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Postnatal early overnutrition dysregulates the intrarenal renin–angiotensin system and extracellular matrix-linked molecules in juvenile male rats☆

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Abstract

Overnutrition during the perinatal period has been associated with susceptibility to obesity and related comorbidities. We examined the effects of postnatal early overnutrition on the development of juvenile obesity and the associated renal pathophysiological changes. Three or 10 pups per mother from rat pup litters were assigned to either the overnutrition or control groups during the first 21 days of life. The effects of overfeeding were measured at 28 days. The smaller male litter pups were heavier than the controls between 4 and 28 days after birth ($P<.05$). By 28 days of age, the kidney weight per body weight ratio decreased in the small litter group ($P<.05$). Circulating leptin levels increased in the small litter rats ($P<.05$). Overnutrition had no effect on renal cell proliferation, apoptosis, macrophages and glomerulosclerosis. In the immunoblots and immunohistochemistry, renin and angiotensin II type (AT) 2 receptor expression increased in the overfed rats ($P<.05$). By contrast, the plasminogen activator inhibitor (PAI)-1 and matrix metalloproteinase (MMP)-9 expression decreased in the overnutrition group ($P<.05$). The AT 1 receptor, tissue inhibitor of MMP-1, monocyte chemoattractant protein-1, tumor necrosis factor-α, osteopontin and adiponectin expression was not changed. Our data showed that postnatal early overfeeding led to hyperleptinemia, juvenile obesity and the acquired reset of renal maturation. Up-regulation of renin and AT2 and down-regulation of PAI-1 and MMP-9 might contribute to abnormal programming of renal growth in rats exposed to postnatal early overnutrition.

Keywords: Extracellular matrix; Kidney development; Obesity; Renin–angiotensin system

1. Introduction

Obesity is becoming a worldwide epidemic. The current epidemic of childhood obesity is possibly the most important threat to the future cardiovascular health in adult life [1]. Excessive weight during childhood exposes the child to high levels of free fatty acids and leptin and low adiponectin levels, and increases many other complications associated with obesity [2]. The acquired reset in early life of key cardiovascular hormone systems such as the renin–angiotensin system (RAS) has been suggested to cause lifelong functional and structural alterations [3]. Although infancy has not been the target of obesity prevention, several observations have shown that rapid weight gain in infancy may influence weight later in childhood as well as the later development of adult cardiovascular disease [4,5].

Obesity not only accelerates the progression of chronic kidney disease but also has been associated with the development of new-onset kidney disease [6,7]. The potential mechanisms include inflammation, lipotoxicity, hemodynamic effects, as well as other unknown mechanisms [8]. Obese individuals commonly have increased circulating levels of RAS components; the activity of RAS has been linked to the metabolic syndrome [9].

All components of the RAS are significantly expressed in the developing kidney both spatially and temporally; RAS-regulated renal growth and development have been reported in neonatal rats [10–12]. The RAS has been implicated also in the progression of renal disease, and the reversal of chronic kidney disease has been achieved in experimental models where the RAS is blocked [13]. Likewise, the remodeling of the extracellular matrix (ECM) is a key event not only in the progression and reversal of kidney disease [14], but also during kidney development. The ECM-related molecules control multiple steps of renal development; changes in the ECM composition can interfere with ordinary nephrogenesis [15].

The present study was performed to determine whether overnutrition during the neonatal period affects the development of early-onset juvenile obesity, and the associated renal pathophysiological changes. To investigate this, first, the effects of postnatal overfeeding on the development of obesity were studied over 28 days in rats. Second, the metabolic complications and changes in renal function were assessed in the neonatally overfed rats. Third, the effects of postnatal overnutrition on changes of renal structure and molecular expression were studied in relationship to obesity and normal kidney development.

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2. Materials and method

2.1. Animal preparation

Virgin Sprague-Dawley rats were timed mated with normal males at the age of 3 months. On the second day of life (D2), male pups were randomly distributed among the mothers to achieve cross-fostering and the litter size was adjusted to 10 male newborns [normal litter (NL), $n=20$] to induce normal feeding or to three male pups [small litter (SL), $n=18$] to induce overfeeding. Rats were weaned at D21, and the body weight was monitored every 3 days from D1 to D28. After weaning, rats had free access to tap water and standard chow. The rats were sacrificed at D28. Their kidneys were harvested and processed for the study. One whole kidney (right kidney) from each rat was used for light microscopy and immunohistochemistry, and the other whole kidney (left kidney) for Western blot analysis. All experimental procedures were approved by the Animal Experimentation Ethics Committee of the Korea University Guro Hospital. All procedures conformed to the Korean national guidelines for the care and handling of animals and the published guidelines from the National Institutes of Health (Bethesda, MD, USA).

2.2. Analysis of kidney function

Systolic, diastolic and mean blood pressure (BP) were determined at D27 in unanesthetized prewarmed rats by tail-cuff plethysmography (Kent Scientific Corp., Torrington, CT, USA). Before the recording, the rats were allowed to adapt to the measurement conditions on three consecutive days for 15 min each sessions. Then, measurements were performed on D27, and the mean value was calculated for each rat. The rats were placed in metabolic cages for urine collection, and urinary albumin was measured with a commercially available enzyme-linked immunosorbent assay (ELISA) kit (Alpco, Salem, NH, USA). Urine creatinine was also determined using an ELISA kit (Assay Designs, Ann Arbor, MI, USA), and the random urine albumin was revised according to the respective urine creatinine. On D28, cardiac blood was obtained from deeply anesthetized rats (inhaled isoflurane, 50 mg/kg ip pentobarbitone sodium). The blood glucose was determined automatically by photometric determination using the glucoseoxidase–peroxidase method (Abbott, Illinois, USA). Blood urea nitrogen (BUN) (Bioassay Systems, Hayward, CA, USA) and serum creatinine (Luminos, Ann Arbor, MI, USA) were also assayed by commercial ELISA kits using standard concentrations ranging from 0.27 to 50 mg/dl and from 0.015 to 8 mg/dl, respectively. Leptin was measured with an ELISA kit (Assay Designs, Ann Arbor, MI, USA) using standard concentrations ranging from 0.024 to 6.3 ng/ml.

