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Decreasing podocyte number during human kidney intrauterine development

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Am J Physiol Renal Physiol 307: F1033–F1040, 2014. First published August 20, 2014; doi:10.1152/ajprenal.00165.2014.—Nephron number at birth has relevant clinical importance with implications for long-term renal health. In recent years, the podocyte depletion hypothesis has emerged as an important concept in kidney pathology. This study was aimed at verifying whether human podocyte number changes significantly during intrauterine life. To this end, 62 subjects with gestational ages ranging from 20 to 41 wk were examined. Kidney sections were stained with hematoxylin and eosin and digitally scanned at ×400 magnification. Subjects were subdivided into fetuses (gestational age ≥24 wk, n = 5), preterms (gestational age ≥25 and ≤36 wk, n = 39), and full-term newborns (gestational age ≥37 wk, n = 18). The average podocyte number of 1,908 ± 645, 1,394 ± 498, and 1,126 ± 256 was, respectively, observed in fetuses, preterms, and full-term newborns. A significant main effect (P = 0.0051) of gestational age on podocyte number was observed with a significantly lower number in full-term newborns than in fetuses (P < 0.01). Intragroup variability was also observed. We speculate that variations in podocyte number could be correlated with factors such as drugs and maternal diet occurring during intrauterine life. In conclusion, this study shows, for the first time, a decreasing trend in podocyte number during gestation.

podocytes; podocytopathies; development; fetal programming; human kidney

IN RECENT YEARS, SEVERAL STUDIES demonstrated that nephron number is highly variable in humans at birth, with glomerular number and kidney size in neonates being associated with birth weight (17). Preterm birth has been suggested to play a major role in nephrogenesis and in nephron number at birth (12), kidneys of preterms being characterized by accelerated maturation and by abnormal morphology of nephrons (35). Moreover, intrauterine growth restriction (IUGR) has been hypothesized to play a major role in the development of nephrons (14, 26). A marked interindividual variability in nephron number at birth has been reported by our group even in newborns with the same birth weight (9). In this latter study, newborns with same gestational age or same body weight showed significant differences in nephron number, suggesting the existence of a high number of factors, including drugs and therapeutic interventions (1, 5, 7, 10, 20), that could alter renal development during gestation and eventually affect nephron number at birth.

The interest of neonatologists and nephrologists in the factors determining nephron number at birth is not simply related to reaching a better knowledge of kidney development. Indeed, nephron number at birth has important clinical relevance (25) with critical implications for long-term renal health (2, 13, 15, 31). Subjects born very prematurely, putatively carriers of congenital oligonephronia, have been reported to have a lower glomerular filtration rate and to be more susceptible to develop microalbuminuria (18), increased albuminuria (38), and hypertension (4, 21, 32, 34) in childhood or in adulthood. Nevertheless, is the interindividual variability restricted to nephron number or is it in regard to the different glomerular cell types? Furthermore, does podocyte number at birth show differences among different gestational ages?

The recent report of increased urinary podocytes in preterms receiving indomethacin in the perinatal period (19), together with the observation of a case of podocyte loss in the kidney of a preterm newborn (28), induced us to investigate glomerular area podocyte number in a series of newborns of different gestational ages.

This study was aimed at verifying whether different subjects characterized by different intrauterine lives and different gestational ages could have a different number of podocytes at birth. We hypothesized that variations in podocyte number could be correlated with both gestational age of the subject and other factors such as drugs, maternal diet, or therapeutic intervention occurring during gestational life (1, 5, 7, 10, 20, 24).

In our work, we investigated the correlation between podocyte number, glomerular area, and gestational age. Finally, differences in podocyte number between glomeruli located in the deepest cortex zone and in the capsular zone were further evaluated.

MATERIALS AND METHODS

Kidney samples were obtained at autopsy from the Neonatal Intensive Care Unit of the Azienda Ospedaliero Universitari and University of Cagliari. Autopsies were performed between 2000 and 2013. Ethics approval for autopsies was obtained from ASL 8 Cagliari Research Ethics Committee.

