Glomerular Ultrafiltration in Normal and Preeclamptic Pregnancy

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Abstract. GFR and renal plasma flow (RPF) decrease in preeclampsia, a serious hypertensive complication of pregnancy. Serial data derived in late pregnancy (LP) and >5 mo postpartum (PP) in 13 healthy controls and 10 preeclamptic women (13 and 5, respectively) returning PP for theoretical analysis of neutral dextran sieving curves (θ_D), are presented and are used to calculate the key determinants of glomerular ultrafiltration. Normal LP hyperfiltration was associated with increases in RPF and the ultrafiltration coefficient (K_f), as well as in the nondiscriminatory shunt pathway (ω_0) and the SD of pore size (S). Preeclamptic LP showed the largest ω_0 and S values, indicating a loss of size-selectivity, accompanying reduced K_f and RPF, both of which are implicated in the relative hypofiltration. Despite a 100-fold increase in urinary albumin excretion (UAE), LP preeclamptic θ_D values were reduced for the equivalent neutral dextran (36Å), providing indirect evidence for a loss of glomerular barrier charge-selectivity. All the determinants of GFR and all modeled parameters were comparable across both groups PP, strong evidence that preeclamptic glomerular dysfunction resolves.

Preeclampsia, a major cause of maternal and fetal morbidity and mortality, is a multisystem disorder affecting, among others, the hepatic, hematologic, and renal systems. The latter include decreases in renal plasma flow (RPF) and GFR as well as proteinuria that may be in the nephrotic range. Impaired RPF and disordered glomerular barrier integrity could both contribute to hypofiltration (1). One goal of our study was to determine the relative contributions of each in preeclampsia; the other being the quantification of glomerular barrier parameters in relation to proteinuria. Renal hemodynamics were analyzed using clearance of PAH and inulin, while fractional dextran clearances (θ_D), combined with mathematical modeling, provided glomerular sieving data, including estimation of the ultrafiltration coefficient (K_f) and glomerular porosity. Preeclamptic and healthy women were studied in late pregnancy (LP) and again 5 mo postpartum (PP).

Materials and Methods

Subjects

Thirteen normotensive, healthy white women with no family history or evidence of renal or cardiovascular disease were studied during LP (36 to 38 wk) and again at least 5 mo PP. Ten untreated preeclamptic primigravidae were also studied in LP, of which 5 returned PP. All met the criteria of the International Society for the Study of Hypertension in Pregnancy (ISSHP) for preeclampsia (*de novo* hypertension >140/90 mmHg; proteinuria >500 mg/24 h) (2). When PP, none were hypertensive, ingesting oral contraceptives, taking medication or breast-feeding. All gave informed written consent to protocols approved by the Joint Ethics Committee of Newcastle and North Tyneside Health Authority and the Universities of Newcastle upon Tyne and Northumbria at Newcastle.

Protocols

On the study morning, each subject ate a light carbohydrate breakfast but avoided tea and coffee. A 24-h urine collection was completed before the morning of the study, from which total protein excretion (TPE) was calculated. With the subject seated in a temperature-controlled room, basal blood tests were taken (full blood count, urea, and electrolytes). A priming infusion of 48 ml of dextran (10% Dextran-40 in 0.9% saline; Baxter Healthcare, UK), 10 ml of inulin (Inutest; Fresenius Kabi, Austria), and 2 ml of p-aminohippurate (PAH; 20% PAH; Merck, Sharp & Dohme) was administered over 10 min followed by a sustaining infusion (10% dextran, 5% inulin, and 2% PAH) at 1 ml/min (3). After an hour to allow equilibration, the volunteer voided and then three 20-min urine collections were made and blood sampled in the mid-period (clearances 1, 2, and 3). Water was ingested to replace preceding urinary losses. BP was measured at 30 min intervals (Datascope).

GFR and RPF were calculated from the mean of three inulin and PAH clearances (1, 2, and 3). The renal PAH extraction rate in pregnancy was assumed to be 0.85 (4). The clearances of neutral dextrans (θ_D) were measured in clearance period 3. Analytical procedures and across-batch coefficients of variation (CV) for inulin, PAH, total serum protein, TPE, urinary albumin excretion (UAE), and dextran have been described elsewhere, as have the formulae used in all the calculations (5). Dextran separation, for radii ranging from 31 to 65Å, was undertaken using gel permeation chromatography with a

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refractive index detector where the integrator divided the chromatogram into 38 integrations per ml of elution volume, with no interference from protein(s) or inulin. Calibration with five standards (6.7, 11.7, 27.0, 42.8, and 78.8 kD) was undertaken every 3 wk, and the 42.8 kD standard was used daily. Further details of chromatography and integration using online analysis programs (PCGCP software) are described in a previous publication (5).

