Determinants of GFR depression in early membranous nephropathy

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Hladunewich, M. A., K. V. Lemley, K. L. Blouch, and B. D. Myers. Determinants of GFR depression in early membranous nephropathy. Am J Physiol Renal Physiol 284: F1014–F1022, 2003. First published January 14, 2003; 10.1152/ajprenal.00273.2002.—We evaluated the glomerular filtration rate (GFR) in 34 subjects with membranous nephropathy (MN) of new onset. We used physiological techniques to measure GFR, renal plasma flow, and oncotic pressure and computed a value for the two-kidney ultrafiltration coefficient ($K_f$). A morphometric analysis of glomeruli in the diagnostic biopsy permitted computation of the single-nephron ultrafiltration coefficient (SNK$_f$). MN subjects were divided into two groups: moderate or severe, according to whether GFR was depressed by less or more than 50%. SNK$_f$ was normal but similar in moderate and severe MN. In contrast, two-kidney $K_f$ was significantly more depressed in severe than in moderate MN. We estimated the total number of functioning glomeruli ($N_g$) by dividing two-kidney $K_f$ by SNK$_f$. Whereas mean $N_g$ was similar in controls and moderate MN (1.5 and 1.4–1.7 $\times$ 10$^6$, respectively), it was significantly lower in severe MN (0.5 $\times$ 10$^6$). This degree of glomerulopenia was not reflected in the rate of global sclerosis. We conclude that a combination of depressed SNK$_f$ (due to foot process broadening) and profound glomerulopenia accounts for GFR depression of >50% early in the course of MN. The cause of the glomerulopenia remains to be elucidated.

glomerular filtration rate; glomerular hemodynamics; ultrafiltration coefficient; glomerular morphometry; glomerular number

THE ONSET AND EARLY STAGES of membranous nephropathy (MN) are often associated with depression of the glomerular filtration rate (GFR) (11, 25–27). This phenomenon has been shown by micropuncture study of early experimental (Heymann’s) MN in the rat to be a consequence of depression of the ultrafiltration coefficient ($K_f$), a measure of the intrinsic ultrafiltration capacity of glomerular capillary walls (1, 13, 29). The net pressure for ultrafiltration, the remaining determinant of GFR, has invariably been found to be elevated in experimental MN, indicating that $K_f$ depression is the sole factor leading to hypofiltration under these circumstances (1, 13, 29).

$K_f$ is the product of the hydraulic permeability of glomerular capillary walls and the surface area available for filtration (9). We studied these determinants of $K_f$ by using a stereological approach to quantify the structural changes in glomeruli obtained by percutaneous renal biopsy from patients with active MN (11, 27). Such studies reveal the autoimmune injury to glomerular epithelial cells (podocytes) that underlies MN to lead to gross deformation of their foot processes. An ensuing decline in the number of filtration slits, through which filtrate gains access to Bowman’s space, impairs the hydraulic permeability of the affected glomerular capillary walls (11). Our morphometric analyses have previously pointed to impaired hydraulic permeability as the only identifiable GFR-lowering factor early in the course of MN (11, 26, 27). In contrast, a recent analysis of serial biopsies revealed that both impaired hydraulic permeability and a loss of filtration surface area contributed to chronic and persistent depression of $K_f$ and GFR after 2–5 yr of MN (26).

GFR depression, presenting as azotemia, at the onset of MN has been identified as an early predictor of eventual progression to end-stage renal failure (6, 20, 22, 30). We thus designed the present study to further elucidate the mechanism of hypofiltration in early MN. We once again used morphometric techniques to examine glomerular structure in the diagnostic biopsies of a large number of patients with MN of new onset. We combined the structural findings with a physiological evaluation of GFR and its hemodynamic determinants. We then used mathematical modeling to estimate ultrafiltration capacity, both at the level of individual glomeruli [single-nephron $K_f$ (SNK$_f$)] and of the aggregate of all functioning glomeruli in the two human kidneys (2-kidney $K_f$). The subject of this report is the relationship among these two quantities and the extent to which GFR was depressed in two groups of subjects with MN of graded severity.

METHODS

Patient Population

The subjects of our study were 34 adult patients who presented to our clinic with a nephrotic syndrome and a
GFR IN MEMBRANOUS NEPHROPATHY

Histopathological diagnosis of MN. The patients varied in age from 17 to 71 yr, and 22 were men. All physiological (clearance) studies were performed within a year of the diagnostic biopsy (median interval = 2 mo). Two groups of healthy individuals were studied to provide control values for the glomerular functional and structural parameters. Control group 1 was composed of 130 healthy volunteers. Their ages varied between 18 and 80 yr, and 89 were men. The under-went renal clearance studies comparable to those performed in the patient population. Control group 2 was composed of 19 living kidney transplant donors. Their ages varied between 23 and 48 yr, and 11 were men. Each underwent a renal biopsy at the time of transplantation. All denied a history of renal disease, hypertension, or diabetes mellitus. At the time of evaluation, each was found to be normotensive and normoglycemic, to have a normal serum creatinine level, and to have a urinary protein excretion rate in the normal range.

