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Effect of gonadectomy on the metabolism of arachidonic acid in isolated kidney of a rat model of metabolic syndrome

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Abstract

Influence of sex on arachidonic acid metabolism, a pathway involved in the link between metabolic syndrome (MS) and renal damage, was studied in isolated perfused kidney. Metabolic syndrome was induced by feeding 30% sucrose solution for 24 weeks to intact and gonadectomized female (Ovx) and male (Cas) rats. Systolic blood pressure, albuminuria, as well as prostaglandin E_2 and thromboxane B_2 from urine and perfusate increased in MS male and MS ovariectomized females; castration reduced them in MS males. Perfusion of arachidonic acid in kidneys from MS males increased perfusion pressure compared with controls. No difference appeared in perfusion pressure between control and MS females. Castration diminished perfusion pressure in MS; the opposite was observed in Ovx MS. Perfusion with arachidonic acid plus indomethacin decreased perfusion pressure in MS male kidneys and in Cas MS. In Ovx MS, arachidonic acid plus indomethacin decreased perfusion pressure, but not in female control, MS, and Ovx control. Increase in perfusion pressure with arachidonic acid in both male MS and Ovx MS was related to cyclooxygenase (COX)-1 and COX-2 overexpression in kidney. Castration reduced the expression of COX-1 and COX-2 in MS to control levels. The results suggest that the alteration in arachidonic acid metabolism associated with changes in the expression of COX-1 and COX-2 induced by sucrose intake, and influenced by sex hormones, may contribute to renal damage.

1. Introduction

The metabolic syndrome (MS) is characterized by the association of various pathologies: hypertension, dyslipidemia, hyperinsulinemia, nephropathy, and cardiovascular alterations [1]. Its prevalence may depend on sex both in humans and in animal models [2,3]. The prevalence of MS in premenopausal women is lower than that of men of the same age; the incidence in women increases after the menopause and may become higher than that in men [4]. One of the organs that may be severely impaired in MS is the kidney [5].

In animal models of chronic renal disease, males show more accelerated progression of renal injury than females [6]. In male rats [7], castration and continuous blockade of the androgen receptor attenuate renal injury, proteinuria, and blood pressure and inhibit renin-angiotensin system (RAS) [8]. Generally, estrogens are a protective factor against the development of nephropathy by diminishing vascular resistance and inhibiting circulating renin and angiotensin-converting enzyme, decreasing circulating angiotensin levels. Estrogen deficiency as a result of menopause or ovariectomy has the opposite effects on the RAS [9].

On the another hand, kidney arachidonic acid (AA) metabolism and the eicosanoids, products of the cyclooxygenase (COX) pathway, participate in the regulation of vascular tone, renin release, sodium metabolism, renal hemodynamics, and blood pressure, among other functions [10].

Eicosanoids are a diverse metabolite group, produced by the enzymatic oxidation of AA and catalyzed through 3 major enzymatic pathways: the COX (with the intervention of the 2 COX isoforms COX-1 and COX-2) [11], the

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lipoxygenase, and the cytochrome P-450 pathways [12]. Cyclooxygenase-1 is constitutively expressed in the kidney; and COX-2 is induced by inflammatory stimuli, but is also constitutively localized in the macula densa and collecting tubules in the renal vasculature [13,14]. The expression of the COX-2 isoform is increased in the kidney during development, hypertension, obesity, and MS [15,16].

Arachidonic acid metabolite levels are determined by factors that include species, tissue, and hormonal background [17,18].

Reaven and Ho [19] developed MS in rats by administration of high-sucrose or high-fructose diets, which induce hypertriglyceridemia, hypertension, nephropathy, and hyperinsulinemia, and increase intraabdominal fat tissue and insulin resistance [20]. We have developed a variant whose abnormalities are induced by continuous administration of sucrose in the drinking water [21,22].

In this study, we investigated the effect of gonadectomy on the metabolism of AA in isolated kidneys of control and MS male and female rats.

2. Materials and methods

2.1. Animal groups

Weanling female and male Wistar rats weighing approximately 40 ± 2 g were used. The controls (C) received tap water for drinking, whereas the MS rats had 30% sucrose solution; both groups were fed commercial rat chow ad libitum for 24 weeks. These groups were further subdivided into 4 subgroups (n = 8 in each subgroup) of C or MS: group 1, intact C female and male; group 2, intact MS female and male; group 3, ovariectomized female (Ovx) C and MS; and group 4, castrated male (Cas) C and MS. The treatment period lasted 6 months. The animals were housed at room temperature of 21°C, with 12-hour cycles of light/darkness.

The care and use of animals followed the institutional guidelines and received institutional approval.

2.2. Gonadectomy

The gonadectomy was carried out at the beginning of the sucrose treatment. Castration and ovariectomy were performed under anesthesia (pentobarbital sodium 63 mg/kg of body weight) as previously described [23,24]. The surgical procedures were as humane as possible and complied with the guidelines for animal care of our institution.

2.3. Measurement of serum sex hormones

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

2.4. Systolic blood pressure

At the end of the treatment, systolic blood pressure (SBP) was measured by a tail-cuff attached to a pneumatic pulse transducer and programmed electrosphygmomanometer (Narco Scientific, Austin, TX), as described previously [23,24]. Briefly, the rats were immobilized in an acrylic device that allowed them to breathe freely, resting on a plate that kept them warm, but left the tail available to apply the tail-cuff. The animals were left to relax for some 15 to 20 minutes. Four measurements of SBP were carried out on each rat or until the readings were reproducible. The data obtained with this technique are not different from either those collected in our database along several years or those obtained by direct arterial cannulation.

