Human fetal kidney morphometry during gestation and the relationship between weight, kidney morphometry and plasma active renin concentration at birth

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INTRODUCTION

There is increasing evidence that not only is being small for gestational age (especially the disproportionately small for gestational age) associated with increased perinatal morbidity [1-6] and mortality [7] but that such infants, when they become adults [8, 9], have a greater risk of developing hypertension, cardiovascular diseases [8, 10], early death from heart attacks, chronic lung diseases [11], and impaired glucose tolerance [5, 12]. With regard to hypertension, it has been suggested that small-for-gestational-age (SGA) infants delivered before 34 weeks gestation are not at risk compared with those delivered after 34 weeks gestation, despite being fed with similar nutritional formulae [13]. The existence of a ‘critical window’ in late gestation for programming the development of high blood pressure has therefore been suggested and, furthermore, it has been hypothesized that birth before this ‘critical window’ may prevent its onset. An understanding of the possible mechanisms predisposing SGA fetuses to these adult diseases may be of great importance in designing therapeutic strategies to limit their occurrence.

It is established that the kidneys play an important part in the regulation of blood pressure in adults through the renin–angiotensin system. In
contrast, the kidney and the renin–angiotensin system have not been well studied in the human fetus. Weiner and Robillard [14] and Tannirandorn et al. [15] demonstrated elevated plasma renin levels in five and two growth retarded fetuses respectively, whereas Kingdom et al. [16] showed elevated angiotensin II levels in 13 growth retarded fetuses delivered at 31 weeks gestation. The hypoxic, growth retarded fetus has been shown to have abnormal renal artery Doppler velocity waveforms compared with the normoxaemic, appropriate size and growth retarded fetus [17]. Hinchliffe et al. [15] demonstrated elevated plasma renin activity. The observations of these workers, that blood pressure in these smaller rat fetuses is higher than that in normally grown rat fetuses during the first few months of life, suggest that the mechanisms which underlie the development of hypertension in the offspring must operate very early in life and possibly in utero.

All these findings suggest that pathological processes occur in the kidneys of the growth retarded fetus in utero which could be implicated in adult hypertension; however, to the best of our knowledge, there are currently no studies linking fetal renal size and the renin–angiotensin system.

Our objectives were therefore (i) to examine in a serial study the changes in renal morphometry with gestation in small and appropriately grown fetuses, and (ii) to examine the relationship between fetal renal morphometry at birth and circulating umbilical vein active renin concentration.

PATIENTS AND METHODS

Serial study

A cohort of 87 healthy pregnant women were studied longitudinally from 20 to 38 weeks gestation. They were recruited after routine booking or anomaly ultrasound scans. Those with medical disorders such as diabetes mellitus, hypertension, thyroid and connective tissue disorders or those whose fetuses had known congenital abnormalities were excluded. To ensure that an adequate number of SGA fetuses was studied in this pilot work, there was a bias towards recruiting patients with risk factors for having SGA fetuses. The study was approved by the ethics committee of Leicestershire Health Authority. Each patient gave verbal consent to participate in the study. All the kidney measurements were performed using an Aloka SSD 650 SL model IP-1230C-TH (Aloka Co. Ltd, Japan) after a radiographer had measured the morphometry of the fetus. The kidney measurements (made at recruitment and fortnightly thereafter until delivery) were made blind to the various morphometric measurements of the fetus. A satisfactory transverse plane of the fetus was first defined at the level of the four chambers of the heart. The fetus was then scanned caudally in this plane until the stomach was seen clearly. Scanning was continued caudally until the kidneys were visualized. The kidneys were measured at a level where the stomach was still visible or just below it. Three measurements from different frames of the transverse (TS) and anterior-posterior (A-P) diameters and circumference of both kidneys were made. The probe was then rotated through 90° and measurements from three frames of the longitudinal axis of the kidneys were taken. The interframe variation in measurements was 2.5–12%. Results are expressed as the means of the three measurements for both kidneys.

