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TRANSLATIONAL PHYSIOLOGY

Decreased endothelial nitric oxide, systemic oxidative stress, and increased sympathetic modulation contribute to hypertension in obese rats

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da Cunha NV, Pinge-Filho P, Panis C, Silva BR, Pernomian L, Grando MD, Cecchini R, Bendhack LM, Martins-Pinge MC. Decreased endothelial nitric oxide, systemic oxidative stress, and increased sympathetic modulation contribute to hypertension in obese rats. *Am J Physiol Heart Circ Physiol* 306: H1472–H1480, 2014. First published March 14, 2014; doi:10.1152/ajpheart.00520.2013.—We investigated the involvement of nitric oxide (NO) and reactive oxygen species (ROS) on autonomic cardiovascular parameters, vascular reactivity, and endothelial cells isolated from aorta of monosodium glutamate (MSG) obese rats. Obesity was induced by administration of 4 mg/g body wt of MSG or equimolar saline [control (CTR)] to newborn rats. At the 60th day, the treatment was started with N^G-nitro-L-arginine methyl ester (L-NAME, 20 mg/kg) or 0.9% saline. At the 90th day, after artery catheterization, mean arterial pressure (MAP) and heart rate were recorded. Plasma was collected to assess lipid peroxidation. Endothelial cells isolated from aorta were evaluated by flow cytometry and fluorescence intensity (FI) emitted by NO-sensitive dye [4,5-diaminofluoresceindiacetate (DAF-2DA)] and by ROS-sensitive dye [dihydroethidium (DHE)]. Vascular reactivity was made by concentration-response curves of acetylcholine. MSG showed hypertension compared with CTR. Treatment with L-NAME increased MAP only in CTR. The MSG induced an increase in the low-frequency (LF) band and a decrease in the high-frequency band of pulse interval. L-NAME treatment increased the LF band of systolic arterial pressure only in CTR without changes in MSG. Lipid peroxidation levels were higher in MSG and were attenuated after L-NAME. In endothelial cells, basal FI to DAF was higher in CTR than in MSG. In both groups, acetylcholine increased FI for DAF from basal. The FI baseline to DHE was higher in MSG than in CTR. Acetylcholine increased FI to DHE in the CTR group, but decreased in MSG animals. We suggest that reduced NO production and increased production of ROS may contribute to hypertension in obese MSG animals.

nitric oxide synthase; endothelium; autonomic; monosodium glutamate

THE PREVALENCE OF OBESITY is increasing globally, both in industrialized and in developing nations. High-density caloric diets and physical inactivity are the main causes of obesity (24). Obesity is commonly associated with diabetes, cardiovascular diseases, and several other comorbidities such as the metabolic syndrome. The metabolic syndrome is a cluster of metabolic perturbations characterized by insulin resistance, abdominal obesity, hypertension, and hypertriglyceridemia (50).

The monosodium glutamate (MSG)-induced obesity is a model that has been described by the literature that mimics, at least in part, the clinical condition of obesity (12, 48). The obesity is induced by subcutaneous administration of monosodium glutamate (MSG model), during the first days of life, in which the blood-brain barrier is not formed, affecting the circumventricular organs, including the hypothalamic arcuate nucleus (ARC) (29). The ARC is one of the main places that regulate energy homeostasis and is suggested to have a role in the alterations associated with MSG-induced obesity (32). It has been observed that the MSG animals have reduced production of growth hormone, hypercorticosteronemia, hyperinsulinemia, hyperleptinemia, insulin resistance, behavioral changes, and increased deposition of visceral fat (2, 14, 18, 38, 47).

Although alterations in the function of the autonomic nervous system are not included in the criteria needed for the diagnosis of metabolic syndrome, it is well known that the sympathetic nervous system activation is implicated in the pathogenesis of specific components of the metabolic syndrome and is associated with increased cardiovascular risk. A common feature of obesity in humans and animal models is the increased sympathetic activity (33, 44), which may lead to increased blood pressure via peripheral vasoconstriction and increased renal tubular reabsorption of sodium (49). The literature has been exploited among the potential mechanisms involved in increased sympathetic activity in obesity. In a previous study, we found an increase in the sympathetic component of the cardiac autonomic modulation (8). We also observed that the MSG animal presented increased renal sympathetic nerve activity compared with controls (10).

Obesity has also been associated with an inflammatory state of low intensity (26) characterized by high circulating levels of cytokines and reactive oxygen species (ROS), resulting in increased oxidative stress (1, 36, 42). We have previously demonstrated that MSG-induced obesity in rats induced a

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moderate level of hypertension in adulthood, with higher levels of prostaglandins compared with controls, suggesting an involvement of inflammation in this model of obesity (7). In the same study, we observed an increase in systemic oxidative stress in these animals. However, we do not know if the vasculature is the generator of this oxidative status.

One source of ROS is uncoupled endothelial nitric oxide synthase (eNOS). The eNOS uncoupling is a process in which eNOS generates O_2^- when the concentration of either L-arginine, the substrate of nitric oxide synthase (NOS), or BH_4, a cofactor of the enzyme, is depleted. This transformation of eNOS from a protective enzyme to a contributor to oxidative stress has been observed in several in vitro models, in animal models of cardiovascular diseases, and in patients with cardiovascular risk factors (22).

NO is a potent modulator of cardiac and vascular function (11). A study of Tokarev and Jezová (60) showed alterations in the nitric oxide (NO) production in MSG rats that involve the cardiovascular function. After acute injection of L-NAME in control and MSG rats the increase in mean arterial pressure (MAP) and decrease in heart rate (HR) were reduced in the MSG obese rats. Also in another work (61) it was demonstrated that eNOS expression was reduced, with a concomitant reduction of mitochondrial biogenesis and function, in white and brown adipose tissue and in the soleus muscle of three different animal models of obesity. However, the involvement of NO in the cardiovascular and autonomic function of MSG obese rats was not evaluated yet.

