Title: Fetal and child health - impact on kidney development and long-term risk of hypertension and kidney disease

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Fetal and child health - impact on kidney development and long-term risk of hypertension and kidney disease

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Developmental programming of non-communicable diseases is now an established paradigm. With respect to hypertension and chronic kidney disease, adverse events experienced in utero can affect development of the fetal kidney and reduce final nephron number. Low birth weight and prematurity are the most consistent clinical surrogates for a low nephron number, and are associated with increased risk of hypertension, proteinuria and kidney disease in later life. Rapid weight gain in childhood or adolescence further compounds these risks. Low birth weight, prematurity and rapid childhood weight gain should alert clinicians to an individual’s life-long risk of hypertension and kidney disease, prompting education to minimize additional risk factors and ensuring follow-up. Birth weight and prematurity are significantly impacted by maternal nutrition and health during pregnancy. Optimization of maternal health and early childhood nutrition could therefore attenuate this programming cycle and reduce the global burden of hypertension and kidney disease in the future.
Key messages:

- Low birth weight and prematurity are significant risk factors for hypertension, proteinuria and chronic kidney disease in later life.
- Low birth weight and prematurity occur in 15% and 9.6% of live births respectively world wide, suggesting a high proportion of the world’s children are at risk of hypertension and kidney disease.
- Low birth weight and prematurity are associated with a congenital reduction in nephron number.
- Low nephron numbers are associated with higher blood pressures and greater susceptibility to kidney disease.
- High birth weight, especially as a result of exposure to maternal diabetes in utero, is associated with increased risk of proteinuria and kidney disease in later life.
- The risk of low birth weight and prematurity is impacted by maternal nutrition and health before and during pregnancy, as well as the mother’s own birth weight, demonstrating the intergenerational effects of programming.
- Upward crossing of weight or body mass index centiles in childhood or adolescence, is associated with increased risk of high blood pressure, progression of renal disease, type 2 diabetes, obesity and cardiovascular disease in later life. These effects can be independent of birth weight.
Hypertension is now the leading risk factor for global disease burden, and is a major cause and consequence of chronic kidney disease (CKD). Global deaths from kidney disease have risen by 83% since 1990. Recognition of the burden of CKD, its’ risk factors, and implementation of prevention strategies are therefore key to saving many lives. Increasingly, fetal and early childhood development are being recognized as important contributors to the development of non-communicable diseases (NCDs). The Developmental Origins of Health and Disease concept is based on the epidemiologic observation of a graded risk for hypertension, type 2 diabetes, cardiovascular and CKD across the range of fetal and early childhood development. Acknowledgement of this paradigm is important because interventions to optimize fetal and child health as strategies to prevent adult NCDs have great potential economic, societal and individual benefit. Many developing countries carry the dual burdens of under- and overnutrition contributing to the vicious cycles of poor maternal health, suboptimal fetal development and unhealthy childhood growth which all augment the risk of adult disease. In this manuscript we will describe how fetal and child health impact kidney development and risk of disease, focusing mainly on human studies, but utilizing animal data where necessary to provide more insight.

IMPACT OF FETAL DEVELOPMENT ON KIDNEY FUNCTION AND OUTCOMES

Twenty five years ago, Brenner and colleagues proposed that a congenital (developmentally programmed) reduction in nephron number may explain why some individuals are more susceptible to hypertension and renal injury than others. A kidney with fewer nephrons was postulated to have a reduced filtration surface area, resulting in limitation of sodium excretion leading to higher blood pressures, as well as a reduced renal adaptive capacity in the setting of injury. This hypothesis suggested a plausible link between higher prevalences of hypertension and renal disease in populations with a higher incidence of low birth weight (LBW), where LBW was expected to be associated with lower nephron number (Figure 1). Consistent with this hypothesis, various animal models have confirmed the association of LBW with later-life hypertension, mediated, in
part, by a reduced nephron number acquired in utero (the pathophysiologic mechanisms impacting nephrogenesis are reviewed elsewhere)\textsuperscript{9}. Similarly, in humans, LBW is a risk factor for hypertension and CKD as will be discussed below\textsuperscript{10}. LBW is a marker of poor fetal growth. Risk factors for LBW vary in developed and developing countries, but the global incidence is 15\%, suggesting that a large number of children born yearly are at risk of hypertension and kidney disease in later life (Figure 2)\textsuperscript{11}. Given the complexity and far reaching impact of developmental programming, understanding the most proximal origins of hypertension and renal disease risk is crucial to developing public health strategies to reduce their global impact\textsuperscript{4}.

LBW is universally defined as a birth weight under 2.5 Kg, whereas high birth weight (HBW) is defined as above 4.0 or 4.5 Kg\textsuperscript{12}. Other terms reflecting neonatal size include intrauterine growth restriction (IUGR), small for gestational age (SGA) and large for gestational age (LGA). For simplicity, we will use LBW to include all those with a birth weight under 2.5 Kg, including those that are SGA, and HBW to include those with birth weights above 4.0 – 4.5 Kg and LGA. A premature infant, \textit{i.e.} born before 37 weeks of gestation, is generally LBW, which may be an appropriate weight for gestational age (AGA) if growth occurred normally until birth, or SGA if growth was restricted\textsuperscript{12}.