2.5. Albuminuria

At the end of the treatment and previous to the collection of urine, the animals were placed in metabolic cages (Nalgene, San Diego, CA) for 5 days, with free access to food and water. The urine was filtered and collected on ice for 24 hours. Albuminuria was measured using bromocresol green reagent; this technique is specific for the quantification of albumin in urine [25].

2.6. Isolated perfused kidney

The rats were anesthetized by intraperitoneal injection of pentobarbital sodium (63 mg/kg body weight). The right kidney was exposed by midline laparotomy, and the mesenteric and right renal arteries were cleared of surrounding tissue. The right renal artery was cannulated through the mesenteric artery to avoid interruption of blood flow; and the kidney was removed, suspended, and perfused at constant flow by means of a peristaltic pump (MasterFlex Easy-load II, no. 77200-50; Cole-Parmer Instrument Co, Vemon Hills, IL) with Krebs solution at 37°C and oxygenated with 95% O_2/5% CO_2. The solution had the following composition (in millimoles per liter): 118 NaCl, 1.2 NaH_2PO_4, 25 $NaHCO_3$, 4.7 KCl, 1.2 $CaCl_2$, 4.2 $MgSO_4$, and 5.5 glucose (pH 7.4). Flow was adjusted to a basal perfusion pressure (PP) of 75 to 90 mm Hg. Mean flow rate of the perfusing solution was 8 to 9 mL/min. Perfusion pressure was measured with a transducer (Grass Telefactor, Grass Technologies, Astro Med, West Warwick, RI), captured, and recorded by means of a Grass model polygraph 79D and online program (Grass PolyView). Data are expressed as changes (Δ) of PP in millimeters of mercury. After at least 15 minutes of perfusion and once a stable PP had been obtained, vasoconstrictor responses to 4 μg/(mL min) AA were determined in the absence and presence of 10 μmol/L indomethacin (Indo) (COX pathway inhibitor), 10 μmol/L NS398 (COX-2 selective inhibitor), or 100 μmol/L ozagrel (pathway inhibitor of thromboxane synthase). These doses were selected from published data as more convenient after dose-response curves were obtained [26].

2.7. Prostaglandins

Prostaglandins were extracted from the urine or kidney perfusate by adding 0.5 mL of water-ethanol 1:4 and 10 μL of glacial acetic acid to 1 mL urine or perfusate. The mixture

was well shaken, and the samples were incubated at room temperature for 5 minutes and centrifuged at 2500*g* for 5 minutes. The supernatant was applied to a Sep-Pak C18 minicolumn (Millipore, Billerica, MA) previously equilibrated with 2 vol of 10% ethanol. The column was then washed with 1 vol of water followed by 1 vol of hexane; and prostaglandins were eluted with 1.50 mL of ethyl acetate. The samples were dried under a nitrogen stream and resuspended in 300 μL phosphate-buffered saline–ethanol (2:1 vol/vol). The concentrations of the 3 prostanoids in urine or perfusate were determined by enzyme immunoassay with kits obtained from Cayman Chemical (Ann Arbor, MI).

2.8. Arachidonic acid

Kidney homogenate AA was extracted as described by Folch et al [27], derivatized to the corresponding methyl esters, and analyzed by gas liquid chromatography (GC-2010 Shimadzu Gas Chromatograph, Shimadzu North America, Pleasanton, CA) as previously described [22,28].

2.9. Microsomes

The left kidney was dissected, cut up, and homogenized in 5 mL cold buffer (250 mmol/L sucrose, 10 mmol/L Tris, 1 mmol/L EDTA [pH 7.4]) in the presence of protease inhibitors (1 mmol/L phenylmethylsulfonyl fluoride, 2 μmol/L pepstatin A, 2 μmol/L leupeptin, and 0.1% aprotinin; Sigma-Aldrich, St Louis, MO); and the homogenate was kept on ice. The homogenate was then centrifuged at 600*g* for 5 minutes at 4°C. The pellet was discarded, and the supernatant was centrifuged at 8000*g* for 10 minutes at 4°C. The pellet was discarded, and the supernatant was centrifuged at 44 000*g* for 1 hour at 4°C; the resultant pellet was resuspended in 250 μL 0.1 mol/L phosphate buffer.

2.10. Immunoblotting

The microsomes (100 μg) were mixed with sample buffer (Tris-HCl [pH 6.5], 1% sodium dodecyl sulfate [SDS], 50% glycerol, and 0.1% bromophenol blue) and boiled for 2 minutes. Proteins were separated on a 10% SDS–polyacrylamide gel electrophoresis gel and transferred to Hybond-C extra nitrocellulose membrane (Millipore). The blots were blocked for 3 hours with Tris buffer solution (TBS) containing 5% nonfat dry milk and 0.5% Tween 20. A rabbit phospholipase A_2 polyclonal antibody, rabbit COX-1 polyclonal antibody, and rabbit COX-2 polyclonal antibody (Santa Cruz Biotechnology, Santa Cruz, CA) were applied individually to each gel, at a dilution of 1:1000, for an entire night. The blots were washed in TBS and incubated with secondary antibody biotinylated-goat antirabbit immunoglobulin G (ZYMED Laboratories, San Diego, CA) at a dilution of 1:5000.

After incubation with the secondary antibody, the membranes were washed with TBS; and the band detection was carried out using 3′3′-diaminobenzidine. Membranes were stripped in a TBS containing 1% SDS and 100 mmol/L β-mercaptoethanol (pH 2), followed by incubation with a 1/2000 α-actin monoclonal mouse antibody. Band intensity was measured densitometrically with a Sigma Scan Pro5 program (Ashburn, VA).