Kidney size and renin study

To study the relationship between kidney size and the renin–angiotensin system, we measured umbilical vein plasma renin in the 87 fetuses at the time of delivery. Immediately after delivery, a loop of the cord was isolated between two clamps. A heparinized blood sample was then obtained from the umbilical vein and promptly placed on ice. Total fetal haemoglobin concentration and oxygen saturation were measured on a sample of the whole blood using an OSM3 Hemoximeter (Radiometer, Copenhagen). The remaining whole blood was centrifuged at 3000g for 15 min, and the plasma separated and stored at −70°C until further analysis. Active renin in the plasma was assayed blind to the obstetric history by the enzyme-kinetic method for measuring renin concentration in human plasma. This was based on RIA of angiotensinogen I generated during incubation of plasma and excess ox renin substrate [21].

Results are presented as mean ± SD. Differences between blood and kidney indices in the appropriate-for-gestational age (AGA) and SGA fetuses were tested for statistical significance by two sample t-tests using Bonferroni correction factor for multiple testing [22] for the kidney indices. The relationships between birthweights, renal size, haemoglobin concentration, oxygen saturation and umbilical plasma renin levels were estimated using Pearson’s correlation coefficient.

RESULTS

After delivery, the 87 fetuses were divided into two groups: AGA (n = 50) and SGA (n = 37). Appropriate for gestational age was defined by birthweight greater than the 10th centile for gestational age and
sex, while small for gestational age was defined by birthweight below the 10th centile for gestational age and sex using the normograms of Wilcox et al. [23] The birthweights of the AGA and SGA fetuses delivered at term (37–40 weeks gestation) were 3374 ± 456 g and 2387 ± 315 g, respectively. Nine of the SGA fetuses were delivered preterm (28 weeks, 3; 31 weeks, 1; 34 weeks, 3; 36 weeks, 2), while five of the AGA fetuses were delivered preterm (33 weeks, 2; 34 weeks, 1; 36 weeks, 2). There were eight caesarean sections (three emergencies and five electives) in the AGA group and nine (six emergencies and three electives) in the SGA group.

### Serial study

Table 1 shows the mean A-P and TS diameters while Table 2 shows the circumference and longitudinal measurements of the fetal kidneys at different gestational ages. The mean measurements were similar in all planes in the AGA and SGA groups between 22 and 24 weeks gestation. The kidneys in SGA fetuses showed a significantly different pattern in the A-P and TS diameter and circumference growth rates from the AGA fetuses after 24 weeks gestation. This difference continued until delivery when, apart from the longitudinal measurements, the other dimensions were significantly smaller in the SGA fetuses.

Figure 1 shows the growth pattern of the A-P diameter of the kidneys. There was a biphasic pattern of growth in the AGA group; an initial accelerative phase between 26 and 34 weeks gestation and then a decelerative phase between 34 and 38 weeks gestation. In the appropriately grown

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**Table 1. A-P and TS diameters of fetal kidneys from 22 to 38 weeks gestation.** Values are given as mean ± SD. *Confidence interval for difference between AGA and SGA. †Using Bonferroni correction factor for multiple testing.

