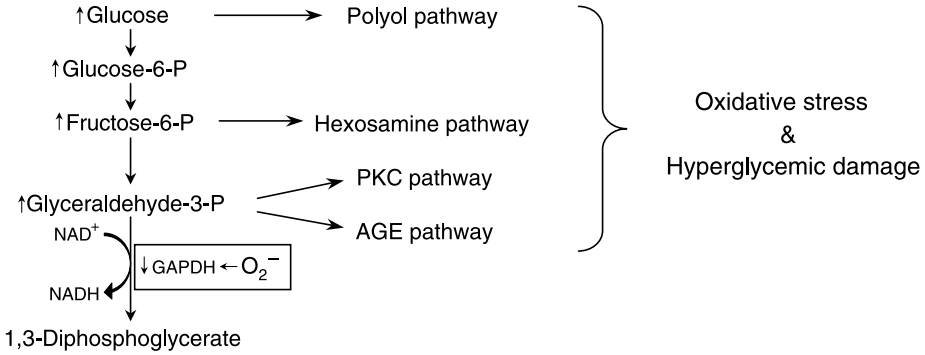

Fig. 1. Brownlee's 'Unifying Mechanism' of diabetes. Hyperglycemia causes excess intracellular glucose levels in specific cell types. Superoxide inhibition of GAPDH suppresses glycolytic metabolism causing glycolytic metabolites to divert into pathways of glucose over-utilization. The resulting four shunt pathways observed in diabetes give rise to oxidative stress and promote the progression of diabetic complications (adapted from Brownlee, 2001)

sorbitol in this pathway, at the expense of consuming NADPH. High glucose levels can deplete NADPH levels and thus impair the regeneration of the intracellular antioxidant, reduced glutathione.

In the Hexosamine pathway, fructose-6 phosphate is converted into glucosamine-6 phosphate, and finally into uridine diphosphate N-acetyl glucosamine. The latter product can modify transcription factors such as Sp1 into increasing expression of transforming growth factor-β (TGF-β) and plasminogen activator inhibitor-1 (PAI-1) (Du et al., 2000; Kolm-Litty et al., 1998).

Both the protein kinase C (PKC) and the advanced glycation end products (AGE) pathways can be derived from glyceraldehyde-3 phosphate (Fig. 1). Induction of PKC can have a number of effects, including the up regulation of TGF-β and PAI-1, and increasing expression of proinflammatory cytokines by activation of NF-κB. PKC will be briefly discussed later in this review.

AGE formation can produce adverse effects through modification of intracellular and extracellular proteins, and extracellular matrix (ECM). The AGE receptor, RAGE, is up-regulated in diabetes. Activated RAGE induces TGF-β (Yan et al., 2003), acts as an endothelial adhesion receptor for leukocyte recruitment (Chavakis et al., 2003), and activates NF-κB (Yan et al., 2003).

The common feature of these four diabetes related pathways is increased oxidative stress, with an increase in mitochondrial reactive oxygen species (ROS) formation (Brownlee, 2001). As can be seen in Fig. 1, ROS impedes the glycolytic pathway by inhibiting the enzyme glyceraldehyde-3 phosphate dehydrogenase (GAPDH) and thus the conversion of glyceraldehyde-3 phosphate into 1,3 diphosphoglycerate. High glucose results in an overabundance not only in glucose, but also its metabolites glucose-6 phosphate, fructose-6 phosphate and glyceraldehyde-3 phosphate. The accumulation of these glycolytic metabolites results in a shunting into the polyol, hexosamine, PKC and AGE pathways (Fig. 1). Inhibition of GAPDH alone by antisense DNA technology results in an elevation of each of the four pathways to the same extent observed with hyperglycemia (Du et al., 2003).

Is mitochondrial ROS responsible for the effects seen on these pathways? Attenuation of ROS through overexpression of either manganese superoxide dismutase (MnSOD), to directly reduce ROS, or uncoupling protein-1 (UCP-1), to collapse the mitochondrial proton gradient and prevent generation of ROS, reduces high glucose activation of these pathways (Brownlee, 2001). Furthermore, Houstis et al. (2006) recently demonstrated a causal role of ROS in both cellular and in vivo models of insulin resistance. Taken together, these data clearly demonstrate that ROS plays a vital role in the progression of these diabetic pathways.

