

## Renal Changes in the Early Stages of Diet-Induced Obesity in Ovariectomized Rats

L. S. B. AMARAL<sup>1</sup>, J. A. SILVA<sup>1</sup>, T. M. TRINDADE<sup>1</sup>, W. B. D. RIBAS<sup>1</sup>, C. L. MACEDO<sup>2</sup>, T. M. COIMBRA<sup>3</sup>, N. O. BELO<sup>1</sup>, A. C. M. MAGALHÃES<sup>1</sup>, T. J. SOARES<sup>1</sup>

<sup>1</sup>Multidisciplinary Institute of Health, Federal University of Bahia, Bahia, Brazil, <sup>2</sup>University of Southwest of Bahia, Vitória da Conquista, Bahia, Brazil, <sup>3</sup>Department of Physiology, University of São Paulo at Ribeirão Preto School of Medicine, Ribeirão Preto, São Paulo, Brazil

Received July 22, 2013

Accepted March 21, 2014

On-line August 26, 2014

### Summary

The relationship between obesity and renal lesions, especially in low estrogen levels, has been less documented. The aim of this study was to assess the renal changes in diet-induced obesity in ovariectomized rats. Wistar rats were ovariectomized or sham-operated and divided into four groups: sham-operated rats fed a standard diet (SSD); ovariectomized rats fed a standard diet (OSD); sham-operated rats fed a high-fat diet (SHFD); ovariectomized rats fed a high-fat diet (OHFD). Body weight and blood pressure were measured weekly. The rats were killed 24 weeks after initiation of standard or high-fat diet treatment, the kidneys were removed for immunohistochemical and histological studies. Blood and urine samples were collected to quantify sodium, potassium and creatinine. OHFD rats presented increases in visceral adipose tissue, serum insulin levels, blood pressure and proteinuria, and a decrease in fractional excretion of sodium as well. Histological and morphometric studies showed focal alterations in the renal cortex. Expression of macrophages, lymphocytes, nuclear factor-kappa B (NF- $\kappa$ B), Proliferating Cell Nuclear Antigen (PCNA), angiotensin II (ANG II) and vimentin was greater in OHFD rats than in control rats. Thus, these results demonstrate that the high-fat diet in ovariectomized rats promoted renal function and structure changes, renal interstitial infiltration of mononuclear cells and increased expression of ANG II and NF- $\kappa$ B.

### Key words

Kidney • Obesity • Diet • Ovariectomy • Renal inflammation

### Corresponding author

T. de Jesus Soares, Instituto Multidisciplinar em Saúde,

Universidade Federal da Bahia, Rua Rio de Contas, 58, Candeias, CEP: 45.029-094, Vitória da Conquista, Bahia, Brazil. Fax: 55-77-3429-2709. E-mail: tesoares@ufba.br

### Introduction

Obesity is a complex disease often associated to hypertension, cardiovascular diseases, and metabolic disorders. The combination of these chronic diseases is called metabolic syndrome (Grundy 2004). However, renal risks induced by obesity, especially its role in initiation and progression of renal diseases, have only been recently recognized (Abrass 2004, Tang *et al.* 2012). Several studies have demonstrated that the association of insulin resistance, hyperinsulinemia and abdominal obesity promotes an increase in the incidence of kidney disease (Hall 1997, Hall *et al.* 2002, Fujita 2008), even when there is no evidence of hypertension, diabetes, or preexisting renal disease. Therefore, metabolic syndrome may induce renal damage before the appearance of hypertension and diabetes (Fox *et al.* 2004, Hsu *et al.* 2006).

Excess weight gain and obesity may cause renal changes, including glomerular hyperfiltration, sodium retention, enlargement of Bowman's space, increased glomerular cell proliferation, mesangial matrix expansion, inflammatory cell infiltration and tubulointerstitial lesions (Coimbra *et al.* 2000, Tang *et al.* 2012). These early renal alterations can progress to more intense and diffuse lesions in the kidneys, such as focal segmental glomerulosclerosis, proteinuria and tubulointerstitial lesions, which are observed in prolonged obesity (Hall *et al.* 2004).

Although the association between obesity and kidney disease has been established, the pathogenic mechanisms involved are still unclear. Metabolic, hemodynamic and inflammatory factors, observed in obesity, may contribute to the development and progression of renal disease. Obesity raises blood pressure by increasing renal tubular reabsorption of sodium, causing volume expansion due to activation of the sympathetic nervous system and renin-angiotensin system (Hall 1997, Hall *et al.* 2002, Rahmouni *et al.* 2005).

