RELATIONSHIPS AMONG PROTEIN AND ALBUMIN CONCENTRATIONS
AND ONCOTIC PRESSURE IN NEPHROTIC PLASMA

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AND ONCOTIC PRESSURE IN NEPHROTIC PLASMA

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ABSTRACT

We determined oncotic pressure (π) by membrane osmometry and assayed total protein (TP) and albumin (Alb) concentrations in plasma of 102 nephrotic subjects and 27 healthy controls. All three quantities were markedly depressed in the nephrotic group. When plasma was serially diluted and concentrated, nephrotic but not control plasma also exhibited a highly variable change point in the non-linear relationship between TP or Alb and π. Absent a unique change point, we developed quadratic models which incorporated TP, Alb and (TP x Alb) to prospectively predict π in unperturbed plasma. The ability of the most successful quadratic model to predict π in afferent or efferent arteriolar plasma was limited; the prediction errors reached 10 mmHg in nephrotic and 6 mmHg in control subjects. The nephrotic model coefficients also differed significantly from control, and pointed to an important influence of non-albumin proteins on π in nephrotic plasma. Investigation of the intrinsic membrane properties of diseased glomerular capillary walls requires precise knowledge of π. For this purpose we recommend that π be directly determined by membrane osmometry rather than calculated from protein concentration(s).

Key words: colloid osmotic pressure membrane osmometry afferent oncotic pressure efferent oncotic pressure quadratic prediction models glomerular filtration rate
INTRODUCTION

The rate at which fluid is exchanged across microvascular walls is strongly influenced by the colloid osmotic (or oncotic) pressure exerted by circulating proteins within the vascular space. The relationship between plasma protein concentration and oncotic pressure is non-linear, and cubic or quadratic equations have traditionally been used to compute the latter from the former (5,7,14,20). It has long been known that the cubic or quadratic relationship between protein concentration and oncotic pressure is strongly influenced by the relative concentrations of albumin and non-albumin proteins, however. For example, the profound alteration in plasma protein composition that attends the nephrotic syndrome has been shown to radically alter the relationship between protein concentration and oncotic pressure in the plasma of nephrotic rats (16). The present study was undertaken to determine whether the same holds true for nephrotic humans, and if so, to characterize the relationships in plasma among total protein and albumin concentrations, and oncotic pressure in this disorder.
METHODS

Study Populations

The experimental population was comprised of 102 patients with nephrotic range proteinuria (>3.5 g/24 hr) and edema. Their ages ranged from 15 to 69 years. Renal biopsy revealed a variety of underlying glomerular diseases. These included minimal change nephropathy (N=8), membranoproliferative glomerulonephritis (N=8), focal and segmental glomerulosclerosis (N=18), membrandous glomerulopathy (N=30) and lupus-associated glomerulonephritis (N=38). Twenty-seven healthy volunteers with no history of renal disease, diabetes or hypertension served as controls. Their ages ranged from 22 to 54 years, and each was found at the time of the study to be normotensive and to have a negative dipstick test for urinary protein.

Study Protocol

Each patient and volunteer was studied after giving consent to a protocol, approved by the Institutional Review Board of Stanford University Medical Center. All subjects were admitted to a clinical research center where they remained recumbent for 30-60 minutes before blood was sampled for estimation of total protein and albumin concentrations and oncotic pressure in plasma. Each was then given a water load (15 ml/kg per 60 min) along with a continuous infusion of inulin and PAH, calculated to maintain the respective plasma concentrations of each marker constant at 20 and 1.5 mg/dl. Four accurately timed urine collections were next obtained by spontaneous voiding. Peripheral venous blood was drawn from an indwelling cannula to bracket each urine collection. The average inulin clearance for the four collection periods was equated with the glomerular filtration rate (GFR). The rate of renal plasma flow (RPF) was calculated by dividing the corresponding PAH clearance by an assumed renal PAH extraction ratio of 0.85 in the healthy controls and 0.7 in the nephrotic patients (3). The filtration fraction was calculated by dividing the GFR by the estimated RPF. Arterial blood pressure was determined by sphygmomanometry, and mean arterial pressure was calculated from the sum of diastolic pressure and one-third of the pulse pressure.
Laboratory Determinations

