

EXPOSURE TO MATERNAL DIABETES INDUCES SALT-SENSITIVE HYPERTENSION AND IMPAIRS RENAL FUNCTION IN ADULT RAT OFFSPRING

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Objective:

Epidemiological and experimental studies have led to the hypothesis of fetal origin of adult diseases, suggesting that some adult diseases might be determined before birth by altered fetal development. We have previously demonstrated in the rat that *in utero* exposure to maternal diabetes impairs renal development leading to a reduction in nephron number. Little is known on the long term consequences of *in utero* exposure to maternal diabetes. The aim of the study was to assess, in the rat, long-term effects of *in utero* exposure to maternal diabetes on blood pressure and renal function in adulthood.

Research Design and Methods:

Diabetes was induced in Sprague-Dawley pregnant rats by streptozotocin on day 0 of gestation. Systolic blood pressure, plasma renin activity, renal function, were measured in the offsprings from 1 to 18 months of age. High salt diet experiments were performed at the pre-hypertensive stage and the abundance of tubular sodium transporters was evaluated by western-blot analysis. Kidney tissues were processed for histopathology and glomerular computer-assisted histomorphometry.

Results and Conclusions:

We demonstrated that *in utero* exposure to maternal diabetes induces a salt-sensitive hypertension in the offsprings associated with a decrease in renal function in adulthood. High salt diet experiments show an alteration of renal sodium handling that may be explained by a fetal re-programmation of tubular functions in association or as a result of the inborn nephron deficit induced by *in utero* exposure to maternal diabetes.

Diabetes mellitus has recently assumed epidemic proportion. Estimates suggest that worldwide, 30 millions women of reproductive age will suffer from diabetes by 2030. Data from birth certificates indicate that some form of maternal diabetes complicates around 3% of pregnancies in US (1; 2). Pre-existing diabetes complicates pregnancy at a rate of 1-3 per 1000 births and increases the rates of obstetric complications, stillbirth, perinatal mortality, congenital malformations, and macrosomia compared with the background population (3; 4). Gestational diabetes is also associated with substantial rates of maternal and perinatal complications. Diabetic-associated malformations result from developmental defects occurring in early organogenesis (5). They include caudal regression syndrome and urogenital abnormalities which can be as severe as renal agenesis (6). Beside these numerous epidemiological data concerning perinatal outcome of pregnancy of diabetic women, little is known on the long-term consequences of *in utero* exposure to maternal diabetes in adulthood (7-9).

Fetal programming refers to observation that an adverse environmental stimulus experienced *in utero* during the critical period of development of organogenesis, can induce long-term effects on developing organism (10; 11). These structural and functional effects predispose the offspring to several diseases in adulthood, i. e. hypertension and cardiovascular diseases. Many epidemiological studies have clearly confirmed the seminal works of Barker et al, that evidenced the inverse relationship between a low birth weight as

a marker of intrauterine stress, and the risk of developing cardiovascular disease, and hypertension (10). Brenner et al. proposed that the inborn nephron deficit associated with this low birth weight predispose to impaired renal sodium excretion and to increased susceptibility to hypertension (12). More recently, several studies have also suggested a similar association between low birth weight and chronic kidney disease (12).

We have previously shown that offspring of streptozotocin-induced diabetic rats have an impaired nephrogenesis with a reduction of 30 % of nephron number (13-15). Our model is characterized by moderate levels of maternal hyperglycemia and by normal gestation and delivery with healthy pups without intrauterine growth retardation or congenital malformation.

Therefore, the first aim of this work was to determine in our rat model whether maternal diabetes programs adult hypertension in the offspring and to address the implication of impaired renal sodium excretion in this process. The second aim of this study was to determine whether inborn nephron deficit alters renal function and to identify glomerular hypertrophy and injury as factors of progression of renal diseases.

Material and Methods

Animals and nephron counting

Pregnant Sprague-Dawley rats, weighing 250-300g, were made diabetic on day 0 of gestation by a single intraperitoneal injection of streptozotocin (Sigma, Saint Quentin-Fallavier, France), 35 mg/kg body weight in 0.4 mol/l citrate buffer, pH 4.5. The diabetic state was checked by measuring the plasma glucose concentration (Accuchek, Roche, France). Only pregnant females whose

plasma glucose ranged between 15 and 20 mmol/l were included in the study. This diabetic status was confirmed every two days until delivery. As previously shown this protocol results in a 30% reduction of the nephron number (13).

On the day of delivery, the newborn rats were weighed. Each litter was then reduced to 10 pups. The present study was restricted to male offsprings. All the animals were maintained in a temperature and light controlled room at 21°C with a 12 hours light cycle. They had free access to food (UAR Laboratory, Villemoisson sur Orge, France) and tap water.

