Renal programming: cause for concern?

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Submitted 1 December 2010; accepted in final form 29 December 2010

Kett MM, Denton KM. Renal programming: cause for concern? Am J Physiol Regul Integr Comp Physiol 300: R791–R803, 2011. First published December 29, 2010; doi:10.1152/ajpregu.00791.2010.—Development of the kidney can be altered in utero in response to a suboptimal environment. The intrarenal factors that have been most well characterized as being sensitive to programming events are kidney mass/nephron endowment, the renin-angiotensin system, tubular sodium handling, and the renal sympathetic nerves. Newborns that have been subjected to an adverse intrauterine environment may thus begin life at a distinct disadvantage, in terms of renal function, at a time when the kidney must take over the primary role for extracellular fluid homeostasis from the placenta. A poor beginning, causing renal programming, has been linked to increased risk of hypertension and renal disease in adulthood. However, although a cause for concern, increasingly, evidence demonstrates that renal programming is not a fait accompli in terms of future cardiovascular and renal disease. A greater understanding of postnatal renal maturation and the impact of secondary factors (genes, sex, diet, stress, and disease) on this process is required to predict which babies are at risk of increased cardiovascular and renal disease as adults and to be able to devise preventative measures.

hypertension; kidney disease; renin-angiotensin system; sympathetic nervous system; nephron endowment

KIDNEY DEVELOPMENT IN HUMANS OCCURS IN UTERO BETWEEN THE 5TH AND 36TH WK. Thus, by the end of gestation, nephrogenesis is complete, and the basic renal architecture including vascular, neural, and hormonal networks is established. The kidney, however, does not begin to function as the primary organ in body fluid homeostasis until after birth. Furthermore, maturation of the human kidney continues for months and, arguably, up to several years after birth. In contrast, nephrogenesis continues postnatally in several species, most notably rodents. As with humans, however, maturation of the kidney in animal species continues during the weaning and early postweaning periods. Given this extensive time frame over which the kidney develops and matures, it is perhaps not surprising that studies in humans and animals have demonstrated how exquisitely sensitive the kidney is to adverse intrauterine and neonatal environments leading to permanent changes in renal structure and function, that is, renal programming. These programming events may manifest grossly as a reduced renal mass (e.g., following intrauterine growth retardation, preterm birth, or unilateral renal agenesis), or more subtly, such as altered activity of the renal renin-angiotensin system (RAS). Indeed, what has become increasingly clear is the magnitude of programmed changes that can be induced across a wide spectrum of kidney cell types. However, regardless of the type of programming a kidney is subjected to, it must compensate for any deficits to allow it to face the normal day-to-day challenges of maintaining homeostasis from birth. Furthermore, the kidney must adapt to the rapid postnatal growth of the individual as it matures to adulthood and adapt when exposed to physiological and environmental stressors. This review examines the programmed kidney, specifically the major changes to structure and function documented in the literature. Furthermore, it examines the consequences of beginning life with a suboptimal kidney for long-term cardiovascular and renal health. Ultimately, we argue that it is how the kidney compensates for programmed changes and its ability to respond to these physiological and environmental pressures that dictates the health and longevity of the organ and thus the individual. We conclude that a poor beginning causing renal programming, though a cause for concern, is not a fait accompli in terms of future cardiovascular and renal disease (Fig. 1).

THE PROGRAMMED KIDNEY

The kidney can be programmed by a variety of intrauterine and neonatal insults including maternal/neonatal undernutrition or malnutrition, placental insufficiency, exposure to maternal disease (hypertension, diabetes), or hormones, and exposure to pharmaceuticals, alcohol, or other toxins (31, 45, 75, 101). The most recognizable evidence of a suboptimal intrauterine or neonatal environment is reduced birth weight or neonatal growth. However, as we and others have demonstrated, programming of the kidney can occur in the absence of...
any effects on birth weight (31, 75, 101). The intrarenal factors that have been most well characterized as being sensitive to programming events are kidney mass/nephron numbers, RAS, renal sodium handling, and the renal sympathetic nerves (Fig. 1), and each will be discussed in turn. However, it is important to note that other factors, such as renal oxidative stress, immune cell infiltration, and altered renal vascular reactivity have also been reported in programming models (128, 141). There also appears to be a synergy between these systems, whereby a change in one may drive or reinforce changes in another or potentially mask the impact. With current technologies it is not possible to examine in detail early programming events or mechanistic adaptations in these systems in humans, thus the bulk of this data comes from animal models. Animal models also allow for dissection into whether programmed changes in the kidney are primary in nature, i.e., present at birth, or whether changes to renal structure and function are likely to be secondary in nature, that is, compensatory to allow for renal function to be maintained.