**Nephron number in humans**

It is now well established that the total number of nephrons in the normal adult human kidney varies widely\textsuperscript{13}. While the average number of nephrons per kidney was assumed to be between 900,000 and 1 million, the observed range is over 10-fold\textsuperscript{13}. In the largest study to date (Monash series), total nephron number among 176 African Americans ranged from 210,332 to 2,702,079 and among 132 white Americans, ranged from 227,327 to 1,660,232\textsuperscript{14}.

Birth weight correlates linearly with nephron number in humans, and nephron number was found to increase by 257,426 per kilogram increase in birth weight, suggesting by extrapolation that nephron numbers would be lower in people of LBW\textsuperscript{15}. Although nephron numbers have rarely been measured in adults of known LBW, nephron numbers
were significantly reduced in LBW infants\textsuperscript{16,17}. It is important to appreciate that the total number of nephrons in an adult human kidney reflects the number of nephrons formed during development (nephron endowment) minus the number of nephrons subsequently lost, therefore cumulative injury over time may contribute to a kidney’s reaching a critically low nephron number, leading to disease\textsuperscript{13}. Nephrogenesis in humans ends at around 36 weeks of gestation, after which no new nephrons can form\textsuperscript{16}. In the Monash series, nephron number in 15 infants who died before the age of 3 months ranged 4.5-fold, from 246,181 to 1,106,062, suggesting that much of the variation in adult human nephron number is determined before birth\textsuperscript{18}.

**Developmental determinants of low nephron number**

The most robust clinical surrogates for low nephron number are LBW and prematurity. Not all factors impacting nephron number result in LBW, however, therefore awareness of risk factors for low nephron number per se is also important (Table 1). The most important risk factors, some of which may be modifiable with public health interventions, include maternal health and nutrition, pre- and post-natal environments, prematurity and genetic predisposition\textsuperscript{19}.

*Maternal factors* impacting risk of LBW and prematurity should be considered risk factors for low nephron number in the offspring (Figure 1)\textsuperscript{20}. Mothers who had themselves been of LBW, compared to non-LBW, had a higher odds of having LBW babies (OR 1.8, 95% CI 1.3-2.5) independent of socioeconomic conditions, suggesting a genetic or epigenetic intergenerational effect\textsuperscript{21}. *Hypertensive disorders* of pregnancy occurred in 8.4 million women worldwide in 2004, and are major risk factors for LBW\textsuperscript{22}. As outlined elsewhere in this series, risk of preeclampsia in a mother is increased by her being of LBW (OR 1.69, 95% CI 1.4 – 2.02), premature (OR 1.95, 95% CI 1.54-2.47), or by her parents being born in pre-eclamptic pregnancies (OR 2.2, 95% CI 2.0 - 2.4) demonstrating the complexity of intergenerational programming\textsuperscript{23-25}. Pre-pregnancy maternal *CKD and hypertension* are also significant risk factors for preeclampsia, LBW
and preterm delivery\textsuperscript{26}. In a Cuban cohort, maternal hypertension was associated with LBW, which in turn was associated with low nephron number\textsuperscript{17}.

The world-wide prevalence of gestational diabetes is poorly documented, but reportedly varies from 0.1 – 25.3\%\textsuperscript{27}. Maternal obesity, now occurring in 15 - 20\% of pregnancies, is a strong risk factor for gestational diabetes, as is maternal prematurity\textsuperscript{25, 28}. In experimental animals, maternal hyperglycemia is associated with reduced nephron number, higher blood pressures, microalbuminuria and reduced GFRs in offspring\textsuperscript{29}. In humans, renal functional reserve was found to be reduced in adult children of diabetic mothers compared to diabetic fathers, suggesting a reduction in nephron number acquired during gestational diabetes exposure\textsuperscript{30}. Maternal diabetes is also associated with a 3 fold increased risk of renal agenesis and dysgenesis, therefore hyperglycemia strongly impacts fetal renal development\textsuperscript{31}. Furthermore, gestational diabetes is often associated with infant HBW, a known risk factor for subsequent hypertension, type 2 diabetes, renal and cardiovascular disease, although the impact on nephron number is unknown\textsuperscript{32}.

Maternal behaviors during pregnancy may impact fetal nephrogenesis. Maternal smoking has been associated with LBW and low nephron number in humans\textsuperscript{17}. Alcohol consumption is associated with dose-dependent increased risk of prematurity and fetal growth restriction\textsuperscript{33}. In animals, gestational alcohol exposure impairs embryonic ureteric bud branching, resulting in low nephron number, and may therefore be a risk in humans\textsuperscript{34}.