Moreover, metabolic changes found in obesity such as insulin resistance, hyperinsulinemia, hyperleptinemia and abnormal lipid metabolism, can also promote podocyte damage leading to proteinuria and renal injury (Coimbra *et al.* 2000, Abrass 2004, Hall *et al.* 2004). Early progressive podocyte damages and macrophage infiltration is associated with hyperlipidemia and type II diabetes mellitus in Zucker rats and precedes both the development of glomerulosclerosis and tubule interstitial lesions (Coimbra *et al.* 2000). Macrophages can produce many cytotoxic products, including reactive oxygen species (ROS), cytokines, chemokines and angiotensin II (ANG II) (Rodríguez-Iturbe *et al.* 2004, Akcay *et al.* 2009), which can activate the nuclear factor-kappa B (NF- $\kappa$ B) pathway (Guijarro and Egido 2001, Zheng *et al.* 2008). Upon stimulation, NF- $\kappa$ B is released from an inhibitory subunit (I $\kappa$ B) and translocates into the nucleus, where it promotes the transcriptional activation of inflammatory genes (adhesion molecules, proinflammatory cytokines and chemotactic factors for macrophages and monocytes). Infiltration of lymphocytes and macrophages is also associated to increased intrarenal ANG II activity, which can induce sodium retention and hypertension (Rodríguez-Iturbe *et al.* 2002, Rodríguez-Iturbe *et al.* 2004).

On the other hand, the prevalence of renal and cardiovascular diseases has been related to gender and blood levels of sex hormones (Dubey and Jackson 2001, Neugarten 2002). Estrogen promotes the accumulation of subcutaneous fat, and the loss of estrogen in menopause or ovariectomy in experimental animals, is associated with an increase in visceral fat (Shi and Clegg 2009). However, there are few studies investigating the relationship between obesity and development of renal lesions, especially in conditions of decreased levels of ovarian hormones. We hypothesized that high-fat diet in ovariectomized rats might contribute to renal changes. The objective of the present study was to evaluate the effects of a high-fat diet on the renal function and structure in ovariectomized rats.

In addition, we evaluated macrophages and lymphocytes infiltration, and ANG II and NF- $\kappa$ B expressions in the renal cortex of these animals.

## Methods

### *Animals, diet and experimental protocols*

A total of 26 female Wistar rats weighing 150-200 g were used in this study. All experimental procedures were conducted in accordance with the recommendations of the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the Ethics Committee in Animal Experimentation of the State University of Feira de Santana. The rats were anesthetized with ketamine (50 mg/kg) and xylazine (5 mg/kg), submitted to ovariectomy or sham surgery and divided into four distinct groups: sham-operated rats fed a standard diet (SSD), n=6; ovariectomized rats fed a standard diet (OSD), n=6; sham-operated rats fed a high-fat diet (SHFD), n=6; ovariectomized rats fed a high-fat diet (OHFD), n=8. All rats were maintained on their diet for 24 weeks. Diets were manufactured by Pragsoluções (São Paulo, SP, Brazil). The composition of the diets is listed on Table 1. The body weight and blood pressure were measured weekly and blood and urine samples were collected for the analysis of renal function. At the end of 24 weeks, the animals were killed by decapitation and their kidneys were removed for histological, morphometric and immunohistochemical analysis. The visceral adipose tissue of each animal was removed and weighed. The trunk blood was collected for analysis of renal function and serum insulin levels.

### *Blood pressure*

For the measurement of blood pressure, conscious rats were individually warmed at 37 °C for 5 min. Blood pressure and heart rate were measured by a programmable tail-cuff sphygmomanometer (LE 5001 Electro-Sphygmomanometer – Panlab, Spain).

### *Plasma insulin and TNF- $\alpha$*

Plasma insulin levels were assayed by a radioimmunoassay kit (Rat insulin RIA kit; Linco Research, St. Charles, MO, USA), through the Count ® kits Coat (DPC – Diagnostic Products Corporation). The plasma TNF- $\alpha$  levels were determined by enzyme immunoassay (ELISA) using commercially available kit (Biosource) according to the manufacturer's instructions.

The TNF- $\alpha$  concentrations were calculated using a standard curve included on the same plate as the samples.