<table>
<thead>
<tr>
<th>Gestation (weeks)</th>
<th>AGA (±1SD)</th>
<th>SGA (±1SD)</th>
<th>95% confidence interval</th>
<th>P value †</th>
<th>AGA (±1SD)</th>
<th>SGA (±1SD)</th>
<th>95% confidence interval</th>
<th>P value †</th>
</tr>
</thead>
<tbody>
<tr>
<td>22</td>
<td>11.68 ± 1.3</td>
<td>11.69 ± 0.4</td>
<td>-0.61 to 0.6</td>
<td>1.0</td>
<td>13.0 ± 1.5</td>
<td>13.1 ± 0.7</td>
<td>-0.83 to 0.63</td>
<td>1.0</td>
</tr>
<tr>
<td>24</td>
<td>12.6 ± 1.0</td>
<td>12.2 ± 1.0</td>
<td>-0.12 to 0.92</td>
<td>1.0</td>
<td>14.3 ± 1.3</td>
<td>15.2 ± 1.6</td>
<td>-0.64 to -0.16</td>
<td>0.18</td>
</tr>
<tr>
<td>26</td>
<td>15.9 ± 1.9</td>
<td>14.5 ± 1.1</td>
<td>0.68 to 2.12</td>
<td>0.002</td>
<td>17.5 ± 2.1</td>
<td>16.0 ± 1.7</td>
<td>0.63 to 2.34</td>
<td>0.01</td>
</tr>
<tr>
<td>28</td>
<td>17.3 ± 2.0</td>
<td>15.7 ± 1.6</td>
<td>0.81 to 2.39</td>
<td>0.001</td>
<td>19.2 ± 2.4</td>
<td>17.6 ± 2.0</td>
<td>0.63 to 2.57</td>
<td>0.01</td>
</tr>
<tr>
<td>30</td>
<td>20.6 ± 2.6</td>
<td>16.2 ± 1.0</td>
<td>3.47 to 6.33</td>
<td>&lt;0.0001</td>
<td>20.5 ± 2.8</td>
<td>18.1 ± 1.4</td>
<td>1.36 to 3.43</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>32</td>
<td>22.3 ± 2.5</td>
<td>17.6 ± 1.7</td>
<td>3.72 to 6.58</td>
<td>&lt;0.001</td>
<td>23.6 ± 2.6</td>
<td>19.5 ± 1.6</td>
<td>3.00 to 5.00</td>
<td>&lt;0.000001</td>
</tr>
<tr>
<td>34</td>
<td>24.9 ± 1.6</td>
<td>18.4 ± 1.7</td>
<td>5.66 to 7.14</td>
<td>&lt;0.00001</td>
<td>23.1 ± 1.7</td>
<td>20.1 ± 1.8</td>
<td>2.22 to 3.78</td>
<td>&lt;0.000001</td>
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<tr>
<td>36</td>
<td>25.5 ± 2.2</td>
<td>19.1 ± 2.4</td>
<td>5.33 to 7.45</td>
<td>&lt;0.00001</td>
<td>24.8 ± 2.7</td>
<td>21.1 ± 2.3</td>
<td>2.51 to 4.69</td>
<td>&lt;0.000001</td>
</tr>
<tr>
<td>38</td>
<td>26.1 ± 2.5</td>
<td>19.8 ± 2.6</td>
<td>5.08 to 7.51</td>
<td>&lt;0.00001</td>
<td>25.6 ± 2.8</td>
<td>22.2 ± 2.7</td>
<td>2.07 to 4.73</td>
<td>&lt;0.000001</td>
</tr>
</tbody>
</table>

**Table 2. Circumference and length of fetal kidneys from 22 to 38 weeks gestation.** *Confidence interval for difference between AGA and SGA. †Using Bonferroni correction factor for multiple testing.