Renal Hemodynamic Measurements

Afferent glomerular oncotic pressure (π_A) was derived from total serum protein concentration (C) in g/dl using the following formula (6):

(1) $\pi_A = 2.1C + 0.16C^2 + 0.009C^3$

Filtration fraction (FF) is calculated from GFR/RPF. The relationship between GFR and its determinants was examined using the following formula:

(2) $GFR = K_f (\Delta P - \pi_{GC})$

π_{GC} is the glomerular oncotic pressure and can be approximated by taking a mean of afferent (π_A) and efferent (π_E) glomerular oncotic pressures (7). This provides an underestimate of K_f because π_{GC} is calculated assuming a linear increase in protein concentration between the afferent and efferent ends of the glomerulus, which is not the case. Methods of estimating π_{GC} are detailed elsewhere (7). π_E is approximated using the following formula:

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

Dextran Sieving Analysis and Heteroporous Membrane Modeling

Dextran-40, a polymer of anhydroglucose encompassing a wide range of molecular weights, is neither secreted nor reabsorbed by the renal tubule with urinary concentration reflecting transglomerular passage, thus providing glomerular size-selectivity data. Mathematical modeling combines fractional dextran sieving data (θ_D) with π_A and renal hemodynamic measurements at an assumed transglomerular hydrostatic pressure difference (ΔP), enabling otherwise inaccessible ultrafiltration parameters to be estimated. Two theoretical models of glomerular function were utilized (7,8,9,10). Each model represents the glomerular capillary wall as a heteroporous membrane characterized by two pore parameters. The isoporous + shunt model assumes that the capillary wall is perforated by a series of restrictive pores of identical radius (r_0) with a parallel shunt pathway (ω_0) that fails to restrict the passage of large molecules. The log-normal model represents the capillary wall as being perforated by a single continuous population of pores with a log-normal distribution of radii, characterized by a mean pore radius (U) and SD (S). Both models take into account the effect of GFR determinants on convective and diffusive transmembrane transport of neutral dextrans and require input values for GFR, RPF, π_A, and ΔP.

It is important to emphasize that ΔP cannot be measured directly in humans, and an assumed range (37 to 43 mmHg) similar to that found in micropuncture studies of rat pregnancy (11) was assigned. These choices for ΔP are arbitrary, but, bearing in mind glomerular physiology, the actual value of ΔP must be greater than π_{GC} for filtration to occur and is likely to be significantly less than systemic arterial pressure.

Each model estimates changes in π_{GC}, volume flows, and fluxes along the glomerular capillary, allowing computation of a series of theoretical θ_D curves, each with values for capillary wall porosity (r_0 and ω_0 or U and S) and K_f. The closeness of fit between measured and theoretical θ_D curves is judged by calculating the sum of χ^2 for the range of dextran sizes between 31 and 65Å. For each assumed ΔP value, the theoretical θ_D curve with the closest fit to the measured θ_D curve identifies the predicted parameters of porosity and K_f. The pitfalls and merits of each of these models are discussed elsewhere (9).

Statistical Analyses

Each woman acted as her own nonpregnant control. Differences in θ_D curves were assessed by first splitting each curve into four bands (31 to 39, 41 to 49, 51 to 59, and 61 to 65Å), and differences among band areas were assessed across test occasions. The significance of changes in GFR, RPF, π_A, and θ_D band area were estimated using an ANOVA for repeated measurements, and significance of differences between each stage of the study was estimated using paired or unpaired *t* tests as appropriate. Wilcoxon rank sum testing was used to assess differences in TPE and UAE. Data are expressed as mean ± SEM. All *P*-values are two-tailed, and results considered significant for $P < 0.05$.

Results

Hemodynamics and Renal Function

Renal hemodynamic data are shown in Table 1. In normal pregnancy, significant increases in GFR, RPF, and FF were evident ($P < 0.001$, $P < 0.001$, $P = 0.02$, respectively), all such changes being absent in preeclampsia.