2.3. Western blotting

Protein extractions and immunoblots were performed as in previous studies [10]. Equal amounts of 5–15 μg of proteins were subjected to 10% SDS-polyacrylamide gels and transferred onto nitrocellulose membranes (KPL, Gaithersburg, MD, USA). The membranes were blocked in 5% skim milk with TBS-T [0.05% Tween 20 in 50 mM of Tris, 150 mM of NaCl and 0.05% NaN_3 (pH 7.4)] at room temperature for 1 h. The membranes were washed two times in TBS-T and incubated for 18 h at 4°C with primary antibodies directed against renin (sc-152, dilution 1:500), angiotensin (Ang) II type 1 receptor (AT1) (sc-57036, 1:400), Ang II type 2 receptor (AT2) (sc-9040, 1:400), plasminogen activator inhibitor (PAI)-1 (sc-8979, 1:500), matrix metalloproteinase (MMP)-9 (sc-10737, 1:250), tissue inhibitor of MMP (TIMP)-1 (sc-21734, 1:200), tumor necrosis factor (TNF)-α (sc-52746, 1:300), monocyte chemoattractant protein (MCP)-1 (sc-21742, 1:200), osteopontin (sc-21742, 1:1000) and adiponectin (sc-26497, 1:200). All of the above-mentioned primary antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Thereafter, the membranes were washed two times with TBS-T and incubated for 40 min with an anti-rabbit IgG (Ap132p, 1:1000, Millipore, Temecula, CA, USA), an anti-mouse IgG (474-1806, KPL, Gaithersburg, MD, USA) and an anti-goat IgG (HAF-109, R&D Systems, Minneapolis, MN, USA) at room temperature. To control for equal loading, α-tubulin (1:1000 dilution; Cell Signaling Technology, Danvers, MA, USA) and anti-mouse IgG conjugated horseradish peroxidase (1:1000 dilution; Millipore, Temecula, CA, USA) were used as primary and secondary antibodies with the same method as described above. The developed X-rays were scanned using the Epson GT-9500 scanner (Seiko, Nagano, Japan), and the results were quantified by a computerized densitometer (Image PC alpha 9; National Institutes of Health).

2.4. Cell proliferation, apoptosis and glomerulosclerosis

The harvested kidneys were treated in 10% formalin solution (Sigma, St. Louis, MO, USA) and embedded in paraffin. The samples were then cut into 4-μm-thick sections and dried onto silicanized slides (Muto-Glass, Japan). At least five rats per group were examined. To detect cellular changes, PCNA and TUNEL staining were carried out as described previously [10,12]. The number of TUNEL-positive apoptotic cells and PCNA-positive cells was calculated by counting 20 areas (25×25 μm) and obtaining the average result. The microscopic identification (×400) of tubules, interstitium and glomeruli in the cortex and medulla was performed using a double-blind method. The count was performed randomly throughout all of the fields analyzed. In order to evaluate the degree of glomerulosclerosis, the sections were also stained with periodic acid-Schiff (PAS) and a semiquantitative score was obtained according to the method described by Ma et al. [16]. Glomerulosclerosis was defined as the collapse and/or obliteration of glomerular capillary tufts accompanied by hyaline material and/or an increase of matrix. Severity of sclerosis for each glomerulus was graded from 0 to 4+ as follows: 0, no lesion; 1+, sclerosis of <25% of the glomerulus; 2+, 3+ and 4+, sclerosis of 25% to 50%, >50 to 75% and >75%, respectively, of the glomerulus. The average semiquantitative score was obtained from at least 30 glomeruli in each kidney section from five individual rats per group under ×400 magnification in a blinded manner.

2.5. Immunohistochemistry

2.5.1. Renin, AT2, PAI-1 and MMP-9

Five kidneys in each group were selected for representative immunohistochemistry of renin, AT2, PAI-1 and MMP-9, using an avidin–biotin immunoperoxidase method (Vectastain ABC kit, Burlingame, CA, USA). Immunohistochemistry was performed for the proteins directed against positive findings in the immunoblots.

The paraffin sections were deparaffinized with xylene, followed by rehydration in a descending series of ethanols. Then, the endogenous peroxidase activity was quenched in 0.6% hydrogen peroxide for 15 min. Antigen retrieval was performed with 0.1% citric acid (DAKO Co., Carpinteria, CA, USA). After quenching and antigen retrieval, the sections were incubated with primary antibodies against renin (1:200), AT2 (1:150), PAI-1 (1:100) and MMP-9 (1:100). All of the above-mentioned primary antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). As negative controls, the primary antibody was substituted with phosphate buffered saline (PBS). The incubation time was overnight at 4°C. After incubation, the sections were washed twice in PBS for 5 min and incubated for 30 min with secondary antibodies (peroxidase-conjugated anti-rabbit IgG; 1:200, Millipore, Temecula, CA, USA). Then, the slides were washed in PBS and incubated for 50 min with the Vectastain ABC reagent. The immunoreaction products were developed using 3,3-diaminobenzidine as the chromogen, at standard development times. The sections were counterstained in 0.5% methyl green solution (Trevigen, Gaithersburg, MD, USA) for 5 min, dehydrated and evaluated using light microscopy (×400).

