Renal Redox Stress and Remodeling in Metabolic Syndrome, Type 2 Diabetes mellitus, and Diabetic Nephropathy: Paying Homage to the Podocyte

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Key Words

Asymmetrical dimethylarginine · Atherosclerosis · Cardiorenal metabolic syndrome · Glomerulosclerosis · Microalbuminuria · NAD(P)H oxidase · Oxidative stress · Podocyte · Reactive oxygen species

Abstract

Type 2 diabetes mellitus has reached epidemic proportions and diabetic nephropathy is the leading cause of end-stage renal disease. The metabolic syndrome constitutes a milieu conducive to tissue redox stress. This loss of redox homeostasis contributes to renal remodeling and parallels the concurrent increased vascular redox stress associated with the cardiometabolic syndrome. The multiple metabolic toxicities, redox stress and endothelial dysfunction combine to weave the complicated mosaic fabric of diabetic glomerulosclerosis and diabetic nephropathy. A better understanding may provide both the clinician and researcher tools to unravel this complicated disease process. Cellular remodeling of podocyte foot processes in the Ren-2 transgenic rat model of tissue angiotensin II overexpression (TG(mREN-2)27) and the Zucker diabetic fatty model of type 2 diabetes mellitus have been observed in preliminary studies. Importantly, angiotensin II receptor blockers have been shown to abrogate these ultrastructural

changes in the foot processes of the podocyte in preliminary studies. An integrated, global risk reduction, approach in therapy addressing the multiple metabolic abnormalities combined with attempts to reach therapeutic goals at an earlier stage could have a profound effect on the development and progressive nature to end-stage renal disease and ultimately renal replacement therapy.

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Introduction

Diabetes mellitus is the leading cause for end-stage renal disease (ESRD) in the US and abroad. In 1999, 31.8% of all registered ESRD cases in the US were attributed to diabetic nephropathy (DN) [1]. In 2001, DN accounted for 43% of new cases with ESRD in the US [2, 3]. DN develops in approximately 35% of type 1 diabetes mellitus (T1DM) and 15–20% of patients with type 2 diabetes mellitus (T2DM). At least 90% or greater of all diabetics have T2DM and in the past decade (due to the exponential growth and improved survival rates following cardiovascular events) T2DM now represents the largest number of patients with ESRD at a cost of 15.6 billion dollars per year.

ESRD in patients with T2DM may be viewed as a disease of medical progress due to the improved treatment

Fig. 1. Glomerulosclerosis: diffuse and nodular H and E photomicrographs. **a** Diffuse glomerulosclerosis. **b** Nodular glomerulosclerosis with classical KW changes described in 1936.

of hypertension and coronary heart disease. More patients with T2DM now live long enough for nephropathy and ESRD to develop [4]. A recent study demonstrated a higher incidence of DN in T2DM than in T1DM (44.4% vs. 20.0%) in a group of 17,256 early-onset Japanese diabetic patients [5]. Patients with T2DM developing ESRD requiring renal replacement therapy are increasing in all countries making this situation one of global importance [4, 6]. Therefore, it is crucial to better understand and take the necessary aggressive-preventive measures to slow this progressive disease process. Most diabetic ESRD patients die of cardiovascular disease (CVD). Thus, we need to realize that intimal redox stress, endothelial dysfunction and accelerated atherosclerosis are increased in these patients [7–9].

Diabetic Nephropathy

Diabetic nephropathy (DN) is a clinical syndrome of albuminuria, declining glomerular filtration rate, hypertension, and an increased CVD risk that is 2- to 4-fold higher in both T1DM and T2DM patients [4, 10–12]. Pathological changes consist of glomerular, renovascular, and tubulointerstitial remodeling (fig. 1, 2). The basic lesion is a pronounced thickening of the basement membrane of glomerular capillaries (GBM), arterioles, collecting tubules and tubulointerstitial fibrosis. Additionally, there are changes of inflammation with monocyte-de-

rived macrophages, as well as, mesangial cell hyperplasia and marked mesangial matrix expansion within the glomeruli. Recently, podocyte cellular remodeling of the endothelial GBM interface has garnered a great interest.

While the large majority of patients with T2DM and microalbuminuria (75–80%) have the classic DN lesions with the associated Kimmelstiel-Wilson changes, approximately 20% have nonspecific vascular (hyaline arteriosclerosis and or hyperplastic arteriosclerosis) changes associated with tubulointerstitial fibrosis and minimal or no glomerular changes. These changes are associated with pre or co-existing hypertension and ischemia due to systemic or intraglomerular hypertension and the associated macrovascular accelerated atherosclerosis (atheroscleropathy). Additionally, renal artery stenosis with associated cholesterol microembolism may play a significant role in this later process [13, 14].

This review focuses on renal redox stress and how this interacts with the metabolic toxicities of insulin resistance, impaired glucose tolerance, obesity, hypertension, and dyslipidemia (i.e. cardiometabolic syndrome or cardiorenal metabolic syndrome) to result in this unique milieu with its associated remodeling of the intra-renal vasculature, the glomerulus, and the tubulointerstitium. Patients with diabetic ESRD (80% or greater) die as a result of CVD. Therefore, it is necessary to relate the parallel processes of diabetic glomerulosclerosis and diabetic accelerated atherosclerosis of T2DM (table 1). This parallel

process is largely due to the similarly shared injurious stimuli to the endothelium, which places the endothelium in a central role of renal redox stress and remodeling (fig. 3) [7–9, 15].

