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Role of biomechanical forces in hyperfiltration-mediated glomerular injury in congenital anomalies of the kidney and urinary tract

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ABSTRACT

Congenital anomalies of the kidney and urinary tract (CAKUT) including solitary kidney constitute the main cause of progressive chronic kidney disease (CKD) in children. Children born with CAKUT develop signs of CKD only during adolescence and do not respond to renin-angiotensin-aldosterone system blockers. Early cellular changes underlying CKD progression to end-stage renal disease by early adulthood are not well understood. The mechanism of maladaptive hyperfiltration that occurs from loss of functional nephrons, including solitary kidney, is not clear. We re-examine the phenomenon of hyperfiltration in the context of biomechanical forces with special reference to glomerular podocytes. Capillary stretch exerts tensile stress on podocytes through the glomerular basement membrane. The flow of ultrafiltrate over the cell surface directly causes fluid flow shear stress (FFSS) on podocytes. FFSS on the podocyte surface increases 1.5- to 2-fold in animal models of solitary kidney and its effect on podocytes is a subject of ongoing research. Podocytes (i) are mechanosensitive to tensile and shear forces, (ii) use prostaglandin E₂, angiotensin-II or nitric oxide for mechanoperception and (iii) use specific signaling pathways for mechanotransduction. We discuss (i) the nature of and differences in cellular responses to biomechanical forces, (ii) methods to study biomechanical forces and (iii) effects of biomechanical forces on podocytes and glomeruli. Future studies on FFSS will likely identify novel targets for strategies for early intervention to complement and strengthen the current regimen for treating children with CAKUT.

Keywords: CAKUT, fluid flow shear stress, hyperfiltration, podocytes, tensile stress

INTRODUCTION

Congenital anomalies of the kidney and urinary tract (CAKUT) including solitary kidney constitute the most common cause of chronic kidney disease (CKD) in children. CAKUT accounts for nearly 57% cases of CKD in children, according to the North American Pediatric Renal Trials and Collaborative Studies registry [1]. The NIH-CKiD (Chronic Kidney Disease in Children Study), USRDS (United States Renal Data System), and several European and Asian registries report the incidence of CAKUT as 49–62% in children with CKD and as 34–43% in those with end-stage renal disease (ESRD) [2–7]. The rate of decline in kidney function is related to modifiable factors such as proteinuria, hyperfiltration, blood pressure and diabetes, and to non-modifiable factors such as age, gender and ethnicity. Among the modifiable factors, hyperfiltration takes temporal precedence over others in modulating the progression of CKD.

In children with CAKUT, lack of postnatal nephrogenesis and hyperfiltration-mediated injury set up a vicious cycle of nephron loss with progressive CKD. Hyperfiltration not only results from but also contributes to ongoing and repetitive glomerular injury [8, 9]. With regard to the prevalence of risk...
Reconsidering hyperfiltration in terms of biomechanical forces provides an opportunity for identifying new facets of CKD progression in children with CAKUT. We discuss (i) the nature of forces and (iii) effects of biomechanical forces on podocytes and progression in children with CAKUT. 

Hyperfiltration is considered a long-known and frequently studied phenomenon, and early work has explored the physiological aspects of glomerular filtration. These studies and principles of fluid engineering suggest that glomerular capillary pressure (PGC) maintains an outward flow of the plasma fluid while causing tensile stress on the capillary wall that is transmitted to podocytes. In parallel, continuous flow of the ultrafiltrate into Bowman’s space generates shear stress on podocytes. Reconsidering hyperfiltration in terms of biomechanical forces provides an opportunity for identifying new facets of CKD progression in children with CAKUT. We discuss (i) the nature of and differences in the response of podocytes to tensile stress and shear stress, (ii) methods available to study biomechanical forces and (iii) effects of biomechanical forces on podocytes and glomeruli.