Reduced Renal Mass and Nephron Endowment

Studies performed over the last 40 years using acid maceration techniques and, more recently, unbiased stereological techniques, have highlighted the vast range of nephron numbers within adult population groups from Australia, Denmark, France, Germany, Senegal, and the United States (39, 64, 72, 96, 97, 107, 120). Furthermore, Zhang et al. (161) have demonstrated that this range in nephron number is present in kidneys obtained from infants (<3 mo old), suggesting the range in adult nephron number reflects a range in nephron endowment and not simply variations in nephron loss in individuals with aging. While these studies have typically demonstrated three- to fourfold range in small cohorts (see Ref. 120), the largest of these studies, of Caucasian and African Americans in Mississippi, of 232 adults has identified almost a 10-fold range in nephron numbers (210,332 to 2,026,541) (39). Indeed, when the 420 kidneys examined in the Monash Series are compiled (Australian, United States, and Senegalese populations), the nephron number range is 13-fold (see Ref. 120). Interestingly, of all the population groups examined, African Americans appear to have the largest range in nephron number representing both the upper and lower limits of this 13-fold range (63, 65, 120). In these human studies, renal mass and nephron endowment have been strongly associated with birth weight, which is often used as a surrogate marker of the intrauterine environment. Analyses of kidneys from fetuses and infants that were preterm, had intrauterine growth restriction or low birth weight, or were small for gestational age have shown marked reductions in kidney mass, and, where studies involved tissues collected at autopsy, significant reductions in nephron number (60, 61, 77, 134). A reduced nephron endowment has, however, been demonstrated in human studies without reductions in birth weight, which is often used as a surrogate marker of the intrauterine environment. Analyses of kidneys from fetuses and infants that were preterm, had intrauterine growth restriction or low birth weight, or were small for gestational age have shown marked reductions in kidney mass, and, where studies involved tissues collected at autopsy, significant reductions in nephron number (60, 61, 77, 134). A reduced nephron endowment has, however, been demonstrated in human studies without reductions in birth weight (60). Furthermore, unilateral renal agenesis has also been associated with fetal programming, particularly as a consequence of maternal diabetes (106).

Despite this large body of evidence linking suboptimal intrauterine environment with reduced renal mass and nephron endowment, identifying the period of gestation in humans during which nephrogenesis and kidney mass is sensitive to insults is difficult. In a serial ultrasound study, Konje et al. (77) compared renal growth in 87 small and appropriate-for-gestational-age fetuses from 22–38 wk of gestation. They found that the greatest reduction in renal growth in the small-for-gestational-age fetuses was from 26–34 wk of gestation, and the reduced kidney mass persisted until birth (77). However, this study in living subjects could provide no evidence as to whether nephrogenesis was affected during this time, or indeed the specific perturbation driving the reduced renal growth. It is for this reason that much of our knowledge of the types of insults driving reduced renal mass and nephron endowment, the time frame of sensitivity to these insults, and the mechanisms by which these insults translate to reduced renal mass and nephron endowment have been generated in animal models.
These studies in animal models have exposed the kidney, and particularly the process of nephrogenesis, as being highly sensitive to a broad range of perturbations to the intrauterine environment even when the insult is insufficient to lower birth weight. Furthermore, studies of glucocorticoid exposure, and to a lesser extent maternal retinoic acid, low protein, and diabetes, highlight that insults need only last for a brief moment in time, as little as 24–48 h, to lead to persistent reductions in kidney mass and nephron number. These studies have shown that the period of gestation when the kidney is most sensitive to insults is that which coincides with the early stages of nephrogenesis when the ureteric bud has entered the metanephric mesenchyme and the first few branching events are occurring. In an elegant series of experiments, Ortiz and colleagues (112, 113) administered the synthetic glucocorticoid dexamethasone to pregnant rat dams for periods of 48 h over days 11–12, 13–14, 15–16, 17–18, 19–20, 20–21 of gestation. They found that treatment only reduced nephron endowment when administered on days 15–16 and 17–18, coinciding with the early phase of kidney development (112, 113). Similarly, treatment of pregnant ewes with dexamethasone for 48 h during the equivalent stage of renal development also resulted in significant reductions in nephron number (152). Furthermore, we demonstrated a similar reduction in nephron endowment when rats were given high, but physiological, levels of the natural glucocorticoid corticosterone during this critical window (135). Amri et al. (4) also demonstrated that infusion of glucose from days 12–16 of gestation led to a 20% reduction in nephron number of offspring but only in those dams whose hyperglycemia was transiently higher (for ~24 h) on day 13. It is important to note that renal programming does not only lead to a reduced nephron endowment. Lelievre-Pegorier et al. (84) demonstrated that a single dose of retinoic acid given to pregnant rats on day 11 of gestation was sufficient to lead to a 20% increase in nephron endowment of offspring.

One of the major pathways by which maternal insults may result in reduced nephrogenesis and renal mass is via a greater exposure of the fetus to high glucocorticoids. Chronic hypoxemia, maternal low-protein diet, and ANG II have all been shown to reduce the level and activity of 11β-hydroxysteroid dehydrogenase type-2 (11β-HSD2) in placental and fetal tissues (80, 83, 104). As 11β-HSD2 is the key intracellular enzyme that inactivates natural glucocorticoids, loss of activity of this enzyme will lead to greater transference of maternal glucocorticoids across the placenta into the fetus that, at that time, has a reduced capacity to deactivate glucocorticoids (45, 139). Ultimately, this may lead to greater availability of glucocorticoids to act at glucocorticoid and mineralocorticoid receptors and impact on kidney development (150, 159). To this end, Dickinson et al. (37) found that maternal dexamethasone treatment stimulated expression of bone morphogenetic proteins and transforming growth factor-β1, genes that inhibit ureteric branching morphogenesis and thus reduce nephron number. Another pathway likely to be involved in the translation of a poor intrauterine environment to a reduced nephron endowment is the glial cell line-derived neurotrophic factor (GDNF)-cRet pathway. Studies of metanephric kidneys and knockout mutant mice have clearly demonstrated the significance of this pathway for induction of kidney development and nephrogenesis (24, 25, 49, 84, 123). Expression of the proto-oncogene cRet, a tyrosine kinase receptor, is strongly regulated by maternal vitamin A levels (49, 84). Low plasma concentration of vitamin A and retinol binding protein, and lower stores of vitamin A have been demonstrated in small-for-gestational-age infants and are associated with alcohol and smoking (see Refs. 49 and 98). Interestingly, Song et al. (138) have recently demonstrated that the activation of the angiotensin receptor type 2 (AT2R) is important for the regulation of GDNF-cRet signaling pathway.