Neonatal Characteristics and Criteria of Inclusion

Sixty-two subjects with gestational ages ranging from 20 to 41 wk were examined. Clinical histories were obtained from the autopsy reports, including gestational age at birth, body weight at birth,
been used (Fig. 1). The selection of glomeruli was chosen to hit at least three glomerular profiles. Each glomerulus was oriented toward the deepest zone of the cortex (30). Each straight line was obtained from the product between the number of podocytes per unit volume and the average caliper diameter of the podocytes $\delta$ (23). $N_p/\delta$ is the ratio between superficial podocyte number and glomerular area.

\[
p_p = \frac{N_p}{\delta}
\]

The total glomerular volume was estimated according to the Weibel-Gomez method (39). This method involves calculating mean glomerular volume $V_g$ from the following formula:

\[
V_g = A_s \frac{1.38}{1.01}
\]

where $A_s$ is the glomerular area, 1.38 is the shape coefficient for a sphere, and 1.01 is the size distribution coefficient assuming a 10% coefficient of variation (22). Total glomerular podocyte number was obtained from the product between the number of podocytes per unit volume and the glomerular volume

\[
N_p = p_p V_g
\]

An average volumetric number of podocytes per kidney was obtained from all 10 profiles observed along the 3 straight lines.

**Image Acquisition and Processing**

All the images were acquired by optical microscopy (Leica Microsystems) at ×400 magnification. Image-processing procedures were performed using Matlab software (Mathworks).

**Statistical Analysis**

All statistical analyses were performed using Prism 5.00 (GraphPad Software).

With the aim to investigate the correlation between podocyte number and body weight, a correlation analysis was undertaken in the three groups. Two-way ANOVA was used to evaluate how the main effect of sex, age, position of glomeruli (capsular or deepest cortex zones), and their interaction effect influence podocyte number and glomerular area. The level of significance was accepted at $P < 0.05$. This was followed by a multiple comparison Bonferroni posttest to determine the differences between groups.

**RESULTS**

**Assessment of Glomerular Area**

The average glomerular area in fetuses, preterms, and full-term newborns was, respectively, 4,373 ± 1,033, 3,790 ± 992 $\mu$m² (Table 2). Two-way ANOVA showed no interaction effect between gestational age and sex on glomerular area. There was no significant main effect of sex. There was no statistically significant main effect of gestational age (Fig. 2A) (Table 3).

The glomerular area in the capsular zone was, respectively, 3,799 ± 997, 3,746 ± 920, and 3,768 ± 766 $\mu$m² in fetuses, preterms, and full-term newborns. The glomerular area in the deepest cortex zone was, respectively, 5,540 ± 1,612, 4,536 ± 1,150, and 4,284 ± 1,107 $\mu$m² in fetuses, preterms, and

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**Table 1. Age, sex, and body weight of fetuses, preterms, and full-term newborns**

<table>
<thead>
<tr>
<th></th>
<th>Fetuses (n = 5)</th>
<th>Preterms (n = 39)</th>
<th>Full-Term (n = 18)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gestational age, wk</td>
<td>22.6 ± 1.52 (20–24)</td>
<td>29.87 ± 3.63 (25–36)</td>
<td>39.42 ± 1.02 (37–41)</td>
</tr>
<tr>
<td>Postnatal age, days</td>
<td>8.25 ± 12.54 (0.25–30)</td>
<td>5.65 ± 7.03 (0.04–30)</td>
<td>18.7 ± 30.69 (0.04–90)</td>
</tr>
<tr>
<td>Sex ratio, M:F</td>
<td>2:3</td>
<td>24:15</td>
<td>9.9</td>
</tr>
<tr>
<td>Body weight, g</td>
<td>495 ± 56 (440–572)</td>
<td>1,396 ± 776 (540–3,070)</td>
<td>3,121 ± 527 (2,170–3,850)</td>
</tr>
</tbody>
</table>

Values are means ± SD with data range in parentheses.