Urinary Protein and Albumin Excretion

Before the infusion study, in normal LP, TPE did not exceed 300 mg/24 h on any test occasion. As expected, TPE was

Table 1. Normal and preeclamptic group serial changes in renal haemodynamic and functional (mean ± SEM, $n = 13$)[a]

	GFR (ml/min)	RPF (ml/min)	FF (%)	MAP (mmHg)	π_A (mmHg)	π_E (mmHg)	U_{Na} (μmol/min)	UAE median (interquartile range) μg/min
Normal LP	158 ± 15	849 ± 50	18.6 ± 1.1	86 ± 3	18.90 ± 0.4	25.24 ± 0.9	0.172	6.1 (4.4 to 10.6)
Preeclamptic LP	105 ± 6[c]	781 ± 38[ns]	13.5 ± 1.0[b]	111 ± 1[d]	17.52 ± 0.7[ns]	19.80 ± 0.5[b]	0.218 ± 0.038[ns]	1817 (1128 to 3455)[d]
Normal PP	92 ± 5	585 ± 27	15.7 ± 0.5	82 ± 7	24.53 ± 0.2	29.33 ± 0.9	0.156	8.0 (4.2 to 9.9)
Preeclamptic PP	108 ± 5	679 ± 46	15.3 ± 1.0	80 ± 6	22.58 ± 0.4	26.66 ± 0.4	0.240 ± 0.027	18.3 (N/A)

[a] LP, late pregnancy; PP, postpartum; SCr, serum creatinine; GFR, glomerular filtration rate; RPF, renal plasma flow; FF, filtration fraction; MAP, mean arterial pressure; π_A, afferent oncotic pressure; π_E, efferent glomerular oncotic pressure; UAE, urinary albumin excretion.

Preeclamptic LP *versus* Normal LP: ns, not significant; [b] $P < 0.05$; [c] $P < 0.01$; [d] $P < 0.001$; Other significant differences are highlighted in the text.

significantly higher in preeclamptic LP (mean, 2.0 g/24 h; interquartile range, 1.6 to 3.1 g/24 h; $P < 0.0001$) with UAE rates (measured during collection period 3) 300 times higher than normal LP but returning to normal PP. Although quantification of UAE in a 20-min collection period is subject to timing and collection errors, there was a moderate correlation between this UAE and 24 h TPE ($r = 0.4322$).

Renal Handling of Neutral Dextrans

Compared with normal LP, measured θ_D for radii 31 to 63Å were reduced in preeclampsia (Figure 1). These reductions were statistically significant for bands 31 to 39Å ($P < 0.0001$) and 41 to 49Å ($P < 0.01$). This is in marked contrast to the PP data in which θ_D values were almost identical between groups.

Theoretical Analysis of Ultrafiltration Parameters: Normal Group

K_f values were increased in LP except at the lowest assumed ΔP (Table 2). The log-normal model predicted reduced LP mean pore size U, offset by an increase in S (the variance around the mean). The isoporous + shunt model predicted similar r_0 values in LP and PP but with the shunt ω_0 almost doubled in LP. χ^2 values were consistently low, with the isoporous + shunt model providing the closest overall fit to the measured θ_D data.

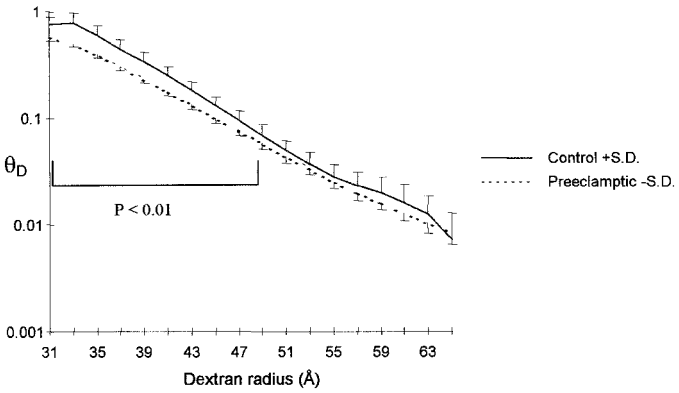

Figure 1. Mean late pregnancy (34 to 36 wk) fractional dextran clearance (θ_D) for normal ($n = 13$) and preeclamptic ($n = 10$) subjects.

Theoretical Analysis of Ultrafiltration Parameters: Preeclamptic Group

K_f was reduced by 50% in preeclamptic LP returning to normal PP (Table 3). The log-normal model predicted reduced U, combined with the largest S. Although the isoporous + shunt model predicted r_0 increases in preeclamptic LP compared with PP, r_0 remained reduced compared with normal pregnancy. Preeclamptic LP revealed an almost twofold increase in ω_0, which normalized PP. Once again, the isoporous + shunt model outperformed the log-normal model, with χ^2 testing giving the best fit on each occasion.