Redox Stress

Oxidation of organic fuels results in the transference of electrons between oxygen species providing energy for each cell. Redox homeostasis describes the normal physiologic process of reduction and oxidation in order to repair unstable, damaging, reactive oxygen species (ROS). This homeostatic balance between ROS and antioxidant capacity is in contrast to redox stress. Redox stress (redox imbalance) implies a loss of this unique homeostasis resulting in an excess production of ROS either through the process of reduction or oxidation. There exists a reductive stress in hyperglycemic conditions of overt T2DM and prediabetes, wherein there is a reductive stress (pseudohypoxia) as NADH is greater than NAD+ [7–9]. Oxidative stress implies a loss of redox homeostasis (imbalance) with an excess of ROS by the singular process of oxidation.

It has been known for some time that ROS are detrimental and toxic to cells and tissues as a result of injury to lipids, nucleic acids, and proteins: (a) Lipid peroxidation of membranes (loss of membrane function and increased permeability) and generation of lipid autoperoxidation reactions. (b) DNA damage leading to mutation and death (apoptosis). (c) Cross-linking or vulcanization of sulfhydryl-rich proteins leading to dysfunctional signaling pathways and stiff aged proteins (specifically, collagen of the extracellular matrix) [7–9, 16]. Throughout this review, we will attempt to remain focused on the relationship between redox stress and ROS in the renal architecture, and how these two interact with the

Fig. 2. Renal glomerular remodeling: Cross-section of a glomerulus to include the mesangial capillary stalk. Transition from normal on left to DN on right. Top: Normal glomerular capillary transitioning to center: mesangial stalk to: bottom: a glomerular capillary with changes of DN and diabetic glomerulosclerosis. Mesangial cell hyperplasia (yellow), expansion of mesangial matrix (pink), thickening of glomerular basement membrane (red), loss of foot processes of the podocyte (blue-purple), podocyte atrophy (orange-red) and eventual loss (podocyturia) or apoptosis. Note the uniqueness of the mesangial cells abutting the glomerular endothelial cells without an intervening basement membrane.

Table 1. A comparison between diabetic atherosclerosis and diabetic glomerulosclerosis

Endothelial injury: both have injurious stimuli in common (fig. 3)

<i>Diabetic atherosclerosis</i>	<i>Diabetic glomerulosclerosis</i>
Arterial endothelium	Glomerular capillary endothelium
Intima	Mesangium (glomerular intima)
Monocyte-derived macrophage	Monocyte-derived macrophage
Smooth muscle cell (mesenchymal)	Mesangial cell (mesenchymal)
Pericyte	Podocyte (visceral epithelium)
<i>These mesenchymal cells lose foot processes from the endothelial cell in diabetes and develop structural remodeling, dysfunction, and loss through apoptosis</i>	
Vulnerable atherosclerotic plaque: rupture	Vulnerable glomerular: collapse
<i>Atheroscleropathy</i>	<i>Glomerulopathy</i>

Fig. 3. Arterial, glomerular intimal (mesangial) remodeling: the endothelial cell's central role. Injurious stimuli: modified LDL-cholesterol, VLDL-cholesterol (triglycerides-free fatty acids), infection and/or chronic inflammation, homocysteine, angiotensin II, hemodynamic stress, glucose, AGE-RAGE and intimal or mesangial redox stress (A-FLIGHT-U toxicities of MetS, IR, and T2DM) all contribute to the remodeling process within the intima and glomerular intima or mesangium. The endothelial cell (a sensory responder cell) directs the arterial, glomerular intima (mesangium) similar to the maestro directing an orchestra. As the glomerular and arteriolar structures remodel, the downstream ischemia and redox stress within the ECM will result in remodeling changes within the tubulo-interstitial structures. The mesenchymal smooth muscle cells of the media are comparable to the mesangial cells.

Table 2. The A-FLIGHT-U acronym
Identification of the multiple metabolic toxicities responsible for ROS generation and redox stress

A	Ang II: activates membrane bound NAD(P)H → ROS and TGF-beta Amylin: activates RAAS (Ang II) Antioxidant-oxidant imbalance Advanced glycation endproducts Advanced fructosylation endproducts Advanced lipoxidation endproducts Atherosclerotic nephropathy Accelerated atherosclerosis – atheroscleropathy Aging
F	Free fatty acid toxicity
L	Lipotoxicity
I	Insulin resistance – insulin toxicity – hyperinsulinemia
G	Glucotoxicity
H	Hypertension toxicity Homocysteine toxicity
T	Triglyceride toxicity
U	Uric acid toxicity via xanthine oxidase

multiplicative effect of the A-FLIGHT-U toxicities (table 2) of the MetS which are responsible for increased ROS production. When redox homeostasis transitions to redox stress, redox signaling ensues in all tissues and organs regardless of the multiple similar or dissimilar etiologies due to redox stress being a redox signaling system [17, 18]. An elegant discussion of the cellular oxidative processes in renal disease has recently been published for a more in-depth view of these complex mechanisms [19].