76 rats issued from 16 control mothers (CMO) and 74 rats issued from 16 diabetic mothers (DMO) were used in this study. Six 1-month-old animals issued from 3 different litters of each group were removed, weighed, and prepared for nephron counting by acid maceration method as previously described (13). The remaining animals were followed from 1 month to 18 months of life either for blood pressure or functional or histological renal evaluation. Some of them, 7 and 6 rats issued from 2 different litters of CMO and DMO groups, were followed after 18 months of life to determine the effect of *in utero* exposure to maternal diabetes on long-term survival. All experiments were conducted in accordance with the institutional guidelines and the recommendations for the care and use of laboratory animals put forward by the French Ministry of Agriculture.

Design of the experimental protocol from 1 month to 18 months

In each group, we have assessed in rats of 1 to 18 months of age: systolic blood pressure, renal function and proteinuria, plasma renin activity (PRA), renal renin

expression (RRE), glomerular histomorphometry and renal histology.

Systolic blood pressure was measured during three successive days in conscious rats by a non-invasive method, tail-cuff plethysmography, using a blood pressure monitor (BP recorder 8005 Narco BioSystems). Before the effective measurements, rats were trained by placing them in restrainers and pressing their tail for several times. Blood pressure records were made several consecutive times, on quiet animals in a silent ambience, at constant temperature under a heating apparatus. Measurements were always performed by the same person.

Renal function was estimated by creatinine clearance and proteinuria. For that purpose, rats were individually housed in metabolic cages (Techniplast, Exton, PA) with free access to food and demineralized water. After 3 days adaptation, food and water intakes were measured every 24-hour period and the urines collected throughout the protocol during three other consecutive days. Urine that was spontaneously excreted during each 24-h period was collected to determine daily urinary creatinine and sodium and protein excretions. At the end of the 3 days experimental study, blood samples were collected for creatinine and PRA measurements.

Kidneys and hearts from 1 to 18-month-old CMO and DMO rats were removed, weighed separately, and were fixed with formalin for either histology or histomorphometry.

High salt-diet protocol

Six 3-month-old rats issued from 2 different litters from CMO and DMO groups were individually housed in metabolic cages and fed with normal salt

diet (0.3% NaCl). Blood pressure and urinary sodium excretion were measured. The rats were then moved on a high salt diet (3% NaCl) and the urinary sodium excretions were measured daily during 3 days. Food intake was measured every 24-hour period. After 7 days, systolic blood pressure was determined, kidneys were then removed for sodium transporter abundances evaluation.

Analytical Procedures

Plasma and urinary analysis were performed by standard methods using a Konelab 20 analyser (Thermo Electron, Courtaboeuf, France) to determine sodium, creatinine and protein concentrations. Urinary creatinine concentration was measured by high phase liquid chromatography (Dionex DX-500, Dionex corp., Voisins le Bretonneux, France). Analysis of plasma renin activity (PRA) was performed from aortic blood for 1-, 6- and 18-month-old rats by radioimmunoassay (GammaCoat Plasma Renin Activity ¹²⁵I RIA Kit, Stillwater, MN). Plasma glucose concentrations were determined immediately after sampling by the glucose-oxydase method using a glucose analyzer (Beckman Instruments, Fullerton, CA).

Histology

Histological analysis was performed on kidney samples taken at 1, 3, 6, 12, 18 and 23 months of age for both groups. Tissues were fixed in formalin, embedded in paraffin and cutted in 4 µm-thick transversal sections. Kidney sections were routinely stained with hematein-eosin, Masson's trichrome and silver staining. Semi-quantitative evaluation of glomerulosclerosis, interstitial fibrosis, tubular atrophy, and vascular lesions were performed in kidney sections from

both groups in a blinded fashion as previously described (16).

Computer assisted-morphometric analysis

Computer assisted-morphometric analysis was performed on kidney samples obtained at 1, 3, 6, 12 and 18 months for both groups, as previously described (17; 18). For each animal, the total glomerular surface area (TGA) was expressed as the mean of the values measured in 50 randomly sampled glomeruli (25 in the superficial cortex and 25 in the juxtamedullary cortex). The TGA, limited by the internal edge of Bowman's capsule, was determined in 1-, to 18-month-old rats. In addition the total area of capillary lumens (TCL) and total mesangial surface area (TMA) were measured in both groups at 6- and 18-month-old rats. The TCL/TGA and TMA/TGA ratio were then calculated for each glomerulus and expressed as the mean of 50 measured glomeruli for each animal.

Immunohistochemistry

Immunohistochemistry with anti-renin polyclonal antibody was performed on kidney sections with a 3-steps avidin-biotin immunoperoxidase method with prior antigen unmasking procedure as previously described (19). Renin immunoreactivity was then evaluated in a blinded fashion using a semi-quantitative scoring system as previously described (19).

