Can rodent models of diabetic kidney disease clarify the significance of early hyperfiltration?: recognizing clinical and experimental uncertainties

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ABSTRACT

In the past, hyperfiltration and increased glomerular capillary pressure have been identified as important determinants of the development of DN (diabetic nephropathy). Recently, some basic research and clinical reviews on DN have omitted identifying hyperfiltration as an important risk factor. At the same time, different rodent models of DN have been described without and with documented hyperfiltration. In the present review, the importance of hyperfiltration is reassessed, reviewing key clinical and research studies, including the first single nephron studies in a mouse model of DN. From clinical studies of Type 1 and Type 2 diabetes mellitus, it is clear that many patients do not have early hyperfiltration and, even when present, its contribution to subsequent DN remains uncertain. Key mechanisms underlying hyperfiltration in rodent models are reviewed. Findings on intrarenal NO metabolism and the control of single-nephron GFR (glomerular filtration rate) in rodent models of DN are also presented. Characterization of valid experimental models of DN should include a careful delineation of the absence or presence of early hyperfiltration, with special efforts made to establish the specific role hyperfiltration may play in the emergence of DN.

INTRODUCTION

Reviews on the pathogenesis of DN (diabetic nephropathy) have differed with respect to the importance of early elevations in GFR (glomerular filtration rate) commonly referred to as hyperfiltration. In the past, hyperfiltration was strongly emphasized as a risk factor with the presumption that the accompanying increase in PGC (glomerular capillary pressure) contributes to the progression of DN with specific glomerular damage [1–4]. In recent clinical reviews, hyperfiltration is often not identified as a risk factor for DN (for example, see [5,6]). Indeed, in an important ASN (American Society of Nephrology) position paper, proposals for future research efforts on DN did not refer to hyperfiltration in early diabetes [7].

In contrast, when hyperfiltration in rodent models of DN is reviewed, it usually is implied, without qualification, that it is a characteristic of the early stages of both clinical and experimental DM (diabetes mellitus). In review articles, we and others have not appropriately emphasized two issues: (i) hyperfiltration may be absent in rodent models, and (ii) its role in contributing to lesions of DN remains unclear [8–11]. In the present

Key words: diabetes mellitus, diabetic nephropathy, glomerular filtration rate, hyperfiltration, nitric oxide, rodent model.

Abbreviations: AA, afferent arteriole; AMDCC, Animal Models of Diabetic Complications Consortium; DM, diabetes mellitus; DN, diabetic nephropathy; GFR, glomerular filtration rate; KO, knockout; l-NMMA, N\(^{O}\)-monomethyl-l-arginine; NOx, nitrate/nitrite; NOS, NO synthase; eNOS, endothelial NOS; nNOS, neuronal NOS; Nx, nephrectomy; ODC, ornithine decarboxylase; PGC, glomerular capillary pressure; SMTC, S-methyl-l-thiocitrulline; SNGFR, single-nephron GFR; STZ, streptozotocin; T1DM, Type 1 DM; T2DM, Type 2 DM; TF, tubular fluid; TGF, tubuloglomerular feedback.

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review, an attempt is made to summarize more carefully the clinical uncertainties regarding hyperfiltration, to identify the characteristics of some key mouse models of DN, including the first single-nephron in vivo mouse results, and to identify uncertainties regarding the risk for the emergence of nephropathy. In my laboratory, we have shown that whole-kidney GFR and SNGFR (single-nephron GFR) [12] are elevated in the T2DM (Type 2 DM) db/db mouse model, where albuminuria and significant glomerular lesions were demonstrated. In another study, hyperfiltration was observed in diabetic mice developing albuminuria (DBA/2J, KK/HJ and FVB/NJ mice) and, importantly, in strains without significant albuminuria [10]. Accordingly, one objective of the present review is to assess the pathogenic importance of hyperfiltration for future research efforts.