Colloid Osmometry

The oncotic pressure in venous plasma was taken to be the same as that in plasma entering the glomerular tuft ($\pi_A$) and measured in a Wescor 4400 colloid osmometer (Wescor Inc., Logan, Utah, USA). This instrument contains a saline-filled reference chamber, and a sample chamber. The chambers are separated by a semipermeable membrane (SS-030, Wescor Inc.) which has a molecular weight cutoff of 30,000 daltons so as to exclude proteins the size of albumin and larger. After injecting 4.5 ml of plasma into the sample chamber, 3 minutes were allowed to elapse, a period by the end of which saline flux from the reference to the sample chamber had come to a halt. A transducer then measured the increment in hydrostatic pressure generated in the sample chamber at equilibrium. The instrument was calibrated with a water manometer (Wescor AC-010) and the oncotic pressure exerted by a 5% albumin solution was used as a calibrating standard. The interassay coefficient of variation for determination of oncotic pressure by this method was found to be 1.8%.

Protein Assays

The concentration of total protein in plasma was determined using the Pierce Assay Reagent, which combines the biuret reaction with bicinehinonic acid. The latter renders the assay less susceptible to interference from lipids. An Immunochemistry systems Analyzer II (Beckman Instruments, Inc., Brea, CA) was used to measure plasma albumin concentration by rate nephelometry. In this system the intensity of light scattered during immunoprecipitation of albumin with a specific antibody is proportional to the concentration of albumin in the solution. The interassay coefficients of variation for the total protein and albumin assays were respectively, 2.5 and 3.8%. The concentration of immunoglobulin G (IgG) in plasma was measured by radial immunodiffusion, and the concentrations of albumin and IgG in urine were determined using an enzyme-linked immunosorbent assay, as described previously (19).
In Vitro Studies

1. Serial Dilution and Concentration

To examine the relationships among total protein concentration, albumin concentration and oncotic pressure in individual subjects, the plasma of 8 nephrotic and 4 control subjects was subjected to serial dilution and concentration. The samples were diluted to lower concentration of protein to 0.1, 0.15, and 0.33 of the original concentration by addition of saline. Each sample was then concentrated to 1.3 and 1.6 times the original concentration by ultrafiltration through a Centricon 10 microconcentrator (Amicon Division, W.R. Grace & Co., Beverly, MA). The concentration of total protein and albumin at each level of dilution and concentration was then reassayed and the corresponding oncotic pressure determined.

2. Simulation of Efferent Oncotic Pressure

Knowledge of oncotic pressure at both the efferent end (\(\pi_E\)) and afferent end (\(\pi_A\)) of the glomerular capillary network is required to estimate the mean intraluminal capillary oncotic pressure opposing the formation of glomerular filtrate. We thus tested the accuracy with which \(\pi_E\) can be predicted from measured values of either total protein and albumin concentrations or \(\pi_A\). To do this we concentrated the plasma of six nephrotic and six control subjects, so as to correct for the fraction of water removed by glomerular ultrafiltration, and thus simulate the concentration of protein prevailing in the efferent arteriole in vivo. This was achieved by concentrating each plasma sample using the equation:

\[
V_E = V_A \times (1-FF)
\]

(1)

where \(V_A\) is the initial and \(V_E\) the final volume of the sample after concentration, and FF is the measured filtration fraction for each of the 12 individual subjects. Total protein and albumin concentrations, along with oncotic pressure were then determined in both the initial, unperturbed and final, concentrated samples of plasma.

