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Cardiac fibrosis and vascular remodeling are attenuated by metformin in obese rats

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ABSTRACT

Background: Human obesity has been associated with alterations of vascular structure, especially in large and medium arteries, but the effects of insulin-sensitizers are not well known.
Methods: Twenty-five male Wistar rats received subcutaneous injections of monosodium glutamate (MSG) or an equivalent volume of vehicle from the second to the sixth day after birth, At 16 weeks of age, five MSG rats started receiving an oral treatment with metformin (300 mg/kg) which was maintained for six weeks, composing five groups: control 16 weeks (CON-16), MSG 16 weeks (MSG-16), control 22 weeks (CON-22), MSG 22 weeks (MSG-22), and MSG plus metformin 22 weeks (MET-22). Systolic blood pressure (BP) was verified weekly. The lumen diameter and media thickness, media cross-sectional area (CSA) and growth index of the intramyocardial arterioles were measured. Cardiac interstitial and perivascular collagen density were also evaluated.
Results: Systolic BP was significantly increased in the MSG-22 comparing to MSG-16 group. Insulin resistance was confirmed by HOMA-IR index and metformin-treated group presented reduction of insulin levels at week 22. The morphology analysis showed greater media-to-lumen ratio and CSA in the obese groups, which were reduced by the metformin treatment. Connective tissue deposition in the perivascular region of the left ventricle was significantly higher in the obese groups which was attenuated by metformin.
Conclusions: Hypertrophic vascular remodeling and cardiac collagen deposition were significantly evident in MSG-induced obese rats. Metformin treatment was able to reduce insulin resistance and attenuated this adverse cardiac and vascular remodeling.

1. Introduction

The prevalence of overweight and obesity has markedly increased over the past two decades, growing rapidly from Western societies to the rest of the world [1,2]. The INTERHEART study demonstrated that obesity is one of the nine modifiable risk factors accounting for more than 90% of myocardial infarction risk [3]. Thus, considering obesity as a risk factor for cardiovascular disease, the comprehension of underlying pathological changes linking obesity with heart disease is essential. A recent evaluation of Framingham Study participants stratified by body mass index and waist circumference indicated that individuals with generalized or abdominal obesity had a high prevalence of subclinical disease which partly contributed to the increased cardiovascular risk associated with excess adiposity in these subjects [4].

Recent studies have shown that human obesity is associated with alterations of vascular structure and function, especially in large and medium arteries [5,6]. Structural alterations of subcutaneous small resistance arteries, as indicated by an increased media-to-lumen ratio, are frequently present in hypertensive and or/diabetic patients. These vascular changes may represent the earliest alterations in these subjects and present a strong prognostic significance [7,8]. In severe obese normotensive subjects, Grassi et al. recently showed that subcutaneous small resistance arteries undergo profound structural modifications characterized by a hypertrophic process, probably secondary to vascular smooth muscle cell growth [9].

The administration of monosodium glutamate (MSG) to newborn rats provokes hypothalamic injury, leading to neuronal loss that impairs insulin and leptin signaling resulting in obesity [10,11]. The MSG-induced obesity model exhibits most features observed in human obesity such as abdominal obesity, insulin resistance, and hyperinsulinemia. In fact, even obese mice induced by MSG have already been considered a useful experimental model for developing insulin resistance [12]. In these animals, fat accumulation and insulin resistance contribute to alterations in microvascular reactivity independently of the presence of type 2 diabetes or hypertension [13].

In addition to its insulin-sensitizing effects, previous data indicate that metformin improves vascular function through a direct mechanism rather than by restoring metabolic abnormalities [14]. Thus, it seems that metformin has a direct effect predominantly on vascular function. The precise mechanism is still unidentified, but it appears that metformin promotes increased peripheral glucose disposal at lower insulin concentrations. Interestingly, the use of metformin in

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nondiabetic obese adults has been demonstrated to cause reduced food intake and weight loss with reduction in fasting plasma glucose, and insulin concentrations [15]. So we hypothesized that metformin is able to attenuate some adverse effects of obesity induced by MSG on cardiac vascular and perivascular structures. Thus, in this study we investigated the effects of metformin on cardiovascular remodeling and collagen deposition in rats that developed insulin resistance and central deposits of fat after MSG treatment.

2. Material and methods

This protocol was approved by the Ethical Committee for Animal Research of the Institute of Biomedical Sciences, University of Sao Paulo (Protocol n. 007/04) and conforms to the Guide for the Care and Use of Laboratory Animals (NIH Publication No. 85-23, revised 1996). Twenty-five male Wistar rats received subcutaneous injections of MSG (4.0 mg/g body weight) dissolved in 0.9% NaCl or an equivalent volume of vehicle from the second to the sixth day after birth. At 16 weeks of age, five MSG rats started receiving an oral treatment with metformin (300 mg/kg) which was maintained for six weeks. Thus, the study was composed by five groups (n = 5 for each): control 16 weeks (CON-16), monosodium glutamate 16 weeks (MSG-16), control 22 weeks (CON-22), monosodium glutamate 22 weeks (MSG-22), and monosodium glutamate plus metformin 22 weeks (MET-22). Systolic blood pressure (BP) was verified weekly in conscious rats through the non-invasive method of the tail cuff plethysmography (Letica LE 5100, Panhab, Barcelona, Spain). The average of three pressure readings was obtained.