Of course, it needs to be recognized that the issues surrounding hyperfiltration are complex. What is the incidence of early hyperfiltration in T1DM (Type 1 DM) and T2DM? When present, how long must hyperfiltration persist to cause adverse effects? In documenting possible adverse effects, how long do patients need to be followed? If a subsequent fall in GFR is an adverse effect, should GFR fall more or less when previous hyperfiltration existed? Is proteinuria as significant as an end point and adverse effect as biopsy-proven lesions of DN? Does the absence of early hyperfiltration in some diabetic patients who develop nephropathy suggest that diabetic rodent models of glomerular disease may be relevant without assessing early hyperfiltration? Do the different pathways to DN and different genetic backgrounds modify the hyperfiltration risk?

In the following sections, from the vast literature available, clinical evidence for hyperfiltration in both T1DM and T2DM and related findings supporting increased risk factors of hyperfiltration for deterioration of GFR and lesions of DN will be selectively reviewed. Clearly, if there is ever a case to be made for modulating early hyperfiltration in diabetic patients, this must be predicated on a clear understanding of what pathogenic mechanisms of DN can be expected to be altered. In this context, several experimental rodent models of diabetes will be described with attention to alterations in SNGFR and whole-kidney GFR and mechanisms normally responsible for the control of SNGFR in these models. Techniques for assessing intrarenal NO will then be considered, and some issues relating to the role of NO in models of experimental DM will be reviewed, and how the rodent diabetic kidney, in the experimental setting, may respond to NOS (NO synthase) inhibition. The effects of a reduction in renal mass on GFR in rodent models of diabetes will be considered with new insights derived from diabetic mouse models. Finally, in the concluding section of the present review, new clinical and rodent studies will be considered which not only provide new insights into mechanisms of DN, but raise new issues related to hyperfiltration and intraglomerular haemodynamic changes in diabetes.

**EARLY HYPERFILTRATION IN HUMAN DM**

In T1DM, hyperfiltration appears to be absent early in the disease in approx. 30% of patients [13], whereas, in T2DM, hyperfiltration is probably absent in approx. 50% of patients [14]. Does the presence of hyperfiltration in diabetes enhance the risk for DN? Two recent clinical reviews have not considered hyperfiltration to be an established risk factor [5,6]. The review by Caramori et al. [6] highlights the complexity of interpreting studies done over the last few decades, identifying renal abnormalities that follow hyperfiltration or other presumed risk factors. The authors note that, after hyperfiltration is established, microalbuminuria, a fall in GFR and the development of demonstrable pathological lesions of DN have been followed in several studies of T1DM patients for as long as 18 years. They conclude that it is still uncertain whether early hyperfiltration is truly an independent predictor of lesions of DN or microalbuminuria. For example, Rudberg et al. [15] have reported that hyperfiltration is a risk factor for developing glomerular changes of DN in T1DM. In contrast, in the T1DM study by Drummond and Maurer [13], it appears those patients with hyperfiltration were not clearly at greater risk for developing DN (duration of the disease from 2–20 years) at least in the early years of observation. Even after 5 years of follow-up, the risk of hyperfiltration was considered unclear [16].

What about early hyperfiltration and subsequent DN in T2DM? Is there a consensus that results on hyperfiltration and its possible adverse risk characteristics are even less certain than in T1DM. However, there appears to be agreement that hyperfiltration is probably absent in approx. 50% of T2DM patients [14]. Moreover, in a study of T2DM Afro-American patients [17], hyperfiltration was not more likely to be associated with a decline in renal function. In summary, in a detailed analysis of the role of hyperfiltration in the development of DN in both T1DM and T2DM, Parving et al. [18] reviewed seven major studies [19–25], concluding that hyperfiltration is only variably present as a marker or risk factor.

What then should be made of the past emphasis of hyperfiltration and associate increased \( P_{\text{GC}} \) as a precursor to DN? In a considered review, acknowledging the seemingly benign nature of isolated hyperfiltration in situations such as pregnancy, Brenner and co-workers [26,27] have proposed a ‘multi-hit’ paradigm and a risk score, wherein hyperfiltration can be evaluated to be a significant risk in a specific clinical setting. Thus, after living donor nephrectomy for transplantation, the donor is considered at increased risk of more rapid progression of renal disease if diabetes appears in subsequent years.
However, in the absence of other risk factors or ‘hits’, the marked hyperfiltration ensuing postnephrectomy is not deemed a sufficient liability to cause glomerulopathy.

