Fetal Programming of Adult Kidney Disease: Cellular and Molecular Mechanisms

Dov Hershkovitz,* Zvi Burbea,† Karl Skorecki,*†‡ and Barry M. Brenner§

*Laboratory of Molecular Medicine and †Department of Nephrology, Rambam Health Care Campus, and ‡Rappaport Faculty of Medicine and Research Institute, Technion Israel Institute of Technology, Haifa, Israel; and §Brigham and Women’s Hospital and Harvard Medical School, Boston, Massachusetts


The role of the kidney in the pathogenesis of hypertension has long been established, although recent studies challenge renal hegemony and suggest an important role for vascular cells as well (1,2). In recent years, the formulation has expanded and now includes the concept that chronic hypertension and kidney disease are also related to events that occur during the prenatal period and usually result in low birth weight (LBW).

A recent overview considered the role of fetal programming in the development of adult kidney disease and hypertension (3). The aim of this review is to present further evidence to support an association between LBW and the increased prevalence of hypertension as well as progression toward chronic kidney disease (CKD) in adult life. Various potential mechanisms for this association are discussed, specifically the low nephron number (LNN) hypothesis and related cellular and molecular mechanisms that have been proposed.

Definition of LBW

LBW infants are defined as infants who weigh ≤2500 g at birth. Infants who are delivered before 37 wk from the first day of the last menstrual period are termed preterm. The LBW infant population can be divided into preterm, appropriate for gestational age (AGA), or small for gestational age (SGA). The predominant cause of LBW infants in the United States is preterm birth, whereas in developing countries, the cause is usually intrauterine growth restriction (IUGR). During the year 2004, 8.1% of births in the United States were LBW, and more than half of these were preterm. During the past two decades, there has been an increase in the prevalence of LBW as higher risk pregnancies progress to term and postnatal survival improves (4) (Figure 1).

Association between LBW and Adult Disease

The size that the fetus can attain depends on the maternal–fetal nutrient supply and the space that the maternal environment can provide (5). Low maternal socioeconomic status and poor maternal nutritional status will reduce nutrient supply to the developing fetus. Similarly, factors that frustrate the passage of nutrients through the placenta, such as smoking and hypertension, are associated with increased risk for LBW. Factors that affect intrauterine space availability, such as primiparity, low maternal height, and mothers who were born SGA, are additional risk factors for LBW. Nearly two decades ago, Barker (6) presented the theory of the fetal origins of adult diseases. The theory is based on observed epidemiologic associations between LBW and increased risk for ischemic heart disease, type 2 diabetes, and hypertension (7–9).

The first associations found were between LBW and later life hypertension and cardiovascular disease (7,10,11). High BP was found to occur at a higher incidence in children and adults who were of LBW (11). A systematic review of 80 studies that described the relationship of BP with birth weight reported that systolic BP was lower by approximately 2 mmHg for every 1-kg increase in birth weight (12). Among Pima Indians, patients who had type 2 diabetes and a history of LBW also displayed an increased risk for developing diabetic nephropathy (13). Moreover, LBW is associated with more rapid progression of other kidney diseases, such as IgA nephropathy, membranous nephropathy, and minimal-change disease, suggesting that the kidneys of LBW infants are more vulnerable to future insults (14–16).

Nevertheless, the strength of association between birth weight and subsequent hypertension remains widely debated. Several studies did not find an association (17,18). Moreover, others claim that the suggested association is the result of inadequate or inappropriate adjustments for confounding factors (19,20). For example, Huxley et al. (19) found a trend toward a weaker association between LBW and hypertension in larger compared with smaller studies. However, it seems that although the relation is not invariant, the totality of evidence does suggest an important direct or indirect interaction between birth weight and subsequent hypertension (21).