Physiological Evaluation

All patients and volunteers underwent a determination of GFR, renal plasma flow, and preglomerular vascular pressures according to a protocol approved by the Institutional Review Board at the Stanford University School of Medicine. Initially, blood was sampled for determination of plasma oncotic pressure (\(\pi_\lambda\)). Urine was voided spontaneously after diuresis had been established with an oral water load (10 to 15 ml/kg). A priming dose of insulin (50 mg/kg) and par-aminobenzoic acid (PAH; 12 mg/kg) was then administered. Thereafter, inulin and PAH were given by continuous infusion to maintain plasma levels constant at ~20 and 1.5 mg/dl, respectively.

Sixty minutes after the priming infusion, arterial blood pressure was determined. Four timed urine collections were then made, each of which was bracketed by a blood sample drawn from a peripheral vein. The GFR was expressed as the average value for the four timed inulin clearances. The rate of renal plasma flow was estimated by dividing the corre-sponding clearance of PAH by an estimate of the prevailing renal arteriovenous extraction ratio for PAH. We showed previously that reductions of GFR and peritubular capillary protein concentration exert an additive effect to lower the PAH extraction ratio in patients with glomerular disease (3). From the relationship observed in that study between the PAH extraction ratio and GFR, we assigned the following values to the subjects of the present study: 0.9 for healthy controls, 0.8 for patients with MN and a normal GFR (>90 ml-min^-1·1.73 m^2), and 0.7 for patients with MN and a depressed GFR. Inulin and PAH were determined with col-orimetric methods using a Technicon Auto Analyzer II (3). Plasma oncotic pressure was measured directly using a Wes-cor 4400 membrane osmometer (Wescor, Logan, UT) and serum creatinine levels by a rate-dependent modification of the Jaffé reaction, employing a Beckman Creatinine Ana-lyzer (model 2, Fullerton, CA).

Morphometric Evaluation

Light microscopy. All glomeruli in a single, 1-µm-thick section stained with periodic acid-Schiff reagent were ana-lyzed at the light microscopic level. On average, 14 (range 4–49) glomeruli were examined in each diagnostic biopsy in the patients with MN. The average number of glomeruli among the 19 control biopsies was 19 (range 5–58). A dedi-cated computer system (Southern Micro Instruments, At-lanta, GA), consisting of a video camera and monitor, micro-scope, and digitizing tablet, was used to perform the mea-surements. The outline of each glomerular tuft in the section was traced onto the digitizing tablet and the mean tuft cross-sectional area was determined using computerized planimetry. The measured tuft area included any parts with segmental sclerosis. We next counted the number of patent (\(N_p\)) and globally sclerotic (\(N_s\)) glomeruli in a single section of cortical tissue. Serial sections were examined to verify the assignment of \(N_s\) in the single section. The percentage of globally sclerotic glomeruli (\(G_s\)) was calculated by

\[
G_s = \frac{N_s}{N_s + N_p}(D_p/D_s) \times 100
\]

where \(D_s\) and \(D_p\) are the mean diameters of globally sclerotic and patent glomeruli, respectively, derived from the tuft cross-sectional areas. The ratio accounts for the difference in the probability of encountering a glomerulus of either type in a random cross section due to their different sizes. Glomer-ular volume (\(V_g\)) was calculated from the average tuft cross-sectional area (\(A_{tuft}\)) as follows

\[
V_g = \frac{\beta}{d}(A_{tuft})^{3/2}(f_s)
\]

where \(\beta\) is a dimensionless shape coefficient (\(\beta = 1.38\) for spheres), \(d\) is a size distribution coefficient (\(d = 1.1\)), which is used to adjust for variations in glomerular size (28), and \(f_s\) is a correction factor for the tissue shrinkage associated with paraffin embedding (\(f_s = 1.64\)) (17). The fractional interstitial area was examined at \(\times 600\) magnification. A 10 × 10-point grid was superimposed over each field in the entire cross section, and the fraction of total area occupied by interstitium was determined by point counting. Interstitial area was defined as that outside of tubular and vascular structures, other than peritubular capillaries.