Preparation of Membrane Fractions and Western blot analysis

Kidneys of both groups were removed from the anesthetized rats and cut into

5mm slices. Membrane fractions from the cortex and the outer medulla (inner stripe) were prepared and Western blot analysis was performed as previously described (20; 21), to identify Na transporters (primary antibodies: anti-NHE3 1:1 000; anti-NCC 1:50 000; anti-BSC1 1:5 000; anti- α ENaC 1:3 000; anti- β ENaC 1:20 000; anti- γ ENaC 1:2 000; anti-Na⁺/K⁺ATPase 1:20 000). After incubation with the appropriate peroxidase-conjugated secondary antibodies, blots were washed and luminol-enhanced chemiluminescence (ECL, Perkin Elmer Life Science Products, Inc. Boston, MA) was used to visualize bound antibodies before exposure to Hyperfilm ECL (Amersham, Arlington Heights, IL). The autoradiography was digitized with the use of a laser scanner (Epson Perfection 1650, Epson), and quantification of each band was performed by densitometry using NIH Image software. Densitometric values were normalized to the mean for the control group that was defined as 100%.

Statistical Analysis

All results are expressed as the mean \pm SEM values. Statistical analysis was performed by unpaired and paired Student's t-test or by analysis of variance (Anova) when appropriate. Statistical significance was defined as $p < 0.05$.

RESULTS

Gestational and delivery conditions and follow up of rats exposed *in utero* to maternal diabetes

Glycemia of the 16 diabetic mothers was constant throughout gestation and 4 fold higher than in the 16 controls (5.27 ± 0.34 mM versus 21.6 ± 0.12 mM). Gestation

occurred normally and the rats delivered spontaneously at term (21 days of gestation). The number of pups per litter was similar in both groups. At birth, pups appeared healthy with similar birth weight as compared to controls. As previously described (13), in our model, *in utero* exposure to maternal diabetes induced nephron deficit in the offsprings. At one month, the reduction of nephron number was about 30% in rats issued from diabetic mothers as compared to controls ($35 \ 133 \pm 507$ versus $25 \ 600 \pm 570$, respectively, $p < 0.0001$, $n=6$ in each group).

As shown in Table 1, mean body weight progressively increased with age and was similar in both groups from the newborn period until 3 months. However, at 6 and 12 months of age, it was significantly higher in DMO group than in CMO group. Kidney and heart weights (relative to body weight) decreased as a function of age but were not significantly different in both groups. Same normal blood glucose levels were observed in CMO and DMO (5.90 ± 0.23 mM versus 5.61 ± 0.70 mM in 6-month-old rats from both groups, $n=6$ from 3 litters).

Effects of *in utero* exposure to maternal diabetes on blood pressure

Systolic blood pressure

Rats exposed *in utero* to maternal diabetes demonstrated higher blood pressure in adulthood (Figure 1). While the systolic BP of 1-month and 3-month-old rats was similar in both groups, it was significantly increased from 6 months of age in DMO as compared to CMO. Hypertension lasted and further increased during the observation period.

Plasma renin activity and renal renin expression

PRA and RRE are given in Table 2. At the pre-hypertensive stage (1 and 3 months rats) PRA and RRE were not significantly different in the two groups. From 6 months of age (hypertensive stage), a significant decrease of PRA was observed in DMO, when compared with their respective age-matched CMO. With ageing, the reduction of PRA and RRE was present in both groups.

Effect of high salt diet on blood pressure and urinary sodium excretion

To study the response to a chronic alteration in sodium balance, both groups of rats were given a high salt diet (3% NaCl diet instead of 0.3%)(Figure 2). Means of the twenty-four-hours diet intake for the 3 days of sodium excretion measurement were similar in the two groups (28.32 ± 0.83 versus 28.03 ± 1.1 g in CMO and DMO group, respectively). Systolic BP was similar in the two groups on normal sodium diet (0.3%). High salt diet (3%) induced a raise of systolic BP in DMO group (respectively 131.7 ± 0.8 mmHg versus 154.5 ± 3.2 mmHg; paired t-test: $p < 0.01$) and had no effect on BP in CMO group.

As expected, high sodium diet led to a significant increase of urinary sodium excretion in both groups. However, this increase was significantly delayed in DMO as compared to CMO, accounting for a larger positive sodium balance.

Effect of high salt diet on relative abundance of renal sodium transporters

We assessed the effects of a high salt diet on sodium transporters protein by semiquantitative immunoblots of membrane fractions from the cortex and

outer medulla (inner stripe) obtained from the DMO and CMO kidney of 3-month-old rats (figure 3). In the cortex, both β - and γ -ENaC subunits were significantly upregulated in DMO as compared to CMO, while α -ENaC protein abundance was unchanged. Na/K ATPase protein abundance was also significantly upregulated in DMO. In the medulla, the high salt diet led to a decrease in BSC1 protein abundance in the DMO group as compared to the CMO group. Protein levels of α -, β - and γ -ENaC were unaffected.

Finally, the protein levels of NHE3 and NCC were not different in DMO receiving a high salt diet, as compared to CMO neither in the cortex nor in the medulla.

