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Maternal undernutrition and the offspring kidney: from fetal to adult life

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Abstract

Maternal dietary protein restriction during pregnancy is associated with low fetal birth weight and leads to renal morphological and physiological changes. Different mechanisms can contribute to this phenotype: exposure to fetal glucocorticoid, alterations in the components of the renin-angiotensin system, apoptosis, and DNA methylation. A low-protein diet during gestation decreases the activity of placental 11ß-hydroxysteroid dehydrogenase, exposing the fetus to glucocorticoids and resetting the hypothalamic-pituitary-adrenal axis in the offspring. The abnormal function/expression of type 1 ($AT1_R$) or type 2 ($AT2_R$) AngII receptors during any period of life may be the consequence or cause of renal adaptation. $AT1_R$ is up-regulated, compared with control, on the first day after birth of offspring born to low-protein diet mothers, but this protein appears to be down-regulated by 12 days of age and thereafter. In these offspring, $AT2_R$ expression differs from control at 1 day of age, but is also down-regulated thereafter, with low nephron numbers at all ages: from the fetal period, at the end of nephron formation, and during adulthood. However, during adulthood, the glomerular filtration rate is not altered, due to glomerulus and podocyte hypertrophy. Kidney tubule transporters are regulated by physiological mechanisms; Na^+/K^+-ATPase is inhibited by AngII and, in this model, the down-regulated AngII receptors fail to inhibit Na^+/K^+-ATPase, leading to increased Na^+ reabsorption, contributing to the hypertensive status. We also considered the modulation of pro-apoptotic and anti-apoptotic factors during nephrogenesis, since organogenesis depends upon a tight balance between proliferation, differentiation and cell death.

Key words: Nephrogenesis; Blood pressure; Angiotensin receptors; Glucocorticoids; Maternal undernutrition

Introduction

The role of the kidney in the pathogenesis of hypertension has long been established, although recent studies challenge renal hegemony and suggest an important role for vascular cells as well (1,2). In recent years, this view has expanded and now includes the concept that chronic hypertension and kidney dysfunction are also related to events that occur during the prenatal period. This relationship between fetal programming of disease and the kidney suggests that several mechanisms can predispose to hypertension during adulthood through impaired nephrogenesis. A recent overview considered the role of fetal programming in the development of adult kidney disease and hypertension (3). The purpose of the present review is to describe some of the mechanisms that can affect the kidney, with special focus on the renin-angiotensin system (RAS). The kidney is an organ that is centrally related to the development of hypertension through its function of renal sodium handling and intravascular fluid volume homeostasis. Importantly, following cross transplants, if the donor is hypertensive, the recipient becomes hypertensive too, since factors intrinsic to the kidney itself affect blood pressure (4,5). In 1968, Zeman (6) first reported that rat offspring of mothers that were severely protein-restricted (6% casein-diet) throughout pregnancy had kidneys that contained fewer glomeruli than offspring of mothers on a normal diet (24% casein). Nephrogenesis requires a fine balance of many factors that can be disturbed by intrauterine growth restriction (IUGR), leading to a low nephron endowment (7).

The aim of this review is also to present further evidence supporting an association between maternal low-protein ingestion and the increased prevalence of hypertension, as well as progression towards kidney dysfunction in adult life. Various potential mechanisms for this association are discussed, specifically the low nephron number hypothesis

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and related cellular and molecular mechanisms that have been proposed. Some factors involved in nephrogenesis, such as glucocorticoid exposure, regulation of components of the RAS, apoptosis, and p53 gene methylation, have been investigated in different low birth weight (LBW) models and will be considered in this review.

