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Relationship between weight at birth and the number and size of renal glomeruli in humans: A histomorphometric study

Reinaldo Mañalich, Leonardo Reyes, Mercedes Herrera, Clara Melendi and Isabel Fundora

National Institute of Nephrology, National Referral Center for Pathology, and "González Coro" Gynecology-Obstetric Hospital, Havana, Cuba

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Background. The number of nephrons in humans varies considerably under normal circumstances, and retarded intrauterine growth has been reported to be associated with a significant reduction in nephron number. Low nephron number may be an independent risk factor for the development of hypertension. We therefore decided to evaluate the relationship between body weight at birth and the number and size of nephron units.

Methods. We examined coronal sections of the kidneys of 35 neonates who died within two weeks of birth because of hyaline membrane, infectious complications, brain hemorrhage, or perinatal hypoxia and had no urinary congenital malformations. Nine of them (5 males and 4 females) were between 36 and 37 weeks of gestation, and the rest had 38 or more weeks of gestation. Eighteen neonates weighed less than 2500 g at birth [low birth weight (LBW); 9 females and 9 males], and 17 had weights above this value [normal birth weight (NBW); 8 females and 9 males]. In each section, glomeruli present in four sequential subcapsular microscopic fields, corresponding to 0.6 mm^2, were counted; in addition, the area of each of 65 consecutive glomeruli was determined by a computerized measurement system. Glomerular volume was calculated from the glomerular area. Linear regression analysis was used to test the relationship between glomerular number and size and the weight at birth.

Results. The number of glomeruli per 0.6 mm^2 of renal cortex was 92.9 ± 4.85 in the LBW and 105.8 ± 3.91 in NBW ($P < 0.0001$). Glomerular volume ($\mu^3 \times 10^{-3}$) was 529.1 ± 187.63 in the LBW group and 158.0 ± 49.89 in the NBW group ($P < 0.0001$). The glomeruli occupied 8.59 ± 1.38% of the kidney area under examination in the LBW group and 14.3 ± 2.75% in the NBW group ($P < 0.0001$). There were significant direct correlations between the weight at birth and the number of glomeruli ($r = 0.870$, $P < 0.0001$) and area occupied by glomeruli ($r = 0.935$, $P < 0.0001$). There were inverse correlations between the number of glomeruli and the volume of the glomeruli ($r = -0.816$, $P < 0.0001$) and the weight at birth and glomerular volume ($r = -0.848$, $P < 0.0001$). These findings were independent of sex and race (black vs. white). Essential arterial hypertension existed in 38.9% of the mothers of children with LBW and in 5.9% of the mothers of children with NBW ($P < 0.05$). Smoking habits existed in 50% of the mothers of LBW children and in 11.8% of the mothers of NBW children ($P < 0.05$).

Conclusion. There are strong correlations between glomerular number (direct) and size (inverse) with LBW in this cohort. Endowment with decreased nephron numbers may be a risk factor for hypertension and the rate of progression of renal disease.

Low birthweight (LBW) is an important public health problem in developing countries [1]. In 1988, Brenner, García, and Anderson noted that the number of nephrons was inversely correlated with the risk of developing hypertension in rats and, taking into account the population-based hypertensive risk in humans, put forth the provocative hypothesis that decreased nephron number is an independent risk for hypertension [2]. Since these classic studies [2], other workers have shown that LBW is associated with a higher incidence of high blood pressure in adulthood [3–8], and highlighted mechanisms, which if present in fetal life, would be relevant in the appearance of essential hypertension [6].

Since low-protein diets are experimentally associated with LBW, a decreased number of nephrons and increased risk of hypertension [9], Mackenzie, Lawler, and Brenner [10] postulated that low nephron number, genetically determined or acquired, is a likely explanation for the increased risk of hypertension in children with low weight at birth. In support of these postulates, studies have shown that spontaneously hypertensive rats are endowed with 10 to 25% fewer nephrons than their normotensive counterparts [10–12].

The present work was done to explore the relationship between weight at birth and the number and size of the nephron units existing in the human kidney.