2.5.2. Ed-1

To analyze the infiltration of monocytes/macrophages into glomeruli, immunohistochemistry against the rat monocyte-specific marker ED-1 (1: 100, Abdserotec, Kidlington, UK) was also performed. ED-1-positive cells were randomly counted in at least 20 fields of cortex per section under ×400 magnification.

2.6. Statistical analysis

The male litter was considered as the unit for statistical analysis. Data are presented as the mean±S.E.M. Differences between the groups were analyzed by t test. Statistical significance was defined as $P<.05$. The SigmaStat version 2.03 for Windows (SPSS Science, Chicago, IL, USA) was used for the analysis.

3. Results

On Day 1 of life, the body weight did not differ between the two groups (NL group 7.53±0.06 g vs. SL group 7.54±0.12 g). However, between 4 and 28 days after birth, the SL rats were significantly overweight compared to the NL group ($P<.05$). At 28 days of age, the SL rats were 39.5% heavier than the NL rats (124.7±4.3 vs. 75.4±1.8 g, $P<.05$; Fig. 1A). The kidney weight was 0.5±0.03 g in the NL group and 0.62±0.03 g in the SL group. The kidney weight per body weight ratio was decreased in the SL group, compared to the NL rats (0.005± 0.0003 vs. 0.007±0.0005, $P<.05$; Fig. 1B) (Table 1).

3.1. Functional findings

By 4 weeks of age, there were no differences in the BP and blood glucose levels between the two groups. Plasma leptin levels at the end of the study were significantly increased in neonatally overfed SL rats (NL vs. SL: 0.78±0.4 vs. 2.84±0.72 ng/mL, $P<.05$). Determination of the BUN and serum creatinine levels revealed no significant differences between the two groups (Table 1). The urinary albumin-to-creatinine ratio did not change (data not shown).

3.2. Cell proliferation, apoptosis, ED-1 and glomerulosclerosis

Overnutrition had no significant effects on renal cell proliferation, apoptosis, numbers of ED-1-positive macrophages and glomerulosclerosis (Fig. 2A–H). The numbers of proliferating cell nuclear antigen

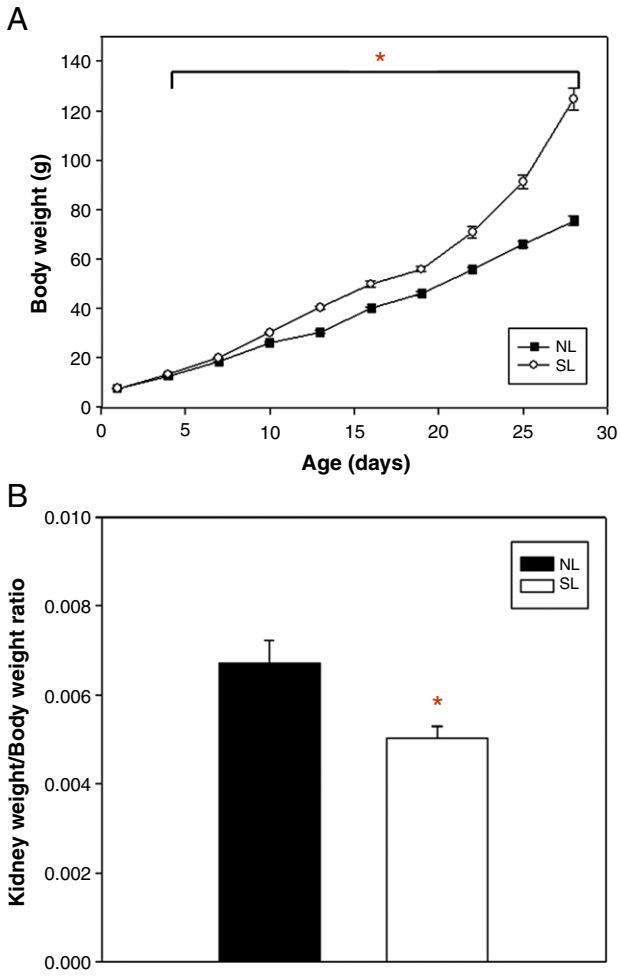


Fig. 1. Body and kidney weights. (A) Significant overweight was observed between 4 and 28 days after birth in the postnatally overfed rats (NL, normal litter, SL, small litter; *P<.05). (B) The kidney weight per body weight ratio decreased in the small litter group, compared to the normal litter rats (*P<.05).

(PCNA)-positive cells, TUNEL-positive apoptotic cells and ED-1-positive macrophages and the average semiquantitative score for glomerulosclerosis did not differ between the two groups (data not shown).

3.3. RAS gene expression

The immunoblots showed that renin/tubulin protein expression significantly increased in the SL group compared to the control rats (P<.05; Fig. 3A). The immunohistochemistry showed that renin expression was detected in juxtaglomerular cells and some tubular epithelial cells in the control kidney (Fig. 3B). The neonatally overfed SL rats had increased renin expression in the medullary and cortical tubular cells including juxtaglomerular cells, compared to the NL control rats (Fig. 3C). A significant increase in the expression of AT2/tubulin protein was also found in the SL group (P<.05; Fig. 4A). Immunohistochemically, AT2 expression was more strongly detected in the juxtaglomerular cells and tubular segments of the SL rats, compared to the NL group (Fig. 4B and C). The immunoblots showed that AT1/tubulin protein expression did not differ between the two groups (Fig. 5A).