2.11. Statistical analysis

The data are presented as mean ± SE. Statistical significance was determined by 2-way analysis of variance test with Tukey honestly significant difference post hoc test (SigmaPlot 11; Systat Software, Chicago, IL). Differences were considered statistically significant at $P < .05$.

3. Results

3.1. Body weight

No difference in body weight was found between MS, C, and Ovx C female rats; but ovariectomy increased it in Ovx MS in comparison with MS females ($P < .05$). Castration significantly reduced body weight in MS and C in comparison with intact rats ($P < .05$).

3.2. Intraabdominal fat

The MS female rats accumulated significantly more intraabdominal fat compared with the C females ($P < .05$); but after ovariectomy, the highest increase was observed in MS compared with intact MS females ($P < .05$).

The accumulated intraabdominal fat in MS male rats was significantly greater than that in C animals ($P < .01$). Castration decreased fat accumulation in Cas MS when compared with intact MS male rats ($P < .01$).

3.3. Systolic blood pressure

The SBP did not differ between C, MS, and Ovx C female rats. In Ovx MS, it increased vs MS females ($P = .01$, Table 1). In MS males, SBP was significantly increased compared with C male rats ($P = .001$), whereas castration decreased it significantly in MS ($P = .001$) but did not affect it in C rats.

In a sample of measurements taken as described in "Materials and methods," the coefficient of variability ranged from 0.16% to 2.8% (n = 70), with no apparent bias in any group.

3.4. Albuminuria

Albuminuria was not altered in C, MS, and Ovx C females; but it did increase ($P = .03$) in Ovx MS rats. In MS male rats, it was significantly increased vs C males ($P = .001$) (Table 1). Castration in MS rats decreased it vs intact MS rats ($P = .001$), whereas castration in C caused no significant change.

3.5. Sex hormones

Undetectable levels of the corresponding sex hormones proved the success of the gonadectomy procedure.

Table 1
General characteristics of experimental rat groups, control, and MS

	Female rats				Male rats			
	C	MS	Ovx C	Ovx MS	C	MS	Cas C	Cas MS
Body weight (g)	363 ± 9	387 ± 13	362 ± 3	455 ± 7*	499 ± 5	480 ± 10	431 ± 1¶	405 ± 3¶
Intraabdominal fat (g)	5.6 ± 0.4	13.3 ± 0.9†	6.0 ± 0.7	20.3 ± 2.8†	5.0 ± 0.1	18.3 ± 1.4#	4.0 ± 0.5	13.1 ± 0.4#
SBP (mm Hg)	111 ± 3	120 ± 5	116 ± 2	139 ± 5‡	123 ± 2	145 ± 3**	128 ± 1	125 ± 1**
Albuminuria (mg/24 h)	3.9 ± 0.4	3.6 ± 1.9	3.7 ± 0.3	10.7 ± 2.3§	6.5 ± 0.7	15.5 ± 1.5††	5.8 ± 1.4	4.0 ± 0.9††
Serum estradiol (nmol/L)	0.28 ± 0.01	0.38 ± 0.003	<0.001	<0.001	0.003 ± 0.001	0.002 ± 0.001‡‡	0.12 ± 0.04‡‡	0.11 ± 0.02‡‡
Serum testosterone (μmol/L)	0.013 ± 0.005	0.008 ± 0.003	0.016 ± 0.003	0.005 ± 0.001‖	0.025 ± 0.006	0.006 ± 0.002§§	<0.001	<0.001

Body weight, intraabdominal fat, SBP, albuminuria, and hormone concentration in C, MS, Ovx C, and Ovx MS female rat groups, and C, MS, Cas C, and Cas MS male rat groups. With the exception of the Ovx C vs Cas C in albuminuria and body weight, all groups showed significant difference between females and males ($P < .01$). Values are means ± SE.

Females:

* Body weight MS vs Ovx MS; $P < .05$.
† Intraabdominal fat C vs SM and MS vs Ovx MS; $P < .05$.
‡ SBP MS vs Ovx MS; $P < .05$.
§ Albuminuria MS vs Ovx MS; $P < .05$.
‖ Serum testosterone MS vs Ovx MS; $P < .05$.

Males:

¶ Body weight C and MS vs Cas C and Cas MS; $P < .01$.
# Intraabdominal fat C and Cas MS vs MS; $P < .01$.
** SBP C and Cas MS vs MS; $P < .01$.
†† Albuminuria C and Cas MS vs MS; $P < .01$.
‡‡ Serum estradiol C and MS vs Cas C and Cas MS; $P < .05$.
§§ Serum testosterone C vs MS; $P < .05$.

Testosterone levels in C and MS female rats showed no difference; but in Ovx MS, testosterone decreased in comparison with Ovx C ($P < .05$).

In MS male rats, basal testosterone was lower than that in C ($P = .01$). Estradiol levels in Cas C and Cas MS showed significant increase in comparison with those in C and MS ($P < .01$, Table 1).

3.6. Arachidonic acid

In kidney homogenate from C, MS, and Ovx C female rats, the concentration of free AA was not significantly different; but it decreased in the kidney homogenate from Ovx MS in comparison with that from MS females (10.8 ± 1 vs 14 ± 0.8 nmol/mg protein, respectively; $P = .03$).

A decrease of AA was observed in the kidney homogenate from MS male rats compared with that from C males (23.2 ± 1 vs 29.4 ± 2.3 nmol/mg protein, respectively; $P = .03$). The concentration of AA increased in Cas C (36 ± 2.5 nmol/mg protein) and Cas MS (32.7 ± 1.3 nmol/mg protein) in comparison with that of C and MS males ($P = .001$, Fig. 1).