2.1. Image analysis of the vascular structure

After sacrifice, the heart was removed and fragments of the left ventricle were placed for 48 h at room temperature in fixative (formaldehyde 4% in 0.1 M phosphate buffer pH 7.2) [16], and then embedded in Paraplast plus (Sigma, St. Louis, USA), sectioned at 5 μm thickness, and stained with hematoxylin–eosin and picro sirius red. Five fields per section, five sections per organ, totaling 125 fields per group were analyzed by video microscopy. The lumen diameter and media thickness of the intramyocardial arterioles that appeared circular on cross section were measured using the Image Pro Plus analysis software version 5.01 (Media Cybernetics, Silver Spring, USA) in an Olympus BX51 microscope with LC Evolution digital camera. Media cross-sectional area (CSA) was obtained by subtraction of the internal CSA from external CSA: $CSA = (\pi/4) \times (De^2 - Di^2)$, where De and Di were external and lumen diameters, respectively. Growth index was calculated as $(CSA_t - CSA_c)/CSA_c$, where CSA_c and CSA_t are media cross-sectional areas of control and treated (MSG ± metformin) vessels, respectively.

2.2. Collagen quantification in the heart

The heart was fixed and processed for paraffin embedding as previously described. Tissue sections were dewaxed with ethanol and stained with 0.5% Sirius red. Perivascular collagen was normalized by dividing the CSA of intramyocardial arterioles occupied by collagen by the luminal area of the vessel. Interstitial collagen density was evaluated in two different regions of the LV, including the subepicardial and the subendocardial myocardium. From each of 3 nonconsecutive serial sections, about 10 fields in each region of the heart were randomly selected and recorded (×20 objective).

3. Data analysis

Values were expressed as mean ± SEM. The values were compared by one-way ANOVA followed by a Tukey post-test. A value of $p<0.05$ was considered statistically significant. All statistical analyses were performed using Prism for Windows, version 5.0 (GraphPad Software, Inc.).

4. Results

The Lee index was statistically higher in MSG groups which also presented greater values of retroperitoneal white adipose tissue when compared to control groups (Table 1). There was no significant difference in systolic BP when comparing MSG to control groups. However, the systolic BP was significantly increased in the MSG-22 comparing to MSG-16 group (122 ± 2 vs 108 ± 2 mm Hg, $p<0.05$), and MET-22 group (118 ± 1 mm Hg) showed lower BP levels without reaching statistical significance (Fig. 1A). At week 16, despite normal plasma glucose levels, insulin resistance was confirmed as indicated by a higher HOMA-IR index (Fig. 1B, D). The metformin-treated group presented reduction of insulin levels and showed improvement in the insulin resistance tests at week 22 (Fig. 1C, D).

4.1. Vascular structure

The morphology analysis showed similar diameter of the intramyocardial arterioles among all the groups and greater media thickness in the obese groups, which was reduced by the metformin treatment. In the obese groups by observing an increased media-to-lumen ratio which was reduced by metformin. Media cross-sectional area was also increased in MSG groups compared to control groups resulting in an elevated growth index suggesting in a hypertrophic vascular remodeling in these animals (Table 1).

4.2. Cardiac collagen deposition

Interstitial connective tissue deposition in the perivascular region of the left ventricle was significantly higher in the obese groups which was attenuated by metformin. Collagen deposition in the subendocardial and subpericardial areas was also higher in MSG-treated rats but did not reach statistical significance (Table 1, Fig. 2).

5. Discusssion

To our knowledge, there is no consistent data about the structure of small arteries in the heart of obese animals yet. The present study

Table 1
Obesity parameters, vascular morphometry of intramyocardial arterioles and cardiac collagen deposition in all groups.

	CON-16	MSG-16	CONT-22	MSG-22	MET-22
Lee index, %	29.0 ± 0.2	30.4 ± 0.2***	29.5 ± 0.1	31.2 ± 0.2***	30.2 ± 0.1##
Retroperitoneal WAT, g/100 g	0.97 ± 0.07	2.81 ± 0.11*	1.13 ± 0.21	2.62 ± 0.43	2.24 ± 0.23
Lumen diameter, μm	32.3 ± 1.7	34.2 ± 3.3	35.9 ± 2.3	34.7 ± 2.4	33.9 ± 1.3
Media thickness, μm	12.6 ± 0.6	14.9 ± 0.9	10.9 ± 0.4	14.5 ± 0.6*	11.5 ± 1.0
Media-lumen ratio, %	30.2 ± 2.0	39.9 ± 3.7*	29.5 ± 1.2	39.8 ± 1.3*	31.5 ± 0.9
Media CSA, $\times 10^3 \mu m^2$	12.5 ± 0.5	14.9 ± 0.9	10.9 ± 0.4	14.5 ± 0.6*	11.5 ± 1.0
Growth index, %	–	19.2	–	33.0	5.5
Myocardial collagen					
Subendocardial, %	2.1 ± 0.8	4.1 ± 0.3	4.0 ± 0.3	4.8 ± 0.8	3.0 ± 0.1
Subpericardial, %	2 ± 0.3	3 ± 0.2	2 ± 0.4	4 ± 0.5	4 ± 0.5
Perivascular, %	0.80 ± 0.05	1.19 ± 0.19	0.83 ± 0.06	1.39 ± 0.06**	1.02 ± 0.04

Data are expressed in mean ± SEM. WAT, white adipose tissue; CSA, cross-sectional area.
* $p<0.05$ vs respective control groups.
** $p<0.01$ vs respective control groups.
*** $p<0.001$ vs respective control groups.
\#\# $p<0.01$ vs MSG-22.