**FLUID MECHANIC PERSPECTIVE OF STRESS AND STRAIN**

The passage of blood through the capillary and the flow of the ultrafiltrate along the podocyte can be explored using principles of fluid mechanics. Capillary loops in the glomeruli appear as a coiled structure. Being hollow, the tubular structure of capillary may be visualized from within as a porous, thin-walled cylinder with holes and bends, with blood flowing under pressure. This structure results in one column of fluid (blood) that moves within the capillary and another (ultrafiltrate) that moves outside of the capillary between podocytes and over cell surface.

Stresses (force/area) are of two types, namely axial and shear. An object experiences strain when subjected to stress defined as the intensity of deformation resulting from the applied stress. Axial stress (σ), tensile or compressive in nature, exerts force perpendicular to the area to which it is applied and is expressed as \( \sigma = \frac{F}{A} \), where \( \Delta l / l \) represents the change in length and \( l \) is the original length. Axial stress could be uniaxial, where force is applied along one of the axial directions or biaxial when force is applied along two perpendicular axes and the object stretches/compresses in two perpendicular directions.

Fluid passing through a tube (e.g. capillary) indicates fluid pressure within a hollow cylinder with closed ends. The closed end is an idealization for the bends in a tube, e.g. looped capillary. Radially directed internal pressure parallel to the wall of the tube (cylinder) and the axial thrust from the closed ends (a bend in the tube) stretch the walls of the cylinder in two axial directions. A tube is considered thin when \( r/t < 10 \), the ratio of the radius (\( r \)) and the thickness of the wall (\( t \)), is less than 10. Circumferential stretch results in a hoop stress in the cylinder wall given by \( \sigma_0 = \frac{p r}{t} \). The axial stress, which is one half of the hoop stress, is given as \( \sigma_a = \frac{p r}{2t} \), where \( p \) is the pressure inside the capillary. The axial/hoop stress causes axial and hoop strains, due to the elastic modulus of components of the capillary wall. Thus, endothelial cells along the lumen, and the glomerular basement membrane (GBM) and podocytes covering the capillary will experience uniaxial tensile stress, and possibly bi-axial tensile stress at the capillary bends (Figure 1).

The second type of stress is shear stress (\( \tau \)) caused by a force parallel to the surface of the object and also defined as force divided by the area. However, shear strain (\( \gamma \)) is defined as change in angle (in radians) of an object exposed to shear stress. It should be noted that shear stress/strain on one surface automatically causes complementary shear stress on all surfaces, thereby changing its shape, e.g. change of a rectangular shape to parallelogram following deformation. Under small deformation and static behavior, the stresses and strains are related as follows: \( \sigma = E \epsilon \) and \( = G \gamma \), where \( E \) and \( G \) are the elastic modulus and shear modulus, respectively. The two moduli are related by the expression \( E = \frac{G (1 + \nu)}{2 (1 - \nu)} \), where \( \nu \) is the Poisson’s ratio of the membrane. The Poisson’s ratio of a material represents the reduction in lateral strain when a membrane is stretched in the longitudinal direction.

Blood flow in capillaries over endothelium and ultrafiltrate flow between podocytes would be analogous to a fluid flowing over surface(s) of an object causing shear stress. Shear stress caused by a fluid column in a parallel plate configuration is given by the equation: \( \tau = \frac{6 q n}{w h^2} \), where \( \tau \) is fluid flow shear stress (FFSS) in dynes/cm², \( w \) and \( h \) are the width and height of the fluid

