Free Radical Research, August 2009; 43(8): 761–771

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healthcare

Association of renal damage and oxidative stress in a rat model of metabolic syndrome. Influence of gender

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(Received 16 February 2009; revised 14 May 2009)

Abstract

This study investigated the association between nephropathy and oxidative stress, by measurement of systolic blood pressure, lipid peroxidation, activities of catalase, manganese- and copper-zinc-superoxide dismutase and endothelial nitric oxide synthase expression and concentrations of nitrates/nitrites in kidneys from rats with Metabolic Syndrome. Weaning female or male rats had 30% sucrose to drink for 24 weeks (Metabolic Syndrome). Modulation by sex hormones was investigated by gonadectomy and hormone replacement. In Metabolic Syndrome, Castrated Metabolic Syndrome + Testosterone males and Ovariectomized Metabolic Syndrome females had increased blood pressure, proteinuria and lipid peroxidation. Nitrates/nitrites and activities of catalase, manganese and copper-zinc-superoxide dismutase decreased vs intact Control, Castrated Metabolic Syndrome males, intact Metabolic Syndrome and Ovariectomized Metabolic Syndrome + Estradiol females. The results suggest that sex hormones modulate the activity of superoxide-dismutase, catalase and endothelial nitric oxide-synthase. Ovariectomy decreased the protection against oxidative stress in females; the opposite occurred in castrated males.

Keywords: *Rat metabolic syndrome, renal damage, antioxidants, sex hormones*

Introduction

High consumption of carbohydrates and/or fat has increased in several world populations; this regime associated with sedentary habits induces pathophysiological alterations such as hypertension, dyslipidemias, hyperinsulinemia, insulin resistance and obesity, constituting what is known as the metabolic syndrome (MS) and, at present, a global epidemic. One of its frequent features is nephropathy which, in animal models, is associated with morphological changes such as tubular necrosis, inflammatory cell infiltration, accumulation of hyaline droplets and sclerosis, resulting in nephrotic syndrome, associated with hypertension [1]. The mechanism by which high intake of sucrose induces renal failure has not been wholly clarified [2,3].

Renal failure is also associated with an increase in reactive oxygen species (ROS) [4], therefore producing oxidative stress and an unbalanced level of cellular antioxidants such as catalase (CAT), superoxide dismutase (SOD), reduced glutathione, as well as other enzymes, i.e. endothelial nitric oxide synthase (eNOS). The antioxidant enzymes contribute to eliminate the radicals $O_2^{-\bullet}$ and H_2O_2, preventing the formation of the very active species $O_2^{-\bullet}$ and HO^- (ROS), which are very damaging to cells, particularly to those of the endothelium [5].

On the other hand, in the prevalence of renal and cardiovascular diseases, some dependence on age, gender and blood levels of sex hormones has been observed [6]. Although there are controversial results, in general terms oestrogens are a protective factor

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ISSN 1071-5762 print/ISSN 1029-2470 online
DOI: 10.1080/10715760903045296

against the development of such diseases: the average age at which symptoms appear in women is older than in men [7], whereas the risk is doubled if the women have undergone hysterectomy and oophorectomy; this does not occur when they preserve their ovaries [8].

Among the hypothesis that may explain the protective effect of oestrogens (E) there are the following: E can suppress the synthesis of collagen and fibronectin, proteins that are increased in nephropathies [9]; E reduce the accumulation of LDL in coronary arteries, they can activate muscarinic receptors, thus stimulating endothelial cells to synthesize nitric oxide (NO) and, consequently, increase the circulating levels of nitrates and nitrites [10]. E reduce the entry of extracellular calcium into the vascular smooth muscle [11], decrease the affinity of receptors to vasoconstrictors and have a relaxing effect on vessels, decreasing levels of endothelin and norepinephrine [12], besides having antioxidant properties against ROS [13,14].

Regarding the role of androgens, Brandes and Mugge [15] have described that male rat aortas generate larger amounts of free radicals than those from females and, therefore, NO released by the endothelium is oxidized to a larger extent by those species.

Concerning the rat model of MS, this has been developed in our laboratory as a variant of that described by Reaven [16] in which high consumption of sucrose induces hypertriglyceridemia, moderate hypertension, hyperinsulinemia, insulin resistance, nephropathy, intra-abdominal accumulation of adipose tissue and increased oxidative stress, among other alterations. In our model, the abnormalities are induced by addition of 30% sucrose in the drinking water, from the time the rats are weaned, for a period of 6 months [17–21].

Considering the previous observations, this work has been focused on the study of sex-hormone modulation, by gonadectomy, of the antioxidant system, particularly that related to CAT and SOD besides the role of eNOS in the development of the renal pathological manifestations in our rat model of MS.

Materials and methods

Weanling Wistar female or male rats were used in groups of eight animals each. Sucrose-fed rats were given 30% sucrose solution to drink for 24 weeks (MS). Controls received plain water; all groups were fed commercial rat chow (Formulab diet 5001). The animals were divided into C (intact controls male or female), Ovx C, Ovx C + E (ovariectomized control females, ovariectomized control females plus estradiol, respectively) and Cas C, Cas C + T (castrated control males, castrated control males plus testosterone, respectively), MS (intact sucrose-fed males or females), Ovx MS, Ovx MS + E (ovariectomized MS females or ovariectomized MS plus estradiol) and Cas MS, Cas MS + T (castrated MS males or castrated MS plus testosterone). Surgical castration was performed at 1 month of age.

Orchiectomy

The rats were anaesthetized with pentobarbital sodium (63 mg/Kg of body weight. Pfizer, Mexico). The area of the scrotum was shaved, cleaned with soap and disinfected with ethanol. A longitudinal incision of ~ 1 cm was made, the efferent ducts of the testicles were ligated and the testicles were removed. After the extirpation the skin incision was sutured [21].

Ovariectomy

This was performed under anaesthesia (pentobarbital sodium 63 mg/Kg of body weight). The abdominal and pelvic area of the back was depilated, cleaned with soap and disinfected with ethanol. A longitudinal incision of ~ 1.5 cm was made, the skin was separated from the muscle and a second incision of ~ 0.5 cm was made in the muscle on both sides of the first, to exteriorize the ovaries. The Fallopian tubes were ligated and cut below the ligature. After the extirpation, the incision was sutured [22].