Maternal-fetal environment and adult disease

The size that the fetus can attain depends on the maternal-fetal nutrient supply and the space that the maternal environment can provide (8). Low maternal socioeconomic level and poor maternal nutritional status will reduce the nutrient supply to the developing fetus. Similarly, factors that frustrate the passage of nutrients through the placenta, such as smoking and hypertension, are associated with an increased risk for the development of the fetal origins of adult diseases. Nearly two decades ago, Barker (9) presented the theory that the maternal environment could induce adult diseases, based on observed epidemiologic associations between LBW and increased risk for ischemic heart disease, type 2 diabetes, and hypertension (10). The first associations found were between LBW and later life hypertension and cardiovascular disease (10-12). High blood pressure occurs at a higher incidence in children and adults who were of LBW (12). A systematic review of 80 studies that described the relationship of blood pressure with birth weight reported that systolic BP is lower by approximately 2 mmHg for every 1-kg increase in birth weight (13). Among Pima Indians, patients who had diabetes and a history of maternal undernutrition also displayed an increased risk to develop diabetic nephropathy (14). Moreover, poor maternal nutrition is associated with more rapid progression of other kidney diseases, such as IgA nephropathy, membranous nephropathy, and minimal-change disease, suggesting that the kidneys of these infants are more vulnerable to future insults (15). Nevertheless, the strength of the association between the maternal-fetal environment and subsequent hypertension remains widely debated. Moreover, some investigators claim that the suggested association is the result of inappropriate adjustments for confounding factors (16). For example, Huxley et al. (13) found a trend towards a weaker association between LBW and hypertension in larger compared with smaller studies (16). However, it seems that, although the relation is not invariant, the bulk of evidence suggests an important direct or indirect interaction between birth weight and subsequent hypertension (17). These observations have led to the formulation of an important conceptual construct that pertains to the fetal origins of adult disease. This theory states that, during development, body organs pass through a period of plasticity and sensitivity to the environment, which leaves a durable imprint that affects subsequent health. Suboptimal intrauterine conditions may result in impaired fetal growth and the production of phenotypes that are better matched to the inadequate intrauterine environment. These adaptive processes are aimed to increase the likelihood of survival *in utero* and after birth, with expected continuation of borderline or inadequate environmental conditions. However, this response may result in adverse long-term consequences later in life, especially when the postnatal environment affords more favorable growth conditions than those experienced *in utero*. One of the most studied aspects with regard to Barker's theory (9) has been the fetal origin of adult diabetes. Numerous reports have demonstrated an association between poor maternal nutritional status and subsequent development of pancreatic endocrine insufficiency and diabetes in experimental animals and humans (18,19). However, a detailed consideration of the relationship between maternal low protein ingestion and diabetes is beyond the scope of this review.

Experimental studies have been conducted using different models of IUGR. Among others, maternal low-protein diet (20), 50% food restriction (21) and glucocorticoid exposure (22) are the focus of many studies because they reflect the situation of people who live in poverty. Both of them result in LBW leading the fetus to develop adult diseases, like diabetes, heart disease and hypertension.

Glucocorticoid exposure *in utero*

In 1993, Benediktsson et al. (22) showed that rats exposed to excess glucocorticoid *in utero* developed adult hypertension. Since that study, the early exposure of the fetus to glucocorticoids has been used as a model of fetal programming in different species (23-25). Fetal protection from maternal glucocorticoid is normally affected by placental 11ß-hydroxysteroid dehydrogenate type 2 (11ß-HSD2), which converts cortisol and corticosterone to inactive products (cortisone, 11-dehydrocorticosterone). The activity of 11ß-HSD2 correlates positively with birth weight and negatively with placental weight in rats (22), suggesting an important role for this enzyme in regulating fetal growth and development.

Glucocorticoids are widely used in the management of women at risk of preterm delivery to accelerate pulmonary maturation. Antenatal glucocorticoids are associated with a reduction in birth weight in human studies (26). Preterm children treated postnatally with glucocorticoid display elevated blood pressure at an early age (27). Dexamethasone (DEX) is a synthetic glucocorticoid non-metabolized by 11ß-HSD2 that readily crosses the placental barrier and has been used to study the effects of fetal glucocorticoid exposure. Another model that mimics the effects of a maternal low-protein diet is the treatment during pregnancy with carbenoxolone, an 11ß-HSD2 inhibitor (28).