**Renal RAS**

Recognition that the RAS plays important roles not only in the regulation of renal function and arterial pressure in the adult but also in the structural and functional development of the fetal kidney has lead to the intrarenal RAS being a focus in studies examining potential mechanisms in programming models of cardiovascular and kidney disease (102). Overall, studies to date suggest that alterations in the RAS play a role in the pathogenesis of hypertension in developmental programming. Yet, the reports have been conflicting with up- and downregulation of almost all components of the intrarenal RAS being reported (see Table 1). In large part this is due to the multiplicity of models employed and the wide age range at which offspring were studied. Broadly, however, the data in programming models taken as a whole suggests a temporal biphasic response with reduced RAS expression in the neonate that becomes normalized with age (Table 1). However, it should be noted that this normalization in the adult may, in fact, represent RAS activation that is inappropriately high for the given level of arterial pressure in the offspring.

Significant progress has been made in terms of understanding the consequences of a reduced intrarenal RAS expression on kidney development in the neonate, with reductions in angiotensin receptor and peptide levels being associated with reduced ureteric branching and a lower nephron endowment (138, 160). Yet, in the majority of studies, the functional impact of a downregulation of the RAS in early life on function in the adult, other than the effect on arterial pressure, was not assessed (Table 1). Those few studies that have examined renal function in association with altered neonatal intrarenal RAS expression have demonstrated that glomerular filtration rate (GFR) per nephron was higher compared with control groups, which suggests indirectly that glomerular hyperfiltration was present (126, 154, 158). In contrast, in some studies in models of programmed hypertension, GFR was found to be lower than in control groups, perhaps suggesting that glomerular hyperfiltration was not a factor in these models or that further nephron loss had occurred with aging since nephron number had been determined earlier in life (53, 136, 137). Finally, a small number of studies have examined renal tissue excretion of sodium and/or protein in models of programmed hypertension with the majority demonstrating disturbed tubular function (3, 137, 140). Thus, while the majority of studies suggest that neonatal suppression of the intrarenal RAS contributes to altered renal structural development [in agreement with studies in which the RAS is directly blocked by angiotensin converting enzyme inhibitors, angiotensin receptor blockers, or genetic deletion (54)], the functional impact has been studied in surprisingly little detail.
Renal Sympathetic Nervous System

Alterations in renal sympathetic nerve activity produce important effects on renal function, including changes in renin secretion, sodium reabsorption, and vascular tone. These factors contribute to the kidney’s main function of regulating body fluid balance. There is strong evidence to suggest that sympathetic overactivity, and, in particular, renal sympathetic activ-

### Table 1. Alterations in the renin-angiotensin system in young and adult offspring associated with different intrauterine insults

<table>
<thead>
<tr>
<th>Model</th>
<th>Species</th>
<th>Timing of Insult</th>
<th>Age and Sex of Offspring at Study</th>
<th>mRNA or Protein Expression</th>
<th>Physiological Response</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucocorticoids</td>
<td>Sheep</td>
<td>Early gestation</td>
<td>40 mo ♀</td>
<td>++ Plasma renin, ANG II or Aogen</td>
<td>↑ Basal MAP, females only</td>
<td>(116)</td>
</tr>
<tr>
<td></td>
<td>Rat</td>
<td>6–7 mo ♂ ♂</td>
<td></td>
<td>++ Renal Aogen</td>
<td>↑ Basal TBP, females only</td>
<td>(108)</td>
</tr>
<tr>
<td></td>
<td>Rat</td>
<td>Mid-to-late gestation</td>
<td>6 mo</td>
<td>↑ Renal ACE and renin in males and females</td>
<td>ND</td>
<td>(159)</td>
</tr>
<tr>
<td>Maternal nutrient restriction</td>
<td>Rat</td>
<td>Mid-to-late gestation</td>
<td>4 and 8 wk ♂</td>
<td>↔ PRA, plasma ANG II, and renal ANG II levels</td>
<td>↑ Basal TBP at 8 but not 4 wk of age</td>
<td>(27)</td>
</tr>
<tr>
<td>or low protein diet</td>
<td>Sheep</td>
<td>Early-to-mid gestation</td>
<td>9 mo old ♂</td>
<td>↑ Renal cortical ACE protein</td>
<td>↑ Basal MAP</td>
<td>(47)</td>
</tr>
<tr>
<td></td>
<td>Rat</td>
<td>Mid-to-late gestation</td>
<td>4–12 wk ♂ ♂</td>
<td>↑ PRA</td>
<td>↑ Basal TBP from 8 wk of age</td>
<td>(11)</td>
</tr>
<tr>
<td></td>
<td>Rat</td>
<td>Throughout gestation</td>
<td>1–5 days and 22 wk ♂</td>
<td>↓ Renal renin mRNA and ANG II levels at 1 to 5 days of age</td>
<td>↑ Basal MAP to 22 wk of age</td>
<td>(154, 155)</td>
</tr>
<tr>
<td></td>
<td>Rat</td>
<td>Throughout gestation</td>
<td>16 wk ♂</td>
<td>↓ Renal AT1R and AT2R protein</td>
<td>↑ Basal MAP, ↓ sodium excretion, ↔ GFR</td>
<td>(99)</td>
</tr>
<tr>
<td></td>
<td>Rat</td>
<td>Throughout gestation</td>
<td>4 wk ♂</td>
<td>↑ Renal AT1R protein</td>
<td>↑ Basal MAP (anaesthetised)</td>
<td>(125, 126)</td>
</tr>
<tr>
<td></td>
<td>Rat</td>
<td>1 to 11 mo ♂ ♀</td>
<td>1–2 mo</td>
<td>↑ Renal renin and ANG II tissue levels</td>
<td>↑ Basal TBP at 8 wk of age</td>
<td>(94, 95, 146, 147)</td>
</tr>
<tr>
<td>Placental insufficiency</td>
<td>Rat</td>
<td>Late gestation</td>
<td>0 to 16 wk ♂</td>
<td>Newborn: ↓ renin and aogen</td>
<td>↑ Basal MAP that was abolished by ACEi</td>
<td>(53, 110)</td>
</tr>
<tr>
<td></td>
<td>Rat</td>
<td>16 wk ♂</td>
<td></td>
<td>↑ Renal renin and Aogen mRNA, ↑ ACE activity</td>
<td>↑ Pressor response to ANG II in presence of ACEi</td>
<td>(31, 32, 92, 93)</td>
</tr>
<tr>
<td>Maternal renal hypertension</td>
<td>Rabbit</td>
<td>Throughout gestation</td>
<td>10–45 wk ♂ ♂</td>
<td>↓ PRA – 5 and 10 wk</td>
<td>↑ Basal MAP at 30 and 45 wk</td>
<td>(31, 32, 92)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>↔ PRA 30 and 45 wk</td>
<td></td>
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</tr>
</tbody>
</table>