Hormonal treatment

Estradiol valerate (Primogyn, Schering, Mexico. 1 mg/Kg body weight) was injected i.m. every 3 days, during the last 4 weeks of the experimental period. Testosterone enanthate (Primoteston, Schering, Mexico. 1 mg/Kg body weight) was injected i.m. every 3 days, during the last 4 weeks of the experimental period.

Measurement of serum sex hormones

Serum testosterone and estradiol were measured using the Diagnostic Products Corporation kit (Los Angeles, CA).

Systolic blood pressure

At the end of the sucrose treatment period systolic blood pressure (SBP) was measured by the tail-cuff method [21,22], with a sensor connected to a pressure transducer and a PC equipped with a program (Grass PolyView) for the capture and processing of data. The results obtained by this method have been compared with those from invasive techniques and no difference has been found.

Proteinuria

A few days before the end of the experimental period the rats were individually housed in metabolic cages (Nalgene, New York) with free access to food and drink; 24-h urine was collected, filtered and stored at -20°C; 10 µl urine were used for the measurement of total protein.

Kidney homogenate

The animals were anaesthetized with pentobarbital (63 mg/Kg), the kidneys were washed *in situ* with saline solution and immediately perfused with a solution containing 0.25 mM sucrose, 10 mM Tris and 1 mM EDTA, pH 7.35, then they were dissected and the capsule was removed. Samples from the organ were taken and homogenized in the same sucrose solution in the presence of protease inhibitors (1 mM PMSF, 2 µM pepstatin A, 2 µM leupeptin and 0.1% aprotinin) and the homogenate kept on ice. The kidney homogenate was centrifuged at $900 \times g$ for 10 min at 4°C. The supernatant was separated and kept at -70°C until required.

Protein concentration in kidney homogenate and urine was determined by the method of Lowry et al. [23].

Lipid peroxidation (TBARS)

TBARS, a marker of damage by free radicals, was measured by a standard method [22]. To 1 mg of protein from the kidney homogenate, 50 µl methanol with 4% BHT plus phosphate buffer pH 7.4 were added. The mixture was shaken vigorously in a vortex for 5 s and then incubated in a water bath at 37°C for 30 min. This was followed by the addition of 1.5 ml of 0.8% thiobarbituric acid and incubation in a water bath at boiling temperature for 1 h. To stop the reaction, the samples were cooled on ice; 1 ml 5% KCl was added to each sample as well as 5 ml n-butanol, they were shaken in a vortex for 30 s and then centrifuged at $416 \times g$ for 2 min. The n-butanol phase was extracted and absorbance was measured at 532 nm in a spectrophotometer. A calibration curve was obtained using tetraethoxypropane (Sigma) as a standard.

Nitrates and nitrites

These were measured as previously described [21]. One milligram of protein from kidney homogenate was incubated with 25 µl Cu-Cd for 30 min and constant shaking, The mixture was centrifuged at $850 \times g$ at room temperature and the supernatant was recuperated; 100 µl of 10% $ZnSO_4$ and 100 µL 0.5 N NaOH were added to the supernatant and centrifuged at $7155 \times g$. The supernatant was recovered and incubated in the presence of 400 µl of Griess reagent (200 µl 1% sulphanylamide and 200 µl 0.1% N-naphtyl-ethyldiamine). The total volume was adjusted to 1 ml. A calibration curve was obtained with solutions of KNO_2 ranging from 10–0.156 nmoles/ml. Absorbance was measured at 540 nm.

Superoxide dismutase activity (SOD)

SOD enzymatic activity was determined in the kidney homogenate by non-denaturing gel electrophoresis and nitro blue tetrazolium (NBT) staining, as described by Flohe and Otting [24]. Twenty micrograms of the resulting extract were applied directly, without boiling, to a non-denaturing 10% polyacrylamide gel. The electrophoresis was carried out at 120 V for 4 h. Following this, the gel was incubated in a 2.45 mM NBT solution for 20 min, then the liquid was discarded and the gel was incubated in a 28 mM TEMED solution containing 36 mM potassium phosphate (pH 7.8), 0.028 mM riboflavin. After 10 min incubation in the dark, the blue NBT stain for O_2 was developed by exposure to UV light for another 10 min.

Riboflavin and TEMED, in the presence of UV light and oxygen, produce superoxide radicals; NBT and SOD compete for them. Where SOD is present the gel remains transparent, whereas reduced NBT turns it purple-blue.

A standard curve was obtained with a serial dilution (2.5, 5, 10, 15, 30 and 60 ng) of SOD from bovine erythrocytes (Sigma Chemical Co.). SOD activity was calculated following the technique described by Chen and Pan [25].

Catalase activity (CAT)

Twenty micrograms of kidney homogenate were analysed by native-gel electrophoresis with 8% polyacrylamide [26]. Staining for detection of CAT activity was performed as follows: the electrophoresed gel was incubated with 30% H_2O_2 for 10 min and then with a mixture of 1% $K_3Fe(CN)_6$ and 1% $FeCl_3 6H_2O$ for 10 min in the dark. The gel was washed three times with water. Staining was absent at sites of H_2O_2 scavenging.

A standard curve was obtained with a serial dilution (2.5, 5, 10, 15, 30 and 60 ng) of CAT from bovine liver (Sigma Chemical Co.). The activity was 25 000 units/mg protein. CAT activity was calculated following the technique described above [25].

The gels of CAT and SOD were analysed by densitometry with an image analyser Sigma Scan Pro 5.

Immunoblotting of eNOS

One milligram of kidney homogenate was mixed with stacking buffer (59% glycerol vol/vol; Tris-HCl pH 6.5; 1% weight/vol SDS, 0.1% weight/vol bromophenol blue; 0.01% 2-mercaptoethanol). The mixture

was heated at 100°C for 1–2 min. The proteins were separated in a 10% SDS/PAGE gel under reducing conditions; at the end of the electrophoresis the gel was transferred to a nitrocellulose (Hybond-P) membrane 0.45 μm (Millipore, Mexico). The membrane was blocked for 3 h with TBS containing 5% non-fat dehydrated milk and 0.05% Tween-20.