Electron Microscopy

For transmission electron microscopy, tissue was fixed in 2.5% glutaraldehyde and 2.0% paraformaldehyde in 0.1 M cacodylate buffer and then embedded in Epon. Toluidine blue-stained sections were surveyed to locate blocks with patent glomeruli present entirely within the block. An ultra-structural analysis was performed on two glomerular profiles in each patient. Ultrathin sections (~90 nm) of the selected glomeruli were stained with lead citrate and uranyl acetate. A complete montage of each glomerulus (~2,850 magnification) was prepared and line-intercept counting was used to calculate the fractional surface density of the peripheral capillary wall by standard stereologic methods (28). Six to eight images of peripheral capillary loops in each of the glomerular profiles were then photographed at \(\times 11,280\) to evaluate the frequency of epithelial filtration slits and the thickness of the peripheral glomerular basement membrane (GBM). Filtration slit frequency was determined by counting the total number of epithelial filtration slits and dividing that number by the total length of the peripheral capillary wall at the epithelial interface (11). The harmonic mean basement membrane thickness (\(\delta_{mean}\)) was calculated for each individual using the method of orthogonal intercepts (14)

\[
\delta_{mean} = \frac{8}{3\pi} \times \delta_{Helm}
\]

where \(\delta_{Helm}\) is the apparent harmonic mean basement membrane thickness. Measured thickness included both normal GBM material and intramembranous deposits. The number of intercepts per individual was between 142 and 192 on average.
Calculations

Glomerular oncotic pressure. We showed that oncotic pressure in nephritic humans rises linearly as plasma flows axially along the glomerular capillaries and water is removed by ultrafiltration (5). We first calculated efferent (postglomerular) oncotic pressure ($\pi_{GC}$) as the arithmetic mean of $\pi_A$ and $\pi_C$.

Two-kidney $K_f$. A mathematical model for the glomerular filtration of water (9, 27) was used to calculate the two-kidney $K_f$, 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, renal plasma flow, and $\pi_A$, as well as an assumed value for the glomerular transcapillary hydraulic pressure difference ($\Delta P$). The latter quantity cannot be directly measured in humans. However, using an indirect curve-fitting technique, we estimated that $\Delta P$ approximates 40 mmHg in the healthy human kidney and assigned this value to both the control and MN groups in the present study (18, 27). Micropuncture determinations in Heymann nephritis, a rodent model of MN, indicate that $\Delta P$ is invariably elevated in this form of glomerular injury (1, 13, 29). Moreover, human MN is accompanied by arterial hypertension (see below). Given that a fraction of the increment in arterial pressure is likely transmitted into glomerular capillaries, it is probable that $\Delta P$ in human MN is also elevated. Thus, an assumption that $\Delta P$ in MN is the same as in healthy controls is a conservative one and should provide an upper bound for the average $K_f$ in this disorder (27). To allow for the effect of possible variations in $\Delta P$ on computed membrane parameters in patients with MN, we performed a sensitivity analysis, repeating all calculations over a hypothetical $\Delta P$ range (35 to 45 mmHg) that brackets the assumed control value of 40 mmHg.

Single-nephron $K_f$. The total filtration surface area in a single glomerular tuft was calculated from

$$S = S_v \times V_G$$

where $S_v$ and $V_G$ are, respectively, the filtration surface density and glomerular tuft volume.

The intrinsic hydraulic permeability of the glomerular capillary wall ($k$) was estimated from the filtration slit frequency (FSF) and basement membrane thickness by using a hydrodynamic model of viscous flow that has been described in detail elsewhere (8, 11). In this model, the capillary wall consists of a large number of repeating structural units, each of which is based on a single filtration slit. The width of such a structural unit (W) is calculated from the FSF by

$$W = \frac{2}{\pi} \times \frac{1}{\text{FSF}}$$

where $2/\pi$ is a stereologic factor that accounts for the random angle of sectioning.

Considering the capillary wall as a system of resistances in series, the overall hydraulic permeability is calculated from the permeabilities of each component layer by

$$k = \frac{1}{k_{in}} + \frac{1}{k_{bm}} + \frac{1}{k_{ep}}^{-1}$$

where $k_{in}$, $k_{bm}$, and $k_{ep}$ are, respectively, the hydraulic permeabilities of the endothelium, basement membrane, and epithelium. Many of the needed structural parameters have not been measured for the human glomerular capillary wall, necessitating substitution of corresponding values derived from rats, as described in detail by us previously (11, 26). The values derived from previous studies in the rat and used in the model calculation include the permeabilities of the endothelium ($k_{in}$, $2.0 \times 10^{-7}$ m·s⁻¹·Pa⁻¹) and of the slit diaphragm ($k_{ep}$, $7.9 \times 10^{-8}$ m·s⁻¹·Pa⁻¹), the filtration slit diaphragm width ($W_s$, 41 nm), and the Darcy permeability of the glomerular basement membrane ($k_{bm}$, 2.7 nm²) (7, 10). A preliminary study in our laboratory showed that the value for $W_s$ in humans is probably quite similar (36 ± 4 nm, n = 4) and does not appear to differ between patients with MN and healthy controls ($n = 2$ each).

The permeability of the epithelial layer was calculated using

$$k_{ep} = \epsilon k_s = \frac{W_s^2 k_s}{W}$$

where $\epsilon$ is the fraction of the basement membrane area occupied by filtration slits and $W_s$ is the slit width ($\epsilon_s = W_s/W$). The permeability of the basement membrane ($k_{bm}$) was calculated using Eq. 21 of Drumond and Deen (10).