<table>
<thead>
<tr>
<th>Gestation (weeks)</th>
<th>AGA (±1SD)</th>
<th>SGA (±1SD)</th>
<th>95% confidence interval</th>
<th>P value †</th>
<th>AGA (±1SD)</th>
<th>SGA (±1SD)</th>
<th>95% confidence interval</th>
<th>P value †</th>
</tr>
</thead>
<tbody>
<tr>
<td>22</td>
<td>38.2 ± 3.9</td>
<td>36.1 ± 2.2</td>
<td>-0.06 to 4.28</td>
<td>0.54</td>
<td>22.0 ± 3.0</td>
<td>21.7 ± 1.8</td>
<td>-0.24 to 2.84</td>
<td>0.81</td>
</tr>
<tr>
<td>24</td>
<td>42.3 ± 4.7</td>
<td>41.7 ± 3.2</td>
<td>-1.78 to 2.98</td>
<td>1.0</td>
<td>22.9 ± 2.7</td>
<td>22.3 ± 1.6</td>
<td>-0.37 to 1.67</td>
<td>1.0</td>
</tr>
<tr>
<td>26</td>
<td>51.0 ± 6.6</td>
<td>47.3 ± 5.9</td>
<td>4.36 to 10.00</td>
<td>0.0001</td>
<td>27.0 ± 1.1</td>
<td>26.8 ± 1.4</td>
<td>-0.35 to 0.75</td>
<td>1.0</td>
</tr>
<tr>
<td>28</td>
<td>55.6 ± 7.2</td>
<td>51.2 ± 7.3</td>
<td>1.28 to 7.52</td>
<td>0.053</td>
<td>32.0 ± 1.1</td>
<td>31.1 ± 2.7</td>
<td>-0.05 to 1.75</td>
<td>0.36</td>
</tr>
<tr>
<td>30</td>
<td>60.2 ± 7.2</td>
<td>52.6 ± 5.0</td>
<td>4.77 to 10.43</td>
<td>0.0001</td>
<td>33.5 ± 5.6</td>
<td>32.2 ± 1.6</td>
<td>0.67 to 3.29</td>
<td>1.0</td>
</tr>
<tr>
<td>32</td>
<td>65.2 ± 9.7</td>
<td>57.5 ± 5.2</td>
<td>4.08 to 11.32</td>
<td>0.001</td>
<td>36.5 ± 2.3</td>
<td>34.8 ± 2.3</td>
<td>0.66 to 2.74</td>
<td>0.06</td>
</tr>
<tr>
<td>34</td>
<td>68.4 ± 4.7</td>
<td>60.4 ± 5.3</td>
<td>5.77 to 10.23</td>
<td>0.0001</td>
<td>38.4 ± 2.5</td>
<td>36.5 ± 1.2</td>
<td>0.79 to 3.01</td>
<td>0.05</td>
</tr>
<tr>
<td>36</td>
<td>72.5 ± 7.6</td>
<td>63.2 ± 7.7</td>
<td>6.14 to 13.26</td>
<td>0.0001</td>
<td>39.7 ± 1.9</td>
<td>37.9 ± 2.5</td>
<td>0.77 to 2.83</td>
<td>0.05</td>
</tr>
<tr>
<td>38</td>
<td>76.0 ± 8.4</td>
<td>66 ± 7.8</td>
<td>6.07 to 13.92</td>
<td>0.0001</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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**Fig. 1. Relationship between A-P diameter of fetal kidney and gestational age. Bars represent ±SD.**
Fig. 2. Differences in A-P diameter growth rate between AGA and SGA fetuses.

Fig. 3. Relationship between renin concentration and fetal haemoglobin at delivery.

Fig. 4. Umbilical vein renin concentration versus oxygen saturation.

Fig. 5. Relationship between birthweight and umbilical vein active renin concentration.

fetuses the velocity of growth was highest between 26 and 34 weeks, while in the SGA group there was no initial accelerative phase — in fact the growth velocity was almost uniformly similar throughout gestation. The differences in growth rate between the AGA and SGA groups are shown in Fig. 2. This confirms that the growth rate in the AGA group was significantly greater than in the SGA group between 26 and 34 weeks gestation. The A-P diameter in the SGA group after this gestation was consistently lower than in the AGA group such that, by the time of delivery, the A-P measurement was significantly higher ($P<0.00001$) in the AGA group ($26.1 \pm 2.5$ mm compared with $19.8 \pm 2.6$ mm). A similar growth pattern was observed for the TS diameter and circumference of the kidneys. The growth in kidney length was similar in both groups although it was slightly slower in the SGA group (Table 2).

**Kidney size and renin study**

Umbilical vein plasma active renin levels in the 87 babies delivered between 28 and 40 weeks gestation varied considerably. The concentration in the 87 fetuses was $225.4 \pm 23.2 \mu$-units/ml plasma. The renin concentration in the AGA group was $164.9 \pm 28.3 \mu$-units/ml plasma compared with $274.4 \pm 32.9 \mu$-units/ml plasma in the SGA group ($P<0.05$). In the two groups, active renin levels were similar in those delivered by caesarean section and those delivered vaginally. However, active renin concentration was significantly higher in SGA fetuses delivered preterm ($n=9$; $443.8 \pm 331.9 \mu$-units/ml plasma) compared with that in those delivered at term ($n=28$; $211.7 \pm 31.9 \mu$-units/ml plasma). There was no statistically significant difference ($P>0.05$) between active renin levels in AGA fetuses delivered preterm and at term.

