REVIEW ARTICLE

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Insights into Glomerular Filtration and Albuminuria

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Chronic kidney diseases affect more than 10% of the world's population, and most cases arise from disorders of the kidney's filtration barrier, which is located within a million microvascular units called glomeruli.[1] Although it has been known for many decades that, in the kidney, glomeruli are the site of plasma ultrafiltration and urine production, both the molecular design and function of the filtration barrier remained elusive until recently.[2,3] Moreover, several decades since the recognition that inhibitors of the renin–angiotensin system are beneficial in reducing proteinuria and slowing the progression of diabetic kidney disease, patients are still at risk for end-stage kidney failure from diabetes and other proteinuric kidney diseases.

Evidence is emerging about the added value of sodium–glucose cotransporter 2 (SGLT2) inhibitors, beyond their glucose-lowering effect, when they are used to treat patients with or without diabetes who have proteinuria and declining kidney function.[4-6] Various mechanisms have been proposed to explain the renoprotective effect of SGLT2 inhibitors,[7] including a reduction in pressure within the glomerular capillaries, with resulting protection of glomerular podocytes, which are the targets of injury in most, if not all, proteinuric kidney diseases. Reduction of the glomerular pressure appears to be mediated by constriction of the afferent arterioles, small vessels that supply the glomerular microcirculation with enormous amounts of blood from the circulation. As discussed below, such observations align closely with our current understanding of the respective roles of glomerular capillary pressure, the glomerular basement membrane (GBM), and podocytes in regulating glomerular permeability to albumin and other proteins.

Kidney function depends on the bulk filtration of large volumes of water and small solutes to clear potential toxins that are derived from intracellular metabolism and gastrointestinal microbial metabolism, as well as to maintain salt and water and acid–base homeostasis. The glomeruli produce as much as 180 liters of glomerular filtrate per day in healthy adults, yet only very small amounts of albumin leak into the urine, the end product, with its much smaller volume.[8] Although estimates of the fraction of albumin in the glomerular filtrate (as compared with in plasma) have varied according to the techniques used to measure it, and some filtered albumin is unquestionably retrieved by tubular reabsorption,[9-11] the amount of plasma proteins that escape with the glomerular filtrate is tiny and depends on the selective permeability of the glomerular filtration barrier.[12]

Diseases that reduce the glomerular capillary surface area available for filtration or that alter the intrinsic permeability of the capillary wall reduce the glomerular filtration rate (GFR). Although downstream compensatory mechanisms maintain the glomerular–tubular balance and regulate fluids, electrolytes, and the acid–base balance at physiologic levels, even small reductions in the GFR are associ-

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ated with increased cardiovascular morbidity and mortality and reduced overall survival.[1,13,14] Albuminuria, another manifestation of diseases that affect the glomerular capillary wall by altering its selective permeability, is also associated with increased cardiovascular morbidity and mortality, even at levels of urine albumin not generally regarded as pathologic and even in the absence of hypertension and diabetes.[15,16] In this review, we discuss current insights, based on classic studies that defined the size- and charge-selective properties of the glomerular filter,[17] to help explain how the unique structure and composition of the glomerular capillary wall maintain highly selective filtration properties when healthy, and how that changes with kidney disease.

EFFECTS OF PODOCYTE DAMAGE

The capillaries in each of the million or so glomeruli in the human kidneys contain a filtration device. Each filtration device consists of three layers: specialized and fenestrated endothelium that lines the luminal side of the capillary wall; an extracellular matrix–based GBM that contains type IV collagen, laminin-521, and nidogen, as well as sulfated proteoglycans; and podocytes that cover the outer surface of the GBM, closely enveloping the glomerular capillaries through extensions (foot processes) that interdigitate with those of adjacent podocytes (Fig. 1).[18,19] The podocyte foot processes of neighboring cells are separated by filtration slits that are bridged by a membrane-like cell junction, called a slit diaphragm[20]; the foot processes are firmly attached to the GBM by various proteins that lead to cell–matrix adhesion.[21] The intricate structure of podocytes allows for ultrafiltration of the large volumes of fluid and small solutes that are necessary for normal clearance of toxic wastes; albumin and most other plasma protein components are retained in the bloodstream.

