Fetal kidney programming by severe food restriction: Effects on structure, hormonal receptor expression and urinary sodium excretion in rats

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Abstract

Introduction: The present study investigates, in 23-day-old and adult male rats, the effect of severe food restriction in utero on blood pressure (BP), and its association with nephron structure and function changes, angiotensin II (AT1R/AT2R), glucocorticoid (GR) and mineralocorticoid (MR) receptor expression.

Materials and methods: The daily food supply to pregnant rats was measured and one group (*n*=15) received normal quantity of food (NF) while the other received 50% of that (FR50%) (*n*=15). Kidneys were processed to AT1R, AT2R, MR, and GR immunolocalization and for western blotting analysis. The renal function was estimated by creatinine and lithium clearances in 12-week-old offspring.

Results: By stereological analyses, FR50% offspring present a reduction of nephron numbers (35%) with unchanged renal volume. Expression of AT1R and AT2R was significantly decreased in FR50% while the expression of GR and MR increased in FR50%. We also verified a pronounced decrease in urinary sodium excretion accompanied by increased BP in 12-week-old FR50% offspring.

Conclusion: The current data suggest that changes in renal function are conducive to excess sodium tubule reabsorption, and this might potentiate the programming of adult hypertension. It is plausible to arise in the current study an association between decreasing natriuresis, reciprocal changes in renal AngII and steroid receptors with the hypertension development found in FR50% compared with age-matched NF offspring.

Keywords

Fetal programming, arterial hypertension, severe food restriction, renal function

Introduction

Fetal programming by maternal malnutrition results in low birth weight and reduction in nephron number[1–3] increasing the risk for adult development of cardiovascular and renal diseases.[4,5] Vikse et al.[6] showed that in humans the low birth weight increased in 70% the risk for end-stage kidney failure in adults. Experiments have been demonstrated that renal programming with nephron number reduction may occur even when birth weight is normal.[7,8] It is important that the consequences of fetal programming are not limited to the first generation and their effects may be prolonged to subsequent generations.[9] The nephron number reduction and hypertension provoked by gestational protein restriction may pass throughout for F1 and F2 generations in both male and female programmed rats.[10] The renal programming has been studied in an extended number of animal models and recently we showed that rats submitted to gestational low protein diet presented 8% lower birth weight and hypertension from the 10th week of life in parallel with

30% reduced nephron number.[11,12] Also, studies in 16-week-old offspring of maternal low-protein rats have shown that renal sodium excretion was 50% lower and angiotensin II receptors were strikingly reduced.[11,12] Different animal models and gestational diet regimes or insults may result in distinct programming effects and also different compensative challenges to maintaining homeostasis from birth to adult life. The renin–angiotensin system (RAS) has been

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implicated in the major renal programming studies.[13] The more discussed mechanism involved in fetal programming is the reduction in expression and/or activity of placental 11βHSD-2 exposing fetus to high concentration of maternal glucocorticoids.[14,15] The action of maternal corticosteroids in mineralo- (MR) and glucocorticoid (GR) receptors may have an impact on renal development.[16,17] After birth, the day-by-day adaptations of programmed rats to keep water and salt homeostasis may involve changes in expression/localization of these receptors. Recently, Rosario et al.[18] have speculated that transplacental down-regulation of nutrients flow; in particular, amino acid transporters may be also evolved in fetal programming by gestational protein restriction. The purpose of the present study was to determine whether maternal undernutrition during pregnancy alters the kidney expression of angiotensin II (AT1R and AT2R), MRs and GRs in 23-day-old and 12-week-old male rats. Since we have verified in a protein restriction model that the long-term changes in renal sodium tubule handling are associated with arterial hypertension development, we hypothesized that undernutrition hypertension may result from decreased urinary sodium excretion in the offspring. Thus, to comparatively analyze these two models, we studied the tubular sodium handling, evaluated by lithium clearance, in conscious maternal undernourished rats compared with their appropriate normal maternal food intake controls.

Material and methods

Animals

The experiments were conducted on age-matched rats of sibling-mated Wistar Hannover rats (0.250–0.300 kg) and allowed free access to water and normal rat chow. The general guidelines established by the Brazilian College of Animal Experimentation (COBEA) were followed throughout the investigation. Our local colonies originated from a breeding stock supplied by CEMIB/Unicamp, Campinas, SP, Brazil. Immediately after weaning at 3 weeks of age, animals were maintained under controlled temperatures (25°C) and lighting conditions (7:00–19:00), with free access to tap water and standard rodent laboratory chow (Nuvital, Curitiba, PR, Brazil) and followed up to 12 weeks of age. Animals were then mated and the day that sperm were seen in the vaginal smear was designated as day 1 of pregnancy. The dams were divided into two groups: throughout the entire pregnancy, the daily food supply of one group (FR50%) was restricted to 50% of the food consumed by the other group (NF), and fed ad libitum. All groups returned to the ad libitum standard rodent chow intake after delivery. Body weight and food consumption were determined every day (subsequently normalized for body weight). The male pups from 15 FR50% and 15 NF different litters were followed and maintained with normal chow ad libitum until 23 days old and adulthood.

Blood pressure measurement

The systemic arterial pressure was measured in conscious 6-, 8-, 9-, 10-, 11- and 12-week-old rats by an indirect tail-cuff method using an electrosphygmomanometer (Narco Bio-Systems, Austin, TX) combined with a pneumatic pulse transducer/amplifier. This indirect approach allowed repeated measurements with a close correlation (correlation coefficient = 0.975) compared with direct intra-arterial recording. The mean of three consecutive readings represented the blood pressure.

Morphometric measurements and stereological estimation of kidney volume, glomerular number and glomerular volume

At postnatal *Day 23*, rats were anesthetized with a mixture of ketamine (75 mg.kg^{-1} body weight, i.p.) and xylasine (10 mg.kg^{-1} body weight, i.p.) and the kidneys were removed and weighed. Left kidneys were weighted and the volume was estimated by Cavalieri's principle. Then, they were longitudinally divided into two halves and 4 mm slices were weighted. The slices were placed in 10% formalin for stereological estimation of total nephron number. Total body weight and the weights of kidneys were determined and NF (n=5) and FR50% (n=7) obtained from different litters. Tissue blocks were exhaustively sectioned at 5 μm and then stained with hematoxilin-eosin. We used the "fractionators" method to estimate the number of glomeruli and estimated the total number of renal corpuscles per kidney considering the analyzed fraction of the kidney corrected to the entire organ as described by Mandarim-de-Lacerda.[19]

Renal function tests

The renal function tests were performed on the last day at 12 weeks of age in unanesthetized, unrestrained NF (n = 15) and RF50% (n = 11) male rats. Creatinine and lithium clearance were performed as standard methodology.[12,20]

Calculations and biochemical analysis

Plasma and urinary sodium, potassium, and lithium concentrations were measured by flame photometry (B262; Micronal, São Paulo, Brazil). Creatinine was determined spectrophotometrically (362; Micronal, São Paulo, Brazil) by the alkaline picrate method. The results are reported as means ± SD per 100 g body weight. Renal clearance was calculated by a standard formula ($C = UV/P$) using the plasma creatinine and lithium levels for each period. C_{Cr} was used to estimate the glomerular filtration rate and C_{Li+} was used to assess proximal tubule output. FE_{Na+} and FE_{K+} were calculated as C_{Na+}/C_{Cr} and C_{K+}/C_{Cr}, respectively. FEP_{Na+} (fractional proximal sodium excretion) and $FEPP_{Na+}$ (fractional post-proximal sodium excretion) were calculated as $C_{Li+}/C_{Cr} \times 100$ and $C_{Na+}/C_{Li+} \times 100$, respectively.[11,20,21]

Tissue extracts

Twenty-three-day-old and 12-week-old male rats from the NF (*n*=5) and FR50% (*n*=5) groups were used. The rats were anesthetized with a mixture of ketamine (75 mg.kg^{-1} body weight, i.p.) and xylasine (10 mg.kg^{-1} body weight, i.p.) and the animal's abdominal cavity was opened to kidney removal. The tissue was minced coarsely and homogenized immediately in 10 volumes of solubilization buffer (10 mL/L Triton-X 100, 100 mM Tris[hydroxymethyl] amino-methane (Tris) pH 7.4, 10 mM sodium pyrophosphate, 100 mM sodium fluoride, 10 mM ethylenediaminetetraacetic acid (EDTA), 10 mM sodium vanadate, 2 mM phenylmethylsulfonyl fluoride (PSMF) and 0.1 mg/mL aprotinin at 4ºC, using a polytron PTA 20S generator (model PT 10/35, Brinkmann Instruments, Westbury, NY, USA) operated at maximum speed for 20 s. The tissue extracts were centrifuged at 11.000 rpm at 4°C for 40 min, and the supernatants used as samples.