Renal effects of *in utero* exposure to maternal diabetes

Renal function and proteinuria

Table 3 reports the follow-up of proteinuria and glomerular filtration rate (GFR) estimated by the creatinine clearance, in 1 to 18 months rats of both groups. The creatinine clearance progressively increased with age in both group and was significantly lower in DMO as compared to CMO. GFR was reduced by about 10% in 3-month-old rats and by 30% from 6 to 18 months period. Proteinuria levels increased in both groups with ageing and were significantly higher in DMO.

Glomerulus histomorphometry

To address the question of a structural glomerular adaptation to the reduction of nephron number, a glomerular histomorphometry study was performed in kidneys taken from 1- to 18-month-old CMO and DMO rats (Figure 4). In each age group no significant differences were

observed between glomeruli measured in either superficial or deep cortex areas between CMO and DMO kidney rats (data not shown). TGA significantly increased with advancing age. TGA was not significantly different between the 2 groups, although it was transitory increased in 6-month-old DMO rats. TCL was significantly increased in the 6-month-old DMO as compared to the CMO. TCL/TGA was similar in both groups. At 18 months of age, TMA and TMA/TGA were similar in DMO and CMO groups.

Renal histopathology

Renal histology was assessed in 1- to 18-month-old CMO and DMO rats. Before 18 months of age, all kidneys were normal and devoided of glomerulosclerosis and interstitial fibrosis (data not shown). At 18 months of age, renal lesions were limited to focal areas of interstitial fibrosis with minimal tubular atrophy and to very few glomeruli with segmental glomerulosclerosis (Figure 5, A-B). Semi-quantitative analysis showed that the extent of both glomerulosclerosis (Glomerulosclerosis index: CMO = 10.08 ± 1.62 , n=13 rats from 3 different litters vs DMO = 8.49 ± 1.91 , n=9 rats from 2 different litters; unpaired t-test ; NS, arbitrary units) and tubulointerstitial lesions (Interstitial fibrosis index: CMO = 5.12 ± 1.69 , n=8 rats from 2 different litters vs DMO = 7.80 ± 4.25 , n=5 rats from 3 different litters; unpaired t-test ; NS, arbitrary units) were not different in the two groups.

Survival study

Survival study showed an increased mortality after 18 months of age in DMO. At 23 months, long-term survival was markedly reduced in the DMO group,

33.3 % as compared to 85.7% in the CMO group. We therefore decided to sacrifice the remaining rats to address late kidney histopathology in rats of the two groups.

A widespread interstitial fibrosis with tubular atrophy and dilatation was present in the kidneys of DMO rats, associated with glomerulosclerosis and glomerular cysts. A scarce interstitial fibrosis and tubular atrophy was present in CMO rats (Figure 5, C-F). Glomerulosclerosis index is 7.25 ± 3.50 , n=6 in CMO versus 87 and 66 in 2 DMO rats and interstitial fibrosis index is 27.5 ± 24.3 , n=6 in CMO versus 230 and 300 in 2 DMO rats. No structural changes in the intra-renal vessels were observed at any age.

Discussion

The present study identifies, in the rat, the long term consequences of *in utero* exposure to maternal diabetes and shows in adulthood that *in utero* exposure is associated with the development of a salt-sensitive systolic hypertension and with a decreased in renal function.

A mild to moderate increase in systolic arterial blood pressure is observed in the offspring of diabetic mothers from 6 months of age, which progressively went worse with the age. This raise of blood pressure is associated with low PRA, suggesting a salt-sensitive hypertension. We further confirm the salt sensitivity with high sodium diet experiments performed at prehypertensive stage: in DMO rats a high sodium diet induced increase of systolic blood pressure and led to a shift to the right of the urinary sodium excretion curve, indicating a delayed sodium excretion. A similar salt-sensitive hypertension has been reported in neonatal uni-nephrectomized rat model by Woods et al. (22). Together, these

results are in accordance with Brenner's hypothesis (23) which states that inborn nephron deficit predispose to reduced renal sodium excretion leading to hypertension susceptibility, especially in the setting of dietary sodium excess. Interestingly, in inbred rat models of hypertension, the relationship between nephron number and blood pressure is still a matter of debate (24-26). In addition, recently Rocha et al. and Magaton et al. (8; 9) reported hypertension in rats issued from diabetic mothers without inborn nephron deficit. However, their model slightly differs from ours: i. the level of maternal hyperglycemia is more pronounced in our model (we have previously shown that inborn nephron deficit correlated with the level of maternal hyperglycemia (13)) ii. we performed direct glomerular counting with the gold-standard acid-maceration method while they used an histological-derived method that is more appropriate to evaluate the density of glomeruli.