The identification of mutations in genes expressed by podocytes as the genetic cause of albuminuria in both familial and sporadic kidney disease has spurred research into podocyte pathobiology and furthered our understanding of the glomerular filtration barrier.[22-28] Such studies started about two decades ago with the identification of the genetic cause of congenital nephrotic syndrome of the Finnish type, a rare autosomal recessive disorder caused by mutations in *NPHS1*.

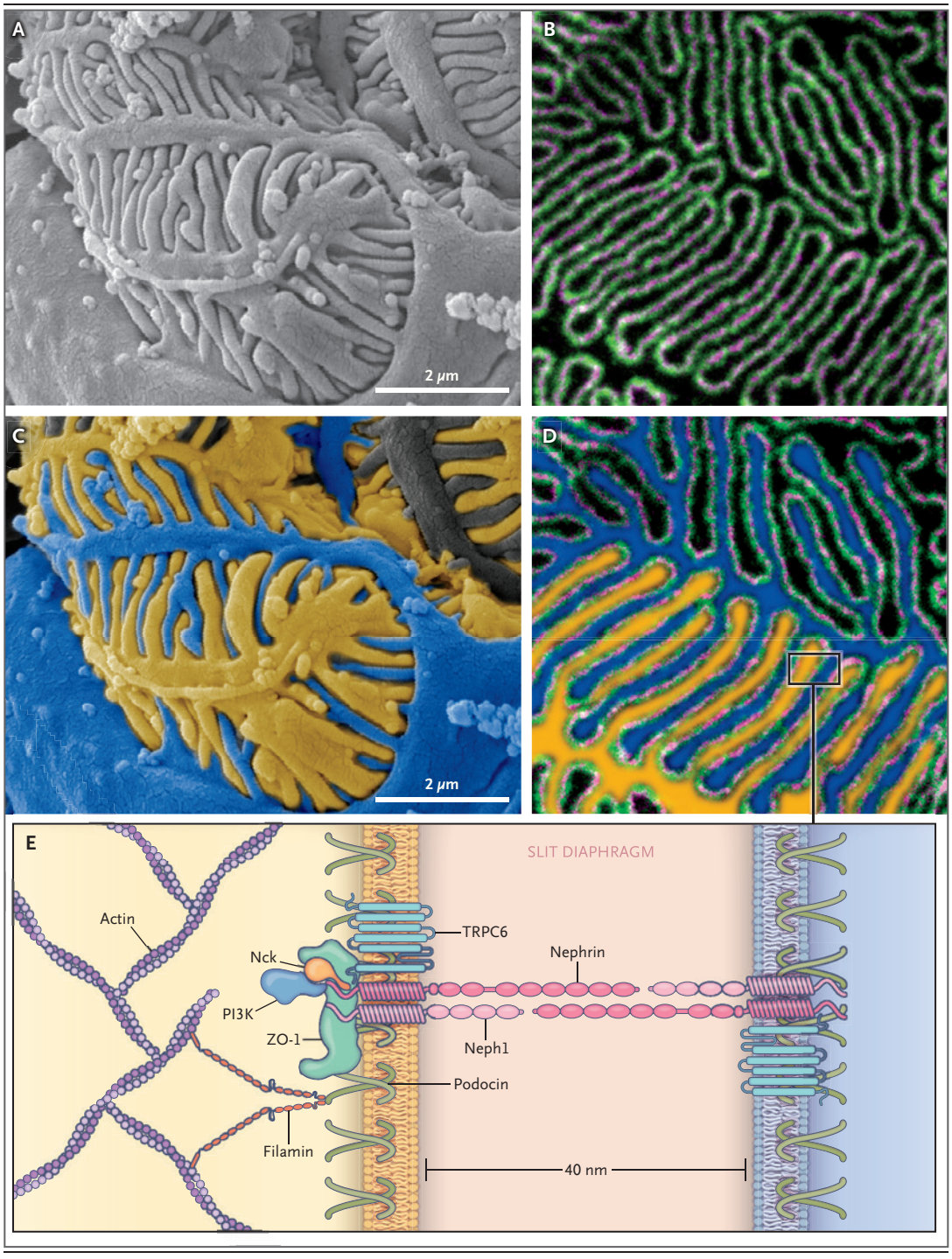
Figure 1 (facing page). Morphologic Features of Podocyte Foot Processes on Ultra-High-Resolution Imaging.