Statistical Methods

We used linear and non-linear regression analyses to devise models that predict oncotic pressure from its relationship with total protein and albumin concentrations in plasma. Sample
reuse techniques, such as the bootstrap and cross-validation were used in examining residuals from predictive models to assess their goodness of fit (3,8,9). We also used various graphical methods, including q-q plots, to assess goodness of distributional fit and the appropriateness of Gaussian assumptions regarding the errors of measurement in a given model (4). Finally, a principal components analysis was used when redundancy of parameterization was observed, so as to reduce the dimensionality of the model predictors (6,8). Student’s two-tailed or the Behrens-Fisher two sample t test were used to evaluate the significance of differences between or within the groups.
RESULTS

Modeling and Predicting $\pi_A$

(a) Perturbed plasma

We first examined the relationships among total protein and albumin concentrations and oncotic pressure in the individual subjects, whose plasma samples had been serially diluted and concentrated. We initially used a graphical approach, plotting oncotic pressure for each individual against the perturbed concentrations of albumin and total protein. Both plots were non-linear. As illustrated in Figure 1, the plot of oncotic pressure against albumin concentration revealed a discrete change point, that is, an elbow at which the nature of the relationship changed. Among control subjects the change point was relatively narrowly distributed around an albumin concentration of 4 g/dl. Among nephrotic subjects the change point varied widely however, ranging from 0.1-5.5 g/dl (Figure 1).

To allow for the variability of the change point we devised a quadratic model which incorporates the change point and which is displayed as equation (2).

$$\pi = \alpha \text{Alb} + \beta \text{TP} + \gamma \text{TP}^2 + \delta \text{Alb} \times \text{TP} + \eta (\text{Alb} - c)^+.$$  \hspace{1cm} (2)

The subscripted + indicates that a negative value has been replaced by zero, Alb = albumin and TP = total protein concentration. While the constants $\alpha, \beta, \gamma, \delta$ are common to all subjects, the parameter $\eta$ and the change-point (c) are subject-dependent. Note that the last term in (2) renders the model "non-linear" in the unknown parameters. This model fitted the serial dilution and concentration data very well, with a multiple correlation coefficient far in excess of 99%. However, there remained the issue of applicability to subsequent subjects for whom the change point is not known without recourse to serial dilution and concentration of plasma. We accordingly used a statistical technique termed "the bootstrap" to assess prospective predictions (3,9). This revealed the data base illustrated in Figure 1 to be inadequate to infer accurately what the true distribution of the change point is, globally for all patients or within the subgroups of interest.
(b) **Unperturbed plasma**

The urinary losses in control and nephrotic subjects of albumin and IgG, the two most abundant proteins in plasma, are summarized in Table 1. The heavy proteinuria in the nephrotic group was accompanied by significant depression of the plasma levels of total protein, albumin and IgG. The oncotic pressure in plasma was depressed in parallel, 14.3±0.4 vs 23.1±0.5 mmHg in nephrotic and control groups, respectively.

The relationships between oncotic pressure and total protein or albumin concentrations in unperturbed plasma of the 27 control and 102 nephrotic subjects are illustrated in Figures 2 and 3. As shown, oncotic pressure varied directly with either concentration, but the relationships were highly variable. By way of example, for respective concentrations of total protein and albumin of 7 and 3.5 g/dl, measured \( \pi \) varied from 17-27 mmHg in the control subjects. The corresponding measured values for \( \pi \) in nephrotic subjects also spanned a range of 10 or more mmHg at a total protein or albumin concentration of 5 and 2.5 g/dl respectively (Figures 2 and 3).

We next used the data in Figures 1, 2 and 3 (for a total of 31 controls and 110 nephrotics) to separately predict oncotic pressure in the control and nephrotic subjects from corresponding albumin and total protein concentrations. As noted earlier, a quadratic equation has been widely used to describe the relationship between total protein concentration and that of oncotic pressure, and the albumin-to-total protein concentration ratio has been shown to influence the oncotic pressure (7,14,16). We therefore devised a model that is fully quadratic in both albumin and total protein and that incorporates synergism (interaction) between these two predictors. This model is displayed as equation (3).