3.4. ECM-related gene expression

The immunoblots showed that PAI-1/tubulin protein expression was significantly decreased in the SL group compared to the control rats (P<.05; Fig. 6A). PAI-1 expression was found in many tubular epithelial cells in the control NL rat kidneys; however, it was decreased in the SL group (Fig. 6B and C). MMP-9/tubulin protein expression was also decreased in the SL rat kidneys (P<.05; Fig. 7A). The immunohistochemistry showed that MMP-9 expression was easily detected in almost all tubular epithelial cells and glomerular cells in the control kidneys (Fig. 7B). In the SL rats, it was more weakly detected in some tubular cells (Fig. 7C). The TIMP-1/tubulin protein expression showed no differences between the two groups (Fig. 5B).

3.5. Other inflammatory cytokines

The immunoblots showed that TNF-α, MCP-1, osteopontin and adiponectin protein expression did not differ between the NL and SL groups (Fig. 5C–E). Immunohistochemistry for these cytokines was not performed.

4. Discussion

In the present study, early postnatal overnutrition led to hyperleptinemia, juvenile obesity and pathophysiological renal changes at the molecular level. Postnatally overfed rats displayed acquired resetting, in early life, of the RAS and ECM genes, which play a key role in renal growth and development. The increase of renin and AT2 and the decrease of PAI-1 and MMP-9 in the juvenile obese rat kidneys suggest that renal development can be abnormally programmed by postnatal overnutrition.

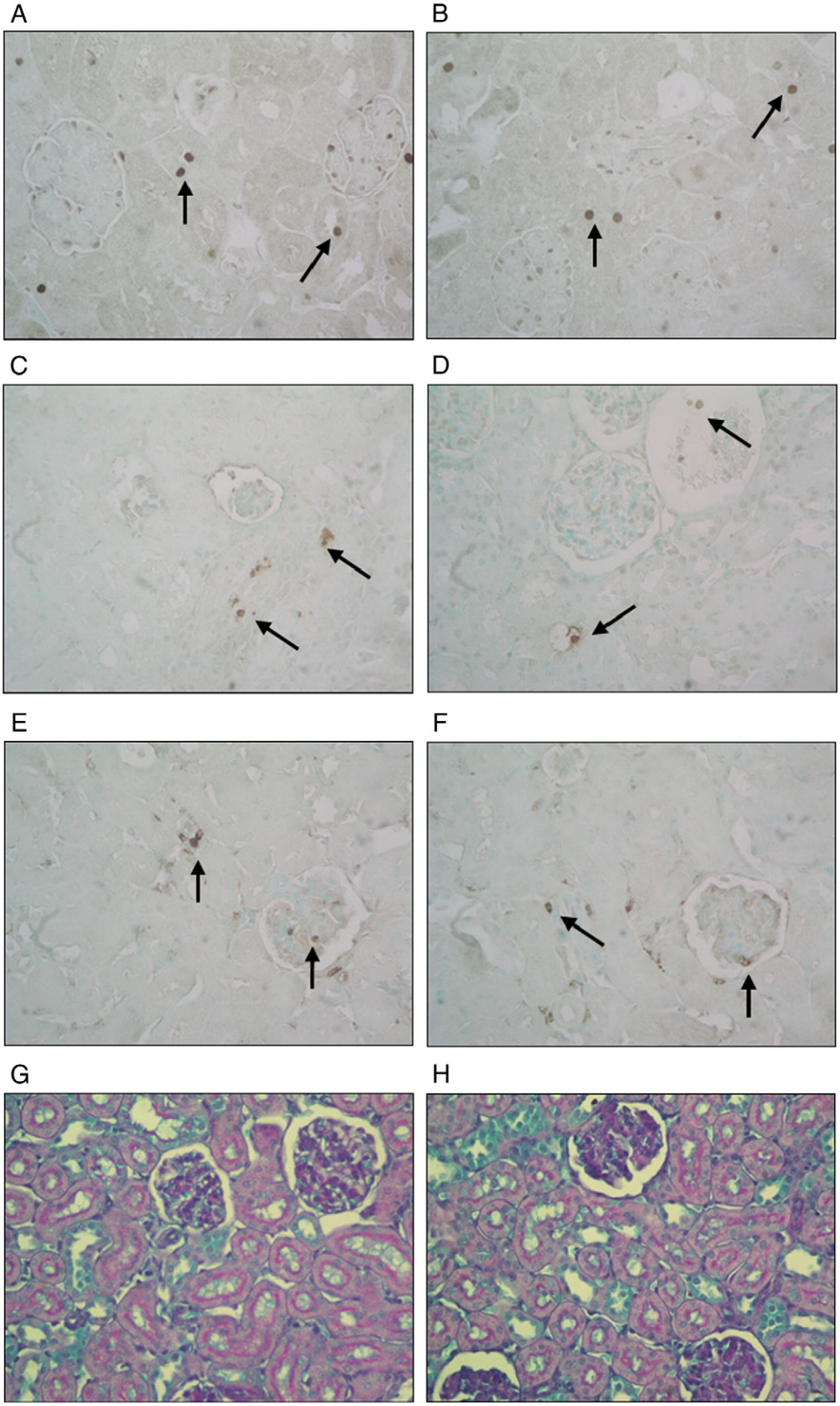
Rats raised in a SL have been confirmed to be a useful experimental model for the study of the consequences of overnutrition during the critical perinatal period [17]. Early postnatal overfed rats have hyperphagia, are overweight and have impaired glucose tolerance, hyperinsulinemia and increased systolic BP later in life [18]. Increased leptin concentrations, or accelerated maturation of the hypothalamic-pituitary-adrenal axis, have been suggested to be associated with increased caloric intake and metabolic disturbances [17,19]. The results of those studies are in accord with the present observation. The overfed SL rats had increased body weights. By 4 days of age, the SL rats were already heavier than the NL pups and, by 28 days of age, they were 39.5% heavier. In contrast, others have reported that a modest litter reduction (by approximately 50%) resulted in postnatal growth restriction, possibly due to removal of the stimulus for maternal milk production [20].