Scanning electron microscopy shows the outer aspect of glomerular capillaries, where plasma ultrafiltration occurs (Panels A and C). Stimulated emission depletion microscopy shows the slit diaphragm connecting adjacent foot processes (Panels B and D) (magenta indicates nephrin, and green, podocin). Color coding of adjacent interdigitating foot processes (Panels C and D) shows the interaction between neighboring podocytes. Also shown is a schematic representation of the slit-diaphragm protein complex that bridges the distance between neighboring foot processes and allows the formation of a filtration slit (Panel E). Nephrin and Neph1 are transmembrane proteins with extracellular domains that connect adjacent foot processes. The cytoplasmic tails of these proteins interact with scaffold proteins such as ZO-1 (zonula occludens 1), signaling adapters such as Nck, and kinases such as phosphatidylinositol 3-kinase (PI3K) to regulate the actin cytoskeleton. The membrane protein podocin clusters at the membrane, interacts with nephrin, and coordinates the protein and lipid environment at the slit-diaphragm protein complex, which renders TRPC6 (transient receptor potential cation channel 6) a mechanosensitive channel.

These mutations result in a severe albuminuria in infants and young children, along with progressive kidney failure.

NPHS1 encodes the immunoglobulin superfamily protein nephrin, a major constituent of the slit diaphragm (Fig. 1). Nephrin molecules bridge the distance between two adjacent foot processes to form a 40-nm membrane-like cell junction.[22,29,30] Part of a large multiprotein complex at the filtration slit (Fig. 1), nephrin recruits adaptor proteins to induce signaling to the podocyte cytoskeleton.[31-35] It is now clear that nephrin-based protein interactions, which are essential for shaping the unique podocyte ultrastructure, mediate signal transduction by responding to mechanical cues and controlling cytoskeletal rearrangements in podocytes (Fig. 1). Moreover, podocin, a product of *NPHS2*, has been shown to interact with nephrin at the slit diaphragm[31] and to organize the lipid environment of the slit-diaphragm complex as a mechanosensor at the filtration slit that also contains ion channels.[26,27,33] Podocin is the most commonly mutated protein in children and adolescents who have "steroid-resistant" nephrotic syndrome (nephrotic syndrome that does not remit with glucocorticoid therapy).

A number of additional podocyte-expressed genes have been identified that, when mutated, cause albuminuria, including the cytoskeletal



genes *ACTN4* and *INF2*[25,36]; these observations are consistent with the critical role of the actin cytoskeleton of podocytes in maintaining the foot-process architecture and the integrity of the glomerular filtration barrier. Studies of these mutations and the resultant mutant proteins have clearly indicated that podocyte injury can cause albuminuria. Moreover, numerous acquired diseases, including diabetic nephropathy, minimal change disease, focal and segmental glomerulosclerosis, membranous nephropathy, hypertensive kidney disease, human immunodeficiency virus–associated nephropathy, and lupus nephritis, also affect podocytes, causing dysfunction of

the filtration barrier and albuminuria. When podocytes are injured, the intercellular junctions and cytoskeletal structure of the foot processes are altered, and the cells are characterized by a simplified architecture, called foot-process effacement.[37,38] These changes are, in principle, reversible, underlining the dynamic structure of podocytes. However, podocytes are postmitotic cells and have a very limited capacity for self-renewal.[39-42] Thus, podocyte loss, whether due to detachment or cell death, results in irreversible damage and scarring of the renal filtration units.[43]