The single-nephron ultrafiltration coefficient (SNKf) was calculated from the product of filtration surface area ($S$) and the local hydraulic permeability of the walls of patent glomerular capillaries ($k$) in the glomeruli that were examined ultrastructurally. In making this calculation, we corrected for the effect of immersion fixation to decrease glomerular dimensions relative to in situ perfused glomeruli (17).

Number of glomeruli. We estimated the total number of functioning glomeruli ($N_f$) in the two kidneys as the quotient of $GFR$ in MN that

$$N_f = 2 - \text{kidney } K_f / \text{single-nephron } K_f$$

Statistical Analysis

Initially, Student’s t-test was used to assess the difference in the GFR between the control group and all patients with MN. Linear regression analysis was used to elicit possible relationships between the GFR and a number of morphometric values derived from previous studies in the rat and used in the model calculation include the permeabilities of the endothelium ($k_{in}$, $2.0 \times 10^{-7}$ m·s⁻¹·Pa⁻¹) and of the slit diaphragm ($k_{ep}$, $7.9 \times 10^{-8}$ m·s⁻¹·Pa⁻¹), the filtration slit diaphragm width ($W_s$, 41 nm), and the Darcy permeability of the glomerular basement membrane ($k_{bm}$, 2.7 nm²) (7, 10). A preliminary study in our laboratory showed that the value for $W_s$ in humans is probably quite similar (36 ± 4 nm, n = 4) and does not appear to differ between patients with MN and healthy controls ($n = 2$ each).

RESULTS

Physiological Assessment

The mean GFR in healthy controls was 101 ± 17 ml·min⁻¹·1.73 m². By contrast, the finding in MN that

$$GFR = 62 \pm 30 \text{ ml·min}^{-1} \cdot 1.73 \text{ m}^2 \quad (P < 0.0001 \text{ vs. controls})$$

indicates that in addition to being depressed, the GFR varied widely (range 10–119 ml·min⁻¹·1.73 m²). As stated above, we used a GFR above or below
50% of the average value in healthy individuals (i.e., 50 ml·min⁻¹·1.73 m²) to categorize the MN as moderate (n = 21) or severe (n = 13), respectively. Judged by the median levels of proteinuria of 6.2 g/24 h (2.2–10.5) and 13.6 g/24 h (4.8–25.9) in moderate and severe MN, respectively, the severe MN group had significantly worse nephrosis (P = 0.001).

Results of our physiological assessment of GFR and its determinants are summarized in Table 1. Whereas GFR in moderate MN tended to be slightly depressed (83 ± 15 ml/min), the corresponding rate of renal plasma flow tended to be elevated, 806 ± 188 vs. 566 ± 128 ml/min in controls (P < 0.01). Furthermore, the marked depression of GFR (29 ± 11 ml/min) according to which subjects were assigned to the severe MN group was not associated with a significant depression of the rate of renal plasma flow (504 ± 382 ml/min; Table 1). Thus, a marked depression of the filtration fraction in each category of MN, 0.11 ± 0.03 in moderate and 0.07 ± 0.03 in severe (vs. 0.18 ± 0.03 in controls), indicates that changes in determinants of GFR other than renal plasma flow must explain the observed level of hypofiltration.

Reflecting the marked hypoproteinemia, afferent oncotic pressure (πₐ) was markedly depressed in moderate MN, 15.2 ± 3.9 vs. 24.4 ± 2.4 mmHg in the control group (P < 0.001). The corresponding value for mean oncotic pressure along the glomerular capillaries (πgc) was proportionately more depressed compared with the control group, mean 16.2 ± 4.2 vs. 27.1 ± 2.6 mmHg, respectively (P < 0.001). πₐ (11.2 ± 3.3) and πgc (11.7 ± 3.6 mmHg) were significantly more depressed in the severe MN group (Table 1). The depression in πgc in MN can be inferred to elevate the net ultrafiltration pressure by ~10 and 14 mmHg in the moderate and severe groups, respectively. Because πgc is the force opposing the formation of filtrate, depression of either ΔP and/or Kf must be invoked to explain the observed hypofiltration.

In an effort to estimate the magnitude of the effect attributable to Kf depression, we first assumed a normal ΔP of 40 mmHg, a value similar to that observed by micropuncture in the normal euvolemic rat (24).

