# Perinatal Nephron Programming Is not So Sweet in Maternal Diabetes

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Development of the permanent, metanephric kidney begins at approximately embryonic day 11 (E11) in mice, E12 in rats, and during the fourth through fifth gestational weeks in humans. During these stages, the ureteric bud projects from the mesonephric duct and enters the metanephric anlage, whereupon buds branch repeatedly and ultimately form the collecting duct system of the mature kidney (and urothelium, including the renal pelvis, ureters, and bladder trigone). At the inception of nephrogenesis, metanephric mesenchymal cells are attracted to and condense around each tip of an advancing ureteric bud branch. Shortly after condensation, the mesenchyme then converts to polarized epithelia, which proceeds through an orderly sequence of nephric structures (termed vesicle, comma- and s-shaped, developing capillary loop, and glomerular stages) that eventually constitute the mature nephron. These nephrogenic processes of ureteric bud growth and branching, mesenchymal cell induction and aggregation, conversion to epithelia, and glomerular differentiation and tubule elongation occur repeatedly until there is a full complement of nephrons. Nephrogenesis concludes approximately 1 wk after birth in rodents and during the 34th gestational week in humans.

Considerable progress has been made in understanding many of the molecular details that underlie the induction of nephrogenesis, and only a few of them can be mentioned here. For example, the "paired box" transcription factor-2 (Pax2) first appears during the caudal descent of the nephric duct, then expresses in uninduced and induced metanephric mesenchyme, where it stimulates expression of glial cell–derived neurotrophic factor,[1] and also expresses in ureteric bud epithelia, where it suppresses apoptosis. Pax2 also increases expression of Wnt-4, a secreted glycoprotein that activates the $\beta$-catenin signaling pathway regulating cell growth.[2] The transcription factor WT1 is expressed in uninduced mesenchyme but is sharply upregulated as cells condense around ureteric bud branches. Wnt-4 is also upregulated in condensing mesenchyme and, together with WT1, expresses through the vesicle and comma- and S-shaped stages, suggesting both of these proteins are key mediators of epithelial differentiation. One of the gene products directly regulated by WT1 is Pax2, which becomes downregulated during s-shaped stages of nephron development.[3] Similarly, reciprocal expression of the receptor tyrosine kinase Ret and its ligand, glial cell–derived neurotrophic factor, by ureteric bud epithelia and metanephric mesenchyme, respectively, induces and maintains ureteric bud branching morphogenesis.[1] As the condensed metanephric mesenchymal cells serially convert to epithelia, the expression of a host of mesenchymal proteins (*e.g.*, neural cell adhesion molecule, vimentin, types I and III collagen) are suppressed, whereas proteins that typify epithelia (E-cadherin, cytokeratin, type IV collagen, and laminin) all upregulate.

Although much has been learned about the induction of nephrogenesis, considerably less is known about mechanisms that conclude the process. Nevertheless, many factors contribute to final nephron endowment, including the extent of ureteric bud elongation and branching, conversion of mesenchyme to epithelia, maintenance of the epithelial nephric figures, and overall rates of metanephric mitosis and apoptosis; some of the genetic regulators of these processes have already been summarized. Furthermore, unbiased stereologic methods show mature human kidney can average from as few as approximately 200,000 to nearly 2 million nephrons.[4] This wide variation in nephron endowment may have profound consequences, however, and there is increasing evidence that individuals with reduced nephron number are prone to develop hypertension, renal failure, and/or other cardiovascular disorders later in life. Notably, mice with a complete absence of *Pax2* lack the caudal portion of the Wolffian duct, from which the ureteric bud originates, and are therefore anephric.[5] Humans who are heterozygous for *Pax2* mutations have renal-coloboma syndrome, which results in ocular colobomas, renal hypoplasia, and renal failure in childhood.[6] Additional evidence shows heterozygous mutations of *Pax2* in mice also result in loss of renal mass with increased apoptosis and decreased branching of the ureteric bud, leading to significantly fewer nephrons.[6]

Several different genetic mutations cause renal growth disorders,[7,8] but there are also many important—and possibly much more prevalent—environmental causes. A growing body of data in humans and experimental animals indicates that maternal malnutrition, placental insufficiency, fetal exposure to certain medications and other toxins, inhibition of the renin-angiotensin system, and/or vitamin A (retinoid) depletion all can result in low birth weight.[9] Although this may not always affect nephron endowment, in many cases low birth weight correlates inversely with a tendency for the development of hypertension, proteinuria, and metabolic syndrome in adulthood.[9] Increasingly, the Barker hypothesis (adult disease has fetal origins), as it relates to certain renal functional abnormalities in maturity, attributes at least some of the harm to events taking place specifically during kidney organogenesis, which in humans normally occurs exclusively *in utero*.