The hypothesis that podocyte loss is a culprit in the development of glomerulosclerosis was formulated more than 30 years ago[39,41] and has subsequently been proved both experimentally and clinically.[44-47] Among persons with steroid-resistant nephrotic syndrome, mutations have also been identified in genes encoding mitochondrial proteins, which lead to mitochondrial dysfunction and impaired respiratory enzyme activity.[48] Such mutations have similarly been observed in a mouse model of proteinuria in which oxygen free radical damage occurs in podocytes.[49] Although numerous mutations involving podocyte proteins have been identified — a list that keeps growing as technological advances are made and more genes are found to modulate the function of podocytes[50] — most forms of podocyte injury are acquired and of these, many are antibody-mediated.

EFFECTS OF PODOCYTE AUTOIMMUNITY

Some of the earliest examples of acquired podocyte autoimmunity were derived from studies in Heymann nephritis, a model of membranous nephropathy in rats in which circulating antibodies bind to the target antigen, megalin, in coated pits on the soles of podocyte foot processes, where they activate complement and cause morphologic changes that are characteristic of human membranous nephropathy. These changes include foot-process effacement, slit-diaphragm dislocation, severe proteinuria, and generation of reactive oxygen species, with disorganization of the GBM through new matrix production and lipid peroxidation of type IV collagen.[51,52] The antigen in most cases of human membranous nephropathy was subsequently identified and was shown to be the target of circulating autoantibodies to the M-type phospholipase A_2 receptor (PLA_2R). PLA_2R is expressed on human podocytes and is shed along with anti-PLA_2R autoantibodies to form subepithelial immune deposits.[53] A growing list of additional podocyte target antigens have subsequently been identified in anti-PLA_2R antibody–negative cases of membranous nephropathy.[54-57] Though much less common than anti-PLA_2R antibodies, these antibodies lead to the same or very similar pathological features and are manifested clinically as nephrotic syndrome or severe albuminuria.

In addition to autoantibodies to podocyte antigens as a cause of glomerulopathy, there are two unusual but highly informative examples of glomerulopathies caused by alloantibodies directed at podocyte proteins. In babies with a truncating mutation of *NPHS1* (Fin-major), the slit-diaphragm protein nephrin is absent and end-stage kidney failure develops early in life as a result. When such patients receive a kidney transplant, nephrotic syndrome sometimes recurs. However, the mechanism is different from that of congenital nephrotic syndrome. In patients in whom nephrin was never expressed, the syndrome is due to the development of antinephrin alloantibodies directed at a neoantigen in the transplanted kidney.[58,59] This observation was recapitulated in a rodent model by injecting antibodies directed at the extracellular region of nephrin.[60]

A second example of alloimmune nephropathy involving a podocyte antigen was described in babies born with nephrotic syndrome whose mothers had a deficiency of neutral endopeptidase (NEP) that was due to sensitization in previous pregnancies with a NEP-positive partner.[61,62] Transplacental passage of the maternal IgG anti-NEP antibodies bound NEP on the fetal podocytes and induced membranous nephropathy in the neonate, manifested as severe proteinuria. Podocyte injury with simplification of the foot processes and secondary changes in the GBM is common to all these conditions.