Afterwards, the membrane was incubated with a 1/1000 dilution of rabbit primary IgG polyclonal antibodies against eNOS (NOS3 antibody rabbit IgG [C-20]: sc-654. Santa Cruz Biotechnology, Inc), overnight at 4°C and continuous shaking. The membrane was rinsed three times with TBS and incubated with a 1/1000 dilution of goat secondary polyclonal antibodies anti-rabbit IgG (ZYMED Laboratories, 65-6140. Biotin-Goat, Anti-Rabbit IgG [H + L] specific antibody. DS Grade) for 3 h at room temperature; 3'3' diaminobenzidine was used to develop it. After identifying the relevant protein the membrane was washed with a buffer of 1% Tris, 1% SDS and 100 mM β-mercaptoethanol, pH 2 for 2 h, followed by a washing with TBS; it was blocked for 3 h and incubated overnight with a 1/2000 dilution of mouse monoclonal biotinilated anti-bodies against α-actin (α-actin [1A4] sc-32251 mouse IgG_{2a} Santa Cruz Biotechnology, Inc) and then it was developed with 3'3'diaminobenzidine. The membrane was analysed by densitometry by means of the program Sigma Scan Plot 5.

Histopathological analysis

Sections for light microscopy were stained with periodic acid-Schiff stain (PAS). A sample of kidney tissue was perfused with a solution of 2% paraformaldehyde and 2.5% glutaraldehyde in cacodylate buffer (pH 7.4) for electron microscopy.

Statistical analysis

The analysis and figures were made with the program Sigma Plot (Sigma Plot, version 10, Jandel Corporation, 1986–2005). Data are shown as the mean ± SE. Statistical significance was determined by Student's *t*-test and One-way ANOVA. $P < 0.05$ was taken as significant.

Results

Systolic blood pressure (SBP)

Values are shown in Figure 1. In female C, MS, Ovx C and Ovx C + E rats, SBP was 111 ± 3, 121 ± 5, 112 ± 2 and 119 ± 1 mmHg, respectively, without significant statistical difference. In Ovx MS, SBP increased to 139 ± 4 vs MS ($p = 0.01$) and Ovx MS + E, SBP decreased to 125 ± 1 vs Ovx MS ($p = 0.04$).

In male rats, SBP in C was lower than in MS animals, 123 ± 2 vs 145 ± 3 mmHg, respectively ($p = 0.001$). Castration and hormonal treatment did not induce any change in C and Cas C + T (125 ± 1, 127 ± 1 mmHg, respectively), but in Cas MS and Cas MS + T, SBP decreased and increased to 128 ± 1 and 142 ± 1 mmHg, respectively, when compared with MS and Cas MS ($p = 0.001$).

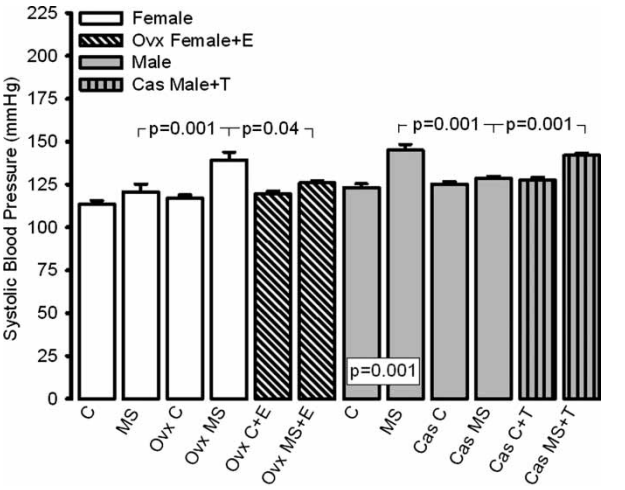

Figure 1. Systolic blood pressure from female and male control and metabolic syndrome rats taken at the end of the sucrose treatment. C = control rats; MS = metabolic syndrome rats; Ovx C or Ovx MS = ovariectomized female rats; Ovx C + E or Ovx MS + E = ovariectomized female rats plus estradiol; Cas C or Cas MS = castrated male rats; Cas C + T or MS + T = castrated male rats plus testosterone. Values are mean ± SE ($n = 8$ different animals).

Proteinuria

Figure 2 shows that urinary excretion of protein in C, MS, Ovx C and Ovx C + E female rats was not statistically different, but there was a significant increase and decrease in Ovx MS and Ovx MS + E, respectively ($p = 0.03$). Male rats showed significantly higher levels of urinary excretion protein in MS vs C

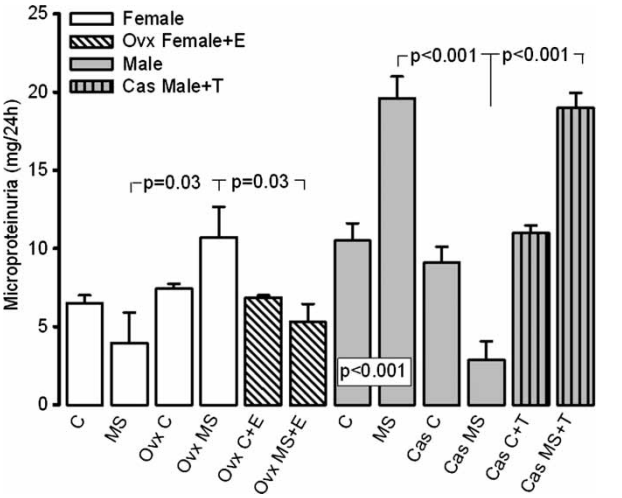

Figure 2. Microproteinuria. Five days before the end of the experimental period the rats were individually housed in metabolic cages: 24 h-urine was collected and protein content was measured. See Figure 1 legend for abbreviations. Values are mean ± SE ($n = 8$ different animals).

and Cas MS + T vs Cas MS ($p < 0.001$). Castration decreased proteinuria in MS when compared with intact MS ($p = 0.001$), but did not modify it with relation to its intact control and Cas C + T.

Lipid peroxidation

In female rats there was no difference in TBARS, (MDA equivalent), between C and MS in kidney homogenate (Figure 3). The ovariectomy increased TBARS in MS ($p = 0.05$) and did not affect it in C. The hormonal treatment in Ovx MS + E decreased them vs Ovx MS ($p = 0.04$) but in Ovx C + E there was no significant change vs Ovx C.

