Glomerular heteroporous membrane modeling in third trimester and postpartum before and during amino acid infusion

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Glomerular heteroporous membrane modeling in third trimester and postpartum before and during amino acid infusion. Am J Physiol Renal Physiol 282: F170–F175, 2001; 10.1152/ajprenal.00195.2000.—Human pregnancy is associated with substantial increments in glomerular filtration rate (GFR) and renal plasma flow (RPF). We have previously demonstrated that permeselectivity to neutral dextrans is altered in pregnancy, theoretical analysis of the dextran sieving curves suggesting that elevated GFR is due to increased RPF and decreased glomerular oncotic pressure (πGC) with no evidence of increased transglomerular hydrostatic pressure difference (ΔP). These conclusions have been challenged, with claims that the rise in GFR is primarily a result of a decrement in πGC. With refined laboratory and infusion protocols, we have reexplored the determinants of ultrafiltration in a serial study of 11 healthy women in late pregnancy (LP) and 4 mo postpartum (PP), both in the baseline state and after increasing GFR and RPF by infusion of amino acids. Results were analyzed using two computer modeling programs. Increased GFR in LP (38%, P < 0.05) was due to a combination of elevated RPF (22%) and a decrement in πGC and associated with an increased ultrafiltration coefficient, without evidence of increased ΔP, and additional amino acid-provoked GFR increments (P < 0.05) produced similar findings. In addition, refined methodology permitted collection of sufficient data on excreted large-radii dextrans (>60 Å) to better define the nondiscriminatory “shunt” pathway (ω0) and the standard deviation of pore size (S) about the mean radius of the distribution. Thus it was possible to demonstrate that the physiological increase in total protein excretion in LP is associated with a prominent shunt and an upward shift in breadth of distribution of pore sizes. This ability to quantify ω0 and S will now permit better evaluation of the pathophysiological changes in the glomerulus associated with pregnancy in women with renal disease and in gravidas developing preeclampsia.

renal hemodynamics; dextran clearance; pregnancy

HUMAN PREGNANCY IS ASSOCIATED with striking increments in renal hemodynamics, often exceeding 50%, yet both glomerular filtration rate (GFR) and renal plasma flow (RPF) increase further when gravidas are loaded with protein or amino acids (2, 29). Such observations raise the question as to whether such increases are potentially harmful to the kidney (5, 19, 22), especially in women with underlying kidney disorders who conceive, many of whom will also manifest gestational increments in GFR and RPF (8, 18). Our approach to these questions has been to adapt techniques used in nonpregnant populations whereby fractional clearances of neutral dextrans (Cdextran/Cinulin = θD) and other input data are analyzed in mathematical models that predict the ultrafiltration coefficient (kD) and glomerular size-selective function and allow assumptions to be made about transglomerular hydrostatic pressure difference (ΔP) (10, 12, 28). In a previous serial study during pregnancy, we demonstrated that permeselectivity to neutral dextrans was altered and theoretical analysis of the sieving curves suggested that, especially in the third trimester, hyperfiltration was due to a combination of increases in RPF and decreased glomerular oncotic pressure (πGC) (28). Glomerular size selectivity appeared to be altered, but there was no evidence of increased ΔP, data reassuring to those concerned that hyperfiltration might be detrimental to the kidney.

Our previous study, however, had problems. There were too little data on excreted large-radii dextrans (>60 Å) to accurately quantify the nondiscriminatory “shunt” pathway (ω0) or the standard deviation of pore size (S) about the mean pore radius (U) that in the “isoporous + shunt” and “log-normal” models, respectively, are especially important for understanding the pathophysiology of the glomerular filtration barrier in patients with a variety of renal diseases (10, 12, 13). Moreover, Lafayette et al. (21) have evaluated women in the immediate puerperium, postcesarean section, and concluded that hyperfiltration in healthy pregnant women is predominantly, if not uniquely, due to a 27% depression of πGC, in essence challenging our conclusion.