With measured values of GFR, renal plasma flow, and this value for ΔP, the ultrafiltration model of Deen et al. (9) yielded a value for two-kidney Kf of 11.0 ± 5.7 ml·min⁻¹·mmHg⁻¹ in healthy controls (Table 1 and Fig. 1). We next used a sensitivity analysis to estimate the influence of ΔP on Kf in each grade of MN. We examined the effects of a ΔP that was the same (40 mmHg), higher (45 mmHg), or lower (35 mmHg) than normal. The computed values indicate that Kf is depressed in MN regardless of the actual value of ΔP within this range. There is negligible overlap among controls, moderate and severe MN under any combination of ΔP values (Fig. 1). Because arterial pressure was elevated in MN (Table 1), we infer that ΔP is in fact likely to be elevated. For purposes of the analysis that follows, however, we made the conservative assumption that none of the increment in arterial pressure was transmitted into glomerular capillaries and that ΔP was equivalent to the control value (i.e., 40 mmHg). Because ΔP and Kf are reciprocally related, this should provide an upper bound for two-kidney Kf in MN relative to the control. This quantity, which we will refer to as Kf150, was only 34% and 10% of control Kf during moderate and severe MN, respectively (Table 1).

Morphological Assessment

Our morphometric analysis is summarized in Table 2. The first finding that is remarkable is that despite the striking differences in GFR and two-kidney filtra-

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**Table 1. Physiological data**

<table>
<thead>
<tr>
<th></th>
<th>Normal</th>
<th>Moderate MN</th>
<th>Severe MN</th>
</tr>
</thead>
<tbody>
<tr>
<td>GFR, ml·min⁻¹·1.73 m²</td>
<td>101 ± 17</td>
<td>83 ± 15</td>
<td>29 ± 11</td>
</tr>
<tr>
<td>RPF, ml·min⁻¹·1.73 m²</td>
<td>566 ± 128</td>
<td>806 ± 188</td>
<td>504 ± 382</td>
</tr>
<tr>
<td>Filtration fraction</td>
<td>0.18 ± 0.03</td>
<td>0.11 ± 0.03</td>
<td>0.07 ± 0.03</td>
</tr>
<tr>
<td>Afferent arteriole, mmHg</td>
<td>24.4 ± 2.4</td>
<td>15.2 ± 3.9</td>
<td>11.2 ± 3.3</td>
</tr>
<tr>
<td>Glomerular capillary, mmHg</td>
<td>27.1 ± 2.6</td>
<td>16.2 ± 4.2</td>
<td>11.7 ± 3.6</td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td>90 ± 9.5</td>
<td>102 ± 14̣</td>
<td>111 ± 15̣</td>
</tr>
<tr>
<td>Kf, ml·min⁻¹·mmHg⁻¹</td>
<td>11.0 ± 5.7</td>
<td>3.7 ± 0.9̣</td>
<td>1.1 ± 0.5̣</td>
</tr>
</tbody>
</table>

Values are means ± SD. GFR, glomerular filtration rate; RPF, renal plasma flow; MAP, mean arterial pressure; Kf, ultrafiltration coefficient; MN, membranous nephropathy. *P < 0.01 vs. normal, †P < 0.01 vs. moderate. ‡P < 0.05 vs. moderate.
tion capacity ($K_{f40}$) between moderate and severe MN, quantitative glomerular morphology was similar in the two grades of injury. The only histopathological finding in the diagnostic biopsy that distinguished severe from moderate injury was a substantial expansion in the former of the interstitial compartment (Table 2). The mean percent global sclerosis was similar in each injury grade (Table 2) as was the prevalence of patients with global sclerosis (8/21 and 5/13 in moderate and severe, respectively; Fig. 2). We determined filtration surface area from the product of glomerular volume and filtration surface density in the patent glomeruli (Table 2). Reflecting a near doubling of glomerular volume (Table 2), filtration surface area was increased in each MN subset (Fig. 3A). Almost all of the resistance to transcapillary water flow is exerted by the glomerular basement membrane and the diaphragms at the bases of the epithelial filtration slits (8, 10, 11). Basement membrane thickness was increased twofold, a phenomenon that is predicted to lower hydraulic permeability (Table 2). Also, broadening of foot processes lowered the frequency of intervening filtration slits to approximately one-third of normal in both in-

Table 2. Morphometric analysis

<table>
<thead>
<tr>
<th></th>
<th>Normal</th>
<th>Moderate MN</th>
<th>Severe MN</th>
</tr>
</thead>
<tbody>
<tr>
<td>Global sclerosis, %</td>
<td>2 ± 4</td>
<td>8 ± 13</td>
<td>8 ± 13</td>
</tr>
<tr>
<td>Fractional interstitial area, %</td>
<td>14 ± 4</td>
<td>19 ± 6</td>
<td>27 ± 13†</td>
</tr>
<tr>
<td>Glomerular volume, $\mu$m³ × $10^9$</td>
<td>2.3 ± 0.8</td>
<td>4.5 ± 1.4*</td>
<td>5.1 ± 1.4*</td>
</tr>
<tr>
<td>Surface density, $\mu$m²/$\mu$m³</td>
<td>0.14 ± 0.03</td>
<td>0.09 ± 0.03*</td>
<td>0.09 ± 0.02*</td>
</tr>
<tr>
<td>GBM thickness, nm</td>
<td>403 ± 62</td>
<td>817 ± 215*</td>
<td>752 ± 237*</td>
</tr>
<tr>
<td>Filtration slit frequency, #/mm</td>
<td>1,324 ± 183</td>
<td>502 ± 191*</td>
<td>367 ± 238*</td>
</tr>
</tbody>
</table>

Values are means ± SE. GBM, glomerular basement membrane. *$P < 0.01$ vs. normal. †$P < 0.01$ vs. moderate.