Feeding a low-protein diet to pregnant rat dams reduces the activity of placental 11ß-HSD2 by 33%, programming hypertension in the adult offspring (29). A major consequence of maternal protein restriction appears to be a resetting of the

hypothalamic-pituitary-adrenal axis function in the offspring (24). Gestational low-protein-exposed rats exhibit increased sensitivity to low-normal concentrations of corticosterone and have increased numbers of glucocorticoid receptors in the adrenals. In an elegant study with adrenalectomized rats, Gardner et al. (30) demonstrated that, in a model of maternal low-protein diet, adrenalectomy reduces high blood pressure when compared with control animals. Corticosteroid replacement restores the high blood pressure in these rats, an indication of a glucocorticoid-dependent phenomenon (30). When cortisol was administered to pregnant sheep, the offspring showed similar changes in the expression of type 1 angiotensin II receptor ($AT1_R$) compared to controls, suggesting that alterations in the renal RAS may be a mechanism by which early prenatal glucocorticoid exposure causes fetal programming (31).

The hypertension programmed by glucocorticoid exposure is linked to pathological changes in kidneys, such as increased sodium transport, alterations in the RAS and low nephron number. Studies with DEX have revealed that the severity of these phenotypes depends on the timing and duration of glucocorticoid exposure (32). The critical "window" of development during which kidneys are more susceptible to DEX exposure is the very early stage of renal development, where the ureteric bud invades the metanephric mesenchyme and begins to branch (33). Singh et al. (32) demonstrated that rat metanephric DEX exposure *in vitro* for 2 days during early nephrogenesis inhibits ureteric branching morphogenesis and nephrogenesis, suggesting that a reduction in ureteric branching morphogenesis may be a key mechanism through which DEX reduces nephron endowment.

Another study has shown that prenatal DEX is associated with an increase in Na^+/H^+ exchange activity in proximal tubules, as well as in the brush-border membrane NHE3 protein (34). Furthermore, a recent study demonstrated that, in offspring exposed *in utero* to DEX, renal denervation at 6 weeks of age resulted in a decrease in NHE3, NKCC2 and NCC protein abundance to levels observed in vehicle-treated sham rats (35).

Renin-angiotensin system

The role of the RAS in the control of blood pressure and homeostasis balance is well recognized. The RAS was accepted to have a role in kidney development, with a role in vasculogenesis, branching morphogenesis and development of hypertension in the adult, as the result of a reduction in nephron number during nephrogenesis (7,36).

All the components of the RAS are expressed in the embryonic kidney and, in rats, the expression can be detected from 12 to 17 days of gestation, being higher in fetuses and newborn rats than in adult rats (36). Pharmacological or genetic alterations in the RAS during kidney development induce gross abnormalities, such as a hypoplastic papilla. Treatment of rats during active nephrogenesis with an AT1 antagonist or with an angiotensin-converting enzyme (ACE) inhibitor leads to decreased nephron number (37), delayed nephron maturation and altered renal water handling (38). Human kidneys of fetuses from mothers that received an AT1 antagonist during gestation show poorly developed tubules, a reduced number of proximal tubules, poorly developed vasa recta and a hyperplastic juxtaglomerular apparatus (39).

Many studies have shown that perturbed maternal nutritional status alters renal renin protein and mRNA levels, as well as renal angiotensin II (AngII) concentration (40) and changes renal expression of AngII receptors and mitogen-activated protein kinase (MAPK) in the pups (41), resulting in higher blood pressure and structural changes in the kidney of adult offspring. Conflicting results regarding RAS among the groups are explained by different levels of protein in the diet, different periods of treatment during gestation, different rat strain and, mainly, by the different methods adopted, such as whole kidney Western blot, cortex Western blot, or whole kidney RT-PCR.