**FIGURE 1:** Stresses, defined as force divided by a unit area, are of two types, namely axial and shear. Axial stress (\( \sigma \)) results from a force perpendicular to the area which it is applied, and shear stress (\( \tau \)) is caused by a force parallel to the surface of the area. Blood flow in glomerular capillary exerts force perpendicular to the capillary wall (two solid arrows) creating an axial (tensile) stress on podocytes localized along the outer aspect of capillary wall. The 3D architecture of podocyte attached to the GBM is highly complex. Each slit junction regulates the flow of a fraction of the total ultrafiltrate that flows into Bowman’s space. Ultrafiltrate emerging through a large number of slit junctions between podocyte processes coalesces to form a fluid column. Dashed arrows represent fluid column from a number of filtration sites that are too numerous to depict in a simple cartoon.
column in cm, \( \eta \) is viscosity in centipoises, and \( q \) is flow rate in mL/s. Similarly, shear stress of a fluid column in a cylinder is given by the equation: \( \tau = \frac{4qv}{rw} \), where \( \eta \) is viscosity in centipoise, \( v \) is the velocity in mL/s and \( r \) is the radius in cm. In the glomerulus, the flowing fluid column (blood) within the capillary will create shear stress on endothelial cells. Similarly, the flowing fluid column (ultrafiltrate) creates shear stress on podocytes (Figure 1).

**Methods to study the effects of biomechanical forces on podocytes and glomeruli**

Published research mentions Flexcell International Corporation (Burlington, NC, USA) and Strex Incorporation (Osaka, Japan) as major suppliers of equipment to study the effect of biomechanical forces. We have described a convenient flow chamber to study the effect of low FFSS on cells grown on glass slides or isolated rat glomeruli [12].

**Tensile stress**

Methods to study tensile or compressive stress (\( \sigma \)) are based on techniques to induce elongation or compression of cells or organelle (e.g. glomerulus). Podocytes are grown on a flexible membrane that constitutes the bottom of specially constructed cell culture dishes. Negative pressure (vacuum) causes a computer-controlled cyclical or sustained stretch in the adherent cell layer. Stretch can be applied in 1D or 2D, resulting in uniaxial or biaxial stress. Other variables include the (i) frequency (cycles/s, Hertz), (ii) magnitude (percentage change), (iii) pattern of application (square wave or sine wave) and (iv) the duration of stretch. Studies of podocytes have used 0.5–1 Hz, 2.5–20% stretch for 30 min to 3 days of continuous tensile stress. Additional methods to study axial stress include application of hydrostatic force, hypsomotic stretch or magnetic pull [13–15].

**FFSS**

Methods to study shear stress (\( \tau \)) use technique(s) for controlled flow of a fluid column over podocytes or glomeruli. Fluid flow over the cells grown on glass slides illustrates a parallel plate geometry, where FFSS \( \tau = \frac{6qv}{rw} \). In these instruments, height, width and viscosity of the fluid column (e.g. cell culture medium) are maintained constant. Therefore, the magnitude of FFSS is proportional to the rate of flow. Fluid column can be patterned as laminar, pulsatile or oscillating. Rotational flow of fluid is generated by placing the cell culture dish on an orbital shaker [16]. FFSS at the bottom of the dish is calculated using the equation \( \tau_{\text{max}} = \alpha(\rho \eta(2\pi f)^{3/2})^{1/2} \), where \( \alpha \) is the radius of orbit rotation, \( \rho \) is the density of the fluid medium, \( \eta \) is the viscosity of the fluid medium and \( f \) is the frequency of rotations per second. We have developed a flow chamber to study the effect of low FFSS on podocytes (0.2 dynes/cm\(^2\)) and glomeruli (0.3 dynes/cm\(^2\)) [17]. Others have described application of low FFSS between 0.015 and 1.75 dynes/cm\(^2\), as well as a high range between 8 and 649 dynes/cm\(^2\) [16, 18, 19].