Fig. 2. Prevalence and extent of glomerulosclerosis in moderate (left) and severe MN (right).

Fig. 3. Box plots comparing filtration surface area ($S$; A), hydraulic permeability ($k$; B), and single-nephron $K_f$ (SNKf; C) in controls, moderate MN, and severe MN. ○ Represent outliers. *$P < 0.01$ vs. controls; †$P < 0.05$ vs. moderate.
jury grades of MN (Table 2), further limiting water flux into Bowman’s space.

We applied the foregoing findings to the mathematical model of viscous flow of Drumond and Deen (10) to calculate a value for local hydraulic permeability. It was profoundly impaired in each category of MN. Surprisingly, however, the impairment of hydraulic permeability was similar in the two injury grades, 8 ± 3 and 6 ± 3, respectively, in moderate and severe MN vs. 22 ± 3 m·s⁻¹·Pa⁻¹×10⁻¹⁰ in controls (Fig. 3B). We next calculated single-nephron $K_f$ for each individual from the product of filtration surface and hydraulic permeability. The determination of single-nephron $K_f$ from morphometric data is completely independent of the physiological determination of $K_{G0}$. It is thus of interest that a striking disparity emerged between computed single-nephron $K_f$ and two-kidney $K_f$ in the two injury grades. Whereas the value for two-kidney $K_f$ was severe < moderate MN < controls (Table 1 and Fig. 1), such a graded reduction of ultrafiltration capacity was not evident at the single-nephron level. Single-nephron $K_f$ for both moderate and severe injury (3.1 ± 1.9 and 3.2 ± 2.3 nl·min⁻¹·mmHg⁻¹, respectively) was similarly depressed below the control value (7.5 ± 2.6 nl·min⁻¹·mmHg⁻¹) (Fig. 3C). In keeping with the group findings, linear regression analysis revealed no significant relationships across the two MN groups, between GFR on the one hand and either hydraulic permeability ($R^2 = 0.11$) filtration surface area ($R^2 = 0.11$) or single-nephron $K_f$ ($R^2 = 0.006$) on the other.

Glomerular Density

Computation of functional glomerular number ($N_g$) from Eq. 9 suggests that more severe glomerulopenia is the reason for the disproportionately low GFR and two-kidney $K_f$ in severe vs. moderate MN (Table 1). Because the numerator (2-kidney $K_f$) and denominator (single-nephron $K_f$) in Eq. 9 were determined in two separate control groups (see METHODS), only a group mean value for $N_g$ in controls could be calculated. This quotient yields a value for $N_g$ of $1.5 \times 10^6$ for healthy controls (Table 3), which is close to the value of $1.2 \times 10^6$ found by direct morphometric analysis of normal kidneys at autopsy (19). The corresponding value of $N_g$ in moderate MN (i.e., assuming $\Delta P = 40$ mmHg) is computed to be $1.7 \pm 1.2 \times 10^6$. Allowing for an elevation of $\Delta P$ in the hypertensive patients with MN to 45 mmHg, the corresponding value in moderate MN for $N_g$ would be $1.4 \pm 1.0 \times 10^6$ (Table 3). Corresponding values for the $N_g$ in those with severe MN are $0.54 \pm 0.5$ and $0.45 \pm 0.4 \times 10^6$, respectively (Table 3), suggesting that $N_g$ in severe MN was considerably lower than would be suggested by the low frequency of global sclerosis. The possibility that resorption of sclerotic glomeruli masked the true extent of glomerular loss is suggested by the finding that severe, but not moderate, MN was accompanied by marked collagenization and expansion of the interstitial compartment (Table 2).

**DISCUSSION**

As might be expected, the extent to which GFR declines early in the course of MN is proportional to the magnitude of depression of two-kidney $K_f$, a measure of the total capacity for ultrafiltration of all functional glomeruli in the two human kidneys. In contrast, no such relationship is apparent between GFR and $K_f$ determined by morphometric analysis of individual glomeruli. The reason for the decline in SNK$_f$ in early MN, is a fall in hydraulic permeability ($k$). We cannot exclude the possibility that molecular rather than structural alterations in the filtration slit diaphragm lower $k$ more in severe than in moderate MN (15). It seems to us, however, that the most plausible explanation for the disparity is that the disproportionate reduction of two-kidney $K_f$ in severe MN is due to a steep reduction in the number of glomeruli.