Maternal hyperglycemia can similarly induce a wide range of developmental abnormalities affecting multiple organ systems in the fetus (diabetic embryopathy), including kidney.[10,11] Indeed, women with pregestational diabetes and fasting hyperglycemia

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have at least a three- to four-fold increased risk for infant malformations.[12] Metabolic changes accompanying chronic hyperglycemia in patients with diabetes include abnormal myoinositol and diacylglycerol levels, stimulation of protein kinase C, nonenzymatic glycation of intracellular and extracellular proteins, and increased production of reactive oxygen species, all of which can progressively damage a wide variety of tissues.[11] Unusually susceptible to injury are molecules with relatively slow turnover, such as DNA and collagen, and one of the hallmarks of diabetes in adults is excessive accumulation and abnormal cross-linking of basement membrane proteins, particularly in renal glomeruli and other vascular structures. In pregnant women with poorly controlled pregestational diabetes, the fetus is exposed to elevated glucose levels throughout pregnancy, including the several weeks after conception, when many developmental processes are especially vulnerable. For example, caudal regression syndrome is a relatively rare congenital defect associated with an absence of the sacrum and defects of the lumbar spine and with a variety of malformations of the lower limbs and central nervous, cardiovascular, gastrointestinal, musculoskeletal, and genitourinary systems. Although the genetic anomaly is undefined, caudal regression syndrome is at least 250 times more prevalent in diabetic pregnancies.[13] Furthermore, studies in diabetic pregnant mice showed they are significantly more prone to generate embryos with caudal regression when they are treated with all-*trans* retinoic acid, a widely known teratogen.[13] These findings suggest maternal diabetes together with environmental factors operate synergistically to potentiate diabetic embryopathy.[13]

A number of studies have examined the adverse effects of hyperglycemia on nephrogenesis specifically. Marked decreases in nephron induction and tubulogenesis are observed when rat metanephroi grown in organ culture are treated with high (30 mM) D-glucose as compared with normal (5 mM) D-glucose.[14] In organ cultured mouse metanephroi,[15] and *in vivo*,[16] high glucose induces excessive *Pax2* gene expression through generation of reactive oxygen species and activation of the NF-κB pathway, which also mediates inflammatory responses and apoptosis. In this issue of *JASN*, new details are provided on the detrimental nephrogenic effects of maternal hyperglycemia.[17] Offspring of diabetic mice have significantly lower body weight, body size, kidney weight, and fewer nephrons than pups from nondiabetic controls. In addition, kidneys from offspring of diabetic mice have increased expression of mRNA encoding angiotensinogen and renin and nuclear localization of the NF-κB isoforms p50 and p65. Importantly, there is also evidence for increased glomerular and tubular apoptosis in kidneys from mice born of diabetic mothers, which may represent the ultimate explanation for reduced nephron endowment in these animals.

Given the relatively lengthy period in which the human kidney undergoes nephrogenesis (from the fourth/fifth gestational week to week 35), perhaps restoration of normal glycemic control from mid- to late-gestational periods can minimize adverse effects of maternal hyperglycemia on kidney development. Along these lines, one study using a growth-restricted, placental insufficiency model found that cross-fostering growth-restricted pups postnatally using normal lactating dams corrected loss of renal endowment and prevented the development of hypertension.[18] Conversely, another study reported that even a brief infusion of glucose into pregnant rats at the inception of nephrogenesis (from E12 to E16) resulted in significant nephron deficits 2 wk after birth.[19] In view of the expanding epidemic of diabetes, a much larger population of infants and mothers will undoubtedly become at risk to the hazards and complications of hyperglycemia during pregnancy.