\[
\pi = \alpha_{Alb} + \beta_{TP} + \gamma_{Alb^2} + \delta_{TP^2} + \eta_{Alb^*TP}
\]  

(3)

It should be noted that the component of equation (3) involving the square of albumin constitutes a potential substitute for the unknown change point in these unperturbed plasma samples.
Equation (3) fitted both control and nephrotic groups very well; multiple correlation coefficients were 99.5% for control subjects and 97.2% for nephrotic subjects (Table 2). Notwithstanding the uncommonly high levels of the multiple correlation coefficients for biological data, none of the regression coefficients in the controls and only one of the five coefficients in the nephrotic subjects was significant at the 5% level according to conventional t tests (Table 2). Thus, the parameterization given by (3) is necessarily redundant.

We accordingly used the technique of principal component analysis to reduce dimensionality by finding a few linear combinations of existing variables that retain nearly all their variability. Computing principal components revealed that only two of the five predictors of equation (3), namely the square of total protein and the product of albumin and total protein, are sufficient to predict oncotic pressure nearly as well as the five predictors in the fully quadratic model. Multiple correlations declined only to 99.2% for the controls and to 94.6% for the nephrotic subjects. Further, all coefficients were significant at less than the 5% level for both groups (Table 2). The now simpler model for prediction can be written thus.

\[ \pi = \alpha TP^2 + \beta Alb*TP \]  

(4)

It is clear from Table 2 that unlike the situation with the fully quadratic model, all terms in the principal component regression model (4) are highly significant, for controls and for nephrotics. Among the four coefficients, the coefficient of \((TP)^2\) for the nephrotics is the most important, being twice as large as the product (interaction) term and having a t-statistic of 10.46. In controls by contrast, the regression coefficient of the product term was larger than that of total protein squared, suggesting that synergism of albumin and total protein is somehow more predictive for control than for nephrotic subjects. Another difference between control and nephrotic subjects was the striking disparity between each of the coefficients in equation (3) and (4) (Table 2). As is the case for the differing change points between the two groups, the disparate coefficients with either model suggest that changes in protein composition in the nephrotic syndrome alter the relationships between \(\pi\), total protein and albumin concentrations in this order.
Since albumin is a component of total protein, we can write TP as a sum of Alb and (TP-Alb), the latter being, obviously, the sum of all non-albumin proteins. The model (4) can then be expressed as a sum of three terms: \((\text{Alb})^2\), \((\text{Alb})(\text{TP-Alb})\), and \((\text{TP-Alb})^2\). A model with these three predictors is intermediate between the fully quadratic model (3) and the reduced model (4). An analysis with principal components suggests that \((\text{Alb})^2\) will not be significantly predictive in this new model with the three predictors, and this is borne out by regressions not reported in detail here. So one may eliminate \((\text{Alb})^2\) from consideration, yielding a reduced model analogous to (4), which we refer to as (4A).

\[
\pi = (\alpha)(\text{TP-Alb})^2 + (\beta)[(\text{Alb})(\text{TP-Alb})].
\]  

(4A)

Whereas the second term in (4) quantifies the synergistic effect of albumin and total protein on the prediction of oncotic pressure, the second term in (4A) now quantifies the synergistic effect of albumin and the non-albumin proteins. Similarly, the first term in (4) quantifies the effect of the square of total protein on prediction; but the first term in (4A) quantifies the effect of the square of only the non-albumin proteins. For both controls and nephrotics, the synergistic second terms of (4A) are enormously significant, with nearly equal values and t statistics that are approximately 21 for both groups (Table 2). The first term in (4A) is also significant for both groups, but note that the signs are reversed. For the controls, \(\alpha\) is negative, which suggests that, if anything, a higher concentration of non-albumin proteins might drive down oncotic pressure. For the nephrotics \(\alpha\) is positive, suggesting that a higher concentration of non-albumin proteins may play a role in maintaining oncotic pressure in these hypo-albuminemic subjects.

