

Developmental Hypertension, Nephrogenesis, and Mother's Milk: Programming the Neonate

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Compelling epidemiologic and experimental evidence indicates that early-life experiences shape risk for disease in later life. Infants who are born smaller, reflecting a slower growth trajectory *in utero*, experience higher incidences of hypertension, obesity, diabetes, and renal disease as adults (1). Birth weight is a crude surrogate for the broad spectrum of specific adverse events that may impair fetal growth in humans; therefore, experimental models have been developed to probe postnatal outcomes after specific interventions that are relevant to human pregnancy, including nutrient deficits and placental insufficiency (2). Attention continues to focus primarily on fetal growth, a period of biologic plasticity in which environmental insults can permanently “program” the fetus and thereby alter the postnatal phenotype by a number of different mechanisms. Impaired growth during critical periods of organ development may have an impact on disease risk by permanently reducing the number of functional units, for example nephrons (3), pancreatic islet cells (4), and/or cardiac myocytes (5). In each case, the organ size adapts appropriately to the limited *in utero* growth milieu and proportionally to the smaller body size; however, if postnatal body size and/or metabolic demands greatly exceed this preset functional capacity, then disease-producing mismatches may ensue. This concept has been most extensively explored in animal models wherein limiting maternal nutrients or placental blood supply is associated with reduced nephron number and later hypertension in offspring (2,3). In other modes of programming, growth restriction *in utero* may permanently alter postnatal activity and/or homeostatic set points of regulatory systems such as the renin/angiotensin system (6) and the hypothalamic/pituitary/adrenal axis (7), leading to altered postnatal function, which may then promote disease susceptibility.

As the field has evolved, its scope has expanded to recognize that the early postnatal period is an additional developmentally plastic phase that is also susceptible to environmental programming of disease vulnerability. Therefore, in epidemiologic studies, when lower birth weight is followed by failure to thrive in infancy, coronary disease risk is enhanced (8,9). When lower birth weight is followed by rapid childhood weight gain, hy-

per-tension (10), obesity, and diabetes (11) risks are further increased beyond those conveyed by low birth weight alone. Clearly, there are *interactions* between consequences of fetal growth restriction and the postnatal environments that are subsequently encountered.

To date, interpretations of both epidemiologic and experimental studies have generally made the assumption that events or interventions that are operative during pregnancy influence nutrient availability for only the prenatal phase of development in the offspring. Wlodek *et al.* (12) in this issue compellingly challenge that assumption: Applying late-gestational placental insufficiency in the rat, these investigators show that maternal mammary gland development is also impaired, thereby superimposing postnatal lactational insufficiency on the prenatally growth-restricted offspring. In this carefully designed study, the investigators use cross-fostering of placentally restricted *versus* normal rat offspring to distinguish the contributions of prenatal *versus* postnatal phases in programming of hypertension and reduced nephron number. Uteroplacental insufficiency (UPI), created at gestational day 18 (of 21), was achieved by ligation of uterine arteries and veins and was accompanied by reduction in litter size from the typical 10 to 14 pups to five pups. Wlodek *et al.* then cross-fostered litters from each group onto either dams with UPI (with impaired mammary gland function) or normal dams. BP (performed serially by tail-cuff methods), nephron number (by classic stereologic methods), and renal angiotensin II (AngII) type 1 receptor (AT1R) gene expression were assessed in 5-mo-old offspring (only male results are reported).

Understanding the implications of these findings for human disease requires that one salient point be addressed up front: Whereas nephrogenesis in humans is complete by gestational weeks 32 to 35 (thus before birth) (13), 80% of nephrons in the rat kidney form in the first 7 to 10 d *after* birth (14). Therefore, the lactational period in the rats under study (from birth to 25 to 35 d of age) encompasses the bulk of the nephrogenic period and would, in a comparable human study design, not influence new nephron formation (although might well influence other aspects of renal growth/maturation). Nonetheless, keeping this important difference in mind, Wlodek *et al.* provide new information that is relevant to early developmental programming with themes that are relevant to human nephrogenesis and to developmental hypertension.