**Table 1.** Ingredients and chemical composition of nonpurified experimental diets.

	Control diet	High-fat diet
<b>Ingredient (g/kg of diet)</b>		
<i>Cornstarch</i>	373.8	138.8
<i>Soybean meal</i>	305	305
<i>Sucrose</i>	65	60
<i>Bovine lard</i>	0	240
<i>Maltodextrin</i>	100	100
<i>Calcium salts of soybean fatty acids</i>	75	75
<i>Cellulose</i>	31.7	31.7
<i>L-cystine</i>	3	3
<i>Choline bitartrate</i>	1.5	1.5
<i>Mineral Mix</i> <sup>1</sup>	35	35
<i>Vitamin Mix</i> <sup>2</sup>	10	10
<i>Tert-butylhydroquinone</i>	0.01	0.03
<b>Chemical composition (Dry matter %)</b>		
<i>Crude protein</i>	15.10	15.00
<i>Fat</i>	6.97	30.85
<i>Total carbohydrates</i>	66.76	42.97
<i>Ash</i>	2.78	2.74
<i>Metabolizable energy (Mcal/kg)</i>	3.99	5.19
<b>% of Metabolizable energy</b>		
<i>Protein</i>	32.36	24.89
<i>Carbohydrates</i>	54.25	23.24
<i>Fat</i>	12.39	51.1
<i>Vitamin</i>	1.00	0.77

<sup>1</sup> Mineral mix AIN-93G-MX, <sup>2</sup> Vitamin Mix AIN-93-VX.

### Renal function

The rats were placed in metabolic cages and urine and blood samples were collected to measure creatinine, sodium and potassium levels. Plasma and urine creatinine was measured by the Jaffé method (Haygen 1953), and glomerular filtration rate (GFR) was determined by creatinine clearance. The levels of sodium and potassium were measured using a flame photometry in order to determine the fractional

excretions of these ions. The quantification of urinary protein was performed by colorimetric method using a commercial kit (Sensiprot; Labtest®, MG, Brazil).

### Renal morphology

The kidneys samples from rats of all groups were fixed in methacarn solution (methanol 60 %, chloroform 30 %, and 10 % acetic acid) and processed for paraffin embedding. Four- $\mu$ m histological sections were stained with Masson's trichrome and examined under light microscopy. Tubulointerstitial injury was defined as inflammatory cell infiltrate, tubular lumen dilation or tubular and interstitial fibrosis. Damage was graded according to Shih *et al.* (1988) on a scale ranging from 0 to 4 (0 = normal; 0.5 = small focal areas; 1 = involvement of less than 10 % of the cortex; 2 = 10-25 % involvement of the cortex; 3 = 25-75 % involvement of the cortex; 4 = extensive damage involving more than 75 % of the cortex). The incidence of glomerulosclerosis was determined by scoring of 100 glomeruli within a section of each kidney. Each glomerular area was graded semi quantitatively and the mean score per kidney was calculated. Each score reflects the extent of the damage: 0 = absent or less than 5 %; 1 = sclerotic area >5 to 25 % (minimal sclerosis); 2 = 25 to 50 % of sclerotic area (moderate sclerosis); 3 = 50 to 75 % of sclerotic area (moderate-severe sclerosis); 4 = 75 to 100 % of sclerotic area (severe sclerosis). Then, the glomerulosclerosis index (GSI) was calculated using the following formula:  $GSI = (1 \times n_1) + (2 \times n_2) + (3 \times n_3) + (4 \times n_4)/n_T$ , where  $n_x$  is the number of glomeruli in each grade of glomerulosclerosis and  $n_T$  is the total number of glomeruli (Saito *et al.* 1987). Morphometric studies of the glomerular tuft were performed with a light camera connected to an image analyser (Kontron Electronic System KS 300, Eching, Germany). Encircled areas were determined by computerized morphometry. This analysis of all the procedures was performed by the investigator blinded to the origin of the slides.

### Immunohistochemical studies

The sections of renal tissue were deparaffinized and incubated overnight at 4 °C with a polyclonal anti-angiotensin II (Peninsula Laboratories, CA, USA) 1/200, a polyclonal anti-NF- $\kappa$ B p65 (Santa Cruz Biotechnology, CA, USA) 1/200, or for 1 h at room temperature with anti-CD43 (Harlan Sera-Lab, USA) 1/200, anti-rat-ED1-macrophages/monocytes (Serotec, UK) 1/1000, anti-

vimentin (Dako, Glostrup, Denmark) 1/500, a marker of tubular injury and anti-PCNA (proliferating cell nuclear antigen) 1/1000 monoclonal antibodies. The reaction product was detected with an avidin-biotin-peroxidase complex (Leica Biosystems Newcastle, United Kingdom). The color reaction was developed with 3,3-diamino-benzidine (Easypath®), the material was counterstained (hematoxylin or methylgreen), dehydrated and mounted. Nonspecific protein binding was blocked by incubation with 20 % goat serum in PBS for 20 min. Negative controls consisted of replacement of primary antibody with equivalent concentrations of normal rabbit IgG or mouse IgG for polyclonal and monoclonal antibodies, respectively.

