Improvement of metabolic parameters and vascular function by metformin in obese non-diabetic rats

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Aims: Metformin is an insulin sensitizing agent with beneficial effects in diabetic patients on glycemic levels and in the cardiovascular system. We examined whether the metabolic changes and the vascular dysfunction in monosodium glutamate-induced obese non-diabetic (MSG) rats might be improved by metformin.

Main methods: 15 week-old MSG rats were treated with metformin for 15 days and compared with age-matched untreated MSG and non-obese non-diabetic rats (control). Blood pressure, insulin sensitivity, vascular reactivity and prostanoid release in the perfused mesenteric arterial bed as well as nitric oxide production and reactive oxygen species generation in isolated mesenteric arteries were analyzed.

Key findings: 18-week-old MSG rats displayed higher Lee index, fat accumulation, dyslipidemia, insulin resistance and hyperinsulinemia. Metformin treatment improved these alterations. The norepinephrine-induced response, increased in the mesenteric arteriolar bed from MSG rats, was corrected by metformin. Indomethacin corrected the reduced sensitivity to acetylcholine, reduced in MSG rats, was also corrected by metformin. Indomethacin corrected the reduced sensitivity to acetylcholine in MSG rats but did not affect metformin effects. The sensitivity to sodium nitroprusside was increased in preparations from metformin-treated rats. Metformin treatment restored both the reduced PGI2/TXA2 ratio and the increased reactive oxygen species generation in preparations from MSG rats.

Significance: Metformin improved the vascular function in MSG rats through reduction in reactive oxygen species generation, modulation of membrane hyperpolarization, correction of the unbalanced prostanoids release and increase in the sensitivity of the smooth muscle to nitric oxide.

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Introduction

Obesity represents a major public health problem (Hill, 2006). This condition is associated with increased risk of type 2 diabetes (Goran et al., 2003). Epidemiological and observational studies have shown that overweight/obese individuals tend to be insulin resistant and become more insulin sensitive with weight loss (Lionetti et al., 2009). These findings suggest that insulin resistance is the link between obesity and the related clinical syndromes, such as type 2 diabetes.

Obesity is associated with endothelial dysfunction (Stapleton et al., 2008), which in turn can be considered the first step in the progression of cardiovascular diseases (Lerman and Zeiher, 2005). A number of experimental and clinical studies have consistently demonstrated impaired arterial function manifested by reduced endothelium-dependent vasodilation in obesity (Tzianabos et al., 2010; Lobato et al., 2010; Zalesin et al., 2008). We have previously demonstrated that obesity induced by monosodium glutamate (MSG) impairs microvascular reactivity in rats. An altered ability of the endothelium to maintain the vascular homeostasis through the release of endothelium-derived relaxing factors (EDRFs) and endothelium-derived contracting factors (EDCFs) was demonstrated in MSG-induced obese rats (Lobato et al., 2010).

The biguanide metformin (dimethylguanidine) is one of the most commonly used drugs for type 2 diabetes treatment (Nathan et al., 2006). There are evidences for a potential role of metformin in the prevention of type 2 diabetes in obese patients (Diabetes Prevention Program Research Group, 2002). Metformin has also been associated with reduction in the progression of the cardiovascular repercussions of obesity (UKPDS, 1998; Kurukulasuriya et al., 1999; Grant, 2003). Therefore, we hypothesized that metformin could have beneficial
effects on the vascular function in obesity independently of the presence of a diabetic condition. Considering that obesity and insulin resistance impair the vascular function before the onset of type 2 diabetes (Goran et al., 2003; Lobato et al., 2010), the aim of the present study was to investigate the effects of metformin on metabolic and vascular alterations in obesity. We used non-diabetic MSG obese rats, that manifests obesity and vascular dysfunction (Lobato et al., 2010), providing a suitable model to investigate the effects of metformin in obesity before the onset of diabetes.

Methods

Animals

The investigation was approved by the Ethical Committee for Animal Research of the Institute of Biomedical Sciences, University of Sao Paulo (Protocol no. 007/04), conforming to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996). Male Wistar rats received subcutaneous injections of MSG [4.0 g/kg body weight; Sigma-Aldrich, Germany] dissolved in 0.9% NaCl (MSG rats) or an equivalent volume of vehicle (control rats), from the second to the sixth day after birth. The breeding conditions were followed as previously described (Akamine et al., 2006).