Prenatal factors: Maternal diets deficient in protein, total calories or iron have all been shown to reduce nephron numbers in experimental animals, most often associated with LBW\textsuperscript{9}. In humans, maternal protein and micronutrient deficiencies are common in developing countries, and maternal malnutrition, underweight, iron deficiency and anemia are all recognized risk factors for LBW\textsuperscript{35}. Vitamin A deficiency is also highly prevalent among pregnant women world-wide (Figure 2)\textsuperscript{36}. In animals, maternal diets deficient in vitamin A, resulting in levels similar to those seen in deficient humans, induce a dose-dependent reduction in nephron number, whereas vitamin A
supplementation augments nephron number\textsuperscript{37}. Importantly vitamin A deficiency alone does not cause LBW, suggesting the impact may be overlooked if a normal birth weight (NBW) is presumed to exclude an adverse developmental environment. The active metabolite of Vitamin A, Retinoic acid, regulates transcription of Ret, a tyrosine kinase receptor critical for kidney development. It is therefore plausible that vitamin A intake may be an important determinant of nephron number in humans. Indeed, maternal vitamin A deficiency was associated with significantly smaller newborn adjusted renal volume in Indian compared to Canadian infants, likely reflecting lower nephron numbers (Table 1)\textsuperscript{38}.

Prematurity occurs in 9.6\% of live births (Figure 2) and is associated with higher blood pressures, renal and cardiovascular disease in later life\textsuperscript{12, 39}. Nephron number correlates with gestational age in premature infants in whom nephrogenesis may continue for a period after birth, although glomeruli are large and abnormal and renal maturation appears accelerated\textsuperscript{16, 40}. Consistent with these morphologic abnormalities, prematurity is a risk factor for acute kidney injury (AKI), which is an independent predictor of mortality and subsequent CKD in very low birth weight (VLBW) infants\textsuperscript{12}.

Post-natal factors: Nephrogenesis in humans is complete at term, however ongoing nephrogenesis has been observed up to 40 days after birth in infants born before 30 weeks of gestation\textsuperscript{40}. A window of vulnerability therefore exists in preterm infants in which kidney development can be impacted (Table 1). Indeed, extrauterine growth restriction was associated with significantly lower GFRs in VLBW children at a mean of 7.6 years of age, and conversely the odds of renal impairment was 33\% lower at 6.4 years among VLBW children who had gained more weight in neonatal intensive care, demonstrating the importance of early nutrition on kidney development\textsuperscript{41, 42}. Nephron number was found to be lower in premature infants who developed renal failure before death, although whether this was a cause or consequence of low nephron number is unknown\textsuperscript{40}.
Many premature infants receive perinatal medications such as non-steroidal anti-inflammatory drugs, glucocorticoids and aminoglycosides. Extrapolating from animal models, these and other medications may impact nephron number as well as increase the risk of AKI in infants\(^43\). Interestingly, however, follow up of individuals exposed to betamethasone for 48 hours prior to birth did not reveal any increase in blood pressure at age 30 years compared to controls\(^44\). Short-term steroids may therefore not impact kidney development, but the effects of such common medications on human nephrogenesis requires further study.

**Genetics:** Rare genetic and congenital abnormalities resulting in renal hypoplasia contribute to about half of all childhood end-stage renal disease (ESRD)\(^45\). Search for common polymorphisms in several genes known to participate in kidney development have shown correlations between altered gene transcription and newborn kidney size, which is proportional to nephron number(Table 1)\(^18\). These studies have been conducted in primarily Caucasian populations, therefore implications in other populations need to be investigated\(^18, 46, 47\). The molecular mechanisms regulating nephrogenesis are expertly reviewed elsewhere\(^48\). Individual permutations of these genetic variants may explain the wide variability seen in human nephron numbers, as some reduce and some augment kidney volume. Interaction between genetic polymorphisms and environmental circumstances during kidney development has not been studied. Gene microarray analysis of neonatal kidneys revealed global downregulation of gene expression in animal models of maternal low protein diet or placental insufficiency\(^49\). It is therefore conceivable that altered levels of gene expression resulting from a polymorphism may become even more amplified under conditions of superimposed maternal nutrient deficiency, further decreasing nephron number\(^50\).

**Clinical surrogates for nephron number**

At present, all reports of human nephron number have come from kidneys obtained at autopsy. Given the current reliance on autopsy specimens, surrogate markers for nephron number are important (Table 2).
Like total nephron number, mean glomerular volume was found to vary up to 10-fold in the Monash Series of 420 kidneys of subjects from five ethnic groups\textsuperscript{51}. Total nephron number consistently varies inversely with mean glomerular volume\textsuperscript{13}. This increase in glomerular volume likely reflects compensatory hypertrophy and hyperfiltration in individual nephrons. Indeed, total filtration surface area is relatively preserved in kidneys with low nephron number, possibly at the expense of increased glomerular pressure, which can accelerate further nephron loss\textsuperscript{14}. Consistent with this, increased glomerular size is a predictor of poorer renal outcomes among African Americans, Pima Indians and Aboriginal Australians, and in the absence of other causes, should be considered a surrogate for low nephron number\textsuperscript{9}. Given the heterogeneity and hypertrophy occurring in glomeruli, kidney size has not been consistently found to correlate with nephron number in adults, but the relationship appears linear in infants under 3 months of age\textsuperscript{18,52}