To validate the fully quadratic and reduced models, we used each to predict \(\pi\) prospectively for the two groups of subjects illustrated in Figures 2 and 3. For comparison we also predicted \(\pi\) from the following "standard" equation taken from the literature and which involves total protein (TP) but not albumin concentration (7).

\[
\pi = 1.645\text{TP} + 0.29\text{TP}^2
\]  

(5)
Because equations (3) and (4) but not (5) were derived from our own data, an optimistic bias could be introduced in attempting a simple validation on the same data. We accordingly used the technique of cross-validation, which entailed our backing individual cases out of the fitting, one at a time, then fitting based on the remaining data; finally, we computed residuals by comparing an observed oncotic pressure with what was fitted from the remaining subjects' data. Histograms that summarize the quality of fits are given in Figures 4 and 5.

Figure 4 summarizes the errors between predicted and measured $\pi$ in control subjects. There was little bias in any of the models. The predicted mode for $\pi$ was within 1 mmHg of the measured value. However, the prediction errors ranged from -7 to +6 mmHg. Moreover, neither the fully quadratic nor the reduced quadratic models were measurably better than the literature model based on total protein concentration only. The bias and errors in predicting oncotic pressure were much more striking in the nephrotic population (Figure 5). The prediction errors range from -13 to +11 mmHg. Although the fully quadratic model was the least biased and yielded the smallest prediction error, it is clearly not accurate enough to replace a direct measurement of oncotic pressures.

Although (4) is far simpler and almost as predictive as (3), we prefer the latter model when direct measurements of oncotic pressure are not available. This is because the residuals from the cross-validatory fits have a nearly normal (Gaussian) distribution for (3) but not for (4) (Figure 6). The same is true for 4A, the errors for which are also distributed in a non-Gaussian fashion. The presence of a Gaussian distribution in errors in (3) suggests that we have not left anything systematically predictive out of our fully quadratic model.

**Modeling and predicting $\pi_F$**

Whereas the mean value for estimated renal plasma flow was similar in the control and nephrotic groups, the mean value for glomerular filtration rate was depressed below the control value by half in the nephrotic group (Table 3). The ensuing lowering of the filtration fraction in nephrotic subjects is predicted to lower protein concentration and oncotic pressure disproportionately at the efferent end of the glomerular capillary network. To estimate $\pi_F$ we
first employed the formulation traditionally used by renal physiologists to estimate total protein and albumin concentrations at the efferent end (TPE, AlbE) from that measured value at the afferent end (TPA, AlbA).

\[
TPE \text{ or } AlbE = TPA \text{ or } AlbA/(1-FF),
\]

where FF denotes the filtration fraction. TPE and AlbE or TPE alone were then used to compute \( \pi_E \) using equations (3) and (5). We also attempted to infer \( \pi_E \) from \( \pi_A \) by substituting oncotic pressure for total protein concentration in equation (7).

\[
\pi_E = \pi_A/(1-FF)
\]
equation (8) assumes that oncotic pressure increases in a linear fashion as plasma flows axially along the glomerular capillaries.

We then compared the value for \( \pi_E \) computed from (3) and (7), or (5) and (7), or more directly from (8) to that measured in simulated efferent arteriolar plasma [equation (1)]. The results are summarized for each individual so studied in Figure 7, which displays the absolute error (whether negative or positive) between the calculated value and the \textit{in vitro} simulation of \( \pi_E \). On average, \( \pi_E \) calculated from (8) differed from simulated \( \pi_E \) by 1.5 mmHg in the six control subjects, and by only 0.4 mmHg in the six nephrotic subjects. The fully quadratic and standard literature models resulted in consistently larger prediction errors, averaging 5.7 and 7.0 mmHg in controls and 5.3 and 3.0 mmHg in nephrotic subjects, respectively. From equation (8) (and assuming that our simulation of \( \pi_E \) was accurate) we estimate \( \pi_E \) to be disproportionately depressed in the nephrotic group (Table 3, Figure 8). Thus, the mean oncotic pressure prevailing along glomerular capillaries in nephrotic subjects is more depressed than what is suggested by the determination of \( \pi_A \) alone.
DISCUSSION

Our analyses confirm that the relationship between total protein or albumin concentration and oncotic pressure is non-linear. However, the point at which the relationship changes appears to be strongly dependent on the composition of the plasma proteins. When the change point is not known, attempts to predict oncotic pressure from protein concentration result in substantial errors.

