Determinants of Glomerular Hypofiltration in Nephrotic Patients With Minimal Change Nephropathy

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ABSTRACT
Physiologic and morphologic techniques were used to eluciate the determinants of the GFR in 25 nephrotic patients with minimal change nephropathy. They were divided into two groups according to the finding of either a normal (Group 1, $N = 13$) or a depressed (Group 2, $N = 12$) inulin clearance. RPF, afferent oncotic pressure, and dextran sieving coefficients were determined. Mathematical models of glomerular ultrafiltration were then used to compute likely upper bounds for the ultrafiltration coefficient and pore area/length ratio (a measure of pore density). The upper bounds for each measure of intrinsic ultrafiltration capacity were depressed below estimated normal values in healthy controls by 55 and 47% in Group 1 patients and by 86 and 83% in Group 2 patients with minimal change nephropathy. A corresponding excess of ultrafiltration pressure (versus control), attributable solely to reduced intracapillary oncotic pressure, was by 10.8 and 11.5 mm Hg, respectively. Glomerular morphometry revealed peripheral capillary filtration surface area to be preserved in both minimal change nephropathy groups. However, a significant reduction in filtration slit frequency due to epithelial podocyte broadening correlated with the computed ultrafiltration coefficient across the two minimal change nephropathy groups ($r = 0.65; P < 0.001$). It was concluded that podocyte deformation invariably lowers the ultrafiltration coefficient and pore area/length ratio in minimal change nephropathy but that an offsetting reduction in intracapillary oncotic pressure prevents the GFR from declining in many cases. However, the models presented here predict that the depression of capillary oncotic pressure is insufficient to compensate when the ultrafiltration coefficient is lowered by substantially more than half and that it is in this circumstance that minimal change nephropathy is most likely to be accompanied by glomerular hypofiltration.

Key Words: Filtration dynamics, glomerular oncotic pressure, ultrafiltration coefficient, dextran sieving, pore density, glomerular morphometry

Minimal change nephropathy (MCN), a common cause of the nephrotic syndrome, is widely regarded as the most benign of nephrotic, glomerular injuries. Although the massive proteinuria points to severe disruption of the filtration barrier within the glomerular capillary wall, most cases are responsive to and completely reversed by immunosuppressive therapy (1,2). Furthermore, the serum creatinine level is usually maintained within the normal range, suggesting that the rate of glomerular ultrafiltration (GFR) remains rapid in this disorder (3).

Several laboratories, including our own, have used the clearance of inulin to accurately estimate the GFR in MCN. Despite normal levels of creatinine in serum, a substantial portion of cases was found to exhibit glomerular hypofiltration (4–8). The depression of GFR in MCN has, in fact, been sufficiently profound to precipitate acute renal failure (9). The latter complication has often been attributed to constriction of the effective blood volume (10). The demonstration in some cases of hyperreninemia (11,12) or coexistent ATN (9,13,14) appears to support a hemodynamic mechanism for hypofiltration, one in which the glomerular perfusion rate and, perhaps, pressure are lowered.

Another potential mechanism for hypofiltration in MCN is a reduction in the intrinsic ultrafiltration capacity of the glomerular capillary walls. Indeed, micropuncture studies of experimental models of MCN in the rat have revealed depression of the glomerular ultrafiltration coefficient ($K_f$)—the product of the hydraulic permeability of the glomerular capillary walls and the filtration surface area. In contrast, the net pressure for ultrafiltration was elevated, suggesting that a low $K_f$ can be uniquely responsible for hypofiltration (15–18). The fact that this may also be true of human MCN is suggested by the consistent demonstration in this disorder of restricted transglomerular sieving of uncharged dextran or polyvinylpyrrolidone macromolecules of in-
termediate size (7.8,19,20). Although this finding is consistent with a reduced $K_r$, a depression of the glomerular perfusion rate or pressure is predicted to result in enhancement and not depression of the transglomerular passage of uncharged macromolecules [21].

The purpose of this study was to explore the contribution of various determinants of GFR to hypofiltration in MCN. To do this, we have estimated glomerular flows, preglomerular vascular pressures, and dextran sieving coefficients in 25 nephrotic individuals whose glomeruli exhibited only minimal or minor changes by light microscopy. We then analyzed the findings with theoretical models of glomerular filtration in an effort to distinguish between possible contributions by reduced ultrafiltration pressure and diminished ultrafiltration capacity to the prevailing GFR. To define the structural correlates of impaired ultrafiltration capacity, we also subjected glomeruli obtained by needle biopsy of the kidney to a morphometric analysis.