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**Table 2. Glomerular volume and number of podocytes in different zones**

<table>
<thead>
<tr>
<th></th>
<th>Fetuses (n = 5)</th>
<th>Preterms (n = 39)</th>
<th>Full-Term (n = 18)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glomerular volume</td>
<td>1.52 ± 0.33 (1.02–2.01)</td>
<td>1.63 ± 0.33 (1.02–2.01)</td>
<td>1.73 ± 0.33 (1.02–2.01)</td>
</tr>
<tr>
<td>Superficial number</td>
<td>1,150 ± 397 (590–1,620)</td>
<td>3,799 ± 997 (3,070–4,536)</td>
<td>5,540 ± 1,612 (2,170–3,850)</td>
</tr>
<tr>
<td>Deep number</td>
<td>920 ± 397 (390–1,350)</td>
<td>3,746 ± 920 (3,070–4,536)</td>
<td>4,536 ± 1,150 (2,170–3,850)</td>
</tr>
<tr>
<td>Total number</td>
<td>2,070 ± 794 (1,340–2,460)</td>
<td>7,545 ± 1,917 (5,070–9,036)</td>
<td>10,076 ± 2,764 (7,210–12,850)</td>
</tr>
</tbody>
</table>

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**Fig. 1. Example of analyses of a glomerulus. A: glomerulus with marked podocytic nuclei. B: glomerulus enclosed in a bounding box.**
full-term newborns (Table 2). Two-way ANOVA showed no interaction effect between gestational age and glomerular position within the kidney on glomerular area. There was a statistically significant main effect of gestational age (Fig. 2) (Table 3). A Bonferroni posttest indicated that glomerular area was significantly lower in the full-term group than in the fetus group \(t(21) = 3.16, P < 0.01\) and preterm \(t(55) = 2.63, P < 0.016\) groups. No differences between preterm infants and fetuses were found. A representation of variation in podocyte number at different gestational ages is depicted in Fig. 6.

Podocyte number relative to the glomerular area was \(0.43 \pm 0.10, 0.37 \pm 0.08, 0.31 \pm 0.06\), respectively, in fetuses, preterm infants, and full-term newborns (Table 2); this parameter was significantly higher in fetuses compared with the full-term group. Two-way ANOVA showed no interaction effect between gestational age and sex on the ratio between podocyte number and glomerular area. There was no significant main effect of sex. There was a statistically significant main effect of gestational age \(F(2, 56) = 6.32, P = 0.0033\) of gestational age (Fig. 5B) (Table 3). A Bonferroni posttest indicated that the ratio between podocyte number and glomerular area was significantly lower in the full-term group than in the fetus \(t(21) = 3.16, P < 0.01\) and preterm \(t(55) = 2.63, P < 0.016\) groups. No differences between preterm infants and fetuses were found. A representation of variation in podocyte number at different gestational ages is depicted in Fig. 6.

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Fig. 3. Intragroup variability. Two glomeruli of subjects who exhibited higher and lower podocyte number in fetuses (A), preterms (B), and full-term newborns (C) are shown.

Fig. 4. Correlation between podocyte number and body weight in fetuses (left), preterms (middle), and full-term newborns (right).
Fig. 5. Decreased podocyte number in fetuses, preterm, and full-term subjects. Total podocyte number (A) and podocyte number relative to glomerular sectional area (B) among fetuses, preterm, and full-term newborns in males (filled bars) and females (open bars) are shown. Values are means ± SD. *P < 0.05, **P < 0.01.