After 16 weeks, rats from the MSG group were divided into two subgroups: 1 – rats from this subgroup received a daily dose of metformin (300 mg/kg, for 15 days), by gavage; 2 – rats from this subgroup received the same volume of vehicle (water) by the same route and for the same period, and will be identified as MSG rats. All experimental groups were studied at 18 weeks of age.

Assessment of water and food consumption was performed by placing the animals in metabolic cages. Rats were acclimated for 72 h and data were collected over the next 24 h.

Blood pressure — tail-cuff method

Blood pressure (BP) was measured in unanesthetized animals by an indirect tail-cuff method (PowerLab 4/S, ADInstruments, Australia). Rats were maintained at 37 °C for 10 min, and then three consecutive stable measurements were averaged.

Intravenous insulin tolerance test

Tail blood samples were collected before (0 min) and 4, 8, 12 and 16 min after an intravenous injection of regular insulin (0.75 U/kg b.w., Biobras, Brazil). The constant rate for blood glucose disappearance during the Insulin Tolerance Test (kITT) was calculated based on the linear regression of the neperian logarithm of blood glucose concentrations obtained during the test.

Blood biochemical assays

For biochemical assays, rats were submitted to food deprivation (5 h) and weighted. After sodium thiopental (50 mg/kg, intraperitoneally, Cristália, Brazil) anesthesia and laparotomy, blood samples were taken from the descending aorta. Glucose levels and the lipid profile were assessed spectrophotometrically using colorimetric method (Celm, Brazil). Insulin was determined using radioimmunoassay (Linco, USA). The Homeostasis Model Assessment (HOMA-IR), an index of insulin resistance (Matsuda, 2010), was calculated from glucose and insulin levels, using the equation: HOMA-IR = fasting insulin (in μU/mL) × fasting glucose (in mmol/L)/22.5. The Lee's obesity index was calculated as follows: body weight$^{1/3}$(g)/nasal-anal length (cm) × 100. Visceral adipose tissue (peripigidymal and retroperitoneal) and the lean mass (soleus and extensor digitorum longus) were removed from each animal. The tissues were weighted after dissection and separation from vessels and other connective tissues.

Vascular reactivity in the perfused mesenteric arteriolar bed

The perfused mesenteric arteriolar bed was prepared as previously described (Lobato et al., 2010). Under anesthesia, the abdominal cavity was opened and a polyethylene cannula was inserted into the superior mesenteric artery. The whole preparation was cut close to the intestinal border and transferred to an organ bath at 37 °C and perfused at a constant flow rate (2 mL/min) by a peristaltic pump with Krebs–Henseleit solution (pH 7.4) containing 5% CO2 and 95% O2. The composition of the solution was (in mM): NaCl 130.0, KCl 4.7, CaCl2 1.6, NaHCO3 14.9, MgSO4 1.17, KH2PO4 1.18, EDTA disodium salt 0.026 and glucose 5.5. Vascular responses were evaluated as changes in the perfusion pressure, measured with a pressure transducer (BP Transducer, ADInstruments, Australia) and recorded on a digital acquisition system (Power Lab, ADInstruments).

After a 45-min equilibration period, concentration–effect curves to norepinephrine (NE, 0.1–100 μM), potassium chloride (KCl, 5–225 mM), a direct acting smooth muscle contracting agent, acetylcholine (ACh, 0.001–30 μM), an endothelium-dependent vasodilator, and sodium nitroprusside (SNP, 0.001–10 μM), a NO donor, were obtained. All curves were performed in the presence of desipramine (inhibitor of NE uptake, 10 nM). The vasodilator responses to ACh and SNP were determined in NE-contracted preparations in a concentration that produced 80% of the maximal contractile response.

In order to determine the role of NO, prostanoids and EDHF on the NE and ACh responses, NO-Nitro-o-arginine Methyl Ester (L-NNAME, 100 μM), a NO synthase inhibitor, indomethacin (10 μM), a cyclooxygenase (COX) inhibitor, or tetraethylammonium (TEA, 2 mM), a non-selective K+-channel blocker, were used. Each drug was added to the perfusing solution 30 min before the concentration–effect curves were performed and was maintained throughout the experiment. The concentrations of the agents used were based on the data in the literature (Lobato et al., 2010).