Tissue processing, histology, and immunohistochemical procedures

Twenty-three-day-old and 12-week-old male rats from the NF (*n*=5) and RF50% (*n*=5) groups were anesthetized with a mixture of ketamine (75 mg.kg^{-1} body weight, i.p.) and xylasine (10 mg.kg^{-1} body weight, i.p.) and the level of anesthesia was controlled by monitoring the corneal reflex. The animals were then perfused with saline containing heparin (5%) for 15 min under constant pressure, followed by perfusion with 0.1 M phosphate buffer (pH 7.4) containing 4% (w/v) paraformaldehyde and 0.1 M sucrose for 25 min. After perfusion, kidneys were removed, weighted, and representative samples were fixed in 4% phosphate-buffered formalin during 24 h for paraffin embedding. For immunohistochemical analysis we used anti-AT1R, AT2R, MCR, and GR antibodies (Santa Cruz Biotech, Inc., CA, USA). Antigen retrieval was performed using 0.01 M citrate buffer (pH 6.0) boiling in a microwave oven (1,300 W) twice for 5 min each. Proteins were immunohistochemically detected using the avidin–biotin–peroxidase method. Briefly, deparaffinized 5-µm-thick heart sections on poly-l-lysine coated slides were treated with 3% H$_2$O$_2$ in phosphate-buffered saline for 15 min, non-fat milk for 60 min, primary antibodies for 60 min, and avidin–biotin–peroxidase solution (Vector Laboratories Inc, CA, USA, 1:1:50 dilution). Chromogenic color was accomplished with 3,3′-diaminobenzidine tetrahydrochloride (DAB, Sigma–Aldrich Co., St. Louis, MO, USA) as the substrate to demonstrate the sites of peroxidase binding. The slides were counterstained with Harris's hematoxylin. Five cortical and five medullar fields of each histological section (*n*=3 for each group) were analyzed and the average of the readings determined the immunoreactivity. Images were captured with photomicroscope and analyzed by the Leica Qwin 3.1 for windows.

Antibodies and chemicals

Protein quantification was performed using the Bradford method. For quantification, both tissue and total extract samples (250 µg protein) were subjected to SDS-PAGE. After electrophoretic separation, proteins were transferred to nitrocellulose membranes and then blotted with specific antibody. The samples were treated with Laemmli buffer containing 100 mM dithiothreitol (DTT), heated in a boiling water bath for 4 min and subjected to 8% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) in a Bio-Rad minigel apparatus (Mini-Protean, Bio-Rad, Hercules, CA, USA). Electrotransfer of proteins from the gel to the nitrocellulose membranes was performed for 90 min at 120 V (constant) in a Bio-Rad miniature transfer apparatus (Mini-Protean). The non-specific protein binding to the nitrocellulose was reduced by preincubating the filter for 2 h at 22°C in blocking buffer (5% non-fat dry milk, 10 mM Tris, 150 mM NaCl, and 0.02% Tween 20). The nitrocellulose blots were incubated at 4°C overnight with primary antibodies diluted in blocking buffer (3% non-fat dry milk, 10 mM Tris, 150 mM NaCl, and 0.02% Tween 20).
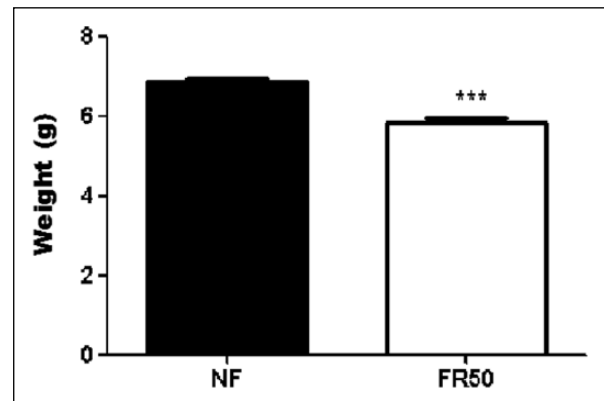

Figure 1. The birth weight in the FR50% group is reduced when compared with NF. NF: normal quantity of food; FR50%: 50% of normal quantity of food received.
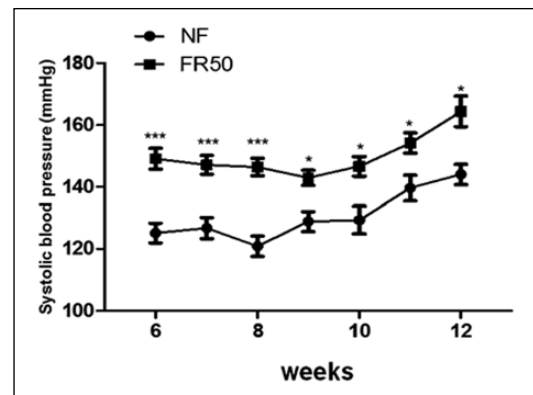

Figure 2. Systolic blood pressure is significantly enhanced from sixth to 12th week of age in FR50% comparatively to NF. NF: normal quantity of food; FR50%: 50% of normal quantity of food received.

Immunoreactive bands were detected using the enhanced chemiluminescence method (RPN 2108 ECL western blotting analysis system; Amersham Biosciences) and were detected by autoradiography using preflashed Kodak XAR film (Eastman Kodak, Rochester, NY, USA) with Cronex Lightning Plus intensifying screen (DuPont, Wilmington, DE, USA) for 10 min. Images of the developed radiographs were scanned (Epson Stylus 3500) and band intensities were quantified by optical densitometry (*Scion Image Corporation*).

Statistical analysis

All data were reported as means ± EPM. Comparisons involving only two means within or between groups were performed using Student's *t*-test. A *p*-value < 0.05 was considered to indicate significance.

Results

FR50% male offspring presented significant reduction in birth body weight (5.67 ± 0.16 g *vs*. 6.84 ± 0.13 g in NF, *p*<0.001) (Figure 1). Systolic blood pressure increased (Figure 2) from sixth to 12th week (sixth, 149.1 ± 3.4 mmHg *vs*. 125.1 ± 3.2 mmHg in NF, *p*<0.001; 12th, 164.4 ± 4.9 mmHg *vs*. 144 ± 3.3 mmHg in NF, *p*=0.02).

By stereological analyses we verified that FR50% offspring present a significant reduction of nephron numbers per kidney (about 35% less: 22 450±1497 units *vs*. 34 630±3560 units in NF, *p*=0.0055, Figure 3) with unchanged cortical (461 000±24 240 *vs*. 452 000±51 630 in NF, *p*=0.88) and glomerular volume (1760±0.22 μL *vs*. 1038±0.14 μL in NF, *p*=0.03) when compared with NF group.