For the evaluation of immunoperoxidase staining for vimentin, each tubulointerstitial grid field of renal cortex (measuring 0.245 mm<sup>2</sup> each) was graded semiquantitatively, and the mean score per kidney (ranging from 0 to IV) was calculated (Coimbra *et al.* 2000). Each score primarily reflected changes in the extent, rather than the intensity, of staining and depended on the percentage of grid field showing positive staining: absent or less than 5 % = 0; 5-25 % = 1; >25-50 % = 2; >50-75 % = 3; and >75 % = 4. This semiquantitative scoring system has been reported to be reproducible among different observers. Thirty consecutive grid fields were analyzed and determined an average score for each kidney (Lewers *et al.* 1970). The number of ED-1, angiotensin II, lymphocyte, PCNA and NF- $\kappa$ B positive cells were counted in 30 grid fields (measuring 0.245 mm<sup>2</sup>) from tubule interstitial compartment of the renal cortex, and mean counts per kidney were calculated.

#### Statistical analysis

Data of urine flow rate, proteinuria, tubulointerstitial lesions, glomerulosclerosis index and vimentin scores were submitted to Kruskal-Wallis nonparametric test with multiple comparisons by Dunn test, and expressed as median and percentile 25 and 75. The other data were submitted to analysis of variance with the Newman-Keuls multiple comparisons test and are expressed as mean  $\pm$  SEM. The level of statistical significance was set at  $p < 0.05$ .

## Results

#### Body weight, visceral fat weight and caloric intake

At the end of 24 weeks of the diet, the OHFD group had significantly higher body weight than all groups

( $p < 0.05$ ) (Table 2). Visceral fat was also higher in the OHFD group compared with the SSD and OSD groups ( $p < 0.05$ ). As expected, due to the greater caloric density of the high-fat diet, caloric intake was higher in the OHFD and SHFD than SSD and OSD groups ( $p < 0.05$ ) (Table 2).

#### Blood pressure and heart rate

The OHFD group showed systolic blood pressure (SBP) (mm Hg) higher than all experimental groups ( $p < 0.05$ ) (Table 2). Mean arterial pressure was also higher in the OHFD when compared to SSD and SHFD groups ( $p < 0.05$ ). There were no significant changes in diastolic blood pressure (Table 2) or heart rate (data not shown) between groups.

#### Plasma insulin and TNF- $\alpha$

The OHFD group showed an increase in plasma insulin levels compared with all other groups ( $p < 0.001$ ) (Table 2). In addition to increased plasma levels of insulin, the OHFD group also presented insulin resistance (data not shown). Plasma TNF- $\alpha$  levels were undetectable in all experimental groups.

#### Renal function

The OHFD group showed a decrease in fractional excretion of sodium (FENa%) compared with other groups ( $p < 0.05$ ), as well as increased urinary protein excretion compared with OSD group ( $p < 0.05$ ) (Table 3). There was no statistical difference in the levels of fractional excretion of potassium (FEK%), plasma creatinine, GFR and urinary flow between the experimental groups (Table 3).

#### Morphological studies

Histological studies showed larger areas of focal lesions in the tubulointerstitial compartment from the renal cortex in OHFD rats compared to the other groups ( $p < 0.01$ ) (Fig. 1A and B, Table 4). These lesions were characterized by inflammatory infiltrates, tubular dilation, tubular atrophy and interstitial fibrosis (Table 4, Fig. 1B). Histological sections of SSD and SHFD rats were not shown due to similarities with OSD group. Similarly, the GSI of OHFD group was significantly higher compared with the GSI of all other groups ( $p < 0.001$ ) (Table 4). Morphometric studies showed increase in glomerular tuft area of cortical glomeruli from OHFD and in SHFD rats compared to SSD and OSD groups ( $p < 0.05$ ) (Table 4). There were no statistical differences in glomerular tuft area of the juxtamedular glomeruli between experimental groups (data not shown).



**Table 2.** Caloric intake, body weight, visceral fat weight, blood pressure and plasma insulin of sham-operated fed a standard diet (SSD), ovariectomized fed a standard diet (OSD), sham-operated fed a high-fat diet (SHFD) and ovariectomized fed a high-fat diet (OHFD) rats.