eNOS and eNO

Endothelial nitric oxide (eNO) is derived from the endothelial nitric oxide synthase (eNOS) enzyme reaction converting *L*-arginine to NO plus *L*-citrulline (fig. 4). eNO is a constitutive, locally produced, naturally occurring chain breaking antioxidant (anti-redox-

Fig. 4. The uncoupling of the eNOS reaction. Oxygen reacts with *L*-arginine and the eNOS enzyme, in which the BH₄ cofactor couples with the NAD(P)H enzyme and the reaction runs to the right, becoming a net producer of protective nitric oxide. When the eNOS reaction uncouples due to myriad causes (redox stress) (fig. 6), the eNOS reaction uncouples and becomes a net producer of the deleterious ROS – superoxide $[O_2^{\cdot-}]$.

idant) that scavenges locally produced ROS. Once conceptualized that a specific and unique gene (eNOS, iNOS, and nNOS) encodes the transcription of nitric oxide in a specific organ system, the opposing faces of this highly specialized, small, bioactive, and readily diffusible gas can be better understood [9]. The healthy endothelium is a net producer of eNO; however, in the metabolic milieu of the MetS and T2DM with endothelial dysfunction there is a net production of ROS associated with DN.

Remodeling: To Alter the Existing Structure

Remodeling is a structural rearranging-adaptive process that occurs in a long-term, chronic response to changes in hemodynamic conditions or the effects of injurious stimuli (fig. 3). The paradox is that this process may become a disease entity, in and of itself, resulting in the actual disease process of fibrotic scarring due to chronic injurious stimuli. This happens usually when there is loss of control of the remodeling process and the extracellular matrix (ECM) always seems to play a pivotal role in this maladaptive process. By correcting the loss of redox and metabolic homeostasis and decreasing the multiple injurious stimuli (A-FLIGHT-U toxicities of the MetS, T2DM and DN), clinicians and researchers may be able

to better control the remodeling changes that are so destructive to the end-organ tissues affected in the clinical syndrome of DN [7–9].

Metabolic Syndrome and Insulin Resistance

Metabolic syndrome (MetS) affects approximately 47 million plus Americans [20]. Of these 47 million, approximately 20% will develop T2DM and the remaining 80% will be able to compensate (at least for a period of time) through the process of beta cell expansion, hypertrophy, and hyperplasia (utilizing the replicative pool of periductal cells) [21, 22]. The resulting hyperinsulinemia, hyperproinsulinemia, and hyperamylinemia (37.6 million) does not come without a price to pay as this compensatory mechanism places these patients at risk for CVD [23, 24]. Insulin resistance (IR) describes the condition, whereby; there is a resistance to insulin-mediated glucose uptake by cells and is central to the clustering of multiple metabolic abnormalities and clinically the MetS (fig.5). The World Health Organization closely parallels the NCEP ATPIII guidelines; however, it includes microalbuminuria that conveys the significance of DN to the metabolic milieu of the MetS [24, 25].

Fig. 5. Major players in the metabolic syndrome. Over the past decade we have come to understand the important environmental role of obesity (specifically visceral or central obesity) and sedentary lifestyle in the MetS. Overnutrition and underexercise are thought to be major contributors to the exponential growth and current epidemic of the MetS and T2DM. The term MetS has evolved since Dr. Reaven's Banting lecture in 1988. In his original description of Syndrome X, Dr. Reaven listed a table with the heading Syndrome X and it consisted of the following:

- I Glucose intolerance.
 - II Hyperinsulinemia.
 - III The lipid triad: Increased very low-density lipoprotein – triglyceride and small dense LDL-C. Decreased high-density lipoprotein cholesterol.
 - IV Hypertension.
- Insulin resistance being central to the clustering syndrome.

Fig. 6. The multiple stressors causing uncoupling of the eNOS enzyme by decreased DDAH and the increased ADMA. Each of the stressors contribute to decreased DDAH and increased ADMA, which competes for the substrate *L*-arginine with resultant eNOS dysfunction, eNOS uncoupling, increased superoxide production, decreased endothelial nitric oxide and endothelial dysfunction.

Obesity

The obesity epidemic is of considerable importance and is closely related to the T2DM epidemic (diabetes). There is accumulating evidence that obesity contributes to glomerular injury. In an obese canine model at 8 weeks

fed a high-fat diet there is considerable glomerular remodeling changes: an increase in kidney weight by $31 \pm 7\%$, a significant increase in Bowman's space, increased mesangial matrix and thickening of glomerular and tubular basement membranes, and an increase in the number of

dividing cells within the glomerulus. These early remodeling changes associated with obesity may therefore be significantly important in the long term remodeling process of glomerulosclerosis and DN. Similar renal changes are found in the Zucker obese model of T2DM discussed later.

The A-FLIGHT-U Acronym (table 2)

The multiple metabolic A-FLIGHT-U toxicities acronym was created to enable the reader to better understand the relationship of the multiple toxicities to the development of renal redox stress, ROS and renal cellular and extracellular matrix remodeling [27–65].

For a detailed discussion of each individual component of the A-FLIGHT-U acronym, the reader is referred to references [7–9, 62–65]. Additionally, it should also be noted that the multiple toxicities found within table 2 apply to each of the multiple complicating diabetic opathies and their respective remodeling adaptations.