Because one mechanism involved in such renal sub-optimal sodium handling may traduce a re-setting of tubular sodium cotransporters expression, we then evaluate at the pre-hypertensive stage and under high-salt diet, the protein abundance of Na transporters and channels in the kidney. In the cortex of DMO rats we found an increase of three different sodium transporter proteins: β ENaC, γ ENaC and Na-K ATPase. Such relative increases without decreases in other sodium transporters would be predicted to result in enhanced tubular Na reabsorption and might play a role in the development or maintenance of elevated blood pressure in these animals (27). The decrease in BSC1 in the medulla may reflect a medullary compensatory effect linked to the increase of cortical sodium transporters. An abnormal expression of

Na transporters have also been reported by Manning et al (28). In their model, maternal protein restriction is associated with low birth weight, development of hypertension at 8 weeks of age and significant increased expression in the sodium co-transporters BSC1 and TSC (28). Together, these results strongly substantiate a perinatal re-programmation of tubular functions regulation in association or as a result of inborn nephron deficit (29).

In the present study, we also show that maternal diabetes is associated with a decreased renal function in adulthood, as assessed by creatinine clearance and proteinuria measurements. According to Brenner et al, a compensatory glomerular hypertrophy and hyperfiltration occur in response to inborn nephron deficit, to sustain adequate renal function (12; 23). Such glomerular adaptation made at the expense of intraglomerular hypertension accelerates injury to the remaining functional glomeruli and perpetuates the ongoing glomerulosclerosis, leading to renal failure. However, in our model where inborn nephron deficit is observed, systematic analysis of renal histology shows that both glomerulosclerosis and interstitial fibrosis are absent in DMO and CMO groups at all stages before 18 months of age. At 18 months, accounting to aging (17), mild renal structural lesions occur but at the same extent in the two groups. In addition, glomerular computer-assisted histomorphometry shows a mild glomerular hypertrophy only at 6 months, at the onset of hypertension in DMO rats. In our model, absence of early increase of glomerular size and of sustained glomeruli hypertrophy with aging does not support a major glomerular adaptation to the inborn nephron deficit. Together these two set of results seem to weight against a compensatory glomerular hypertrophy

and hyperfiltration as factors of alteration and worsening of the renal function until 18 months of age. Concerning the study of Rocha et al., one must note that although decreased nephron number was not observed in young animals, the number of nephrons was reduced at 12 months in DMO rats (8). However, since no histological data was provided in their study to evaluate the extent of glomerulosclerosis, the mechanisms of ongoing nephron loss and its implication in the decline of renal function has not been elucidated in their model.

In the other hand, a dramatic impairment of renal histology with widespread glomerular and tubulo-interstitial lesions was observed in kidneys issued from 23-month-old DMO rats. Such late alterations of kidney structures compared with the early impairment of renal function could hardly be explained by the consequences of a congenital nephron deficit alone. Indeed, the link between the congenital nephron deficit and the adverse effect of compensatory hyperfiltration on the remaining glomeruli leading to glomerulosclerosis has been unambiguously demonstrated (and quantitatively assessed) in models with severe reduction in the nephron number or in models with toxic renal impairment that associates both *in utero* glomerular reduction and tubulo-interstitial lesions (30; 31). In our present model, the mild (30%) congenital nephron deficit might not be sufficient *per se* to induce glomerular lesions, at least during the first 18 months of life.

Another issue is to consider the kidney as a target organ of hypertension. In our model, although hypertension is present from 6 months of age, high level

of systolic blood pressure is only achieved at 18 months. It is well known that the increase of blood pressure is limited and slowly increases with age in the majority of the model of perinatal programming of hypertension as compared to other rat models of hypertension (32; 33). This may explain why we observe the histopathological renal consequences of hypertension only in the 23-month-old rats. However, the implication of hypertension alone in the renal lesions of our model is questionable, since, even at 23 months, intra-renal vascular hypertensive lesions are nearly absent at histology, and myocardial (other target organ of hypertension) histology is similar in both groups (data not shown).

This study thus identifies maternal diabetes as a novel risk factor for fetal programming of adult hypertension and impairment of renal function. Alteration of renal sodium handling, observed in our model, may be explained by a fetal re-setting of tubular functions, as a consequence or in association with congenital nephron deficit.

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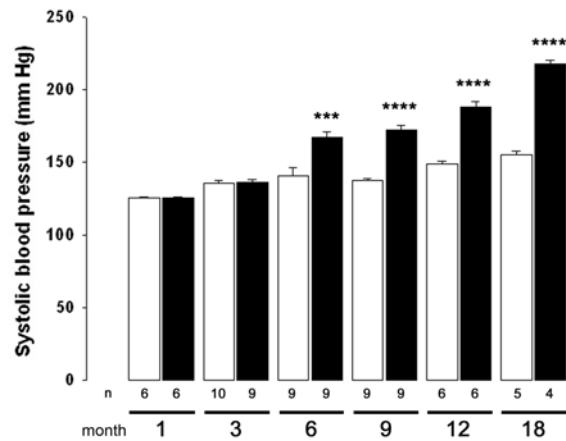


Figure 1: Effect of maternal diabetes on blood pressure levels in the offspring.