Recent studies by Kaysen and his co-workers have shown that the nephrotic syndrome has disparate effects on the synthetic rates of albumin and IgG, the two most abundant proteins in plasma (1,12). The rate of synthesis of albumin by hepatocytes is stimulated but insufficiently to compensate for massive urinary albumin losses, with the result that the serum albumin concentration falls. By contrast, the synthetic rate of IgG, a product of plasmacytes, is not altered. As a result, only moderately heavy urinary losses of this protein cause serum IgG to decline in parallel with serum albumin (1). Current evidence suggests that the oncotic pressure of plasma perfusing the liver is a major regulator of hepatic protein synthesis (22). This has led Kaysen to propose that the low oncotic pressure that attends the nephrotic syndrome could cause a generalized stimulation of hepatic protein synthesis (1). Enhanced levels in plasma of several hepatic-derived non-albumin proteins could then help to restore the prevailing oncotic pressure, thereby offsetting the effects of the low serum albumin and IgG concentrations that typify the nephrotic syndrome (Table 1).

The identity of non-albumin proteins that could potentially enhance oncotic pressure in nephrotic plasma remains to be established. Nevertheless, several of the predictions of our quadratic models are consistent with Kaysen's hypothesis. These include the striking disparity between controls and nephrotic subjects in virtually all coefficients, regardless of which model is used (Table 2). In particular, the inference from the reduced quadratic model (4) that synergism between albumin and total protein is greater among controls than nephrotic subjects is noteworthy. Of even greater pertinence is the inference from the reduced model (4A) that the square of the concentration of non-albumin proteins exerts a positive influence on oncotic
pressure in nephrotic subjects but not in controls (Table 2). That non-albumin proteins indeed exert an important effect on oncotic pressure in nephrotic plasma appears to be borne out by the in vitro relationship between albumin concentration and oncotic pressure in plasma that has been serially diluted and concentrated. Whereas the change point in this relationship was narrowly distributed among controls, it varied widely in nephrotic subjects (Figure 1). Taken together the foregoing predictions and observation suggest that a major contribution by unidentified non-albumin proteins could account for the unsatisfactory predictability of oncotic pressure from total protein and albumin concentrations in patients with the nephrotic syndrome.

Precise knowledge of the oncotic pressure exerted by proteins as plasma flows axially along glomerular capillaries is of particular importance to students of chronic glomerular injury. In addition to causing barrier dysfunction and proteinuria, chronic diseases of the glomerulus have been shown to invariably lower the glomerular ultrafiltration coefficient ($K_f$), a measure of the intrinsic ultrafiltration capacity of the glomerular capillary walls (2,10,11,15,17,24,25). The ensuing increase in resistance to transcapillary water flux serves to lower the GFR. However, coexistent proteinuria often lowers intraluminal glomerular capillary oncotic pressure, thereby enhancing net ultrafiltration pressure and offsetting the effect of reduced $K_f$ to lower GFR. Because $K_f$ reduction is the primary abnormality in chronic glomerular diseases, a number of mathematical models have been derived to accurately determine this intrinsic property of glomerular capillary walls (7,13,21,23). All require knowledge of the driving hydraulic and opposing oncotic pressures that determine the net pressure for ultrafiltration (18). With only rare exceptions investigators who use such models to elucidate the pathophysiology of glomerular diseases have used total protein concentration to predict intraluminal, glomerular oncotic pressure. Our analysis suggests that such predictions may be in error by up to 13 mmHg, an error that approximates the actual value for net ultrafiltration pressure in glomerulopathic rats (15). Clearly errors of this magnitude would lead to correspondingly large errors in the calculation of $K_f$. 
Based on the present findings we propose that the practice of predicting oncotite pressure from plasma total protein concentration alone be abandoned, particularly in the study of nephrotic humans or animals (16). Substitution of our fully quadratic model [equation (3)], after determining the appropriate regression coefficients for a given species, is likely to yield predictions with less bias and smaller errors. Better still, we propose that direct determination of oncotite pressure be substituted for the currently used indirect, predictive methods. In our hands the determination of oncotite pressure in plasma, while avoiding prediction errors, is also more accurate and reproducible than that of total protein and albumin concentrations (see Methods). Furthermore, in the study of humans, from whom the relatively large plasma samples (~5 ml) required for oncotite pressure determination can be easily and safely obtained, the necessary equipment and laboratory supplies are considerably less costly than those required for the assay of total protein and albumin in serum. For all these reasons, we recommend that investigators of glomerular pathophysiology routinely adopt colloid membrane osmometry to estimate oncotite pressure. We submit that this methodological approach will enhance the quality of investigations designed to evaluate the intrinsic ultrafiltration properties of both healthy and diseased glomerular capillary walls.
ACKNOWLEDGEMENTS