TBP, tail artery pressure; MAP, mean arterial pressure; PRA, plasma renin activity; ACE, angiotensin converting enzyme; ACEi, angiotensin converting enzyme inhibition; aogen, angiotensinogen; ND, not done; GFR, glomerular filtration rate; RBF, renal blood flow. AT1R, angiotensin receptor type 1.
ity, is involved in the pathogenesis of hypertension (36). Furthermore, the renal sympathetic nerves are implicated in the programming of arterial pressure, although this aspect has not been extensively studied. The seminal work of Alexander et al. (2), demonstrated that renal denervation attenuated the development of hypertension in offspring of mothers with placental insufficiency. In another report, Dagan et al. (29) demonstrated a similar effect of renal denervation in a prenatal dexamethasone model that was associated with increased arterial pressure, low nephron number, and increase in renal sodium transporters. These effects of denervation are supported by findings of selective renal increases in renal vascular reactivity in response to adrenergic agonists (128) and increased sympathetic nerve activity in female offspring of rat dams with placental insufficiency (68). Furthermore, we have demonstrated that female rabbit offspring of hypertensive mothers, which have increased arterial pressure as adults, have an increased renal noradrenaline content, an index of renal sympathetic innervation, at birth (92). Taken together these studies support the hypothesis that exposure of the fetus to adverse stimuli alters renal sympathetic innervation and contributes to the development of adult hypertension via increases in renal vascular tone and sodium reabsorption (see Ref. 5).

The mechanisms leading to alterations to the renal sympathetic nervous system in these programming models are less clear. Knowledge of the growth and development of the renal nerves is still rudimentary, although in a broader context more is known about the development of the sympathetic nervous system (50). What is known is that the renal nerves and blood vessels develop in close association and the gene networks orchestrating the wiring of these systems are intertwined (16, 51). In the rat, neuronal precursor cells are present early in kidney development (70). The nerves proper do not enter the kidney until mid- to late gestation. They reach the outer cortical renal arterioles 1–2 wk after birth, and functional maturation continues well into postnatal life (10, 121) (Fig. 2).

In humans, nerves arrive at the renal arterioles earlier, but functional maturation still continues after birth (143). Studies have demonstrated that both fetal renal RAS and fetal glucocorticoid levels may influence development of the renal sympathetic innervation. One of the major roles of AT2R, at least in the central nervous system, is to promote neurite outgrowth and elongation, and under some conditions induce neuronal cell death (46). Increased renal AT2R expression at birth has been reported in models of programmed hypertension, and it is postulated that this may influence renal development, as AT2R are densely distributed in the metanephric mesenchyme (146, 150). Thus increased AT2R expression may influence nerve growth and maturation in the offspring of hypertensive mothers. Furthermore, it has also been demonstrated that fetal glucocorticoid levels modulate tissue RAS expression, which may have flow-on effects in terms of nerve growth (20).

Close association between the renal nerves and renin cells during development. In the developing kidney, renin-containing cells extend along the arcuate arteries to the afferent arterioles. As development proceeds, the renin cells retract until only those cells adjacent to the glomerulus are renin secreting: the adult phenotype (121, 132). At the same time as the renin secretory cells regress, the renal sympathetic nerves grow into the kidney following the track of the blood vessels (121, 132) (Fig. 2). Thus, development of the renal nerves may be influenced by changes in the fetal RAS. We have established that at least two distinct populations of sympathetic nerves innervate the kidney (Fig. 2) and that these populations differentially innervate the renal effector cells (smooth muscle, renin, and tubular cells) (41, 87, 88). These findings are supported by evidence that different renal neuroeffectors cells release discrete neurotrophic factors (70). Most significantly, we have also demonstrated physiologically that these nerve populations may be capable of controlling different aspects of renal function selectively (34, 35). Thus, there is strong evidence that distinct populations of neurons innervate the kidney and differentially control renal function. Of note, neuropeptide Y (NPY)-positive neurons extend beyond the renin-containing cells and innervate the efferent arterioles and renal medulla, whereas NPY-negative neurons do not (41). This further suggests that renin cells may play an important role in influencing the growth and development of the renal nerves and might perhaps also influence the subpopulations of nerves within the kidney differentially with consequences for renal function. On this basis, it is intriguing, that 1) the renal vasculature can revert to the fetal phenotype, such that renin granulation has been noted to extend back along the renal vasculature following chronic stimulation of renin release in response to low sodium diet or angiotensin converting enzyme inhibition in the adult (33); 2) vascular reactivity to acetylcholine and ANG II is enhanced in these phenotypically altered vessels (33); and 3) that the innervation density of the afferent arterioles is altered by chronic ACE inhibition (89). These data suggest that renal innervation density and vascular function can be influenced postnatally, and this may offer an opportunity to reverse some of the adverse consequences of a poor in utero environment. In support of such an argument, postnatal ACE inhibition or angiotensin receptor blockade has been shown to attenuate the development of hypertension in spontaneously hypertensive rats (55, 73, 74, 103) and offspring of mothers with placental insufficiency (53) and low protein diet (95).