Prostanoid release measurements

The isolated mesenteric arteriolar bed was allowed to equilibrate for 30 min. The ability of the preparations to release TXA2 (estimated from measurements of 11-dehydro-TXB2) and PGI2 (estimated from measurements of 6-keto-PGF1α) was assessed in 1 mL samples of perfusate collected before and after stimulation with NE (100 nM) or ACh (30 nM) using enzyme immunoassay kits (Cayman Chemical, USA).

Measurement of nitric oxide production in mesenteric arteries

Nitric oxide (NO) production was determined using 4,5-diamino-fluorescein diacetate (DAF-2), a NO-sensitive fluorescent dye (Lobato et al., 2010). Mesenteric arteries were dissected, embedded in a freezing medium and frozen. Transverse arteriolar cryostat sections (20 μm) were collected on glass slides and incubated at 37 °C with 8 μM DAF-2 in phosphate buffer (0.1 M, pH 7.4) containing CaCl2 (0.45 mM). After 30 min, the sections were stimulated with ACh (100 μM) in the absence/presence of BH4 (1 mM). Digital images were collected on a microscope (Carl Zeiss, Germany) equipped for epifluorescence and with a fluorescein filter. The images were analyzed with the Image software (KS-300, Zeiss) by measuring the mean optical density of the fluorescence in the endothelium.

Reactive oxygen species generation in mesenteric arteries

Reactive oxygen species (ROS) generation was determined by hydroethidine (Lobato et al., 2010). Transverse mesenteric arteries
were obtained as described for measurement of NO production and incubated at 37 °C with hydroethidine (2.5 μM) in phosphate buffer (0.1 M, pH 7.4). Images were collected on a microscope equipped for epifluorescence and with a rhodamine filter. The mean optical density of the fluorescence in the vessel wall was measured. To evaluate superoxide (O$_2^·$) production and the participation of NOS in the ROS generation, mesenteric arteries were treated with SOD (150 IU/mL) or l-NAME (100 μM), respectively, 30 min before the tissues were frozen.

**Results**

**General characteristics of metformin-treated rats**

General and biochemical characteristics of the different groups are presented in Table 1. The food intake was not different among groups. The higher Lee index and fat mass weight found in MSG rats were significantly reduced by metformin treatment. The lean mass weight of metformin-treated MSG rats was not different from that of control rats or from MSG rats. The levels of cholesterol, triglycerides and low density lipoprotein (LDL) cholesterol, increased in MSG rats, were restored to the control levels after metformin treatment. In addition, metformin treatment promoted increase in high density lipoprotein (HDL) cholesterol, restoring the levels of this lipoprotein to values observed in control rats. Although similar serum glucose levels were found among groups, MSG rats displayed enhanced HOMA-IR index, and hyperinsulinemia. Metformin treatment restored these parameters to values observed in control rats. No difference in BP levels was found among groups.

**Vascular reactivity in the mesenteric arteriolar bed**

Similar basal perfusion pressure was found in preparations from all experimental groups (around 20 mm Hg). Metformin treatment did not alter the contractile response induced by KCl (in mm Hg, control = 75.2 ± 4.1, MSG = 76.2 ± 5.7, MSG-Met = 78.6 ± 6.1). However, the vasoconstrictor response to NE, significantly increased in MSG rats, was corrected by metformin (Fig. 1A). Perfusion of the mesenteric arteriolar bed with Krebs–Henseleit solution containing l-NAME or TEA for 30 min further increased the response to NE in all experimental groups (Fig. 1B and C). Indomethacin corrected the enhanced contractile response to NE in MSG rats. The effect of metformin correcting the enhanced contractile response observed in MSG rats was not altered by indomethacin (Fig. 1D).

Metformin treatment restored the reduced sensitivity (lower pD2) to ACh observed in the mesenteric arteriolar bed from MSG rats. Perfusion of the preparations with l-NAME decreased the maximum response to ACh in all experimental groups (Fig. 2B). Perfusion with TEA decreased significantly the maximal vasodilator response to ACh in MSG rats. On the other hand, in preparations from metformin-treated rats, similar to that observed in control rats, TEA did not reduce the response to ACh (Fig. 2C). The sensitivity to ACh in the mesenteric arteriolar bed from control rats was not modified by indomethacin (pD2, control = 7.7 ± 0.1, Control + Indo = 7.6 ± 0.2, Fig. 2D), however, in MSG rats this agent was able to correct the reduced sensitivity to ACh (MSG = 7.0 ± 0.1, MSG + Indo = 7.8 ± 0.05, p < 0.05 vs. respective group in the absence of the inhibitor, Fig. 2D). In preparations from metformin-treated rats, similar to that observed in control rats, the response to ACh was not affected by perfusion with indomethacin (MSG-MET = 7.6 ± 0.1, MET + Indo = 7.9 ± 0.1, Fig. 2D).