While an in-depth discussion of each of the A-FLIGHT-U toxicities is not within the scope of this review, a discussion of antioxidant enzymes, glucotoxicity, endothelial dysfunction-endothelial nitric oxide, proteases, hypertension, and electron micrographic observations of podocyte foot processes in an over expressing angiotensin II model (Ren-2) and the Zucker obese model were felt necessary to better understand the role of MetS, IR and T2DM in renal redox stress and remodeling in DN.

Anti-Oxidant Enzymes: Anti-Oxidant Reserve Compromised

In addition to the excess generation of the ROS seen in T2DM and the MetS, there exists an impaired generation of endogenous antioxidants. Superoxide dismutase (SOD) [66], glutathione reduced (GSH) [67], and ascorbic acid (vitamin C) [68] are all decreased and associated with endothelial dysfunction and DN. Moreover, there is evidence of a diminished capacity of other antioxidants such as vitamin E with a reduced activity of catalase and glutathione peroxidase [69]. The exact mechanisms of impairment are still not completely understood but two hypotheses exist.

Protein glycation with resultant advanced glycation end-products (AGEs) may be a mechanism that damages the protein within the primary antioxidant enzymes, and the antioxidant enzymes, which are co-dependent on one another, may be dysfunctional if one or the other is being consumed by an overactive demand.

Absence of Network Antioxidant Enzymes: Endothelial Nitric Oxide Synthase (eNOS)

The absence of network antioxidant enzymes could play an additional role. In an eNOS knockout mouse model insulin resistance, hyperlipidemia, and hypertension were present in mice lacking the specific isoform eNOS [70]. This represents the loss of the naturally occurring oxygen free radical scavenging – antioxidant effect of eNO. In the MetS and T2DM, this would not require a complete absence of the eNOS enzyme and there is evidence of a gene polymorphism in humans. Investigators have been able to demonstrate that a gene polymorphism (Glu²⁹⁸→Asp in exon 7 of the eNOS gene) was associated with coronary spastic angina, myocardial infarction and found further evidence for this gene polymorphism in the statistically significant association with the development of essential hypertension in two separate Japanese populations [71]. Additionally, another group has shown a significant association of the Glu²⁹⁸→Asp gene polymorphism and ESRD in T2DM, which once again points to the importance of the endothelial cell and the eNOS reaction in DN [72].

Endogenous Inhibitors of the eNOS Reaction

Asymmetrical dimethylarginine (ADMA), initially found in patients with renal insufficiency, has recently been shown to be associated with endothelial dysfunction and increased risk of CVD [73–80]. Researchers have been able to demonstrate a positive correlation with impaired insulin-mediated glucose disposal and elevated levels of ADMA [73]. Plasma ADMA concentrations are increased in insulin-resistant subjects independent of hypertension and act as a competitive inhibitor of the *L*-arginine substrate in the eNOS reaction. Increases in plasma ADMA concentrations may contribute to the endothelial dysfunction observed in insulin-resistant individuals. Elevated levels of ADMA have been observed in insulin resistance – hyperinsulinemia, hypertension, dyslipidemia, hyperhomocysteinemia, and chronic kidney disease. ADMA is formed by protein arginine N-methyltransferases (PRMT) and LDL-cholesterol (both native and oxidized) upregulates PRMTs increasing ADMA [73, 74]. Under physiologic conditions, eNOS is the endothelial constitutive (rate-limiting) enzyme responsible for the conversion of *L*-arginine to NO and *L*-citrulline. It requires a cofactor tetrahydrobiopterin (BH₄). There is a paradoxical uncoupling of the eNOS enzyme that allows this above reaction to be capable of producing superoxide

if there is insufficient BH₄, *L*-arginine, or if there is direct interference with and/or defect in the eNOS enzyme. Uncoupling of the eNOS enzyme results in the production of damaging superoxide, which adds to the oxidative stress within the arterial vessel wall and the glomerular capillary endothelium. Causative factors for eNOS uncoupling are as follows: Increased O₂⁻ and peroxynitrite, elevations in glucose, native LDL-cholesterol and oxidatively modified (mmLDL-cholesterol), hyperhomocysteinemia, decreased or impaired cofactor BH₄, decreased *L*-arginine, increased ADMA, and C reactive protein (fig. 6). Additionally, the diabetic endothelium has been shown to be a net producer of superoxide instead of eNO resulting in a decreased ratio of eNO/ROS (fig. 4, 6), [9, 75–79]. Uncoupling of the eNOS reaction is likely an important process contributing to renal redox stress and remodeling [76–81].

Glucotoxicity

DN is associated with both type 1 and type 2 diabetes mellitus and similarly shares oxidative-redox stress. In the MetS and early T2DM the compensatory hyperinsulinemia, hyperproinsulinemia, and hyperamylinemia due to insulin resistance result in an activation of a local-tissue renin-angiotensin-aldosterone system (RAAS) and specifically angiotensin II (Ang II) with resultant redox stress. Once hyperglycemia has developed, it is responsible for not only a reductive stress (NADH greater than NAD⁺) but also the increase in local-tissue Ang II, which induces oxidative stress and endothelial damage associated with vasoconstriction, thrombosis, inflammation and renovascular remodeling associated with DN.