In the present study, the overfed rats were found to have a decreased kidney/body weight ratio. There were yet no histological or functional changes, including renal cell proliferation, apoptosis, glomerulosclerosis, serum creatinine, albuminuria and BP in juvenile obese rats (at 28 days of age). The blood glucose levels did not differ between the two groups; however, the plasma leptin levels were

Table 1
Body and kidney weights, blood pressure and metabolic parameters (Day 28) in rats raised in small litters (SL) compared to rats raised in normal litters (NL; controls)

	NL (n=20)	SL (n=18)
1st day of life body weight (g)	7.53±0.06	7.54±0.12
28th day of life body weight (g)	75.4±1.8	124.7±4.3 *
Kidney weight (g)	0.5±0.03	0.62±0.03
Kidney weight/body weight	0.007±0.0005	0.005±0.0003 *
Blood pressure (mmHg)		
Systolic	98.2±5.0	107±1.9
Diastolic	61.6±3.4	71±3.8
Mean	73.2±2.69	83±2.52
Blood glucose (mg/dl)	141.5±4.7	154.4±7.5
Plasma leptin (ng/ml)	0.78±0.4	2.84±0.72 *
Blood urea nitrogen (mg/dl)	4.09±0.38	3.59±0.32
Serum creatinine (mg/dl)	0.26±0.05	0.33±0.17

Data are means±S.E.M.
* Significant between-group comparison (P<.05, t test).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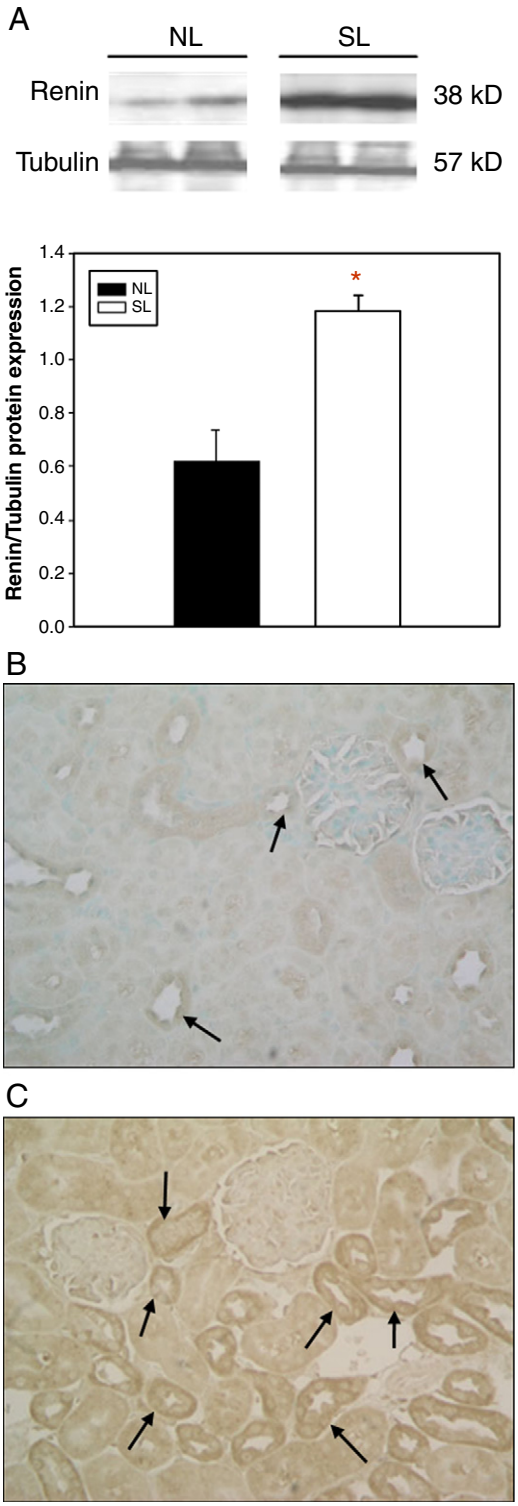

Fig. 3. Intrarenal renin expression significantly increased in the SL group compared to the control rats. (A) Immunoblots, (B) cortical expression of the control group (arrows), (C) cortical expression within juxtaglomerular cells and tubular epithelial cells of the obese rats (arrows) (n=5 for each group) (*P<.05) (B and C, original magnification ×400).