These observations have led to the formulation of an important conceptual construct that pertains to the fetal origins of adult disease. This theory states that during development, body organs pass through a period of plasticity and sensitivity to the environment, which leaves a durable imprint that affects sub-
the scope of this review. Beyond the connection with type 2 diabetes and hence diabetic nephropathy, the contribution of various additional factors has been invoked to explain the apparent greater risk and more rapid progression of adult kidney disease and the higher incidence of hypertension as a result of LBW. Among these, the major focus of attention has been on the role of LNN (33,34). LBW has been associated with LNN in human and animal studies. The induction of IUGR by restriction of protein in the diet resulted in reduced nephron number and impaired renal function in rats (35,36). Nephron number and GFR were significantly lower, even after correction for body weight, in piglets with IUGR compared with those with normal weight (37). Reduced nephron number has been achieved using other experimental models of LBW, such as calorie restriction and partial ligation of the uterine artery (38–41). Reduced glomerular number and function were found in spontaneous LBW as well as induced LBW in a rat model (42).

The findings in the animal models accord well with data from human studies. Hinchcliffe et al. (43) examined nephron number of stillborns and infants who died within 1 yr of birth and found that nephron number was significantly lower in the infants with IUGR compared with the AGA infants. A more recent study examined the kidneys of neonates in relation to their birth weight and found a lower nephron number and an increased glomerular volume in the growth-restricted group (44). A similar correlation was found in adults (45), and these correlations were found to be independent of gender (46). A significant correlation between birth weight and nephron number has been reported in white Americans, whereas no such correlation could be demonstrated for black Americans, although the study may not have been sufficiently powered to achieve statistical significance (47). In adult aboriginal Australians, a population with a high prevalence of LBW and kidney disease, fewer glomeruli and larger mean glomerular volume were found compared with the non-aboriginal Australian population. Glomerular number correlated with adult height, suggesting a relationship with birth weight (48). It should be noted that normal birth weight does not automatically suggest a normal nephron number, and some individuals with normal birth weight may have a low or suboptimal nephron number (silent nephron deficit) (49).

Ultrasound studies that examined the fetal kidney have supported the findings in the aforementioned histologic studies. Konje et al. (50) reported that during the third trimester, the fetal renal growth in SGA fetuses was slower and resulted in thinner kidneys of normal length, compared with AGA kidneys. This might suggest impairment of nephron number in spontaneous LBW as well as induced LBW in a rat model (42).

Why Would LBW Infants Have LNN?

The relation between birth weight and hypertension may be the result of fetoplacental, maternal, or genetic factors. A twin study of 653 individuals found that the relationship is due mainly to fetoplacental factors and that genetic and maternal factors play a minor role (52). Similarly, in a cohort of female twin pairs, the differences in BP were suggested to result from delayed intrauterine growth (53). Other twin studies, however, have indicated an important contribution of genetic factors to adult hypertension in the LBW setting (54,55). A recent study sought to distinguish between the effects on kidney growth of preterm birth versus the SGA state. It was found that weight for gestational age is an important determinant of kidney size at birth and at 18 mo of age. The authors suggested that weight for gestational age rather than birth weight should be used in analysis of the impact of fetal growth on renal function (56).
Similarly, in a study of 422 individuals who were 19 yr of age and were born preterm (<32 wk), birth weight was related to renal outcome. However, no convincing relation between gestational age and renal function was found, suggesting again that the important determinant of kidney function is the extent of IUGR (57).

The most prevalent animal models for LBW-induced adult disease are protein and calorie restriction. The “life history theory” states that if the total amount of energy to an animal is limited, then increased allocation of energy to one organ system must reduce allocation to one or more other organ systems (58). For example, the undernourished fetus protects its brain development by diverting more blood to the brain at the expense of blood supply to other organ systems. Lesser growth of the kidney, for example, might be a “trade off” to protect brain growth. Several cellular and molecular mechanisms have been suggested as contributing to the consequent impaired nephrogenesis.