3.7. Effect of AA on PP in female rat kidney

Perfusion pressure was measured in perfused isolated kidneys from C, MS, Ovx C, and Ovx MS female rats, with 4 μg of AA, in the presence or absence of Indo, NS398, or ozagrel. No difference in PP was found between C, MS, and Ovx C groups (19.4 ± 4.1, 15.4 ± 3.7, and 20.4 ± 2 mm Hg, respectively). Gonadectomy increased PP in Ovx MS rats (31.7 ± 5.3 vs 15.4 ± 3.7 mm Hg, $P = .02$) in comparison with that in intact MS (Fig. 2A). The perfusion of 4 μg of AA plus 10 μmol/L Indo decreased PP in kidney of Ovx MS by 74% ($P = .05$), but did not modify PP in the kidney from C, MS, and Ovx C rats, when compared with PP without Indo.

Fig. 2A shows PP in the female rat groups, when 4 μg of AA plus 10 μmol/L of NS398 was perfused in the kidney from Ovx MS rat. NS398 reduced the PP by 43% in comparison with MS ($P = .05$). In the kidneys from C, MS, and Ovx C rats, NS398 had no effect on PP. There was no change between groups when AA was perfused in the presence of 100 μmol/L ozagrel.

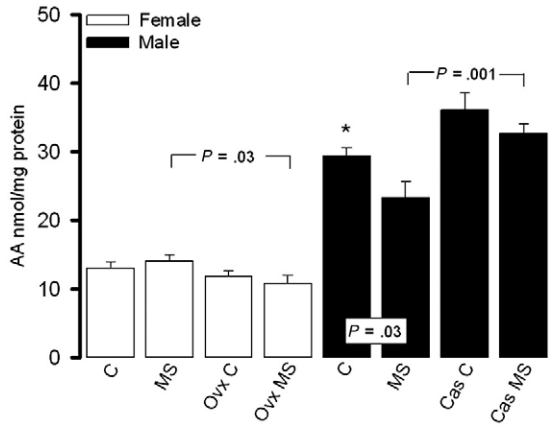

Fig. 1. Nonesterified AA concentration in the kidney homogenate of experimental rat groups. C vs Cas C; $P < .03$ (*). In all the cases, the difference between females and males was significant; $P < .001$. Values are means ± SE.

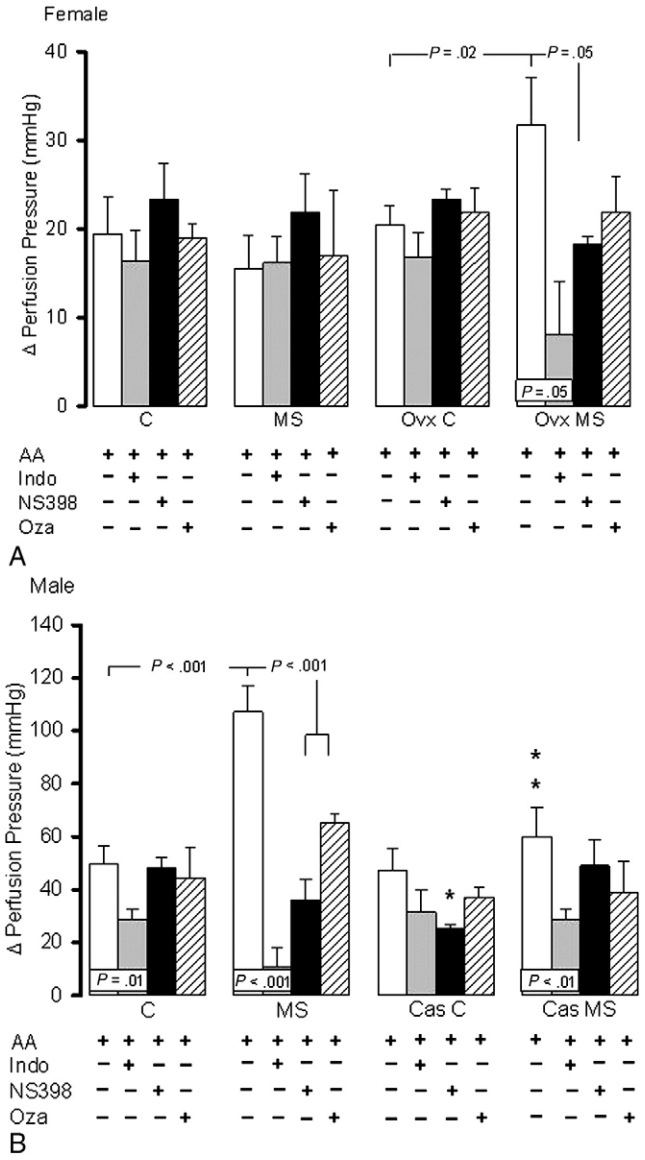

Fig. 2. Renal PP in isolated kidney. The C, MS, Ovx C and Ovx MS female rats (A) and C, MS, Cas C and Cas MS male rats (B) were perfused with 4 μg of AA in the absence or the presence of inhibitors. Arachidonic acid 4 μg, AA 4 μg plus 10 μmol/L Indo, AA 4 μg plus 10 μmol/L NS398, and AA 4 μg plus 100 μmol/L ozagrel. Male: Cas C plus AA vs Cas C plus AA + NS398 (*), SM vs Cas MS; $P < .001$ (**). When comparing female against male rats, the only groups that showed significant differences were Ovx C vs Cas C with ozagrel ($P < .001$) and MS female vs MS male with AA ($P < .01$). Values are means ± SE.