**METHODS**

**Patient Population**

The subjects of our study were 25 adult patients who presented consecutively to our clinic with a nephrotic syndrome and a histopathologic diagnosis of MCN. The latter was based on the electron microscopic demonstration of an isolated or disproportionate injury to glomerular epithelial cells, with simplification and broadening of their podocytes. By light microscopy, the glomeruli appeared entirely normal or exhibited only minor changes (22). The most common minor change was modest mesangial cell hyperplasia; this was observed in 14 biopsy cores. In two cases, an additional minor finding was the collapse of a single segment of an isolated glomerular tuft (1 out of 20 and 37 glomeruli, respectively). In neither case was the segmental collapse accompanied by an accumulation of extracellular matrix or hyaline material. The patients varied widely in age (15 to 69 yr), and 16 of 25 were male patients. In five instances, the presenting nephrotic episode represented a relapse of MCN, which had been diagnosed by needle renal biopsy 9 to 36 mo previously. The presenting episode of nephrosis was the first for the remaining 20 subjects.

One hundred one healthy volunteers underwent an identical evaluation of glomerular function and serve as a control group. They spanned an age range (18 to 80 yr) similar to that observed in the patients with MCN and were also predominantly men (64 of 101). All denied a history of renal disease, hypertension, and diabetes. At the time of evaluation, each was found to be normotensive and normoglycemic and to have a negative dipstick test for urinary protein. The relationship between GFR (inulin clearance) and age in the healthy volunteers was used to divide the patient population with MCN into two groups (Figure 1). Group 1 was composed of 13 patients in whom the age-adjusted GFR was within or above the normal range; Group 2 was composed of the remaining 12 subjects, in whom the age-adjusted GFR was depressed (Figure 1).

**Physiologic Evaluation**

Patients and volunteers consented to undergo differential solute clearances according to a protocol that had been approved previously by the Institutional Review Board at the Stanford University School of Medicine. Each was admitted to a clinical research center on the morning of study. Antihypertensive agents were withdrawn 48 h before admission in five patients with MCN who were receiving such therapy. Urine was voided spontaneously after diuresis had been established with an oral water load (10 to 15 mL/kg). A priming dose of inulin (50 mg/kg) and para-aminobiphenyl acid (PAH; 12 mg/kg) was then administered. In a subset of 19 volunteers (age 18 to 51 yr) in 18 patients with MCN, this was followed by a priming infusion of dextran 40 (130 mg/kg per 10 min). Dextran infusion was withheld in the remaining seven subjects with MCN because each reported a previous episode of penicillin hypersensitivity, a phenomenon that we have found to be predictive of anaphylactoid reactions to dextran. Thereafter, inulin and PAH were given by continuous infusion to maintain levels in plasma constant at 20 and 1.5 mg/dL, respectively. Dextran 40 was infused constantly at half the rate calculated for inulin.

![Figure 1. GFR in patients with MCN is plotted as a function of age. The normal age-adjusted range (mean ± 1 SD per decade) is based on determinations of inulin clearance in 101 healthy volunteers and is represented as a shaded band. Patients with MCN who fall either above or below 1 SD below the mean value have been assigned to Group 1 (circles) or 2 (triangles), respectively.](image-url)
Sixty minutes after the priming infusion, arterial blood pressure was determined and blood was sampled for an examination of plasma oncotic pressure (πA) and plasma protein concentration. Four timed urine collections were then made, each of which was bracketed by a blood sample drawn from a peripheral vein. GFR was expressed as the average value for the four timed inulin clearances. The rate of RPF was estimated by dividing the corresponding clearance of PAH by an estimate of the prevailing renal arteriovenous extraction ratio for PAH (EpaH). We have shown previously that reductions of GFR and peritubular capillary protein concentration exert an additive effect to lower EpaH in patients with glomerular disease (23). The relationship observed in that study between EpaH and GFR, given either a normal or a depressed efferent arteriolar oncotic pressure (a surrogate measure for efferent protein concentration), is illustrated in Figure 2. It was used to select the value for EpaH that was most representative for each group. These were (to the first decimal place) 0.9 for healthy controls and 0.8 and 0.7 for Groups 1 and 2 MCN, respectively.

The filtration fraction (FF) was calculated by dividing the GFR by the estimated RPF. The efferent oncotic pressure (πE) was calculated by use of the equation

$$\pi_E = \pi_A/(1 - FF)$$  

Equation 1

Equation 1 assumes that the removal of water by ultrafiltration results in a linear increase in oncotic pressure as plasma flows axially along the glomerular capillaries (24). Fractional dextran clearances (θD) were calculated with the equation:

$$\theta_D = (U/P)_D/(U/P)_m$$  

Equation 2

where (U/P)_D and (U/P)_m refer to the urine-to-midpoint plasma concentration ratio of dextran and insulin, respectively.

