Editorial Review

Osmotic polyuria: an overlooked mechanism in diabetic nephropathy

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Abstract
Tubulo-interstitial pathology in diabetic nephropathy is thought to be caused by cell injury that is induced by high ambient glucose levels and increased proportions of glycated proteins. Other mechanistic hypotheses engage glomerular ultrafiltration of proteins and bioactive growth factors and their effects on tubular cells. Some scholars promote tubular ischaemia due to reduced peritubular blood flow as a response to glomerular injury. All of these mechanisms contribute to renal tubulo-interstitial injury in diabetic nephropathy. However, they do not well explain observations that have been made in studies of experimental animals and evaluations of human biopsies showing dilated collecting ducts in early diabetic nephropathy. Dilatation of distal nephron segments is routinely seen in human biopsies or in histological sections from experimental diabetic nephropathy and is reminiscent of similar findings in obstructive nephropathy. Moreover, they are these dilated tubules that are the primary source for pro-inflammatory and pro-fibrogenic cytokines and regulators. Based on this large body of observations from this laboratory and the published literature this narrative develops a novel hypothesis where hyperglycaemic, osmotic polyuria play important contributory roles in the initiation and progression of tubulo-interstitial injury in diabetic nephropathy.

Keywords: Diabetes; diabetic nephropathy; polyuria; shear stress; tubular pressure

Introduction
Diabetic nephropathy is a common and severe complication that occurs in ~40% of patients with a long-standing type II diabetes mellitus and in a lesser proportion of subjects with type I diabetes. It occurs usually concomitantly with other microvascular complications of diabetes such as diabetic retinopathy and/or neuropathy that let some scholars view diabetic nephropathy as one of several clinical syndromes resulting from diabetic injury to microvessels. Abnormal microalbuminuria has emerged as a clinical risk factor for renal failure in diabetic renal disease as well as for cardiovascular disease in general and specifically in diabetes. These epidemiological observations together with the experimental recognition that glomerular visceral epithelial cells (podocytes) are an early target in diabetic renal injury have given rise to the view that diabetic nephropathy may, in fact, be initially and primarily a glomerular disease and tubulo-interstitial nephropathy may be secondary to glomerular proteinuria and/or ischaemia given that peritubular capillaries are located downstream of efferent arterioles.

Clinical–pathological correlations have drawn attention that renal failure in diabetic nephropathy is more closely determined by tubulo-interstitial injury and fibrosis than by diabetic nodular and/or diffuse glomerular sclerosis [1,26]. Findings from experimental in vitro studies and in vivo observations in animal models have derived a series of hypotheses as to the mechanisms of tubulo-interstitial injury. Most of these invoke high glucose levels and the increased proportion of glycation of structural and regulatory cell proteins and extracellular proteins (such as glycated albumin and modified ultrafiltered proteins) at the initiation of an injury cascade. Responses of tubular cells to glycemic injury include the elaboration of chemokines (MCP-1, RANTES, etc) as well as several cytokines (such as TGFβ, PDGF and several others). Chemokines attract and activate immigrating macrophages that, in turn, elaborate many bioactive molecules. Cytokines act by autocrine modes on tubular cells initiating a pro-fibrogenic gene expression programme but also in paracrine modes on resident fibroblasts and pericytes causing pro-fibrogenic transformation of these two latter cells (Figure 1). Most mechanistic hypotheses describing the development and progression of interstitial injury in diabetic nephropathy use all or some of these paradigms. Clinical observations showing that tight glycemic control reduces the onset and progression of diabetic nephropathy are compatible with these theories but also support other mechanistic views. Moreover, several observations do not appear to be well compatible with the notion that glycemia and glycated proteins induce interstitial...
fibrogenesis in diabetic nephropathy through mechanisms that are, in fact, identical to those thought to cause tubular atrophy and progressive interstitial fibrosis in virtually every other renal disease leading to complete renal failure. In this narrative we attempt to develop another hypothesis of tubulo-interstitial injury in diabetic nephropathy that is based on polyuria that is associated with poor glycaemic control.