Mechanisms
Role of Telomere Integrity and Renal Cell Turnover
The ends of linear chromosomes are capped by complex nuclear protein structures, which include stretches of repetitive DNA sequences (TTAGGG)n, known as telomeres (59). Human telomeres shorten with each round of cell division, through various mechanisms, in a process that might have an important role in cellular senescence and organism aging (60). The starting telomere length after conception in humans is usually in the range of 12 to 15 MB (61). Replicative arrest is associated with telomere shortening to approximately 2 to 5 kb, for at least one or more chromosomes. In the absence of telomerase, abnormalities in DNA replication or sister chromatid separation are among the mechanisms that have been implicated to compromise genomic integrity in senescent cells with short telomeres (62). Most organ systems undergo continuous cellular turnover in health, and this process is often accelerated as part of the response to injury. It is postulated that a subset of cells (referred to as adult or tissue stem cells) may be retained in the postnatal state for this purpose. Accordingly, lifelong organ system maintenance depends on the number of renal cell divisions that are required during fetal organ development and growth, subsequent cellular turnover in health and disease, the rate of telomere shortening, and the action of mechanisms to preserve or restore telomere length/integrity. These factors are organ system and life history specific and expected to be markedly affected by intrauterine growth and subsequent postnatal catch-up growth. Indeed, after IUGR, longevity was reduced in male rats that experienced postnatal catch-up growth, compared with those that did not. Examination of telomeres correspondingly revealed shorter postnatal catch-up growth. In this study, IUGR caused the increased expression of proapoptotic factors such as p53 and Bax. In the case of p53, this occurred through hypomethylation of the promoter. In addition, heightened p53 activity induced the downregulation of antiapoptotic factors, such as Bcl-2. As a consequence, an increase in the ratio between proapoptotic and antiapoptotic factors promotes renal cellular apoptosis, possibly leading to LNN in the offspring (72).

Additional Mechanisms in the Regulation of Nephron Cell Mass
The cellular mass of organ systems during development depends on a careful balance between cell proliferation and apoptosis. In animal models, IUGR was associated with evidence of higher levels of renal cellular apoptosis. Rat offspring that were born to a mother that was supplied a low-protein diet during pregnancy had fewer glomeruli than normal. Molecular and morphologic analysis found increased metanephric apoptosis in the group with IUGR. The authors suggested that fewer glomeruli were the result of increased apoptosis, leading to a reduction in renal progenitor cells (67). Several molecular mechanisms have been proposed for IUGR-induced renal cellular apoptosis.

The renin-angiotensin system (RAS) and the paired homeobox 2 gene (Pax-2) are critical factors in nephrogenesis (68–70). Angiotensin II was shown to increase Pax-2 gene expression, a process that might be important in renal development and repair. In rats with IUGR, the RAS is downregulated, leading to reduced Pax-2 expression, thereby causing a shift from proliferation toward apoptosis and resulting in LNN (71).

Bilateral partial ligation of the uterine arteries of the pregnant rat, another model for IUGR, resulted in reduced nephron number and an increase in apoptosis in the kidneys of juvenile rats. In this study, IUGR caused the increased expression of proapoptotic factors such as p53 and Bax. In the case of p53, this occurred through hypomethylation of the promoter. In addition, heightened p53 activity induced the downregulation of antiapoptotic factors, such as Bcl-2. As a consequence, an increase in the ratio between proapoptotic and antiapoptotic factors promotes renal cellular apoptosis, possibly leading to LNN in the offspring (72).

Mechanisms that involve mitochondria have also been postulated in the relationship among LBW, LNN, and adult disease. Mitochondrial dysfunction can give rise to abnormalities in any organ or tissue (73). In the kidneys, mitochondrial DNA (mtDNA) mutations have been associated with hypertension and ESRD (74,75). A mitochondrial gene variant was found to be more prevalent in a cohort of 58 black individuals with hypertension-associated ESRD compared with 58 normotensive
individuals (74). Other mitochondrial mutations have been associated with hypertension and renal pathologies (75). In rats, IUGR may cause mitochondrial dysfunction. Protein malnutrition in utero causes changes in mtDNA content, impaired cell development, and a reduced insulin secretory response to glucose (76). In addition, partial ligation of both uterine arteries resulted in increased accumulation of mtDNA mutations and reduced mitochondrial activity in the pancreatic cells of offspring. The deterioration in mitochondrial function leads to a decline in cell function and the development of diabetes (77). Uteroplacental insufficiency was also shown to cause reduced mtDNA and mitochondrial protein production in the brain of juvenile rats (78). In another IUGR model, protein malnutrition in fetal life resulted in reduced mtDNA content in liver and skeletal muscle (79). Therefore, it seems likely, as has been shown in the case of pancreatic β cells, that mtDNA mutations and mitochondrial dysfunction might play a role in the development of LNN in the kidney with IUGR, leading to the subsequent emergence of hypertension and kidney disease.