Renal function data

The data for renal function in the 12-week-old male offspring of both (NF and FR50%) groups are summarized in Figure 4. The urinary flow rates and the glomerular filtration rates, estimated by C_{Cr}, did not significantly differ among the groups during the renal tubule sodium handling studies (147±40 μL/min/100 g b.w. *vs*. 136±27 μL/min/100 g b.w. in NF, *p*=0.39). Fractional urinary sodium excretion ($FE_{Na}{}^+$) was significantly lower in FR50% rats when compared with the NF age-matched group, as follows: 0.2±0.07% *vs*. 0.6±0.4% in NF, *p*=0.03). The decreased $FE_{Na}{}^+$ in RF50% rats was accompanied by unchanged proximal sodium excretion ($FEP_{Na}{}^+$: 34±11% *vs*. 44±23% in NF) and fractional potassium excretion ($FE_K{}^+$) compared with the NF age-paired control group. This decreased FE_{Na+} occurred in parallel with significant reduction in $FEPP_{Na}{}^+$ (FR50%: 7±4% *vs*. 17±11% in NF; Figure 4).
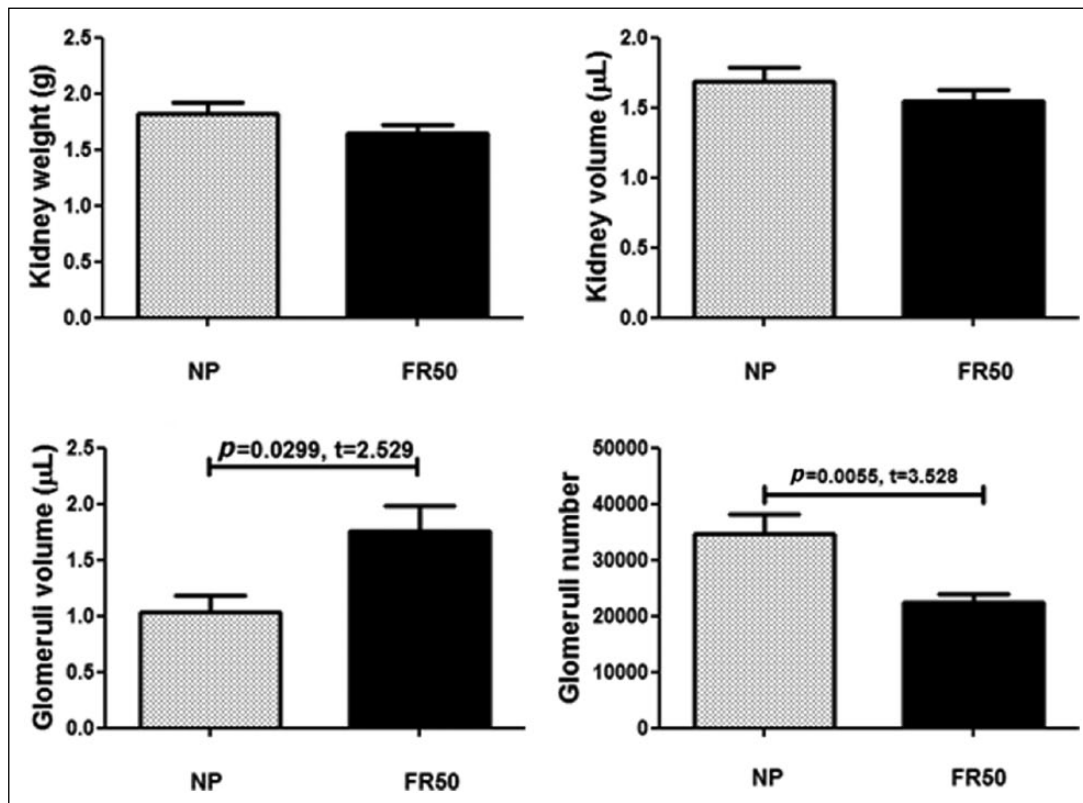

Figure 3. FR50% offspring present a significant reduction of nephron numbers per kidney when compared with NF. NF: normal quantity of food; FR50%: 50% of normal quantity of food received.
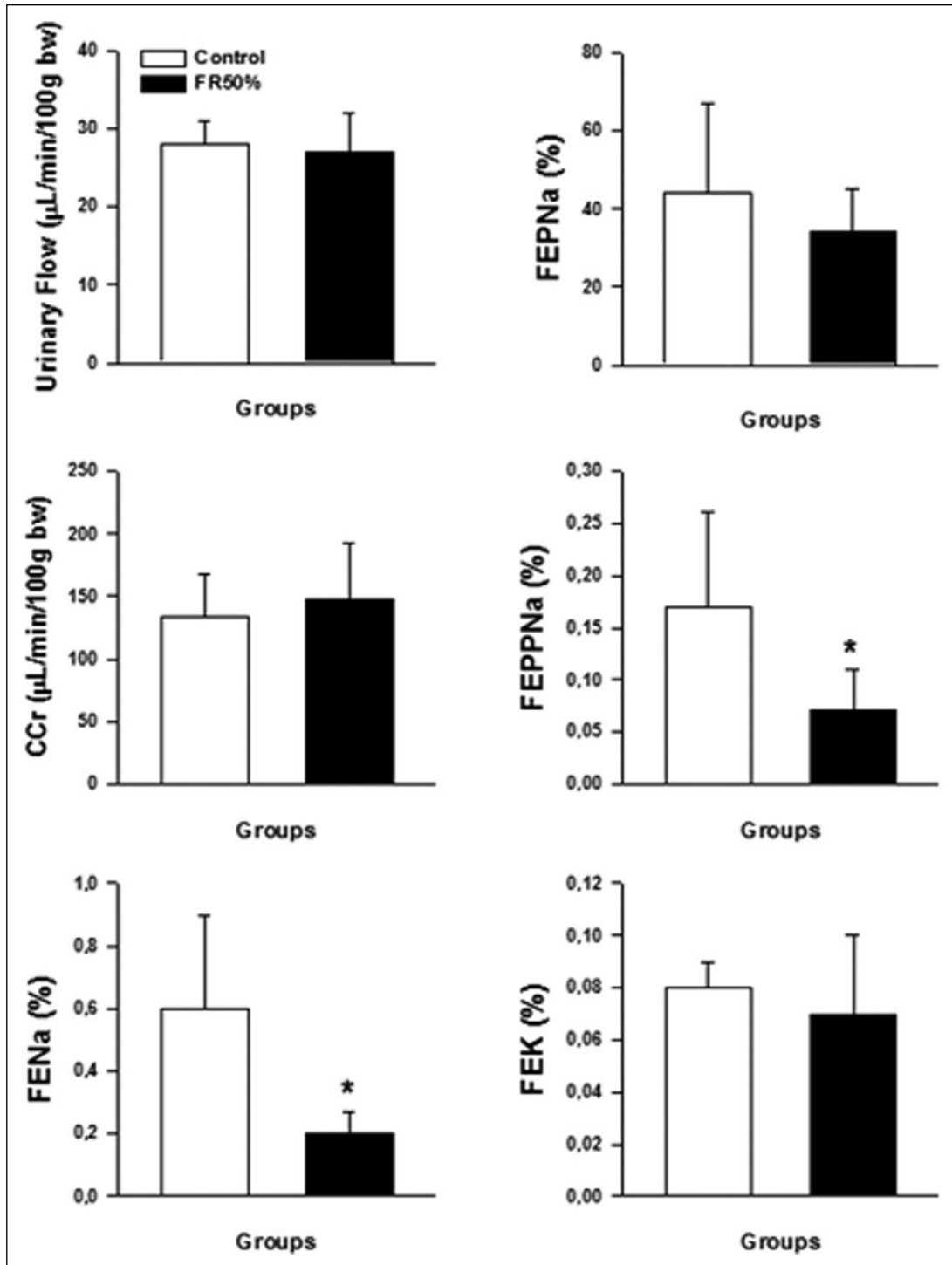
Figure 4. Renal function. Creatinine clearance (C_{Cr}), fractional sodium excretion (FE_{Na+}), proximal (FEP_{Na+}) and post-proximal ($FEPP_{Na+}$) fractional sodium excretion and fractional potassium excretion (FE_{K+}) in NF and 12-week-old FR50% offspring. The data are reported as the means ± SEM. *$p \leq 0.05$ versus control (Student's t-test). NF: normal quantity of food; FR50%: 50% of normal quantity of food received.