Key words: low birthweight, arterial hypertension, smoking, progressive renal disease, intrauterine growth.

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METHODS

Coronal sections of kidneys from 35 children who died in the neonatal period from nonrenal causes and had no renal or urinary malformations were obtained from the Hospital Docente Gineco-Obstétrico "Ramón González Coro" in the period from January 1984 to January 1998. The gestational age of the infants at birth ranged from 36 to 41 weeks. Eighteen infants had a LBW (<2500 g), and the remaining 17 had a normal birthweight (NBW; >2500 g). The causes of death were hyaline membrane ($N = 15$), neonatal sepsis/pneumonia ($N = 10$), hypoxic complications ($N = 9$), and brain hemorrhage ($N = 1$). Estimates of the dietary protein intake in the mothers could not be made.

The kidneys in these infants had been fixed in formalin 15% and embedded in paraffin at the time of autopsy. For the present study, the kidneys were retrieved, and four serial coronal sections 2 μ thick were done with a vertical Leitz microtome. These sections were stained with hematoxylin and eosin for morphometric studies.

Histomorphometric analyses were done by a renal pathologist (C.M.) who was blinded as to the origin of the biopsy. Analyses were done with the help of a computerized area measurement system (DIGIPAT; Eicisoft, Cuba, 1995) supported by Windows 3.1 that includes capture of the image from an Olympus BM-2 microscope and digitalization of this image (BLASTER video card). The computerized system is commercially available has been officially validated (registry #TO220020621200).

The glomeruli counted were from the cortical area immediately under the capsule. Four consecutive fields to the right, representing an area of 0.6 mm^2, were chosen in each instance. The amplified image was used in the computer to delineate the Bowman capsule. At least 65 glomeruli, which could be completely delineated, were used for analysis in each case. The system analyzes directly the glomerular area, the maximal diameter, and the percentage of the total area occupied by the glomeruli.

The glomerular volume was calculated with the use of the following formula:

$$Vg = (\beta/\kappa \times A_G)^{3/2}$$

where $\beta = 1.38$ is the shape coefficient of spheres, and $\kappa = 1.1$ the size distribution coefficient. A_G is the glomerular volume [13].

Statistical comparisons between the LBW and NBW groups were done by nonparametric Mann–Whitney tests and Fisher's exact test with the help of a commercial statistical program (GraphPad InStat®) and the relationships between variables were explored with a commercial statistical graphs package (GraphPad Prism®).

Table 1. General data in the infants with low (LBW) and normal (NBW) weight at birth

	LBW ($N = 18$)	NBW ($N = 17$)
Gestational age *weeks*	37.0 ± 1.05[a]	38.9 ± 1.29
Sex *males/females*	9/9	8/9
Race *black/white*	10/8	7/10
Causes of death		
Hyaline membrane	9	6
Neonatal sepsis	5	5
Hypoxia	4	5
Brain hemorrhage	0	1
Glomerular number	92.9 ± 4.85[a]	105.8 ± 3.91
Glomerular volume $\mu^3 \times 10^{-3}$	529.1 ± 187.6[a]	158.0 ± 49.89
Area occupied by glomeruli %	8.59 ± 1.38[a]	14.3 ± 2.75

Data shown are mean ± SD.
[a] $P < 0.0001$ vs. NBW

RESULTS

Table 1 shows the comparison of the LBW and NBW groups with respect to weight, sex, gestational age and number of glomeruli, and glomerular morphometric data. All of the children had more than 36 weeks of gestation. Prematurity was present in nine children (5 males and 4 females). Arterial hypertension was found in 16 mothers of the children in this study. Eight of them were previously normotensive and had hypertension associated with gestation; in the remaining eight, essential hypertension was present prior to pregnancy. Essential hypertension existed in seven mothers of the children with LBW (38.8%) and in one mother of the children with NBW (5.9%. $P < 0.05$). Gestational hypertension occurred in five and three mothers of the LBW and NBW children, respectively (P = NS). Nine mothers of children in the LBW group (25.7%) were smokers, while only two mothers of the NBW children were smokers (5.7%, $P < 0.02$). The information about the fathers' hypertension and smoking was not available. There were no significant sex or race (black vs. nonblack) differences between the LBW and the NBW groups.