3.8. Effect of AA on PP in male rat kidney

The perfusion of 4 μg AA in MS males increased PP in comparison with C rats (107.0 ± 10.0 vs 49.5 ± 6.8 mm Hg, respectively; $P < .001$). In Cas C rats, PP was not modified (47.0 ± 8.2 mm Hg) in comparison with that in C; but in Cas MS rats, PP diminished (59.8 ± 11.1 vs 107.0 ± 10.0 mm Hg, $P < .001$) in comparison with that in MS rats (Fig. 2B).

The perfusion of 4 μg AA plus 10 μmol/L Indo decreased PP in C rat kidney by 41% ($P = .01$) and by 89% in kidney from MS rats ($P < .001$). However, the perfusion of Cas MS rat kidneys with 4 μg AA plus Indo at the same concentration only diminished it by 51% ($P < .01$), as in control animals.

The addition of 4 μg AA plus 10 μmol/L NS398 or 100 μmol/L ozagrel did not affect PP in C, Cas C, and Cas MS; but PP in MS rat kidney was decreased by 66% and 39% in the presence of NS398 and ozagrel, respectively ($P < .001$, Fig. 2B).

3.9. COX-1 and COX-2 expression

Kidney microsomes from Ovx MS female rats had greater COX-1 protein expression than those from MS female rats ($P = .01$, Fig. 3A). In male kidney microsomes from C, there was a significantly lesser expression than that in MS. In Cas MS males, a decrease in COX-1 protein expression was observed in comparison with that from MS male rats ($P = .03$ and $P = .01$, respectively).

Cyclooxygenase-2 protein expression mostly showed a similar trend to that of COX-1 (Fig. 3B).

Kidney microsomes from MS female rats had significantly lesser COX-2 protein expression than those from Ovx MS rats ($P = .009$). In the kidney microsomes from C and Cas MS male rats, a decrease of COX-2 protein expression was observed in comparison with that from MS male rats ($P = .02$).

3.10. Levels of AA metabolites in kidney perfusate

Table 2 shows no difference in the levels of thromboxane (TX) B_2 between C and MS in female kidney perfusate. Gonadectomy increased it in MS ($P = .01$) but not in C.

In MS males, TXB_2 was significantly increased compared with C male rats ($P = .004$), whereas castration decreased it significantly in MS ($P = .004$) but did not affect it in C rats.

In the levels of prostaglandin (PG) E_2 and 6-keto-$PGF_{1\alpha}$, no significant difference was observed among any of the female groups; but in MS male rats, PGE_2 was significantly increased in comparison with that in C ($P = .04$). Castration normalized the levels of PGE_2 in MS ($P < .04$), but did not change those in C male rats, whereas 6-keto-$PGF_{1\alpha}$ was not changed in any of the groups of male rats.

3.11. Prostaglandin level in urine

The level of 6-keto-$PGF_{1\alpha}$ in urine from female rats did not show significant differences in any of the groups; but in MS males, it decreased compared with C and Cas MS ($P < .01$, Fig. 4A).

The level of PGE_2 increased in Ovx MS vs MS females ($P = .04$). In Cas MS and C male rats, the PGE_2 was decreased in comparison with MS males ($P = .04$ and $P = .01$, respectively; Fig. 4B).

In Ovx MS, TXB_2 levels increased significantly in comparison with those from MS and Ovx C ($P = .01$). In males, the highest level of TXB_2 was found in MS; and castration reduced it to the level found in C ($P < .01$ and $P = .01$, respectively; Fig. 4C).

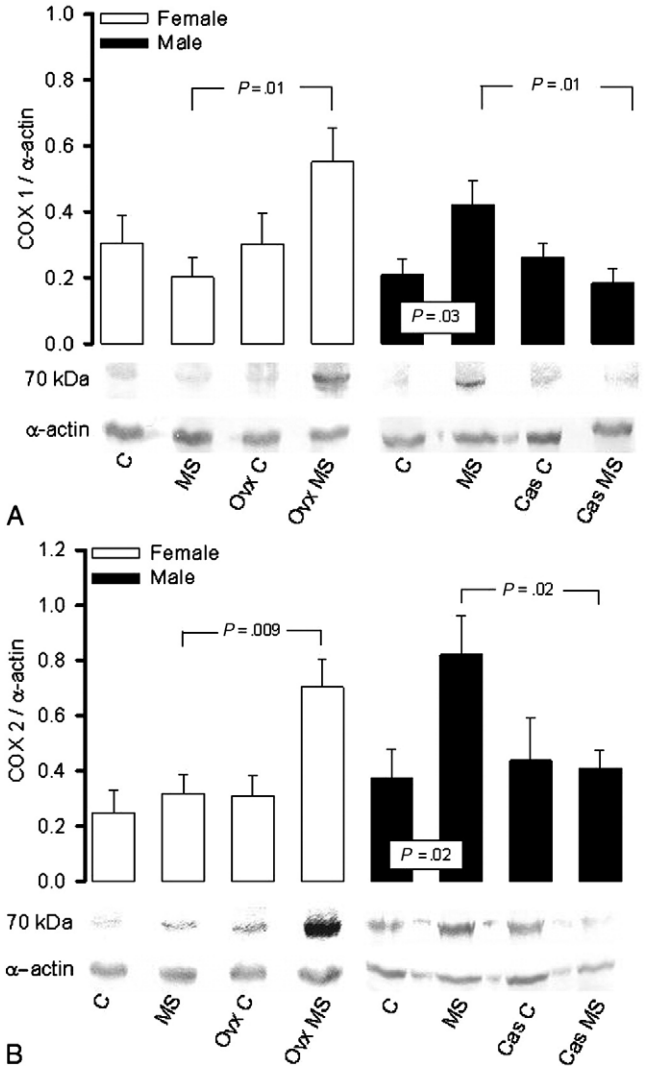

Fig. 3. Densitometric analysis of Western blots of COX-1 and COX-2 protein expression in kidney microsomes from female and male rats. Cyclooxygenase-1 expression (A). When comparing female against male rats, the only groups that showed significant difference were MS female vs MS male ($P < .05$) and Ovx MS vs Cas MS ($P < .001$). Cyclooxygenase-2 expression (B). The comparison between female and male rats showed that the groups significantly different were MS female vs MS male ($P < .001$) and Ovx MS vs Cas MS ($P < .01$). Values are means ± SE.