Western blot analysis

Western blot analysis in male offspring of NF and RF50% rat kidney yielded a single band at the expected weight of corresponding proteins. The MR expression in 23-day-old and 12-week-old male rats was not statistically different between NF and FR50% (23 days: 873.3 ± 22 vs. 642.6 ± 51.6 in NF; 12-week-old: 1151± 42 vs. 987.7 ± 70.2 in NF).
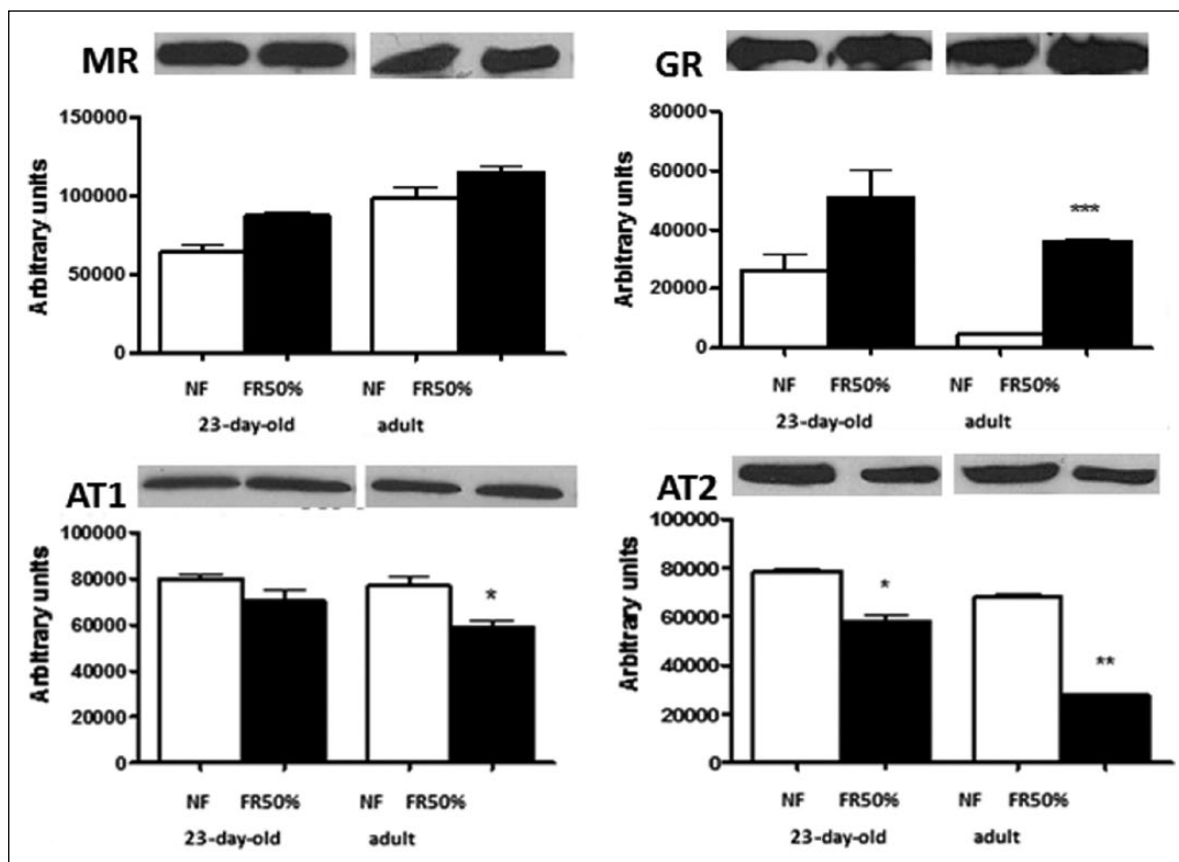
Figure 5. Western blotting of kidneys from NF and FR50% rats of 23 days and 12 weeks of age. The MR expression was not significantly altered. GR expression was enhanced but only in adult rats this difference was significant. The AT1R-reduced expression was only significant in adult rats and AT2R reduction was significant in both ages studied. NF: normal quantity of food; FR50%: 50% of normal quantity of food received; GR: glucocorticoid receptor; MR: mineralocorticoid receptor.

The expression of GR increased in FR50% but this rise was significant only in 12-week-old rats (23 days: 507 ± 91.02 *vs.* 262.6 ± 56.16 in NF, $p=0.15$; 12-week-old: 444.6 ± 36.45 *vs.* 360 ± 78.15 in NF, $p=0.0007$), shown in Figure 5. The expression of AT1R was reduced in FR50% being significant only in 12-week-old rats (23-day-old: 702.1 ± 46.01 *vs.* 799.3 ± 18.44 in NF, $p=0.19$; 12-week-old: 590.8 ± 27.09 *vs.* 770 ± 35.91 in NF, $p=0.05$) (Figure 5). AT2R expression was significantly decreased in 23-day-old and 12-week-old rats from the FR50% group (23 days: 580 ± 23 *vs.* 786 ± 91 in NF, $p=0.01$; 12-week-old: 275 ± 95.5 *vs.* 678.7 ± 15 in NF, $p=0.001$) (Figure 5).

Immunohistochemical analysis

Although by western blot we have not found significant differences in the expression of MR in 23-day-old rats, by immunohistochemistry we verified significantly increased immunoreactivity of this receptor in the basolateral membrane of the cells of the proximal convoluted tubules in animals of the FR50% group (Figure 6). In 12-week-old male rats we found increased cytosolic expression in glomerular cells and in the parietal epithelium of Bowman's capsule

(Figure 7(a) and (e)). This increase was also observed in the nuclei and citosol of post-proximal segments of both cortex (Figure 7(b) and (f)) and medulla (Figure 7(d) and (h)). In the proximal segments the immunoreactivity was enhanced in the brush border (Figure 7(c) and (g)). For GR expression we observed a significant difference in the immunoreactivity that is raised in the basolateral membrane of all tubular segments, being more significant in post-proximal segments in 23-day-old rats (Figure 8). In 12-week-old rats only medullar significant enhance was observed (Figure 9).

We verified a significant reduction in AT1R expression in 23-day-old rat kidneys of FR50% by immunohistochemistry. This reduction occurred in the apical surface of tubules, in mesangium and macula dense cells (Figure 10). In accordance with immunoblot results, the AT2R reactivity was also reduced in the FR50% group of 23-day-old rats (Figure 11).