The concentrations of insulin and PAH were determined by the use of an automated assay (25). Concentrations of dextran were assayed with anthrone after the component molecules of dextran 40 in urine and deproteinized plasma were separated into 2 Å fractions by gel permeation chromatography with precalibrated Ultraloe AcA44 columns (LKB, Pleasant Hill, CA). Concentrations of albumin and immunoglobulin G (IgG) in serum and urine were determined by immunochemical methods, which have been described in detail elsewhere (26). Plasma oncotic pressure was measured directly with a Wescor 4400 membrane osmometer (Wescor Inc., Logan, Utah). Serum and urinary creatinine levels were determined by a rate-dependent modification of the Jaffe reaction, with a Beckman Creatinine Analyzer (Model 2; Beckman Instruments, Fullerton, CA).

**Theoretical Analysis of Filtration Data**

A mathematical model for the glomerular filtration of water (27) was used to calculate Kf, which is defined in this study as the product of glomerular hydraulic permeability and the total filtration surface area of all glomerular capillaries in the two human kidneys. The input values for the model included the measured values of GFR, RPF, and πA, and an assumed value for the glomerular transcapillary hydraulic pressure difference (ΔP). The latter quantity cannot be measured directly in humans. However, using an indirect curve-fitting technique, we have estimated that ΔP approximates 35 mm Hg in the healthy human kidney and have assigned this value to the control and each MCN group in this study (28). Micropuncture determinants in rodent analogs of MCN indicate that ΔP can be elevated but is never depressed in this form of glomerular injury (15–16). Given that human MCN is accompanied by arterial hypertension (vide infra), it is probable that a fraction of the increment in arterial pressure is transmitted into the glomerular capillaries and that ΔP is likely, if anything, to be elevated. Thus, an assumption that ΔP in MCN is the same as in healthy controls is a conservative one and should provide an upper bound for the prevailing level of Kf in this disorder (27). We have accordingly designated the latter computation in MCN as "maximum" Kf (Kfmax).

We also used sieving profiles of uncharged and nonreabsorbable dextrans of broad size distribution to compute additional membrane parameters for the 18 individuals with MCN who received an infusion.
of dextran, as well as for the normal controls. For this purpose, we used a mathematical model that represents the glomerular capillary wall as a heterogeneous membrane, one containing two parallel populations of pores of widely varying sizes (29). According to this model, the membrane is dominated by a population of small and restrictive cylindrical pores of identical radius \( r_0 \). The second and parallel pore population is composed of pores that are large and nondiscriminatory, serving as a "shunt pathway" through the membrane. The shunt pathway is characterized by a parameter, \( \omega_0 \), that governs the fraction of the total filtrate volume passing through the nonrestrictive portion of the membrane. The membrane barrier to the filtration of water and uncharged macromolecules in this "isoporous plus shunt" membrane model is characterized fully by the values of \( r_0 \), \( \omega_0 \), and \( K_e \). An additional membrane parameter that can be derived from \( K_e \) and \( r_0 \) is the ratio of effective pore area-to-pore length \( (S'/l) \), which is closely related to the apparent number of restrictive pores in the two kidneys (\( N \)). More precisely,

\[
N = S'/\pi r_0^2 \quad \text{Equation 3}
\]

Thus, if \( l \) and \( r_0 \) are nearly constant, changes in \( S'/l \) are very nearly proportional to changes in the total number of restrictive pores (21).

The approach used for modeling the intrinsic membrane parameters separates their effects on dextran sieving coefficients from those due to purely hemodynamic changes (29). To allow for the effect of possible variations in AP on computed membrane parameters in patients with MCN, we performed a sensitivity analysis, repeating all calculations over a hypothetical range of AP values (30 to 40 mm Hg) that brackets the assumed control value of 35 mm Hg.

**Morphometric Evaluation**

Tissue blocks suitable for morphometric analysis were available for 17 patients (Group 1, \( N = 9 \); Group 2, \( N = 8 \)) in whom a kidney biopsy had been performed at the time of the clearance study. Biopsies performed on 10 living kidney donors at the time of renal transplantation were used to provide control values for the morphometric quantities of interest. Each of these transplant donors had been shown during a routine preoperative evaluation to have normal renal function and anatomy. They varied in age between 30 and 47 yr and 6 of the 10 were men.