Glomerular number has been estimated in the dog kidney. Glomerular density in a kidney biopsy core of known volume was extrapolated to cortical volume, as assessed by MRI. Subsequently, a fractionator method used for validation after nephrectomy demonstrated good agreement (2). However, this technique has not yet been applied to estimate glomerular number in humans. Because there is no technique available that is sufficiently sensitive to directly image human glomeruli in vivo at present, we estimated the number of functional glomeruli in our experimental subjects from the quotient, two-kidney $K_f$/single-nephron $K_f$. Our estimate in control subjects is in good agreement with the number of glomeruli estimated by morphometric analyses of kidneys of subjects coming to autopsy with no evidence of renal disease (19). The number was similar in patients with moderate MN. Our estimate in subjects with severe MN, however, is far lower, averaging only 500,000 glomeruli. Although we cannot exclude the possibility that the latter subjects may have been endowed with only a small number of glomeruli at birth, this seems unlikely to us for the following reasons. First, the estimated glomerular number is over two standard deviations below the mean value for normal individuals in the aforementioned autopsy study (mean = 1,234,000; coefficient of variation = 0.25). Second, glomerulopenia of similar magnitude has been demonstrated at autopsy in subjects with severe diabetic nephropathy (4). Finally, marked expansion and collagenization of the interstitial compartment in severe
but not moderate MN point to an advanced stage of chronic renal injury in the former. Taken together, these observations suggest that severe MN predisposes to glomerulosclerosis, perhaps as a result of sclerosis and resorption of heavily damaged glomeruli.

We acknowledge that our estimate of $N_g$ has limitations as neither of the values needed for the estimate, namely two-kidney $K_f$ and single-nephron $K_f$, is precisely known. The most notable error in calculating two-kidney $K_f$ is likely to arise from discrepancies between our assumed $\Delta P$ value of 40 mmHg and the actual value of $\Delta P$, which cannot be determined in humans. Several factors could lead to errors in calculation of SNKf. For example, the use of fixed correction factors for the glomerular shrinkage associated with paraffin embedding and immersion fixation could compromise the accuracy of our estimation of glomerular volume and hence filtration surface area (17). Similarly, the need to use data from rats could compromise the accuracy with which we estimated hydraulic permeability (8).

Whereas we are able to determine the dimensions of major glomerular structures in humans morphometrically, the characteristics of several “nanostructures” have to be extrapolated from reported data for the rat. These latter include the width of the filtration slit (w_s) and the fractional area of fenestrae (r_f), both of which we showed to be similar in the human glomerulus. As stated in METHODS, we find w_s in both normal human subjects and those with MN to average 36 ± 4 nm, a value quite similar to the 41 nm reported for rats (10). Similarly, using scanning electron microscopy, we showed that r_f in humans averages 0.16 vs. 0.20 in the rat (16). Because of their large dimensions, the resistance imposed by endothelial fenestrae accounts for only 1–2% of total resistance to water flow. Thus, the small aforementioned difference between humans and the rat should have a negligible influence on computed $k$ (8, 10).

A key example of a nanostructure that has not been validated in humans is the dimensions of the apertures in the filtration slit diaphragms, as determined in the normal rat by Rodewald and Karnovsky (23). Given that MN results primarily from an injury to podocytes, it is possible that changes in their foot processes could alter the dimensions of the apertures. That the latter do not influence model predictions strongly, however, has been shown in minimal change nephropathy, a glomerular injury characterized by essentially identical changes in foot processes to those seen in MN. Drumond and Deen (10) used micropuncture determinations of $K_f$ and a morphometric determination of filtration surface area in rats with adriamycin nephrosis, an analog of minimal change nephropathy, to compute an experimental value of $k$ ($k_{\exp}$) for this disorder (10). They showed that model predictions for $k$ were within the same range as $k_{\exp}$.

We also provided similar evidence to validate the model in humans with minimal change nephropathy (11). We computed $k_{\exp}$ from the above-described physiological determination of two-kidney $K_f$, an assumed value of $1.2 \times 10^6$ glomeruli and morphometrically determined filtration surface area. Once again, there was remarkably good agreement between $k_{\exp}$ and $k$ predicted by the model ($r = 0.71$, $P < 0.001$). Thus, alterations in foot processes do not seem to cause large enough changes in epithelial permeability ($k_{\exp}$) to influence the value of $k$ computed by the model using the normal rodent dimensions of the apertures in the filtration slit diaphragm. It appears that a reduction in fractional area of filtration slits, in turn a function of reduced filtration slit frequency, rather than changes in intrinsic slit diaphragm structure, is responsible for lower $k_{\exp}$ and hence $k$ under these circumstances (11).