Discussion

We have shown that the normal pregnancy increments in GFR and RPF were not evident in preeclampsia, thus endorsing our previous work (3,5) that in normal LP GFR increments are RPF-driven with contributions from both decreased π_A and increased K_f. In preeclamptic LP, there was a 50% reduction in K_f, and GFR values were almost identical to those PP. The proteinuria correlates with a loss of glomerular barrier size selectivity and almost certainly reduced charge selectivity too.

It is important to acknowledge that theoretical modeling of glomerular permselectivity makes major assumptions (7,9), and circumspection is mandatory when interpreting results. Both theoretical models were, however, in close agreement (Tables 2 and 3), each predicting gestational increases in those parameters linked to clearance of larger dextrans (a widening of the pore distribution S and an increase in the nondiscriminating shunt ω_0), maximal in preeclamptic LP. Compared with PP values, K_f was increased in normal LP contrasting with the 50% reduction seen in preeclampsia. Although the inability to measure ΔP in humans precludes a precise estimate of K_f, the inferred reduction in preeclampsia was so great that there was little doubt that glomerular ultrafiltration would be compromised.

Of particular interest are the similarities of these results to nonpregnant human nephrotic syndrome (12), where K_f reductions were proportional to structural changes on renal biopsy

Table 2. Predicted values of K_f and porosity using theoretical models in normal late pregnancy (36 to 38 wk) and postpartum (>5 mo) (mean ± SEM)[a]

	Assumed ΔP (mmHg)	Derived K_f ($ml \cdot min^{-1} \cdot mmHg^{-1}$)	r_0 (Å)	ω_0	Sum of χ^2	U (Å)	± S (Å)	Sum of χ^2
Late Pregnancy	37	11.20	63.19	0.0050	1.271	56.25	1.064	1.328
	40	8.94	62.66	0.0063	1.116	56.30	1.152	1.536
	43	7.475	63.02	0.0064	1.238	56.37	1.152	1.598
Postpartum	37	12.99	62.58	0.0024	1.350	57.36	1.130	1.025
	40	7.93	62.94	0.0030	1.015	58.43	1.121	1.184
	43	6.04	63.00	0.0036	1.110	58.63	1.119	1.310

[a] ΔP, transglomerular hydrostatic pressure difference; K_f, ultrafiltration coefficient pressure; r_0 and ω_0, predicted values using isoporous+shunt model for pore radius and shunt contribution, respectively; U and S, predicted values using log-normal model for mean pore radius and standard deviation of pore radius about the mean, respectively.

Table 3. Predicted values of K_f and porosity in preeclamptic late pregnancy (36 to 38 wk) and postpartum (>5 mo) (mean ± SEM)[a]

	Assumed ΔP (mmHg)	Derived K_f ($ml \cdot min^{-1} \cdot mmHg^{-1}$)	r_0 (Å)	ω_0	Sum of χ^2	U (Å)	± S (Å)	Sum of χ^2
Preeclamptic	37	5.48	61.66	0.0061	0.6954	52.70	1.180	0.8337
Late pregnancy	40	4.64	61.68	0.0071	0.7287	52.82	1.179	0.8826
	43	4.02	61.68	0.0075	0.9527	52.88	1.179	0.9171
Preeclamptic	37	10.34	60.89	0.0035	0.7902	54.73	1.145	1.218
Postpartum	40	8.06	60.92	0.0042	0.9182	54.87	1.144	1.359
	43	6.60	60.92	0.0048	1.001	55.07	1.142	1.448

[a] ΔP, transglomerular hydrostatic pressure difference; K_f, ultrafiltration coefficient; r_0 and ω_0, predicted values using isoporous+shunt model for pore radius and shunt contribution, respectively; U and S, predicted values using log-normal model for mean pore radius and standard deviation of pore radius, respectively.

associated with reduced glomerular hydraulic permeability to water (*k*), ascribable to reduced filtration slit frequency.

Reduced K_f with significant proteinuria appears paradoxical; structural changes hindering the ultrafiltration of water might reasonably be expected to equally retard the transmural passage of proteins. Drumond *et al.* (12) proposed two possible explanations, and each could be applied to our data. First, broadened epithelial foot processes capable of reducing *k* may, in a small proportion, lead to complete disruption of the slit diaphragms, allowing for a high loss of protein across small pockets of the glomerular wall while contributing only small increases to the net flux of water. Increases in ω_0 would reflect such a change, as seen in preeclamptic LP. Second, reduced charge selectivity could increase proteinuria despite a reduced area available for ultrafiltration.