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FIGURE LEGENDS

Figure 1. Relationship between oncotic pressure and albumin concentration in serially diluted and concentrated plasma (O) of control (upper panel) or nephrotic subjects (lower panel). The change point in the relationship is indicated by a solid square symbol. Note that the squares depicting the two lowest change points in the nephrotic group (at an albumin concentration of 0.2 and 0.4 g/dl, respectively) are partially obscured by a cluster of data points for the two most dilute plasma samples.

Figure 2. Relationship between oncotic pressure and total protein concentration is unperturbed plasma. Control subjects are depicted by an open, and nephrotic subjects by a closed symbol.

Figure 3. Relationship between oncotic pressure and albumin concentration in unperturbed plasma of control (O) and nephrotic subjects (●).

Figure 4. Prediction errors of equations (3) (upper), (4) (middle) and (5) (lower panel) in control subjects.

Figure 5. Prediction errors of equations (3) (upper), (4) (middle) and (5) (lower panel) in nephrotic subjects.

Figure 6. Quantile plots of residuals for the fully quadratic (o) and reduced quadratic models (Δ). Controls are on the left and nephrotic subjects on the right. The relative linearity of the plot for the fully but not reduced quadratic model indicates that residuals in the former have a Gaussian distribution.
Figure 7. Comparison of absolute prediction error for $\pi_E$ with equations (3) (open bars), (5) (hatched bars) and (8) (closed bars) in control (upper) and nephrotic subjects (lower panel). The subjects have been sorted in ascending order according to the absolute prediction error of equation (8).

Figure 8. Box plots of measured $\pi_A$ and $\pi_E$ calculated from equation (8) in controls (left) and nephrotic subjects (right). The horizontal lines represent (from the top downwards) the 75th, 50th, and 25th percentiles. The vertical lines represent the 90th (upper) and 10th percentiles (lower), respectively. Note that the 50th percentile for oncotic pressure increases less between afferent and efferent ends of the glomerular tuft in nephrotics than controls.
<table>
<thead>
<tr>
<th></th>
<th>Controls (n=27)</th>
<th>Nephrotics (n=102)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urinary albumin excretion¹ (µg/min)</td>
<td>6.00 (2-51)</td>
<td>5053 (2003-26300)</td>
</tr>
<tr>
<td>Urinary IgG excretion¹ (µg/min)</td>
<td>1.10 (0.2-3.9)</td>
<td>350 (22-2719)</td>
</tr>
<tr>
<td>Plasma albumin concentration (g/dl)</td>
<td>4.02 (0.07)</td>
<td>2.01 (0.08)*</td>
</tr>
<tr>
<td>Plasma IgG concentration (g/dl)</td>
<td>0.91 (0.04)</td>
<td>0.60 (0.05)*</td>
</tr>
<tr>
<td>Serum total protein concentration (g/dl)</td>
<td>6.61 (0.08)</td>
<td>5.08 (0.09)*</td>
</tr>
</tbody>
</table>

¹ Urinary protein excretion rates are expressed as the median (and range). The remaining data are expressed as a mean (± Standard Error).