Although the endocrine, biochemical, and even molecular aspects of MSG-induced obesity have been extensively studied, the involvement of NO and ROS in cardiovascular and autonomic parameters is less understood. Based on this evidence, we tested the hypothesis that: *1*) NO would be involved in the increased blood pressure levels and sympathetic outflow in obesity; *2*) obesity would increase plasma oxidative stress; and *3*) obesity would reduce NO and would increase ROS in endothelial cells.

MATERIALS AND METHODS

Animal care. All experiments were performed in male Wistar rats supplied by the central animal house of the State University of Londrina in Brazil. The animals were housed in perspex cages in a room with a 12:12-h light-dark cycle. Food and water were always available except during the experiments. All experimental protocols were performed in accordance with the *Guide for the Care and Use of Laboratory Animals* and the Ethical Principles for Animal Experimentation established by the Brazilian Committee for Animal Experimentation and were approved by the animal experimentation Ethics Committee of the State University of Londrina, process number 33645/2010-29.

Induction of MSG obesity. The male Wistar rats were either given subcutaneous injections of MSG [4 mg/g of body wt (Sigma, St. Louis, MO)] or an equimolar saline [controls (CTR)] during their first 5 days of life (15). MSG obesity was characterized by calculating the Lee Index for each rat in the 90th day of life using the following formula: $\sqrt{}$body wt/naso-anal length $\times$ 1,000, and also the weight of the retroperitoneal fat and perigonadal where body weight and length are given in gram and centimeters, respectively.

Pharmacological blockade with nonselective constitutive nitric oxide synthase inhibitor. Rats received saline or glutamate in the neonatal period and were treated by the intraperitoneal route with the nonselective constitutive nitric oxide synthase (cNOS) inhibitor N^G-nitro-L-arginine methyl ester (L-NAME, 20 mg/kg) (37) or 0.9% saline (vehicle control). The treatment started at 60 days of life, and the drug was administered every day until the animals were 90 days old. In the present study the animals were divided in the following four groups: saline control, L-NAME control, saline MSG, and L-NAME MSG.

Evaluation of cardiovascular parameters. In the 90th day of life, control and MSG animals were anesthetized intraperitoneally with tribromoethanol (250 mg/kg; Sigma) for chronic catheterization of the femoral artery and vein, with the purpose of monitoring blood pressure and administration of drugs if necessary. The catheter segments were constituted by welded segments of polyethylene PE-10 (4–5 cm) and polyethylene PE-50 (12–13 cm), which were filled with 0.9% saline and anticoagulant (15 U/ml heparin saline solution) and blocked with an occluder. After catheter implantation, they were exteriorized at the dorsal region subcutaneously and fixed to the skin by surgical suture. Following surgery, the animals returned to individual cages throughout the postoperative period. After 24 h, the baseline MAP and HR were recorded.

The MAP and HR were recorded in awake and freely moving animals. The arterial cannula of the animal was attached to a pressure transducer (Powerlab model MLT0380) connected to a computerized recording system (Powerlab/ADInstruments). During the period of recording the animals were kept in individual boxes in a quiet environment as described in previous studies from our laboratory (13, 41, 43). After 1 h of recording MAP and HR, the animals were disconnected from the recording apparatus and remained in their home box.

HR and blood pressure variability. The baseline blood pressure recorded during a 30-min period was processed by customized computer software, which applies an algorithm to detect cycle-to-cycle inflection points in the pulsatile arterial pressure (AP) signal, thus determining beat-by-beat values of systolic and diastolic pressures. Beat-by-beat pulse interval (PI) series from pulsatile AP signal were also generated by measuring the length of time between adjacent systolic waves. From the baseline 30-min recording period, the time series of PI were divided into contiguous segments of 300 beats, overlapped by half. After calculating the mean value and variance of each segment, they were submitted to a model-based autoregressive spectral analysis as described elsewhere (40, 53, 59). Briefly, a modeling of the oscillatory components presented in stationary segments of beat-by-beat time series of PI was calculated based on Levinson-Durbin recursion, with the model order chosen according to the Akaike criterion (40). This procedure allows automatic quantification of the center frequency and power of each relevant oscillatory component present in the time series. The oscillatory components were labeled as very low frequency (VLF: 0.01–0.20 Hz), low frequency (LF: 0.20–0.75 Hz), or high frequency (HF: 0.75–2.50 Hz). The powers of LF and HF components of the systolic arterial pressure (SAP) were expressed in absolute units ($mmHg^2$). The powers of LF and HF components of heart rate variability (HRV) were expressed in absolute (ms^2) and also in normalized units, obtained by calculating the percentage of the LF and HF variability with respect to the total power after subtracting the power of the VLF component (frequencies 0.20 Hz). The normalization procedure tends to minimize the effect of the changes in total power on the absolute values of LF and HF variability (40, 53, 59).

Measurement of plasma lipoperoxidation by chemiluminescence reaction. Lipoperoxidation induced by *tert*-butyl hydroperoxide was evaluated by chemiluminescence assay, according to Zimiani and collaborators (64). Plasma lipoperoxidation was evaluated by adding 125 μl of sample in 865 μl of buffer, 10 mM phosphate, pH 7.4 (NaCl 0.9%), with addition of 10 μl of *t*-butyl 3 mM solution. The reading of the reaction was carried out in a Glomax luminometer (TD 20/20 Turner Designers). The results were expressed in relative light units, and the entire curve obtained was used as an indicator of lipoperoxidation.