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This study was therefore designed to reexplore the determinants of ultrafiltration in late pregnancy both in the baseline state when GFR is elevated and after increasing renal hemodynamics further by infusing amino acids. In addition, we refined our methodology in a manner permitting better quantification of both \( \omega_0 \) and S in normal pregnant women. The results confirm that the gestational increase in GFR is due to a combination of increased RPF and decreased oncotic pressure, and again modeling produced no evidence that either the basal or amino acid-provoked GFR increments were associated with increases in \( \Delta P \).

**MATERIALS AND METHODS**

**Subjects.** Eleven normotensive healthy Caucasian women with no evidence or family history of renal or cardiovascular disease were studied during late pregnancy (LP: 36–38 wk gestation) and again 4 mo postpartum (PP), when none were breast feeding or ingesting oral contraceptives. All gave informed consent in writing to protocols approved by the Joint Ethics Committee of the Universities of Newcastle upon Tyne and Northumbria and the Newcastle upon Tyne and North Tyneside Health Authority.

**Protocols.** There were no limitations on diet and/or activity before investigation, but on the morning of each study subjects were requested to ingest a light carbohydrate breakfast and to avoid tea or coffee. A 24-h urine collection to measure total protein excretion (TPE) and urinary albumin excretion (UAE) was completed on the very morning of the study. On arrival the subjects were seated comfortably, basal blood samples were collected, and then a priming infusion containing 48 ml of dextran (10% dextran-40 in 0.9% saline; Baxter Healthcare, Thetford, UK), 10 ml of inulin (25% Inutest; Fresenius Kabi, Linz, Austria) and 2 ml of p-a minohippurate (PAH; 20% PAH; Merck, Sharp & Dohme, Hoddesdon, UK) was administered over 10 min followed by a sustaining infusion (which contained 264 ml of dextran, 75 ml inulin, and 36 ml of PAH) set at 60 ml/h. Sixty minutes later the volunteer voided, and three 20-min urine collections combined with midperiod blood sampling were obtained (clearance periods 1, 2, and 3). After these “control” collection periods, an amino acid infusion (Vamin 9; Kabi Pharmacia, Milton Keynes, UK) was administered at 4 ml/min as described previously (29), with a further 60 min of equilibration elapsing, and then clearance periods 4, 5, and 6 were measured (as described for the control collections). To minimize errors due to incomplete bladder emptying, an adequate diuresis was ensured by ad libitum oral tap water intake. Blood pressure was measured at 30-min intervals.

GFR and RPF were calculated as the mean of three inulin and PAH clearances, respectively (1, 2, and 3 then 4, 5, and 6). The renal PAH extraction rate in healthy pregnancy was assumed to be 0.85 (1, 6). The clearances of neutral dextrans (\( \theta_p \); radii, 30–65 Å) were measured in clearance periods 4 and 6. Analytical procedures and across-batch coefficients of variations for inulin, PAH, routine biochemistry, total serum protein, TPE, UAE, and dextran have been described elsewhere, as have the equations used in calculations (28).

Dextrans separation was undertaken by gel permeation chromatography (GPC) with a refractive index detector where the integrator divided the chromatogram into 60 slices/min, with no interference from protein(s) or inulin. Calibration with five standards (6.7, 11.7, 27.0, 42.8, and 78.8 kDa) is undertaken every 3 wk, and the 42.8-kDa standard is used daily. Further details of chromatography and integration using on-line analysis programs (PCGCP software) are described in a previous publication (28).

\[
\pi = 2.1 C + 0.16 C^2 + 0.009 C^3
\]  

The relationship between GFR and its determinants was examined using Eq. 2

\[
\text{GFR} = K_e (\Delta P - \pi) \text{GC}
\]

where \( \pi_{GC} \) is the intraglomerular oncotic pressure and can be approximated by taking a mean of the afferent (\( \pi_A \)) and efferent (\( \pi_E \)) glomerular oncotic pressures (11). The value of \( \pi_E \) was calculated by Eq. 3

\[
\pi_E = \pi_d (1 - FF)
\]

where FF is the filtration fraction.