Although such studies clearly support the critical role of podocytes in maintaining a functional kidney filtration barrier, defects in the GBM, as well as injury to glomerular endothelial cells, can also cause albuminuria, reinforcing the concept that all three layers of the filtration barrier are required for permselective glomerular ultrafiltration. The contribution of the GBM may be exemplified by the fact that mutation of components of laminin-521 in Pierson's syndrome,

an inherited mutation in the laminin β_2 chain,[63] as well as mutations in the α_3, α_4, and α_5 chains of type IV collagen in Alport's syndrome,[64] results in albuminuria and progressive kidney disease. Moreover, damage to the glomerular endothelium can also cause albuminuria. For example, in preeclampsia, interference in vascular endothelial growth factor (VEGF) signaling to the glomerular endothelial cells causes albuminuria and nephrotic syndrome.[65] Preeclampsia, which affects 5 to 10% of pregnant women in the United States, is a complex hypertensive disease characterized by overexpression of soluble fms-like tyrosine kinase 1 (sFlt-1), a soluble VEGF receptor that binds and neutralizes VEGF. The resultant lack of VEGF leads to maternal vascular dysfunction and organ damage.[66,67] Similarly, anti-VEGF therapy with bevacizumab in patients with cancer can cause albuminuria, hypertension, and glomerular disease.[68,69]

A BIOPHYSICAL MODEL OF GLOMERULAR ULTRAFILTRATION

Despite decades of research on the glomerular filtration barrier, a biophysical model to explain how the kidney filter allows extensive fluid filtration while restricting the sieving of macromolecules was lacking until relatively recently.[12,70] Several decades ago, studies with electron microscopy that localized different tracers of the size of albumin or larger indicated an important role of the GBM in retaining proteins in plasma while allowing free filtration of water and solutes, since the tracers did not enter the GBM but instead were restricted to its subendothelial surface.[71,72] Damage to podocytes mediated by puromycin, an antibiotic that inhibits protein synthesis and is used to study models of proteinuria, resulted in consecutive penetration of the tracers into the GBM and uptake by podocytes.[73,74] In contrast, other injected tracers appeared to pass through the GBM but were impeded at the level of the podocyte slit diaphragm, an observation that led to the conclusion that slit diaphragms are the primary barrier of the selective filter.[75,76] For decades, the controversy over control of filtration could not be resolved, and the interpretations based on a coarse filter at the GBM followed by a fine filter at the slit diaphragm did not explain why the glomerulus does not clog with partially filtered proteins.[77]

Given the abundance of evidence that podocyte injury underlies most, if not all, proteinuric kidney diseases, new technologies, including ultrahigh-resolution imaging and genetically engineered mouse models of human disease, were used to examine the glomerular filtration barrier under conditions not previously possible with ultrastructural tracers and conventional light and fluorescence microscopy. These advances led to the development of an experimentally validated biophysical model of glomerular ultrafiltration.[78] Filtration across the glomerular capillary is driven by a net filtration pressure of roughly 20 mm Hg, derived from a hydrostatic pressure gradient of about 40 mm Hg minus the oncotic pressure of the plasma (about 24 mm Hg as blood enters the glomerular capillary), which acts to restrain filtration (Fig. 2).[80] The remarkable luminal pressure exerts physical forces on