Table 1. *Effects of MSG-neonatal administration on body composition in adult rats pretreated with saline or L-NAME*

	CTR Saline (n = 7)	MSG Saline (n = 8)	CTR L-NAME (n = 5)	MSG L-NAME (n = 6)
Weight, g	374 ± 16.17	316 ± 6.94*	384 ± 16.69	281 ± 15.95*#
Naso-anal length, cm	23 ± 0.39	21 ± 0.32*	24 ± 0.25	20 ± 0.48*
Lee Index	0.31 ± 0.004	0.33 ± 0.005*	0.30 ± 0.001	0.32 ± 0.003*
Periepididymal fat, g	3.24 ± 0.42	6.36 ± 0.81*	2.92 ± 0.50	4.38 ± 0.57*
Retroperitoneal fat, g	3.60 ± 0.46	7.08 ± 0.51*	3.06 ± 0.69	5.09 ± 0.51*#
Heart, g	0.9 ± 0.10	0.8 ± 0.03	1,12 ± 0.06	0.76 ± 0.06*

Data are shown as means ± SE; n, no. of rats. CTR, control; MSG, monosodium glutamate; L-NAME, N^G-nitro-L-arginine methyl ester. *Different from control group ($P < 0.05$). #Different from MSG group ($P < 0.05$).

Functional study of vascular reactivity. Rats were killed by decapitation under anesthesia. The thoracic aorta was quickly removed and cut into rings (4 mm length). The aortic rings were placed between two stainless steel stirrups and connected to an isometric force transducer (Letica Scientific Instruments, Barcelona, Spain) to measure tension in the vessels. The rings were placed in the organ chamber containing Krebs solution with the following composition (in mM): 130.0 NaCl, 4.7 KCl, 1.2 KH_2PO_4, 1.2 $MgSO_4$, 14.9 $NaHCO_3$, 5.5 glucose, and 1.6 $CaCl_2$. The solution was maintained at pH 7.4 gassed with 95% O_2 and 5% CO_2 at 37°C. The rings were initially stretched to a basal tension of 1.5 g and then were allowed to equilibrate for 60 min. Endothelial integrity was assessed qualitatively by the degree of relaxation induced by acetylcholine (ACh, 1 μM) in the presence of contractile tone induced by phenylephrine (PE, 0.1 μM). It was discarded if relaxation with ACh was not 80% or greater. Concentration-effect curves for ACh were constructed in nonobese (CTR) and obese (MSG) rat aortas, treated or not with L-NAME. The potency (pD_2) and maximum effect (ME) of ACh in inducing relaxation were evaluated.

Measurement of NO and ROS production in isolated endothelial cells. Aortas were isolated from control and MSG rats, dissected, and longitudinally opened. Endothelial cells were mechanically isolated from the vessels by gentle friction with plastic stem in plates containing Hanks solution. The cell suspension was centrifuged at 1,000 rpm for 5 min, and the cells pellet was suspended in 2.0 ml of Hanks solution with the following composition: 145 mM NaCl, 5 mM KCl, 10 mM dextrose, and 10 mM HEPES, pH 7.4 (4). The endothelial cells were isolated carefully and characterized by flow cytometry after incubation at 37°C for 1 h with endothelial cell marker antibody PE-mouse anti-rat platelet-endothelial cell adhesion molecule-1/CD31 (1:250; BD Pharmingen) in a method adapted from DeLisser et al. (17).

Isolated endothelial cells from control and MSG aortas were first separated into aliquots in the following three groups: blank, basal, and stimulated by ACh. Cytofluorographic analysis was performed by using a Becton-Dickinson FAC/Scanto (San Jose, CA). Intracellular O_2^- and NO were monitored separately by measuring changes in fluorescence intensity (FI) emitted by dihydroethidium (DHE) (16)