**Elastic modulus and shear modulus**

Biophysical properties of podocytes and glomeruli [20, 21] include the elastic and shear moduli. Atomic force microscopy (AFM) and microindentation equipment are employed to measure the elastic modulus using information on the indentation depth, unloading slope and the type of indenter. The Oliver–Pharr methodology is generally used to determine the elastic indentation modulus. AFM revealed that the stiffness of HIV-infected podocytes is considerably lower than that of a normal podocyte [20]. Wyss et al. [21] measured the elastic properties (compressive modulus and shear modulus) of isolated mouse glomeruli using ‘capillary micromechanics’ and detected much lower Young’s modulus in glomeruli from mouse models of Alport syndrome or HIV-associated nephropathy compared with normal mouse glomeruli.
calculated by the equation $\tau = \frac{6 \eta q}{\pi r^4}$ for rectangular flow or $\tau = \frac{4 \eta v}{r}$ for cylindrical flow, indicating that FFSS is determined by the viscosity of the ultrafiltrate, flow rate [i.e. single nephron glomerular filtration rate (SNGFR)], and the dimensions of the fluid column. Endlich and Endlich [21] calculated the FFSS at the site of filtration slit diaphragm and the apical surface to be 8 Pa and 0.05 Pa, respectively. Friedrich et al. [18] calculated FFSS over the surface of the podocyte to be 0.3 dynes/cm² (or 0.0003 grey) using three separate models for $s$.

Using the equation $\tau = \frac{6 \eta f SNGFR}{\pi s^4(s + 2R_f)} \cdot \frac{z}{H^2} (1 - \frac{z}{C_0})$ to estimate FFSS on the surface of podocytes, we showed a 1.5- to 2-fold increase in the calculated FFSS over podocytes in animal models of solitary kidney (Figure 2) [24]. We also found that: (i) increased SNGFR, not filtration fraction, was the basis of increased FFSS over podocytes in solitary kidney and (ii) the glomerular hypertrophy that accompanies adaptation to hyperfiltration did not compensate for the increased FFSS from increased SNGFR. No similar calculations have been made to estimate FFSS over podocytes using the equation $\tau = \frac{4 \eta v}{r}$.

**Podocytes are intrinsically mechanosensitive**

Mechanical stretch was found to cause narrowing of podocytes cell body, elongation of cytoplasmic processes and hypertrophy without proliferation [25]. Tensile stress (5% linear strain at 0.5 Hz for 24–72 h) decreases transversal stress F-actin filaments but microtubules and intermediate filaments appear unaffected. An increase in radial stress fibers, which converge on actin-rich centers, occurs in almost all cells by Day 3 and reversed to baseline within 1 week post-tensile stress [26, 27]. Actin cytoskeletal changes apparently involve $\alpha$-actinin-4 [28] as well as Rho kinase activity, since Rho kinase inhibitor (Y-27632) blocks cellular changes. Tensile stress renders the actin cytoskeleton more sensitive to exogenous PGE$_2$ [29]. Overall, tensile stress alters podocyte morphology gradually through changes in the actin cytoskeleton that revert back to normal when tensile stress is stopped.

Conversely, podocytes are highly sensitive and respond immediately to FFSS [19]. FFSS increases membrane ruffling and macropinocytosis activity, diminishes transversal stress fibers and induces cortical actin within minutes. These changes can be blocked by pre-treating podocytes with indomethacin and re-capitated by treating podocytes with PGE$_2$ for 24 h without FFSS [19, 30].

Biomechanical forces may also activate pathways for cell death. Thus, while tensile stress up to 10% stretch or FFSS up to 2 dynes/cm$^2$ do not induce apoptosis in podocytes [19, 27, 30], tensile stress of 20% stretch [31] or FFSS of 10 dynes/cm$^2$ result in apoptotic changes [16].

**Mechanoperception in podocytes**

Deformation of the surface glyocalyx with the underlying stiff core proteins may mediate the perception of biomechanical forces in podocytes [32]. Among the known sensors of flow, primary cilia have been observed only in cultured podocytes and embryonic rat glomeruli. Slit diaphragm linked to adaptor molecules and actin cytoskeleton, and TRPC6 (ion channel) with BK$_{Ca}$ (stretch channel) may also act as sensors for tensile stress [23, 33].