Contributions of increased tubular fluid content to kidney and nephron size

Even in advanced renal failure in diabetic nephropathy kidneys have normal or near-normal size and appearance on imaging studies. Simple renal ultrasonography demonstrates that kidneys in patients with advanced renal failure from diabetic nephropathy are typically normal in length and parenchymal thickness. This is much in contrast to most other renal diseases where kidneys in advanced, chronic renal failure are more or less size-reduced with parenchymal atrophy (Figure 2). Renal ultrasonography allows also for the assessment of echogenicity. Increased echogenicity of the renal parenchyma is caused by inflammatory infiltration, advanced fibrosis and/or severe interstitial oedema [14,17]. Sonographic hypoechogeticity is seen when tubules contain relatively large amounts of fluid [14]. Under this condition, even in the presence of advanced interstitial fibrosis, the renal echogenicity may be normal or only slightly increased. Whereas end-stage renal disease under most conditions presents sonographically with small and hyperechoic kidneys, this is different in advanced diabetic nephropathy; kidneys have usually normal size and normal or only modestly increased echogenicity (Figure 2). This unusual sonographic appearance is caused by an increased nephron fluid content in addition to nephron enlargement by cell hypertrophy and hyperplasia.

The diabetic milieu, like hyperglycaemia and elevated interstitial fluid glucose levels per se, causes hypertrophy and perhaps moderate hyperplasia of the nephron [21,22]. Experimental studies have shown that this nephron enlargement occurs early after the onset of diabetes/hyperglycaemia (<1 week) and is reversible by tight glycaemic control [21]. There are two requirements for induction and maintenance of tubular cell enlargement, namely increased cell protein synthesis (surpassing protein catabolism) and reduced cell cycle transition through G1/S. Current models for glycemic cell hypertrophy are based on glucose-induced activation of the mammalian target of rapamycin complex-1 (mTORC-1) that increases ribosomal translational activity and raises levels and activity of the cyclin inhibitors p21\textsuperscript{Cip1} and p27\textsuperscript{Kip1} also downstream of mTORC-1, both of which are inhibited by rapamycin therapy of the diabetic animals [23]. It is one of the pathophysiological enigmas that cell enlargement does not occur in other epithelial organs or tissues; perhaps, the difference is that tubular fluid glucose levels in poorly controlled diabetes are much higher compared to blood levels and it is these superglycaemic levels that are causative in tubular epithelial cell hypertrophy. Nephron enlargement is an early event in diabetes but by all means does not account for the maintenance of a normal or supra-normal kidney size at later stages of progressive renal failure in diabetic nephropathy because nephron dropout and interstitial fibrosis prevail. In addition to tubular cell enlargement, dilatation and increased fluid content of tubules, mainly collecting ducts, contribute substantially to the maintenance of kidney size in diabetic nephropathy with advanced chronic renal failure.

Osmtic polyuria as a mechanistic determinant of tubulo-interstitial injury and progression of renal failure in diabetic nephropathy

The earliest clinical renal symptom in untreated or poorly controlled diabetes in addition to glucosuria is, in fact,
osmotic polyuria. The term 'diabetes' was coined by the Alexandrian physician Aretaeus of Cappadocia from the Greek ‘δια’ (through) and ‘βαιη’ (to pass, to walk). The diagnosis was for many centuries based on observing the urine volume or flow rate; the differential diagnosis of diabetes was made with the medieval version of urinalysis utilizing the taste buds as 'mellitus' (Latin 'mellis', honey) or 'insipidus' (Latin, tasteless). The degree of polyuria, and hence, the accelerated tubular fluid flow rate, is defined by the glucose concentration—dependent osmotic forces, especially in the distal nephron.

Effect of osmotic polyuria on tubular fluid pressure

Polyuria due to osmotic forces changes the rheology of tubular flow and increases flow rate, especially in the distal nephron where flow rates and flow velocity are physiologically low. Three decades ago Marsh and Martin examined the tubular pressure effects of acute osmotic polyuria by micropuncture in hamsters receiving an intravenous injection of mannitol [15]. Compared to baseline the osmotic challenge raises tubular fluid pressure in all tubular segments (Figure 3). However, the greatest pressure gain occurs in the distal nephron, especially in cortical-collecting ducts suggesting that this segment provides the greatest resistance to increased flow. These effects of osmotic diuresis are also well demonstrated with intra-vital microscopy (Figure 4). Recent studies by Simeoni and co-workers are most illustrative in this context [24]. Figure 4 depicts a sequence of images before and at 5, 10 and 15 min after the administration of mannitol to a rat [24]. Shown is a proximal tubule segment in the left upper corner of each image and a distal tubule in the center (Figure 4). During the 15-min period of time after the osmotic challenge, the diameter of the proximal tubule changes little, if any (Figure 4). In contrast, the luminal opening of the distal tubule segment increases at least 3-fold (Figure 4) [24]. This observation nicely illustrates the effect of osmotically increased fluid flow rates on the distal nephron. These findings are also illustrative of relatively good compliance of the distal tubule, at least acutely, and the relatively high resistance of the collecting ducts, and thus, confirm data from Marsh and Martin [15].