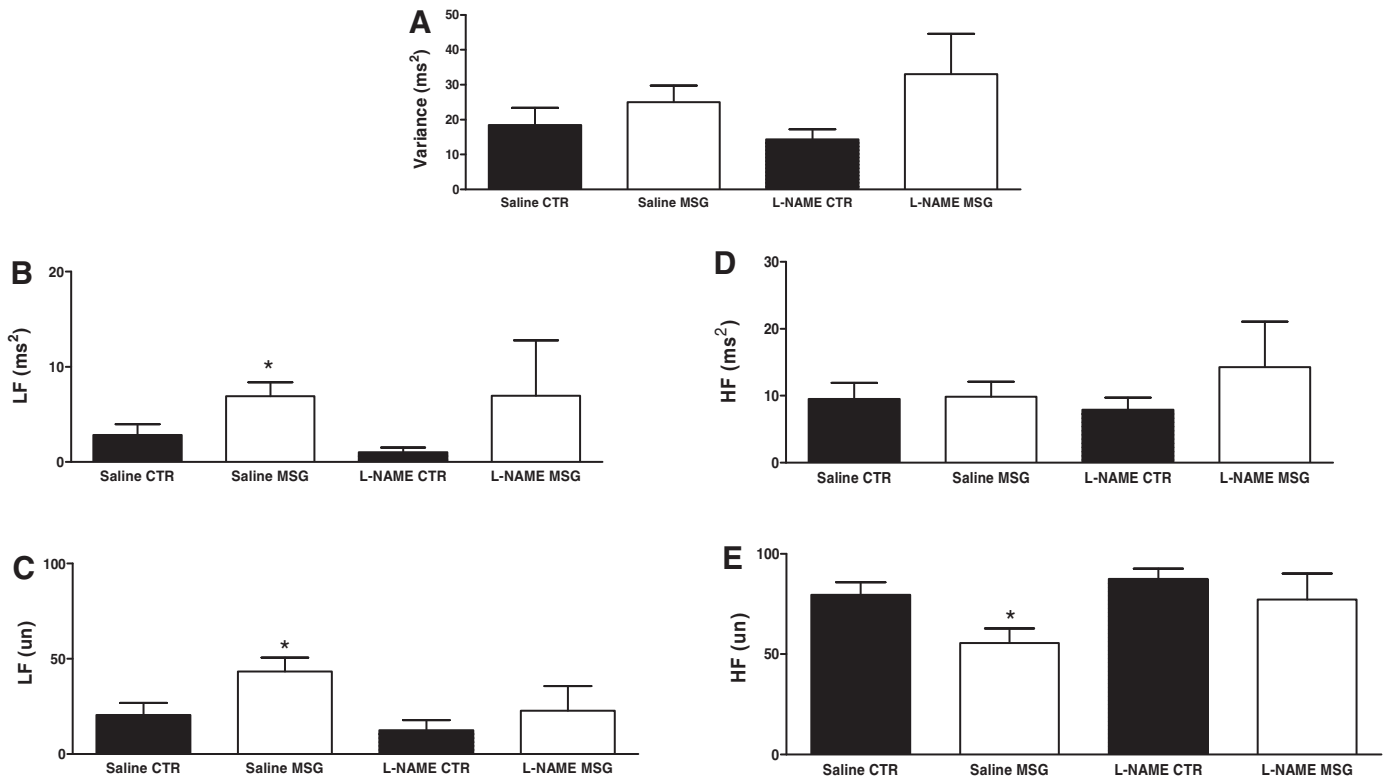

Fig. 1. Spectral parameters of pulse interval (PI) calculated from time series using autoregressive spectral analysis in control (CTR) and obese [monosodium glutamate (MSG)] rats treated previously with saline or N^G-nitro-L-arginine methyl ester (L-NAME). *A*: bar graph representing the total variance of PI; *B*: low-frequency (LF) band in absolute units; *C*: LF band in normalized units; *D*: high-frequency (HF) band in absolute units; *E*: HF band in normalized units. Data are shown as means ± SE. *Different from control group ($P < 0.05$).

and 4,5-diaminofluoresceindiacetate (DAF-2DA) (31) probes, respectively.

Initially, a first group of endothelial cells was analyzed at the flow cytometer without probes (blank) as a control to ensure that there was not interference of emitted fluorescence by probes. After that, the second group of cells was incubated only with DHE (2.5 μM) or DAF-2DA (10 μM) for 20 min (basal). The third group of cells was stimulated with ACh (1 μM) for 10 min, respecting the time of incubation (20 min) for both probes. A similar protocol (blank, basal, and stimulus with ACh) was performed for endothelial cells isolated from CTR and MSG rats treated with the nonselective NOS inhibitor (L-NAME). Cells were initially characterized as endothelial cells by incubating them at 37°C for 1 h with mouse anti-rat PE-CD31 (1:250; BD Pharmingen). About 60% of the isolated cells were CD31 positive. Although we cannot guarantee only CD31-positive cells were analyzed for DAF/DHE due to fluorescence interactions between the D31 marker and DAF/DHE probes, FI for DAF/DHE was made in cells gated for endothelial cell characteristics (cellular size and complexity). Acquisition was set at 5,000 gated cells, and the results were expressed as means ± SE of FI.

Statistical analysis. Statistical analysis of results was performed using the statistical program INSTAT (GraphPad, San Diego, CA). The results were expressed as means ± SE. The differences between the experimental and control groups were analyzed by the analysis of variance test for multiple comparisons followed by the Tukey-Kramer or Student's *t*-test, considering $P < 0.05$ to assess differences between groups of animals.

RESULTS

General characteristics. MSG rats displayed a higher Lee Index, a greater accumulation of retroperitoneal and perigonadal fat, lower body weights, and shorter nasal-anal lengths compared with the control rats as shown in Table 1. These alterations characterized the MSG obesity, which were also documented in other studies of our group (7, 8, 10, 28). The pretreatment with pharmacological blockade of nonselective cNOS inhibitor reduced the body weight and the retroperitoneal fat in MSG rats (Table 1).

Cardiovascular parameters. The evaluation of baseline MAP and HR in obese and control animals showed that MSG rats treated with saline presented an increase in MAP (saline MSG: 138 ± 4 mmHg, $n = 8$, $P < 0.05$) compared with saline-treated control animals (saline CTR: 118 ± 2 mmHg, $n = 7$), with no change in HR (saline CTR: 350 ± 12 beats/min; saline MSG: 384 ± 15 beats/min). Treatment with L-NAME increased MAP in the control animals (L-NAME CTR: 144 ± 7 mmHg, $n = 5$, $P < 0{,}001$) but caused no changes in the MSG animals. HR was unchanged by L-NAME administration.

Autonomic modulation. The autonomic modulation of PI by spectral analysis showed that MSG-obese animals presented an increase in the LF component in absolute units (saline CTR = 2.85 ± 1.11, $n = 7$; saline MSG = 6.91 ± 1.46, $n = 8$, $P < 0.05$) (Fig. 1*B*) and normalized units (saline CTR = 5.69 ± 18.30; saline MSG = 38.49 ± 6.27, $P < 0.05$) (Fig. 1*C*) and a decrease in HF in normalized units (saline CTR = 71.48 ± 6.22; saline MSG = 50.94 ± 7.03, $P < 0.05$) (Fig. 1*E*). None of these parameters was changed with L-NAME treatment (Fig. 1).

In the SAP analysis, no statistical difference was found between control and obese rats (Fig. 2); however, the treatment with L-NAME increased the absolute LF component only in control animals (saline CTR = 8 ± 1, $n = 7$; L-NAME CTR = 14 ± 1, $n = 5$, $P < 0.05$) (Fig. 2*B*) without changes in the MSG group.