There was a significant ($P < 0.0001$) positive correlation between weight at birth and the number of glomeruli (Fig. 1), as well as the relative area occupied by the glomeruli in the renal cortex (Fig. 2). There were significant ($P < 0.0001$) negative correlations between the weight at birth and the glomerular volume (Fig. 3) and between the number and the volume of the glomeruli (Fig. 4). The findings of glomerular volume and glomerular number were independent of sex and race.

DISCUSSION

The total number of nephrons is a biological variable that is defined prior to birth. Approximately 60% of the nephron population develops during the third trimester of pregnancy, up to 36 weeks [14]. No new nephrons are formed after birth [14], and the total number of nephrons

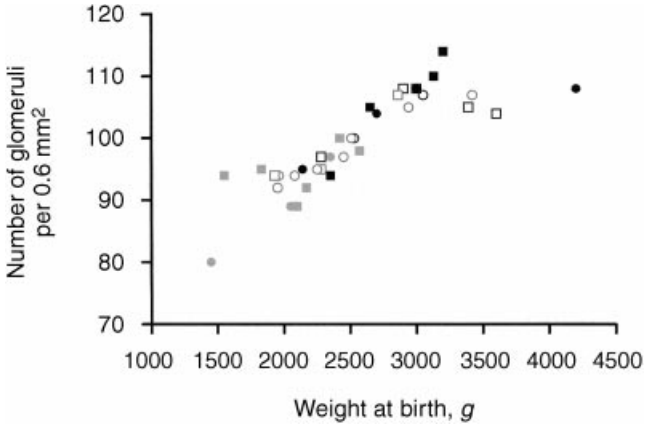

Fig. 1. Relationship between the weight at birth and the number of glomeruli. There is a significant ($r = 0.870$, $P < 0.0001$) relationship between the number of glomeruli in the subcapsular cortex and the weight at birth. It appears that the number of glomeruli increase progressively until the weight at birth reaches 3 kg and remains steady thereafter. Symbols are: (●, ○) females; (■, □) males; (●, ■) black race; (○, □) white race; (◍, ▣) gestation <38 weeks.

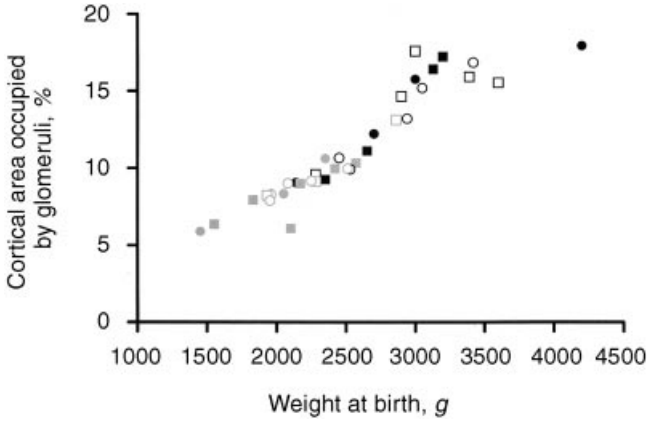

Fig. 2. Positive correlation between the percentage area of the renal cortex occupied by glomeruli and the weight at birth ($r = 0.935$, $P < 0.0001$). Symbols are: (●, ○) females; (■, □) males; (●, ■) black race; (○, □) white race; (◍, ▣) gestation <38 weeks.