Discussion

Early-life events during rat kidney development may lead to a long-term disorder in renal sodium and water handling
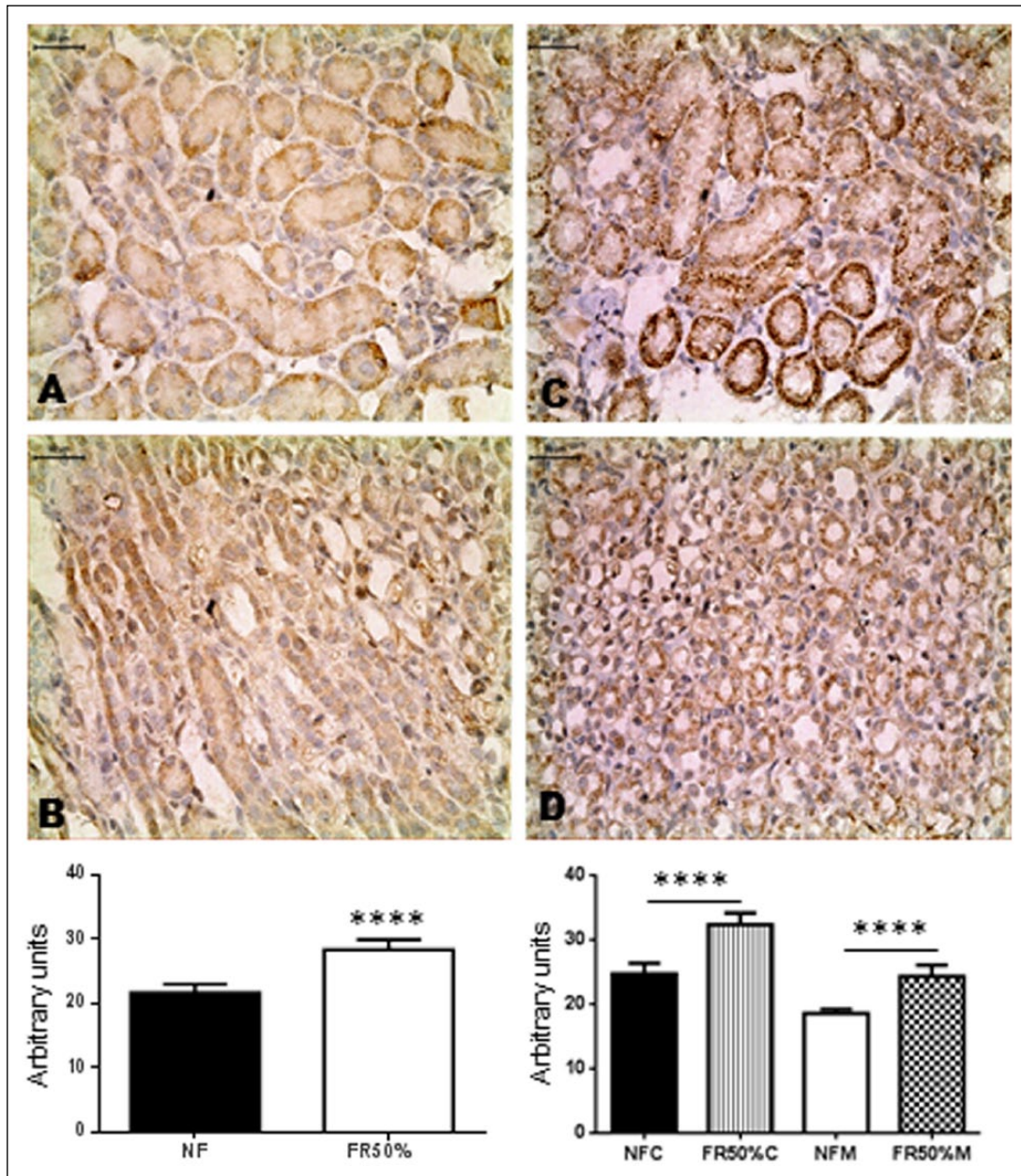
Figure 6. MR kidney expression by immunohistochemistry in NF (a and b) and FR50% (c and d) groups in 23-day-old rats. Arrows indicate the basolateral cell membrane. The graph on the left represents a total kidney staining quantification in NF and FR50% offspring and the graph on the right is cortical (NFC and FR50%C) and medullar (NFM and FR50%M) quantification. ****$p<0.0001$. MR: mineralocorticoid receptor; NF: normal quantity of food; FR50%: 50% of normal quantity of food received.

that seems to be associated with arterial hypertension development at adult age.[11,12] Here, in a severe maternal food-restricted model, we focus on adult hypertension as an outcome, and its association with nephron numbers, kidney dysfunction, and RAS and steroid receptor renal expression. The result obtained in the present study suggests that the kidney is an organ in which there are permanent functional and humoral changes that underlie the developing hypertension. The low birth weight widely observed in fetal programming models induced by maternal malnutrition was confirmed in the current study, where we found a decreased offspring birth weight of 17% in maternal severe food intake restriction. We previously demonstrated that in a gestational low protein (LP) diet model, the birth weight reduced by nearly 8.5%; however, here this reduction was much more accentuated. We also found in FR50% animals, a graded and significant increase in blood pressure from the sixth week of life while, in LP offspring, the enhanced blood pressure was observed beyond the 12th week of age.[11,12]
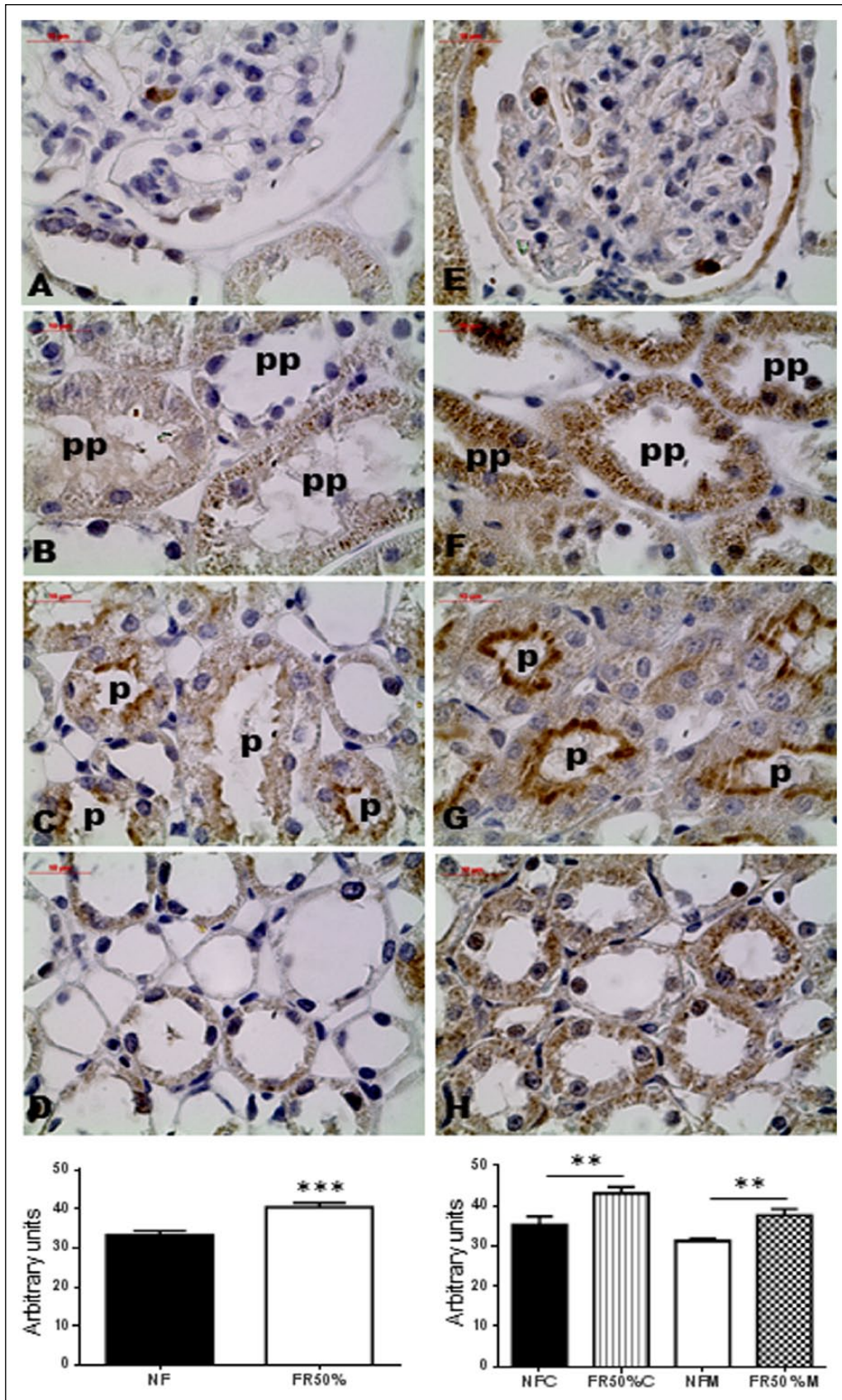
Figure 7. MR kidney expression by immunohistochemistry in NF (a, b, c, and d) and FR50% (e, f, g, and h) groups in adult rats. The graph on the left represents a total kidney staining quantification in NF and FR50% offspring and the graph on the right is cortical (NFC and FR50%C) and medullar (NFM and FR50%M) quantification. $^{***}p=0.0001$, $^{**}p<0.005$. Pp: post-proximal segment; p: proximal segment; MR: mineralocorticoid receptor; NF: normal quantity of food; FR50%: 50% of normal quantity of food received.
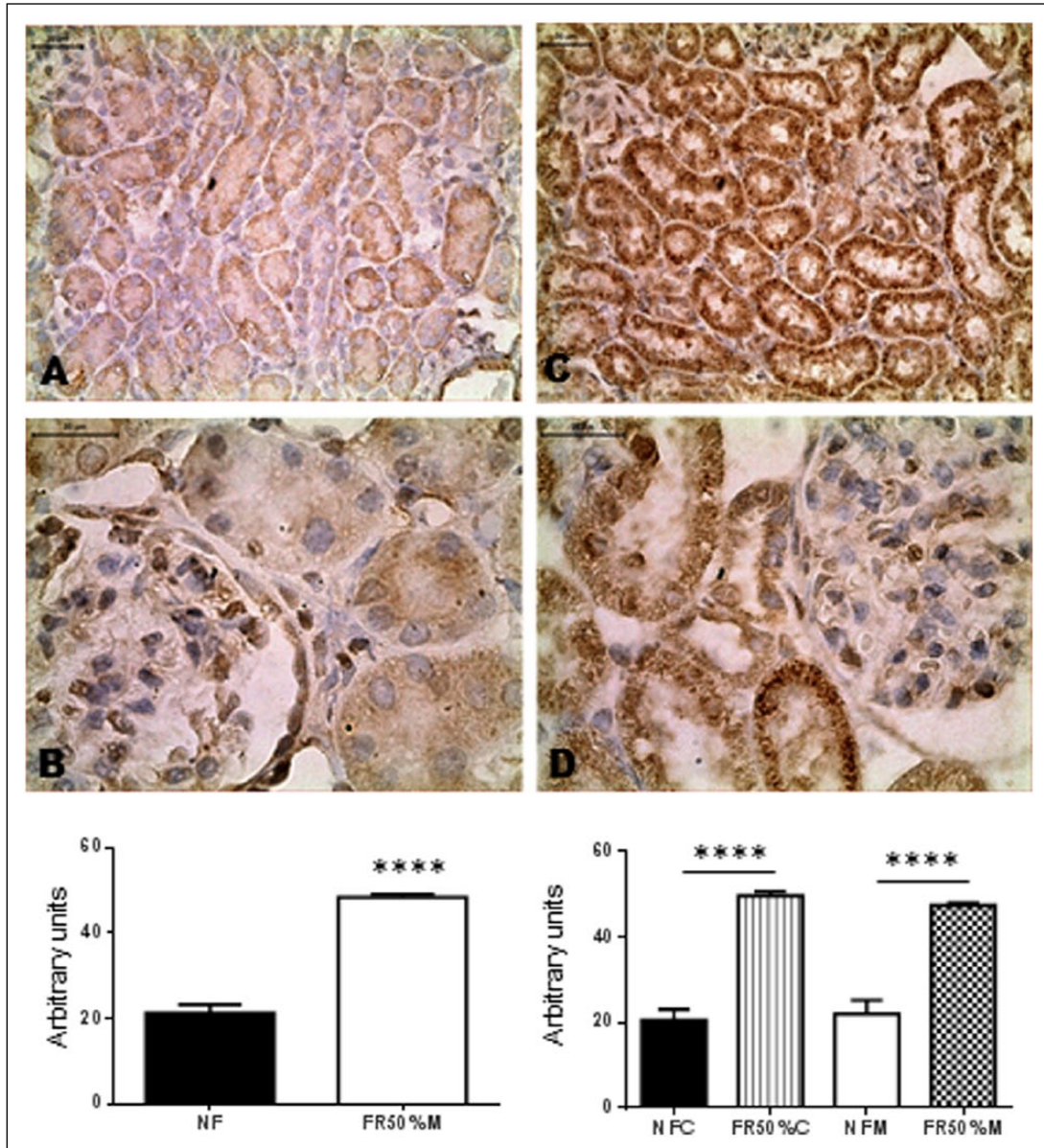
Figure 8. GR kidney expression by immunohistochemistry in NF (a and b) and FR50% (c and d) groups in 23-day-old rats. The graph on the left represents a total kidney staining quantification in NF and FR50% offspring and the graph on the right is cortical (NFC and FR50%C) and medullar (NFM and FR50%M) quantification. ****$p < 0.0001$. GR: glomerular receptor; NF: normal quantity of food; FR50%: 50% of normal quantity of food received.