In male rats, TBARS were significantly increased in MS vs C ($p = 0.01$), while castration of MS decreased it significantly compared with intact MS ($p = 0.05$). Castration and treatment with testosterone had no effect on TBARS in control animals, but hormonal treatment increased it in comparison with Cas MS ($p = 0.01$).

Antioxidant enzymes

Catalase (CAT). There was no difference in the activity of CAT between female C, MS or Ovx C kidney homogenate. However, a significant decrease in CAT activity was observed in Ovx MS when compared with intact MS ($p = 0.04$) (Figure 4A).

The treatment with estradiol induced a significant increase in Ovx MS + E vs Ovx MS ($p = 0.05$) (Figure 4B).

Increased activity was found in C vs MS male rats ($p = 0.02$) as well as in Cas MS vs MS males ($p = 0.05$). No difference was observed between male C and Cas C (Figure 4A). In males the hormonal treatment with testosterone decreased the activity of CAT in Cas C + T and Cas MS + T in comparison with Cas C and Cas MS, respectively ($p = 0.001$) (Figure 4B).

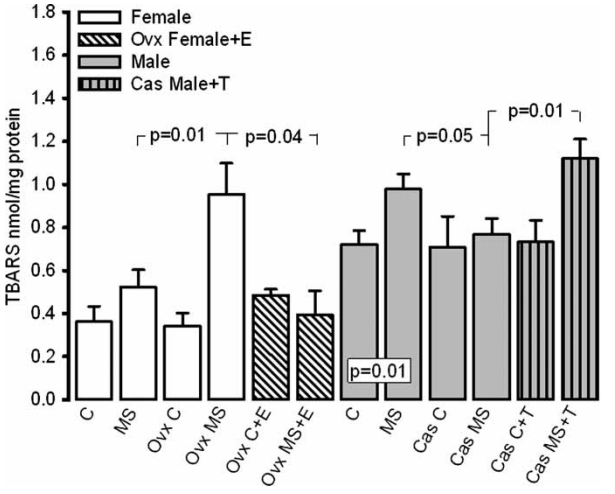

Figure 3. TBARS. Lipid peroxidation was measured in kidney homogenate. See Figure 1 legend for abbreviations. Values are mean ± SE ($n = 8$ different animals).

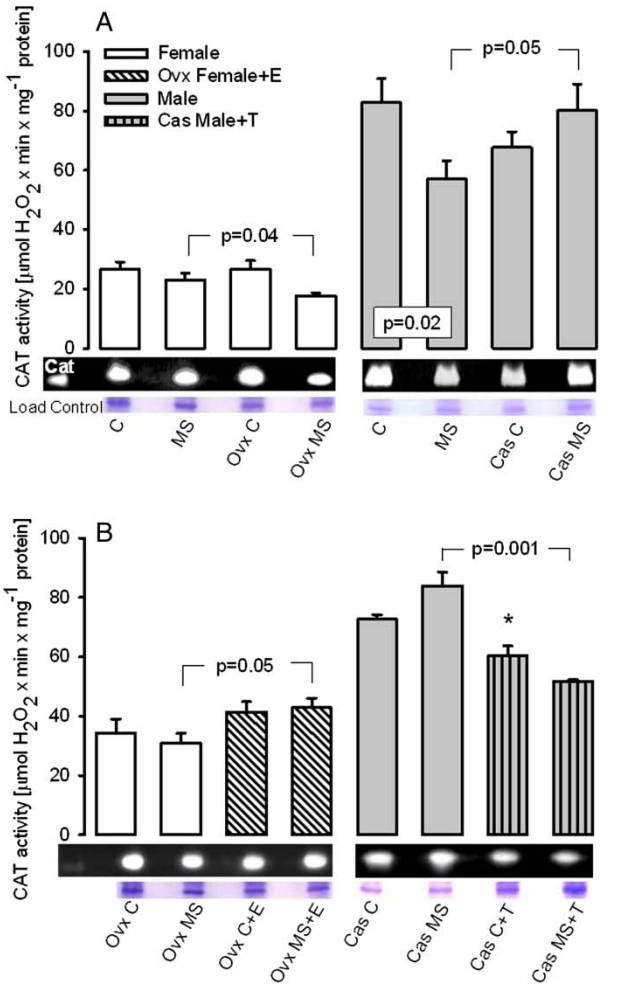

Figure 4. Catalase activity in kidney homogenate. Native-gel electrophoresis with 8% polyacrylamide. (A) Intact and gonadectomized control and metabolic syndrome rats. (B) Gonadectomized female and male rats plus hormone treatment (E = estradiol, T = testosterone). *Cas C vs Cas C + T, $p = 0.001$. See Figure 1 legend for abbreviations. Values are mean ± SE ($n = 8$ different animals).

Superoxide dismutase (Mn-SOD). There were no differences in its activity except between MS vs Ovx MS, the latter being reduced ($p = 0.04$) (Figure 5A), but treatment with estradiol significantly increased it in Ovx MS + E vs Ovx MS ($p = 0.03$) (Figure 5B).

The activity was lowest in MS vs C males, with significance ($p = 0.03$) and Cas MS ($p = 0.05$). No difference was found between C vs Cas C. The testosterone supplement in Cas MS + T decreased activity in comparison with Cas MS ($p = 0.03$) (Figure 5B).

Cu-Zn-SOD. (Figure 5A and B). There was no significant difference among the female groups. In male rats Cas MS and Cas MS + T showed a higher and low level than MS and Cas MS, respectively ($p = 0.03$).