The predominant cause of LBW in the United States is preterm birth. Nephron development in humans begins in the ninth week of gestation and ceases during the 36th week. During the last trimester, there is a rapid rise in nephron number (80). A histomorphometric analysis of 56 extremely preterm infants demonstrated postnatal nephrogenesis. However, fewer glomeruli were generated, and glomerulogenesis ceased after 40 d. Moreover, it was noted that renal failure caused further inhibition of kidney development (81). Therefore, it is tempting to postulate that the extremely preterm infant will not experience an equivalent degree of kidney growth ex utero as compared with in utero and that this by itself would be a cause of LNN. However, as stated earlier, weight for gestational age rather than gestational age per se has been found to be associated with kidney function.
LNN as an Important Determinant of Hypertension and CKD

LNN is associated with an increased risk for hypertension and renal disease, an effect whose pathogenesis is attributed at least in part to glomerular hyperfiltration and/or hypertrophy (82). With reduced nephron mass, the remaining nephrons adapt and undergo hypertrophy to minimize the overall loss of function. Consequently, hyperfiltration occurs in each of the remaining nephrons to compensate for reduced nephron mass and function. This hyperfiltration has pathophysiologic implications: The glomeruli function under increased intracapillary hydraulic pressure, which over time damages the capillary walls. These abnormal conditions have been shown to lead to the development of proteinuria, FSGS, and a progressive decline in the GFR (82).

The postmortem quantitative analytical examination of kidneys of accident victims found that those with hypertension had fewer glomeruli and greater glomerular volume than those of matched normotensive controls (47,83). In animal models, lower renal mass has also been associated with a larger increment in BP in response to increased daily salt intake (84). In genetic models of hypertension in rats, diminished nephron endowment has been associated with hypertension and an increased tendency toward glomerulosclerosis (85–88).

Clinical support for the impact of LNN on future risk for hypertension and renal disease can also be deduced from studies of patients with solitary kidneys. In patients with a single kidney, as a result of either unilateral agenesis or nephrectomy, the incidence of proteinuria (>300 mg/24 h), hypertension, and chronic renal failure was significantly increased compared with control subjects (89–91). Reduced nephron number in the transplanted solitary kidney may likewise play a role in chronic allograft failure, especially when donor nephron number is not appropriately matched to the recipient. In support of this formulation is the finding that the frequency of graft loss 3 yr after transplantation was higher when the donors were women, individuals of African ancestry, or of age <3 or >61 yr (92). In a study of 31,515 cadaveric kidney transplant recipients, graft failure was significantly associated with donor age, gender, and population ancestry and recipient body surface area (93). Transplants into recipients who weighed >100 kg was associated with lower 1- and 3-yr graft survival rates (94). The findings are supported by animal studies that demonstrated the importance of nephron mass in the development of chronic allograft nephropathy (95–99).

The foregoing suggests that factors that are associated with lower donor nephron mass (child or elder donor, female to male) or increased recipient needs (obese recipients) play a significant role in kidney transplant loss. Furthermore, these findings indicate that the number of viable nephrons (i.e., “nephron dose”) is an important determinant of renal survival in both transplanted and native kidneys. Unfortunately, this consideration has not been granted sufficient attention in setting kidney transplantation policy and practice guidelines.

The increased prevalence of CKD and hypertension in adults who are born at LBW can be explained by understanding the concepts of the developmental origins of adult chronic disease.