* p <0.05 nephrotics vs controls
## TABLE 2

**COEFFICIENTS FOR PREDICTION MODELS**

With corresponding t-statistics and multiple correlations

<table>
<thead>
<tr>
<th></th>
<th>Controls (n=31)</th>
<th>Nephrotics (n=110)</th>
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<tbody>
<tr>
<td><strong>Equation (3)</strong></td>
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</tr>
<tr>
<td>$R^2 = 0.995$</td>
<td>$R^2 = 0.972$</td>
<td></td>
</tr>
<tr>
<td>$\alpha$</td>
<td>33.25 (1.67)</td>
<td>2.58 (0.96)</td>
</tr>
<tr>
<td>$\beta$</td>
<td>-18.53 (-1.48)</td>
<td>1.76 (1.67)</td>
</tr>
<tr>
<td>$\gamma$</td>
<td>-2.86 (-1.41)</td>
<td>-1.85 (-4.05)</td>
</tr>
<tr>
<td>$\delta$</td>
<td>2.01 (1.28)</td>
<td>-0.25 (-1.08)</td>
</tr>
<tr>
<td>$\eta$</td>
<td>-1.09 (-0.41)</td>
<td>1.45 (2.03)</td>
</tr>
<tr>
<td><strong>Equation (4)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$R^2 = 0.992$</td>
<td>$R^2 = 0.946$</td>
<td></td>
</tr>
<tr>
<td>$\alpha$</td>
<td>0.28 (3.09)</td>
<td>0.42 (10.46)</td>
</tr>
<tr>
<td>$\beta$</td>
<td>0.41 (2.72)</td>
<td>0.21 (2.35)</td>
</tr>
<tr>
<td><strong>Equation (4A)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$R^2 = 0.993$</td>
<td>$R^2 = 0.952$</td>
<td></td>
</tr>
<tr>
<td>$\alpha$</td>
<td>-1.15 (-5.49)</td>
<td>0.13 (2.24)</td>
</tr>
<tr>
<td>$\beta$</td>
<td>3.03 (20.91)</td>
<td>2.07 (21.73)</td>
</tr>
</tbody>
</table>

1 Multiple correlation ($R^2$) is provided above, and the t-statistic in parentheses alongside each model coefficient.
### TABLE 3
DETERMINANTS OF GLOMERULAR FILTRATION RATE

<table>
<thead>
<tr>
<th></th>
<th>Controls (n=27)</th>
<th>Nephrotics (n=102)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SE</td>
<td>Mean ± SE</td>
</tr>
<tr>
<td>GFR (ml/min/1.73m²)</td>
<td>107 (3)</td>
<td>55 (3)*</td>
</tr>
<tr>
<td>Renal plasma flow (ml/min/1.73m²)</td>
<td>572 (36)</td>
<td>548 (30)</td>
</tr>
<tr>
<td>Filtration fraction</td>
<td>0.19 (0.01)</td>
<td>0.11 (0.01)*</td>
</tr>
<tr>
<td>Mean arterial pressure (mmHg)</td>
<td>85 (2)</td>
<td>106 (2)*</td>
</tr>
<tr>
<td>πₐ (mmHg)</td>
<td>23.1 (0.5)</td>
<td>14.3 (0.4)*</td>
</tr>
<tr>
<td>πₑ (mmHg)¹</td>
<td>28.6 (0.7)</td>
<td>16.2 (0.5)*</td>
</tr>
</tbody>
</table>

¹ πₑ has been calculated from equation (8)

* p <0.05 nephrotics vs controls
Fully quadratic model

Reduced quadratic model

Literature quadratic model

Prediction errors in control subjects (mmHg)
Prediction errors in nephrotic subjects (mmHg)