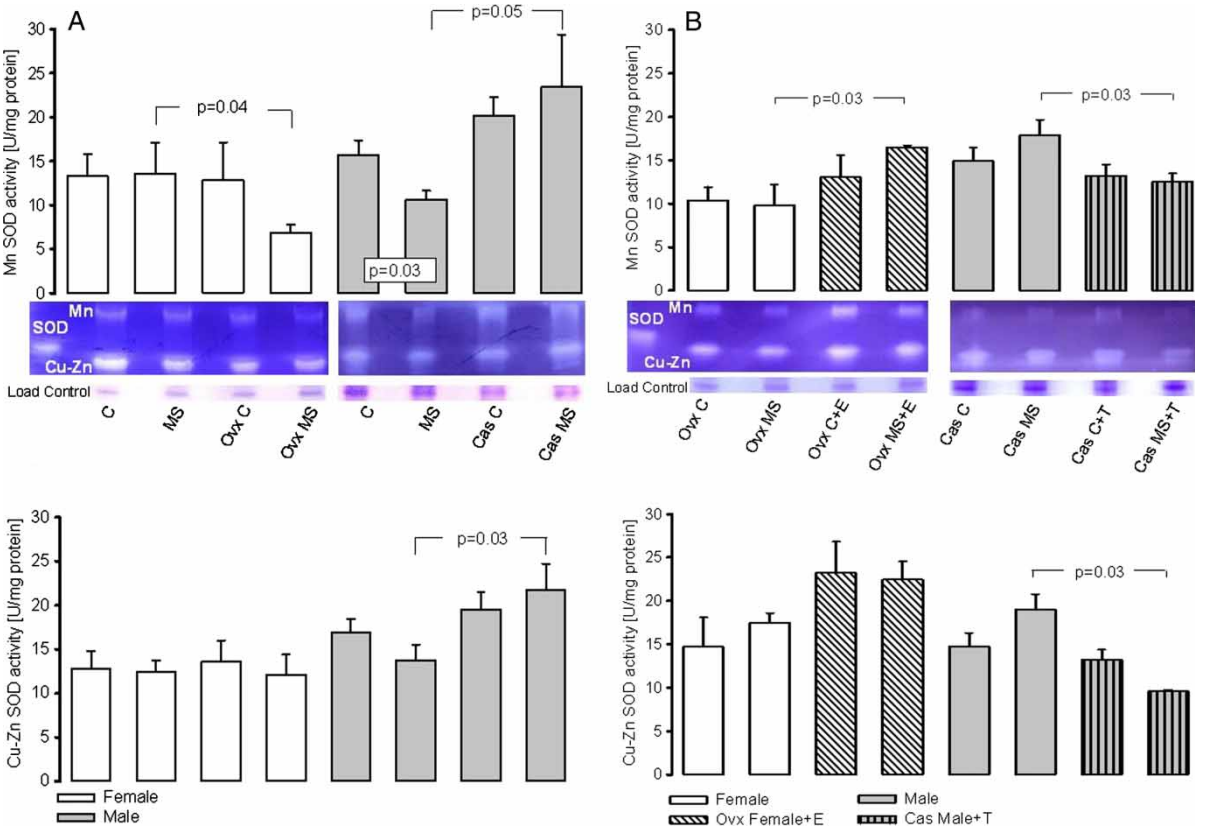

Figure 5. Native-gel electrophoresis with 10% polyacrylamide. Mn and Cu-Zn Superoxide dismutase activity in kidney homogenate from (A) intact and gonadectomized rats and (B) gonadectomized and gonadectomized plus hormone treatment rats. See Figure 1 legend for abbreviations. Values are mean ± SE ($n = 8$ different animals).

Endothelial nitric oxide synthase. Figure 6A shows a decreased level in the expression of eNOS in Ovx MS and no difference among C, Ovx C and MS in female rats.

Figure 6B shows an increased level in the expression of eNOS in Ovx MS + E in comparison with Ovx MS female rats ($p = 0.05$).

The expression of eNOS in MS males was significantly lower in comparison with C and Cas MS ($p = 0.05$); in Cas C it was not significantly different from intact C (Figure 6A). In male Cas MS + T decreased level expression of eNOS was observed in comparison with Cas MS ($p < 0.01$) (Figure 6B).

Nitrates and nitrites (stable metabolites of NO). Figure 7 shows that the concentration of the metabolites in females was the same in C, MS, Ovx C, Ovx C + E and Ovx MS + E, but significantly decreased in Ovx MS ($p = 0.03$). The levels in male rats were not significantly different among C, Cas C, Cas MS and Cas C + T, except for MS and Cas MS + T which were the lowest and significantly decreased ($p < 0.01$ and $p = 0.03$, respectively).

Micrographs

Figures 8 and 9 show micrographs of glomerulus and capillary loops from C and MS male rat kidneys, respectively. Significant changes were observed in MS samples.

Sex hormones

Table I shows that there was no significant change in the serum concentration of estradiol in C, MS, Ovx C + E or Ovx MS + E in female rats. Estradiol was undetectable in Ovx C and Ovx MS. In Cas C and Cas MS males the levels were significantly increased in comparison with their respective control rats.

In females the levels of testosterone concentration were significantly lower in Ovx MS vs Ovx C ($p = 0.05$). Serum testosterone concentration in MS and Cas MS + T male rats was significantly decreased in comparison with C and Cas C + T, respectively ($p < 0.05$).

Testosterone was undetectable in Cas C and Cas MS.

Discussion

The purpose of this study was to establish the relationship between the renal damage found in a rat model of metabolic syndrome, induced by high intake of sucrose [27], and the alterations in the levels of the antioxidant enzymes CAT and SOD, as well as TBARS and eNOS. In addition, we investigated the