4. Discussion

The main purpose of this study was to determine the effects of gonadectomy on AA metabolism of the isolated perfused kidney in a rat model of MS.

The high-sucrose diet increased fat deposits in both male and female rats, the effect being more pronounced in males. In male MS rats, the increased intraabdominal fat was associated with decreased serum testosterone levels, as has also been reported in android obesity, inversely associated with testosterone levels [29]. Moreover, castration of MS rats diminished the intraabdominal fat proportion because testosterone diminishes the activity of the hormone-sensitive lipase in adipocytes and increases the proportion of corporal fat and insulin resistance [30]. These observations suggest that testosterone concentration is an important factor in the development of MS associated with fat accumulation. In female MS rats, the increased intraabdominal fat accumulation by ovariectomy is associated with the loss of circulating estradiol. In women, the loss of circulating estrogen after menopause is associated with an increase in central body fat; and this effect is attenuated by estrogen treatment [31].

Systolic blood pressure in MS male rats was observed to be high after about 4 to 5 months of sucrose treatment, in comparison with MS female rats, in which the increase took place later [32]. In the present study, we observed a similar tendency. The increase and decrease of SBP in Ovx MS female and Cas MS male rats, respectively, suggest that sex hormones are involved in the regulation of SBP in our model; other investigators have observed that, in spontaneously hypertensive (SHR) rats, males have higher SBP than females [33]. A significant decrease in serum testosterone concentration was found in MS male rats when compared with C males. This might be due to its high metabolism rate resulting from the increased amount of intraabdominal fat in MS males. The partial loss of testosterone in MS animals can be associated with increased vasoconstriction and SBP, as described elsewhere [34]. However, the total elimination of testosterone from the circulation by castration improves some variables found altered in MS male rats, such as high SBP, fat accumulation, albuminuria, and COX expression,

Table 2
Levels of AA metabolites in perfusate from kidney

(ng/mL)	Female rats				Male rats			
	C	MS	Ovx C	Ovx MS	C	MS	Cas C	Cas MS
TXB_2	0.38 ± 0.13	0.40 ± 0.05	0.45 ± 0.07	0.97 ± 0.23*	0.49 ± 0.14	1.48 ± 0.17†	0.46 ± 0.13	0.43 ± 0.18†
PGE_2	0.49 ± 0.04	0.42 ± 0.06	0.48 ± 0.11	0.78 ± 0.24	0.64 ± 0.13	1.10 ± 0.15‡	0.66 ± 0.10	0.65 ± 0.06‡
6-keto-$PGF_{1\alpha}$	0.51 ± 0.15	0.51 ± 0.08	0.38 ± 0.06	0.53 ± 0.21	0.62 ± 0.14	0.54 ± 0.10	0.55 ± 0.17	0.30 ± 0.15

In Ovx MS female rats and MS male rats, TXB_2 is the metabolite that shows higher participation in vascular-renal vasoconstriction followed by PGE_2 in MS male rats. Castration normalized it in Cas MS. Significant change was present in TXB_2 and PGE_2 in MS female vs MS male rats; $P = .002$. Values are means ± SE.

* Female: MS vs Ovx MS, male: C and Cas MS vs MS; $P < .01$.

† Male: C and Cas MS vs MS; $P = .004$.

‡ Male: C and Cas MS vs MS; $P = .04$.