All glomeruli up to a maximum of 30 in a single 1- \( \mu \)m section stained with periodic acid–Schiff reagent were analyzed at the light microscopic level. On average, 19 glomeruli per biopsy were examined in each patient with MCN (range, 7 to 30). The average number of glomeruli among the 10 control biopsies was also 19 (range, 13 to 30). The numbers of open and occluded glomerular tufts were recorded; occluded glomeruli were defined as those exhibiting global sclerosis. A dedicated computer system (Southern Micro Instruments, Inc., Atlanta, GA), consisting of a video camera, a screen, a microscope, and a digitizing tablet, was used to perform measurements (7). The outline of each glomerular tuft in the cross-section was traced onto the digitizing tablet at a magnification of \( \times 900 \), and the tuft cross-sectional area \( (A_0) \) was computed by area perimeter analysis. Glomerular volume \( (V_{G0}) \) was calculated from \( A_0 \) as described previously (7). An equation that takes the reduced diameter of globally sclerotic glomeruli into account was used to calculate their actual prevalence \( (G_1) \). The fractional interstitial area was then determined by point and intercept counting in 12 different cortical fields that were selected at random (30).

For transmission electron microscopy, tissue was fixed in 2.5% glutaraldehyde and embedded in epon. Toluidine blue–stained sections were then surveyed to locate the two open glomeruli closest to the center of each section. Ultrathin sections (60 to 70 nm) of the selected glomeruli were next stained with lead citrate and photographed. A complete montage of each glomerulus was used to calculate the peripheral capillary filtering surface density \( (S_v) \) by point and intercept counting at low magnification \( (\times 2,820) \). The filtration surface area \( S \) (in square micrometers per glomerulus) was defined as the interface between the peripheral capillary wall and the epithelium and was calculated as the product of \( S_v \) and the corresponding value of \( V_{G0} \) for the open glomeruli determined by light microscopy as defined above. Six to eight high-power electron photomicrographs were then obtained from each of the two glomerular profiles and printed at a magnification of \( \times 11,280 \) to evaluate the frequency of epithelial filtration slits and the thickness (harmonic mean) of the peripheral glomerular basement membrane (GBM). Filtration slit frequency was determined by dividing the total number of epithelial filtration slits by the total length of the GBM that was captured on the electron photomicrographs (7,30). Each high-magnification electron photomicrograph was overlaid by a grid of intersecting lines. The GBM thickness was then measured by the orthogonal intercept method (31).

**Statistical Analysis**

Differences among the two groups with MCN and the control subjects were evaluated by analysis of variance with Scheffé's test for intergroup comparisons. The values for the excretion rates and the fractional clearances of albumin and IgG and for the filtration slit frequency were skewed, and these values were logtransformed before being subjected to
analysis of variance. Results are expressed as the mean ± SE, except for those mentioned above with non-Gaussian distributions, which are presented as the median value and the range.

RESULTS

Clinical Features and Proteinuria

The patients of Group 2, in whom MCN was accompanied by the depression of GFR, were significantly older than those of Group 1. The respective median ages were 54 (range, 15 to 69) and 27 (range, 18 to 62). The excretion rates and circulating levels of albumin and IgG were similar in the two groups (Table 1). The fact that glomerular permeability to albumin and IgG was nevertheless greater in Group 2 than in Group 1 MCN is suggested by the significantly higher values for the fractional clearance of each protein (Table 1).

GFR and Determinants

The depressed GFR according to which Group 2 MCN was selected averaged 46 ± 7 mL/min per 1.73 m², versus a corresponding level of 106 ± 3 in Group 1 MCN. Hypofiltration in Group 2 was associated with a higher serum creatinine level than in Group 1 subjects, 1.47 ± 0.22 versus 0.80 ± 0.05, respectively (P < 0.001). The two groups of patients also differed in that RPF was significantly lower in Group 2, 393 ± 68 versus 731 ± 44 mL/min per 1.73 m², respectively (P < 0.001). Comparison to the controls reveals RPF to have been selectively elevated in Group 1 MCN and depressed less than in proportion to GFR in Group 2 MCN (Table 2). As a result, the FF was similarly depressed in the two MCN groups, 0.15 ± 0.01 and 0.13 ± 0.02, respectively, versus 0.19 ± 0.01 mm Hg in controls.

In keeping with their hypoalbuminemia, πₐ was similarly depressed in Groups 1 and 2 MCN, 14.3 ± 1.0 and 13.4 ± 1.7 mm Hg, respectively, versus a control value of 24.0 ± 0.2 mm Hg. Reflecting the low FF, πₐ was even more subnormal than πₐ in each group (Table 2). Averaging πₐ and πₐ in each individual, we estimate that the mean intraluminal oncotic pressure (πₒc) was depressed below the control value by 10.8 and 11.5 mm Hg, on average, in Groups 1 and 2 MCN, respectively (Figure 3). Because πₒc is the force opposing filtrate formation, this finding on its own should lead to an increase and not a decrease in GFR or FF in MCN. The mean arterial pressure