Systolic blood pressure significantly increased from 6 months in the offspring from diabetic mothers (DMO group, black bars) as compared to CMO group (open bars). Results are expressed as mean \pm SEM, n = 4 to 10 animals in each group, issued from 2 or 3 litters. (Two-way Anova: Age effect, $p < 0.0001$; group effect, $p < 0.0001$; age X group interaction: $p < 0.0001$ and unpaired t-test between age-matched groups : *** $p < 0.001$, **** $p < 0.0001$).

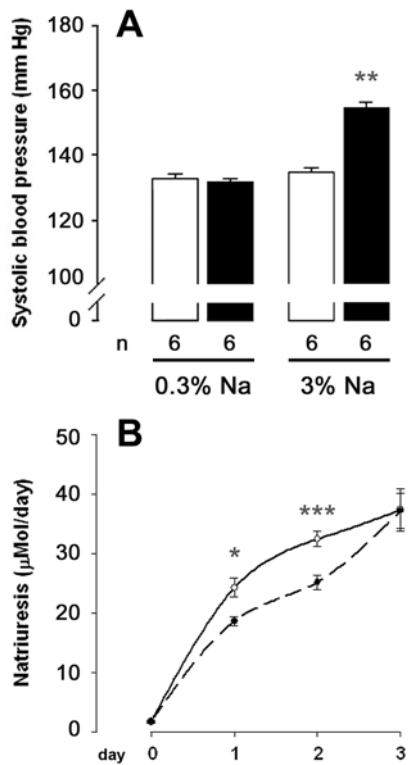


Figure 2: Effect of High sodium diet on systolic blood pressure and on sodium urinary excretion.

Systolic blood pressure (panel A) was measured in 3-month-old rats under 0.3% sodium diet (0.3% Na) and after 7 days of sodium rich diet (3% Na) in CMO (open bars) and DMO (black bars). Sodium-rich diet induced a significant increase of systolic blood pressure in 3-month-old DMO rats ($n=6$ in each group, issued from 2 litters, paired t-test: ** = $p<0.01$) and remains ineffective on systolic blood pressure of CMO rats. Natriuresis (panel B) was followed for 3 days after the onset of high-sodium diet in the same groups of animals (CMO: plain line, DMO: hatched line). Na urinary excretion was impaired DMO rats as compared to CMO rats ($n=6$ in each group, issued from 2 litters, two ways anova: time effect: $p<0.0001$; group effect: $p<0.05$; time X group interaction: $p=0.09$ and unpaired t-test in age-matched animals: * $p<0.05$, *** $p<0.001$).