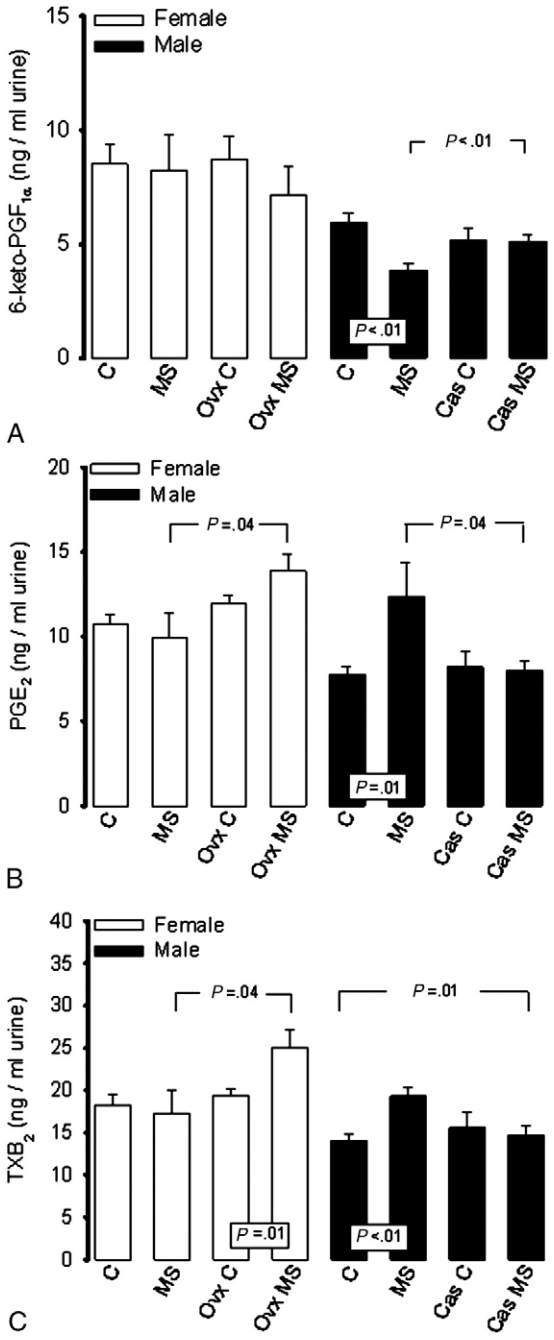

Fig. 4. Levels of 6-keto-$PGF_{1\alpha}$, PGE_2, and TXB_2 in rat urine. 6-keto-$PGF_{1\alpha}$ (A). In all the cases, the difference between females and males was significant ($P < .01$). Prostaglandin E_2 (B). With the exception of the MS female vs MS male, all cases showed significant difference between females and males ($P < .001$). Thromboxane B_2 (C). With the exception of MS female vs MS male, all cases showed significant difference between females and males ($P < .01$). Values are means ± SE.

suggesting that there is a threshold of testosterone level for the development of the MS in our animals. Besides, in Ovx MS females, the lowest level of testosterone corresponds with the highest BP. This supports the notion that there is a threshold for testosterone to contribute to higher BP and levels of albuminuria in Ovx MS females.

The increased levels of estradiol in both Cas C and MS males can be explained by extragonadal aromatization in adrenal glands and adipose tissue, as suggested by several authors [35,36].

The larger vasoconstriction found in kidneys from MS males and attributed to the altered AA metabolism, mediated by the COX pathway, may be associated with the concentration of testosterone. This proposal is supported by work which demonstrated that testosterone is essential for the development of endothelial dysfunction and hypertension, secondary to insulin resistance, suggesting a facilitating role for testosterone in increasing SBP in fructose-fed male rats [37].

Estradiol can decrease the synthesis and release of vasoconstrictor prostaglandins [38,39], whereas testosterone has an opposite effect [40]. Androgens are necessary for the development of hypertension induced by fructose diet in male rats because testosterone can regulate the thromboxane pathway, stimulating thromboxane synthase [41,42]. A testosterone threshold level may be necessary to manifest the vasoconstrictor effect of thromboxane in MS male rats. The decrease of SBP in Cas MS male rats and its increase in Ovx MS female rats suggest effects of sex hormones on prostanoid-potentiated vascular contraction [43]. Testosterone can indirectly stimulate sodium reabsorption via the proximal kidney tubule [44] and also via components of the RAS [45], influencing increase of SBP and thus contributing to renal failure. Estradiol can downregulate the RAS [18,46-48].

The high-fructose or high-sucrose diet–induced high SBP in animal models of MS is associated with renal damage [21]; obesity may also contribute to nephropathy [49].

Proteinuria is an early predictor of rapid progression of renal damage [50]. Our results show that sucrose intake increases albuminuria in male but not in female rats.

The rate of progression of renal disease evidenced by the decline in renal function, increase of glomerular permeability, proteinuria, and glomerulosclerosis [51] appears more rapidly in men than in women [52]. Androgens may mediate sex differences in renal hemodynamics, SBP regulation, and proteinuria in male SHR; nevertheless, castration preserves renal function [33] and reduces proteinuria [53]. Fortepiani et al [54] investigated renal failure in SHR, caused by aging and androgen suppression, demonstrating that castration improves glomerular filtration, renal-vascular resistance, and proteinuria. The increased albuminuria in Ovx MS female rats and MS male rats, respectively, in comparison with their controls, and its decrease in Cas MS male rats suggest that albuminuria is BP-related and that sex hormones may modulate kidney failure in our MS model.

In kidney from MS males, but not in that from MS female rats, AA perfusion produced an increment in PP in comparison with that in C rats, whereas Indo inhibited it to a large extent. This indicates that AA metabolites, released through COX isoform pathways, contribute to increased vasoconstriction in the MS male. The attenuation by NS398

of increased PP induced by AA alone in MS male rats vs C suggests that COX-2 participates in the regulation.

The suppression of sex hormones by gonadectomy reduced or increased PP in kidneys in Cas MS and Ovx MS rats, respectively; and the difference in PP registered in kidneys from MS was larger than that in Cas MS male rats. Consequently, testosterone and estradiol may participate in the regulation of this metabolic pathway [18,41]. Ozagrel attenuated PP increase by AA in MS male rats; this indicated that COX-2 isoform/thromboxane pathways are up-regulated by a decrease in testosterone levels in MS males, but not in MS females. In experimental models of hypertension and MS induced by fructose, it was found that vascular production of TXA_2, a product of COX isoforms [55], is increased [41]. In Cas MS male rats, PP did not change significantly in the presence of ozagrel, but did so with Indo and NS398; this suggested that the total suppression of testosterone by castration could modify the thromboxane pathway.

In gonadectomized fructose-fed male rats, TXA_2 concentration decreases in comparison with that in sham-operated rats [56]. A study in diabetic patients treated with ozagrel showed a decreased urinary albumin excretion, indicating that there is TXA_2 participation in renal failure [57]. Furthermore, PGE_2 and TXA_2 induce transcription of type IV collagen, laminin, and fibronectin [58] in the glomerular basement membrane, which are involved in renal damage [52,59]. In addition, testosterone induces increases in PGE_2 and TXA_2 [53,60]. Dyslipidemia associated with renal failure may also contribute to its progression [61].