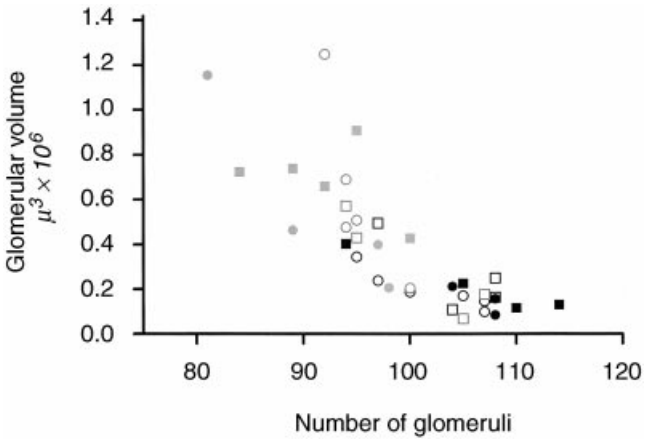

Fig. 3. Negative correlation between the weight at birth and the glomerular volume ($r = 0.840$, $P < 0.0001$). Symbols are: (●, ○) females; (■, □) males; (●, ■) black race; (○, □) white race; (◍, ▣) gestation <38 weeks.

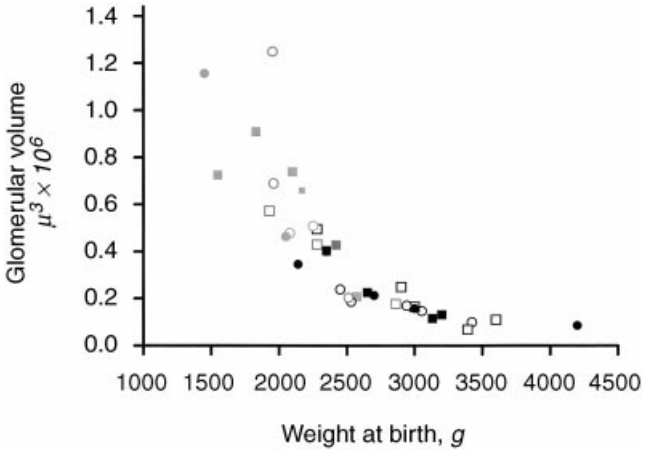

Fig. 4. Negative correlation between the number of glomeruli and glomerular volume ($r = 0.816$, $P < 0.0001$). Symbols are: (●, ○) females; (■, □) males; (●, ■) black race; (○, □) white race; (◍, ▣) gestation <38 weeks.

in humans ranges between 300,000 and 1.1 million, with a mean of approximately 600,000 [15–17]. The number of nephrons is a critical variable in the progression to chronic renal failure, because reductions in nephron number result in glomerular hypertension in the remaining nephron population, which, in turn, triggers a vicious cycle of progressive loss of functioning units [15]. Reduced number of nephrons at birth may be associated with a diminished resistance to any mechanism of renal damage in adult life.

Brenner and coworkers have recruited impressive evidence in favor of the theoretical construct that low nephron number is a risk factor for essential hypertension [10, 18, 19]. For instance, demographic studies have shown that populations with a very high incidence of essential hypertension have a relatively small kidney size, suggesting a diminished number of nephrons [20, 21]. The African American population, known to have a high incidence and increased severity of arterial hypertension (abstract; Falkerner et al, *J Am Soc Nephrol* 7:1549, 1996), appears to be endowed with smaller numbers of larger glomeruli (abstract; ibid) [22], changes considered to be evolutionary because in tropical conditions, sodium conservation would be an adaptive priority [19, 23, 24].

Our findings are in agreement with the observations of others, in that smoking and arterial hypertension in the parents are risk factors for intrauterine growth retardation and LBW [25–27]. Studies have shown that marked retardation in intrauterine growth exerts pro-

found effects in renal development. LBW is associated with a decreased number of nephrons and hypertension in adulthood. In full-term pregnancies with LBW caused by delayed fetal growth, there is a decrease of 20% in the number of nephrons [3, 24]. In rats, similar effects have been noted [11]. In the present work, we have documented a 20% reduction in nephron number in children with LBW (Table 1). In addition, we have found a close positive relationship between weight at birth and the number of glomeruli, as well as a negative correlation between number and volume of glomeruli. Future research should be directed to clarify the relationship between dietary protein intake in pregnancy, LBW, and nephrogenesis.

Reprint requests to Reinaldo Mañalich, M.D., c/o Bernardo Rodríguez-Iturbe, Apartado Postal 1430, Maracaibo 4001-A, Venezuela. E-mail: bri@iamnet.com

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