Distal nephron dilatations due to increased pressure are the hallmark of acute obstructive nephropathy as mimicked by ureteral ligation in the rat. In this renal disease model, distal nephron luminal pressure increases. This causes dilatations of collecting ducts and pressure-induced 'activation' of tubular epithelial cells that respond with the elaboration of pro-inflammatory mediators and profibrogenic cytokines including TGFβ. Studies using this model of renal injury have demonstrated the relative importance of this cytokine in renal fibrogenesis of obstructive nephropathy similar to diabetic nephropathy [10,16]. In fact, there are also several structural similarities between obstructive and early experimental diabetic nephropathy (Figure 5).

Hyperglycaemia-induced polyuria with increased tubular fluid pressure in the distal nephron, especially collecting ducts, is quite obviously the cause of tubular dilatations that are observed in rodent models of diabetes mellitus. Figure 6 depicts a collection of immunohistological images from this laboratory obtained from rats with streptozotocin-induced diabetes mellitus with plasma glucose levels between 300 and 400 mg/dl. The daily diuresis in these diabetic animals ranged from 25 to 60 ml/day (compared to ~8 ml/day in normal, normoglycaemic controls). The daily urine volume by weight corresponds to up to 20% of body weight of the diabetic rats. Given that osmotic diuresis induces increased
Fig. 6. Appearance of cortical tubules in a kidney section immunostained with anti-αSMA in a normal rat (A), a section stained with anti-CTGF in a diabetic rat at 15 weeks (B), a section from a diabetic rat at 30 weeks stained with anti-TGFβ (C) and a section from a diabetic rat (30 weeks) stained with anti-αSMA (D). Anti-VEGF fluorescence-stained sections from a normal (E) and a diabetic mouse (16 weeks) (F) are also shown. All sections from diabetic animals show substantial tubular dilatations of collecting ducts at earlier stages and some additional nephron segments (D) at later stages (30 weeks of diabetes). Note that the various immunostains show increased expression primarily or exclusively in dilated tubular sections. In (G) a high power view of a dilated collecting duct from a diabetic rat is shown indicating a normal or even increased number of cells.

tubular fluid pressure in collecting ducts, it is not surprising that in hyperglycaemic rats, collecting ducts are dilated (Figure 6B, C, D, F and G) and the number of dilated tubules increases with time of hyperglycaemia (Figure 6D). It is highly remarkable that cells in these dilated tubules and not in other non-dilated (proximal) tubules express those cytokines that have been shown to play important roles in renal interstitial fibrogenesis. Examples shown in Figure 6 include TGFβ (Figure 6C) and CTGF (Figure 6B) as well as αSMA (Figure 6D) (which may suggest a gain of a partially myofibroblast phenotype) and VEGF (Figure 6F). These dilated collecting ducts are not atrophic; if any, there may be some degree of hyperplasia reminiscent of cystogenesis (Figure 6G). These observations that molecules thought to be pathogenic in diabetic tubulo-interstitial nephropathy are expressed preferably or exclusively in dilated segments of the distal nephron have not only been made in this laboratory but are, in fact, shown in numerous publications for decades. For example, Chow et al. described that db/db mice have increased tubular expression of the chemokines MCP-1, MIF and M-CSF, and the accompanying images depict an expression of these inflammatory regulators exclusively in dilated distal nephron segments (collecting ducts) [5]. The literature is replete of similar findings demonstrating that the tubular expression of pro-inflammatory and pro-fibrogenic molecules in early experimental diabetic nephropathy occurs mainly in dilated tubules of the distal nephron and only few examples can be cited in this review [4,12]. In most of these publications authors do not note that the expression of molecules of interest is limited to the dilated distal nephron segments and virtually never is this observation discussed in a mechanistic context. This finding is not limited to experimental rodent models of diabetes but is also observed in vivo in human diabetic renal disease (Figure 7) [11]. Thus, at least in earlier stages of diabetic nephropathy, the expression of pathogenic regulatory molecules occurs primarily in nephron segments that have undergone mechanical dilatation due to increased fluid pressure likely caused by osmotically increased tubular fluid flow.