<table>
<thead>
<tr>
<th>TABLE 1. Renal handling of protein⁹</th>
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<tbody>
<tr>
<td><strong>Controls</strong> (N = 101)</td>
</tr>
<tr>
<td>Albumin Excretion Rate (µg/min)</td>
</tr>
<tr>
<td>(2–32)</td>
</tr>
<tr>
<td>IgG Excretion Rate (µg/min)</td>
</tr>
<tr>
<td>(0.1–4)</td>
</tr>
<tr>
<td>Serum Albumin Conc (g/L)</td>
</tr>
<tr>
<td>Serum IgG Conc (g/L)</td>
</tr>
<tr>
<td>θ Albumin* (×10⁻⁵)</td>
</tr>
<tr>
<td>(0.1–1)</td>
</tr>
<tr>
<td>θ IgG* (×10⁻⁵)</td>
</tr>
<tr>
<td>(1–4)</td>
</tr>
</tbody>
</table>

⁹ Mean or median values that are not different are designated with the same letter (b, c, or d).

⁹ Values are median (range). θ, fractional clearance.

<table>
<thead>
<tr>
<th>TABLE 2. Determinants of GFR⁹</th>
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<tbody>
<tr>
<td><strong>Control</strong> (N = 101)</td>
</tr>
<tr>
<td>RPF (mL/min per 1.73 m²)</td>
</tr>
<tr>
<td>FF</td>
</tr>
<tr>
<td>Mean Arterial Pressure (mm Hg)</td>
</tr>
<tr>
<td>πₐ (mm Hg)</td>
</tr>
<tr>
<td>πₐ (mm Hg)</td>
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</tbody>
</table>

⁹ Mean values that are not different are designated with the same letter (b, c, or d).
tended to exceed the control value in each group of patients with MCN, with the average excess of 18 mm Hg reaching statistical significance in Group 2. This makes it likely that ΔP was also elevated in MCN, particularly in Group 2. On the basis of the conservative assumption of an equivalent value for ΔP of 35 mm Hg in controls and each MCN group, we compute the likely upper bound for \( K_f/K_{f_{\text{max}}} \) to be depressed below the corresponding control value by 55 and 86% on average in Group 1 and 2 MCN, respectively (Figure 4). As illustrated in Figure 4, there was little overlap of the individual values for \( K_{f_{\text{max}}} \) among the three groups of subjects. Thus, according to this theoretical analysis, a \( K_f \)-lowering effect of MCN in Group 1 is offset by the elevation of RPF and the depression of \( \tau_A \), with the result that GFR is maintained in the normal range. In Group 2 MCN, however, a more profound lowering of \( K_f \) combines with a modest depression of RPF to lower GFR, notwithstanding the offsetting depression of \( \tau_A \).

**Other Intrinsic Membrane Properties of the Glomerular Capillary Wall**

The configuration of the sieving profile for dextran molecules in the 26 to 60 Å radius interval was similarly altered from control in each group of patients with MCN (Figure 5). Sieving coefficients at the low radius end of the MCN profiles were markedly depressed, whereas sieving coefficients for the largest dextran molecules examined were elevated. The Group 2 MCN sieving profile differed from that in Group 1 MCN in that it intersected the control profile at a lower radius (50 versus 58 Å) and in that there was greater enhancement of the passage of the largest dextrans examined (Figure 5).

The use of the isoporous plus shunt membrane model to integrate the dextran sieving profiles reveals a similar alteration of membrane-pore structure in each MCN group (Table 3). Both pore density (\( S'/l \)) and restrictive pore radius were markedly reduced in MCN. In contrast, as judged by the substantial enhancement of \( \omega_o \), shuntlike pores were, respectively, 10- and 3-fold more prominent in Groups 2 and 1 MCN than in controls.

Linear regression analysis revealed \( \omega_o \) to be correlated with the fractional clearances of both albumin (\( r = 0.70; P < 0.001 \)) and IgG (\( r = 0.71; P < 0.001 \)), suggesting that the impairment of barrier size selectivity contributes to proteinuria. Further, \( \omega_o \) and \( S'/l \) were inversely related (\( r = -0.70; P < 0.01 \)). Thus, the greater membrane dysfunction seen in Group 2 than in Group 1 appears to reflect a greater reduction
of pore density, along with a shift in a small fraction of such pores from the restrictive to the shuntlike portion of the membrane.