Plasma lipoperoxidation. The analysis of plasma lipoperoxidation levels showed that MSG rats presented higher levels of lipoperoxidation compared with the controls. The treatment

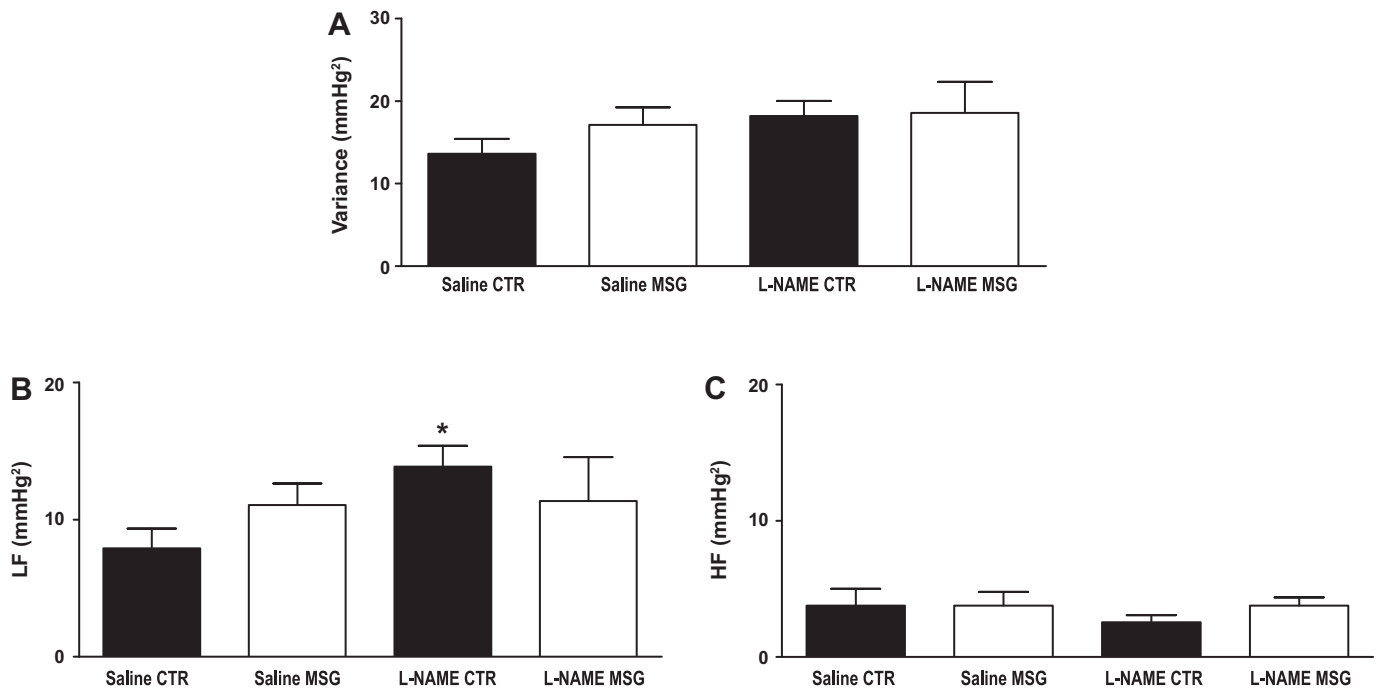

Fig. 2. Spectral parameters of systolic arterial pressure (SAP) calculated from time series using autoregressive spectral analysis in control (CTR) and obese (MSG) rats treated previously with saline or L-NAME. *A*: bar graph representing the total variance of SAP; *B*: LF band; *C*: HF band. Data are shown as means ± SE. *Different from saline control group ($P < 0.05$).

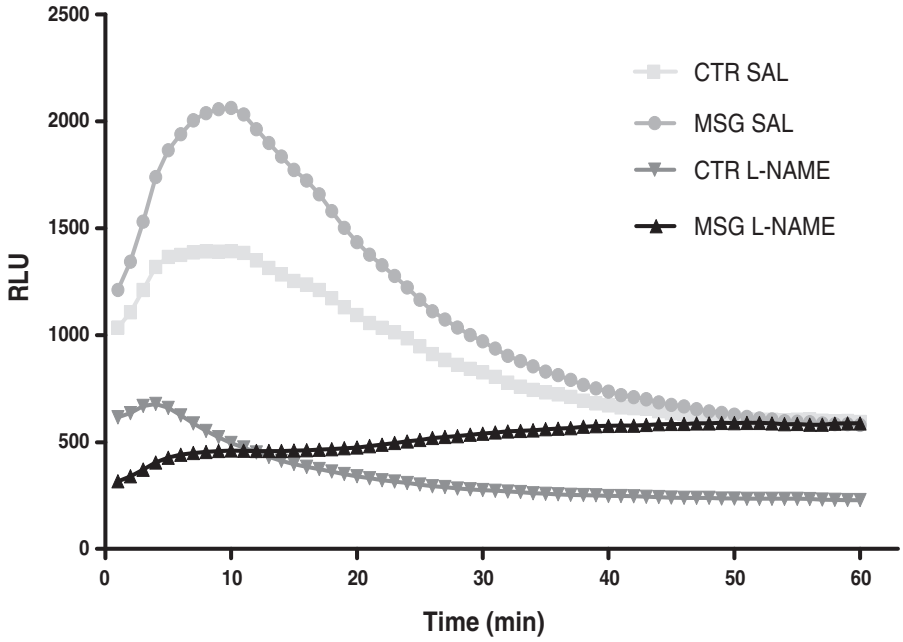

Fig. 3. Effect of treatment with the nitric oxide synthase (NOS) inhibitors on the time course of hydroperoxide-initiated chemiluminescence in plasma of all groups studied. Each graphic is representative from 8–10 animals. The samples were analyzed from all animals individually and represented as means and SE for each group. These data are representative from 2 distinct experiments. The curves were analyzed by using the two-way ANOVA test, which indicated that the curves are significantly different, with P values of 0.001. RLU, relative light units.

with the NOS inhibitor reduced this parameter in both groups (Fig. 3). However, this reduction was greater in the MSG rats.