Specific studies with maternal protein restriction during gestation have shown different modulations of RAS components. Sahajpal and Ashton (42) showed that offspring of mothers that received a low-protein diet during the entire gestational period presented, at 4 weeks of age, fewer glomeruli per g kidney weight and the $AT1_R$ protein level was 24% greater in low-protein pups when compared with normally nourished pups. In another study, the same group showed that $AT1_R$ and $AT2_R$ was greater in the cortex (62 and 35%, respectively) in low-protein pups at 4 weeks of age and renal renin activity and tissue AngII concentrations were not lower in these animals (43). Vehaskari et al. (44) compared the levels of RAS protein and mRNA at 1 day and 28 days of age and found that, at 1 day, $AT1_R$ and $AT2_R$ proteins were decreased, but that $AT2_R$ mRNA was increased. The same variation found by Sahajpal and Ashton (42) was verified at 28 days, when $AT1_R$ and $AT2_R$ proteins were increased, but $AT1_R$b and $AT2_R$ mRNA did not differ, suggesting that the ontogeny of the intrarenal RAS is altered throughout the perinatal and early postnatal period (44).

Our group recently found that $AT2_R$ protein is down-regulated in kidneys of 16-week-old rats born to females that received a low-protein diet (9%) throughout gestation. These animals presented a total absence of $AT2_R$ in the glomeruli and this receptor was localized preferentially associated with intercalated cells of the distal and collecting segments. $AT1_R$ and its JAK-2/SOCS3 pathway proteins are also down-regulated in these experimental animals, as shown by Western blot and immunohistochemistry (20). It is important to note that Sahajpal and Ashton (42) and Vehaskari et al. (44) used 4-week-old rats, whereas we used adult male rats. At 4 weeks of age, rats are normotensive, but become progressively more hypertensive at 6 to 8 weeks

of age (42-44). For this reason, the cited groups studied the role of RAS in the pathogenesis of hypertension and our group focused on the effects of hypertension on renal RAS, obtaining opposite results for $AT1_R$.

We also found that podocytes appear to be larger and crushed in low-protein rats (Figure 1), suggesting that changes in renal functions favor excess hydroelectrolyte re-absorption by the kidney, and as such might potentiate the programming of adult hypertension. These morphological changes could be attributed to an adaptation to the reduced nephron number and, consequently, to glomerular hyperfiltration and overflow in low-protein offspring, and could account for the breakdown in optimal glomerular filtration barrier function. To confirm this hypothesis, ultrastructural analysis by electron microscopy is necessary.

In another study, whole kidneys from 12-day-old animals were prepared for Western blot and revealed that $AT1_R$ and $AT2_R$ are down-regulated in gestational low-protein-exposed animals (Figure 2) and that the same offspring present a low nephron number when compared with offspring of mothers that received normal diets during gestation (Table 1). Data demonstrating fewer glomeruli in these animals confirm reports from other groups that have obtained similar findings in different age groups and models (45,46).

Figure 3 presents an overview of our results regarding kidney offspring status during the fetal period, postnatal and adult life after maternal protein restriction during gestation, taking into account the RAS, nephron number and renal function. This Figure depicts results from different experiments, in which dams were fed a 6% casein diet and were compared with dams fed a 17% casein diet (normoproteic). It may be observed that, in spite of a reduced nephron number from the fetal period onwards, the adult low-protein offspring presented a similar glomerular filtration rate (GFR) to that observed in normal-protein rats, which may be explained by the larger filtration area and larger glomerular tuft volume in parallel to podocyte hypertrophy. Another factor that can contribute to the similar GFR is that $AT2_R$ is down-regulated in the efferent arteriole, which leads to vasoconstriction, thus increasing the interglomerular pressure.

Other groups obtained similar GFR results when they studied young rats (42,47), but when renal function was analyzed during old age, low-protein rats presented a significant decrease in GFR, which means that the age-related decline in GFR was manifested earlier in low-protein rats than in controls (47).