The concentration of fetal haemoglobin in the SGA group was $17.79 \pm 3.1$ g/dl compared with $15.57 \pm 2.2$ g/dl in the AGA group ($P<0.05$). Figure 3 shows a significant linear correlation between fetal haemoglobin concentration and umbilical active renin concentration ($r=0.73$, $P<0.001$; 95% confidence interval 0.46 to 0.88). The haemoglobin oxygen saturation in the umbilical vein of the AGA fetuses was $73.3 \pm 19.6\%$ compared with $63.0 \pm 15.1\%$ in the SGA group. Figure 4 shows that there was a significant inverse correlation ($r=-0.65$, $P<0.001$; 95% confidence interval $-0.84$ to $-0.51$) between umbilical vein haemoglobin oxygen saturation and plasma active renin concentration.

Figure 5 shows the relationship between umbilical vein plasma active renin concentration and birth-
Kidney morphometry and active renin levels

There was an inverse relationship with a correlation coefficient of \(-0.55\) \((P<0.001, 95\% \text{ confidence interval } -0.75 \text{ to } -0.42)\). The relationship between the A-P diameter of the fetal kidney measured just before delivery and umbilical vein active renin concentration in babies delivered after 38 weeks gestation \((n=65)\) is shown in Fig. 6. This shows a statistically significant inverse correlation \((r = -0.67, P < 0.001, 95\% \text{ confidence interval } -0.79 \text{ to } -0.51)\) between the kidney A-P diameter and umbilical vein plasma active renin levels. A similar inverse correlation was observed between renin concentration and the TS diameter of fetal kidneys at delivery \((r = -0.56)\).

**DISCUSSION**

Our results showed that changes in kidney size during gestation in SGA fetuses differed from that in their AGA counterparts. The differences began as early as 26 weeks gestation manifesting as a slowing in growth velocity and diminished A-P, TS and circumference dimensions. By 34 weeks gestation, however, the growth velocity had returned to normal, but because of the deceleration in the growth velocity between 26 and 34 weeks, the A-P, TS and circumference of the kidneys remained significantly smaller in the SGA group for the remainder of pregnancy. Although the kidney length was smaller in the SGA fetuses, the difference was not statistically significant. The exact mechanism responsible for this differential growth pattern is unknown. However, Vyas et al. [17] have shown that renal artery Doppler velocity waveforms are reduced in hypoxaemic SGA fetuses compared with their AGA counterparts. This suggests a reduction in renal perfusion which, during the period of rapid growth, may be associated with a reduction in kidney size. It is well recognized that the hypoxic kidney tends to produce more erythropoietin, hence a higher haemoglobin concentration in hypoxic fetuses [24, 25]. The higher concentration of haemoglobin in our SGA group would suggest that at least some were hypoxaemic. The observed lower oxygen saturation levels in the SGA fetuses and their inverse relationship with renin concentrations would support this.

In this study, we have considered whether the smaller kidney in the SGA fetus is of functional significance to the baby. A functional effect is suggested by the higher mean renin concentration in the SGA fetus at term, and there was an inverse relation between renin concentration and the A-P diameter of the fetal kidney at birth. What could be the possible mechanism for the high renin levels in the SGA fetus? Increased production of renin by the juxta-glomerular apparatus is stimulated by hypoxia, raised prostacyclin and catecholamine levels and increased sympathetic activity, while increased renal afferent arteriolar vascular pressure, high angiotensin II and vasopressin levels inhibit production [26]. In the SGA fetus, hypoxaemia is common [27], while sympathetic nerve activity and catecholamine production are high [14]. These factors may be responsible for the high plasma renin levels that were found in the SGA fetuses, independently of the fact that the kidneys were smaller in these fetuses.