Four subsections are important in the discussion of glucotoxicity and include advanced glycosylation end-products, receptor for advanced glycosylation end-products (the AGE-RAGE connection), glucose auto-oxidative reactions, the polyol-sorbitol pathway and the diacylglycerol-proteinkinase C isoform (DAG-PKC) pathway, and the direct scavenging of glucose on nitric oxide via glucotoxicity [7–9]. Each is important in the production of excessive ROS contributing to the multiple microvascular complications and specifically DN.

Hypertension

While an in-depth discussion of hypertension (Htn) is not within the scope of this review, it plays an extremely important role in renal redox stress and remodeling [82, 83].

Htn is associated with increased redox stress (ROS) and is associated with ROS mediated vascular damage, which is closely associated with the activation of Ang II and its effect on the vascular NAD(P)H oxidase-superoxide generating enzyme [31, 32]. The major enzymatic sources are NAD(P)H oxidase, xanthine oxidase and paradoxically the eNOS enzyme (in the presence of oxidative stress or deficiency of *L*-arginine or tetrahydrobiopterin, and eNOS uncoupling) [84, 85]. It is important to note that glucotoxicity is closely associated with activation of the RAAS at the local interstitial and tissue levels. Recently, amylin has been implicated as being elevated in patients who have a positive family history associated with Htn and is elevated prior to the onset of Htn when insulin remains at normal levels. Thus, amylin levels may become a screening tool for the development of future Htn [86]. Htn is associated with the clustering phenomenon of the MetS and its importance to the overall picture of redox stress is not to be underestimated, as it contributes significantly to renal remodeling and the overall morbidity and mortality associated with T2DM and DN [87–90].

Endothelial Dysfunction and DN

Each of the major players in the MetS (fig. 5) plays a significant role in the development of CVD, T2DM, and DN. Central to each diabetic complication is the presence of vascular abnormalities of the endothelium and associated endothelial dysfunction (ED).

Other factors that may be of importance are: oxidized LDL-cholesterol, low HDL-cholesterol, interferon gamma, TNF- α , VEGF, TGF- β , PDGF, IGF-1, and IL-1 [91]. Each has been shown to play a detrimental role in the development of DN.

IR, Htn, and T2DM, along with progression to microalbuminuria (MAU) and DN are associated with progressive ED. The vascular and glomerular endothelium normally controls VSMC and mesangial cell tone, and limits leukocyte adhesion, generation of ROS, and inflammatory activity. This is mediated in part by the actions of endothelial derived factors such as NO, prostaglandins, endothelin, von Willebrand factor, soluble thrombomod-

ulin, soluble adhesion molecule and plasminogen activator inhibitor (PAI-1) [91].

ED is present when these functions are altered and allow for the vascular endothelium to be more permeable to the pro-inflammatory modulators and have increased interactions with leukocytes, adhesion molecules, and chemoattractants. Once ED is present, this can initiate and allow for accelerated atherosclerosis throughout the vasculature. DN produces a pro-inflammatory state to allow endothelial dysfunction (both in the glomerular endothelium and the systemic vasculature) to result in an overall increased risk for CVD and renal redox stress and remodeling [91].

Redox Stress and Matrix Metalloproteinases

Matrix metalloproteinase(s) (MMP) are redox sensitive and are upregulated and activated from their inactive latent form when exposed to an elevated tension of redox stress [62, 65, 83, 92–95].

Elevated redox stress is associated with an increase in matrix MMP activity, especially the inducible MMP-9 [96]. The observation that MMPs are activated when eNO is decreased and MMPs are quiescent when eNO is in the normal physiologic range is of great interest when considering eNOS dysfunction.

Cells are dependent on integrin matrix ligand-binding sites and MMP-9 is a basement membrane degrading enzyme capable of clipping the integrin matrix ligands. Cells are constantly re-establishing new integrin matrix binding sites; however, if there is a complete disconnection of integrin matrix binding sites due to a robust increase in MMP-9, the cell may undergo apoptosis. Not only is MMP-9 elevated in diabetes but also redox stress was shown to play a very important role [96].

A robust activation of MMP-9 may result in a complete disconnection of the renal cells within the glomerulus (endothelial cell, mesangial cell and podocyte) and the proximal renal tubule resulting in atrophy, dysfunction, apoptosis, cytoskeleton rearrangement and ECM remodeling. Hearts of mice treated with alloxan have demonstrated increased endothelial cell apoptosis and decreased endothelial cell density [96]. Decreased NO, increased peroxynitrite, and ROS in these same animals were found and thus linked the importance of vascular cellular apoptosis, MMP-9 and redox stress. In contrast, alloxan-induced diabetes in MMP-9 knockout mice did not have induced endothelial cell apoptosis and did not have decreased endothelial cell density when compared to wild

type alloxan-induced diabetes. In this same model there was an interesting unpublished observation: The wild-type mouse displayed decreased glomeruli, whereas glomeruli in the MMP-9 knockout mouse were preserved. With a robust activation of MMP-9, the basement membrane and supporting ECM could be degraded allowing a cellular disconnect to occur. These findings may help explain the structural changes of podocyte foot processes, their atrophy, apoptosis and shedding. The partial or complete disconnect of glomerular cells could contribute to the collapse of the glomerulus if there were a robust activation of MMP-9. These observations could relate redox stress, inflammation, MMP-9 and cellular disconnect together resulting in glomerular failure and collapse as observed with cellular separation and disconnect in hooves of laminitic equine models [97]. Additionally, the SHR rat model demonstrated that MMP-2 and MMP-9 were activated in the kidney [82].