**Clinical impact of nephron mass**

**Birth weight, prematurity and blood pressure**

Studies among monozygotic twins, where the lighter twin was found to have higher subsequent blood pressures, suggest that environmental programming may be more crucial than genetic factors\textsuperscript{53}. LBW and prematurity have consistently been associated with increased risk of higher blood pressure in later life (Table 3)\textsuperscript{54, 55}. A recent meta-analysis of 27 studies reported a 2.28 mmHg (95% CI, 1.24-3.33 mmHg) increase in systolic blood pressure in subjects who had a birth weight below compared to above 2.5 Kg\textsuperscript{55}. Unfortunately most studies to date have not distinguished between LBW as a result of growth restriction at any gestational age, or prematurity with AGA. The potential for unmeasured confounding or effect modification by gestational age and/or growth restriction, therefore, must be borne in mind\textsuperscript{12}.

A systematic review of 10 studies including preterm subjects born at a mean gestational age of 30.2 weeks, with a mean birth weight of 1280 g, showed that blood pressures in later life were 2.5 mmHg higher (95% CI, 1.7 – 3.3 mmHg) than in subjects born at term\textsuperscript{54}. Prematurity has predominantly been associated with higher, but still normal,
blood pressures as cohorts studied are still relatively young. Overt hypertension has been documented in 2 studies of premature subjects, one of 2 years olds where the prevalence of hypertension (defined as a systolic or diastolic blood pressure > 95th percentile) was 30% overall, and another of pregnant women aged 25 years, where chronic hypertension was present in 1.4% of those born preterm compared to 0.8% born at term (OR 1.7; 95% CI, 1.32-2.20)25,56. Several studies have tried to dissect the relative roles of prematurity and growth restriction. Some authors suggest prematurity alone is the predominant risk factor, whereas others have found SGA to be more important in determining risk of higher blood pressure and kidney disease57. These differences likely reflect the complex interplay of intra- and extra-uterine events and timing of insults which vary considerably in premature infants12.

Given that the odd ratios for risk of higher blood pressures were similar in the meta-analysis of LBW and the systematic review of prematurity, and that not all LBW is due to prematurity, both LBW and prematurity must be considered important risk factors for high blood pressure. The association starts in early childhood and becomes augmented in adulthood, at which stage blood pressures often reach hypertensive ranges, suggesting compounding of the programming effects by growth, age and lifestyle.

**Nephron number and blood pressure**

In rodents nephrogenesis continues for up to 7-10 days after birth, providing a window, as in human premature infants, where post-natal events may impact nephron number. In rats, rescue of nephron number in LBW rats, by optimization of post-natal nutrition abrogated the development of subsequent hypertension, and conversely, undernutrition of normal birth weight (NBW) rat pups after birth led to lower nephron numbers and higher blood pressure58, 59. These data are consistent with a role of nephron number in hypertension. In humans, among a cohort of German adults who died in accidents, nephron numbers were significantly lower among those with hypertension compared to normotensive controls60. Low nephron numbers have also been associated with higher blood pressures among Australian aboriginals and US and Australian whites, although birth weights were unknown61. The relationship between nephron number and blood
pressure among subjects of African origin appears less clear, but glomerular volume is a significant independent predictor of higher blood pressures in this population\textsuperscript{61}. Additional factors likely contribute to hypertension among those of African origin, but the impact of birth weight, or a contribution of nephron number to severity of hypertension, for example, cannot be excluded.

The observations in humans of normalization of glomerular filtration surface area despite low nephron numbers would tend to argue against the initial hypothesis suggesting a pure limitation of sodium excretion in this setting. Interestingly, however, in both humans and experimental animals, LBW and low nephron number have been associated with a salt-induced increase in blood pressure (Table 3)\textsuperscript{62, 63}. This salt sensitivity in young adults and children correlated inversely with birth weight, independent of GFR, suggesting a primary defect in renal sodium handling\textsuperscript{64, 65}.

Evidence that normalization of nephron number in some experimental models does not eliminate subsequent hypertension, however, points to additional factors participating in developmental programming of hypertension\textsuperscript{66}. Elegant experiments have shown alterations in renal tubule sodium transporter expression, as well as systemic changes in vascular function, neuroendocrine adaptations to stress, insulin sensitivity and sympathetic nervous system activity\textsuperscript{66}. Nephron number, therefore, is not the sole programmed risk factor for hypertension, but it is likely to exacerbate any risk and contribute to kidney disease.

**Renal function, birth weight and nephron mass**

*Glomerular filtration rate:* In the absence of compensatory hyperfiltration, a kidney with a reduced nephron number should have a reduced GFR. Indeed, GFR extrapolated from Amikacin clearance on day 1 of life, preceding any compensatory adaptation, was significantly reduced in premature and LBW infants compared to term controls\textsuperscript{67}. GFR measured by inulin clearance was significantly lower at age 7.6 years in children who had been premature and had severe growth restriction compared to non growth restricted controls\textsuperscript{41}. In this study, the effects were similar in children who experienced growth
restriction prenatally or post-natally in intensive care, again demonstrating the importance of early nutrition. Meta-analysis of 8 studies reported an odds ratio of 1.79 (95% CI, 1.31 – 2.45) for a reduced GFR with LBW\textsuperscript{10}.