The current study also has demonstrated a reduction of 35% in the nephron number, against a 27% reduction in the LP model.[12,22] There is evidence that insult such as maternal food restriction, alters the total number of nephrons and also causes late-onset hypertension.[23–25] In the current study, despite a pronounced reduction of nephron number (35%) we did not observe any significant difference between FR50% and NF glomerular volume and glomerular filtration rate, estimated by CCr. Additionally, 12-week-old male offspring from 50% food-restricted mothers presented a pronounced decrease in fractional urinary sodium excretion accompanied by intensive sodium

reabsorption in distal segments of the nephron in spite of unchanged CCr and FEPNa+. In this study, the reduced number of nephrons is not responsible, *per se*, for the elevated time-dependent blood pressure response in FR50% offspring, since we did not observe any difference between FR50% and NP creatinine clearance. This is consistent with the fact that permanent and severe reduction of nephron units does not cause enhanced arterial blood pressure in the adult rats.[26] Thus, it is plausible to hypothesize that increased kidney sodium transport in FR50% offspring occurs by maladaptive renal tubule development during the ontogenetic period. We may speculate that intrarenal
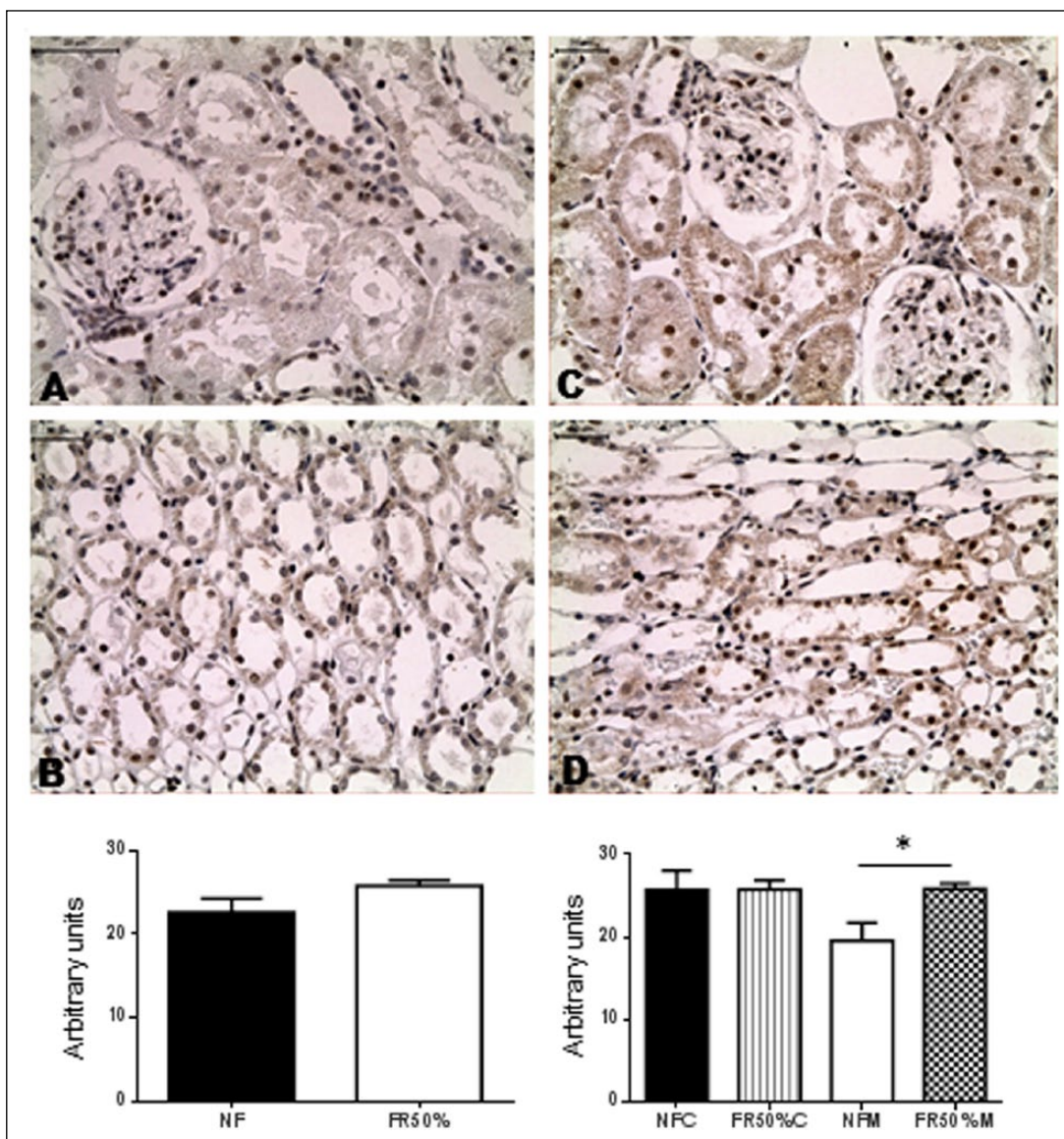
Figure 9. GR kidney expression by immunohistochemistry in NF (a and b) and FR50% (c and d) groups in adult rats. The graph on the left represents a total kidney staining quantification in NF and FR50% offspring and the graph on the right is cortical (NFC and FR50%C) and medullar (NFM and FR50%M) quantification. *p*=0.01. GR: glomerular receptor; NF: normal quantity of food; FR50%: 50% of normal quantity of food received.