Vascular reactivity. The cumulative addition of ACh in CTR and MSG aortic rings induced relaxation in a concentration-dependent way. The relaxing response induced by ACh was not different between CTR and MSG as shown by the values in CTR (ME: 100.6 ± 2.6%, pD_2: 7.25 ± 0.13, $n = 4$) and MSG (ME: 99.2 ± 2.5%, pD_2: 7.42 ± 0.13, $n = 5$). The treatment of the CTR rats with L-NAME did not alter the relaxation induced by ACh (ME: 99.8 ± 3.9%, pD_2: 7.14 ± 0.19, $n = 4$). However, the treatment with L-NAME reduced the potency to ACh in MSG rat aorta (ME: 99.9 ± 8.0%; pD_2: 6.86 ± 0.14, $n = 4$; $P < 0.05$). The dose-response curves of ACh of the four groups studied are presented in Fig. 4.

Measurement of NO production in isolated endothelial cells. The basal NO production in aortic endothelial cells from control (5,362.0 ± 256.5, $n = 5$) was higher than in MSG rats (1,324.2 ± 46.4, $n = 4$, $P < 0.001$) (Fig. 5*A*). ACh stimulus increased NO production in both control and MSG rats (CTR: 7,260.0 ± 98.2, $n = 5$, MSG: 2,331.5 ± 98.8, $n = 4$, $P < 0.001$). However, the NO production remained higher in control rats compared with MSG rats.

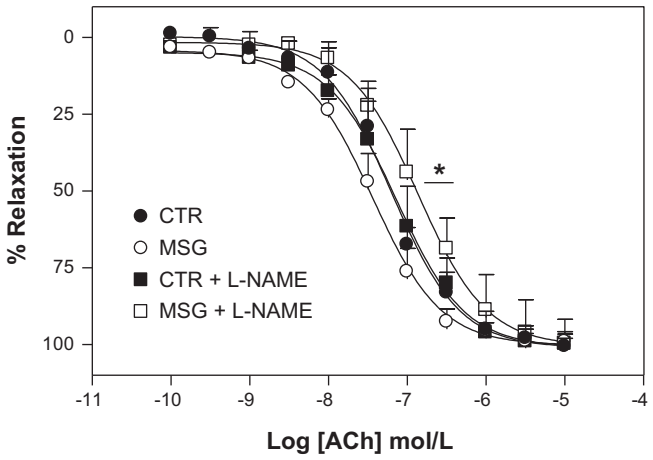

Fig. 4. Vascular relaxation induced by acetylcholine (ACh) in aorta from control rats (CTR) and obese rats induced by MSG, treated or not with the nonselective NOS inhibitor (L-NAME). Data are shown as means ± SE (n = 4–5). *Difference between MSG and MSG + L-NAME.

In isolated endothelial cells from CTR and MSG rats treated with L-NAME, the NO production stimulated with ACh was not different in the CTR (FI: 2,991.3 ± 374.5, $n = 4$) and in MSG (FI: 4,299.3 ± 524.9, $n = 4$) compared with the basal levels (CTR: 2,979.3 ± 509.7, $n = 4$, MSG: 3,948.7 ± 524.8, $n = 4$), as observed in the Fig. 5*B*.

Measurement of ROS production in isolated endothelial cells. The basal O_2^- production was higher in aortic endothelial cells from MSG (48,544.0 ± 1,225.3, $n = 4$, $P < 0.001$) compared with CTR (28,357.4 ± 461.1, $n = 5$) (Fig. 6*A*) rats. However, superoxide production was increased after stimulus with ACh in CTR rats, whereas in MSG rats this production was reduced compared with the respective baseline values. Although the stimulus with ACh reduced the levels of O_2^- in endothelial cells from MSG, these levels remained higher in MSG (40,225.2 ± 634.0, $n = 4$, $P < 0.001$) compared with CTR (31,090.4 ± 1,030.7, $n = 5$) rats.

In isolated endothelial cells from CTR and MSG rats treated with L-NAME, the ROS production stimulated with ACh was not different in the CTR (FI: 41,786.5 ± 572.4, $n = 4$) and in MSG (FI: 42,734.5 ± 925.2, $n = 4$) compared with the basal levels (CTR: 43,274.7 ± 770.9, $n = 4$, MSG: 43,269.7 ± 760.9, $n = 4$) as observed in Fig. 6*B*.

DISCUSSION

Recent studies have demonstrated that rats induced obese by neonatal administration of MSG presented a moderate level of hypertension in adulthood, an increased sympathetic modulation on HRV, increased renal sympathetic nerve activity, altered baroreflex sensitivity, and an increased status of oxidative stress (7, 8, 10, 28). The current study extends these previous findings showing that MSG rats presented reduction in NO