Kidney growth and hyperfiltration

At the onset of diabetes, before complications have set in, there is an increase in kidney size and glomerular filtration rate (GFR) (Rasch and Norgaard, 1983). The escalation in GFR results in hyperfiltration and is the central tenant for eventual glomerular damage and later downstream complications (O'Bryan and Hostetter, 1997). Proteinuria, azotemia, tubulointerstitial fibrosis and eventual ESRD do not occur until many years later.

Abnormalities in glomerular vascular control have been purported to lead to vasodilation and hyperfiltration in early diabetes. In this scenario it is thought that kidney growth is a compensatory response to the increased load imparted by hyperfiltration. Thus, increased tubule growth would allow an increase in reabsorption (movement of salt and fluids from the lumen back into the plasma com-

ponent) capacity to prevent salt and fluid losses that could result from the increased load.

An alternative view is that increased kidney growth, primarily in the proximal tubule, disrupts the tubuloglomerular feedback (TGF) system. As discussed further below, an abnormal increase in salt reabsorption due to this growth can establish a negative cascade where the feedback system signals to increase GFR rather than reduce it. In this scenario, growth is primary to hyperfiltration, and not a consequence of it.

Kidney GFR and tubuloglomerular feedback (TGF)

The nephron comprises the functional unit of the kidney. Blood is filtered in the glomerulus and the filtrate is passed along a tubular system, which again makes contact with its own glomerulus (Fig. 2A). It is at this point where specialized, macula densa cells sense the salt concentration within the distal tubule and signal for an inverse change in GFR. That is, high salt passing in the distal tubule will signal for a decrease in GFR, whereas low salt will elicit an increase in the GFR of that nephron (Fig. 2B). Thus a feedback loop within each nephron allows fine regulation of fluid and electrolyte delivery to the downstream nephron segments in response to GFR and reabsorption upstream in the tubule system (Vallon, 2003).

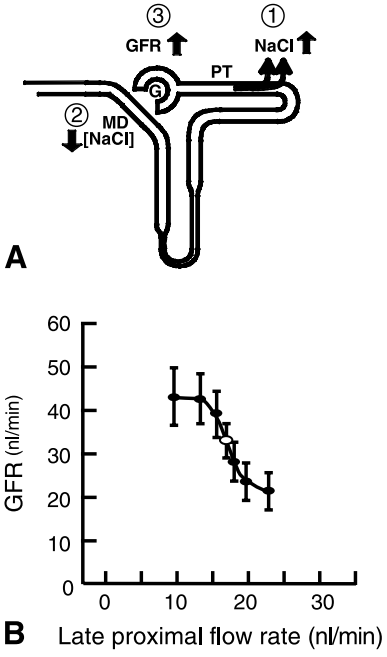

Fig. 2. Early kidney growth and hyperfiltration in diabetes. **A** A schematic of a nephron with *G* glomerulus, *PT* proximal tubule, and *MD* macula densa of the distal tubule. (*1*) Diabetes increases the size and salt reabsorption of the proximal tubule away from the lumen and back into the plasma. (*2*) The salt delivered to the macula densa decreases due to the increased salt reabsorbed at the proximal tubule. (*3*) Decreased macula densa salt signals the TGF system to increase single nephron GFR. Loss of salt and fluid due to abnormal reabsorption at the proximal tubule also increases the hydrostatic pressure gradient between the glomerulus and the proximal tubule. GFR would increase in an attempt to normalize this gradient. These mechanisms which increase GFR in an environment of high GFR contributes to diabetes-induced glomerular hyperfiltration (adapted from Vallon, 2003). **B** The TGF function describes the inverse relationship between single nephron filtration rate (SNGFR, or GFR presented here) and late proximal tubular flow rate. The latter correlates directly with the concentrations of Na^+, K^+ and Cl^- at the macula densa, i.e., high flow = high salt. Ambient values are depicted as an open circle (adapted from Vallon et al., 1995)