Precis of theoretical analyses of transglomerular capillary water and dextran flux and calculation of determinants of ultrafiltration. Two theoretical models of glomerular function were assessed for analysis of alterations in renal hemodynamics and transcapillary dextran flux in pregnancy (10, 13, 24–27). Each represents the glomerular capillary wall as a heterogeneous 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 (\( r_0 \)) and has a parallel shunt pathway (\( \omega_0 \)) that fails to restrict the passage of large molecules (10, 11). The shunt contribution (\( \omega_0 \)) represents the fraction of the filtrate passing through the shunt. 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 the mean pore radius (\( U \)) and the standard deviation of pore sizes (\( S \)) about the mean distribution of pore sizes (10–12). Both models take into account the effect of GFR determinants on convective and diffusive transmembrane transport of varying sized neutral dextrans and require input values for GFR, RPF, \( \pi_A \), and \( \Delta P \).

It must be emphasized that Eq. 2 used to examine relative changes in \( K_f \) and \( \Delta P \) is an oversimplification and, as such, will result in a small underestimation of \( K_f \) because \( \pi_{GC} \) 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 \( \pi_{GC} \) are detailed elsewhere (11).

It is vital to emphasize that \( \Delta P \) cannot be measured directly in humans and for the purposes of modeling, in line with the approach used by others (24), an assumed range of \( \Delta P \) values (37–43 mmHg), similar to that found in micropuncture studies of the rat (3), was assigned. It is important to realize that the choice of \( \Delta P \) range is arbitrary, but with glomerular physiology borne in mind, the actual values of \( \Delta P \) must be greater than \( \pi_{GC} \) for filtration to occur and it is likely to be significantly less than systemic arterial pressure.

Each model then estimates changes in \( \pi_{GC} \), volume flows, and fluxes along the glomerular capillary, allowing computation of a series of theoretical \( \theta_p \) curves, each with values for capillary wall porosity (\( r_0 \) and \( \omega_0 \) or \( U \) and \( S \)) and \( K_f \). The “closeness of fit” between measured and theoretical \( \theta_p \) curves is judged by calculating the sum of chi-square (\( \chi^2 \)) for the range of molecular sizes between 30 and 65 Å. For each assumed \( \Delta P \) value, the theoretical \( \theta_p \) curve with the closest fit to the measured \( \theta_p \) curve identifies the predicted parameters of porosity and \( K_f \).
RESULTS

Seven of the 11 women were nulliparas. All had uneventful pregnancies. Maternal age was 27.1 ± 1.6 (SE) yr, while weight during and after gestation was 76.2 ± 2.2 and 68.1 ± 2.7 kg, respectively.

Renal function. Serum creatinine (S_{Cr}), renal hemodynamics, estimated π_{A} and π_{E}, TPE, UAE, and mean arterial pressure (MAP) are summarized in Table 1. Compared with PP values, both GFR and RPF were significantly greater in LP (38 and 22%, respectively; \( P < 0.05 \)), associated with a corresponding decrease in S_{Cr}, illustrating normal pregnancy-induced hyperfiltration. In LP, π_{A} was decreased (−17.1% vs. PP) as was the calculated value for π_{E}.

In LP and PP, there were significant increases in GFR and RPF in response to amino acid infusion. GFR increased from 139 ± 11 to 150 ± 10 ml/min in LP (\( P < 0.05 \)) and from 101 ± 6 to 111 ± 9 ml/min PP (\( P < 0.05 \)), increments of 8 and 10%, respectively, while RPF increased from 785 ± 55 to 827 ± 38 ml/min in LP (\( P < 0.05 \)) and from 643 ± 36 to 705 ± 32 ml/min PP (\( P < 0.05 \)), increments of 11 and 10%, respectively (Table 1). During amino acid infusion, there were nonsignificant decreases in π_{A} for LP (3.8%) and PP (4.1%), as was also the case for the calculated values of π_{E} (6.0 and 4.4%, respectively).