In addition to being collagenolytic these gelatinases are elastinolytic and in the presence of redox stress could contribute to mesangial stalk weakening and collapse along with the loss of structural support of the podocyte and its strengthening foot processes supporting the glomerular mesangium.

It will be interesting to see if MMP-9 gene polymorphisms may be playing a significant role in the development of DN. A recent study indicates that a Japanese patient population with T2DM who have the A21 allele of the MMP-9 gene may be protected from the development and progression of DN [98]. Also, it will be interesting in the near future to see if the newly developed MMP-9 inhibitors or the tetracycline family (specifically doxycycline) could have a positive clinical outcome in preventing proteinuria, podocyturia and ESRD.

An association of MAU and epithelial dysfunction in diabetics has recently been reported and furthermore it was determined that increased plasma MMP-9 preceded the occurrence of MAU in diabetic patients [99, 100]. Additionally, it has been reported that endothelial dysfunction preceded MAU in diabetics [101] and MMP-9 expression correlated with the period of proteinuria in Heymann nephritis [102]. A direct link seems to exist between glomerular and epithelial cell proteolytic activities associated with the loss of glomerular permselectivity and proximal tubule reabsorption of protein. It has been speculated that one of the sources of increased plasma MMP-9 levels may be epithelial cells, and diabetic patients with podocyturia revealed elevated plasma MMP-9 concentrations [99].

ANG II and TGF- β

The paradox of a low to normal plasma renin state in diabetes and the renoprotection conferred by angiotensin converting enzyme inhibitor(s) (ACEI) and angiotensin receptor blocker(s) (ARB) is largely due to a blockade of the increased activity of a local -tissue RAAS and excess Ang II activity in renal tissue in DN [103].

The roles of Ang II and TGF- β play a significant role in renal redox stress, cellular and ECM remodeling. Ang II contributes to the excess local renal production of TGF- β and is the most potent stimulus for the production and activation of the membranous NAD(P)H oxidase enzyme resulting in oxidative stress-ROS within the renal architecture. Each of their roles has been extensively studied and their effects within the glomerulus and the tubulointerstitium regarding the remodeling of the ECM are well known [104–110].

TGF- β 1 has been observed to increase the activities of MMP-2 (3-fold at 10 ng/ml) and MMP-9 (25-fold at 10 ng/ml) from differentiated podocytes but did not enhance the secretion of its inhibitor, TIMP-2 [111]. This finding supplements the proposals set forth in the preceding section regarding MMPs and redox stress. Recently, we have been able to demonstrate cellular structural remodeling of the podocyte foot processes.

Paying Homage to the Podocyte

Podocytes (visceral epithelial cells) share characteristics of both a mesenchymal and an epithelial cell and are thought to be terminally differentiated [112]. ROS are known to induce an epithelial-mesenchymal transition of both the podocytes, as well as the proximal tubule and are known to possess AT-1 receptors [113], which are subject to the remodeling actions of the RAAS-Ang II and ROS [114]. We have chosen to examine the podocyte in contrast to the mesangial cell (with no disrespect to the earlier held mesangiocentric view, as podocytopathy in DN only adds to the mesangial cell story in diabetic glomerulopathy) in this paper in some detail due to its recent emergent importance in glomerular filtration and observational findings of early cellular remodeling in DN. The podocyte slit diaphragm is the final filtration barrier of albumin in the glomerular endothelial basement membrane-podocyte interface. Indeed, podocytopathy is present early on in the natural history of DN and plays an important role in micro- and macroalbuminuria. Podocyturia reflects multiple metabolic toxicity mechanisms

and both cellular and extracellular matrix remodeling due to redox stress [115]. The podocyte is subject to multiple metabolic toxicities in DN including: hyperglycemia, AGE, TGF- β , Ang II, mechanical stress (increased flow and pressure-HTN) and ROS. These toxicities may result in the clipping of β_1 -integrins and connexons by inducible MMP-9 and result in podocyte dysfunction, injury, broadening of the foot processes with eventual effacement and separation from its matrix with resultant apoptosis and podocyturia while at the same time promoting micro- and macroalbuminuria.

Observational Findings of Cellular Remodeling in the Ren-2 Model

Podocytes: The ‘Foot-Soldiers’ of the Glomerular Filtration Barrier

The foot-soldier is the first to be lost in military battle and the podocytes ‘foot cells’ are the first to be lost in the battle against redox stress and ROS. Thus, the podocyte foot processes may be termed the ‘foot-soldiers’ of the glomerular endothelium in regard to their role in maintaining the filtration barrier within the glomerulus. The podocyte foot processes (in health) stand to attention in an orderly formation and thus have become major players in the development of DN (fig. 7) [116].

Cellular structural remodeling of the podocyte is known to be present in DN and consists of cellular swelling, retraction and effacement of the distal foot processes, vacuole formation, fusion of foot processes, displacement of slit diaphragms, and detachment from the glomerular BM and podocyturia. These changes are known to be present at the onset of microalbuminuria and probably occur even earlier in the natural history of DN [117]. In preliminary studies, we have recently observed the structural remodeling of podocyte foot processes and the glomerular filtration barrier interface. Using the Sprague-Dawley (SD) rat as a control, we compared the transmission electron-microscopic (TEM) findings to the transgenic hypertensive Ren-2 model of tissue Ang II overexpression (TG(mREN-2)27) control-untreated to Ren-2 models treated with an angiotensin receptor blocker (ARB).