Salt and fluid reabsorption increase in the proximal tubule in rats with experimental type 1 diabetes (Thomson et al., 2001; Vallon et al., 1995) as well as in early type 1 diabetes in humans (Vervoort et al., 2005). The absolute proximal reabsorption (APR) of salt increases in diabetes, as does the fractional proximal reabsorption (FPR), which is the APR normalized for GFR. In normal, non-diabetic rats increasing GFR would correspondingly increase APR, but the FPR would decrease. Thus, the increase in FPR in the proximal tubules of diabetic rats demonstrates that the rise salt reabsorption is not a consequence of glomerular hyperfiltration. The increase in salt reabsorbed out of the tubule and back to the plasma results in a low salt signal to the macula densa cells. This in turn elicits a signal to increase the GFR of the nephron in an environment where the GFR is already too high. In addition, growth dependent increases in reabsorption, i.e., loss of salt and fluid from the lumen of the tubule, will decrease the hydrostatic pressure within the proximal tubule. This will result in an increase in the gradient of hydrostatic pressures between the glomerulus and the proximal tubule. To normalize this gradient the nephron will increase GFR.

In summary, diabetic kidney growth elicits an increase in GFR via TGF and hydrostatic pressure gradients thereby creating a negative cascade of increasing GFR. This negative cascade results in progressive hyperfiltration. A review article by Thomson et al. (2004) discusses the topic of TGF in diabetes in detail.

Diabetic kidney growth

Diabetic kidney growth was the focus of a previous review (Satriano and Vallon, 2006). Due to its relevance to the current topic, it will be briefly overviewed here.

A number of growth factors are associated with early diabetes. The most studied of these include including insulin-like growth factor (IGF-1), hepatocyte growth factor (HGF), platelet-derived growth factor (PDGF), fibroblast growth factor (FGF), epidermal growth factor (EGF), vascular endothelial growth factor (VEGF), and diacylglycerol

(DAG). Inhibition of these growth factors demonstrated beneficial effects in models of diabetes, and all induce ornithine decarboxylase (ODC) activity, the first and rate-limiting enzyme of polyamine biosynthesis. ODC mRNA and protein expression increase in the first few days of disease in a model of type I diabetes (Deng et al., 2003). Enzyme activity also increases during this period, and is completely inhibited with difluoromethylornithine (DFMO), a selective inhibitor of ODC (Thomson et al., 2001). Indeed, recent studies establish a strong link between growth factor expression and ODC activity in diabetes (Deng et al., 2003; Pedersen et al., 1992; Thomson et al., 2001).

Growth of the diabetic kidney is associated primarily with proximal tubule hypertrophy. However, growth is biphasic with a period of hyperplasia preceding hypertrophy (Huang and Preisig, 2000; Rasch and Norgaard, 1983). High glucose treatment of a kidney mesangial cell line stimulates an early cell proliferative phase (24–48 h), and a later growth inhibitory phase (72–96 h) (Wolf et al., 1992). 5-bromodeoxyuridine (BrdU) staining, a marker of S-phase DNA synthesis, in animals administered streptozotocin (STZ) demonstrated high incorporation at day 3, but normalized BrdU incorporation by day 7 (Deng et al., 2003). DFMO completely inhibited this increase in day 3 BrdU staining. Thus, the very early growth phase is dependent upon ODC activity. STZ is a naturally occurring chemical that is selectively toxic to the insulin producing beta cells of the pancreas. It is used to produce an animal model of type I, or insulin dependent, diabetes mellitus. Because disease begins with the administration of the drug, type I diabetes is often the animal model of choice in delineating the events associated with the early stages of disease initiation and progression.

The kidney demonstrates constitutive ODC activity principally in the proximal tubule (Levillain and Hus-Citharel, 1998). This activity is increased markedly with steroids (Pass et al., 1982). In STZ diabetic rats, ODC expression appears primarily in the distal, not proximal, tubules of the kidney cortex (Deng et al., 2003). This leads to an interesting hypothesis of polyamines acting as paracrine factors produced in the distal tubule and transferred to the proximal tubules for growth in the diabetic kidney. It is not known why the proximal tubules do not increase ODC expression in this model.