**Proteinuria:** Microalbuminuria is one of the earliest signs of glomerular hyperfiltration and transition to macroalbuminuria is consistent with ongoing renal injury. Hoy et al., first reported an odds ratio of 2.8 (95% CI, 1.26 – 6.31) of macroalbuminuria among Aboriginal Australians who had been of LBW compared to NBW\textsuperscript{68}. In this population, albuminuria was associated with a significant increase in cardiovascular and renal deaths, emphasising its’ clinical relevance\textsuperscript{69}. Since then, many studies have confirmed this association, reflected in an odds ratio of 1.81 (95% CI, 1.19 – 2.77) for albuminuria with LBW reported in a meta analysis of 9 studies\textsuperscript{10}. Among Pima Indians, however, the relationship is U-shaped, with increased risk of proteinuria with birth weights both below 2.5 Kg or above 4.5 Kg\textsuperscript{32}. This population has a high incidence gestational diabetes, exposure to which was the strongest predictor of proteinuria in the HBW subjects\textsuperscript{32}.

**Chronic kidney disease and ESRD:** Several early studies found increased progression of primary renal diseases among subjects who had been of LBW (reviewed in (Luyckx, 2010 #1432)). Among subjects with diabetes, prevalence of nephropathy has been associated with LBW and HBW, as well as short stature\textsuperscript{32, 70}. All of these observations suggest that abnormal fetal growth increases long-term renal disease risk (Table 3). Consistent with this, the odds ratio for CKD, including ESRD, associated with LBW, was 1.73 (95% CI, 1.44 – 2.08) among 18 studies\textsuperscript{10}. Population-based studies have reported a U-shaped relationship between birth weight and risk of CKD or ESRD, suggesting HBW is also important\textsuperscript{8, 71, 72}. Interestingly, in some studies the programmed risk of CKD appears greater in males\textsuperscript{71}. The differential impact of gender on renal programming is, however, quite variable and requires further study\textsuperscript{72, 73}.

As with programming of blood pressure risk, nephron number is unlikely to be the sole factor contributing to renal disease risk, and a low nephron number is unlikely sufficient
to cause renal disease in the absence of additional “hits”. Moreover, developmental programming of related conditions, e.g. type 2 diabetes, cardiovascular disease, insulin resistance and obesity, may further increase renal risk\(^4\)\(^\text{, }12\). Programming of these disorders may occur simultaneously in a developing fetus, depending on timing and nature of the insults. All infants subjected to adverse intrauterine conditions should therefore be considered at risk for all of these disorders.

**IMPACT OF CHILDHOOD WEIGHT GAIN ON RISK FACTORS FOR CHRONIC KIDNEY DISEASE AND KIDNEY FUNCTION**

As discussed above, especially in premature and LBW infants, post-natal malnutrition and clinical circumstances may impact nephrogenesis, childhood renal function and long-term renal disease risk (Figure 1)\(^\text{12, }40,\)\(^\text{, }42\). In LBW rats, low nephron numbers were restored to normal, and the development of hypertension abrogated by provision of adequate post-natal nutrition\(^58\). When LBW rats were overfed post-natally, nephron numbers remained low, and they developed obesity, hypertension and renal injury with time\(^74\). In post-natally overfed NBW rats, despite a higher than normal nephron number, blood pressure, proteinuria and glomerulosclerosis were all increased in adulthood\(^75\). Taken together, these data suggest that normalization of post-natal nutrition may be beneficial, but overfeeding is likely deleterious. In humans, post-natal weight gain and nutrition have also been implicated in developmental programming of adult disease\(^76\).

“Catch-up” growth in children who had been of LBW has long been advocated especially in developing countries, improving an infant’s resilience against infections and reducing the risk of undernutrition, stunting and cognitive impairment\(^5\). Increasingly, however, in many populations world-wide, accelerated weight gain or increase in BMI, even in children with NBW, has been consistently associated with increased risk of adult hypertension, type 2 diabetes, and cardiovascular disease\(^76\)\(^-\)\(^78\). This effect increases with increasing age of the child; upward crossing of weight or BMI centiles in mid-childhood or adolescence is associated with strong adverse effects on later risk, while upward
crossing in infancy (below the age of 1 year) has no or minimal effect on later blood pressure and may protect against diabetes\textsuperscript{78-80}. Importantly, the gain in childhood BMI associated with increased risk of adult disease is not always ‘excessive’ in terms of absolute number. In many developing countries, children that experience rapid growth may still be small by international weight standards, but upwards crossing of BMI centiles appears the critical factor\textsuperscript{78-80}. A single cross-sectional measurement of a child’s weight or BMI therefore may be misleading in such circumstances, emphasizing the need for growth tracking in early childhood.