	SSD (n=6)	OSD (n=6)	SHFD (n=6)	OHFD (n=8)
<i>CI</i>	60.5±4.7	62.9±3.1	81.3±3.6 <sup>#</sup>	79.1±3.1 <sup>#</sup>
<i>BW</i>	316.3±19.1	356.6±16.7	343.8±12.9	423.9±16.4*
<i>VFW</i>	3.87±0.73	4.32±0.63	6.08±0.58	7.13±0.77 <sup>#</sup>
<i>SBP</i>	109.7±1.70	109.1±3.12	111.6±1.05	120.6±2.25*
<i>DBP</i>	89.4±1.5	92.6±4.3	86.6±2.9	96.2±2.9
<i>MAP</i>	96.4±1.7	97.7±3.8	94.5±2.2	105.0±2.3 <sup>#</sup>
<i>P<sub>insulin</sub></i>	0.262±0.07	0.256±0.06	0.242±0.06	1.084±0.16***

Data are expressed as mean ± SEM. CI, caloric intake (kcal/day); BW, Body weight (g); VFW, visceral fat weight (g/100 g BW); SBP, systolic blood pressure (mm Hg); DBP, diastolic blood pressure (mm Hg); MAP, mean arterial pressure (mm Hg); *P<sub>insulin</sub>*, plasma insulin concentration (ng/ml). \*p<0.05 versus SSD, OSD and SHFD; #p<0.05 versus SSD and OSD; \*\*\*p<0.001 versus SSD, OSD and SHFD.

**Table 3.** Parameters of renal function of sham-operated fed a standard diet (SSD), ovariectomized fed a standard diet (OSD), sham-operated fed a high-fat diet (SHFD) and ovariectomized fed a high-fat diet (OHFD) rats.

	SSD (n=6)	OSD (n=6)	SHFD (n=6)	OHFD (n=8)
<i>P<sub>creat</sub></i>	0.54±0.11	0.74±0.04	0.65±0.09	0.60±0.10
<i>GFR</i>	0.46±0.12	0.35±0.08	0.32±0.03	0.39±0.06
<i>FE<sub>Na+</sub></i>	0.38±0.07	0.42±0.05	0.40±0.07	0.18±0.02*
<i>FE<sub>K+</sub></i>	70.14±14.3	49.24±17.6	50.19±7.21	48.82±11.6
<i>UFR</i>	3.4 (2.1; 18.9)	5.7 (2.5; 6.9)	3.3 (2.4; 6.9)	6.0 (4.5; 6.9)
<i>Proteinuria</i>	5.6 (2.6; 9.3)	3.3 (2.3; 3.4)	4.9 (2.9; 7.3)	12.9 (5.4; 24.3) <sup>#</sup>

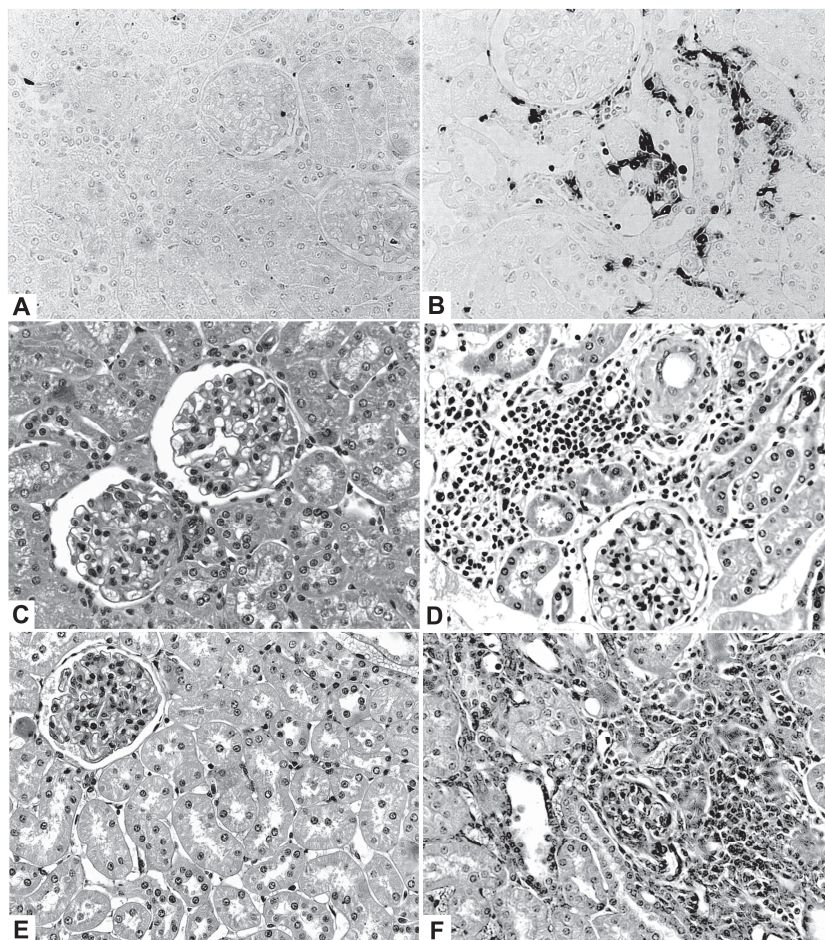
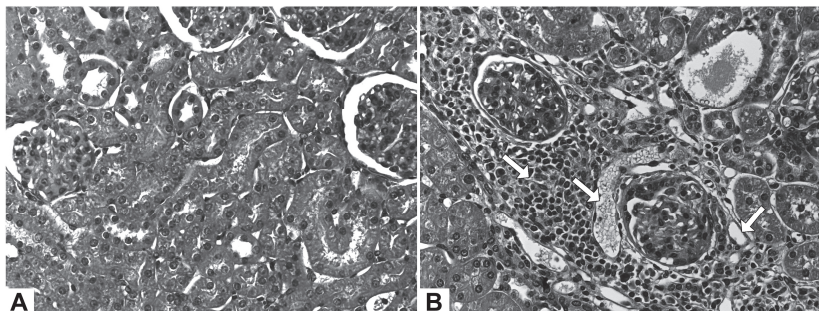
Data are expressed as mean ± SEM, except the data urine flow rate and proteinuria, which are expressed as median and percentile 25 and 75. *P<sub>creat</sub>*, plasma creatinine (mg/dl); GFR, glomerular filtration rate (ml/min/100 g); FE, fractional excretion (%); UFR, urine flow rate (μl/min); proteinuria (mg/24 h). \*p<0.05 versus SSD, OSD and SHFD; #p<0.05 versus OSD.