As would be anticipated from these studies, DFMO inhibition of ODC produces beneficial effects in the early stages of diabetes (Pedersen et al., 1992, 1993). Unfortunately, long-term administration is ineffective in preventing downstream complications (Pedersen et al., 1995). Compensatory polyamine uptake in DFMO treated animals (Bogle et al., 1994), which can substitute for *de novo* polyamine biosynthesis (Seiler and Dezeure, 1990), likely explains the lack of a positive, long-term DFMO effect.

ODC activity and kidney physiology at the onset of diabetes

DFMO inhibition of ODC decreases growth of the diabetic kidney (Pedersen et al., 1993). Importantly, DFMO also decreases GFR in diabetic rats 7 days after STZ induction of diabetes (Thomson et al., 2001). This decrease in GFR correlates with the decrease in kidney growth in diabetic rats administered DFMO (Fig. 3A). Increases in APR and FPR observed with diabetes are also decreased

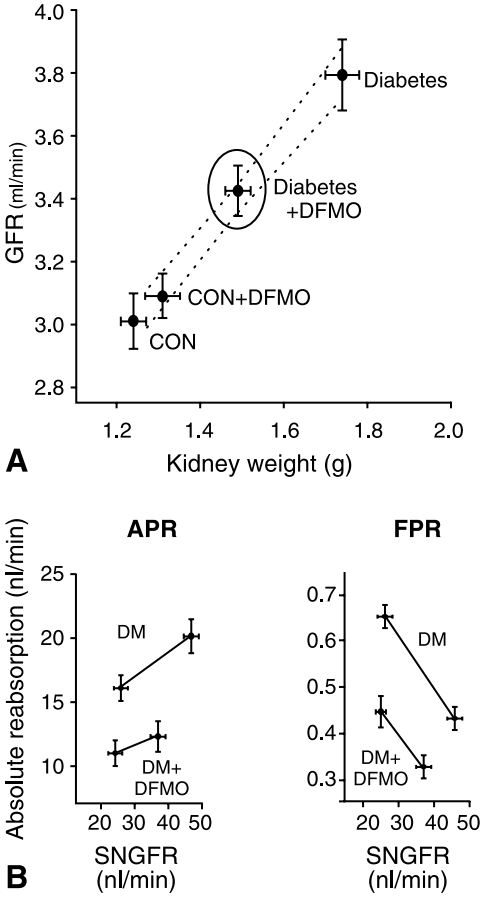

Fig. 3. Kidney growth correlates with GFR in early diabetic rats. **A** STZ diabetes (Diabetes) causes an increase in kidney weight and GFR relative to untreated animals (CON). DFMO, a selective ODC inhibitor, suppresses kidney growth in 7-day STZ-diabetic rats (Diabetes + DFMO; within circle), relative to STZ diabetic controls. Reduction of kidney weight (growth) in DFMO treated diabetic animals corresponds to a reduction in GFR. GFR is for two kidneys. Kidney weight is wet-weight of left kidney. Dashed lines are 95% confidence intervals for linear regression. $r^2 = 0.996$. **B** Absolute proximal reabsorption (APR) and fractional proximal reabsorption (FPR) are increased in diabetes. Suppression of kidney growth abrogates these increases (adapted from Thomson et al., 2001)

in DFMO diabetic rats (Fig. 3B). These data demonstrate the impact of diabetic tubular growth on increasing GFR, and thus hyperfiltration and kidney function at the onset of diabetes. Reduction of APR and FPR of diabetic rats administered DFMO reveals a reduction of the abnormally high proximal tubule reabsorption is associated with suppressed tubule growth. Reduced proximal tubule hyperreabsorption would normalize the TGF response leading to a reduction in GFR. Thus the tubular hypothesis of diabetic hyperfiltration is set into motion by the induction of ODC and aberrant tubular growth.