We accordingly submit that our estimate of $k$ should yield a reasonable approximation of SNKf and thus a reasonable estimate of glomerular number. That this is indeed the case is suggested by the relatively good agreement between the mean number of glomeruli estimated in our control subjects from the quotient two-kidney $K_f$/SNKf and values determined directly in nonnephropathic individuals by using unbiased stereologic techniques at autopsy (19). The rather normal value for estimated $N_g$ in moderate MN is consistent with a relatively low frequency of global glomerulosclerosis. We infer that Eq. 9 should thus be no less successful in estimating $N_g$ in severe MN and that the marked reduction that we calculate in this setting is likely to be real, if not absolutely precise.

Our computation of greater depression of two-kidney $K_f$ in severe compared with moderate MN is influenced by the assumption of a value of $\Delta P$ of 40–45 mmHg in each grade of injury. An alternative explanation for the greater depression of GFR in severe MN is that there was marked reduction in $\Delta P$ in that group. Given known values for GFR, $\pi_A$, renal plasma flow, and single-nephron $K_f$, one can then use the ultrafiltration model of Deen et al. (8, 9) to estimate the extent to which $\Delta P$ would have to be depressed to explain the observed hypofiltration in severe MN, assuming that the premorbid number of glomeruli in both moderate and severe MN was the same as in controls, i.e., $1.5 \times 10^6$ (21). This calculation revealed that a reduction of $\Delta P$ to 18 mmHg in severe MN vs. 34 mmHg in moderate MN would be required to account for the greater depression of GFR observed in those with severe injury at baseline. There are two reasons that make this possibility unlikely, however. As stated previously, micropuncture studies in rat analogs of MN have invariably revealed afferent arteriolar dilatation with an ensuing elevation of $\Delta P$ (1, 13, 29). Even if segmental renovascular resistance in human MN differs from that in the rat, it is hard to conceive how $\Delta P$ could have been depressed by over 20 mmHg in our subjects with severe injury. Arterial pressure in these subjects exceeded normal by 21 mmHg, (Table 1). Transmission of even a minor fraction of this increment into glomerular capillaries should have elevated and not reduced $\Delta P$. By exclusion, this points to a reduction in glomerular number as the most likely explanation for the disproportionate depression of GFR in severe MN.
Another potential alteration of glomerular hemodynamics that could potentially contribute to greater GFR depression in severe than moderate MN is the significantly lower rate of renal plasma flow in the former, 504 ± 382 vs. 806 ± 188 ml·min⁻¹·1.73 m², respectively (P < 0.01). That this is unlikely to be the case is suggested by two findings, however. The first is that renal plasma flow in severe MN is not significantly different from the normal control value (566 ± 128), despite the finding that GFR is depressed by ~70% on average in the former (Table 1). Also, the significantly lower filtration fraction in severe than in moderate MN, 7 ± 3 vs. 11 ± 3%, respectively (P < 0.001; Table 1), points to a GFR-lowering effect by a determinant other than renal plasma flow. Greater Kf depression owing to glomerulopenia in severe MN could be such a determinant of the lower GFR than in moderate MN. Dividing the observed rate of total renal plasma flow by the calculated number of glomeruli in Table 3 yields a rate of renal plasma flow per nephron (assuming ΔP = 40 mmHg). Whereas the latter quantity is 816 ± 558 nl·min⁻¹·nephron⁻¹ in moderate MN, it is almost two-fold higher in severe MN at 1,679 ± 1,989 nl·min⁻¹·nephron⁻¹. Thus, if as we propose, glomerulopenia indeed contributes to the lower GFR in severe MN, the relative depression of total renal plasma flow in this circumstance simply represents a loss of capacity by the cortical microvascular bed and not a reduction in the actual glomerular perfusion rate.

We conclude that the onset of MN is accompanied by a severe depression in hydraulic permeability of the glomerular capillary wall (Fig. 3B). This is partially offset by enhancement of filtration surface area (Fig. 3A) and by profound depression of glomerular oncotic pressure (Table 1). As a result, GFR initially remains in the normal range or is depressed by <50% in moderate MN. In severe MN, by contrast, we propose that equivalent depression of hydraulic permeability in patent glomeruli is compounded by a marked reduction in functional glomerular number (Nf). Together, these two phenomena lower two-kidney Kf to a level where increases in filtration surface area in remnant glomeruli and depression of oncotic pressure can no longer adequately compensate and the GFR falls by >50%. We showed previously that a progressive reduction of GFR in MN over the medium term is a consequence of declining Kf (26). The latter is attributable, in part, to an increasing prevalence of global glomerulosclerosis, and in part to a progressive loss of filtration surface area in remnant glomeruli, with an ensuing decline in single-nephron Kf. We propose that superimposition of these medium-term changes on a markedly reduced number of glomeruli likely accounts for the subset of patients with MN, who progress rapidly to end-stage renal failure. Advances in imaging that will permit human glomeruli to be counted in vivo will be required to validate our proposal and to confirm that glomerular number is indeed depressed early in the course of severe MN.

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