HYPERFILTRATION IN RODENT MODELS OF DN (TABLE 1)

**Rat models**

Most experimental results on hyperfiltration in diabetes derive from the commonly used STZ (streptozotocin) rat model of T1DM. After STZ injection, insulin deficiency and hyperglycaemia develop, and usually maintenance daily insulin injections are used to prevent ketosis, although protocols vary widely. In evaluating conclusions derived from STZ rat studies, which often utilize maintenance insulin doses [28], it is important to note that this is a model of T1DM and, therefore, may not be applicable to T2DM, which is associated with hyperglycaemia, significant circulating insulin levels and insulin resistance. It is appropriate to acknowledge, however, the development of other diabetic models, such as that using neonatal STZ to develop rats with features of T2DM [29].

STZ has been reported to be variably nephrotoxic or may elicit renal effects independent of insulin deficiency. Maeda et al. [30] suggest that the reduction in nNOS (neuronal NOS) activity reported in the STZ-treated rat is a function of STZ itself, rather than of the diabetic state. Lubec et al. [31] compared lipid peroxidation, aromatic hydroxylation of phenylalanine and glycoxidation in both genetically determined diabetic mouse strains (db/db, KK and the diabetic BB rat) with the STZ-treated rat. Aromatic hydroxylation was significantly elevated only in the STZ-induced diabetic state. Lubec et al. [31] concluded that studies in STZ diabetes models may be confounded by the presence of products, reaction and tissue damage generated by aromatic hydroxylation, reflecting hydroxyl radical attack due to STZ. Palm et al. [32] have shown that a portion of the proteinuria in STZ-treated rats is due to STZ itself, rather than the diabetic state. It is also important to note that hypertension is a highly prevalent accompaniment of diabetes and DN. It is clear that, when hypertension is present in STZ rodent models of DM, renal lesions are more pronounced [33–35] and presumably better simulate clinical presentations.

**Mouse models**

Imaeda et al. [36] have documented STZ toxicity in mice, and Breyer et al. [11,37,38] have provided detailed critical reviews of mouse models of DM, noting evidence for direct renal effects of STZ and that the use of high- and low-dose STZ may result in differing diabetic characteristics in different mouse models. Detailed functional and renal pathology results are provided on several important inbred mouse strains [10]. Despite our initial attraction to the STZ model of T1DM in the rat [28], in view of the foregoing concerns regarding non-diabetic STZ renal effects we favour other models, such as the db/db mouse [12]. The STZ model will be referred to again in the discussion of NO results in diabetic rodents.

With respect to the validity of experimental models of DN, it is of interest to review the recent AMDCC (Animal Models of Diabetic Complications Consortium) criteria [37] for appropriate murine models of diabetic kidney disease and demonstrating accompanying hyperfiltration and nodular glomerular mesangial expansion. The db/db mouse T2DM model has been known to be associated with kidney hyperfiltration since 1978.