Diabetic hypertrophy

Is growth alone sufficient to cause the negative cascade in TGF leading to hyperfiltration, glomerular damage and the downstream complications of diabetes? Other models of kidney growth, including unilateral nephrectomy (Humphreys et al., 1988), high protein diet (Kaysen et al., 1989) and steroids (Bettuzzi et al., 2001) all increase kidney ODC activity and growth. However, none result in the long-term complications associated with diabetes. Thus, other factors related to diabetic growth must be involved to explain this disparity. Factors that appear important contributors to the progression of the early kidney hypertrophic process to the late, irreversible changes include protein kinase C (PKC), TGF-β, oxidative stress and eventual apoptosis. In addition to the effects on tubular reabsorption and hyperfiltration, suppressing early growth in diabetes would diminish the oxidative response leaving the cells with higher protective thiol pools and less susceptible to progressive oxidative effects (Wassef et al., 2004).

An important pathway contributing to diabetic complications is PKC. PKC-β expression is associated with the proximal tubule and its activation up regulated in response to STZ diabetes (Pfaff and Vallon, 2002). Pharmacologic inhibition of PKC suppresses diabetic parameters from early glomerular hyperfiltration to late structural damage (Koya et al., 2000; Tuttle and Anderson, 2003). One downstream effector of PKC activation is increased ODC activity (Hsieh et al., 1989), which plays a vital role in early diabetic kidney growth. Another effector molecule of PKC is TGF-β (Koya et al., 1997). TGF-β is an important factor in diabetes and its expression is up regulated along with its type II receptor in both type I and type II diabetes (Ziyadeh et al., 2000). TGF-β contributes to ECM production (Ziyadeh et al., 1994), and suppresses degradation of ECM through inhibition of matrix metalloproteinases. Interestingly, cultured tubule cells of TGF-β knock-out mice not only demonstrate reduced levels of the ECM protein fibronectin, but do not display hypertrophy in response to high glucose (Chen et al., 2004). The effect on hypertrophy may be explained by TGF-β induction of $p27^{KIP1}$ (p27) expression and promotion of cell cycle arrest (Kamesaki et al., 1998).

Early hypertrophy is thought to occur at around day 4 in STZ diabetes with a transition from the hyperplastic growth phase to growth arrest (Huang and Preisig, 2000). This concurs with the time frame of hyperplasia we observe using BrdU incorporation (Deng et al., 2003). Induction of the cyclin dependent kinase inhibitor (CKI) p27 is a key factor in this growth arrest (Monkawa et al., 2002; Wolf et al., 2001). Cells stimulated to divide yet arrested in the G1 phase of the cell cycle by CKI gives rise to hypertrophic cells (Fig. 4).

High glucose activation of mitogen-activated protein kinases (MAPK) extracellular signal-regulated protein kinases (ERK1,2) phosphorylate p27, stabilizing the protein and increasing its expression (Wolf et al., 2003b). Angiotensin II (Ang II) is another factor up regulated in