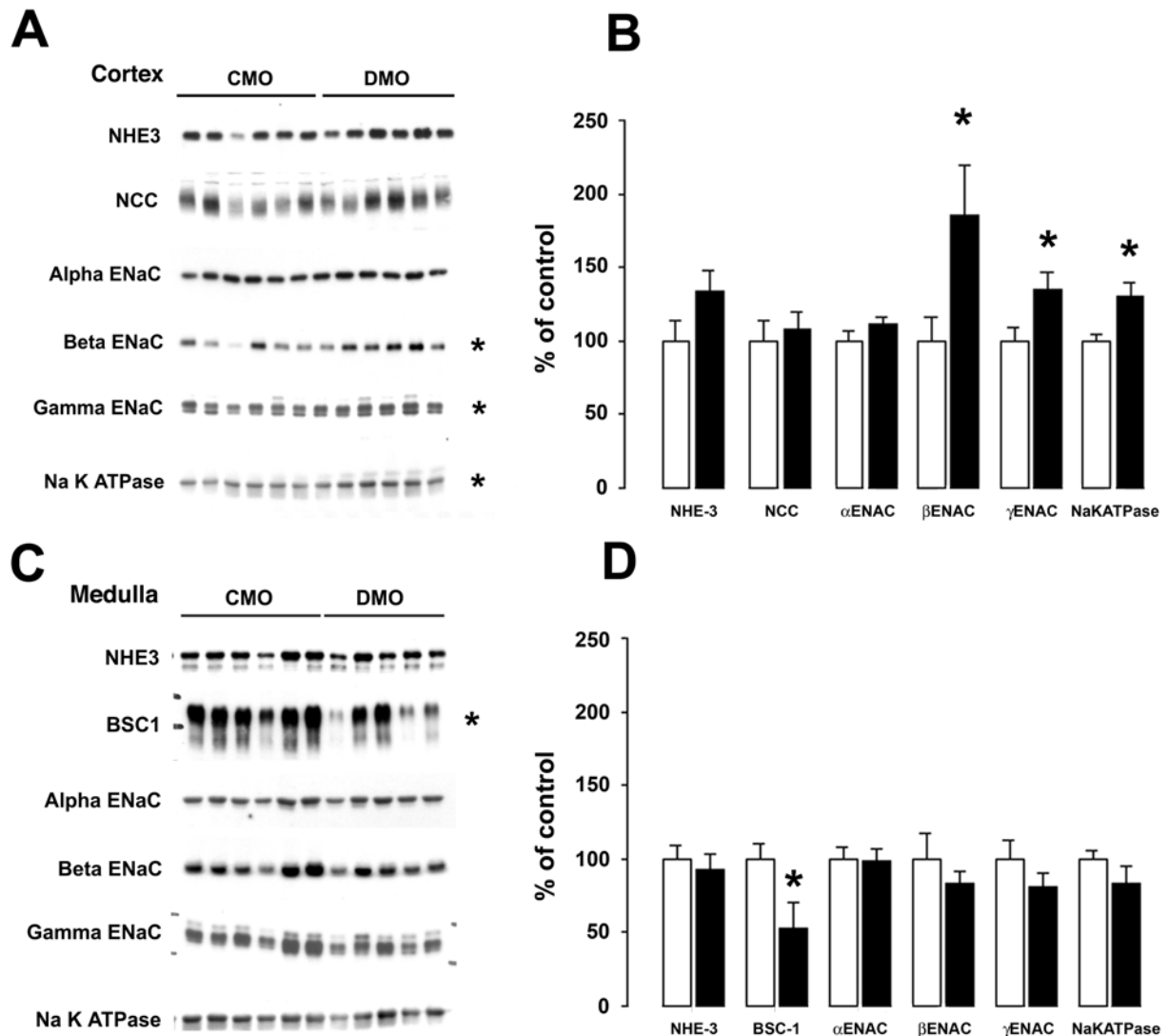


Figure 3: Cortical and medullar renal sodium transporter abundance profile in high-sodium rich diet.

Effect of dietary NaCl loading for 10 days on major renal sodium transporter proteins abundance in DMO and CMO kidney. Semiquantitative immunoblots of membrane fractions from the cortex (A) and outer medulla (inner stripe) (C) obtained from DMO and their CMO. For each blot, each lane was loaded with a homogenate from a different rat ($n = 6$ for both CMO and DMO in cortex; $n = 5$ for CMO and $n = 6$ for DMO in the medulla). Densitometric analyses revealed a significant increase in β - and γ -EnaC and Na/K ATPase abundance in DMO compared with CMO in the cortex (panel B) and a significant decrease in BSC1 in the outer medulla (panel D). The results are expressed as the percentage of the control values. Bars represent means \pm SEM (unpaired t-test between DMO and CMO groups: * $p < 0.05$).

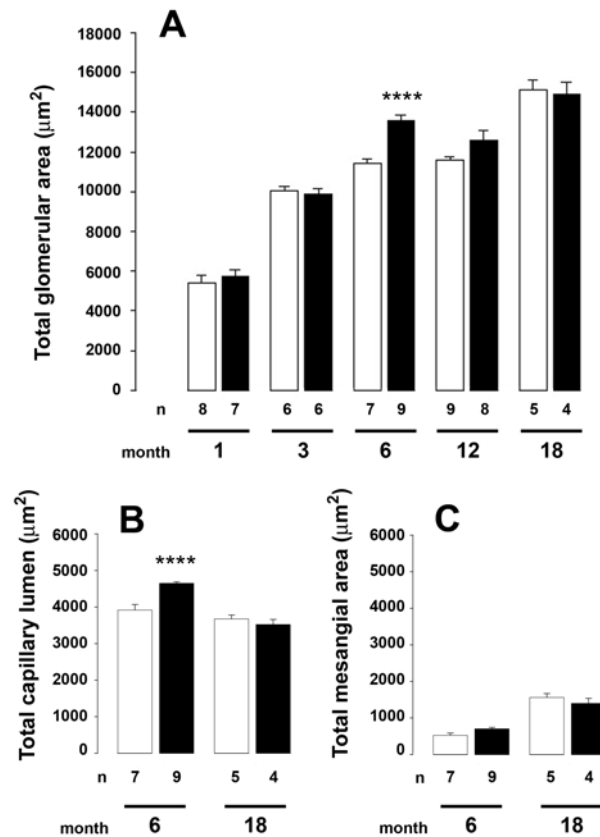


Figure 4: Effect of maternal diabetes on glomerular morphometry.

Total glomerular area (A) increased with ageing in CMO group (open bars) and in DMO group (black bars). Results are expressed as mean \pm SEM, n = 4 to 9 animals in each group, issued from 2 to 3 litters (two-way Anova: age effect: $p < 0.0001$; group effect: $p < 0.01$, age X group interaction: $p < 0.01$). Total glomerular area was significantly increased in 6 month-old DMO rats as compared to age-matched CMO rats while the difference did not achieve statistical significance at 12 months. Total capillary lumen surface (B), total mesangial area (C) were measured at 6 and 18 months in CMO and in DMO. Total capillary lumen surface was increased in 6-months-old DMO rats as compared to CMO rats (unpaired t-test between age-matched groups: ^{****} $p < 0.0001$).