<table>
<thead>
<tr>
<th>Rodent</th>
<th>Model</th>
<th>DM type</th>
<th>Hyperfiltration demonstrated</th>
<th>Comment and references</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat</td>
<td>Single STZ injection (usually)</td>
<td>T1DM</td>
<td>Elevation in both whole-kidney GFR and SNGFR</td>
<td>Widely studied model. Lesions of DN documented. Evidence for hyperfiltration contributing to lesions. Limitations: only relevant to T1DM, possible confounding effects of STZ [3,4,30–32,94].</td>
</tr>
<tr>
<td>Mouse</td>
<td>STZ injections</td>
<td>T1DM</td>
<td>Whole-kidney GFR was elevated</td>
<td>Controversy regarding confounding STZ effects. Pathological changes of DN often not severe [10,11,36–38].</td>
</tr>
<tr>
<td>Mouse</td>
<td>db/db (C57BL6 background), obese and insulin resistant</td>
<td>T2DM</td>
<td>Whole-kidney GFR and SNGFR were elevated</td>
<td>Lesions of DN not as robust as in rat STZ T1DM model or in db/db with C57BLKSJ background. First demonstration of increased SNGFR in any DM mouse model [10,12,95].</td>
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<tr>
<td>Mouse</td>
<td>C57BL/6J-Nos3 null (eNOS-KO mice) with STZ injections</td>
<td>T1DM</td>
<td>GFR was not measured</td>
<td>Classic lesions of nodular glomerulosclerosis. Possible confounding effects of STZ controlled. Future documentation and modulation of early hyperfiltration desirable [41].</td>
</tr>
<tr>
<td>Mouse</td>
<td>eNOS−/− mice on the C57BL6 background crossed with db C57BLKSJ mice</td>
<td>T2DM</td>
<td>GFR was not measured until 26 weeks</td>
<td>Moderate hypertension present. Classic lesions of DN: arteriolar hyalinosis, mesangial expansion, thickening of GBM, and focal segmental and early nodular glomerulosclerosis. Documentation and possible modulation of early hyperfiltration in this excellent model of T2DM is of great interest [40].</td>
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and has been used as a surrogate for human T2DM nephropathy, notwithstanding the milder lesions when compared with the human disease [37]. We have extensively studied whole-kidney GFR and SNGFR and related parameters in db/db mice (B6.Cg-mx/+ Leprdb/j) at approx. 10 weeks of age [12]. At 30 weeks of age, these mice have marked glomerular enlargement and marked mesangial expansion with aneurysmal capillary loop dilation. Vascular changes of arteriolar hyalinization and luminal narrowing were also present. At 10 weeks of age, whole-kidney GFR was greater in these db/db mice than that of the db/m heterozygote or wild-type littermates. This increase in GFR was significantly different whether expressed in absolute terms or per g of kidney weight. SNGFR was also significantly higher in db/db mice. Importantly, we could demonstrate a functioning TGF (tubuloglomerular feedback) system, despite the elevated SNGFR, suggesting the system was reset (also see below). It also appears reasonable to express GFR per g of kidney weight, which provides some correction, albeit far from perfect, for the massive obesity in some models or age-related kidney growth. Thus the comparison of whole-kidney GFR in db/db mice whose obese body weight is vastly greater than either the db/m heterozygote or the wild-type in one study [12] would conceal hyperfiltration if expressed per g of body weight with fat included, rather than kidney weight. Indeed, even when comparing different rodent strains, GFR/kidney weight appears a reasonable parameter.

Rodent models of DN where hyperfiltration has not been assessed

In the introductory paragraphs, it was asked what significance should be attached to important demonstrations of human-like lesions of DN in models of T2DM where early hyperfiltration has not been assessed. In two recent papers, classical DN lesions are reported in two models of eNOS (endothelial NOS)-KO (knockout) mice [40,41] with either db/db KS background or a C57BL6 background and the use of STZ. As it is usually accepted that NO derived from eNOS contributes to diabetic hyperfiltration [35,42], might it be speculated that DN lesions progressed rapidly despite the presumed absence or reduction of early hyperfiltration? This question is considered in depth in the final section of the present review.

INTRARENAL NO IN RODENT MODELS OF DM

The role of NO in DN is complex, and research results in this area are often described as confusing and contradictory [43,44]. Therefore comments will be restricted to our technique of directly measuring intrarenal gaseous NO in living rodents and results from diabetic mice and rats. NO, a labile gas, is generated within the kidney and has been the subject of intense study for more than 15 years. Komers and Anderson [43] in their aptly entitled paper, ‘Paradoxes of nitric oxide in the diabetic kidney’, address several key issues. If it is true that derangements in NO metabolism accompany and possibly sustain hyperfiltration in the db/db mouse with early diabetes [43], attempts to reduce SNGFR using an NOS inhibitor, such as SMTC (S-methyl-l-thiocitrulline) [12], might appear justified. In particular, it would be interesting if a decrease in hyperfiltration by altering NO concentrations could mitigate the lesions of DN. Indeed, as noted above, for more than 15 years, the hyperfiltration observed in early diabetes has been suggested to be related to alteration of afferent arteriolar tone, and many experimental studies have focused on mechanisms sustaining this hyperfiltration and means of preventing it [45,46]. There is evidence that the intrarenal NO system is involved in increases in GFR, and decreased NO with nNOS inhibition acutely reduces hyperfiltration [43,47]. Attention below is focused on NO aspects of early diabetes in rodent models, rather than on longer-term events, where there is evidence of a low NO state. For example, in the T2DM chronically hyperglycaemic obese Zucker rat, there is decreased renal cortical NO production associated with increasing renal injury [48].