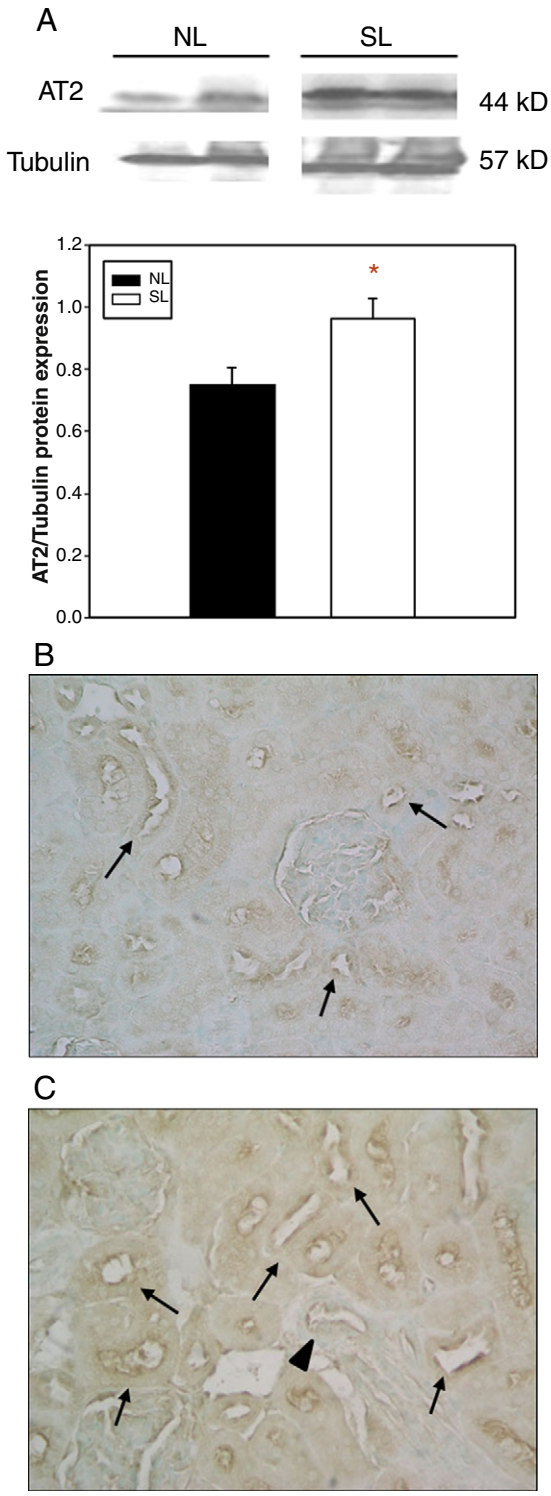

Fig. 4. AT2 expression significantly increased in the SL rat kidneys compared to the control rats. (A) Immunoblots, (B) cortical expression of the control group (arrows), (C) cortical expression within juxtaglomerular and tubular cells (arrows) and vascular endothelial cells (arrowhead) of the obese rats (n=5 for each group) (*P<.05) (B and C, original magnification ×400).

Fig. 2. Overnutrition had no significant effects on renal cell proliferation, apoptosis, numbers of ED-1-positive macrophages and glomerulosclerosis. (A, B) PCNA-positive cells (arrows), (C, D) TUNEL-positive apoptotic cells (arrows), (E, F) ED-1-positive macrophages (arrows), (G, H) PAS stain for glomerulosclerosis (n=5 for each group) (A, C, E, G, normal litter groups; B, D, F, H, small litter groups).

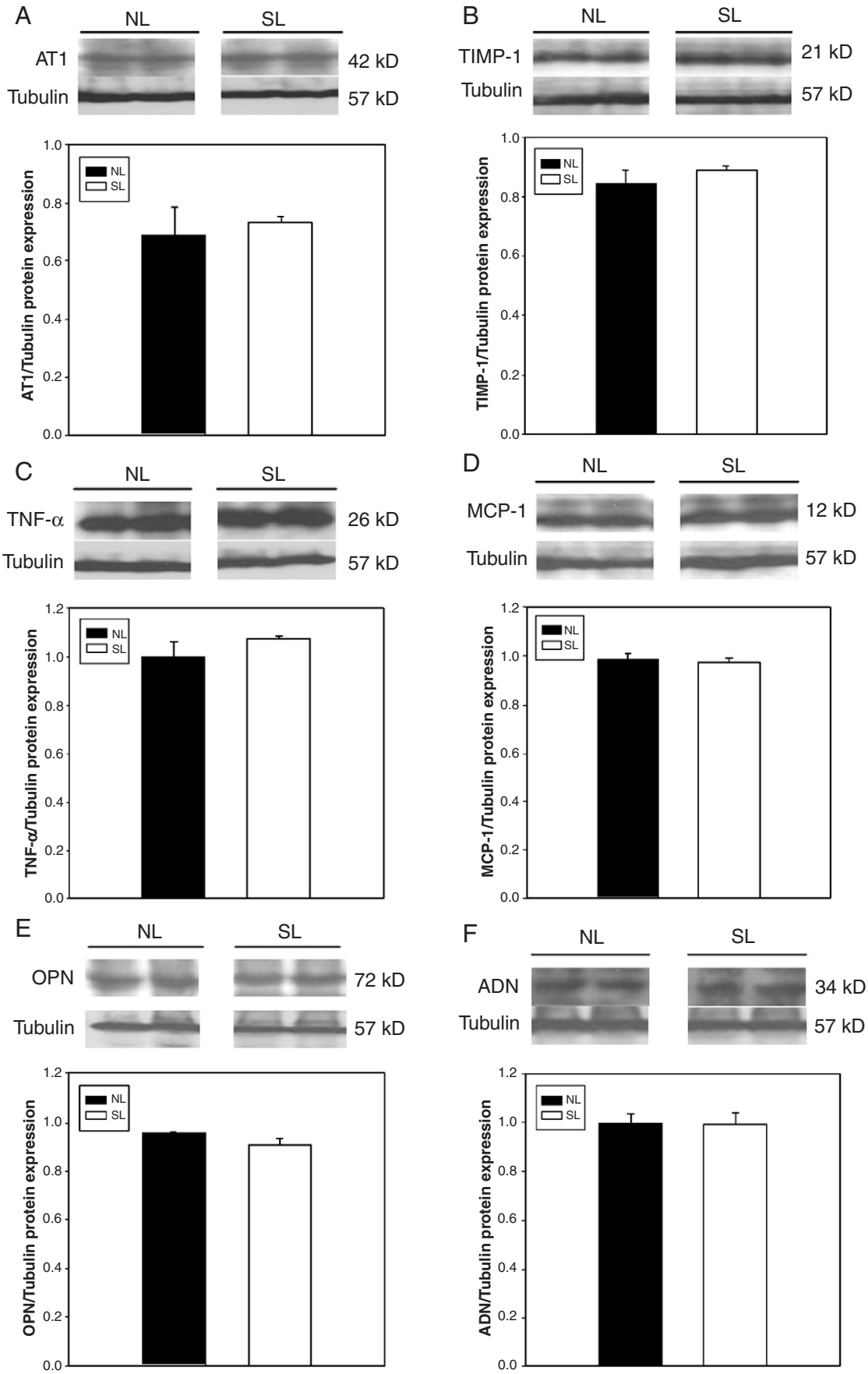

Fig. 5. The immunoblots showed that AT1 (A), TIMP-1 (B), TNF-α (C), MCP-1 (D), osteopontin (E) and adiponectin (F) expression did not differ between the two groups (n=5 for each group).