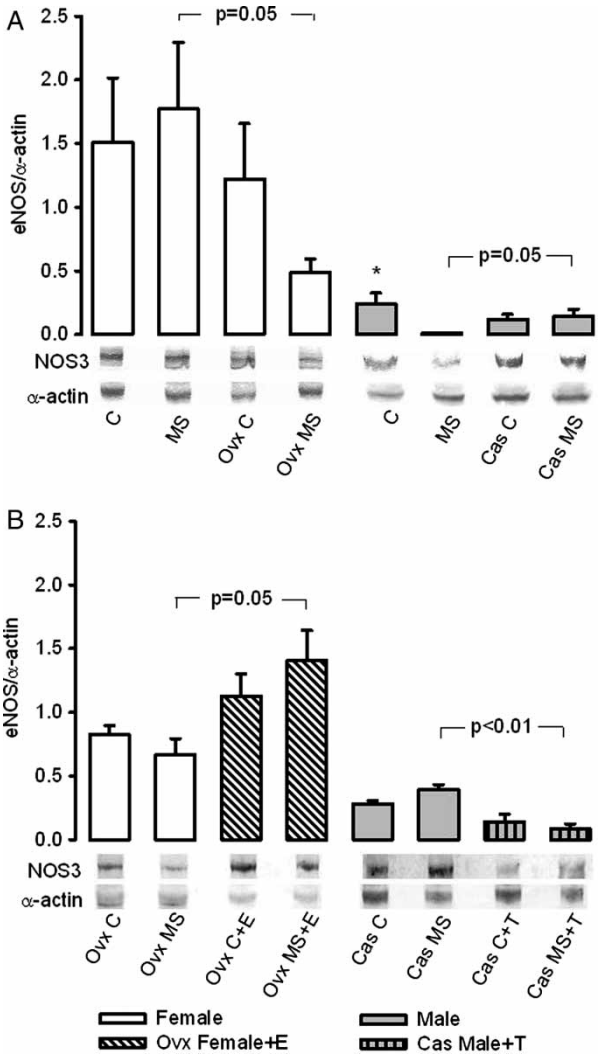

Figure 6. Endothelial nitric oxide synthase expression in kidney homogenate (Western-blot). (A) Intact and gonadectomized control and metabolic syndrome rats. *C vs MS, $p=0.05$. (B) Gonadectomized female and male rats plus hormone treatment (E = estradiol, T = testosterone). See Figure 1 legend for abbreviations. Values are mean ± SE ($n=8$ different animals).

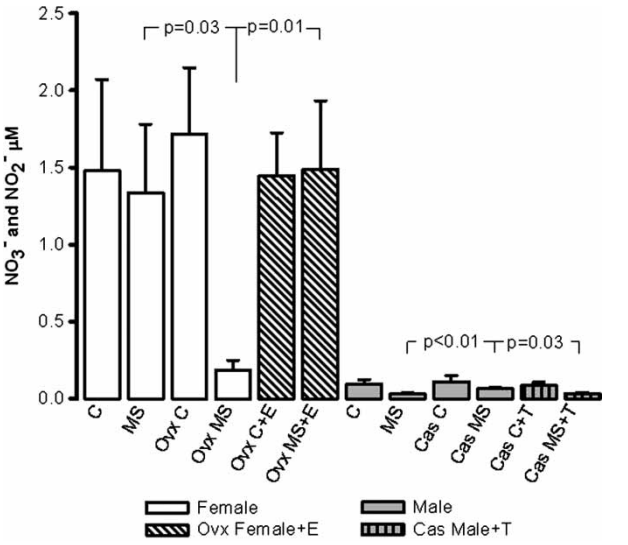

Figure 7. Stable metabolites from nitric oxide NO_3^- and NO_2^- concentration in kidney homogenate. See Figure 1 legend for abbreviations. Values are mean ± SE ($n=8$ different animals).

modulation by castration and hormonal treatment of the antioxidant system in the nephropathy present in the model.

One of the characteristics of endothelial dysfunction found in the metabolic syndrome [21] is the decrease in the synthesis and release of vasodilating agents and increase of vasoconstrictors [28]. It has been suggested that the imbalance between vasoconstriction and vasodilatation and the development of high SBP and MS may be partly due to an increase in vasoconstrictors dependent on the sympathetic system, as well as a deficiency in the expression of eNOS and in the antioxidant system [11,12,29].

Sullivan et al. [30] have confirmed that male animals have higher SBP and proteinuria when compared with females of the same age. Sex dimorphism in blood pressure and protein excretion was abolished by orchiectomy. A significant increase in SBP in Ovx MS in comparison with MS females and Ovx MS + E, respectively, and MS males, Cas MS + T male rats in comparison with Cas MS males was observed, these results support the hypothesis that testosterone participates in the regulation of SBP and

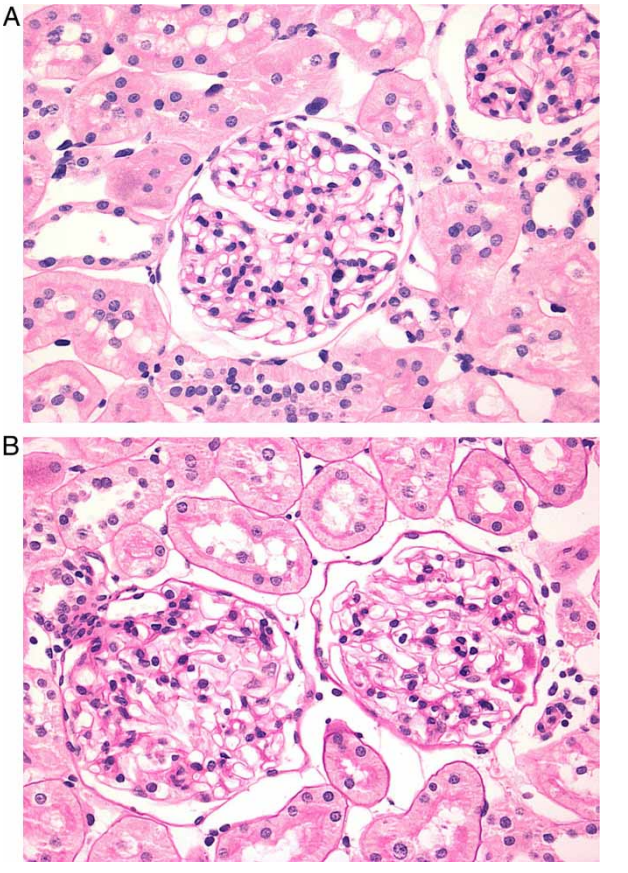

Figure 8. Light microscopy. Periodic acid-Schiff stain. 40×. No abnormalities shown in (A). In (B) (MS) the alterations are: Mesangial zones with increased matrix and discreet hypercellularity; Early sinechia; Closed capillary lumina; Zones of sclerosis.