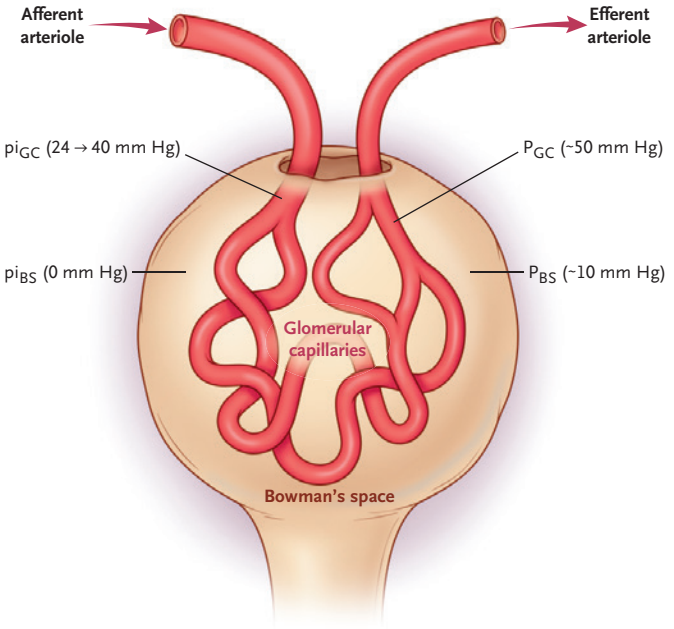


Figure 2. Pressure Gradients Driving and Inhibiting Kidney Filtration.
Filtration across the glomerular capillary is driven by a hydrostatic pressure gradient of about 40 mm Hg (the difference between glomerular capillary pressure [P_{GC}] of about 50 mm Hg and the Bowman's space hydrostatic pressure [P_{BS}] of 10 mm Hg), minus the oncotic pressure of the capillary plasma (pi_{GC}) (about 24 mm Hg as blood enters the glomerular capillary), which acts to restrain filtration. The luminal pressure exerts physical forces on the capillary wall that are counteracted by the glomerular basement membrane (GBM) and by podocytes. The pi_{GC} starts off at the value of normal arterial blood and rises as ultrafiltration removes fluid from the capillary. The oncotic pressure in Bowman's space (pi_{BS}) is constantly close to 0 mm Hg. Adapted from Giebisch and Windhager.[79]

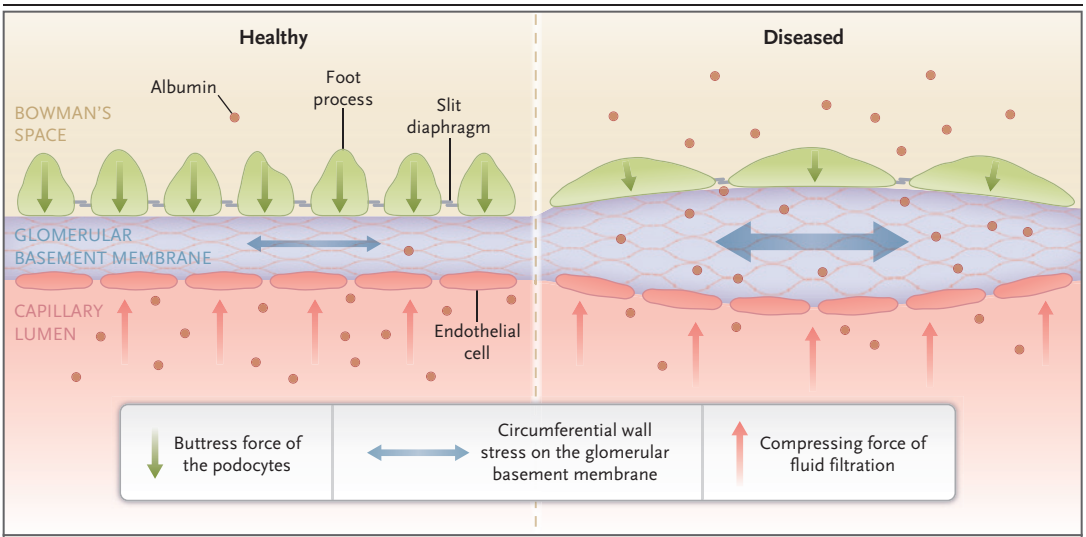


Figure 3. Gel Compression Model of Glomerular Ultrafiltration.

Interdigitating podocyte foot processes counteract the physical forces of hydrostatic pressure in the glomerular capillaries, compressing the gel-like structure of the GBM. The altered biophysical properties of the GBM act as a selectively permeable filter. When podocytes are injured, they take on a more simplified architecture and the slit-diaphragm length is greatly reduced. Concomitantly, the buttressing force of podocytes is lost and the compression force of the GBM is reduced, which increases the permeability to albumin. Adapted from Butt et al.[78]

the capillary wall that are counteracted by the GBM and by podocytes. Specifically, interdigitating podocyte foot processes serve as buttresses[81] against the physical forces of hydrostatic pressure in the glomerular capillaries, compressing the gel-like structure of the GBM (Fig. 3).[82,83] With these altered biophysical properties, the GBM acts as a permselective filter[78] and restricts the permeability to macromolecules transported by diffusion and bulk flow. Thus, the sophisticated foot-process architecture of podocytes not only maximizes the area available for the filtration of water and small solutes but also provides the mechanical resistance against blood pressure that compresses the GBM, preserving permselectivity and preventing loss of albumin and other macromolecules (Fig. 4).[78]