**Mechanotransduction in podocytes**

Molecules activated in response to perturbation of the plasma membrane include stretch channels and small effector molecules such as nitric oxide, Ca$^{2+}$, angiotensin-II (Ang-II), Adenosine triphosphate (ATP) and PGE$_2$ [26, 34–36]. The following sections summarize the molecular changes that contribute to conversion of mechanical force into biochemical signals.

**Tensile stress-induced molecular changes in podocytes**

Both human and mouse podocytes show an increase in Ang-II secretion and expression of Ang-II Type 1 Receptor (AT1R) with a 50% reduction in nephrin expression when exposed to tensile stress for 24–72 h [26, 35]. The downregulation of nephrin could be blocked by AT1R antagonists or anti-Ang-II antibody. However, Liebau et al. [36] did not observe an increase in Ang-II associated with tensile stress in an earlier study.

Calcium mobilization is an important mechanism for transmitting rapid changes. Podocytes do not express voltage-gated Ca$^{2+}$ channels. Endlich et al. [27] showed that tensile stress-induced changes in actin cytoskeleton were not blocked by gadolinium, an inhibitor of stretch-activated channel, but could be blocked by nickel, a non-specific calcium blocker. These
observations suggest the significance of calcium influx and indicate the role of an unidentified channel.

Tensile stress increases the expression/release of secreted protein acidic and rich in cysteine (SPARC, osteonectin or BM-40) and osteopontin, but results to-date appear conflicting. SPARC is a matricellular protein that alters actin cytoskeleton through focal adhesion complexes and plays a role in wound healing, tissue remodeling and fibrosis. Tensile stress induces the expression and release of SPARC [37]. SPARC−/− podocytes resist detachment by tensile stress compared with SPARC+/+ cells [38]. Tensile stress upregulates osteopontin expression in cultured podocytes, and in the DOCA salt-sensitive rat model of glomerular hypertension [39]. Gene deletion in mice showed osteopontin to be mechanoprotective. Osteopontin coating increases podocytes motility, accelerates actin cytoskeleton changes and diminishes cell loss in response to stretch [40]. The protective effects of osteopontin were mediated by increased αvβ3, αvβ5 and αvβ1 integrins. However, others observed that tensile stress increases cell detachment mediated by downregulation of αvβ3 integrin [31].

Intracellular signaling induced by stretch is not well understood. Some researchers have observed that in vitro application of stretch resulted in upregulation of p38 MAPK, ERK1/2 and JNK, but not Akt and GSK3β. Other researchers, however, did not observe such changes [29, 37, 40–42].

Tensile stress activates arachidonic metabolism by cyclooxygenase-2 (COX-2) enzyme. Martineau et al. [29] found that tensile stress (1–8% stretch) resulted in increased expression of COX-2 but not COX-1, and EP3 but not EP1. A small increase in cAMP following tensile stress was significantly enhanced by exogenous PGE2 with a greater disruption of the actin cytoskeleton.

**FFSS-induced molecular changes in podocytes**

Using laminar flow at 0.25–1.75 dynes/cm², Friedrich et al. [19] showed enhanced α-actinin at cell junctions, decreased vinculin, cortical actin binding to cell margin, and unchanged ZO-1 expression. While broadband tyrosine kinase inhibitors (TKI) such as genistein and AG 82 (Tyrophostin A25) caused podocyte loss, specific inhibitors such as PP2 (src family TKI) or PD153035 (EGF receptor TKI) did not. The authors concluded that specific TKs play a protective role in podocytes against FFSS.

Huang et al. [16] applied rotational fluid movement with maximum FFSS of 10 dynes/cm². They demonstrated increased phospholipase D (PLD) activity, c-Src phosphorylation and mTOR activation, suggesting the importance of c-src/PLD1/mTOR pathway in podocytes exposed to FFSS.