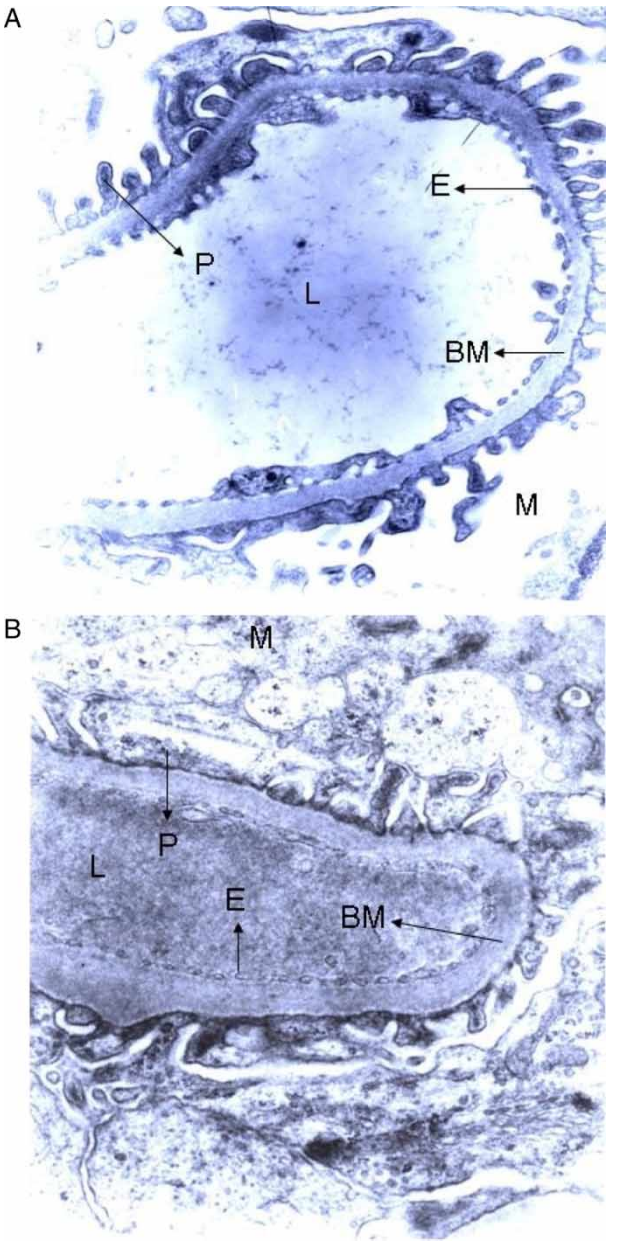

Figure 9. Male rat kidney. Control (A) and MS (B) capillary loops. Electron micrographs 16 000×. No abnormalities shown in (A). In (B) (MS) the alterations are: Areas of obliteration; Glomerular BM irregularly thickened; Loss of fenestration in endothelium; Podocytic processes with more evident obliteration (due to ageing); Blebs in podocytes. BM: basal membrane, E: endothelium, L: lumen, M: mesangium, P: podocyte.

contributes to renal damage in MS male rats, partly through the reduction in the synthesis of the enzymes of the antioxidant system [31]. Some evidence has also been found, for instance, in a rat model of MS induced by a diet of 60% fructose during 9 weeks with and without ovariectomy, in which the SBP increased in ovariectomized fructose-fed rats in comparison with those that only had fructose, indicating the relationship that exists between sex hormones and the regulation of SBP, particularly the contribution of estradiol [32]. On the other hand, Song et al. [33] have described that castration in a fructose-fed rat model prevented hypertension and insulin resistance to develop, stressing the fact that the presence of androgens was required

Microproteinuria is regarded as a marker of renal damage [34]. The increase in protein urinary excretion in Ovx MS female rats, MS males and Cas MS + T, when compared with their respective controls, intact MS females and C males, as well as the decrease in Cas MS males suggests that sex hormones participate in the progression of renal damage associated with the MS.

Sex hormones probably participate in the synthesis and degradation of the components of the extracellular matrix, associated with the normal function of the glomerular basal membrane [35,36]. Our results show that castration decreased the microproteinuria, but treatment with testosterone re-established it, while ovariectomy in MS female rats increased it and treatment with estradiol reduced it.

The deterioration in the renal function, as evidenced by proteinuria, decreased glomerular permeability and glomerulosclerosis, is more rapid in men than in women of the same age. Reckelhoff et al. [37] have shown that androgens modify renal haemodynamics, as well as participating in the elevation of SBP and proteinuria in male spontaneously hypertensive rats, but castration decreases SBP and proteinuria and preserves renal function.

On the other hand it has been reported that oestrogens stimulate the synthesis of NO by overexpression of eNOS, but testosterone has the opposite effect [38]. Furthermore, NO induces vasodilatation in renal arterioles, thus contributing to renal function [38]. The results showed that the increase or decrease of eNOS expression and NO metabolites in Cas MS and Cas MS + T, respectively, is probably due to the elimination of testosterone by castration or its re-establishment by hormonal treatment, because testosterone has been described to induce a decrease of NO metabolites, associated with the development of renal injury [39,40]. Ovariectomy leads to a decrease in the expression of eNOS and nitrates/nitrites, but hormonal replacement re-establishes it. The loss of oestrogens by ovariectomy in Ovx MS rats may have an important role in the decrease observed in the expression of eNOS. Vasudevan et al. [41] have described a decrease in levels of plasma NO metabolites, associated with endothelial malfunction, in a fructose-fed male rat model; gonadectomy reversed this effect. Thus, NO deficiency combined with antioxidant/oxidant imbalance and influenced by gender, appears to be a primary factor in the development of renal damage in this model. High-fructose diet increases NO inactivation, secondary to enhanced formation of superoxide [42].

Our results show that a sucrose-rich diet in MS male rats decreases the expression of eNOS, with the

Table I. Sex hormone concentration in serum of experimental rats groups.

	Female rats					
	C	MS	Ovx C	Ovx MS	Ovx C+E	Ovx MS+E
Estradiol (pg/ml)	4.6±2.3	8.2±2.9	≤0.01	≤0.01	3.9±2.4	10.1±4.5
Testosterone (ng/ml)	0.037±0.02	0.019±0.01	0.048±0.01	0.018±0†	≤0.01	≤0.01
	Male rats					
	C	MS	Cas C	Cas MS	Cas C+T	Cas MS+T
Estradiol (pg/ml)	0.6±0.1	0.5±0.02	34.9±12**	32.6±7.46**	≤0.01	≤0.01
Testosterone (ng/ml)	8.0±0.2	1.1±0.06*	≤0.01	≤0.01	6.9±0.2	0.4±0.04*

C = control rats; MS = metabolic syndrome rats; Ovx C or Ovx MS = ovariectomized female rats; Ovx C+E or Ovx MS+E = ovariectomized female plus estradiol rats; Cas C or Cas MS = castrated male rats; Cas C+T or MS+T = castrated males plus testosterone rats. Values are mean±SE (n = 8 different animals).

†Ovx C vs Ovx MS, $p = 0.05$.