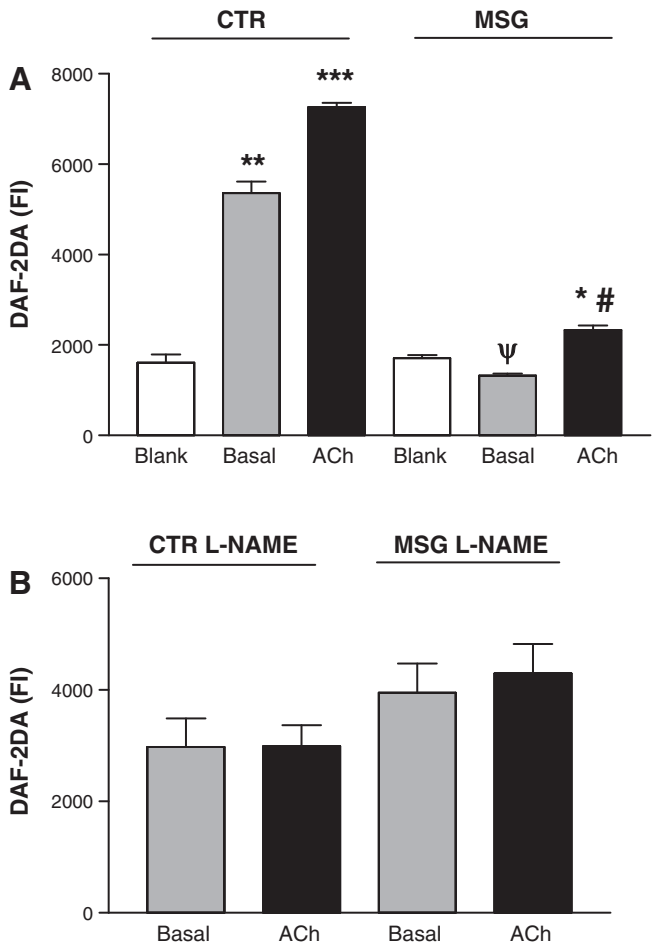

Fig. 5. Detection of nitric oxide (NO) by fluorescence intensity (FI) emitted in the absence of the probe (blank) or in the presence of the probe 4,5-diaminofluoresceindiacetate (DAF-2DA) in endothelial cells isolated from controls (CTR) and MSG aorta (*A*), treated with the nonselective NOS inhibitor L-NAME (*B*), stimulated or not (basal) with ACh. Data are shown as means ± SE (n = 4–5). ***Difference between basal from CTR and blank (CTR) and basal (MSG) ($P < 0.001$). ψDifference between ACh and basal from CTR ($P < 0.001$). **Difference between ACh and basal from MSG ($P < 0.01$). #Difference to ACh between MSG and CTR ($P < 0.001$).

production and increases in ROS generation in rat aortic endothelial cells, without change in vascular reactivity in the aorta. The treatment of obese animals with the nonselective cNOS inhibitor L-NAME decreased body weight and retroperitoneal fat in addition to impaired vasorelaxation induced by ACh. This treatment also increased MAP and the absolute LF component in the SAP spectral analysis only in control animals and attenuated the plasma levels of lipoperoxidation in both groups. Therefore, our data suggest that the hypertension observed in MSG obese rats may be caused by lower production or bioavailability of endothelial NO in addition to increased levels of vascular ROS generation.

The results about characterization of MSG rats in the present study confirm developmental alterations that already have been presented previously in the literature on an MSG-induced obesity model as, for example, attenuated growth, reduced body weight, and an increased weight of visceral fat (39, 52, 63). However, our study adds the information that NO can be related with an alteration in body composition in the MSG-induced obese model.

NO is a radical produced from L-arginine, a reaction mediated by the enzyme NOS, which plays an important role in cardiovascular function and in inflammatory processes (19). It is known that NO levels are diminished in hypertensive humans, providing support for the notion that NO levels play an important role in the pathogenesis of essential hypertension (45). The literature shows that experimental chronic blockade of NOS with L-NAME leads to hypertension accompanied by an increase in sympathetic activity (56, 62). In our study control animals treated with L-NAME also presented an increase in baseline MAP values and an increase in sympathetic modulation in the SAP spectral analysis only in control animals, with no change in obese animals. The increase in the LF component in the SAP analysis indicates the important role played by NO in modulating the AP variability corroborating the literature (3, 57). In contrast to control animals, MSG rats showed no alteration in cardiovascular parameters after treatment with L-NAME. After this treatment, control and MSG obese rats are no longer different in spectral analysis of PI. Because endothelial NO appears to be one of the important

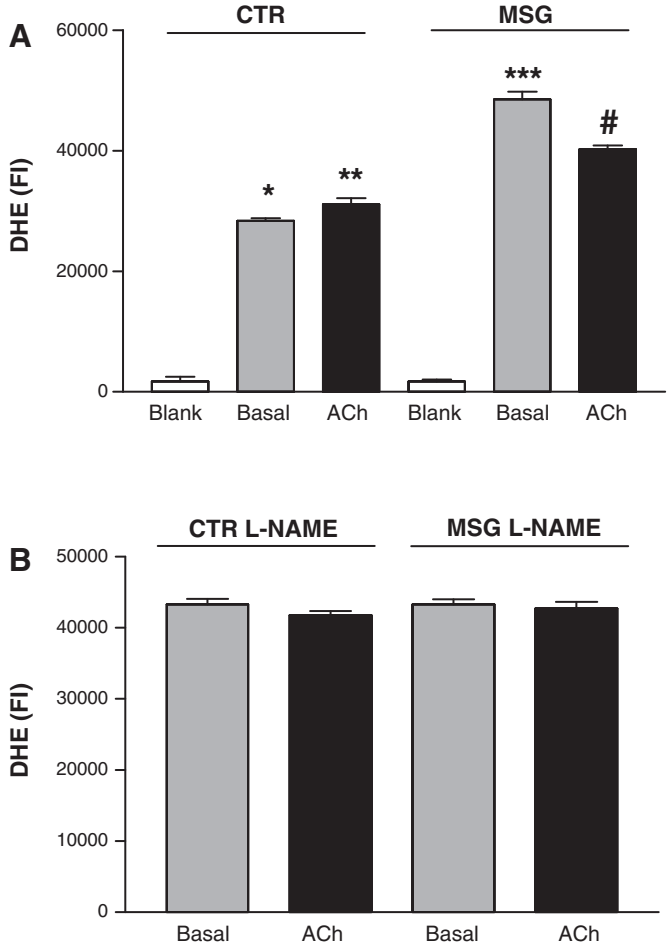

Fig. 6. Detection of O_2^- by FI emitted in the absence of the probe (blank) or in the presence of the probe dihydroethidium (DHE) in endothelial cells isolated from controls (CTR) and MSG aorta (*A*), treated with the nonselective NOS inhibitor L-NAME (*B*), stimulated or not (basal) with ACh. Data are shown as means ± SE (n = 4–5). *Difference between ACh and basal from CTR ($P < 0.05$). **Difference between basal and blank from CTR ($P < 0.001$). ***Difference to basal between MSG and CTR ($P < 0.001$). #Difference to ACh between MSG and CTR and between ACh and basal from MSG ($P < 0.001$).