The mesenteric arteriolar bed from metformin-treated rats was more sensitive (lower EC50, represented by pD2 values) to SNP when compared to both MSG and control preparations (pD2, control = 6.6 ± 0.1, MSG = 6.5 ± 0.2, MSG-MET = 7.1 ± 0.1, p < 0.05, Fig. 2E).

**Prostanoid release from the mesenteric arteriolar bed**

Metformin treatment restored the reduced PGI2/TXA2 ratio observed in unstimulated, NE- and ACh-stimulated preparations from MSG rats (Fig. 3).

**Measurement of nitric oxide production in mesenteric arteries**

The reduced basal and ACh-stimulated NO production found in arteries from MSG rats was not affected by metformin treatment (Fig. 4). The addition of exogenous BHa, an NO synthase cofactor that enhances NO production or l-NAME, an NO synthase inhibitor, to preparations from MSG rats and MSG-MET rats stimulated with ACh, fully corrected NO production (Fig. 4).

**Superoxide anion generation in mesenteric arteries**

The increased ROS generation observed in mesenteric arteries from MSG rats was corrected by metformin treatment. The incubation
with l-NAME or SOD reduced the ROS generation in MSG rats to values similar to those obtained in MSG-MET rats (Fig. 5).

Discussion

In the present study we demonstrated that metformin had beneficial effects in non-diabetic MSG rats correcting the insulin resistance, the hyperinsulinemia and the altered lipid profile. We also demonstrated that metformin treatment was associated with reduction in Lee Index as well as in visceral fat accumulation. Previous studies performed in both humans or in experimental models of type 2 diabetes have demonstrated improvement of metabolic parameters as well as reduction in body weight after metformin treatment (Diabetes Prevention Program Research Group, 2002; UKPDS, 1998; Hundal et al., 2000), reinforcing the potential role of this drug as an early therapeutic intervention to prevent the development of the comorbidities associated with type 2 diabetes.

Oral treatment with metformin led to a decrease in Lee index, an accurate index that correlates highly with body fat. This effect is not related to the food intake since we did not observe difference in this parameter among groups. Metformin is widely recognized to have either little effect on body weight or to facilitate modest weight loss in type 2 diabetic patients (UKPDS, 1998; Hundal et al., 2000). Similarly, metformin has shown to induce weight loss in obese non-diabetic individuals (Nichols and Gomez-Caminero, 2007; Glueck et al., 2001), although long duration studies in this population are scarce. The weight loss by metformin treatment in diabetic patients has been related to reduction in fat mass (Kurukulasuriya et al., 1999). Accordingly, we observed that metformin treatment was able to reduce the fat accumulation observed in MSG rats along with reduction in the Lee Index, an accurate index that determines the body mass gain corrected by the length.

Metformin had also advantageous effects on lipid profile besides its effects on whole-body insulin sensitivity in MSG rats. In type 2 diabetic patients the correction of the dyslipidemia after metformin treatment has been associated with decreased synthesis and increased clearance of VLDL. This may provide an indirect mechanism by which metformin improves the metabolic changes in these patients as described by Wiennsperger and Bailey (1999). Considering that fat accumulation can contribute to changes of lipid profile in obesity by increasing the release of free fatty acids (FFAs), the effect of metformin reducing visceral fat and its insulin sensitizer effect in MSG rats might contribute to the correction of the dyslipidemia observed. Another notable effect of metformin was the increase in HDL cholesterol levels in MSG rats. Even in patients, it has been shown that metformin increases HDL cholesterol levels in overweight, diet-controlled type 2 diabetic patients (Abbasi et al., 1999). Low fasting plasma HDL cholesterol has been reported to be associated with insulin resistance (Abbasi et al., 1999). Based on this, the correction of the insulin resistance after metformin treatment might also be involved in the increase of HDL cholesterol in MSG rats.