We could locate no other antenatal estimates of K_f in preeclampsia. The only other work we are aware of to date are those from Lafayette *et al.* (13). These investigators undertook morphometric analysis of glomeruli by biopsy postpartum both in healthy gravidas and those with preeclampsia, studies performed immediately post-cesarean section under regional anesthesia after volume loading. From their renal hemodynamic measurements, there was an approximately 40% reduction in preeclamptic GFR, with no difference in RBF compared with the control group. SNGFR was reduced by approximately 40% or approximately 23% depending on estimated reductions in *k*, resulting from subendothelial fibrinoid deposits. Their hypothetical preeclamptic K_f values were approximately 44% lower than similarly derived control values (4.8 *versus* 8.6 ml/min per mmHg, respectively) and were in close agreement with our modeled LP K_f values (4.64 *versus* 8.94 ml/min per mmHg) at an assumed ΔP of 40 mmHg. Whether such reductions in K_f can be held solely responsible for glomerular hypofiltration is a matter of controversy, as it ignores the profound depression of RBF consistently seen in preeclampsia (1,14) and again confirmed by this investigation.

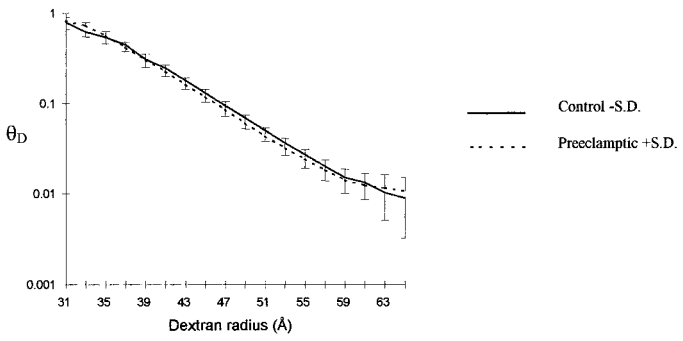

Figure 2. Mean postpartum (>5 mo) fractional dextran clearance (θ_D) for normal ($n = 13$) and preeclamptic ($n = 5$) subjects.

Our θ_D data were derived for dextran of radii up to 65Å (Figures 1 and 2), beyond which the number of molecules that breach the glomerular filtration barrier are so limited that measured θ_D approaches zero. Nevertheless, the large radii dextran θ_D values (>55Å) are crucial for modeling, exerting a significant influence on derived values for S and ω_0 (8,15,16). In the normal group, there were no statistically significant differences between θ_D curves on any test occasion. In preeclamptic LP, there were significant reductions in the θ_D of smaller dextrans (33 to 37Å), which, when compared with normal LP, were even greater and seen for all dextran molecules, reaching significance across the range 31 to 49Å (Figure 2). The subsequent return to almost identical θ_D curves in both groups PP (with normalization of K_f values and S and ω_0) provides strong evidence for reestablishment of glomerular barrier integrity by 5 mo PP.

Reduced preeclamptic θ_D is compatible with increased protein excretion because θ_D curves, while providing detailed size-selectivity data, provide limited information about the actual amount of dextran excreted. Also, the majority of proteins, such as albumin, are anionic and repelled by the negatively charged glomerular barrier. Proteinuria arising from reduced charge-selectivity will not, therefore, be revealed by the clearance rates of neutral dextran. Such changes in barrier charge are implicated in diabetic nephropathy (17), minimal change, and membranous glomerulonephritis (16,18) and have been confirmed by biopsy studies in early-onset preeclampsia (19). Our data *indirectly* support proteinuria due to a loss of charge-selectivity, as preeclamptic LP UAE was increased 30-fold to 100-fold, whereas θ_D at the equivalent neutral dex-

tran radii (36Å) was reduced ($P < 0.01$). This leaves loss of glomerular barrier charge selectivity as the major if not the only explanation for high LP preeclamptic UAE in the presence of reduced θ_D and similar GFR.

In conclusion, this study confirms that normal gestational increases in renal hemodynamics are blunted in preeclampsia and that the reductions in both RPF and K_f can be implicated in the reduced GFR. The glomerular barrier parameters, ω_0 and S, which increase in normal pregnancy, were even larger in preeclampsia, concomitant with increased protein excretion. All changes appear to resolve by 5 mo PP, suggesting that glomerular barrier integrity is fully restored.

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