*C and Cas MS+T vs MS, $p < 0.05$. **Male Cas C and Cas MS vs C and MS, $p < 0.001$.

ensuing decrement of its metabolites, while testosterone reduces it, evidencing the modulating role of sex hormones in the pathogenesis of oxidative stress.

Furthermore, the androgen-induced renin secretion increases angiotensin II levels, promotes vasoconstriction directly and indirectly by increasing the generation of superoxide anion, leading to enhanced NO inactivation toward peroxynitrites [35], on account of a damaged endothelium [22]. The kidney alterations found in our MS model demonstrate the participation of this organ in the regulation of SBP. Levels of eNOS in the kidney were measured in groups of male or female rats, intact, castrated, oophorectomized or oophorectomized with estradiol replacement therapy. The results indicate that gender and levels of sex hormones can modulate renal medullary synthesis of NO [39].

In a recent study we demonstrated that ovariectomy in MS female rats diminishes the concentration of NO metabolites while MDA increases it and that hormone therapy reverts this pathological condition of the endothelium [22].

TBARS is a marker of damage to the lipids in the cell membrane by oxidative stress [19]. The indices of TBARS in the female Ovx MS rat and in MS male and Cas MS+T kidney homogenates, when compared with their respective controls, were found increased, but they decreased in Ovx MS+E and Cas MS; this suggests that the oxidative profile can be modified by gonadectomy.

Consumption of sucrose negatively affects the balance between free radical production and antioxidant defense in female Ovx MS, Ovx MS+E and male MS, Cas MS+T rats, leading to increased lipid susceptibility to peroxidation.

It has been suggested that oestrogens can suppress free radical-induced peroxidation chain reactions; this antioxidant capacity may be a mechanism whereby oestrogens limit renal damage in females [43].

It has been reported that the difference in the incidence of cardiovascular diseases between sexes can be attributed, in part, to the modulation of the lipoprotein metabolism by oestrogens and their antioxidant properties [43]. It has been observed that oestrogens and cathecholoestrogens inhibit microsomal lipid peroxidation stimulated by Fe and NADPH [44].

In rat aortas from SHR, an increase in free radicals with a subsequent increase of oxidative stress damages the endothelium, but castration decreases this damage [45]. El Hafidi and Baños [19] have described that male rats, either C or treated with Fe-dextran, have higher indices of MDA than comparable female rats.

The antioxidant cellular system includes enzymatic components such as SOD, CAT, GPX, ascorbic acid peroxidase and glutathion reductase, as well as other non-enzymatic molecules such as ascorbic acid, cysteine, glutathione, α-tocopherol, hydroquinone, carotenoids and polyamines [46]. High consumption of fructose has been associated with numerous metabolic abnormalities found in MS, both in humans and in animal models, on account of the oxidative stress induced by the diet [47]. Diets high in carbohydrates, such as fructose, sucrose or both can induce hypertriglyceridemia and a reduction in the antioxidant reserves, which represents a risk for severe pathological conditions, particularly as a result of inactivation of antioxidant enzymes [48]. Our MS model presents hypertriglyceridemia and retroperitoneal-fat-accumulation [17,21,49], that may induce increased oxidative stress and diminished antioxidant capacity, particularly in MS male rats; MS females are protected by mechanisms that involve oestrogens. Castration in males significantly increased the levels of serum estradiol, possibly due to extra-gonadal aromatization in adipose tissue, as previously described [21].

Recently, it has been demonstrated that administration of diets rich in sucrose or fructose, during an acute or chronic period have a pro-oxidant effect, which can contribute to the pathoaetiology of MS

[50]. Furthermore, it has been demonstrated that fructose can inactivate CAT and SOD *in vitro* [51]. The protective effects of oestrogens, described in humans and also in animal models, suggest that oestrogens can suppress lipid peroxidation by free radicals, due to their similarity in chemical structure with vitamin E, as opposed to androgens which have a pro-oxidant effect [52]. Our results show a significant decrease in the activity of CAT in the Ovx MS, when compared with intact MS and Ovx MS + E females, also in MS and Cas MS + T male rats in comparison with their C and Cas MS, respectively, suggesting that CAT, key enzyme in the antioxidant system, has an altered activity in the MS male, not evident in the female rats and it is dependent on the hormonal profile. In male fructose-fed rats in which the treatment lasted 2 weeks, a decrease in the expression of CAT mRNA and Cu-Zn SOD mRNA was found in liver, as well as CAT mRNA in heart [53].

On the other hand, the activity of SOD has been found low in male rats that have had a diet rich in sucrose in comparison with castrated males. In a previous study it was observed that the activity of Cu-Zn SOD diminished in the heart of MS rats [54].

Busserolles et al. [50] demonstrated the effects of short-term administration of sucrose to male and female rats on the activity of SOD and oxidative stress in the heart, finding a decrease in the group of male rats. We found the same tendency in the activities of Mn and Cu-Zn SOD; both C and MS females had higher levels than males, which decreased after ovariectomy, but that treatment with estradiol normalized them. The same authors reported the loss of that effect against oxidative stress in ovariectomized rats and its recovery when they were treated with estradiol [50].

SOD can reduce endothelial injury in renal vessels, indirectly implicating the participation of O_2^- associated with high sucrose intake [55]. In a previous study we demonstrated that sucrose feeding significantly increased oxidative stress and decreased SOD activity in the heart of MS male but not in MS female rats [18]. Studies in human erythrocyte Cu-Zn-SOD have shown that it undergoes a gradual decrease in activity on incubation with glucose [55].

Conclusions

Our results show that female rats are protected against the pro-oxidant effects of a high-sucrose diet on the renal system, partly by estradiol, but they lose this protection after ovariectomy. Castration of males, on the other hand, is beneficial. All of which evidences the modulating role that sex hormones have and their participation in the development of renal damage.

Acknowledgements

Our thanks to the Postgraduate Section in the Department of Biological Sciences of the National Autonomous University of Mexico (UNAM) for a scholarship to I. Pérez and for the technical support from Rodrigo Velázquez to carry out the Immunoblotting.

This work was partially supported by grant No. 53617 from the National Council for Science and Technology (CONACyT), Mexico to G.B.

***Declaration of interest*:** The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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This paper was first published online on iFirst on 11 June 2009.