Renal Sodium Transporters

Aberrant renal sodium handling is associated with human hypertension and thus has been examined in animal models of

Fig. 2. Depiction of the development of two populations of renal [blue, neuropeptide Y (NPY)-negative; orange, NPY-positive] nerves growing into the kidney in concert with the retreat of vascular smooth muscle cell expression of renin (red). Arc, arcuate artery; ila, interlobular artery; Aa, afferent arterioles; Ea, efferent arterioles; glomeruli.
renal programming, particularly maternal glucocorticoid exposure and low protein diets. These studies have noted increased protein abundance, mRNA levels, and/or activity of several sodium channels including Na\(^+\)/H\(^+\) exchanger-3 (NHE3), Na-K-Cl cotransporter 2 [NKCC2 (BSC1)], Na\(^+\)/Cl cotransporter (NCC), and thiazide-sensitive Na-Cl cotransporter (26, 28, 29, 93). Furthermore, this has been supported by data demonstrating increased proximal tubule reabsorption and medullary thick ascending limb chloride transport (26, 28). As discussed above, these models do demonstrate reductions in nephron number with normal or moderately reduced GFR, indicating significant single-nephron GFR (SNGFR). Thus one might consider these tubular transport changes to be appropriate compensatory mechanisms to adjust for the larger tubular flow rates, particularly as they have been performed in 4- to 6-wk-old animals. However, there is evidence that these changes may be primary and/or linked to increased renal sympathetic drive. Manning et al. (93) found that mRNA for BSC1 was elevated in 1-day-old rat pups of dams exposed to maternal low protein diet. Dagan et al. (29) found that offspring of dexamethasone-exposed rat dams had increased protein abundance of NHE3, NHCC2, and NCC at 8 wk of age. With renal noradrenaline contents elevated in 3-wk-old dexamethasone-exposed offspring, they performed renal denervation studies to determine the role of the renal sympathetic nervous system in these changes. They found that denervation at 6 wk of age abolished the increased protein levels of these sodium transporters and the elevated arterial pressures (29). Thus renal programming models may have an early, inappropriately high sodium reabsorption that could impact on adult cardiovascular and renal health.

CHALLENGING THE PROGRAMMED KIDNEY

Birth and Maturation of the Programmed Kidney

Although the fetal kidney produces urine, it does so to maintain appropriate amniotic fluid volumes. It is the placenta, rather than the kidney that acts as the primary organ for fetal fluid homeostasis in utero. Thus the process of birth and separation of the newborn from the placenta marks a major shift in the role of the kidney. At birth, GFR and renal blood flow increase markedly, facilitated by increases in systemic arterial and renal ultrafiltration pressure, and falls in renal vascular resistance (see Ref. 90). It is important to note that the kidney, and in particular the renal tubules, continue to mature postnatally (see Ref. 62 for full review). The proximal tubules undergo further differentiation with increased infolding of apical membranes to increase the surface area of microvilli for reabsorption, and increased basal infolding and mitochondria number for ATP generation to drive Na\(^+\)-K\(^+\)-ATPase (62). Furthermore, isoforms and distributions of transporters shift from fetal to adult form (62). Indeed, it is considered that human kidneys take up to 18 mo (6) and rodent kidneys 6 wk (62) for the maturation of sodium and water reabsorption systems in the tubules to occur, and thus until this time they have a limited capacity to regulate salt and water excretion. This is significant when one considers that the infant kidney must also maintain a positive sodium balance to allow for somatic growth (12). Given this period of rapid maturation of human kidneys postnatally, one must also consider that the kidney may be sensitive to renal programming ex utero as demonstrated in rodents (86, 124, 153).

Newborns that have been subjected to an adverse intrauterine environment may thus begin life at a distinct disadvantage in terms of renal function. As discussed above, prematurity (born at < 34 wk gestation) is associated with a reduced kidney mass and nephron number. Furthermore, as nephrogenesis would normally be still occurring in these preterm infants, and many tubular systems do not begin to mature until after the onset of filtration, these infants are born with compromised glomerular and tubular function (9, 145). Indeed, Vanpee et al. (145) found that renal function of preterm (25–30 wk) infants was markedly reduced in the neonate and still reduced at 9 mo. While this study found renal function had normalized by early childhood (145), other studies demonstrate that low birth weight and intrauterine growth restriction increase the risk of end-stage renal disease (148). Prematurity and low birth weight are clearly major insults to the kidney, and thus it is of no surprise that renal function in these infants is impacted. However, as discussed above, more subtle intrauterine events that do not affect birth weight or gestation length can have similar consequences for renal programming events (reduced renal mass and nephron number, altered RAS, sympathetic nervous system, tubular transporters, etc.). Thus a greater number of newborns are at risk of renal dysfunction than that described by the incidences of prematurity and low birth weight.