A sensitivity analysis of the effects of variations of \( \Delta P \) on \( K_f \) and pore density, the two most sensitive measures of intrinsic ultrafiltration capacity, is illustrated in Figures 6 and 7. Even in the unlikely event that \( \Delta P \) was lowered by 5 mm Hg (to only 30 mm Hg) in our hypertensive subjects with MCN, the \( K_f \) and pore density would still be lower than in healthy controls, more so in Group 2 than in Group 1 MCN. In the more likely event that \( \Delta P \) in MCN is similar to or elevated above control values, a greater loss of ultrafiltration capacity becomes evident, and there is no longer any overlap in the computed values for \( K_f \) and \( S' / l \) among the three groups in this circumstance (Figures 6 and 7). Thus, although potential contributions to the low FF in Group 1 MCN and the low GFR in Group 2 by the depression of \( \Delta P \) cannot be excluded, the impairment of the intrinsic ultrafiltration capacity of the glomerular capillary walls appears to be an invariable feature of the glomerular injury in this disorder.

### Glomerular Morphometry

The percentage of glomeruli exhibiting global glomerulosclerosis (G1) and the fractional interstitial area were slightly higher in Group 2 MCN than in either Group 1 MCN or controls (Table 4). These alterations were exclusively attributable to three elderly members of Group 2 (63 to 69 yr) who had a long history of preexisting hypertension, suggesting an association with aging and underlying occlusive renovascular disease, rather than with MCN. Because the oldest kidney donor in the control group was only 47 yr of age, we are unable to distinguish aging-associated effects on glomerular and interstitial function.

### TABLE 3. Membrane parameters

<table>
<thead>
<tr>
<th></th>
<th>Controls (N = 19)</th>
<th>Group 1 MCN (N = 11)</th>
<th>Group 2 MCN (N = 9)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Restrictive Pore Radius</strong> ( (r_0, \text{Å}) )</td>
<td>57.2 ( \pm ) 0.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>51.9 ( \pm ) 0.5&lt;sup&gt;c&lt;/sup&gt;</td>
<td>52.7 ( \pm ) 0.8&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Pore Area/Pore Length</strong> ( (S'/l, K_m) )</td>
<td>314 ( \pm ) 33&lt;sup&gt;b&lt;/sup&gt;</td>
<td>169 ( \pm ) 13&lt;sup&gt;c&lt;/sup&gt;</td>
<td>54 ( \pm ) 3&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Shunt Parameter</strong> ( (\omega_s, \times 10^{-2}) )</td>
<td>1.3 ( \pm ) 0.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.7 ( \pm ) 0.4&lt;sup&gt;c&lt;/sup&gt;</td>
<td>13.4 ( \pm ) 3.9&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> Means that are not different from one another are designated with the same letter (b, c, or d).
Figure 7. Effect of hypothetical variations of ΔP on computed pore density (S'/l) in MCN patients who received dextran: Group 1, N = 10; Group 2, N = 8. Like K (Figure 6), the S'/l is moderately depressed below control in Group 1 and severely depressed in Group 2, regardless of whether ΔP is depressed below (30 mm Hg), the same as (35 mm Hg), or elevated above (40 mm Hg) the assumed control values. The asterisks indicate a significant difference from controls by analysis of variance with the Wilcoxon rank sum test.

Figure 8. Relationship between \( K_{\text{max}} \) and filtration slit frequency (FSF) across the two MCN groups. The solid line is the regression line. PBM, peripheral GBM.

### Table 4. Morphometric analysis

<table>
<thead>
<tr>
<th></th>
<th>Controls ((N = 10))</th>
<th>Group 1 MCN ((N = 9))</th>
<th>Group 2 MCN ((N = 8))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prevalence Globally Sclerosed Glomeruli ((G, %))</td>
<td>2 ± 1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1 ± 1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3 ± 2&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Fractional Interstitial Area ((%))</td>
<td>14.5 ± 1.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>12.5 ± 0.7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>18.1 ± 2.0&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Volume Patent Glomeruli ((V, \mu m^3 \times 10^6))</td>
<td>1.37 ± 0.14&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.11 ± 0.43&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.93 ± 0.15&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Capillary Filtering Surface Area ((S, \mu m^2 \times 10^3))</td>
<td>203 ± 23&lt;sup&gt;b&lt;/sup&gt;</td>
<td>279 ± 59&lt;sup&gt;b&lt;/sup&gt;</td>
<td>239 ± 19&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Filtration Slit Frequency (slits/mm PBM length)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1,152&lt;sup&gt;b&lt;/sup&gt;</td>
<td>411&lt;sup&gt;c&lt;/sup&gt;</td>
<td>184&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>GBM Thickness (nm)</td>
<td>(1,523–1,016)</td>
<td>(616–292)</td>
<td>(799–68)</td>
</tr>
</tbody>
</table>

*Means that are not different from one another are designated with the same letter (b, c, or d).

*Values are median (range).
GFR determinants other than \( K_f \) could explain the stronger relationship between filtration slit frequency and \( K_{f\text{max}} \) than between GFR and this morphometric measure of epithelial podocyte alteration in MCN.