First, when UPI offspring were maintained with their bio-

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logic UPI dams during lactation, both hypertension and reduced nephron number were evident in the adults at 5 mo of age. However, when UPI offspring were cross-fostered onto normal dams, nephron number was comparable to controls and BP remained normal. Accordingly, the reduced nephron number in UPI offspring was eliminated by a normal lactational environment. The authors argue that the bulk of nephron deficit in the restricted offspring was likely generated during the *in utero* period and thus that postnatal nephrogenesis was "accelerated" by cross-fostering with nutritional rescue in the subsequent lactational period. However, given the distribution of normal rat nephrogenesis predominantly in the postnatal period, it seems equally plausible that a major fraction of the nephron deficit was in fact generated during the lactationally deficient postnatal phase. Schreuder *et al.* (14), applying postnatal nutrient restriction in normal rats *via* doubling the litter size, found a 20% reduction in nephron number. If so, then it is perhaps less surprising that adequate nutrition in this period largely ameliorated the problem in the UPI offspring. Because no direct evidence is provided to support or refute either view in the UPI model, future studies that examine nephron number at birth and at specific postnatal time points will be required to clarify whether nutritional rescue primarily prevented nephron deficit or instead overcame a largely preexisting deficit. Whatever the answer, one point is clear: Late gestational induction of nutrient/oxygen restriction *via* UPI in the rodent is not alone sufficient to yield reduced nephron number, and the nephron deficit that is induced by UPI can be offset by restoration of nutritional requirements postnatally.

How does this apply to human nephrogenesis? Postnatal interventions in term infants who are born small for gestational age would not be expected to influence nephron number, because the equivalent period in human development would occur in the first two thirds of the third trimester. However, the findings of Wlodek *et al.* may have important relevance for the very preterm human infant, regardless of whether it is born small for gestational age. Rodriguez *et al.* (15) estimated nephron number by morphometric radial glomerular counts and identified active nephrogenesis by the presence of S-shaped bodies in very preterm infants (*i.e.*, in infants who were born before completion of nephrogenesis). They found that nephrogenesis may continue after birth in infants who do not experience renal failure and further reported that active nephrogenesis could be observed for up to—but not beyond—40 d postnatally (15). The findings of Wlodek *et al.* suggest important issues for such very preterm infants: (1) What constitutes optimal nutritional management for fostering continued postnatal nephrogenesis? (2) Can non-nutritional components of current neonatal intensive care unit care be altered to support better ongoing nephron formation in these challenging patients?

A second set of observations by Wlodek *et al.* may prove to be equally important to our understanding of developmentally induced hypertension. If investigators had chosen to study only the nutritionally rescued UPI offspring (UPI offspring that were cross-fostered onto UPI *versus* normal dams), then we would not have recognized these informative complexities. That is, the

offspring of UPI dams that suckled their own mother exhibited both nephropenia and adult hypertension, whereas UPI offspring that suckled normal dams exhibited neither nephropenia nor hypertension. Because nutritional rescue prevented both nephropenia and hypertension, we would have been sorely tempted to tuck this finding away in the "of course" file. Fortunately for science, Wlodek *et al.*, in their particularly thoughtful experimental design, studied two groups of cross-fostered control groups: Normal offspring were cross-fostered onto UPI dams not only in normally sized litters ($n = 10$) but also in litters that were reduced to equal the spontaneously low litter size that was observed in restricted litters ($n = 5$). Outcomes in these normally born offspring were quite different: Whereas normal-sized litters of normal offspring that suckled UPI dams sustained no hypertension, the small-sized litters of normal offspring that suckled UPI dams unexpectedly developed hypertension. *Neither group exhibited reduced nephron number.* Accordingly, two key points emerge: (1) Programmed hypertension can be induced by a purely postnatal intervention, and (2) programmed hypertension can be dissociated from reduced nephron number in the UPI model.

Because the suckling pups in both of these control-on-UPI groups were normal at birth, there was clearly an interaction between the placentally restricted dam/lactational factors and litter size. The authors, on the basis of their unpublished observations, suggest that the tactile stimulation that was provided by the larger litters induced mammary gland recovery in the placentally restricted dams, thereby (counterintuitively) providing adequate milk/nutrition and normal growth for the larger litter of offspring. This is supported by the significant growth restriction that developed late only in the lactation period in the small-litter normal offspring that suckled UPI dams. As a consequence, the latter experienced postnatal growth restriction followed by postweaning accelerated growth, a pattern that has been shown to enhance risk for hypertension in both normal and low birth weight infants (10). Why the normally born offspring that were subjected to postnatal lactational deficit did not sustain loss of nephron number is not clear but suggests that the *in utero* UPI insult created nephrogenic susceptibility to the postnatal lactational insufficiency. For example, greater vigor of normal (*versus* UPI) offspring may have led to a less severe nutritional stress despite equally reduced litter sizes.

That a prospective postnatal intervention that is sufficient to reduce growth rate in an otherwise normal neonate is capable of inducing programmed hypertension has not been previously reported. Epidemiologic observations in human cohorts have supported the importance of infant growth rate but have been discordant with respect to its impact on later cardiovascular disease risk. Slowed infant growth in human birth cohorts has been linked to later stroke (16), coronary disease (9), and insulin resistance (17) but, interestingly, not to hypertension when studied in young adults (18). However, in a new report by Eriksson *et al.* examining outcomes in 2003 members of an Helsinki birth cohort at an average age of 62 years (19), two distinct growth patterns preceded development of hypertension. In one of those, a normal birth size was followed by

impaired growth in infancy and childhood, suggesting that solely postnatal growth failure may in fact lead to late-life hypertension in humans. The epidemiologic evidence, together with results of Wlodek *et al.*, makes clear that the early postnatal period is a time of continuing susceptibility to environmental programming of later life disease. The lactational insufficiency of UPI dams specifically raises the important possibility that mother's milk may not *always* be the complete solution to infant nutrition, particularly when maternal factors impair lactational quality or quantity during susceptible periods of infant development.