Consequences for Adult Cardiovascular and Renal Health

The kidney, by maintaining an appropriate balance between fluid intake and renal excretion, plays a dominant role in the long-term regulation of arterial pressure. As such, changes to the capacity/fidelity of the programmed kidney to respond to intra- or extrarenal signals may affect this balance and lead to the development of renal dysfunction, renal disease, and hypertension (Fig. 1). Literature in this field had initially suggested that kidney disease and hypertension resulting from the programmed kidney were a fait accompli (15). Animal models of maternal low-protein diet and dexamethasone exposure demonstrated marked systolic hypertension (tail cuff plethysmography) as early as 4–6 wk (28, 82). Furthermore, this animal work was supported by seminal studies in human tissue, demonstrating that a reduced nephron endowment was associated with the development of hypertension (66, 72). However, it has become increasingly clear that even a very low nephron number is not always associated with renal disease or hypertension, indeed some reports of renal programming models have demonstrated hypotension (14, 109). Furthermore, several human studies have failed to show an association between reduced nephron endowment and hypertension, particularly in African Americans (66, 67). This has led to the suggestion that additional factors or insults are required for phenotypic expression of hypertension and kidney disease following renal programming (105). These factors include the degree to which the kidney can compensate for the initial deficits and an individual’s genetics, including sex (Fig. 1). Furthermore, modifiable risk factors, such as diet and stress also appear to contribute to measured adult outcomes of renal programming (Fig. 1).

Compensatory Renal Growth and Hyperfiltration

One of the more overt signs of renal programming is a reduced renal mass (2 small kidneys or unilateral renal agen-
esis/dysgenesis) and a reduced nephron endowment. Studies have highlighted that the kidney compensates for these deficits by hyperfiltration and glomerular and tubular hypertrophy. Furthermore, these studies have shown that many of these processes appear to begin prior to birth and continue into the neonatal period. Given the invasive nature of such measurements, much of this data comes from animal studies of reduced nephron endowment and nephrectomy. Several studies in animal models and humans demonstrate that the degree of compensatory renal growth is greater the earlier the nephrectomy is performed due to a longer period of compensatory growth (7, 17, 71). These nephrectomy studies are consistent with human sonography data of fetuses with a unilateral functioning kidney secondary to unilateral renal agenesis or unilateral multicystic kidney. These studies have detected renal hypertrophy in the functioning kidney as early as 22 wk, resulting in values for total renal mass that were over 80% of values for control fetuses (see Ref. 58). Consistent with this early response of unilateral functioning kidneys, Douglas-Denton et al. (38) found that in utero unilateral nephrectomy of fetal sheep at 100 days of gestation (term 150 days) resulted in marked renal hypertrophy of the remaining kidney such that total kidney mass near term was only ~30% reduced compared with control fetuses with two kidneys. Of great significance, this group found that the final nephron number of this kidney was 45% greater than single kidneys of control animals suggesting the possibility that the nephron deficit in humans with unilateral renal agenesis may not be 50% as predicted (38). However, in a mouse genetic model of unilateral renal agenesis, we found that the deficit was >50% (123). To date, there has been no estimation of nephron number in human cases of unilateral renal agenesis/dysgenesis to determine the degree of nephron deficit.

Glomerular. Clearly a reduction in nephron endowment leads to a reduction in the glomerular capillary surface area available for filtration and tubular surface area, available for reabsorption, secretion, and metabolism. Thus it is not surprising that the renal hypertrophy documented in these models is due to compensatory hypertrophy of both glomerular and tubular compartments. In many models of reduced nephron endowment, glomerular hypertrophy can be sufficient to normalize total glomerular volume and thus glomerular capillary surface area available for filtration (123, 152, 158). However, we and others have shown that normalization of filtration surface area is not necessary to maintain normal GFR in the presence of marked reductions in nephron number, even in the neonatal period (19, 123). Celsi et al. (19) found that 20-day-old rats uninephrectomized at 5 days of age had SNGFR double that of sham rats with no change yet in filtration surface area. Furthermore, we found that GDNF Het mice born with unilateral renal agenesis had normal GFR at 1 yr of age despite the filtration surface area being 50% reduced (123). This suggests that despite marked nephron deficits, normal whole animal GFR can be maintained, even by the immature kidney, by increased glomerular capillary pressure and/or glomerular capillary ultrafiltration coefficient-mediated elevations in single-nephron GFR (19, 123).

The factors driving glomerular hypertrophy remain unclear particularly as the hypertrophic process can begin in utero when the kidney is not regulating fluid homeostasis and has a low flow and low perfusion pressures (90). Elevated systemic and glomerular capillary pressures elevated shear stress resulting from increased single-nephron perfusion and GFRs, and elevated ANG II has been implicated in glomerular hypertrophy secondary to nephrectomy (91). However, we have found no role for systemic pressure or ANG II in normal glomerular growth or glomerular hypertrophy in a genetic model of reduced nephron endowment (74, 133). A greater understanding of the mechanisms underlying the control, both the initiation and cessation, of compensatory glomerular capillary growth and glomerular hypertrophy is warranted.

Although elevated glomerular capillary pressure can be sufficient to increase SNGFR and maintain whole animal GFR, the chronic exposure of the delicate glomerular capillaries to elevated pressures increases the risk of damage. This is most clearly demonstrated by the presence of glomerulosclerosis that, as highlighted by Brenner et al. (15), may further decrease glomerular filtration surface area, initiating a vicious cycle of progressive renal injury and deterioration of renal function. Consistent with this hypothesis, several models of renal programming, particularly those in which nephron endowment is reduced, demonstrated significant glomerulosclerosis, reduced GFR, and/or albuminuria (113, 124, 129, 156, 158). However, as will be discussed later, this is not always the case. Although albuminuria is often considered secondary to glomerular capillary damage, recent evidence suggests that albuminuria more often reflects an imbalance between filtered load and tubular reabsorption (23). Thus the presence of albumin in the urine may simply reflect a greater filtered load secondary to marked hyperfiltration (rather than damage) that exceeds the reabsorptive capacity of the tubules.