LBW children in an environment of adequate nutrient supply tend to experience rapid weight gain\textsuperscript{5}. Among 22-year-old British subjects, systolic blood pressure was 1.3 mmHg (95\% CI, 0.3 -2.3 mmHg) higher for every standard deviation decrease in birth weight, and increased by 1.6 mmHg (95\% CI, 0.6 – 2.7 mmHg) for every standard deviation increase in childhood weight gain between 1 and 10 years\textsuperscript{81}. Many other studies have confirmed an association of rapid childhood weight gain with higher blood pressures and increased arterial stiffness, often already evident in childhood\textsuperscript{79}.

Among 216,771 Scandinavian subjects, those with a birth weight of 2.5 Kg and a BMI of 17.7 Kg/m\textsuperscript{2} (overweight) at age 7, had a 44\% increased risk of cardiovascular disease in adulthood compared with those with a median birth weight of 3.4 Kg and a BMI of 15.3 Kg/m\textsuperscript{2}\textsuperscript{77}. The highest risk of higher blood pressures and cardiovascular disease therefore occurred in children with LBW who become heavy\textsuperscript{77}. Importantly, BMI was strongly positively associated with cardiovascular disease risk in this study, independent of birth weight, demonstrating the importance of childhood obesity itself as a risk factor for adult disease.

Childhood overweight and obesity are increasing world wide (Figure 2)\textsuperscript{82}. Risk factors include HBW, exposure to gestational diabetes and early post-natal weight gain among many others\textsuperscript{83}. Such risk factors are also independently associated with altered nephrogenesis and increased risk of hypertension, type 2 diabetes and renal dysfunction (Figure 1). In addition, obesity per se is a risk factor for progression of renal disease,
therefore superimposition of the burden of obesity on a small kidney with fewer nephrons is likely to compound the risk and act as a “second hit” accelerating renal disease progression\textsuperscript{84}. How best to optimize post-natal growth and positively impact subsequent disease risk, especially in LBW infants is not yet clear. Avoidance of obesity appears a safe guiding principle.

*Early growth and kidney function*

The association of rapid childhood weight gain with the renal disease risk factors of higher blood pressures, diabetes and obesity is likely to compound any primary programmed renal risk. Indeed, in a retrospective analysis of 80 children with proteinuric kidney disease, renal disease progressed fastest among those who had been premature and became obese\textsuperscript{85}. Glomerular size was increased in all obese children, whether premature or term, whereas kidney size remained smaller among all those who had been premature independent of obesity. Similarly, among VLBW infants who developed neonatal AKI, excessive weight gain was a predictor of poorer renal function at a mean age of $7.5 \pm 4.6$ years\textsuperscript{86}. How infant weight gain impacts long-term renal function remains unknown.

Several potential mechanisms have been suggested to explain the amplification of renal and cardiovascular risk by rapid weight gain following growth restriction. One possible link is the development of premature senescence\textsuperscript{87}. Cellular senescence is a state of growth arrest induced by upregulation of the cell cycle inhibitors p53, p21 and p16\textsuperscript{INK4a}\textsuperscript{88}. Upregulation of these genes may be induced by progressive telomere shortening, which occurs with cell replication and is a robust marker of aging, and by reactive oxygen species induced by cellular stress\textsuperscript{88}. Chronic cardiovascular and renal diseases are associated with increased expression of senescence markers\textsuperscript{89}. In animals, LBW followed by rapid postnatal weight gain was associated with shorter telomeres and increased expression of senescence markers in kidneys, hearts and aortas, as well as premature death, all consistent with accelerated aging\textsuperscript{87, 90, 91}. Interestingly, LBW was also associated with a higher renal and cardiovascular mortality in an Australian Aboriginal cohort, which may be consistent with these experimental findings\textsuperscript{92}. Premature
senescence in the kidney may result from ongoing hyperfiltration injury in kidneys with fewer nephrons, compounded by a rapid increase in body size. In humans, leukocyte telomere length was not different between LBW and NBW British newborns, but among 5 year old children from Bangladesh, telomeres were significantly shorter in those who were of LBW. Senescence is linked to oxidative stress. In children born SGA compared to controls, markers of oxidative stress were higher in those who experienced catch-up growth. The link between nephron numbers, catch-up growth, premature senescence and the development of hypertension and renal disease in humans therefore appears plausible, but has not yet been confirmed.