modulators of vascular smooth muscle tone, the absence of effect in blood pressure observed in MSG rats suggests decreased NO production in endothelium to regulate smooth muscle relaxation in those animals. Our data provide evidence that MSG rats have lowered NO production and suggest that some of the cardiovascular changes in MSG-induced obesity may be due to abnormal function of the NO system, corroborating results of the literature (60). Also, the absence of effect of L-NAME to produce a further increase in MAP and SAP analysis of MSG obese rats may be explained by the decrease in body weight after the treatment. This possibility is corroborated by the literature that observed that obese animals submitted to bariatric surgery lost weight and decreases MAP (51).

An increased production of superoxide and the decreased expression of endothelial NO production may increase peroxynitrite in persons with obesity and high blood pressure, diminishing the availability of NO and causing vasoconstriction (21). In the present study we observed that oxidative stress is systemically present in obese MSG animals. The plasma levels of lipoperoxidation were significantly greater in MSG rats, and the treatment with L-NAME attenuated the lipoperoxidation in both groups, but the degree of attenuation was higher in the obese group. There are several mechanisms by which obesity produces ROS. The first of these is the mitochondrial and peroxisomal oxidation of fatty acids, which can produce ROS in oxidation reactions, whereas another mechanism is overconsumption of oxygen, which generates free radicals in the mitochondrial respiratory chain that is found coupled with oxidative phosphorylation in mitochondria. Lipid-rich diets can also induce ROS generation because they can alter oxygen metabolism (20, 21, 27).

Our data showed that NO production in isolated aortic endothelial cells was reduced, and the basal O_2^- production was increased in MSG rats. The decreased NO production in endothelial cells may contribute to a reduced vasodilatation in MSG rats, whereas ROS generation can reduce endothelium-dependent vasodilatation by impairing NO bioavailability. As previously shown by Förstermann and Münzel (22), the eNOS uncoupling is one of the most important sources of ROS. It is a process in which eNOS generates O_2^- when the concentration of either L-arginine, the substrate of NOS, or BH_4, a cofactor of the enzyme, is depleted. Therefore, our data when integrated with those earlier findings support the hypothesis of the involvement of the production of NO and ROS in aortic endothelial cells isolated from MSG rats could be due to eNOS uncoupling. In a similar way, other studies have shown the increased ROS production in resistance vessels of MSG rats (34, 35).

The mechanisms of hypertension in obesity have been studied by a great number of researchers since several models of obesity also present hypertension (6, 9, 55). The hyperactivity of the sympathetic nervous system is proposed as an important mechanism of hypertension and cardiovascular disease (23, 58). Also, alterations in hemodynamic, metabolic, and inflammatory pathways acting individually or in conjunction probably are involved in the pathophysiological mechanisms responsible for obesity-induced hypertension (54). Data in our laboratory have examined one inflammatory mechanism involved in the hypertension in the MSG-obesity (7). In that study, we demonstrated that the MSG-induced obese rat had higher levels of prostaglandins and an increase in plasma lipid peroxidation. Chronic treatment with the cyclooxygenase-2 inhibitor celecoxib attenuated the hypertension and the oxidative stress. In another study, we observed that celecoxib was not able to reverse the increased cardiac sympathetic modulation that occurred in the obese rat (8). However, in the present study our data suggest that the decreased production of NO in the endothelium may be in part responsible for the hypertension in MSG rats. Therefore, it seems that hypertension in MSG rats is derived from a diminished NO production in the vasculature, and another mechanism involved may be oxidative stress, which has been implicated in many types of hypertension (30, 46). In fact, accumulating evidence suggests that an imbalance in NO (decrease) and ROS (increase) activates the sympathetic nervous system, and this mechanism seems to be involved in pathogenesis of neurogenic aspects of hypertension (5, 25,37).

In conclusion, our data showed that obesity reduces NO and increases ROS production in endothelial cells. Those blood vessel alterations may contribute to the enhancement in sympathetic outflow and blood pressure levels in obesity. Further studies to evaluate antioxidant therapy in this model of obesity may contribute to clarify the physiopathology of hypertension-derived obesity.

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DISCLOSURES

The authors declare that there are no conflicts of interest.

AUTHOR CONTRIBUTIONS

Author contributions: N.V.d.C., P.P.-F., L.M.B., and M.C.M.-P. conception and design of research; N.V.d.C., C.P., B.R.S., L.P., and M.D.G. performed experiments; N.V.d.C., P.P.-F., C.P., B.R.S., L.P., M.D.G., L.M.B., and M.C.M.-P. interpreted results of experiments; N.V.d.C., C.P., and L.P. prepared figures; N.V.d.C., P.P.-F., C.P., B.R.S., L.P., M.D.G., L.M.B., and M.C.M.-P. approved final version of manuscript; P.P.-F., C.P., B.R.S., L.P., M.D.G., L.M.B., and M.C.M.-P. analyzed data; L.M.B. and M.C.M.-P. edited and revised manuscript; M.C.M.-P. drafted manuscript.

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