Tubular. Concomitant with increased single-nephron GFR in those with a reduced nephron endowment is increased tubular flow rates. However, as noted earlier, the tubular system is relatively immature at birth with an impaired ability to regulate water and sodium balance. Thus the immature kidney with fewer functioning nephrons may filter solutes at a rate that exceeds the capacity of the proximal tubule to reclaim it. Indeed Al-Dahhan et al. (1) found that GFR exceeds tubular sodium reabsorption capacity in infants born before 33 wk of gestation. Furthermore, if adverse intrauterine events have also impacted on tubular maturity, transporter number/distribution/isoeform type or responsiveness, reabsorption could be further impacted. Alterations to this glomerulotubular balance can have major consequences for fluid homeostasis and long-term blood pressure control (13). However, this has not been examined in any great detail in the immature kidneys of programming models. Specifically, very little is known of segment-specific compensatory growth in models of reduced renal mass/nephron endowment or, as discussed earlier, the changes in renal tubular transporters at birth in these models. Again, the only insight we have is the response to unilateral nephrectomy. In the late 1960s Hayslett et al. (56) concluded that following adult nephrectomy there is a predominance of growth in the proximal convoluted tubule (35% length, 17% lumen diameter) compared with the distal tubule (17% and 12%, respectively). Using 20-day-old rats nephrectomized at 5 days of age, Celsi et al. (18) found that the proximal tubule of the immature kidney can respond to increased filtered load by initially increasing the density of transporters, and that by 60 days of age, there are increases in proximal tubule length. Clearly knowledge of the growth and maturation of specific
tubular segments are required in programmed renal models to identify the consequences for early fluid balance.

Genetics

It has been long recognized in the literature that race, and thus genetic make-up, underpins susceptibility to, and progression of, a variety of diseases, including hypertension and kidney disease. In utilizing rodents of different strains, it has become clear that the outcomes of adverse intrauterine events are indeed impacted by genetics. Pausova et al. (115) found that prenatal nicotine reduced renal mass and increased blood pressure in spontaneously hypertensive rats but not the Brown Norway rat. While Schwedler et al. (131) found that gentamicin exposure reduced nephron number in the ROP strain of mice but not C57 mice. Furthermore, the capacity of the kidney to adapt to deficits induced by programming events is also affected by genetics. Striker and colleagues have performed a variety of experiments examining the impact of a reduced nephron endowment induced by Os mutation in two strains of mice, the ROP and C57. They found that the level of reduction in nephron endowment in the two strains was the same, as too was the degree of compensatory glomerular hypertrophy and cellular proliferation. However, the ROP Os mice had severe glomerulosclerosis associated with increased glomerular type IV collagen, laminin, and tenascin, while the C57 Os mice had minimal evidence of glomerular disease (57). Similar findings of exacerbated glomerulosclerosis were demonstrated in ROP mice in response to surgical nephron reduction and streptozotocin-induced diabetes (42, 162). Lenz et al. (85) found that the severity of glomerulosclerosis in ROP mice was determined by at least 8–10 loci. These studies support the hypothesis that glomerular hypertrophy per se is not a determinant of progressive glomerulosclerosis (79) and suggest the genetic background of an individual, particularly their susceptibility to undergo glomerulosclerosis is a major contributor to renal outcomes of programming events.

Sex Differences in the Incidence of Cardiovascular Disease in Programming Models

Epidemiological studies have consistently shown higher arterial pressure levels in men than women (151). These differences in arterial pressure emerge during adolescence and persist throughout adulthood until menopause (59, 78). Epidemiological evidence also suggests a role for sex differences in the pathophysiology of cardiovascular disease and hypertension as premenopausal women characteristically have a lower incidence and severity of hypertension than men. This protection from cardiovascular disease is, however, lost postmenopause (111, 117). Sex differences in arterial pressure observed in humans are also seen in animal models, with arterial pressure reported to be higher in normotensive male compared with female dogs (100), rats (130), and rabbits (32). The mechanisms responsible for these sex differences in arterial pressure are not well understood, but sex hormones (40, 122) and sex chromosome complement (69) likely contribute, and certainly it is well recognized that there are sex differences in the regulation of arterial pressure by the RAS (127).

Similarly, sex differences in the fetal programming of hypertension and renal disease have been reported, with the reports predominantly, though not exclusively, demonstrating that males are more adversely affected than females (32, 76, 113, 158). Excellent reviews of this literature have been reported recently, and we refer the reader to these (48, 53). One explanation for these differences include the intrinsic sex differences in the regulation of arterial pressure and renal function spoken of above, which confer protection from cardiovascular disease in females of reproductive age. Another is that female fetuses may be less vulnerable to adverse in utero environments and therefore less likely to develop disease in adulthood. There is certainly evidence to support this hypothesis. It has been suggested that a common pathway in many models of fetal programming of hypertension is a reduction in placental 11β-HSD2 expression and thus an increased exposure of the fetus to maternal glucocorticoids (45). It has also been documented that the placenta of female fetuses have higher 11β-HSD2 levels than those of males, meaning that males may be exposed to higher levels of glucocorticoids than females and therefore may be more greatly affected (22). However, in our own studies in offspring of rabbit mothers with secondary hypertension, the females had a greater increase in arterial pressure as adults than the males (32, 92). While in this original study there was no statistical difference in litter size or the sex ratio of the pups, it became apparent when the litters from several cohorts (15 litters from hypertensive and 15 from control mothers) were pooled that there was a reduction in litter size in the hypertensive compared with control mothers due to a lower number of male offspring at birth (2.53 ± 0.31 vs. 3.53 ± 0.26; P < 0.05). This is in accord with literature on humans documenting a reduced survival of male babies of hypertensive pregnancies (149), and a 50% greater survival of preterm female than male infants (43).