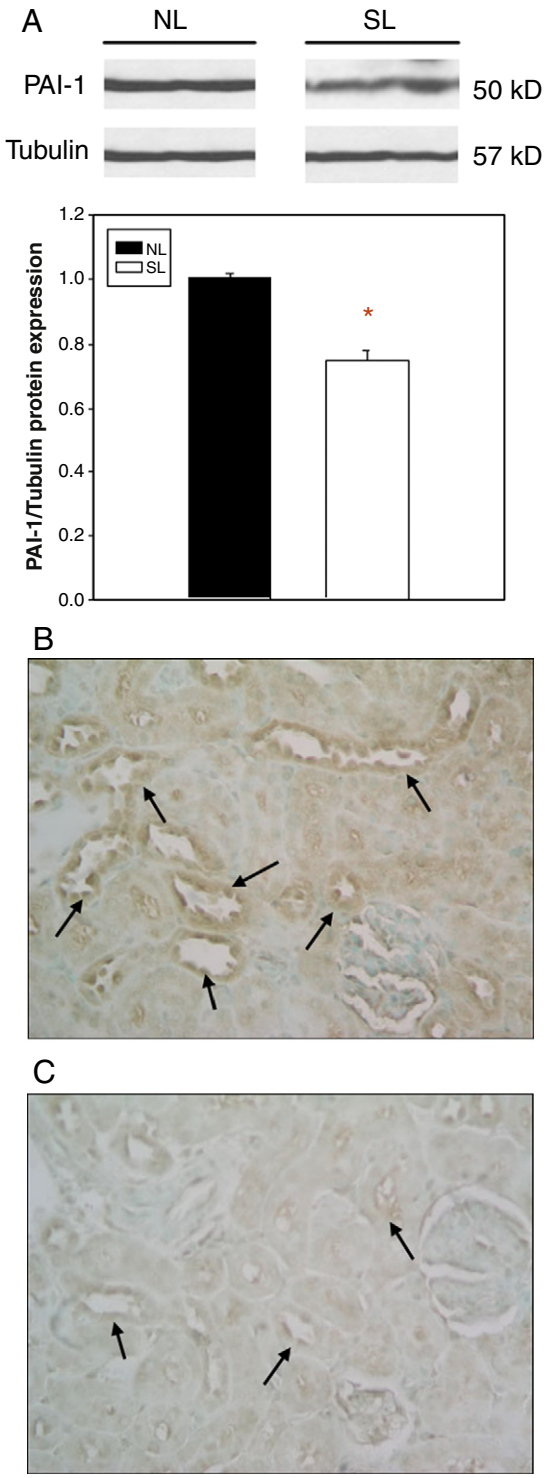

Fig. 6. Intrarenal PAI-1 expression was significantly decreased in the SL group compared to the control rats. (A) Immunoblots, (B) cortical expression within tubular epithelial cells of the control rat kidneys (arrows), (C) cortical expression of the obese rats (arrows) (n=5 for each group) (*P<.05) (B and C, original magnification ×400).

significantly increased in the SL rats. The concentration of leptin was 3.6-fold greater in the obese rats at 28 days of age. The short form of the leptin receptor is abundantly expressed in the kidneys [21]; several studies have implicated leptin as central to the link between obesity and renal disease [22,23].

Nephrogenesis in rats continues until Day 18 postnatally; several peptides have been implicated in this process. The RAS has a significant impact on the growth and maturation of the kidneys during the critical perinatal period [24]. Under conditions of obesity, activation of the RAS has been associated with sympathetic stimulation, hemodynamic alterations and the synthesis of RAS components by visceral fat [8]. Samuelsson et al. [25] have shown a threefold increase of renal renin mRNA expression in the juvenile