Direct real-time intrarenal NO measurements

Komers and Anderson [43], among others [49], have emphasized the limitations of deriving NO data by indirect means, such as the use of urinary NOx (nitrate/nitrite) excretion as well as by non-specific NOS inhibitors. These workers stressed the need for direct intrarenal NO measurements. Similarly, Weight and Nicholson [50] also noted that conflicting experimental NO results could only be resolved by direct intrarenal NO measurements. Our initial speculation was that macula-densa-generated NO could not only modulate SNGFR, as already noted above, but also, by rapid diffusion, might modulate TF (tubular fluid) [NO] in the adjacent tubular systems. Gaseous NO derived from macula densa cells could conceivably diffuse directly to the AA (afferent arteriole). This has been suggested [51] by results derived from the NO-sensitive fluorescent dye DAF-FM DA (4-amino-5-methylamino-2′,7′-diaminofluorescein diacetate), showing NO labelling in the extraglomerular mesangium, despite the NO scavenging detailed by Wilcox and Welch [52]. We speculated that multidirectional diffusion of NO might also modulate TF [NO] of distal and proximal tubules in close proximity to macula densa cells. Indeed, direct in vivo observation (under low-power microscopy) of the surface of the pulsating hyperaemic kidney of a T2DM STZ rat suggests broad vascular dilation consistent with an NO effect. Because of our prior experience building micro pH electrodes,
we wondered if we might develop an NO electrode to measure TF [NO] reliably \emph{in vivo}. Could these TF [NO] measurements mirror the alterations in [NO] in the vicinity of macula densa cells and the contiguous juxtaglomerular interstitium? Working with Dr X. Zhuang (World Precision Instruments, Sarasota, FL, U.S.A.), we extensively modified an approx. 7-\(\mu\)m-tip-diameter electrode and have reported TF [NO] results in different preparations [53–55]. In these studies, several real-time online recordings have been published, including the details of calibration and the \emph{in vivo} demonstration of a simulated calibration within a tubule as well as the decrease in TF [NO] following close renal artery \(1\)-NMMA (N\(\upgamma\)-monomethyl-L-arginine) infusion. We also demonstrated that in the nNOS-KO mouse, TF [NO] was almost one-third less than that measured in control littermates [54].

Our initial TF [NO] measurements in early diabetes were encouraging: in the hyperfiltering STZ rat, TF [NO] was clearly elevated. Although these findings were clear and reproducible, we found that, in the STZ-treated diabetic mouse, which we presumed to display hyperfiltration, TF [NO] was decreased compared with the non-diabetic state. In the \(db/db\) mouse with T2DM, where we have repeatedly demonstrated hyperfiltration [12], we could find no TF [NO] differences when compared with \(db/m\) or wild-type littermates [54]. It is also of interest that the TF [NO] concentrations in mice are in a much higher range than the presumably less hyperbolic rats. Thus the striking directional changes in TF [NO] in the T1DM STZ Sprague-Dawley rat model compared with the STZ B6129G2/J mouse and the lack of TF [NO] change in the \(db/db\) mouse model suggests that the validity of inferences regarding the role of NO in early diabetes may be restricted not only by the type of diabetes studied, but by species dependency as described for arginine metabolism and responses to NOS inhibition in different mouse and rat strains [56,57]. Of course, we now accept that it is unlikely that our TF [NO] measurements reflect events in the confined area within the juxtamedullary apparatus or in immediate proximity to afferent or efferent arterioles. Indeed, there may be an insulated microparacrine [8] environment around the arterioles that senses deviations of luminal NaCl concentration [64].

Despite the anatomical proximity of the nephron's macula densa segment and the AA of the glomerulus, changes in NaCl delivery into the late part of the nephron can alter glomerular arteriolar vasmotor tone and modulate GFR of the same nephron, a process referred to as TGF. Thus SNGFR tends to vary inversely with the [NaCl] appearing at the macula densa site. In the rodent models of T1DM and T2DM with demonstrable hyperfiltration, several possible formulations have suggested either altered NaCl delivery or TGF responsiveness. Could the increase in SNGFR be due to decreased delivery of NaCl to the macula densa site with a normally functioning TGF mechanism? Or perhaps NaCl delivery is normal or elevated with a decrease in TGF sensitivity, resulting in a decrease in afferent arteriolar tone and increased \(P_{GC}\) and hyperfiltration.