We applied laminar FFSS (2 dynes/cm²) to podocytes and found increased secretion and synthesis of prostaglandin E2 (PGE2) during and after FFSS treatment [12, 30, 43]. Increased PGE2 synthesis appears to be a specific response of podocytes to FFSS, as neither Martineau et al. [29] nor we detected increased PGE2 in podocytes subjected to tensile stress (Figure 3). PGE2 interacts with four membrane localized receptors, namely EP1−EP4 of which only EP2 was upregulated by FFSS [43]. Changes in the actin cytoskeleton, COX-2, PGE2 and EP2 receptor induced by FFSS were blocked by indomethacin [12].

Hyperfiltration induced by unilateral nephrectomy of sv29 mice also increases the expression of COX-2 and EP2 in the remnant kidney [12]. A similar increase in COX-2 expression and PGE2 synthesis in renal-collecting duct cells treated with FFSS was shown by Rohatgi et al. [44, 45]. Preliminary results of our recent study suggest that application of FFSS induces an EP2-mediated Akt-GSk3β-β-catenin and not the cAMP-PKA signaling pathway (unpublished observations) (see Figure 4).

**Major differences between tensile stress and FFSS-induced changes in podocytes**

Tensile stress (i) causes formation of actin-rich centers and radial stress fibers, (ii) upregulates COX-2 without increasing PGE2 levels, (iii) upregulates EP4, but not EP2 and (iv) activates p38-MAPK and ERK1/2 pathways. On the other hand, FFSS (i) disrupts actin stress fibers with the formation of a cortical actin ring, (ii) upregulates COX-2 with increased PGE2 levels, (iii) upregulates EP2, but not EP4 and (iv) activates Akt-GSk3β-β-catenin and c-src/PLD1/mTOR pathways. Thus, tensile stress and FFSS differ with regard to their effects on the actin cytoskeleton and specificity for PGE2 receptors.
Effect of FFSS on glomeruli

Prolonged FFSS on podocytes alters glomerular filtration barrier characteristics. We have developed and used an in vitro method to study changes in glomerular albumin permeability (P_{ab}) as a surrogate of changes in glomerular function [46]. We exposed isolated decapsulated rat glomeruli to FFSS at 0.3 dynes/cm^2 for 2 h in a flow chamber designed in our laboratory. FFSS-induced increase in P_{ab} was replicated by incubation with exogenous PGE_2 without FFSS, and blocked by pre-treatment with indomethacin [12]. Thus, the COX2–PGE_2–EP2 pathway appears to participate in cellular response to FFSS.

SIGNIFICANCE OF BIOMECHANICAL FORCES IN CAKUT

As previously mentioned, CAKUT is the most common cause of CKD, with hyperfiltration as a major driver of glomerular dysfunction in affected children. RAAS blockers do not effectively delay the progression of disease [11]. Approximately 50% of children born with solitary kidney progress to ESRD as young adults [47, 48]. The Italian (ItalKid) study found that most CAKUT children progressed to ESRD post-puberty [11].

Traditional risk factors of CKD including hypertension, diabetes or proteinuria do not arise until late puberty. Children with a solitary kidney manifest hypertension (16–26%), proteinuria (19–21%) or estimated GFR <60 mL/min/1.73 m^2 (6–10%) at a median age of ~15 years [49, 50]. We submit that while persistent maladaptive hyperfiltration results in increased FFSS as well as tensile stress, FFSS is mainly responsible for early renal changes leading to progressive CKD in children born with CAKUT. While RAAS inhibitors can address glomerular hypertension, we believe ameliorating the effect of FFSS on podocytes will complement the current treatment approach.

SUMMARY AND FORWARD THOUGHTS

The term hyperfiltration-mediated injury has been used for decades but the mechanism of glomerular injury by hyperfiltration remains unclear. We have developed a paradigm that connects hyperfiltration-mediated podocyte injury to biomechanical forces. Advances in understanding mechanoperception and mechanotransduction of shear stress on podocytes will lead to novel targets to develop treatments for children with CAKUT.

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CONFLICT OF INTEREST

None declared.

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