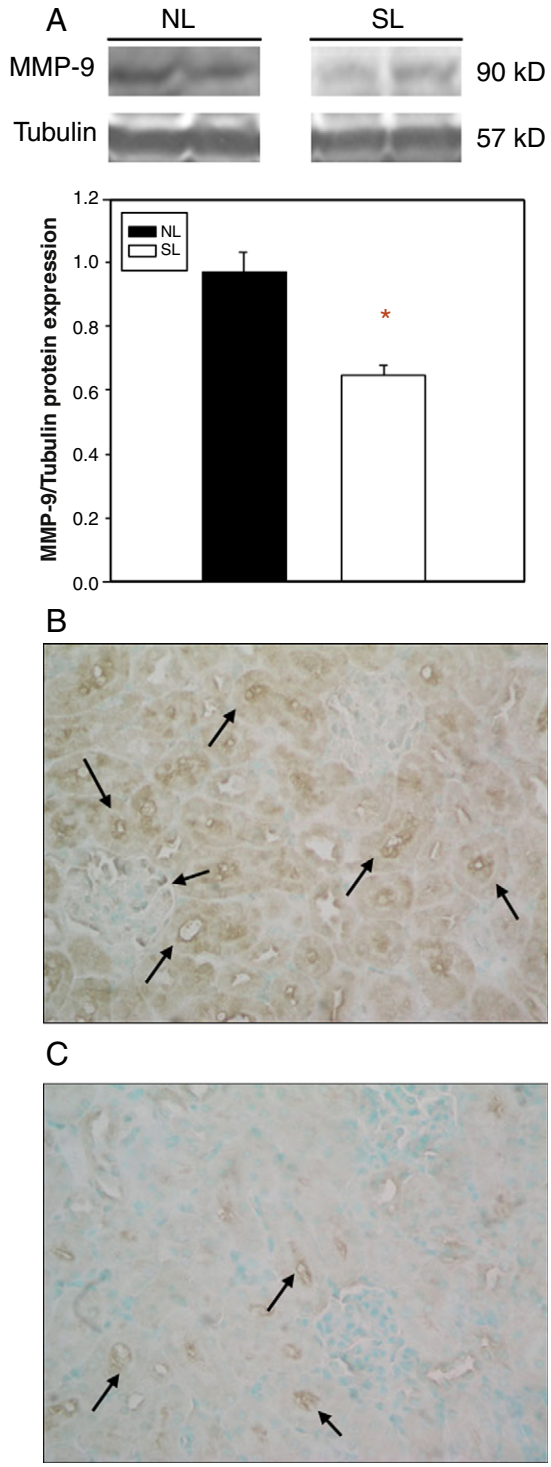

Fig. 7. MMP-9 expression was significantly decreased in the SL rat kidneys. (A) Immunoblots, (B) cortical expression within tubules and glomeruli of the control rat kidneys (arrows), (C) cortical expression of the obese rats (arrows) (n=5 for each group) (*P<.05) (B and C, original magnification ×400).

offspring of obese dams. Perinatal protein restriction has been shown to cause suppression of intrarenal renin and Ang II levels in developing offspring, as well as a reduced number of nephrons [26]. In this study, the overfed SL rats showed increased renin protein expression and amplified its binding sites in the juxtaglomerular and tubular cells in the immunohistochemistry.

The major effector hormone of the RAS is Ang II, which signals via two principal receptors, AT1 and AT2. AT1 plays a pivotal role in renal development by mediating the mitogenic effects of Ang II and promoting the deposition of ECM [27]. AT2 also appears to be critical for normal nephrogenesis and is expressed at very high levels in the developing kidney. AT2 has the opposite effects of AT1, antiproliferative and antigrowth, which have been reported in the cardiovascular system as inhibition of cardiac fibroblast growth, ECM formation and vascular smooth muscle cells, as well as prevention of Ang II-induced growth of neonatal rat myocytes [28,29].

We have shown that AT2 protein levels, not AT1, apparently increased as a result of early postnatal overfeeding in the juvenile obese rat kidneys. The changes in receptor expression occurred earlier in life, and disturbance of the normal AT1/AT2 patterns of expression might have interfered with the growth and maturation of the kidneys. Although AT1 emerged to be important in mediating the programming effects of undernutrition in fetal life [30], exposure to a maternal low-protein diet *in utero* not also increased expression of glomerular AT1 receptors but also reduced AT2 receptor expression in the young rat [31]. Moreover, McMullen et al. [32] noted that AT1 mRNA expression in the kidney at 4 weeks of age was not influenced by perinatal nutrition; a significantly lower renal expression of AT2 mRNA in the offspring rats exposed to maternal low-protein diets was found. In this study, early postnatal overnutrition influenced and augmented the renal expression of AT2, which might have an effect on the abnormal development of the kidneys. The AT2 binding sites were increased on the juxtaglomerular apparatus, tubules and vasculature of the overfed rat kidneys. These results provide some evidence that perinatal nutrition can alter the RAS and renal development.

In the present study, PAI-1 and MMP-9 expression decreased in the kidneys of the neonatally overfed SL rats. MMPs and PAI-1 have been the focus of recent studies because of their important interactions with the RAS and potential role in ECM regulation [16,33]. PAI-1 is the primary inhibitor of plasminogen activators and regarded as a powerful fibrosis-promoting molecule. It has many biological functions including regulating cell proliferation, adhesion, migration and signal production pathways [34]. The ECM accumulation associated with a model of lung fibrosis was shown to be PAI-1 dose dependent [35]. Increase in PAI-1 inhibited degradation of the ECM, resulting in accumulation of the ECM and promoting glomerulosclerosis [36].

The MMPs are a large family of zinc-requiring matrix-degrading enzymes. They can degrade all ECM components and are involved in a variety of pathophysiological processes. Among them, MMP-9 has been implicated in renal development, renal tubule physiology and glomerular pathophysiology [15]. It is thought to play a key role in the balance between ECM synthesis and degradation [37]. MMP-9 metanephric expression was significantly decreased in the kidneys of fetuses exposed to maternal diabetes. The impaired activities of MMP-9 might be involved in the alteration of nephrogenesis caused by maternal diabetes, through abnormal ECM turnover, leading to decreased ureteric bud branching [38].

Our results demonstrated decreased PAI-1 and MMP-9 expression in the obese rats. Inhibition of ECM accumulation and altered ECM turnover can be induced by changes in the molecular expression of PAI-1 and MMP-9. Expression of TIMP-1 and other inflammatory cytokines was not found to be changed by early postnatal overfeeding.

Taken together, the findings of this study suggest that postnatal overnutrition may be a crucial modulator involved in early-onset juvenile obesity and altered renal development. Postnatal early overnutrition plays a significant role in the acquired reset of key intrarenal hormone systems in this experimental model, up-regulation of renin and AT2, and down-regulation of PAI-1 and MMP-9. Application to the human condition cannot be directly inferred, but this study shows that adequate nutrition during early postnatal period is important for determining the homeostatic pathways involved in obesity and kidney maturation.

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