The hypothesis that the fetal kidney is programmed to inappropriately retain Na^+ in later life has been corroborated by different studies (48,49). Alwasel and Ashton (50), in a study of renal function and Na^+ transporters in 4-week-old male rats born to low-protein diet dams, found that GFR was unchanged, suggesting that single nephron GFR may be increased. In addition, these investigators reported that Na^+ transporter protein was unchanged, while the Na^+/K^+-ATPase-α1 subunit was absent in the kidney of low-protein rats. Interestingly, in a Western blot study, we found that, at the end of nephrogenesis, the ß1 subunit of the Na^+/K^+-ATPase protein was also down-regulated in male low-protein rats, but that at 16 weeks of age the protein was increased in these animals when compared with the normal group (Figure 4). This result corroborates the suggestion of do Carmo Pinho et al. (47) that the transcriptional up-regulation of Na^+ transport could give origin to hypertension in this

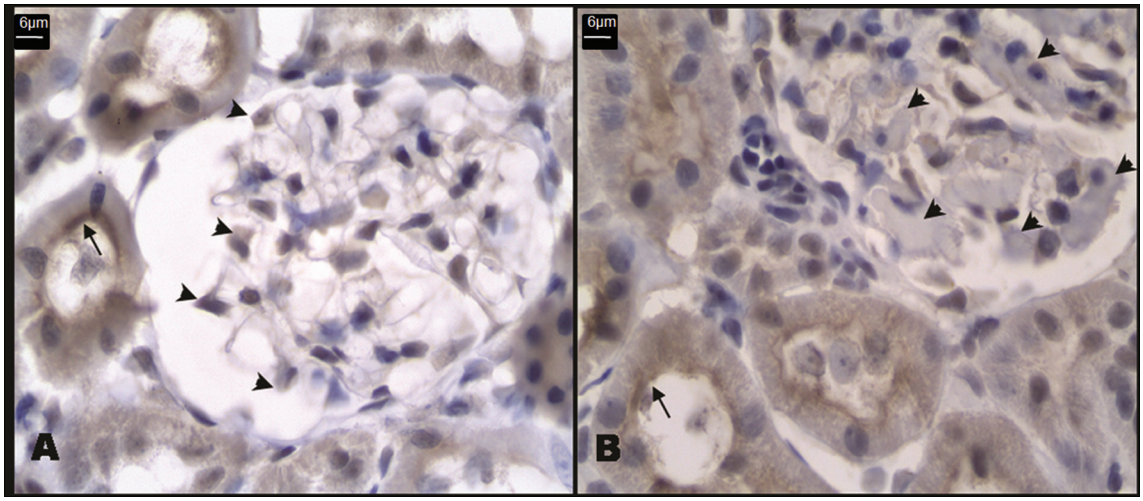

Figure 1. Effects of maternal protein restriction on podocyte morphology. *A*, Glomeruli from a rat born to a dam fed a normal-protein diet. *B*, Glomeruli from a rat born to a dam fed a low-protein (LP) diet. Immunoperoxidase assay was performed against $AT1_R$ antibody (brown - arrow). The arrowheads show podocytes in *A* and *B* but that appears to be enlarged in the kidney of an LP rat (B).

model. AngII $AT2_R$ is an effective inhibitor of Na^+/K^+-ATPase (51) and it is possible that the down-regulated RAS found in 16-week-old low-protein males (20) might lead to a lack of inhibition of Na+/K+-ATPase, explaining results obtained by Western blotting, and the low sodium excretion rate, since Na^+/K^+-ATPase participates in Na^+ reabsorption in the basolateral membrane (Figure 4). However, it is also possible that the high Na^+/K^+-ATPase expression does not indicate that all the excess protein is on the basolateral surface. Fekete et al. (52) demonstrated that AngII promotes Na^+/K^+-ATPase-α1 subunit translocation from the cytoskeletal fraction into the cytosol under experimental circumstances and we must take this into account.

Previous experiments demonstrated the involvement of AngII in the regulation of Na^+ transporters; however, the influence of fetal programming on the physiological function of these transporters is still unclear. Dagan et al. (34) demonstrated that fetal programming by DEX in rats increases proximal tubule transport in part by stimulating Na^+/H^+ exchanger activity and Tiwari et al. (53) recently showed that gender and age result in differential regulation of NaCl antiporter and ENaC in AngII-infused mice.