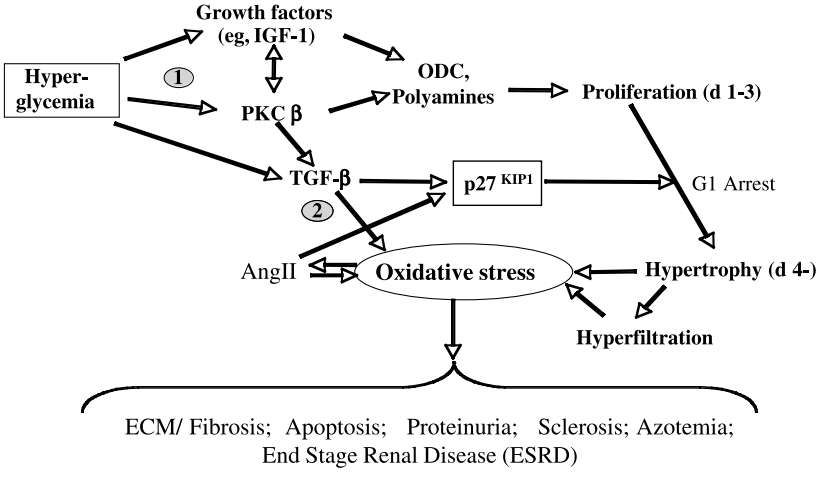

Fig. 4. Components of early diabetic kidney growth. (*1*) Diabetic hyperglycemia induction of growth factors leads to early proliferation. (*2*) TGF-β/$p27^{KIP1}$ induction in diabetes occurs within a few days of onset. Angiotensin II (AngII) and other factors also contribute to $p27^{KIP1}$ induction, which mediates a G1 arrest that initiates the switch from hyperplasia to hypertrophy, as well as contributing to the ROS/oxidative stress burden of the cell. TGF-β and ROS also suppresses ODC activity and induces the formation of extracellular matrix (ECM) and fibrosis

diabetes. High glucose induced Ang II generation is dependent upon reactive oxygen species (ROS) (Hsieh et al., 2002), and both Ang II and high glucose can further enhance ROS and activation of PKB and associated pathways in glomerular podocytes (Kim et al., 2006). NADPH oxidase appears to mediate these effects as antisense oligonucleotides for Nox4 reduced NADPH oxidase dependent ROS generation, kidney hypertrophy and fibronectin generation in STZ diabetic rats (Gorin et al., 2005). Ang II plays a role in mediating proteinuria and increased protein reabsorption that contributes to tubular inflammation and fibrosis (Wolf et al., 2003a).

Thus, hypertrophic cells are associated with high mitochondrial oxygen consumption and ROS production via NADPH oxidase. ROS increases phosphorylation of 4E binding protein-1 (4E-BP1), an inhibitor of eukaryotic initiation factor 4E (eIF-4E) (Feliers et al., 2006). The translation factor eIF-4E increases ODC (Shantz et al., 1996) and VEGF (Feliers et al., 2005) expression. Phosphorylation of 4E-BP1 increases in diabetic p27+/+ mice (Awazu et al., 2003) and in mesangial cells at 5 days exposure to high glucose. Phosphorylation inhibits 4E-BP1 binding to eIF-4E, which activates eIF-4E and increases ODC expression. Phosphorylation of 4E-BP1 decreases in diabetic p27−/− mice, relative to diabetic p27+/+ animals (Awazu et al., 2003), which should decrease free eIF-4E and thus ODC and VEGF expression. ROS generation and increased oxidative stress could also result in the nitrosylation and inhibition of ODC activity (Satriano et al., 1999), thereby suppressing growth. How these factors affect ODC temporally in disease models has not yet been evaluated. We do know the ODC is transiently and markedly increased in very early STZ diabetes (Deng et al., 2003; Pedersen et al., 1992). The resultant oxidative stress from ROS production can also induce p27 expression, and thus hypertrophy (Hannken et al., 1998). Therefore, for the diabetic kidney we can add one more pathway to the Bownlee 'Unifying Mechanism' of diabetes; generation of hypertrophic kidney growth.

The role of the cyclin dependent kinase inhibitor $p27^{KIP1}$ and hypertrophy in diabetes

ODC dependent growth is up regulated in hyperglycemia and diabetes. The reaction to this aberrant growth is induction of CKI, including p27. How do we know that this is an important series of events in diabetes? Inhibiting of any one of the four pathways of the Brownlee 'Unifying Mechanism' hypothesis in clinical trials may provide some benefit, but does not prevent the disease. However, this hyperplastic to hypertrophic growth response occurs very early, and may impact these other, downstream pathways associated with diabetes. Two recent papers utilizing p27 deficient mice demonstrate the significance of this sequence of events.

Loss of the p27 gene does not affect diabetic hyperglycemia relative to p27 wild type (p27+/+) mice. That is, the increase in blood glucose levels are not different between the diabetic p27+/+ and the p27 knock-out (p27−/−) mice (Awazu et al., 2003; Wolf et al., 2005). To observe cell proliferation, Awazu et al. (2003) assessed proliferating cell nuclear antigen (PCNA) staining in control and diabetic conditions with both p27+/+ and p27−/− mice. PCNA increases markedly in both p27+/+ and p27−/− animals at week three after STZ administration in both the glomerular and tubulointerstitial fractions. At week 12 week these values return to close to normal levels. This correlates with the ODC activity and hyperglycemia induced growth occurring very early and tapering off.