As previously noted, the values of \( K_{f} \) and \( \Delta P \) cannot be directly measured in humans and an arbitrary series of \( K_{f} \) values was used to calculate a range of possible \( \Delta P \) values using Eq. 2. Figure 1 depicts the

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**Table 1. Serial changes in renal function in late pregnancy and postpartum**

<table>
<thead>
<tr>
<th></th>
<th>MAP (mmHg)</th>
<th>S_{Cr} (mmol/l)</th>
<th>GFR (ml/min)</th>
<th>RPF (ml/min)</th>
<th>FF</th>
<th>π_{A} (mmHg)</th>
<th>π_{E} (mmHg)</th>
<th>TPE (mg/24 h)</th>
<th>UAE (mg/24 h)</th>
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<tbody>
<tr>
<td><strong>LP Control</strong></td>
<td>88 ± 2</td>
<td>65 ± 2</td>
<td>139 ± 11</td>
<td>785 ± 55</td>
<td>22 ± 1</td>
<td>18.2 ± 0.3</td>
<td>23.3 ± 0.6</td>
<td>195 ± 40</td>
<td>10.0 ± 1.5</td>
</tr>
<tr>
<td><strong>Amino acids</strong></td>
<td>150 ± 10†</td>
<td></td>
<td>872 ± 38†</td>
<td>20 ± 1</td>
<td>17.5 ± 0.5</td>
<td>21.9 ± 0.6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>PP Control</strong></td>
<td>83 ± 2</td>
<td>75 ± 3</td>
<td>101 ± 6‡</td>
<td>643 ± 36‡</td>
<td>19 ± 1</td>
<td>21.9 ± 0.4§</td>
<td>27.0 ± 0.6§</td>
<td>95 ± 11*</td>
<td>12.0 ± 2.1</td>
</tr>
<tr>
<td><strong>Amino acids</strong></td>
<td>111 ± 9¶</td>
<td></td>
<td>705 ± 32‡</td>
<td>18 ± 1</td>
<td>21.0 ± 0.4</td>
<td>25.8 ± 0.8</td>
<td></td>
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Values are means ± SE; \( n = 11 \); LP, late pregnancy (36–38 wk); PP, postpartum (4 mo); MAP, mean arterial pressure; S_{Cr}, serum creatinine; GFR, glomerular filtration rate; RPF, renal plasma flow; FF, filtration fraction; π_{A}, afferent glomerular oncotropic pressure; π_{E}, efferent glomerular oncotropic pressure. *\( P < 0.05 \); †\( P < 0.05 \) vs. LP Control. ‡\( P < 0.05 \) vs. PP Control. §\( P < 0.05 \) vs. LP Control.
relationship between $K_f$ and ΔP during LP and PP, both before and during amino acid infusion. Above a minimum value of $K_f$ (~5 ml·min$^{-1}$·mmHg$^{-1}$), there was little difference in the value of ΔP between LP and PP, even after GFR was increased further by amino acid infusion.

Renal handling of neutral dextrans. Fractional clearances of smaller dextrans (30–49 Å) were significantly decreased in LP ($P < 0.05$). The decrease in the clearance of larger dextrans was not significant. Amino acid infusion further reduced clearance of all Radii dextrans, attaining significance only for radii 40–49 Å in LP ($P < 0.05$) but for radii 40–59 Å in PP ($P < 0.05$) (Table 2).

Urinary protein and albumin excretion. TPE was greater in LP (195 ± 40 mg/24 h) than PP (95 ± 11 mg/24 h) ($P < 0.05$), but there were no differences in UAE between LP and PP. Ongoing studies in our laboratory demonstrate that UAE continues to decrease throughout the peripartum, not attaining non-pregnant levels (<5 mg/24 h) until well after 5 m PP (Davison JM, unpublished observations).

Theoretical analysis of membrane parameters. The isoropous + shunt and log-normal models (10) were used to predict membrane parameters for the mean dextran sieving curves (Table 2). If ΔP was held constant, then the minimum value of $K_f$ was increased in LP compared with PP, with values similar to those obtained in our earlier investigation (Table 3). Such a gestational change would correspond with increased filtration surface area or membrane permeability. Amino acid infusion produced a nonsignificant decrease in $K_f$ in LP and a nonsignificant increment in $K_f$ when PP. Both models predicted a decreased pore size ($r_p$ and $U$) in LP compared with PP (in line with the dextran sieving curves), attaining significance only for $U$ ($P < 0.05$), with amino acid infusion having the effect of further decreasing pore size in both LP and PP ($P < 0.05$ PP). The isoropous + shunt model predicted the presence of a ($\theta_0$) in LP still present PP and the log-normal model predicted widening of the standard deviation of pore sizes ($S$) in LP compared with PP. With amino acid infusion, values for both $\theta_0$ and $S$ further increased but attained significance only for $S$ when PP ($P < 0.05$). As judged by a sum of chi-square, both models predicted $\theta_0$ accurately at each stage of the study.