Diet

Several groups have examined whether the risk of developing cardiovascular and renal disease secondary to adverse intrauterine environment could be modulated by diet. The greatest body of literature in this area has examined dietary salt intake. In general, rodent models of maternal low-protein diet, dexamethasone exposure, and intrauterine growth retardation demonstrate salt sensitivity of blood pressure and elevated albuminuria in response to high-salt diets (8, 129, 140, 142, 158). These findings are consistent with the limited human data that demonstrate that salt sensitivity of blood pressure in adults is inversely correlated with birth weight (30). Additionally, Simonetti et al. (134) have taken it a step further, showing that in children (~11 yr old), salt sensitivity of blood pressure correlated strongly with kidney length and volume. This suggests that a reduced renal mass/nephron number leads to salt sensitivity. Indeed, Woods et al. (157) have shown that rats that underwent neonatal unilateral nephrectomy demonstrated significant salt-sensitive hypertension in adulthood. Sanders et al. (129) also found significant salt sensitivity of blood pressure in intrauterine growth-retarded rats with reduced nephron number. Consistent with this hypothesis, we have shown that the rise in blood pressure in response to 1 wk of high-salt diet was related to the degree of nephron deficit (123). Interestingly, this rise in arterial pressure was not related to filtration surface area (123), suggesting tubular elements are likely to be contributing to the rise in blood pressure.
Recent studies have also suggested that this salt-sensitive phenotype can be modulated early in life (95, 140). Stewart et al. (140) have found that offspring of dams fed a low-protein diet were hypertensive on normal chow, normotensive on low-salt chow, and had exacerbated hypertension on high-salt chow; but interestingly, they found that they could completely prevent the development of hypertension for up to 52 wk when rats were placed on a low-salt diet for just 3 wk postweaning (140). Furthermore, this short manipulation ameliorated glomerulosclerosis, and the rats no longer showed a salt-sensitive phenotype (140). Together these studies suggest that dietary sodium intake can not only exacerbate the cardiovascular and renal outcomes of renal programming but also ameliorate and/or prevent the expression of a hypertensive or renal disease phenotype.

Other dietary factors have also been implicated in the initiation and progression of renal disease and hypertension, including protein intake and body mass in general. Of note, human studies of those born with a single kidney (unilateral renal agenesis) or living with significantly reduced renal mass (unilateral or subtotal nephrectomy) have found that a high body mass index was associated with the presence and progression of renal dysfunction (52, 119). This has lead some authors (21, 118) to suggest that the hyperfiltration associated with obesity on a background of established single-nephron hyperfiltration (secondary to reduced nephron number) leads to an exhaustion of renal functional reserve and the initiation of glomerular disease. Interestingly this paradigm has not been pursued in animal models of renal programming to any great extent.

Stress and Blood Pressure

Early studies of renal programming models induced by maternal low-protein diet, malnutrition, or dexamethasone exposure demonstrated marked increases in systolic blood pressure of as much as 30 mmHg in animals as young as 4 wk of age when measured by tail cuff (28, 82). However, with greater use of radiotracelemetry techniques it has become increasingly apparent that resting blood pressure in these animals is often not different from control animals or only moderately elevated even when followed into old age (8, 44, 81, 144). Some of these studies even show low blood pressures compared with control offspring (14, 81, 109). These radiotracelemetry studies have reinforced that it is the presence of secondary stressors, whether this be in the form of physical/psychological stress (e.g., restraint stress associated with tail cuff), physiological stress (e.g., dietary salt intake), or presence of coexisting disease (e.g., diabetes) that may be crucial for the translation of renal programming events into adult hypertension (Fig. 1). It also reinforces the concerns of using the tail cuff in programming models in which an altered stress response induced by concomitant changes to the HPA axis may be contributing to the blood pressure measurements made. These conclusions are supported by human studies using the Dutch Famine cohort. Painter et al. (114) found that although baseline blood pressures were not different between individuals exposed to famine compared with those not exposed, the exposed individuals had a greater rise in blood pressure in response to psychological stress. This effect was greatest in those exposed early in gestation.

CONCLUSION

In summary, human and animal kidneys are susceptible to environmental pressures during development that lead to permanent alterations to renal structure and function that is renal programming. This review has highlighted the main renal programming events published in the literature but should not be considered a complete list. At birth, the kidney must rapidly compensate functionally and structurally to meet the demands of ex utero life. We have highlighted the enormous capacity of the kidney to compensate for deficits to maintain homeostasis and suggest that in an environment devoid of further insults or stress that renal function and arterial pressure are well maintained. We suggest that the presence of these secondary factors, particularly a sclerosis-prone genetic make-up or dietary salt, is crucial in mediating adverse outcomes for renal and cardiovascular health.

Perspectives and Significance

What is missing from the literature is a deeper understanding of how these programmed kidneys (within most cases, a reduced nephron complement) undergo maturation after birth and respond to physiological challenges (hemorrhage, exercise, volume loading) and whether intrinsic (e.g., tubuloglomerular feedback, pressure natriuresis) or extrinsic (e.g., neural control of renal function, impact of vasopressin on urine concentrating ability) control mechanisms are impaired. A greater understanding of the role of epigenetics in control of renal function in programmed kidneys is also required. Furthermore, these studies need to be temporal in nature if we hope to be able to predict which babies are at risk of increased cardiovascular and renal disease as adults and be able to devise preventative measures.

GRANTS

M. Kett is funded by National Heart Foundation Research (NHMRC) Fellowship CR-07M3337 and Project Grant 491042. K. Denton is funded by NHMRC Research Fellowship 490918, NHMRC Research Project Grant 490919, and National Heart Foundation Grant-in-Aid G-09M-4316.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

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