It is also possible that other cellular elements (including the juxta-glomerular apparatus) of the SGA kidneys, in response to reduced nephron numbers [18] undergo compensatory proliferation, the end result being an increased production of renin by a greater number of juxta-glomerular cells. Paradoxically, Kingdom et al. [28] have recently shown (using immunocytochemical localization techniques) that the concentration of renin granules in growth retarded stillborn kidneys is lower. Their findings are consistent with ours and may reflect a higher rate of secretion of stored renin into the plasma, resulting in higher plasma renin levels in SGA fetuses.

The possibility that the higher renin levels in SGA fetuses may be of functional significance is supported by the recent observation that hypoxic SGA fetuses have a higher blood pressure in the neonatal period [29]. The higher blood pressures observed in these neonates returned to normal within a few weeks of delivery, a finding which is comparable to a fall in plasma renin concentrations secondary to improving oxygenation after birth.

While organogenesis of most organs is completed by the 12th week of gestation, that of fetal kidneys continues until 36 weeks gestation when induction of nephron number ceases [18]. The period between 26 to 34 weeks gestation identified in this study appears to be a 'critical' one for fetal kidney growth. It is the period of most rapid growth and differences in renal size at this stage persist until delivery. In fact, McPartlin et al. [30] have shown that maternal folic acid (necessary for cell division) requirements are maximal during the period 26–32 weeks. Any adverse factor during this period of rapid growth will therefore affect kidney growth. Since there is an
inverse relationship between the A-P diameter and plasma active renin levels at birth, it is possible that active renin levels may be elevated in SGA fetuses as early as 26 to 34 weeks gestation when the marked difference in growth occurs. In fact, the finding of elevated angiotensin II levels in the plasma of growth retarded fetuses delivered at 31 weeks by Kingdom et al. [16] and high renin levels in babies delivered before 34 weeks gestation in this study will support this.

Folkow [31] has proposed that the aetiology of hypertension has two components: an initiating process which raises the blood pressure and a second component which amplifies the effect throughout life. In the human fetus the initiation process is most likely to date back to in utero. It has been shown that the blood pressure of low birth-weight neonates as early as 1 week is elevated [32]. In fact, similar observations have been made by Langley and Jackson [20] in smaller fetal rats delivered to mothers fed with low protein diets. Law et al. [32] have gone further to suggest that the initiation process of hypertension in humans stems from changes in vascular structure, promoted by alterations to fetal and placental blood flow. The alterations in fetal blood flow suggested by Law et al. [32] may be responsible for the deviant kidney development observed in this study. Findings of altered Doppler flow velocity waveforms in the renal artery of growth retarded fetuses [17] support this hypothesis. We believe that these alterations in blood flow do not directly alter the vascular tree but activate the renin–angiotensin system leading to an increased production of angiotensin II, a well recognized vascular growth promoting factor which stimulates proliferation and hypertrophy of the blood vessels thus resulting in thickening of the vessel wall and narrowing of the lumen [33]. It is therefore the increased activity of angiotensin-converting enzyme in growth retarded rats with hypertension, elevated angiotensin II levels in growth retarded fetuses and our own observation of elevated active renin levels in SGA fetuses, that stimulate vascular hypertrophy and proliferation resulting in hypertension. Although the resulting hypertension is transient [29], we speculate that the blood vessels which by now are less compliant remain ‘primed’; thus any subsequent adverse effect results in an exaggerated response and subsequent development of hypertension.

The exact mechanisms which produce the adverse factors that lead to impaired renal development are unknown. However, Godfrey et al. [34], Langley and Jackson [20] and Langley-Evans et al. [35] have suggested that an adverse ‘nutritional plane’ may be responsible. Whatever the mechanism, in order to conclusively link our observations with the development of adult hypertensive disorders in low birthweight infants, a long-term follow-up study of blood pressure and the renin–angiotensin system in these babies and infants is being planned. In addition, the possibility of investigating peripheral vascular response to high circulating renin levels is being explored.

We would, however, like to caution that a certain cause and effect relationship between kidney size and renin levels cannot be made, because of the relatively small sample size of very-small-for-gestational-age fetuses in this study and the significant overlap of renin levels between AGA and SGA groups. A population study (with significantly larger numbers of very-small-for-gestational-age fetuses) is necessary to confirm our observations.

ACKNOWLEDGMENTS

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