In diabetes kidney growth outpaces body growth. An increase in kidney weight to body weight ratio is a standard measurement of aberrant, diabetic kidney growth. Three weeks after administration of STZ, kidney weight to body weight ratio increases in both diabetic p27+/+ and p27−/− mice, relative to their respective non-diabetic controls (Awazu et al., 2003). However, diabetic p27−/− mice at either 6 weeks (Wolf et al., 2005) or 12 weeks (Awazu et al., 2003) after STZ administration do not demonstrate kidney weight to body weight ratios that are different from non-diabetic control animals, even though these ratios remain elevated in diabetic p27+/+. Interestingly, early diabetic kidney growth in the p27−/− mice, unlike the p27+/+ mice, is not via hypertrophic growth. Glomerular volume (Awazu et al., 2003) and mesangial cell volume (Wolf et al., 2005), two indices of kidney hypertrophic growth, increase in diabetic p27+/+ mice, but not in p27−/− mice. At these time points kidney growth would be expected to be hypertrophic, as seen in the p27+/+ group. In vitro mesangial cell cultures from the p27+/+ and p27−/− mice confirmed the lack of hypertrophic growth in p27−/− cells grown in high glucose (Awazu et al., 2003). Thus, the disparity between these groups appears to be the conversion to a hypertrophic phenotype in the diabetic p27+/+ animals, but not in the diabetic p27−/− animals.

Does the lack of hypertrophic growth in diabetic p27−/− animals translate into beneficial effects relative to the p27+/+ animals? Albuminuria, a parameter of diabetic nephropathy, is determined by urine albumin excretion. In studies by Awazu et al. (2003) and Wolf et al. (2005), urine

albumin excretion is extensively enhanced in diabetic p27+/+ mice relative to non-diabetic controls. In contrast, urine albumin excretion in p27−/− diabetic mice is significantly reduced from the p27+/+ levels. In addition, Wolf et al. (2005) demonstrate intermediate results with heterozygous, p27+/−, animals. Abrogation of albuminuria shows a beneficial effect in this functional parameter in diabetic p27−/− mice. Moreover, morphologic parameters of the glomerulosclerosis index, tubulointerstitial index, and vascular damage index are significantly lower in p27−/− mice compared to the levels observed in p27+/+ mice, relative to their respective non-diabetic controls (Wolf et al., 2005). This evidence places p27 as a decisive factor of renal injury in diabetic nephropathy, and links these diabetic parameters with hypertrophic growth.

TGF-β induces p27 expression and also contributes to ECM production in diabetes (Fig. 4). Atypical ECM production is the precursor of diabetic fibrosis and sclerosis. Does lack of p27 affect TGF-β and/or ECM expression? As would be anticipated, TGF-β expression is up regulated in diabetes to an equal extent in both p27+/+ and p27−/− mice (Awazu et al., 2003; Wolf et al., 2005). Interestingly, expression of ECM components fibronectin (Awazu et al., 2003), laminin and collagen type IV (Wolf et al., 2005) are all reduced to near control, non-diabetic levels p27−/− animals. Another parameter in diabetes, mesangial expansion, represents the earliest morphological change in diabetic nephropathy. Mesangial expansion is due to an increase in ECM deposition and glomerular cell hypertrophy. An increase in the mesangial matrix is an indicator of fibrosis and eventual sclerosis. Whereas the relative mesangial area, the calculated mesangial area normalized by glomerular area, increases 270% in diabetic p27+/+ over non-diabetic controls, the increase in p27−/− mice is only 60% (Awazu et al., 2003). It is not clear why there is no change in TGF-β, yet ECM production and mesangial expansion are decreased. Perhaps there is an effect on TGF-β receptor expression. This has not yet been evaluated. Another possibility is potential cell cycle independent functions of p27. P27 can reduce activity of the RhoA pathway (Besson et al., 2004). RhoA activity increases in p27−/− cells, resulting in a reduction of cellular migration via increases in stress fiber formation and focal adhesions (Besson et al., 2004). Recently, the concept of epithelial-to-mesenchymal transition (EMT) as a cause of fibrosis has been promoted (Kalluri and Neilson, 2003). In this scenario EMT plays a role in the development of fibrosis in various pathologies, including diabetic nephropathy. A distinctive feature of EMT is the migration of newly formed mesenchymal fibroblast cells from the kidney tubular epithelium to the interstitial space. In the interstitium they increase ECM production. Lack of p27 could inhibit cellular migration and impair these effects. This intriguing hypothesis set forth by Wolf et al. (2005) has yet to be experimentally confirmed. ROS associated with hypertrophy, as with Brownlee's 'Unifying Mechanism', can also contribute to the excess connective tissue deposition observed in diabetes.