**Conclusion**

The association between fetal and childhood development and increased risk of adult disease is now quite convincing. LBW and prematurity are associated with higher blood pressures and decreased renal function manifesting in early childhood, which may progress to overt disease in adulthood. Nutrition is a cornerstone of this association. Maternal nutrition and health prior to and during pregnancy are crucial in determining fetal growth and development of a kidney with enough nephrons to maintain homeostasis in response to dietary and metabolic stresses, as well as sustain function in the face of superimposed nephron loss. Early postnatal nutrition, especially after premature birth is also crucial in determining renal development. Upward crossing of weight or BMI percentiles after the infant period further programmes risk of hypertension and renal disease. The intergenerational impact of developmental programming is illustrated by LBW increasing the risk of maternal preeclampsia, obesity and gestational diabetes, which all in turn further compound future risks in their offspring. The growing knowledge and understanding of the pathophysiology of developmental programming has identified populations that are “at risk”, and thus should be targets for screening and interventions to interrupt this cycle. Identification of nutritional deficiencies within populations (e.g. vitamin A deficiency) should prompt public health interventions to correct these deficiencies well before pregnancy. Adequate antenatal care should identify women who develop preeclampsia and gestational diabetes, optimize their care during
pregnancy, but also lead to lifestyle education and life-long screening of these women for later disease. Currently a minority of women worldwide are screened for gestational diabetes\textsuperscript{27}. With the rising prevalence of obesity, such screening should be more widespread. Similarly, it has been estimated that only 60\% of all children are weighed at birth (www.childinfo.org), this number should increase. Several interventions to reduce maternal and childhood malnutrition, where prevalent, have been shown to be effective: supplementation of protein energy intake during pregnancy reduced risk of term LBW by 32\%; supplementation of multiple micronutrients in pregnancy reduced LBW by 16\%; implementation of malaria prophylaxis reduced risk of LBW by 37\%\textsuperscript{96}. Vitamin A supplementation did not affect birth weight, but reduced neonatal mortality (RR 0.8, 95\% CI 0.66-0.96), whereas the relative risk of preeclampsia was 0.45 (95\% CI 0.31–0.65) with maternal calcium supplementation\textsuperscript{96, 97}. Thus far no study has followed the offspring of such supplemented pregnancies to determine whether risk of adult disease has been altered, it would be logical to assume a positive impact. In later life, regular cardiovascular exercise was shown to abrogate the metabolic consequences of being small at birth among adult men\textsuperscript{98}. Identification of at-risk pregnancies and offspring, both high and low birth weight, therefore should prompt maternal education to optimize childhood nutrition and activity to prevent obesity. Prematurity and LBW are among the top ten contributors to the global burden of disease, calculations which may not always have included the long term costs of programmed adult NCDs\textsuperscript{1, 22}. Acknowledgement of the role of developmental programming in hypertension and renal disease risk, and implementation of locally adapted preemptive strategies in individual countries, should have significant long term benefits in terms of future health, productivity and cost savings world wide.

Search strategy and selection criteria: Articles reviewed for this manuscript were identified by searching the PubMed database, using the search terms “nephron number”, “nephron endowment”, “nephron mass”, “nephrogenesis”, “birth weight”, “low birth weight”, “high birth weight”, “prematurity”, “preterm birth”, “developmental programming”, “developmental origins of adult health and disease”, “catch-up growth”,
“growth restriction”, “SGA”, “IUGR”, with other key words including “kidney”, “kidney mass”, “kidney size”, “kidney volume”, “diabetes”, “gestational diabetes”, “cardiovascular disease”, “obesity”, “human”, “hypertension”, “hypertensive disorders in pregnancy”, “preeclampsia”, “vitamin A deficiency”, “maternal diet”, “maternal nutrition”. In addition, a snowball strategy was employed utilizing bibliographies of existing manuscripts, textbooks and websites. Data, references and links were also identified by searching the WHO, UNICEF and Google Scholar websites using key words “low birth weight”, “preeclampsia”, “gestational diabetes”, “maternal and newborn health”, “nutrition”, “childhood obesity”. We largely included publications from the last 5 years, but also included older references that have been seminal in the field. Some references were included with animal data when these were considered necessary to explain concepts strongly supporting the pathophysiology but not yet proven in humans.

Conflicts of interest: We declare that we have no conflicts of interest.

References

Table 1: Developmental factors associated with nephron number, kidney size and function

<table>
<thead>
<tr>
<th>Timing</th>
<th>Condition</th>
<th>Effect on kidney</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Prenatal</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Maternal vitamin A deficiency</td>
<td>Small infant kidney size</td>
</tr>
<tr>
<td></td>
<td>Low birth weight</td>
<td>Decreased nephron number</td>
</tr>
<tr>
<td></td>
<td>Growth restriction</td>
<td>Reduced GFR at 7.5 years</td>
</tr>
<tr>
<td></td>
<td>Prematurity</td>
<td>Decreased nephron number</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Reduced kidney size in growth restricted children</td>
</tr>
<tr>
<td>Genetics</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>RET</em> (1476A) polymorphism</td>
<td>10% reduction newborn kidney volume</td>
</tr>
<tr>
<td></td>
<td><em>PAX2</em> AAA haplotype</td>
<td>10% reduction newborn kidney volume</td>
</tr>
<tr>
<td></td>
<td>Combined <em>RET</em> (1476A) polymorphism and <em>PAX2</em> AAA haplotype</td>
<td>23% reduction newborn kidney volume</td>
</tr>
<tr>
<td></td>
<td><em>I/D ACE</em> polymorphism</td>
<td>8% reduction newborn kidney volume</td>
</tr>
<tr>
<td></td>
<td><em>BMPRIA</em> rs7922846 polymorphism</td>
<td>13% reduction newborn kidney volume</td>
</tr>
<tr>
<td></td>
<td><em>OSRI</em> rs12329305(T) polymorphism</td>
<td>12% reduction newborn kidney volume</td>
</tr>
<tr>
<td></td>
<td>Combined <em>OSRI</em> and <em>RET</em> polymorphisms</td>
<td>22% reduction newborn kidney volume</td>
</tr>
<tr>
<td></td>
<td>Combined <em>OSRI</em> and <em>PAX2</em> polymorphisms</td>
<td>27% reduction newborn kidney volume</td>
</tr>
<tr>
<td><strong>Postnatal</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Renal failure</td>
<td>Decreased nephron number</td>
</tr>
<tr>
<td></td>
<td>Growth restriction</td>
<td>Reduced GFR at 7.5 years</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Increased odds of renal dysfunction at 6.4 years</td>
</tr>
<tr>
<td></td>
<td>“Catch-up” growth, childhood and adolescent overweight/obesity</td>
<td>Increased glomerular volume</td>
</tr>
<tr>
<td>Prenatal Genetics</td>
<td>Faster progression of renal disease</td>
<td></td>
</tr>
<tr>
<td>-------------------</td>
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<td></td>
</tr>
<tr>
<td><em>ALDH1A2</em>&lt;sup&gt;27169280(G)&lt;/sup&gt; polymorphism</td>
<td>22% increase newborn kidney size</td>
<td></td>
</tr>
</tbody>
</table>