Fig. 6. Glomeruli of subjects at week 23 (fetus; A), week 36 (preterm; B), and week 41 (full term; C). Podocyte number significantly decreased with increasing gestational age.
DISCUSSION

In recent years, a strong link has been emerging between our life as a fetus and kidney-related diseases occurring in adulthood. This evidence strongly reinforces the theory of the developmental origins of adult kidney diseases (27). Developmental (or fetal) renal programming is rapidly becoming accepted as a key factor in the etiology of adult kidney diseases, including hypertension, nephrotic syndrome, and chronic kidney injury (3). Until now, researchers focused on variability in nephron number at birth (9), and the vast majority of studies in this field underlined the clinical importance of the nephron mass at birth as a key factor in determining our susceptibility to kidney disease in adulthood (4, 25). The “nephron number” hypothesis was confirmed in a mouse model by the finding that maternal treatment with glucocorticoids at midgestation might reduce nephron number of the offspring at birth (6). In that study, reduced nephron number did not necessarily lead to development of hypertension, thus suggesting that the mechanisms linking nephron deficit and renal insufficiency have not yet been completely clarified (8). The podocyte depletion hypothesis has emerged as an important concept in childhood and adulthood kidney pathologies (29). Consequently, the estimation of podocyte number represents a significant component of studies on progressive renal disease.

In this study, through a series of analyses, we have observed a decreasing trend in podocyte number during gestation. Podocyte number was significantly lower in full-term newborns than in fetuses, whereas variations in podocyte number were less evident between nearby groups. Furthermore, glomerular area analyses have shown no differences in glomerular area correlated with variations in gestational age. Taking into account the ratio between podocyte number within each glomerulus and its glomerular area, we observed a reduction in podocyte number; this reduction was due to variations in podocyte number rather than to variations in glomerular area.

The decreasing trend of podocyte number suggests that podocytes might undergo a programmed cell death (apoptosis) or alternatively transdifferentiation during glomerular growth, the high cell number in the first step of kidney development being correlated with the presence of both podocytes and podocyte precursors.

Moreover, by focusing on each group of subjects, our results strongly suggest an important intragroup variability in podocyte number. Subjects with the same gestational age occasionally have shown marked differences regarding podocyte number. The cause at the basis of this striking variability in podocyte number remains unclear. We speculate that variations in podocyte number could be correlated with factors such as drugs, maternal diet, or therapeutic intervention occurring during intrauterine life. These factors, as observed in nephron development, could be the basis of the differences in podocyte number between subjects at birth (1, 5, 7, 10, 20, 24).

In favor of this hypothesis, we investigated how variations in body weight, associated with events occurring during gestation, involve an adverse intrauterine environment and affect podocyte number. Preterms have proved to be more susceptible to variations in podocyte number related to variations in body weight: subjects with low body weight showed a higher podo-
cyte number than subjects with higher body weight, giving the idea of lower renal maturation.

The analyses concerning the glomerular position in the kidney showed how glomeruli closer to the renal capsule have a smaller area than those located in the deepest cortex zone. In addition, glomeruli in the deepest part of the cortex showed the higher number of podocytes. Changes in glomerular area and podocyte number related to the spatial location within the kidney could be associated with different stages of glomerular development (30).

The analyses regarding intraindividual variability in podocyte number have shown different average standard deviations associated with each individual. This aspect was observed in each class of gestational age.

However, caution should be considered in interpreting our results. The use of a single section method (39) may induce an overestimation of cell number (23). Using 5-µm sections could underestimate the podocyte capillary diameter (37), overestimating the absolute podocyte number. In addition, even if the podocyte detection performed by two experienced pathologists looks reliable, podocyte nuclear staining (e.g., WT1 or TLE4) would have been more appropriate to avoid errors in cellular identification. Finally, it is important to emphasize that the process of fixation may be responsible for the presence of artifacts (33, 40).

In conclusion, our study shows that podocyte number significantly differs during human fetal development, being significantly higher in fetuses than in full-term newborns. Importantly, a marked intragroup variability was observed even in subjects with the same gestational age. Moreover, further studies using specific markers of podocyte development are needed to assess possible causes of the variation in podocyte number. A deficient podocyte number may represent a predisposing factor for the development of podocytopathies later in life, in favor of the hypothesis of fetal programming of adult kidney disease.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS


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