When podocytes are injured, they take on a more simplified architecture and the slit-diaphragm length is much reduced, resulting in a reduction in the filtration slit area and a decline in the glomerular filtration rate of water and small solutes (Fig. 4). Concomitantly, the buttressing force of podocytes is lost and the compressive force of the GBM is reduced, which increases the permeability to albumin (Fig. 3). This construct explains the conundrum of how the GFR may decline while permeability to albumin is increased, a phenomenon elegantly studied and documented in humans with proteinuric kidney disease.[84] Although the contribution of additional factors, such as electrokinetic forces at the GBM[85] and a repelling function of the charged glycocalyx of endothelial cells,[86] may also play a role, the biophysical model explains how the glomerular filter optimizes hydraulic conductivity for the filtration of enormous amounts of fluid by maximizing the filtration area (defined by the length of the filtration slit) while retarding passage of proteins through compression of the GBM.

These new data concerning glomerular filtration underscore the importance of regulated glomerular hemodynamics and have fundamental clinical implications beyond a better understanding of the beneficial effects of angiotensin-converting–enzyme inhibitors or angiotensin-receptor blockers. The length of the slit diaphragm is markedly reduced in early albuminuric disease.[78] Since the width of the filtration slit is thought to be fixed and determined by the interacting molecules that bridge the distance between adjacent foot processes, shortening the filtration slit appears to result in a reduction of the filtration area. In this scenario, the filtration rate is at least partially maintained by angioten-