The RAS balance is disrupted in kidneys from offspring of low-protein-fed mothers during different ages, and AngII receptors are expressed in all segments of the nephron, contributing to the increased sodium re-absorption in proximal tubules and to the final sodium excretion rate (20).

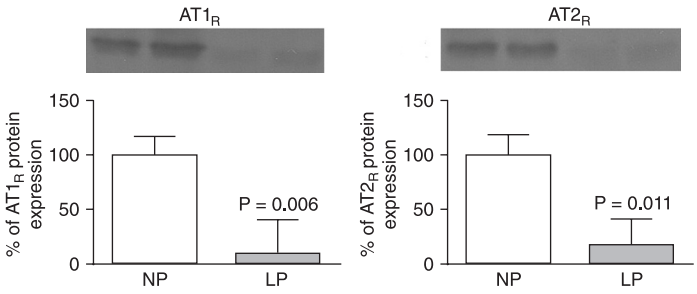

Figure 2. Effects of maternal protein restriction on expression of kidney renin-angiotensin system-associated proteins, at 12 days of age. The figure shows the results obtained in whole-tissue extracts that were immunoblotted for $AT1_R$ and $AT2_R$, as a percentage of protein content, in the NP and LP kidneys. Scanning densitometry results are reported relative to NP, with a value of 100% being assigned to the NP rats. Columns and bars represent the mean ± SEM. $AT1_R$ and $AT2_R$ = type 1 and type 2 angiotensin II receptors; NP = normal protein; LP = low protein. *P < 0.05, NP *vs* LP (Student *t*-test).

Table 1. Weight, number of glomeruli, cortical volume, and mean glomerular volume, at 12 days of age of rat pup kidneys born to mothers fed a 17% protein diet (normal diet, NP) and a 6% protein diet (low-protein diet, LP).

	Rat weight (g)	No. of glomeruli	Absolute cortical volume (mm^3)	Mean glomerular volume (μm^3)
NP	26.96 ± 0.78	9382 ± 1171*	256.29 ± 63.7	160.70 ± 16.7*
LP	25.80 ± 0.88	4980 ± 880	314.56 ± 26.07	414.71 ± 35

Data are reported as means ± SEM. *P ≤ 0.05 *vs* LP (Student *t*-test).