**Table 4.** Number of ED1, ANG II, NF-κB and PCNA-positive cells, lymphocytes, immunostaining score for vimentin, tubulointerstitial lesions (TIL), glomerulosclerotic index (GSI) and glomerular tuft area of cortical glomeruli (area CG) of sham-operated fed a standard diet (SSD), ovariectomized fed a standard diet (OSD), sham-operated fed a high-fat diet (SHFD) and ovariectomized fed a high-fat diet (OHFD) rats.

	SSD (n=6)	OSD (n=6)	SHFD (n=6)	OHFD (n=8)
<i>Lymphocytes</i>	2.5±0.9	4.3±0.4	4.3±0.5	11.4±1.9*
<i>ED-1</i>	5.2±0.8	5.3±0.6	6.4±0.2	21.4±3.2***
<i>ANG II</i>	1.38±0.32	2.49±0.62	1.70±0.48	5.52±0.82**
<i>NF-κB</i>	1.96±0.42	1.57±0.37	2.20±0.27	6.74±1.48**
<i>PCNA</i>	3.1±0.4	2.5±0.5	3.9±0.5	8.3±1.2*
<i>Vimentin</i>	0.3 (0.19;0.33)	0.16 (0.13;0.20)	0.4 (0.3;0.47)	0.8 (0.3;1.1)δ
<i>TIL Score</i>	0.2 (0;0.25)	0.1 (0.03;0.2)	0.13 (0.07;0.21)	0.42 (0.35;0.6)**
<i>GSI</i>	0.07 (0.04;0.11)	0.06 (0.04;0.09)	0.02 (0.01;0.05)	0.23 (0.21;0.37)***
<i>Area<sub>CG</sub></i>	5843±311.5	5744±232.4	6830±317.8 <sup>#</sup>	6835±208.6 <sup>#</sup>

Scores per 0.245 mm<sup>2</sup> grid field, area CG (μm<sup>2</sup>). Data are expressed as mean ± SEM, except the data TIL score, GSI and vimentin, which are expressed as median and percentile 25 and 75. ED1, macrophages/monocytes; ANG II, angiotensin II; NF-κB, nuclear factor-kappa B; PCNA, proliferating cell nuclear antigen. \*\*\*p<0.001 versus SSD, OSD and SHFD, \*\*p<0.01 versus SSD, OSD and SHFD. \*p<0.01 versus SSD and OSD, #p<0.05 versus SSD and OSD, δp<0.05 versus OSD.

**Fig. 1.** Histological sections stained with Masson's trichrome examined under light microscopy (original magnification x200) of OSD (**A**) and OHFD (**B**) rats. Note in B inflammatory infiltrates, tubular dilation and tubular atrophy.