interactive actions of AngII contribute to increased tubular sodium reabsorption, including constriction of postglomerular arterioles, which alter peritubular capillary dynamics and renal medullary blood flow, combined with direct action of AngII on tubular epithelial cell transport.

Surprisingly, in the current study, the expression of AngII receptors (meanly AT2) in both 23-day and 12-week-old maternal food-restricted rats, compared with NF offspring was dramatically down-regulated, suggesting other mechanisms of antinatriuresis in these animals. In the present study, previously observed for us in the LP model study,[11,12] was found a higher AngII AT1/AT2 receptor ratio expression in FR50% offspring when compared with

NF kidneys. Since both AT1 and AT2 receptors are expressed in arteriolar and glomerular cells,[27] these may modulate the renal blood flow and glomerular filtration rate[27,28] increasing the fractional filtration rate, at least partially responsible for enhanced sodium reabsorption in proximal segments of the nephron in FR50% rats. Despite decreased nephron numbers, the unchanged glomerular filtration rate in FR50% offspring is caused, at least in part by higher glomerular AT1/AT2 receptor expression ratio (plus 110% in 16-week-old FR50% rats compared with unchanged AT1/AT2 ratio in NF), presumably decreasing the vasodilatation response in postglomerular arterioles. These data support the notion that AngII predominantly
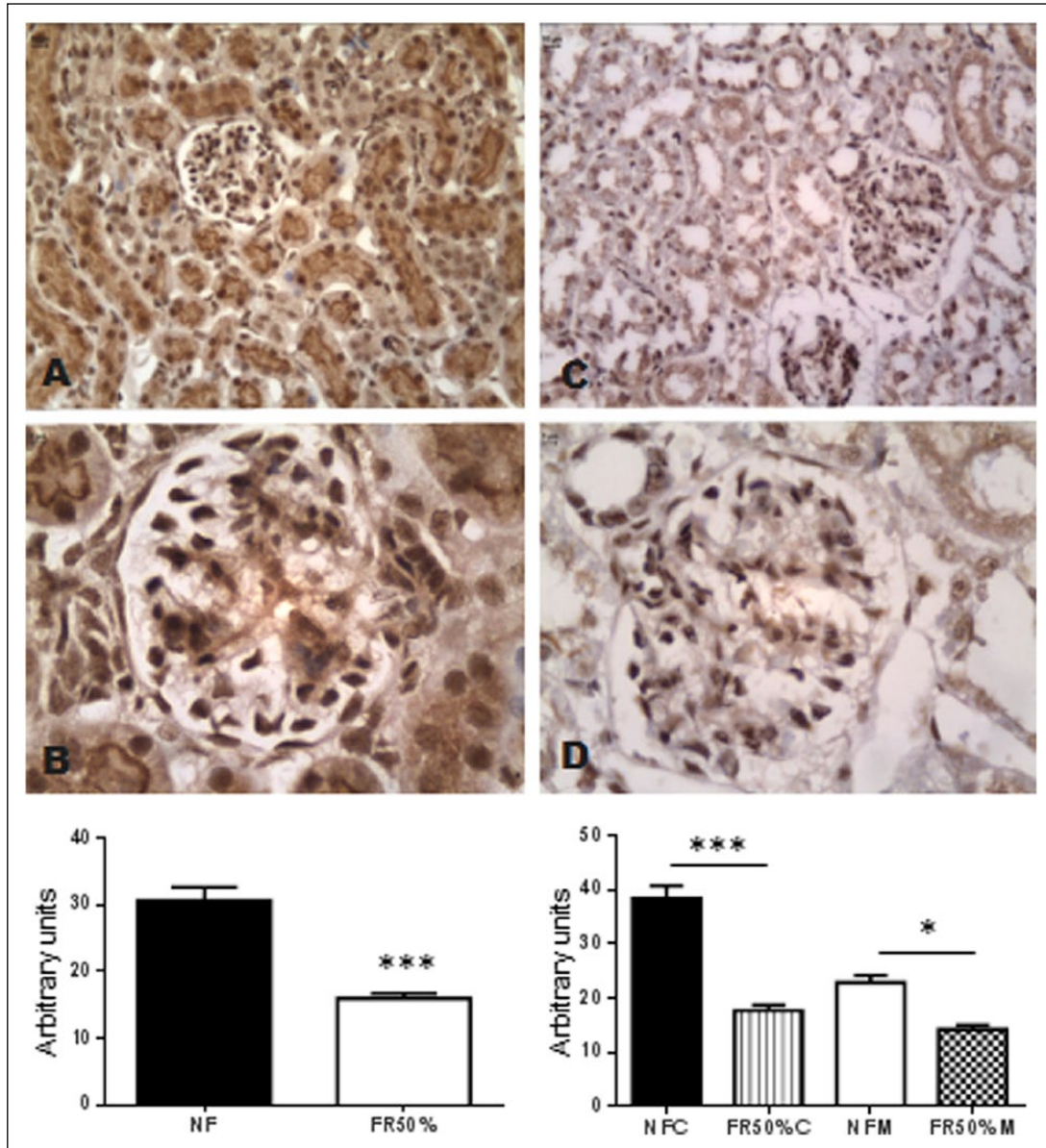
Figure 10. AT1R kidney expression by immunohistochemistry in NF (a and b) and FR50% (c and d) groups in 23-day-old rats. The graph on the left represents a total kidney staining quantification in NF and FR50% offspring and the graph on the right is cortical (NFC and FR50%C) and medullar (NFM and FR50%M) quantification. ****$p<0.0001$, ***$p<0.001$ and *$p=0.01$. NF: normal quantity of food; FR50%: 50% of normal quantity of food received.

constricts the postglomerular arterioles.[29,30] By the current data, we cannot also rule out any specific changes of hydraulic parameters involved in the glomerular ultrafiltration coefficient. In fact, it is also possible that indirect physical mechanisms associated with overexcitability of the sympathetic nervous system induced by the renin–angiotensin system could underlie the decrease in renal sodium excretion and, consequently, enhance arterial pressure in FR50% offspring.