A substantial amount of evidence has consistently demonstrated that obesity is closely related to impaired endothelial function either in animals (Lobato et al., 2010), or in patients (Stapleton et al., 2008; Lerman and Zeiher, 2005; Tziomalos et al., 2010). Considering that metformin has beneficial effects on vascular function in type 2 diabetes (Bailey, 2008), we evaluated in metformin-treated MSG rats, the endothelium-dependent vasodilator response, tested with ACh, and the vasoconstriction induced by NE, that has its response negatively modulated by the endothelium. In fact, metformin promoted beneficial effects in MSG rats correcting the increased response to NE and the lower response to ACh observed in the mesenteric arteriolar bed.

Endothelial dysfunction is usually associated with reduction in NO production and/or increase in NO metabolism (Feletou and Vanhoutte, 2006). It has been suggested that the vasculoprotective effects of metformin are mainly due to improvement of NO signaling (Bailey, 2008). Interestingly, an important finding in our study was that the correction of the endothelial dysfunction with metformin treatment in MSG rats is not due to improvements in NO signaling. This is supported by the fact that metformin did not correct the reduced endothelium-dependent NO production in mesenteric arteries from MSG rats.
It is well documented that endothelial NO synthase (eNOS) uncoupling, a process in which eNOS generates \( \text{O}_2^- \) instead of NO when the concentrations of either l-arginine, the substrate of NOS, or tetrahydrobiopterin (BH4), a cofactor of the enzyme, are depleted, may mediate decrease in NO bioavailability (Förstermann and Münzel, 2006). \( \text{O}_2^- \) generation can be involved in the reduction of endothelium-dependent vasodilatation, by impairing NO bioavailability (Hopps et al., 2010). We have previously demonstrated uncoupling of eNOS in mesenteric arteries from 16-week-old MSG rats (Lobato et al., 2010). In 18-week-old MSG rats, incubation of mesenteric arteries with BH4 corrected the reduced NO production. Furthermore, the treatment of these arteries with either SOD, an \( \text{O}_2^- \) scavenger or l-NAME, a NOS inhibitor, reduced ROS generation, confirming the role of eNOS as a source of \( \text{O}_2^- \) production. Interestingly, although metformin did not correct the reduced NO production in MSG rats, it exerted an antioxidant effect by decreasing the \( \text{O}_2^- \) production. Taking the above findings together, we speculate that metformin's beneficial effects on endothelial dysfunction in MSG rats may be at least partly due to the suppression of the oxidative stress. The antioxidant effect of metformin has been demonstrated in previous studies (Gallo et al., 2005; Mahrouf et al., 2006; Ouslimani et al., 2005); however, the role of it on the beneficial effects of metformin in obesity has not been described before.

In the present study, an additional mechanism involved in metformin effects appears to be the modulation of membrane hyperpolarization. The substantial decrease of the ACh-induced relaxation after perfusion of the mesenteric arteriolar bed from MSG rats with TEA, a K⁺ channel blocker, revealed the major participation of the hyperpolarization for the vasodilator response, which could be a compensatory mechanism for the reduced NO production observed in this model. The correction of this alteration might be suggested in our study, since TEA was not able to decrease the ACh-induced response in metformin-treated rats, similarly to that observed in control rats. Accumulating evidences suggest that alterations in the production/release of prostanoids by the endothelium directly contribute to the endothelial dysfunction in vascular diseases (Yuhki et al., 2010; Ilíquez et al., 2008). In fact, the increased vasoconstriction and the reduced vasodilatation in MSG rats were corrected after COX inhibition. Additionally, MSG rats displayed decreased PGI₂/TX₂ ratio in the mesenteric arteriolar bed. Therefore, the unbalanced

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**Fig. 2.** Concentration–response curves to acetylcholine in mesenteric arteriolar beds from control, monosodium glutamate-induced obese rats (MSG) and metformin-treated MSG rats (MSG-MET) in the absence (A) or presence of l-NAME (B, 100 μM), TEA (C, 2 mM) or indomethacin (D, Indo 10 μM). E – concentration–response curves to sodium nitroprusside in mesenteric arteriolar beds from control, monosodium glutamate-induced obese rats (MSG) and metformin-treated MSG rats. * p < 0.05 vs. control. # p < 0.05 vs. respective group in the absence of blockade. Relaxation (measured by percentage of contraction reduction) is presented as the mean ± S.E.M of eight independent experiments.
release of vasodilator/vasoconstrictor prostanoids derived from COX might explain the endothelial dysfunction in MSG rats. Interestingly, an important finding in this study was that the decreased PGI2/TXA2 ratio in MSG rats was not detected after metformin treatment, indicating that metformin improves the endothelial function in MSG rats by restoring the balance in the synthesis/release of prostanoids.