Conclusions

Diabetes is a global pandemic largely due to our inability to prevent or control the disease. Contemporary management of patients with diabetes often begins at an advanced stage. Treatment at this stage can slow, but not eradicate, disease progression. Defining the physiologic mechanisms at the onset of diabetes and the subsequent network of responses these mechanisms set in motion would be a considerable step forward in addressing the disease. To diagnose and treat diabetes at an early stage would lead to a better response, increased life span and increased quality of life in the patient population. This information may also yield new targets from which can evolve therapeutics for intervention at these early stages.

Evidence presented in this review points to atypical diabetic kidney growth evoking a CKI mediated hypertrophy as a primary event in the pathogenesis of diabetes. For the kidney, it is clear that this aberrant growth is a parameter that could be evaluated as a marker of early diabetes, and targeted to temper disease progression. A good candidate for both of these parameters is ODC (Fig. 4). Type I and type II diabetes at first appear as separate entities, that is, insulin dependent and insulin resistant diabetes, respectively. However, basic pathophysiologic mechanisms involved in pathogenesis and progression of the disease are similar (Parving et al., 2000). Although the window for ODC as an early marker for diabetic kidney growth may be short in experimental models of type I diabetes, duration and elevation of ODC activity has yet to be assessed in models of type II diabetes, which would have a longer onset of disease. DFMO is ineffective as a therapeutic in the STZ induced type I model of diabetes mellitus (Pedersen et al., 1995) (unpublished results), likely due to the up regulation of compensatory polyamine transport, but it would have promise if combined with an in vivo inhibitor of polyamine transport. Alternatively, translational induction of ODC antizyme, which inhibits both ODC and polyamine transport (Hayashi et al., 1996), could have beneficial effects in the early stages of the disease, prior to downstream com-

plications. Polyamine analogs are coming of age where they can begin to exploit this latter direction (Mitchell et al., 2002). Efficacy of this approach to limit diabetic growth can be seen in the ability to partially attenuate diabetic nephropathy by blocking individual growth factors such as IGF-1 (Flyvbjerg, 2001), VEGF (Flyvbjerg et al., 2002), or EGF (Wassef et al., 2004). Downstream from the growth factors, within the Akt effector pathways, is the mammalian target of rapamycin (mTOR). Recently this too has proven a beneficial target in diabetic models (Lloberas et al., 2006). ODC/polyamines are downstream from mTOR in this sequence and may prove more selective targets. Advances in ODC/polyamine transport inhibitors will provide us the tools to test these concepts.

Brownlee's 'Unifying Mechanism' hypothesis presents the overall burden of ROS as the principle factor responsible for the progression of diabetic complications (Fig. 1). The current literature further validates this concept. In the kidney, hypertrophic growth constitutes an ODC/polyamine dependent hyperplastic growth followed by a CKI dependent arrest, leading to hypertrophy. Hypertrophy of the proximal tubules and glomerular mesangial cells constitutes a response to hyperglycemia which increases oxidative stress at a very early stage of the disease (Fig. 4). Limiting early growth, and thus the early oxidative stress imposed by hypertrophy, holds the potential to impact later stages of disease progression.

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Author's address: Joseph Satriano, UCSD and VASDHS, 3350 La Jolla Village Dr. (9111H), San Diego, CA 92161, USA,
Fax: +1-858 642-1438, E-mail: jsatriano@ucsd.edu