If the increased distal nephron fluid flow causes dilatation of collecting ducts one might expect that non-osmotic polyuric states are also associated with this histological finding. The only other diseases that fall into this category are caused by non-osmolar water diuresis such as psychogenic polydipsia and diabetes insipidus. There is very limited literature on structural renal consequences of these water-diuresis states. However, dilated collecting ducts are also observed under these conditions in humans [18]. Dilations of distal nephron segments, mainly collecting ducts, are also seen in lithium-induced nephrogenic diabetes insipidus in the rat [6].

There is little experimental study on the effects of mechanical forces, especially elevated hydrodynamic pressure on tubular cell biology. Recently, some evidence has emerged that increased pressure alters some aspects of the gene expression programme consistent with a pro-inflammatory and pro-fibrogenic change in tubular cell phenotype. Broadbelt et al. recently demonstrated that the exposure of cultured tubular cells to static pressure increases the expression of iNOS and soluble guanylate cyclase [2]. Increases and especially rapid changes in
cortical-collecting duct flow causing cell stretch and collecting duct dilatation are associated with an increase in the intracellular Ca²⁺ in both principal and intercalating cells indicating that the mechanical force of stretch is indeed translated into an intracellular signal. The observations cited earlier in this narrative that pressure and stretch dilatation in osmotic diuresis are greatest in the distal nephron, mainly the collecting duct, may account for the observation that these dilated portions of the nephron in experimental diabetes express increased levels of fibrosis-regulating proteins rather than other portions of the nephron that are exposed to the same level of ambient glycemia and glycated proteins.

**Tubular flow velocity and shear stress** In addition to distal nephron dilatation and stretch, and elevated luminal pressure, tubular cells are also exposed to a second physical force that results from the increased nephron flow in osmotic polyuria of diabetes, namely shear stress. There is a large body of literature on the effects of shear stress on the vasculature and on endothelial cells demonstrating important effects on the cytoskeleton, cell signals and gene expression, but little is known about the effects of this mechanical force on tubular cells. Nevertheless, there is evidence from recent experimental observations that tubular cells in vivo and in vitro recognize and respond to fluid flow velocity and the associated shear stress. In proximal tubules brush border microvilli have a mechanosensory function and fluid dynamic torque is translated into modulations of Na⁺ absorption [7]. It is noteworthy that in experimental diabetes in rats proximal tubular microvilli height is significantly reduced, perhaps in response to the constant exposure to increased tubular fluid flow rates [25]. In cultured tubular cells transient exposure to shear stress at the apical membrane induces a substantial re-arrangement of the actin cytoskeleton. In this experiment shear stress is also associated with increased transcription factor binding to the shear stress response element (SSRE), suggesting changes in the regulation of gene expression that may not be dissimilar to those that occur in shear stress-challenged endothelial cells [8]. SSREs with the general palindromic sequence GAGACC are found in many transcription factors such as NFκB, AP1, SP1, Oct1 and Egr1/Sp1 and shear stress upregulates the transcriptional activity of these (and other) factors [9]. In collecting duct cells, increased shear stress raises nitric oxide release suggesting activation of one or more NOS-isoforms [3]. Microperfusion or superfusion of cortical collecting ducts induce rapid increases in the intracellular Ca²⁺ concentration in both principal and intercalated cells [13,27]. There is evidence that the apical primary cilium is a flow/shear-stress sensor and increased bending activates Ca²⁺ entry through the polycystin-2 Ca channel [19,20]. However, cell responses to flow and shear stress may not solely be sensed by the cilium as indicated by the fact that intercalated cells that do not display cilia also respond to shear stress with an increase in intracellular Ca²⁺ [13].

Overall, experimental findings showing that mechanical forces (luminal pressure and shear stress) cause alterations in tubular cell phenotype and their gene expression program is highly compatible with in vivo observations that increased the expression of pro-inflammatory and pro-fibrogenic regulators occur in dilated distal nephron segments in diabetic nephropathy.

In summary, in the present narrative we review observational evidence supporting an important role of tubular fluid pressure and flow dynamics as additional cause of early tubular cell injury in diabetic renal interstitial nephropathy. Increased tubular pressure and shear stress occur especially in the distal nephron (collecting ducts) as a result of hyperglycemic osmotic polyuria due to poorly controlled diabetes. Evidence suggests that these mechanical forces are important but overlooked mechanisms contributing to the induction and progression of tubulo-interstitial fibrogenesis in diabetic nephropathy.

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**References**


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