In preliminary studies, utilizing the SD model as a control animal without Htn, we observed an orderly structural arrangement of the podocyte foot processes (fig. 7). We then examined the foot processes of the Ren-2 model control and found cellular remodeling of the foot processes (fig. 8, 9). We then compared the TEM findings of the Ren-2 model control with that of the Ren-2 model treated with an ARB. The Ren-2-treated model, while still

Fig. 7. Sprague-Dawley control: Transmission electron-microscopic image 10K magnification. Note the orderly-uniform alignment of the foot processes ‘foot soldiers’ and filtration slits, which make up the glomerular filtration barrier (white boxed in area). P = Podocyte; E = endothelial cell. Note: Where the tissue is cut on the bias, the foot processes will appear more disorderly.

Fig. 8. Ren-2 untreated: Note the disordered appearance of the foot processes (boxed in black) along this straight run of the GBM, loss of podocyte foot processes, fusion of foot processes (arrow), shortening of foot processes, the variation of the width of the filtration slits, the broadening of foot processes with a reduction of the number of filtration slit processes per unit length, and the multiple inclusion cysts or vacuoles within the cytoplasm of the podocyte (arrowheads). Magnification: 10K.

structurally abnormal demonstrated a marked improvement in the appearance of the foot processes and preserved filtration slits (fig. 9). While these preliminary findings are only observational structural remodeling changes, they point to the importance of Ang II within the glomerulus and the cellular remodeling of the podocyte foot processes. Additionally, they point to a possible

preventive structural change utilizing an ARB. The abrogation of the structural changes may help explain the protective role of ARBs in recent clinical trials, as it has been previously shown that an ARB abrogated the oxidative stress in the Ren-2 model and resulted in improved insulin sensitivity [118].

Fig. 9. Ren-2 treated with ARB. **a** Sprague-Dawley control. Note the uniformity of foot processes and the filtration slits and the slit diaphragm (the dark black line connecting each podocyte. Magnification: 60K. **b** Ren-2 untreated: Note the disordered appearance of the foot processes and the laying down of the foot processes with fusion and loss of filtration slits and slit diaphragms (the dark particulate background contamination does not interfere with the structure portrayed). Magnification: 60K. **c** Ren-2 untreated: Note the fusion of foot processes and the absence of slit diaphragms. Magnification: 80K. **d** Ren-2 treated ARB: Note the foot-soldiers-foot processes are now upright and standing at attention. Slit dia-

phragms are now present. Even though the Ren-2 (ARB)-treated animals show definite improvement there still remain abnormalities when one views many glomeruli and podocyte foot processes. ARB treatment appears to abrogate most of the abnormal TEM changes associated with this model of excess Ang II in the untreated model. The more upright position of the podocyte foot processes in the ARB treated animals may represent an improvement in the actin cytoskeletal β_1 -integrin matrix ligand binding complex to the adjacent ECM of the glomerular basement membrane. Magnification: 60K.

Observational Findings of Cellular Remodeling in the Zucker Diabetic Fatty (ZDF) Model, Which Represents the Effects of Multiple Metabolic Toxicities: A-FLIGHT-U

Preliminary observational studies examining the podocyte endothelial GBM interface remodeling by TEM in the Zucker obese model revealed even more destructive structural-remodeling changes when compared to the Ren-2 model (fig. 10). This model, like human T2DM is characterized by the changes associated with IR, MetS, Htn and eventually overt T2DM with the associated multiple metabolic toxicities (A-FLIGHT-U). These toxicities act synergistically to result in an even greater remodeling of the glomeruli and the podocyte GBM interface. These changes may even antedate the development of micro and macroalbuminuria. This remodeling occurs early in the natural history of DN and after observing these profound

TEM changes in the Zucker model one must consider the important role in taking a clinical global risk reduction approach in T2DM and DN [119–122] (table 3).

Conclusion

Throughout this review, we have tried to remain focused on the relationship between redox stress and ROS in the glomerular intima (mesangium), arterial intima, and how these two interact with the multiplicative effect of the A-FLIGHT-U toxicities of MetS, IR, prediabetes, and overt T2DM to induce DN and atheroscleropathy. Redox stress and ROS operate through similar mechanisms and will operate in a similar fashion in other chronic disease states, which result in cellular and extracellular matrix remodeling.

Fig. 10. Comparison of the Zucker diabetic fatty rat to the Zucker lean model. **a** Zucker lean control at 16 weeks. Magnification: 40K. Note the ordered and upright position of the podocyte foot processes. **b** Sprague-Dawley control 16 weeks. Magnification: 10K. Again note the ordered and upright position of the podocyte foot processes. **c** Zucker diabetic fatty at 16 weeks. Magnification 10K. Note the complete effacement of the podocyte foot processes (asterisks) and the variable electron dense inclusion cysts in the adjacent podocyte (P). Overall there is noted a hypocellularity due to podocyte loss (podocycturia and apoptosis) when viewing this model. Additionally, we have noted some disorder of the endothelial cell fenestrations in this model, which will require further observation and understanding.