The involvement and local expression of all components of the renin–angiotensin system during nephrogenesis have been remarkably demonstrated by several studies.[1,11,12,31]

The perinatal treatment of rats with high doses of the AT1R antagonist caused, in adult life, a striking reduction in the nephron number associated with arterial hypertension.[32] Here, in the FR50% offspring, we demonstrate a decreased AT1R expression by immunoblot (minus 30%) and immunohistochemistry in all nephron segments accompanied with early and pronounced reduction of nephron units. On the other hand, the type 2 angiotensin II receptors are expressed at high levels in embryonic tissues, and decrease rapidly after birth.[33] These receptors antagonize many physiological AT1R-mediated effects by inhibiting cell growth, and by inducing apoptosis and vasodilatation.[34]
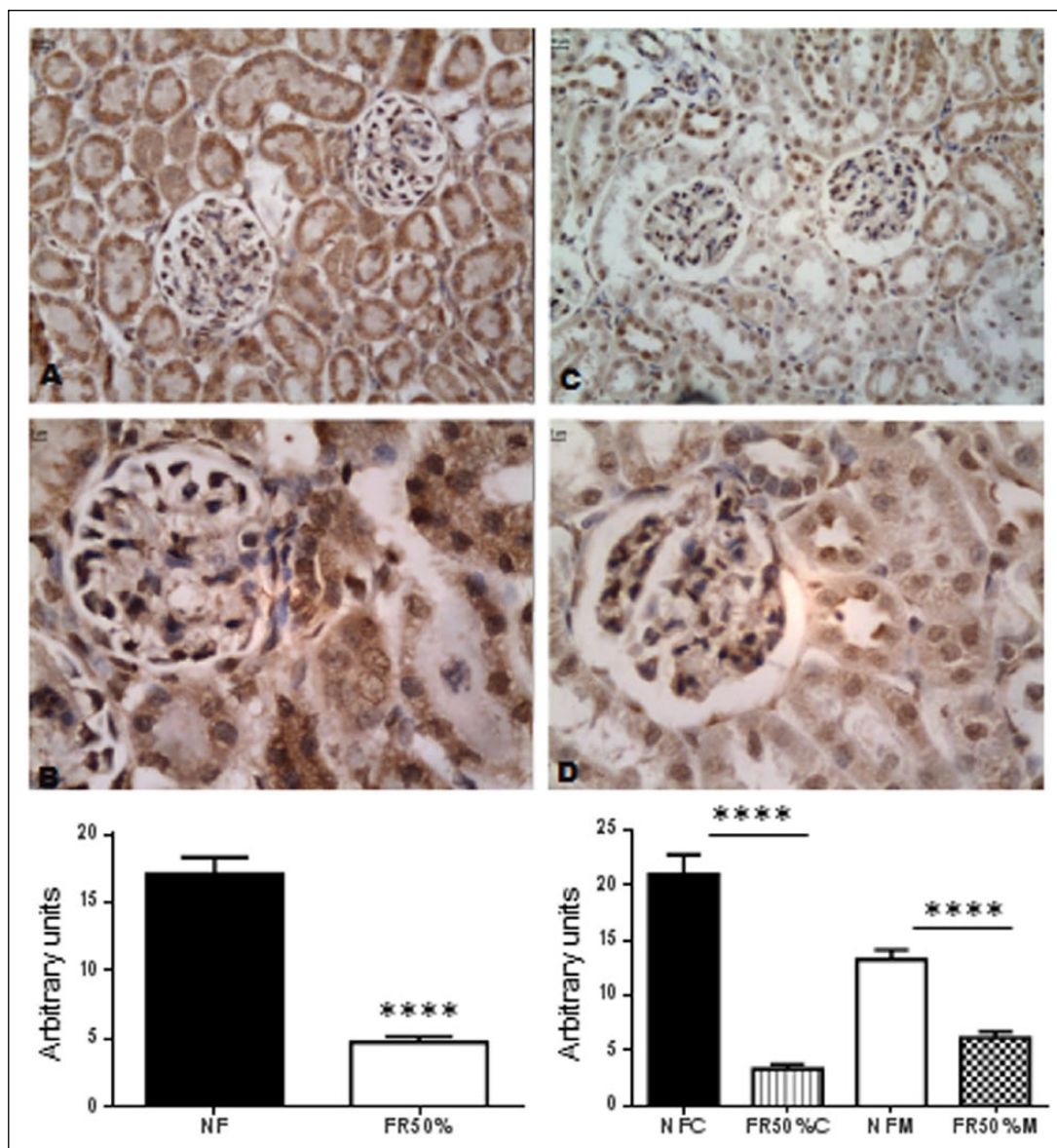
Figure 11. AT2R kidney expression by immunohistochemistry in NF (a and b) and FR50% (c and d) groups in 23-day-old rats. The graph on the left represents a total kidney staining quantification in NF and FR50% offspring and on the right is cortical (NFC and FR50%C) and medullar (NFM and FR50%M) quantification. ****$p<0.0001$. NF: normal quantity of food; FR50%: 50% of normal quantity of food received.

In the current study, the renal expression of AT2R presented a striking decrease (about 25% to 23-day and 60% to 12-week-old offspring), which is consistent with a previous report showing reduced renal AT2R mRNA in food-restricted rats.[11,12,23,35] We cannot rule out that the decreased renal AngII receptor expression in the FR50% progeny may be simply an adaptive downregulation response that limits the fetal renal growth and post-natal sodium transport.

Conversely, the expression of the MRs and GRs in 23-day-old and adult FR50% kidneys was significantly increased, when compared with the age-matched control groups. The most well-defined physiological action of

minerado- and glucocorticoid is the regulation of electrolyte and volume balance through its renal action on the distal tubule and cortical collecting duct. The amino acid sequences of the MR and GR have a high degree of homology. The identity is near 94% at the center of the molecule in the DNA-binding domain and 55–60% in the C-terminal ligand-binding domain. Most effects of mineralocorticoid are mediated by a specific nuclear receptor, the MR. Stimulation of MR in these cells results in sodium reabsorption and potassium excretion. This steroid binding to its cognate receptor enhances the transcription of steroid-inducible proteins and the synthesis of the subunits of the

apical epithelial sodium channel and of the basolateral Na/K-ATPase are increased.[36,37] Moreover, the nongenomic effects of mineralocorticoid, such as the methylation of the sodium channel, may contribute to the increased steroid-mediated sodium reabsorption.[37,38] It has been observed that aldosterone and cortisol have similar binding affinities for the MR in vitro.[39] Circulating levels of cortisol in humans and corticosterone in rodents are 2- to 3-fold higher than aldosterone levels. However, in vivo, only aldosterone acts as a physiologic agonist of the MR. This apparent paradox was solved with the discovery of the microsomal enzyme 11βHSD.[40–42] In mineralocorticoid target tissues, the type 2 isoenzyme converts the biologically active 11-hydroxy-steroid to their inactive 11-keto-steroid forms, thus conferring ligand specificity on the MR. In the current study, the increased reabsorption of sodium in distal portions of nephron may be associated with enhanced expression of MR and GR in these sites and/or to decreased tubule expression of 11βHSD2 as demonstrated in other tissues in food-restricted animals.[43]

Based on these comparisons, we may suppose that a severe gestational reduction of all food nutrient and calories content accentuate the renal and cardiovascular disorders in adult life, when compared with the maternal protein-restricted intake group at the same period. Moreover, the psychological stress caused by extreme food restriction may also contribute to exacerbate the effects of concomitant nutritional stress. Although the precise mechanism responsible for the subsequently enhanced sodium retention response in 12-week-old FR50% offspring is not completely known, the current data suggest that changes in renal function are conducive to excess sodium tubule reabsorption that is associated with presumable higher AngII AT1/AT2 receptor ratio expression in peripheral vessels, which might potentiate the programming of adult hypertension.

Conclusion

In fact, it is plausible to deduce in the current study an association between decreased natriuresis, reciprocal changes in renal AngII and steroid receptors with the hypertension development found in severe food-restricted offspring when compared with age-matched NF rats. In conclusion, we suppose that, under extreme food restriction, the fetus responds with adaptations accommodating to intrauterine conditions. After birth, the overload for an economical kidney version may result in decreased urinary sodium excretion and increased blood pressure. This overload can result in premature nephron senescence in parallel with functional loss.

Conflict of interest

None declared.

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