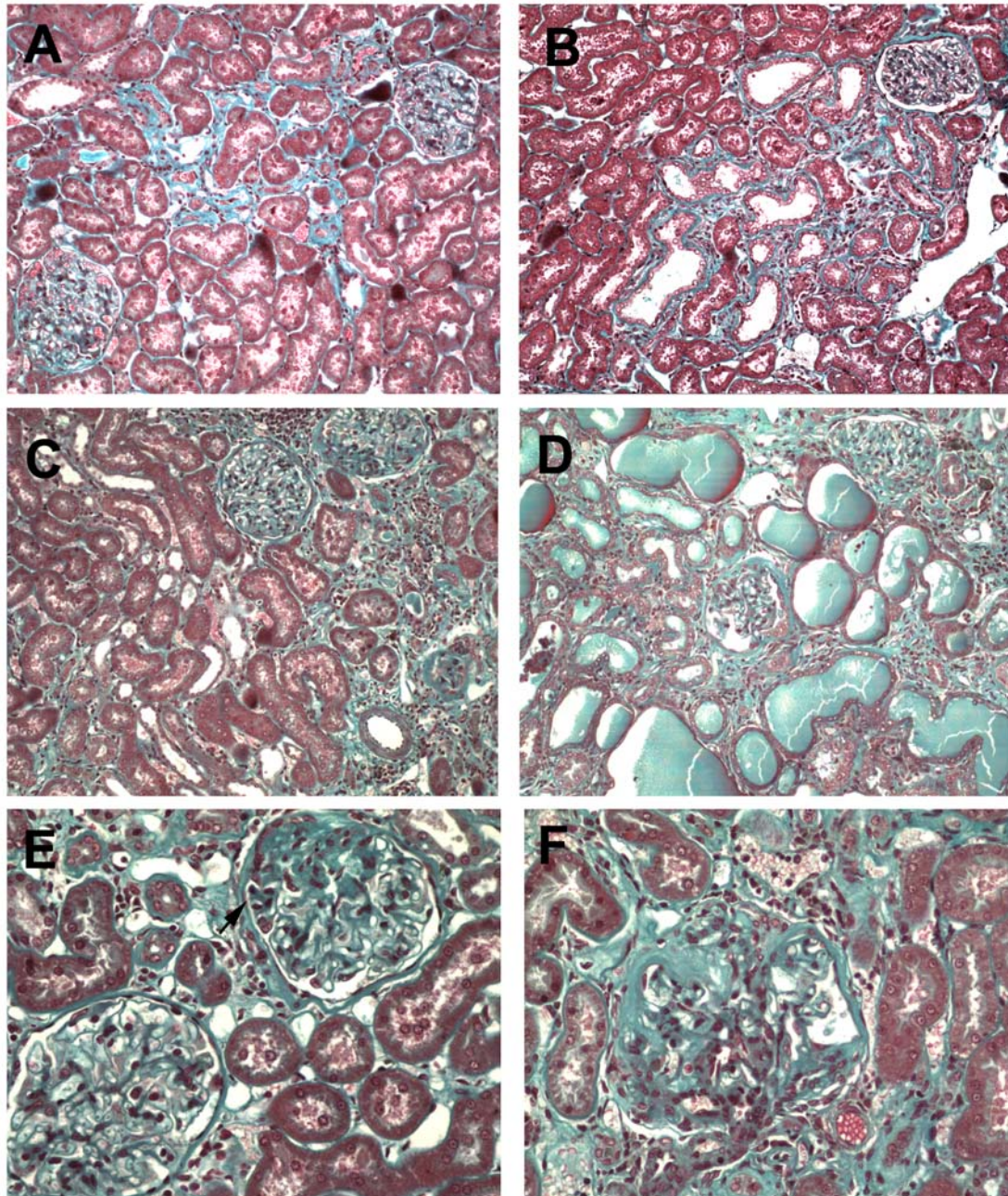


Figure 5: Renal histopathology in 18- and 23-month-old rats.

Histopathology was performed at 18 months (panels A-B) and at 23 months (panel C-F) in kidney from CMO (panels A, C, and E) and from DMO (panels B, D, and F). Similar focal area of interstitial fibrosis and tubular atrophy in CMO (panel A) and DMO (panel B) 18-month-old rats. Widespread interstitial fibrosis and tubular atrophy with severe glomerulosclerosis were present in 23-month-old DMO rats (panel D and F) as compared with limited alteration in CMO (panel C and E, segmental glomerulosclerosis: arrow). Masson's trichrome. Magnification A-D: X200; C-F: X800.