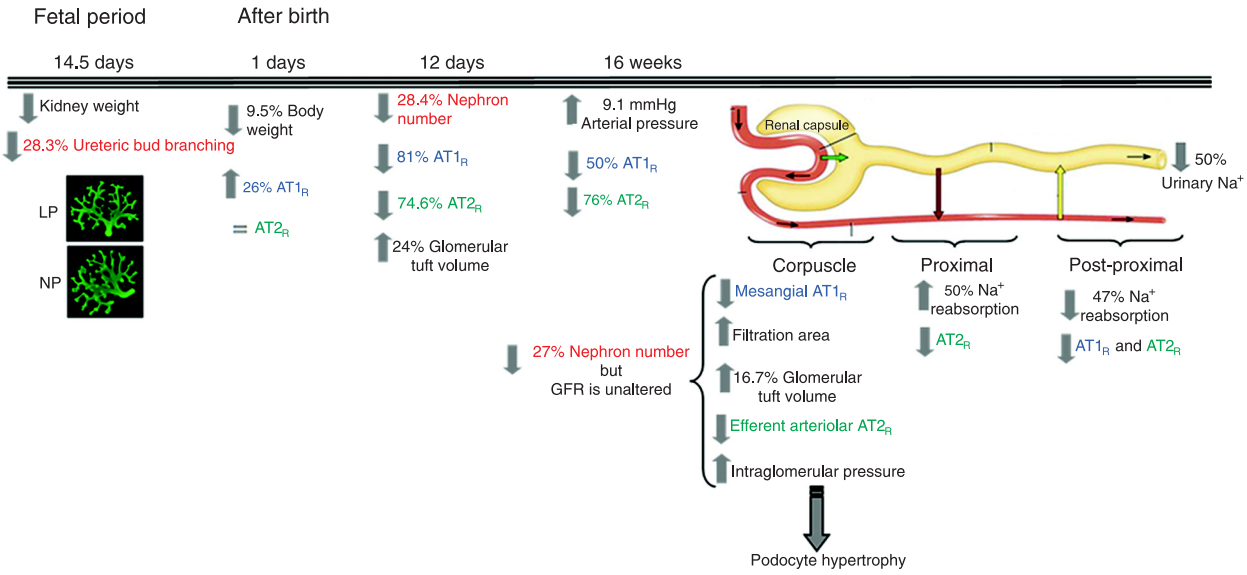

Figure 3. Schematic figure showing an overview of the interaction of the renin-angiotensin system (RAS) and the kidney from the fetal period to adult life. Ages are specified on the line. $AT1_R$ = type 1 angiotensin II receptor; $AT2_R$ = type 2 angiotensin II receptor; GFR = glomerular filtration rate; LP = low protein; NP = normal protein.

Our low urinary sodium excretion data may also be explained by previous findings showing that excess fetal glucocorticoid, such as observed in maternal undernutrition status programs, reduced the expression of placental, central nervous system and renal 11ß-HSD2 (see section above). As discussed above, the renal 11ß-HSD2 normally serves to prevent illicit access of glucocorticoids to the renal mineralocorticoid receptors, thereby maintaining mineralocorticoid receptors specificity for aldosterone, and, accordingly, humans with 11ß-HSD2 deficiency exhibit hypertension (54). A programmed reduction in renal 11ß-HSD2 would be expected to increase the ability of glucocorticoids to activate in many different tissues both glucocorticoid receptors and mineralocorticoid receptors, resulting in increased transcription of both, with a consequent increase in blood pressure. This increase in glucocorticoid sensitivity may be exacerbated by overactivation of the hypothalamic-pituitary-adrenal axis, as several previous studies have shown that prenatal glucocorticoids elevate adult plasma corticosterone (55), apparently driven by increased expression of hypothalamic corticotrophin-releasing hormone (56). Consistent with the proposal that increased renal glucocorticoid sensitivity underlies programmed hypertension, expression of the renal glucocorticoid-responsive genes, Na^+/K^+-ATPase-α1, or (as shown in the aforementioned and other studies) the Na^+/K^+-ATPase-ß1 subunit, ACE and renin were all elevated in 16-week-old offspring of low-protein mothers. These programmed changes would be expected to increase renal sodium retention and thereby elevate plasma volume and, thus, blood pressure. A similar mechanism seems to underlie hypertension in a rat maternal low-protein programming model in which adult offspring have higher renal expression of Na^+/K^+-ATPase-ß1 and/or also -α1 (50); however, the Na^+/K^+-ATPase expression were not confirmed by the present data in 16-week-old offspring (20).

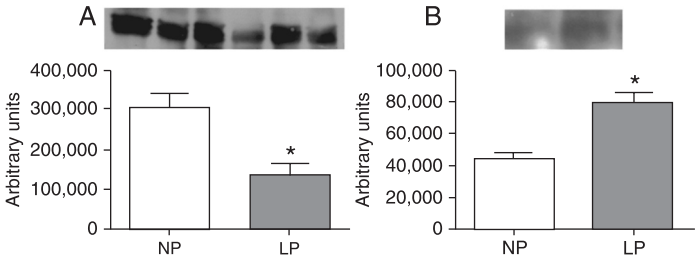

Figure 4. Effect of maternal protein restriction on kidney gene expression at 12 days of age (A) and 16 weeks of age (B). The figure shows the results obtained in whole-tissue extracts that were immunoblotted for Na^+/K^+-ATPase in the NP and LP kidneys. Columns and bars represent the mean ± SEM. NP = normal protein; LP = low protein. *P < 0.05, NP *vs* LP (Student *t*-test).