Even in the non-diabetic state, where a very substantial TGF literature exists, it is difficult to develop a complete formulation of specific steps detailing how NO derived from macula densa cells exerts its afferent arteriolar effects across the juxtaglomerular interstitium, thereby modulating the TGF response [63]. In rodent models of diabetes, differing results in TGF studies may be related to differences in the duration of the diabetes, the severity of hyperglycaemia and insulin use [54]. However, a key postulate supported by T1DM STZ rat studies is that diabetic hyperfiltration could result from a reduction in the TGF signal as a result of enhanced absorption in segments proximal to the macula densa [64]. This important demonstration of enhanced proximal tubule salt and water reabsorption has generated appropriate research interest. Inhibition of sodium/glucose co-transport resulted in a much greater increase in distal electrolyte concentrations and decrease of nephron GFR in diabetic than control rats. Increased proximal TF absorption in diabetic animals may be mediated by the polyamine-generating enzyme ODC (ornithine decarboxylase), since an ODC inhibitor attenuated the increases in the TGF signal as a result of enhanced absorption in segments proximal to the macula densa [64].
in proximal fluid absorption, hyperfiltration and hypertrophy [65]. It is also possible there may be a reduction in TGF responsiveness that would render GFR more susceptible to vasodilator influences [66].

With respect to dietary salt, in early STZ-induced diabetes, GFR was significantly lower in rats drinking saline [67], whereas NaCl restriction, after induction of STZ diabetes, reduced renal vascular resistance and increased GFR [66]. In a key human study, it has been shown that giving a low-salt diet to patients with T1DM increased GFR [68]. This unexpected response to salt intake suggests that the expected resetting of TGF function does not occur in early diabetes; however, contradictory results have been reported in STZ diabetic rats in which salt restriction led to a fall in GFR [69,70].

In early diabetes, in STZ rats, there is a decreased compensatory efficiency of the TGF system [66], whereas at a later stage of diabetes TGF activity is reported to be increased [71]. In rats with superficial glomeruli in which electrolyte concentrations can be measured much closer to the macula densa, markedly lower concentrations of Na⁺, K⁺ and Cl⁻ were found in diabetic rats than in control animals [64]. These and other results suggest that an appropriate dilatory response to a reduced macula densa [NaCl] amplified by structural adaptations is the cause of diabetic hyperfiltration. Is it possible that NO is involved in these adjustments? Probably not, since stimulation of transport and an unaltered TGF function are unlikely consequences of increased levels of NO. However, inhibition of nNOS by injection of SMTC into the abdominal aorta reduced diabetic hyperfiltration [72]. It is important to note that enhanced nNOS- and eNOS-based NO synthesis and clearer responsiveness of GFR to NO inhibition has been observed in several studies [35,72–74].

In the db/db mouse model of early T2DM, we have shown that whole-kidney GFR and SNGFR is elevated, is responsive to extracellular fluid volume changes and can be modulated by acute nNOS inhibition [12]. Importantly, despite the elevated SNGFR, there is an intact TGF system functioning, which is presumably reset to allow hyperfiltration to continue despite somewhat elevated distal flow rates [12]. In these mice, we have demonstrated glomerular hypertrophy, mesangial expansion and arteriolar hyalinization [12].

Although substantial experimental evidence characterizes early diabetes as a high NO state, there are also proponents of the opposite view that diabetes is a state of real or functional NO deficiency. Although this appears reasonable to explain results in late renal insufficiency, the view that early diabetes may be a low NO state is perhaps surprising, as discussed in the final section of the present review. Other studies have stressed that diabetes causes abnormalities in endothelium-dependent regulation of vasomotor tone [75], and endothelial dysfunction may be related, in part, to increased formation of ROS (reactive oxygen species) and enhanced scavenging of NO [76,77].