Although the accurate mechanisms involved in metformin effects on the endothelium have not been elucidated, recent studies point to the role of COX-2 (Matsumoto et al., 2008). In fact, we have demonstrated that 16-week-old MSG rats displayed increased expression of COX-2 that contributed to the increased contractile response to NE as well as to the decreased relaxation in MSG rats (Lobato et al., 2010). Considering that metformin corrected the decreased ratio PGI2/TXA2 observed in the mesenteric arteriolar bed from MSG rats, we suggest that metformin restored the COX-2-derived production of prostanoids in this model of obesity.

The correction of the insulin resistance can also contribute to explain metformin effect on COX-derived vasoactive products, because insulin resistance, increasing endothelial FFAs, can reduce arterial prostacyclin synthase activity. FFAs commonly observed in insulin resistant patients promote increased O$_2^-$ production in endothelial cells, by providing increased electron donors (NADH and FADH2) to the mitochondrial electron transport chain. The FFA-induced overproduction of O$_2^-$ activates a variety of proinflammatory signals and inactivates two important enzymes, PG12 synthase and eNOS (Du et al., 2006; Kuboki et al., 2000), thus decreasing the endothelium-dependent vascular relaxation.

Restoration of endothelium-derived factors release might not be the only factor that accounted for metformin effect since SNP, which acts via direct stimulation of vascular smooth muscle cells independently of an intact endothelium, had its response increased by metformin treatment. SNP and NO, which is released by endothelial-dependent vasodilators, share a final common pathway to produce vasodilatation, stimulating guanylate cyclase and increasing cGMP levels. Thus, it is possible that a direct effect of metformin on vascular
smooth muscle cells, improving the responsiveness to NO, could contribute to the correction of the reduced vasodilatation in MSG rats. A vasodilating effect of metformin has been reported in ex vivo preparations of vascular smooth muscle cells, possibly due to altered calcium handling (Domínguez et al., 1996; Abbasi et al., 1998). Therefore, other mechanisms might be involved in the beneficial effects of metformin on vascular function, as demonstrated in the present study.

One limitation of the present study was the use of an animal model that may not necessarily reproduce the same pathophysiologic abnormalities of human obesity. The cause of obesity in MSG rats is not common among humans, although the phenotype parallels human obesity in many ways. Other limitations of this study include the short duration of metformin treatment, the use of only one vascular preparation (mesenteric arteriolar bed), that is not necessarily reflective of other circulatory beds and the absence of experiments to evaluate the direct action of this drug in the vessel. Thus, additional studies are required to investigate metformin effects in other models of obesity with additional time of treatment as well as in different vascular beds, both in vivo and in vitro.

Conclusion

In summary, the present study demonstrated that the metformin treatment improved the vascular function in MSG rats through reduction in ROS generation, modulation of membrane hyperpolarization, correction of the unbalanced prostanoids release and increase in the sensitivity of the smooth muscle to NO. Our findings support the beneficial effects of metformin previously demonstrated in large intervention studies in type 2 diabetic patients and also offer a credible evidence for the beneficial effects of this drug in obesity.

Conflict of interest

The authors declare that there are no conflicts of interest.

References


Fig. 5. Reactive oxygen species generation in mesenteric arteries from control, monosodium glutamate-induced obese rats (MSG) and metformin-treated MSG rats (MSG-MET) in the absence and presence of SOD (150 IU/mL), an O2 scavenger, or L-NAME (100 µM), an NO synthase inhibitor. A — Bar graphs show reactive oxygen species generation, measured as hydroethidine-positive nuclei fluorescence. *p<0.05 vs. control and MSG. #p<0.05 vs. MSG. B — Representative fluorographs of transverse sections of mesenteric arteries with hydroethidine-positive nuclei. The mean optical density of the fluorescence is expressed as mean±SEM of six independent experiments. Scale bar: 20 µm.

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