The dissociation of programmed hypertension from reduced nephron number in the normal offspring that were exposed to impaired lactation provides a final key point: Reduced nephron number *per se* does not mediate all programmed hypertension and may not necessarily explain the programmed hypertension in the nephropenic UPI offspring. Because the UPI offspring that were from a reduced litter and suckled UPI dams experienced the same postnatal nutritional deficits that induced later hypertension in normal offspring, we are forced to ask whether the hypertension in the nephropenic group is in fact due to the low nephron number, to the same postnatal factors that are operative in the normal offspring/small litter/UIP dam group, or perhaps to both. It is interesting that the magnitude of hypertension was very similar in the group with normal nephron number *versus* the nephropenic group, suggesting that early postnatal factors that operate independent of nephrogenesis may have generated the hypertension in both cases.

Low nephron number as the proximate cause of hypertension after intrauterine growth restriction has advanced to a veritable mantra, albeit for compelling theoretic reasons (3). The results of Wlodek *et al.* suggest that this confidence has perhaps evolved prematurely. All previously published studies that linked reduced nephron number and hypertension in fact documented merely an association, never a cause–effect relationship, and none has resolved the caveat that late-life nephron loss only rarely leads to hypertension (3). Moreover, a few studies have directly challenged the prevailing dogma (20–22). Wlodek *et al.*, although also failing to define a cause–effect link between hypertension and low nephron number, are nonetheless the first to bring the question into such bold relief by their carefully crafted controls.

Finally, in the normal offspring with postnatal nutritional deprivation, accelerated postweaning growth, and hypertension despite normal nephron number, what mechanisms might contribute to the adult hypertension? Wlodek *et al.* find increases in intrarenal AT1R gene expression in this group and reasonably suggest that intrarenal renin/angiotensin activation may contribute. However, these findings appear in already hypertensive animals, raising the question of cause *versus* effect. Moreover, increased AT1R mRNA was also apparent in the large litter/normal offspring that suckled UPI dams, a group that did not develop hypertension. Another emerging theme in the field of developmental hypertension is enhanced oxidant stress as a long-lived consequence of early nutritional programming (23). Moreover, antioxidant interventions in the early postnatal period after prenatal nutrient restriction in rats

have been remarkably effective in preventing later vascular dysfunction and hypertension (24), again suggesting a developmental window postnatally during which programming is mediated by oxidant stress and is also potentially reversible. It is tempting to postulate that nutritional deprivation during this early postnatal window generates permanent changes in pro-oxidant processes and alters vascular function in ways that predispose to sustained hypertension with aging. Because AngII vasoconstriction involves stimulation of oxidant pathways *via* the AT1R (25), differential expression of hypertension in the two groups of normal offspring with high AT1R expression in adulthood could be due to enhanced downstream oxidant-dependent signaling by AngII only in those that were previously exposed to postnatal nutrient deprivation.

In summary, the carefully crafted studies of Wlodek *et al.* in the rat UPI model compel major shifts in our thinking about materno-fetal oxygen/nutrient deprivations that encompass late gestation. First, interventions during pregnancy may potentially influence not only fetal nutritional status but also postnatal nutrient availability to offspring *via* alterations in maternal mammary gland function. Second, postnatal nephrogenesis—which occurs normally in the rat—can be modified by nutritional manipulation, a finding with potential relevance to very preterm infants who continue to form nephrons postnatally and whose well-being mandates that we precisely define the nutritional and pharmacologic pros and cons of supporting successful nephrogenesis in this setting. Third, the early postnatal period is susceptible to nutritional programming of hypertension independent of nephron number, and its contribution to hypertension in published studies of nutrient deficits or UPI applied in late gestation requires reevaluation. Moreover, the need to evaluate maternal lactation adequacy and to define the developmental implications of impaired milk quantity or quality in the early postnatal period for all infants, whether preterm or term, may take on greater urgency than we have previously recognized. Finally, the precise contribution of reduced nephron number needs to be readdressed using approaches that go beyond mere association to identify to what degree nephropenia is a mediator, a modulator, or merely a parallel event in the pathogenesis of developmental hypertension.

Disclosures

None.

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See the related article, "Normal Lactational Environment Restores Nephron Endowment and Prevents Hypertension after Placental Restriction in the Rat," on pages 1688–1696.