**Might early hyperfiltration be explained solely by alteration of myogenic tone of the AA?**

It is clear that, in the above discussion detailing the mechanisms whereby the TGF system might alter SNGFR, it is assumed that AA myogenic tone is normal. However, myogenic tone could conceivably be selectively impaired resulting in hyperfiltration. Loutzenhiser et al. [78] have reviewed various aspects of renal autoregulatory mechanisms. It is suggested that altered AA myogenic tone could act closely in concert with the TGF system to maintain autoregulation in the face of changes in distal salt delivery. It is also plausible that a new ‘balance’ could be achieved between an altered myogenic tone and the TGF response to permit hyperfiltration, with the TGF system functioning in a different range of SNGFR, as we have shown (see above). Although there have been some attempts to directly identify a selective change in AA myogenic tone, for example [79], in the rat model of STZ DM, it is still unclear to what extent this may be present in other preparations.

**EFFECTS OF REDUCED RENAL MASS ON SNGFR IN EARLY DN**

The usual model of renal ablation in rodent research involves removal of renal mass surgically or by selective vascular ablation. SNGFR of surviving nephrons is increased, resulting in elevated flow throughout the nephron [80,81]. Although TGF changes are minimal immediately after reduction in mass, subsequently there is clear adaptation [82,83]. Our findings suggest a role for NO in supporting glomerular hyperperfusion of surviving nephrons. Our real-time direct TF [NO] measurements [55] show a 4-fold increase in TF [NO]. As we noted above, this result is probably not inconsistent with ‘low NO’ in remnant kidneys [84,85]. Local TF NO levels may not reflect whole kidney l-arginine metabolism or urinary excretion of NOx and, despite decreases in nNOS activity or abundance, enhanced substrate delivery per surviving nephron may enhance [NO] in the remnant tissue. In addition, changes in degradation or diffusibility of NO in the remnant kidney may be such that even reduced rates of NO formation may achieve higher steady-state levels. Hypertrophy of macula densa cells and increases in NO derived from eNOS around the surviving nephron may also contribute to the increase in its TF [NO].

To date, there have been no publications of single nephron mouse results examining, *in vivo*, the effects of renal mass reduction in DM. To characterize SNGFR and TGF responses to reduction in renal mass and chronic nNOS inhibition in the db/db mouse, we recently performed unilateral Nx (nephrectomy) and sham Nx in 11-week-old T2DM db/db mice [86]. SNGFR and TGF responses were then defined approx. 3 weeks later without and with SMTC in the drinking water. Whole-kidney GFR
in db/db Nx mice was significantly higher than in sham db/db Nx mice, with SNGFR significantly increased after Nx with TGF responses intact. SMTC ingestion neither altered SNGFR nor TGF response.

**FUTURE RESEARCH DIRECTIONS AS SUGGESTED BY RECENT CLINICAL AND RODENT STUDIES**

The AMDCC has offered valuable considerations for improving the scope of rodent research including issues related to hyperfiltration (see http://www.amdcc.org, and Breyer et al. [11,37,38]). In the present review, hyperfiltration issues in both clinical studies and rodent research models have been examined in detail. We believe the role of hyperfiltration and associated changes in TGF and NO metabolism should be considered by all investigators seeking to delineate factors contributing to DN. Special efforts should be made to document the absence or presence of early hyperfiltration in all models of DN. In particular, when robust lesions of DN are demonstrable, the absence or presence of hyperfiltration, and its duration, is of particular interest. It is possible that when hyperfiltration is present adverse metabolic pathways may be stimulated. If hyperfiltration can be shown to be absent in carefully defined DN models, where glomerular pathology is well documented, there may be valuable implications for intraglomerular hemodynamics, NO metabolism and possible insights into the pathogenesis of the glomerular lesions.