There is a relationship between lipid reduction and both the preservation of normal glomerular filtration and the absence of proteinuria [62].

In our MS, there is hypertriglyceridemia, intraabdominal fat, and an increase of all circulating free fatty acids [28] except for free AA, whose concentration was reduced, which may contribute to renal failure. The concentration of nonesterified AA in kidney homogenate was also found to be decreased in MS males vs C [28,63]. The reduction in nonesterified AA biosynthesis could be a determining factor that may account for altered synthesis of the derived prostaglandins, and also the increase of SBP and albuminuria in MS [63,64].

Castration increased the concentration of nonesterified AA in both Cas MS and Cas C male rats in comparison with C and MS males. In MS female rats, ovariectomy decreased nonesterified AA in comparison with MS female rats. The effect of ovariectomy on AA concentration may be due to the lack of estradiol, which increases phospholipase A_2 activity, a key enzyme for AA release from membrane phospholipids [65,66].

The increment in the expressions of kidney COX-1 and COX-2 in both Ovx MS females and MS males, and the decrease in the expressions in Cas MS male rats in comparison with controls, respectively, may contribute to the changes observed in the amount of nonesterified AA.

In Cas C and Cas MS male rats, the increment of nonesterified AA in kidney homogenate is probably due to the decrease in the expression of COX isoforms, resulting in AA accumulation in the cellular membrane phospholipids (analysis not performed in this study).

The interstitial cells located in the renal medulla present abundant vacuoles, rich in polyunsaturated fatty acids, among them AA [13]. The transplant of renal medulla, but not of cortex, reduces the SBP in hypertensive animals [67].

Gonadectomy in MS males vs MS intact males decreased COX-1 and COX-2 expressions, which are related to a decrement in the TXB_2 (stable metabolite of TXA_2) and PGE_2 in kidney perfusate. The overexpression of COX-1 and COX-2 in MS males is associated with significant increases of both TXB_2 and PGE_2 in the kidney perfusate. In addition, TXB_2 increased by 200% whereas PGE_2 only increased by 57%, suggesting that TXB_2 participates more than PGE_2 in our MS male model. In the MS female group, gonadectomy significantly increased the overexpression of COX isoforms, which was associated with a significant increase in TXB_2 by 142%; PGE_2 tended to increase without reaching significant difference. These results suggest that the lack of estradiol due to ovariectomy and the reduced levels of testosterone by extragonadal biosynthesis in adipose tissue, as previously described [22], in Ovx MS can modulate the metabolism of AA toward the biosynthesis of TXB_2 rather than of PGE_2. PGE_2 and TXB_2 in the renal medulla cells [68], which are involved in renal hemodynamics, participate in the regulation of the vascular tone and in the balance of water, salt, and renin [50,55]. There is an inverse association of COX-1 metabolite (6-keto-$PGF_{1\alpha}$) [69] with the decrease of SBP and renal damage in experimental fructose-fed models [19,60,70].

Our results show a decrease of 6-keto-$PGF_{1\alpha}$ in urine of MS males in comparison with C and Cas MS male rats, but without change in kidney perfusate. The change in concentration of urine 6-keto-$PGF_{1\alpha}$ may be due to altered systemic metabolism in the MS male model.

However, it has been described that postmenopausal women undergoing estrogen replacement therapy have elevated urinary levels of TXA_2 and prostacyclin [43]. Our results show that, with the exception of intact MS females, in comparison with intact MS males, the concentrations of PGE_2 and TXB_2 were significantly increased in all female animals vs all male animals in urine but not in kidney perfusate; this variation in prostaglandin concentration may be due to an effect of estrogens on the systemic metabolism in female rats.

The estrogen effect of increased TXB_2-induced vasoconstriction in females has not been explained. However, the results suggest that TXB_2 does not have such effect in female rats when estrogens are present; but when they are eliminated by ovariectomy, it may exercise its vasoconstrictor effect. Vascular TXA_2 production has been described as important only in pathophysiologic states such as hypertension in males, but not under normal conditions or in females [43,71].

The results suggest that COX isoform pathways are altered and shift toward an increase of TXB_2 and PGE_2 in MS male rats; sex hormones can modulate them, thus improving renal function [6,60].

On the other hand, AA can be metabolized through different pathways, that is, those of lipoxygenase and cytochrome P-450, to produce other prostanoids like 12-, 15-, 20-HETEs and EETs, which may participate in inducing renal damage in MS. Sex differences may regulate the expression of CYP and derived metabolites, associated with renal failure in rodent models [72,73]. This suggestion needs further investigation [12,68,73,74].

We have evaluated aortic reactivity in a variety of male and female rats: intact, castrated, and castrated with replacement of testosterone and estradiol, in control and MS, including the effect on them of Indo in the preparations. The results indicate significantly beneficial effects caused by the presence of estradiol and by the absence of testosterone. Participation of the COX pathway metabolites is evidenced [23,24].

In conclusion, the results suggest that there is a close association between MS and renal dysfunction, mediated by alteration of the AA metabolism through the COX pathway and influenced by sex hormones. In MS female rats, gonadectomy indicates a protective effect by estrogens against the increase of PP and COX isoform expression. In MS male rats, a threshold level of testosterone is required to develop the MS. The absence of testosterone in MS male rats modifies this alteration with a protective effect, decreasing PP and COX isoform expression and contributing to improve renal function.

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