**Fig. 2.** Immunolocalization of ED-1 (**A and B**), ANG II (**C and D**) and NF- $\kappa$ B (**E and F**) positive cells, examined under light microscopy (original magnification x200), from tubular interstitial compartment of the renal cortex of OSD (**A, C and E**) and OHFD (**B, D and F**) rats.

#### Immunohistochemical studies

The immunohistochemical studies showed increased ED-1 ( $p < 0.001$ ) (Fig. 2A and B), lymphocytes ( $p < 0.01$ ), PCNA ( $p < 0.01$ ), angiotensin II ( $p < 0.01$ ) (Fig. 2C and D), and NF- $\kappa$ B ( $p < 0.01$ ) (Fig. 2E and F) staining, with focal distribution in the tubulointerstitial compartment from the renal cortex of OHFD rats, predominantly located in damaged areas (Table 4). The results also revealed larger vimentin immunostaining from the renal cortex of OHFD rats when compared to OSD rats, showing the presence of lesions of the tubule cells ( $p < 0.05$ ). Immunolocalization of ED-1, ANG II and NF- $\kappa$ B positive cells of SSD and SHFD rats were not

shown due to similarities with OSD group.

#### Discussion

Our data show that ovariectomized rats fed a high-fat diet throughout 24 weeks, presented increases in body weight, visceral adipose tissue, SBP and proteinuria as well. In addition, we observed a decrease in fractional excretion of sodium. These animals also had histological changes in glomeruli and in the tubulointerstitial compartment, macrophages and lymphocytes renal infiltration, and increased expressions of ANG II and NF- $\kappa$ B in the renal cortex.

Excessive accumulation of adipose tissue in the intra-abdominal adipose depot is associated with an increased risk of developing cardiovascular problems, type 2 diabetes mellitus and other disorders, like the metabolic syndrome. Reductions in estrogen levels are associated to changes in body weight and fat distribution in post-menopausal women and experimental animals (Clegg *et al.* 2006, Brown and Clegg 2010). In our study we observed that the high-fat diet and ovariectomy, when analyzed individually in SHFD and OSD rats, did not promote significant increases in body weight compared to the control animals. However, the association of high-fat diet and ovariectomy resulted in significant increase in body weight and visceral adipose tissue.

The association of high-fat diet and ovariectomy in OHFD group rats was also able to elevate the SBP. Our results are in accordance with other studies that used metabolic syndrome models induced by a high-fructose diet and high-sucrose diet, in which the authors observed increase in SBP only in rats with metabolic syndrome associated to ovariectomy (Galipeau *et al.* 2002, Pérez-Torres *et al.* 2009). Low levels of plasma estradiol induced by ovariectomy have been associated with increased expression of angiotensin converting enzyme, the synthesis of AII, and plasma renin levels, while hormone replacement therapy contributes to the reversal of these effects (Dubey and Jackson 2001, Xu *et al.* 2008). On the other hand, abdominal obesity may contribute to the development of hypertension due to abnormal production of cytokines and proinflammatory adipokines, including TNF- $\alpha$ , leptin, interleukin-6 (IL-6) and resistin (Gregor and Hotamisligil 2011, Tang *et al.* 2012). Postmenopausal women with metabolic syndrome had high levels of TNF- $\alpha$ , positively correlated with blood pressure levels and IL-6 positively correlated with waist circumference (Chedraui *et al.* 2012). However, in our study, were not found detectable plasma TNF- $\alpha$  levels.

Our data demonstrated alterations in renal function in OHFD group, characterized by increased proteinuria and decreased FENa<sup>+</sup>. Insulin resistance, hyperinsulinemia, hyperleptinemia and abnormal lipid metabolism induced by obesity can also promote podocyte damage, proteinuria and renal injury (Coimbra *et al.* 2000, Abrass 2004). Pérez-Torres *et al.* (2009) demonstrated a positive relationship between low levels of estradiol in obesity and the microproteinuria. Sodium retention and volume expansion are also present in diet-induced obesity in experimental animals (Hall 1997,

Fujita 2008) and in obese humans (Chagnac *et al.* 2008, Sarzani *et al.* 2008). The mechanisms that are probably involved in reduced renal sodium excretion and weight gain are related to increased activity of the sympathetic nervous system, the renin-angiotensin-aldosterone system (RAAS) and reduced plasma levels of ANP (atrial natriuretic peptide) (Chagnac *et al.* 2008, Sarzani *et al.* 2008, Andrade *et al.* 2011).