Adapted with permission from 99. RET – tyrosine kinase receptor; PAX2 – paired box gene 2; ACE – angiotensin converting enzyme; OSR – Odd-Skipped related; BMPR – bone morphogenic protein receptor; *ALDH* – aldehyde dehydrogenase. Individual references listed in appendix.
### Table 2: Clinical surrogates for nephron number

<table>
<thead>
<tr>
<th>Clinical surrogate</th>
<th>Association with nephron number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low birth weight</td>
<td>↑ of 257 426 glomeruli per kidney, per Kg increase in birth weight</td>
</tr>
<tr>
<td>Prematurity</td>
<td>↓ glomerular number, proportional to gestational age, in premature compared to term infants</td>
</tr>
<tr>
<td>Gender</td>
<td>Nephron number 12% lower in females</td>
</tr>
<tr>
<td>Age</td>
<td>↓ 3676 glomeruli per kidney per year of age &gt; 18 years (nephron loss)</td>
</tr>
<tr>
<td>Adult height</td>
<td>↑ 28 000 glomeruli per centimeter increase in height</td>
</tr>
<tr>
<td>Kidney mass</td>
<td>↑ 23459 glomeruli per gram of kidney tissue (in infants)</td>
</tr>
<tr>
<td>Glomerular volume</td>
<td>Inverse correlation between glomerular volume and nephron number</td>
</tr>
<tr>
<td>Ethnicity</td>
<td>↓ in Aboriginal Australians compared to US white and black</td>
</tr>
</tbody>
</table>

#### Possible correlates

- Gestational diabetes exposure ↓ renal functional reserve in offspring of diabetic mothers vs. diabetic fathers

Adapted with permission from 99. Individual references listed in appendix.
Table 3: Clinical associations with programming of kidney function

| Low birth weight and/or prematurity          | Increased blood pressure  
|                                            | Salt sensitivity          
|                                            | Proteinuria               
|                                            | Reduced GFR               
|                                            | Reduced renal functional reserve  
|                                            | Accelerated progression of primary renal disease  
|                                            | Chronic kidney disease    
|                                            | End-stage kidney disease  
|                                            | Death                     
| Low nephron number                          | Increased blood pressure  
|                                            | Increased glomerular volume  
|                                            | Possible predisposition to renal failure in neonates  
| Reduced renal size                          | Increased blood pressure  
|                                            | Salt sensitivity          
|                                            | Reduced GFR               
| High birth weight/ maternal diabetes        | Proteinuria               
|                                            | Reduced renal functional reserve  
|                                            | End-stage kidney disease  
| Rapid increase in weight/BMI in childhood and adolescence, especially after low birth weight | Faster progression of renal disease  
|                                            | Larger glomeruli          
|                                            | Higher blood pressure     
|                                            | Diabetes, impaired glucose tolerance  
|                                            | Cardiovascular disease and death  
|                                            | Obesity                   
|                                            | Metabolic syndrome        

Adapted with permission from 99
Figure 1: Schematic diagram of factors impacting developmental programming of hypertension and kidney disease
Figure 2: Worldwide percent prevalences of factors impacting programming of renal disease, by United Nations region

Error bars represent range of percentage by region. *Vitamin A deficiency numbers exclude countries with 2005 GDP ≥ USD 15 000, where deficiency presumed absent. #Obesity figures for North America and Europe extrapolated from data in “developed countries”. Childhood obesity defined as ≥ 2 SD from weight for height median. Data pooled from references 11, 36, 39, 82, 100
Please see main manuscript, the only change was reducing reference to 100.

Thank you
Response to reviewers:

We are grateful for your accepting our manuscript. As discussed, the reference number has now been reduced to 100 and an appendix with additional references is included as a web appendix.

I have no new tracked version