T2DM, atheroscleropathy, and DN remain a heterogeneous and manifold disease not only in their etiology but also in their manifold A-FLIGHT-U metabolic toxicities associated with MetS, IR and T2DM. If the current trend continues, due to the current epidemic of obesity-diabetes in our adolescent youth and the aging baby boom generation, these patients are going to be seen with increasing frequency and we can only expect dialysis clinics to undergo their own remodeling expansion.

Currently, potential prevention or delaying the progression of DN may be accomplished through aggressive global risk reduction of the manifold toxicities by using the currently available treatment modalities we now have at our disposal [119, 123]. Primary prevention of diabe-

tes, secondary prevention of diabetic complications, and tertiary prevention of morbidity and mortality from established diabetic complications utilizing multiple drug targets in the management of T2DM are becoming more realistic clinically [124, 125]. Therefore, a multidisciplinary (global risk reduction) approach to utilize and implement the RAAS acronym and treatment to known goals may be in order, as this approach has seemed so successful in achieving lipid goals in chronic hemodialysis patients [126] (table 3).

We should consider the pleiotropic effects of HMG-CoA reductase inhibitors (statins), ACEI-ARBs, and 5-methyl tetrahydrofolate (folic acid) and tetrahydrobiopterin. Each aid in the increase of the endogenous, locally

Table 3. The RAAS acronym: stablization of the vulnerable glomerulus and intima in T2DM through redox stress reduction

R	Reductase inhibitors (HMG-CoA); decreasing modified LDL cholesterol, i.e. oxidized, acetylated LDL cholesterol; decreasing VLDL cholesterol; improving endothelial cell dysfunction; thus, decreasing the oxidative-redox stress to the arterial vessel wall, the islet, and the glomerulus
A	ACEi-prils; ARBS-sartans: both inhibiting the effect of angiotensin-II locally as well as systemically; affecting hemodynamic stress through their antihypertensive effect as well as the deleterious effects of angiotensin II on renal cells at the local level – injurious stimuli; decreasing the A-FLIGHT toxicities; plus the direct/indirect antioxidant effect within the islet, the arterial vessel wall, and the glomerulus Aspirin antiplatelet, anti-inflammatory effect; adrenergic (blockade) antihypertensive, partially blocking the conversion of prorenin-renin
A	Aggressive control of diabetes; decreasing modified LDL cholesterol, i.e. glycated LDL cholesterol; improving endothelial cell dysfunction; also decreasing glucose toxicity and the redox stress to the arterial intimal, pancreatic islet and the glomerular intima – mesangium Aggressive control of blood pressure; combination therapy is necessary (three or more) Aggressive control of Hcy with folic acid – with its associated additional positive effect on re-coupling the BH4 cofactor with the eNOS reaction to produce eNO Aggressive control of uric acid levels with xanthine oxidase inhibitors
S	Statins: Improve plaque and glomerular stability (pleiotropic effects) independent of cholesterol lowering; improving endothelial cell dysfunction and preventing the angiogenesis associated with arterial vascular remodeling which destabilizes the unstable atherosclerotic plaque and the unstable glomerulus; plus, the direct/indirect antioxidant anti-inflammatory effects within the islet, the arterial vessel wall, and the glomerulus; promote stabilization of the unstable, vulnerable glomerulus, islet, and the arterial vessel wall Style: Lifestyle modification: stop smoking: lose weight, exercise, and change eating habits

scavenging, naturally occurring antioxidant (antiredoxinant): eNO. While awaiting the availability of newer antioxidant molecules, such as the new probucol-like (phenolic analog) AGI-1067 antioxidant under development [127], tetrahydrobiopterin (BH₄) [128], and the SOD and catalase mimetics, it has been suggested that thiazolidinediones, statins, ACE inhibitors, and AT1 blockers should also be used because they are effective causal antioxidants [129]. Furthermore, it has just been reported that probucol (a potent antioxidant) delays the progression of DN and thus supports the concept of renal redox stress and remodeling [130].

Table 4. ROS are redox-signaling molecules: specific example within the high glucose-stimulated mesangial cell

1	A-FLIGHT-U-derived ROS activate PKC, which in turn activates MAPK
2	MAPK activates the nuclear transcription factors: NFkappa B, Sp 1, and AP-1, which in turn activates:
3	TGF-β 1 and PAI-1, which in turn promotes:
4	The CCN2 family – connective tissue growth factor and interacts with insulin like growth factor (IGF-1) promoting:
5	Extracellular matrix synthesis and decreases ECM degradation with resultant ECM expansion within the mesangium of the glomerulus and tubulointerstitium

When redox homeostasis transitions to redox stress, redox signaling ensues in all tissues and organs regardless of the multiple similar or dissimilar etiologies, as redox stress is a redox signaling system resulting in cellular and ECM remodeling [17, 18, 113] (table 4). In order to understand function, it is important to understand structure (remodeling) as a result of metabolic alterations and specifically renal redox stress. The cardiometabolic syndrome goes by many names; however, to honor renal redox stress and remodeling we propose yet another name: The Cardiorenal Metabolic Syndrome.

Competing Interests

None.

Authors Contributions

M.R. Hayden conceived the idea to write this paper and co-authors contributed equally in the writing, and editing of the manuscript.

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