**DISCUSSION**

These data confirm those previously described by us, that the increment in GFR in late pregnancy (38%) is
due to a combination of elevated RPF (22%) as well as a significant contribution from a decrement in $\pi_A$ with an increment in $K_f$. By modeling we again suggest that hyperfiltration in LP is not associated with increments in $\Delta P$. The results extend our previous observations (28) by demonstrating that the increased proteinuria of normal pregnancy is associated the presence of a non-discriminatory shunt pathway ($\omega_0$) in the isoporous + shunt model and a wider standard deviation of pore sizes ($S$) about the mean in the log-normal model.

We also infused amino acids during and after pregnancy. Here too increments in GFR were primarily RPF driven, with $K_f$ relatively unchanged. Thus even in an already hyperfiltrating gravida one need not invoke an increase in $\Delta P$ to explain further increments in GFR.

In a recent study by Lafayette et al. (21), GFR and RPF in nonpregnant women were compared with those from healthy gravidas, the latter studied immediately after a cesarean delivery while still receiving epidural anesthesia. The authors were aware of the various assumptions made regarding factors influencing glomerular ultrafiltration. Moreover, in another publication (20) these authors cite data contrasting with those they utilize here.

In the present study, we took into account a recent critique and an accompanying editorial on methodological considerations from several publications on glomerular permselectivity to neutral dextrans (15, 26). Our infusion regimen avoids even modest volume expansion, and our Gpc laboratory methodology permits the application of membrane modeling for glomerular permselectivity function over a wide range (30–65 Å) of neutral dextran fractions.

Of interest, basal GFR and RPF values had attained the nonpregnant range at the time of our tests at 4 mo PP, as had other barrier parameters except for $\omega_0$. As previously noted by us (9) and by others (16), TPE has virtually halved when 4-mo PP values are compared with LP measurements, but UAE was similar at the two time points. However, as mentioned earlier, in contrast to others (16 and reviewed in 2, 8) our accruing unpublished data indicate that UAE determinations at 4 mo PP may be too early, as there is a slow decline during the puerperium, reaching truly non-pregnant levels (<5 mg/24 h) only well after PP week 20. In the present study, the log-normal model detected changes in $S$ distribution that correlated with differences in TPE between LP and PP, while no change in $\omega_0$ was apparent. Again, however, our ongoing unpublished serial studies indicate that decrements in $\omega_0$ (compared to LP values) are not detectable until 6 mo PP, at a time when UAE is approaching truly non-pregnant levels.

Also of interest is that the excretion of albumin, which is anionic, cannot be accounted for by its size selectivity alone (36 Å), indicating that although $\omega_0$ and $S$ as theoretical concepts correlate overall with proteinuria one must also take into account other factors such as membrane charge, the configuration and charge of the protein molecule, and alteration in tubular function and not just porosity (7, 23, 26). Indeed, although quantification of $\omega_0$ and $S$ in the nonpregnant...
population correlates with glomerular structural changes, thus endorsing the membrane modeling approach as representing glomerular function, it has been emphasized that changes in sieving behavior toward dextrans are not simply and directly related to the rate at which proteins are excreted (12, 24, 26).

In conclusion, this study, using improved methodology, suggests that the increased GFR in LP is due to a combination of elevated RPF and decreased $\pi_{GC}$. Within the limitations of analysis of dextran sieving data, there appears to be no increase in $\Delta P$. Raising GFR further by amino acid infusion produces similar findings. The data further indicate that the physiological increase in proteinuria in pregnancy is associated with the presence of a shunt pathway ($\omega_0$) in the isoporous + shunt model and a wider standard deviation of mean pore size ($S$) in the log-normal model. These methodological improvements now permit us to focus on the effects of pregnancy in women with parenchymal renal disease and/or preeclampsia.

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