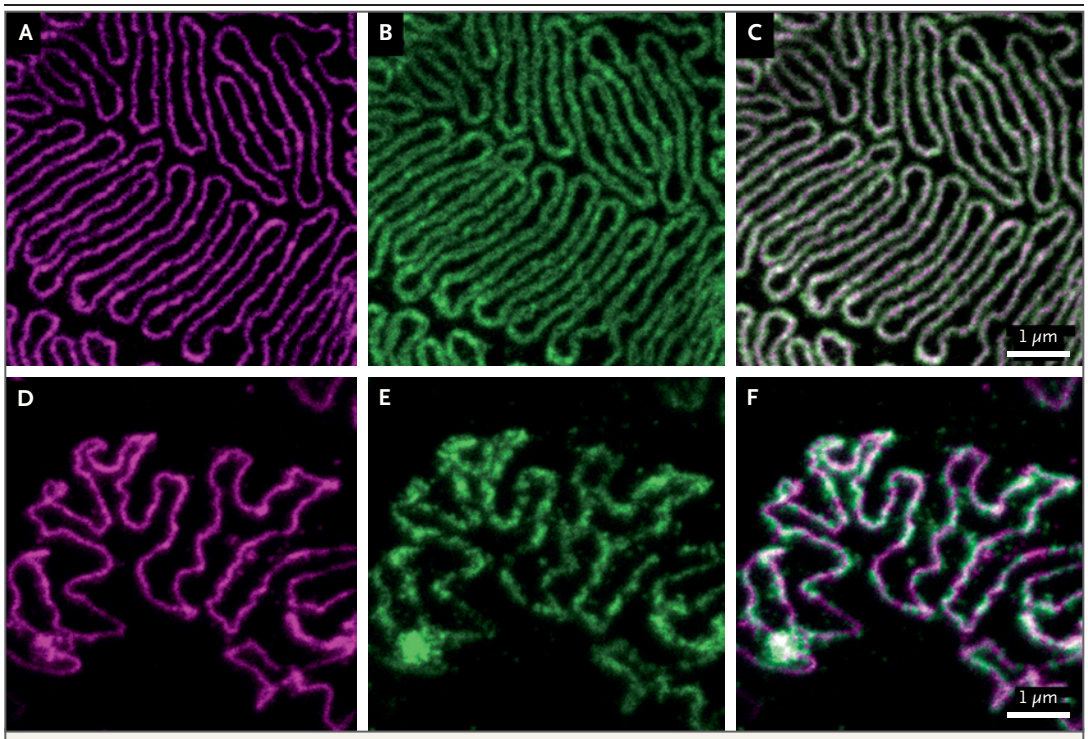


Figure 4. Damaged Podocytes Characterized by Rounded Processes and a Shortened Slit Diaphragm.

The sophisticated foot-process architecture of podocytes not only maximizes the area available for the filtration of water and small solutes but also provides the mechanical resistance against blood pressure that allows the compressed GBM to maintain selective permeability. The structure is lost in glomerular disease, resulting in a shortened slit diaphragm. Panels A, B, and C show the morphologic features of the slit diaphragm in a healthy state (in wild-type mice), and Panels D, E, and F show the altered morphologic features early in the course of the disease (with the *Nphs2*$^{R231Q/A286V}$ mutation). Magenta in Panels A and D indicates nephrin, and green in Panels B and E indicates podocin, with the overlaid colors shown in Panels C and F.

sin II–mediated contraction of the efferent arteriole, which has detrimental effects that offset the benefits of maintaining the GFR. First, the increased capillary pressure cannot be fully counteracted by the defective podocytes, which leads to an increase in proteinuria and, potentially, further injury. Second, maintaining the GFR while the filtration area is decreased drastically increases local fluid flow at the barrier, which exposes podocytes to considerable transverse shear stress and leads to loss of podocytes through detachment, as well as potential scarring of the glomeruli.[43,87] Preventing angiotensin II–mediated constriction of the efferent arteriole by blockade of the renin–angiotensin system is the cornerstone of antiproteinuric therapy to limit progressive podocyte injury and loss in diabetic and nondiabetic kidney disease.

However, hyperfiltration also occurs through loss of regulation of the afferent arteriole. Several studies have shown the mitigating effect of SGLT2 inhibitors on renal outcomes such as progression to end-stage kidney disease, doubling of the serum creatinine level, or death from renal causes in patients with diabetic (and potentially those with nondiabetic) kidney disease,[4,5,88] an effect that is thought to be primarily mediated through constriction of the afferent arteriole and prevention of hyperfiltration.[7] SGLT2 inhibition reduces reabsorption of glucose and sodium within the proximal tubule, which reestablishes sodium delivery to the macula densa and leads to a correction of hyperfiltration through tubuloglomerular feedback and afferent vasoconstriction.[89] Dysfunctional podocytes cannot sufficiently counteract elevated glomerular capillary pressure, suggesting that SGLT2-mediated afferent arteriole vasoconstriction may be beneficial (Fig. 2). The effect of SGLT2 inhibitors appears to be consistent across all levels of kid-

ney function, down to an estimated GFR of 30 ml per minute per 1.73 m^2 of body-surface area, whereas glucose-lowering effects are directly proportional to glomerular filtration and are substantially decreased when kidney function declines,[90] underscoring the importance of regulating glomerular hemodynamics in progressive renal disease.

CONCLUSIONS

Our understanding of the function of the glomerular capillary filter and the mechanisms underlying albuminuria has evolved during the past 20 years. After decades of research, there is now an opportunity to develop mechanism-based therapies that regulate glomerular hemodynamics, on the one hand, and protect mechanically sensitive podocytes, on the other hand, to prevent the progression of chronic kidney disease.

Disclosure forms provided by the authors are available with the full text of this article at NEJM.org.

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