It is not possible to rule out other mechanisms involved in the inappropriate retention of sodium. The maternal protein restriction model for IUGR in rats resulted in the up-regulation of two critical Na^+ transporters. The Na^+/K^+-2Cl and the Na-Cl co-transporters, but not the Na^+/H^+ nor the Na^+ channel, were significantly increased in the rats with IUGR (48). These alterations led to a lower rate of urinary sodium excretion, associated with sodium retention and hypertension.

Apoptosis

Nephrogenesis is a complex process involving cell integration, cell growth and apoptosis. The rapid remodeling of structures requires massive apoptosis. Bcl-2 is an anti-apoptosis protein that attenuates the effect of cytochrome *c* release from the mitochondria and counters the effects of the pro-apoptosis protein, Bax. Bcl-2 and Bax contribute to the signaling pathways that activate caspase-3, an enzyme that is necessary for the condensation of chromatin and fragmentation of DNA that characterize apoptosis (57). Bax and Bcl-2 are known to be expressed during normal kidney development, and their expression is known to be deregulated under certain conditions associated with perturbed nephrogenesis and apoptosis (58).

Using real-time PCR in E13.0 metanephroi, Welham et al. (59) observed a step-wise increase in the expression of both Bax and Bcl-2 in the low-protein group. The increase was greater for the pro-apoptotic gene, Bax, versus the anti-apoptotic gene, Bcl-2, perhaps suggesting that a low-protein diet shifts the balance of expression to up-regulate the death of metanephric precursor cells. In a prior study (58), the same group showed that low-protein diets reduce the final numbers of glomeruli, in association with the increased deletion of precursors at the start of metanephric development, and present a significant increase in the numbers of apoptotic nuclei per unit area at E13, almost totally restricted to the renal mesenchyme.

In a model of uteroplacental insufficiency and the subsequent IUGR, a study observed increased renal p53 hypomethylation in association with increased p53 and Bax mRNA protein, as well as decreased Bcl-2 mRNA, which leads to increased caspase-3 activity (57). Alterations in DNA methylation and histone acetylation in IUGR rats (60) suggest a molecular mechanism by which a low-protein diet and IUGR induce fetal renal apoptosis, with a resulting permanent loss of glomeruli. It would be interesting to better understand how the maternal environment alters the methylation status and the transcriptional rate of genes such as Bcl-2 and Bax.

Our group's recent findings have shown that, in a small study, metanephroi extracted from undernourished rats and grown in culture for 48 h have fewer ureteric branches when compared with normal metanephric development in culture (Mesquita FF, Arena D, Ewen-McCullen L, Gontijo JA, Bertram J, Boer PA, Armitage J, unpublished data).

Perspectives and Conclusion

There is an increasing amount of evidence from human studies and experimental animal models demonstrating that perturbations of the intrauterine environment are one of the main causes of the morphological and physiological changes that occur in different organs, following undernutrition *in utero*. In this brief review, we have concentrated on one organ, the kidney, and shown that adult hypertension, programmed by maternal exposure to a low-protein diet, is linked to marked changes in the renal expression of the glucocorticoid receptor, 11ß-HSD2, and components of the RAS. The function of the kidney depends on the hormonal cascade, physiological mechanisms and morphological patterns that work in synchrony; as such, any alterations in this balance can cause the development of disease during adult life. We also show that renal development, particularly nephron number, can be influenced by a number of environmental factors, and especially by exposure to a low-protein diet during critical periods, which are surprisingly early in fetal life. It is suggested, but remains to be proven, that whenever nephron number is suboptimal, there are maladaptive adjustments to gene expression and long-term kidney function that may lead to the development of cardiovascular disease. It is unlikely that renal malfunction alone is responsible for the development of hypertension. We suggest that central (brain) regulation of blood pressure may also be altered. This is an important area for future investigation.

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