The activation of the RAAS in obesity may be caused by the production of angiotensinogen in the adipose tissue, contributing to increased plasma angiotensin II and sodium retention (Rahmouni *et al.* 2005). Moreover, inflammation of the renal parenchyma leads to sodium retention due to local production of angiotensin II by immune cells, such as macrophages and T cells (Rodríguez-Iturbe *et al.* 2004). Furthermore, inappropriate activation of the intrarenal RAS is an important contributor to the pathogenesis of hypertension and renal injury (Kobori *et al.* 2007). In our study, increased expression of ANG II in the renal cortex of OHFD group rats may explain, in part, the reduction of the fractional sodium excretion observed in these animals. In addition, previous studies from our laboratory showed lower plasma levels of ANP and increased expression of NPR-C receptor responsible for clearance of ANP in renal tissue in obese ovariectomized rats (Andrade *et al.* 2011).

Hyperinsulinemia may cause renal damage by increasing sympathetic activity, oxidative stress, renal reabsorption of sodium, enhance the vascular response to AII and reduce the activity of ANP (Sarafidis and Ruilope 2006, Fujita 2008). Our data demonstrate an increase in plasma insulin levels OHFD group compared to other groups, showing that obesity these animals was paralleled by hyperinsulinemia. In a model of diet-induced obesity in mice, obese animals showed a positive correlation between hyperinsulinemia and changes in the renal structure and function (Deji *et al.* 2009). Chen *et al.* (2011) demonstrated a positive correlation between the levels of insulin and increased urinary excretion of protein. All these evidences support the hypothesis that hyperinsulinemia may also contribute to the development of changes in renal structure and function in OHFD group.

Histological studies revealed discrete and focal morphological changes in glomeruli and in tubulointerstitial compartment of the renal cortex in OHFD group as well. Tubulointerstitial lesions were characterized by inflammatory cell infiltrate, interstitial

fibrosis, tubular atrophy and tubular dilation. Studies show that obesity is related to the development of structural changes, including renal remodeling, proliferation of extracellular matrix, adhesion of Bowman's capsule, infiltration of inflammatory cells and glomerular hypertrophy (Hall 1997, Coimbra *et al.* 2000, Danilewicz and Wagrowska-Danielwicz 2009, Aresu *et al.* 2010). In the present study, morphometric data showed also an increase in glomerular tuft area in animals treated with a high-fat diet, although there was no significant increase in GFR.

The immunohistochemical study also showed increases of ED-1 (macrophages/monocytes) and ANG II positive cells and lymphocytes in the renal cortex of the OHFD group rats. Obesity and hypertension are characterized by the presence of interstitial infiltrate of macrophages and monocytes, and abnormal production of proinflammatory cytokines in renal tissue (Rodríguez-Iturbe *et al.* 2002, Ohtomo *et al.* 2010). The renal interstitial inflammation associated with hypertension is related to increased local production of ROS and ANG II in both infiltrating inflammatory cells and cells of the renal parenchyma (Rodríguez-Iturbe *et al.* 2002, 2004). Therefore, in our study, the reduction of the fractional sodium excretion observed in OHFD group may be explained, at least in part, by an increase of infiltration of macrophages/monocytes, lymphocytes and ANG II in the renal cortex of these rats. The vimentin expression was also increased in the renal cortex of OHFD group, indicating that the tubular cells of the renal cortex of these animals were in the process of cell regeneration after recent injury (Gröne *et al.* 1987). Our study also demonstrated increased expression of PCNA in the renal cortex of these rats, showing an increase of cell proliferation.

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Increased expression of NF- $\kappa$ B p65 was observed in the interstitial area and in the nucleus of the tubular cells of renal cortex of OHFD rats. It has been suggested that, in many types of kidney diseases, NF- $\kappa$ B activation plays an important role by inducing the synthesis of inflammatory substances, which cause kidney damage, including cytokines, growth factors and chemotactic factors for macrophages and monocytes (Guijarro and Egido 2001). Percy *et al.* (2009) show increased expression of NF- $\kappa$ B in a model of obesity and aging in rats. This study demonstrates that obesity is associated with kidney fibrosis, tubular apoptosis and macrophage infiltration in the renal tissue.

In conclusion, our results support our hypothesis that a high-fat diet associated with ovariectomy caused increased body weight, abdominal fat and systolic blood pressure, and renal functional and structural changes. These alterations can represent a precursor condition for the development of more intense renal damage, particularly if the hypertension is present during this process.

## Conflict of Interest

There is no conflict of interest.

## Acknowledgements

This work was supported by the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq, Brazil) and Fundação de Amparo à Pesquisa do Estado da Bahia (FAPESB). The authors thank Josmara B. Fregoneze and Heloísa D. C. Francescato, for taking part in some aspects of these studies, and Cleonice G. A. da Silva and Rubens F. de Melo for the skillful technical assistance.

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