The LBW infant begins life at a renal disadvantage, as a result of LNN. This individual is then vulnerable to developing overt nephropathy in the event of postnatal “second hits,” such as diabetes or other forms of renal injury. Combined with nephron “overload” that is expected during catch-up growth, the risk for progression of these renal injury states toward CKD is enhanced (Figure 3).

Additional Mechanisms for LBW-Related Hypertension

Additional mechanisms have been studied in terms of the relationship between LBW and adult kidney disease and hypertension. Endocrine mechanisms have been proposed to be involved in the intrauterine programming of adult hypertension and renal disease. A number of hormones have an essential role in the regulation of fetal growth and fetal tissue development (100). Spontaneous and experimentally induced IUGR have been shown to induce postnatal changes in hormonal regulation. Of the hormones that are known to be involved in fetal development, the glucocorticoids are the most likely to cause tissue programming in utero (101).

Reduced fetal nutrient availability increases levels of glucocorticoids, which may induce endocrine changes with long-term consequences on tissue growth and development. Maternal exposure to glucocorticoids during pregnancy caused hypertension and insulin resistance in the offspring rat and sheep models (102–105). The hypertensive effect of in utero glucocorticoids may be the result of cellular and molecular changes in various tissues. For example, in sheep, glucocorticoids induce functional changes in the kidneys and the brain by altering the expression of ion transporters and components of the RAS (104,106,107). Glucocorticoids and other hormones have a key role in regulating intrauterine development and therefore may significantly influence the evolution of adult disease.

Another possible mechanism involves the inappropriate retention of sodium. The maternal protein restriction model for IUGR in rats resulted in upregulation of two critical Na transporters. The Na-K-2Cl and the Na-CI co-transporters but neither the Na/H nor the Na channel was significantly increased in the rats with IUGR (108). In addition, expression of the glucocorticoid receptor, as well as α1 and β1 subunits of Na-K ATPase, was found to be increased after maternal protein restriction (109). These alterations lead to a lower rate of urinary sodium excretion with attendant sodium retention and hypertension.

Conclusion

The increasing prevalence of patients with CKD at all stages presents a significant challenge to population health worldwide. Primary and secondary prevention measures have demonstrated beneficial effects when targeted to “high risk” groups (110,111). LBW is a risk factor whose prevalence and importance are also increasing. The LBW infant may have experienced during pregnancy significant obstacles that the embryo confronted during development, and success in overcoming these challenges may have untoward consequences in adult life.

Animal and human studies have shown impaired kidney
maturation (LNN), “endocrine programming,” and telomere-limited exhaustion of progenitor pools for cell turnover to be key factors in the development of adult disease in LBW infants. Various genetic and hormonal factors are altered as the fetus adapts to an unfavorable intrauterine environment. Among these are key regulators of nephrogenesis and nephron size. These adjustments result in the development of kidneys whose long-term integrity is compromised.

As indicated, LBW followed by catch-up growth is associated with adult hypertension and renal disease. Keeping in mind the importance of LBW and IUGR as risk factors for the development of hypertension and adult kidney disease, it is of utmost importance to follow these LBW patients longitudinally over time and try to reduce disease incidence.

It is said that “a very long journey begins with a small step.” In the journey of life, special attention should be given to those who began with too small a step so that we can help them have a healthier journey.

Acknowledgments
We acknowledge the support of the Barrie and Carol Rose Fund of the Canadian Technion Society for generous support.

Disclosures
None.

References
2. Mendelsohn ME: In hypertension, the kidney is not always the heart of the matter. J Clin Invest 115: 840–844, 2005
12. Huxley RR, Shiell AW, Law CM: The role of size at birth and postnatal catch-up growth in determining systolic


28. Hughson M, Farris AB 3rd, Douglas-Denton R, Hoy WE, Bertram JF: Glomerular number and size in autopsy kid-


63. Jennings BJ, Oznane SE, Dorling MW, Hales CN: Early growth determines longevity in male rats and may be related to telomere shortening in the kidney. FEBS Lett 448: 4–8, 1999

64. Hales CN: Suicide of the nephron. Lancet 357: 136–137, 2001


80. Hinchliffe SA, Sargent PH, Howard CV, Chan YF, van