**Outcome of Glomerular Injury**

Each patient with MCN was treated for 6 to 12 wk with either prednisone or cyclosporine. Each member of Group 1 exhibited a complete remission of proteinuria. The proteinuria in 8 of the 12 members of Group 2 also remitted completely in response to the above therapy. In each case, either the GFR or the serum creatinine level was restored to normal after the proteinuria had remitted. One Group 2 patient with unremitting proteinuria died of septicemia 3 wk after the initiation of prednisone therapy. Proteinuria failed to remit and GFR remained depressed after the completion of a full course of therapy in the remaining three nonresponders in Group 2. These included the two patients in whom the segmental collapse of a single glomerulus was observed in the biopsy (see Methods) and who were aged 63 and 67 yr, respectively. Morphometric findings that distinguished these two latter patients were the highest prevalence of global glomerulosclerosis (13 and 17%) and the largest fractional interstitial areas (22 and 31%) observed in this study. Thus, although we have attributed these findings to unrelated occlusive renovascular disease, we cannot exclude the possibility that their nephrotic illness was associated with focal and segmental glomerulosclerosis that was not detected by our biopsy sample (22). There were no associated diseases or unusual morphologic features in the fourth member of Group 2 who failed to respond to therapy. This patient, a 20-yr-old woman, presented with a GFR of 18 ml/min per 1.73 m\(^2\) in June 1991 and received treatment with first prednisone and then cyclosporine for 3 mo each, but she has required dialytic therapy for irreversible end-stage renal failure since November 1991.

**DISCUSSION**

The finding in nephrotic patients of glomeruli that appear virtually normal by light microscopy was used to diagnose MCN in this study. Among 25 consecutive patients bearing this diagnosis, 12 were found to have a depressed GFR. A morphometric analysis confirmed that the overwhelming majority of glomeruli were patent and that the glomerular capillary surface area available for filtration was preserved, if not slightly enhanced (Table 4). As stated previously, we cannot exclude the possibility that three patients in Group 2 whose proteinuria was unresponsive to a full course of immunosuppression had undiagnosed focal and segmental glomerulosclerosis. However, our failure to detect any trace of this process in up to as many as 37 glomeruli per patient makes it unlikely that it could have been sufficiently extensive to significantly compromise filtration surface area in these individuals. By exclusion, we infer that either reduced hydraulic permeability of glomerular capillary walls or diminished ultrafiltration pressure (or some combination of the two) must provide the basis for the hypofiltration observed in this study (27).

The net pressure for ultrafiltration represents the imbalance between the glomerular transcapillary hydraulic pressure difference (\( \Delta P \)) and the opposing oncotic pressure exerted by retained proteins as plasma transits axially along the glomerular capillary tuft (\( \pi_{\text{OC}} \)). Although we were unable to determine the value for \( \Delta P \), our findings indicate that \( \pi_{\text{OC}} \) must have been profoundly lowered in MCN. Not only was the oncotic pressure of plasma entering the glomerular tuft (\( \pi_{\text{A}} \)) severely depressed, but the extent to which intraluminal protein concentration, and hence oncotic pressure, could rise during axial plasma flow was severely limited by the low FF observed in this disorder (Table 2).

We have shown (24) that glomerular capillary oncotic pressure in nephrotic humans rises in a linear fashion as water is removed by ultrafiltration; it follows that \( \pi_{\text{OC}} \), the mean oncotic pressure prevailing along the lumen of glomerular capillaries, is the arithmetic mean of \( \pi_{\text{A}} \) and \( \pi_{\text{E}} \). This estimate of \( \pi_{\text{OC}} \) was depressed in Groups 1 and 2 MCN to only 16.4 ± 1.4 and 14.9 ± 2.0 mm Hg, respectively, versus a corresponding value of 27.7 ± 0.5 mm Hg in healthy controls (Figure 3). A similar magnitude of \( \pi_{\text{OC}} \) depression can be computed from the theoretical model of glomerular ultrafiltration used in this study. The model calculates \( \pi_{\text{OC}} \) from the intraluminal total protein concentration, which is predicted to increase exponentially during axial plasma flow along the glomerular capillaries (27). Dividing the GFR by the computed value for \( K_{f\text{max}} \) yields the corresponding net pressure for ultrafiltration in each group: 18.2 ± 1.3 and 20.1 ± 2.0 mm Hg in Group 1 and 2 MCN, respectively, versus 8.6 ± 0.7 mm Hg in controls. Because \( \Delta P \) is assumed to be 35 mm Hg in all three groups (see Methods), the excess above control of the ultrafiltration pressure in Groups 1 and 2 MCN (9.6 and 11.5 mm Hg, respectively) can be attributed solely to the extent to which \( \pi_{\text{OC}} \) was lowered in MCN. Thus, at the low FF observed in humans, the average magnitude of \( \pi_{\text{OC}} \) depression in MCN can be inferred to approximate 10 to 13 mm Hg, regardless of whether the actual axial rise in this quantity is linear or exponential.