**eNOS compared with nNOS as a possible mediator of hyperfiltration and lesions of DN**

With these considerations in mind, let us consider two important recent studies wherein classic severe DN glomerular lesions were demonstrated in eNOS-KO mice with diabetes either induced by STZ [41] or already present in db/db mice with a C57BLK6/J background [40]. It has been suggested [41,87] that, in these studies, the mesangial expansion, basement membrane thickening and nodular sclerosis may involve VEGF (vascular endothelial growth factor)/NO uncoupling with excessive endothelial cell proliferation coupled with altered autoregulation related to the development of preglomerular arteriolar disease. In both studies [40,41], early hyperfiltration was not assessed; all measurements were made in older mice (at age 26 weeks in the db/db mice, or 12 and 20 weeks after STZ induction of diabetes in 8-week-old mice). It is intriguing, however, to consider the possible effects of the absence of NO derived from eNOS on hyperfiltration in these studies. Quaggin and Coffman [87], in commenting on these eNOS-KO mice studies, note that eNOS contributes to hyperfiltration. Indeed, Veelken et al. [35] have argued that eNOS can be an important contributor to hyperfiltration, not withstanding the clear role of nNOS-derived NO, reviewed above and elsewhere [43–45,88,89]. Accordingly, if in these diabetic rodent preparations [40,41] eNOS contributes importantly to hyperfiltration, these results strongly imply that the proliferation of classic DN lesions occurred in the absence of hyperfiltration or at least with important reductions in hyperfiltration. Of course, as noted in previous sections of the present review, there is clear evidence that nNOS-derived NO can contribute to the modulation of afferent arteriolar tone and hence SNGFR via TGF responses [8,46,88]. However, there are also suggestions that NO may modulate efferent arteriolar tone: in diabetes, NO could preferentially dilate the efferent arteriole, reducing mean arterial pressure and filtration fraction. If in an older STZ rat study, l-arginine supplementation in the drinking water (possibly generating more intraglomerular NO) mitigated both proteinuria and reduced hyperfiltration, although the glomerular lesions were unchanged [62]. However, most relevant to the possibility that NO may mitigate hyperfiltration is a clinical study on hyperfiltration patients with T2DM by Pistrosch et al. [90]. Evidence is presented that rosiglitazone, an insulin-enhancing agent, reduces filtration fraction, hyperfiltration and microalbuminuria, while increasing intrarenal NO availability. This conclusion was based on GFR and renal plasma flow measurements before and after NO inhibition with l-NNMMA. The proposed mechanism is that rosiglitazone elicited increased NO bioavailability, thereby reducing GFR and albumin leakage. Two animal studies using a compound similar to rosiglitazone, troglitazone, have proposed similar efferent arteriolar responses [91,92]. Also relevant are the findings by Patzk et al. [93], indicating that NO can modulate AngII (angiotensin II) constriction of efferent arterioles in eNOS-KO mice.

Thus it is possible that the absence of eNOS-derived NO contributes to hyperfiltration by reducing an efferent arteriolar vasodilatory tone. Clearly, knowledge of early GFR in younger diabetic eNOS-KO mice would direct assessments of intrarenal NO metabolism, as noted above. Put more simply, in early diabetes settings, we need more insight into the relative proportion of intraglomerular NO derived from nNOS or eNOS, and the specific response of afferent and efferent arterioles. If early hyperfiltration is present, and nNOS-derived NO is likely to be the major contributor, specific nNOS inhibition with SMTC might lessen the severity of the lesions. It is also clear that defining the TGF responses in single nephrons of diabetic mice should be undertaken in diabetic eNOS-KO mice to determine not only baseline SNGFR, but also feedback responses to altered loop of Henle flow rates and ambient luminal NaCl at the macula densa site. As noted above, we have already profiled TGF responses in db/db mouse [12] with some evidence that nNOS contributes
to hyperfiltration. Importantly, the more comprehensive approach of Vallon et al. [63] used to assess TGF vascular control in non-diabetic nNOS-KO mice would be of particular interest in the analysis of eNOS-KO diabetic mice.

In summary, the frequency with which early hyperfiltration occurs in diabetic patients and evidence for its association with DN have been reviewed. Rat and mouse models of DN have been described, reviewing features of hyperfiltration when present, as well as the implications for intrarenal NO metabolism and TGF mechanisms in maintaining the hyperfiltration. Our own recent studies on the direct in vivo measurement of NO in TF of diabetic rats and mice have been outlined, as well as our single nephron observations in db/db diabetic mice, without and with renal mass reduction, examining factors associated with hyperfiltration. The documentation both of hyperfiltration and a description of its sustaining mechanisms should be considered as an essential component of rodent DN research.

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