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## DISCLOSURES

None.

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See related article, "Maternal Diabetes Modulates Renal Morphogenesis in Offspring," on pages 943–952.

# Toward the Promise of Renal Replacement Therapy

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In this issue of *JASN*, Tumlin *et al.*[1] report results of a revolutionary phase 2 multicenter, randomized clinical trial comparing 72 h of continuous venovenous hemofiltration (CVVH) with and without a bioartificial kidney (referred to as a renal tubule assist device [RAD]) in the management of severe acute kidney injury. Fifty-eight patients were randomly assigned: 40 to CVVH + RAD and 18 to CVVH alone. Multiple outcomes were evaluated, including the standard metric for clinical trials in critical care with 28-d survival as the primary outcome. All-cause mortality at 90 and 180 d, time to recovery of kidney function, time to intensive care unit and hospital discharge, and safety parameters were also examined. Mortality rates at 28 and 180 d were marginally lower among patients who were randomly assigned to CVVH + RAD.

One could easily criticize aspects of the design, implementation, and analysis of the trial and its reporting. First, there was no documentation of the expected effect size, except in the context of the investigators' estimated improvement (stratified as <10, 10 to 23.3, and >23.3%) that would guide the conduct of subsequent trials. Regardless, the study was hopelessly underpowered. If one were to consider a comparison of two strategies directed toward the management of severe acute kidney injury in the intensive care unit and estimate the 28-d mortality in CVVH-treated patients as the midpoint of the range cited by the authors (60%), the sample size required to detect a reasonable and clinically meaningful reduction in mortality (10% absolute, 16.7% relative) would be 768 with 80% power or 1028 with the 90% power typically recommended for substantive interventions. Corresponding sample sizes would be 188 and 252 with a larger, arguably unrealistic effect estimate (20% absolute, 33% relative). Of note, these sample size estimates do not account for loss to follow-up or dropout. We previously highlighted the pitfalls of conducting underpowered clinical trials, even when results are conventionally significant.[2]

Second, only 10 of 40 patients who were randomly assigned to CVVH + RAD completed the planned 72 h of therapy. The rationale for discontinuing the RAD intervention for clinical improvement or deterioration was not provided.

Third, the primary result (28-d mortality in 13 [33%] of 39 CVVH + RAD– *versus* 11 [61%] of 18 CVVH alone–treated patients) was not statistically significant and failed to consider the patient who was assigned to CVVH + RAD and died before RAD therapy was instituted; that is, the comparison was performed in an as-treated rather than an as-randomized "intention-to-treat" sample.[3]

Fourth, at least seven outcomes were assessed (death at three discrete time points, recovery of kidney function at two discrete time points, and time to death and time to recovery of kidney function) without consideration of the statistical implications of multiple comparisons. Moreover, the authors failed to offer a compelling hypothesis for why a nonsignificant effect in the short term might be expected to produce a significant benefit in the longer term, particularly when the intervention lasted at most 72 h. Finally, numerous nonprespecified subgroup analyses were conducted; for example, with and without sepsis or with higher and lower APACHE II and SOFA scores.

Despite these limitations, the investigators should be commended for having extraordinary vision, courage, and creativity to invent and legitimately test a bioartificial renal device. Although conventional dialysis technologies have been developed and refined with the primary goal of enhancing the clearance of metabolic waste, hazardous electrolytes, and excess extracellular fluid, they have failed to address many, if not most, of the broad-ranging functions of the kidney, as the authors articulate. Although some investigators have questioned whether critically ill patients die *with* or *from* acute kidney injury, epidemiologic evidence strongly suggests that patients with acute kidney injury experience an excess of death directly attributable to the kidney injury itself,[4–6] although it seems unlikely that azotemia, hyperkalemia, hypervolemia, or other dialysis-remediable abnormalities are culpable. Indeed, one might look toward the dialysis *versus* transplantation experience in ESRD as an informative analogy.

The provision of dialysis itself, although sustaining life, fails to restore health to the majority of patients who have ESRD. Patients who have ESRD and receive a kidney transplant enjoyed markedly prolonged survival and enhanced health-related quality of life relative to patients who remain on dialysis, despite the multiplicity of assaults on

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