Analyses of segmental vascular resistance in experimental models of MCN in the rat by the use of the micropuncture technique have revealed resistance in afferent arterioles to be lowered in proportion to, or proportionately more than, that in efferent arterioles. In contrast, the fractional capillary pressure difference with respect to glomerular capillaries in the rat is increased, and it is possible that this increased pressure difference with respect to glomerular capillaries might contribute to the increased glomerular filtration rate in these experiments. However, these studies were performed on contralateral kidneys, and it is possible that the arteriolar response to the glomerular lesion is influenced by the presence of the control kidney.
arterioles (15–18). A comparable change in segmental resistance in human MCN would facilitate the transmission into glomerular capillaries of arterial pressure, which we have found to be elevated in the patients of this study (Table 2). The glomerular capillary hypertension, in turn, would serve to elevate and not depress ΔP. Although we cannot exclude a species difference in segmental vascular resistance in humans with MCN, it is difficult to conceive of a decline in ΔP of sufficient magnitude to offset the depression of the opposing $\pi_{oc}$ of 10 mm Hg or more. This is particularly true of Group 2 MCN, in which the arterial pressure was elevated by 18 mm Hg (Table 2). We are accordingly led to the conclusion that net ultrafiltration pressure is likely to have been elevated in Group 2 MCN and that the profound depression of $K_f$ was the proximate cause of the hypofiltration observed in these subjects.

We have already defined $K_f$ as the product of hydraulic permeability and the surface area available for filtration. As stated previously, the latter quantity was numerically larger in MCN than in controls, a consequence of a trend toward glomerular hypertrophy in this disorder (7,33) (Table 4). By exclusion, a reduction in hydraulic permeability due to alterations along the extracellular pathway for transcapillary water flux appears to be implicated as the predominant cause of the computed depression of $K_f$.

The GBM and the interpodocytic diaphragms have recently been estimated to each account for approximately 50% of the hydraulic permeability of glomerular capillary walls (32). The normal thickness of the GBM in MCN appears to point to the interpodocytic diaphragms as the most likely site for increased resistance to transcapillary water flow (34). The effacement of the podocytes severely curtails the frequency of filtration slits and thus could be the basis of an increased resistance to water flow. The diaphragms at the base of each filtration slit are perforated by apertures (so-called "slit pores") through which ultrafiltrate must ultimately pass to gain access to Bowman’s space (35). Inasmuch as the curtailment of the slit diaphragms and the slit pores could lower hydraulic permeability in MCN, our computation from dextran sieving coefficients that pore density is reduced in parallel with computed $K_f$ in MCN is consistent with this interpretation (Figure 8).

Of interest is that micropuncturists have observed a direct relationship between $\pi_a$ and $K_f$ in the normal rat (36). The fact that these quantities may also be related in humans with MCN is consistent with our recent observation that the remission of proteinuria in this disorder is accompanied by a parallel increase in both $\pi_a$ and computed $K_f$ (7). It is thus conceivable that either hypoproteinemia per se or the associated reduction in $\pi_a$ might contribute to the lowered hydraulic permeability in MCN by aggravating the injury to epithelial foot processes. The fact that this may well be so is suggested by a recent report of foot process broadening and effacement in children with kwashiorkor, who exhibited hypoproteinemia, but no proteinuria (37). Kwashiorkor has also been shown in rats chronically deprived of dietary protein to lower the $K_f$ (38). These latter findings raise the possibility that some biophysical influence associated with hypoproteinemia contributes to the observed alteration of foot process architecture and plays a role in the extent of $K_f$ depression in MCN.

We conclude that broadening of the epithelial podocytes lowers $K_f$ in patients with MCN but that a profound reduction in $\pi_{oc}$ serves to maintain a high ultrafiltration pressure in this disorder. Under the conditions of hemodynamic stability and arterial hypertension that prevailed in this study, we estimate that the epithelial cell alterations that typify MCN would have to lower $K_f$ by much more than 50% before a measurable decline in GFR will eventuate. On the other hand, a superimposed hemodynamic insult could depress ΔP acutely, thereby permitting GFR to fall at more modest levels of $K_f$ depression. Because hemodynamic instability is expected to contribute only transiently to hypofiltration in patients with MCN (9,11), we submit that the profound depression of $K_f$ along with a modest reduction in RPF, accounts for the sustained lowering of GFR that is observed in some patients with this disorder.

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