Diet-induced obesity causes metabolic, endocrine and cardiac alterations in spontaneously hypertensive rats

Silvio A. Oliveira Junior\textsuperscript{1,2}, Maeli Dal Pai-Silva\textsuperscript{3}, Paula F. Martinez\textsuperscript{2}\textsuperscript{10}, Ana P. Lima-Leopoldo\textsuperscript{2}, Dijon H. S. Campos\textsuperscript{2}, André S. Leopoldo\textsuperscript{2}, Marina P. Okoshi\textsuperscript{2}\textsuperscript{10}, Katashi Okoshi\textsuperscript{2}\textsuperscript{10}, Carlos R. Padovani\textsuperscript{4}\textsuperscript{16}, Antonio C. Cicogna\textsuperscript{2}\textsuperscript{13}\textsuperscript{15}

\textsuperscript{1} School of Physical Therapy, Federal University of Mato Grosso do Sul, Campo Grande, MS, Brazil
\textsuperscript{2} Department of Internal Medicine, Botucatu Medical School, São Paulo State University, Botucatu, SP, Brazil
\textsuperscript{3} Department of Morphology, Botucatu Biosciences Institute, São Paulo State University, Botucatu, SP, Brazil
\textsuperscript{4} Department of Biostatistics, Botucatu Biosciences Institute, São Paulo State University, Botucatu, SP, Brazil

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Summary

Background: Although obesity has been associated with several effects in rodents, few investigations have evaluated the metabolic, endocrine, and cardiac parameters of spontaneously hypertensive rats (SHR) with dietary-induced obesity. The current study analyzed the influence of dietary-induced obesity on metabolic, endocrine, and cardiac characteristics in SHR.

Material/Methods: Male SHR were distributed in 2 groups: C-SHR (n=10) and OB-SHR (n=10). While C-SHR received a standard commercial diet (CD; 3.2 kcal/g), OB-SHR were submitted to a hypercaloric diet (HD; 4.6 kcal/g) for 20 weeks. Nutritional, metabolic, and endocrine evaluation involved measurement of calorie intake, dietary efficiency, body weight, adiposity, glycemia, triacylglycerol, insulin, and leptin. Cardiovascular evaluation integrated systolic blood pressure (SBP), echocardiography, gross and ultrastructural morphology, and myosin heavy chain (MHC) analyses of the myocardium.

Results: Animals in OB-SHR had greater values of BW, adiposity, triacylglycerol, and leptin and impaired glycemic tolerance compared with the C-SHR group. In the cardiovascular context, dietary-induced obesity increased interstitial collagen, the cardiomyocyte area, and the relative expression of β-MHC, and the relative expression of β-α isoform ratio of MHC. Likewise, OB-SHR showed ultrastructural morphologic alterations, with loss and disorganization of myofilaments, lipid droplets, severe mitochondrial damage, and T-tubule dilatation. Concerning the in-vivo cardiovascular profile, although SBP and systolic function were unchanged by dietary-induced obesity, echocardiography results evidenced impaired diastolic function in OB-SHR in relation to their control counterparts.

Conclusions: Diet-induced obesity was associated with endocrine alterations, and it accentuated cardiac remodeling, promoting diastolic dysfunction of restrictive filling pattern in the SHR strain.

key words: obesity • diet • spontaneously hypertensive rat • cardiac remodeling

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Author's address: Oliveira Junior S. A., Departamento de Clínica Médica, Faculdade de Medicina de Botucatu, UNESP, Distrito de Rubião Júnior, s/n, 18618-000; Botucatu, SP, Brasil, e-mail: oliveirajr.ufms@gmail.com
Background
Cardiac remodeling involves an adaptive process to maintain myocardial performance in response to stress conditions, including mechanical and volumetric overloads [1]. Remodeling is characterized by time-dependent evolution and includes several adaptive changes, clinically represented by modifications of cardiac form, size, and function [1,2]. Cardiac phenotypic plasticity involves myocyte hypertrophy, interstitial fibrosis, ultrastructural disorganization [2], and altered expression of α- and β-isoforms of myosin heavy chain (MHC) [3].

Essential arterial hypertension configurations an important promoting condition of myocardial remodeling [4], because approximately 75% of human heart failure has a hypertensive cause [5]. In studies on cardiac remodeling, spontaneously hypertensive rats (SHR) are often used experimentally because they develop similar arterial hypertension conditions to those found in humans [6,7]. During maturation, SHR present progressive myocardial hypertrophy, interstitial fibrosis, ultrastructural degeneration, β-myosin heavy chain isoforms up-regulation, and ventricular dysfunction [6–8]. Among 18 to 24 months of life, these animals present signals of ventricular dysfunction and, subsequently, they can develop heart failure with several similar characteristics to human conditioning [6,8].

Furthermore, SHR are also genetically susceptible to nutritional and metabolic abnormalities, such as glucose intolerance, insulin resistance, central obesity, and dyslipidemia [9], frequently manifested from hypercaloric dietary interventions richer in lipids [10–12] and sugars [13–15].

Despite this, the information on nutritional, endocrine, and cardiovascular profiles in SHR with dietary-induced obesity are limited and conflicting. In experimental models, few studies documented glycemic [10,16] and lipid [10,11] changes; in the cardiovascular aspect, accentuated myocardial hypertrophy [14,15,17] was accompanied by a reduction [14] or increase [15,17] in blood pressure. Majane and associates [18] observed unchanged responses of glycermia and systolic blood pressure with cardiac hypertrophy and disturbances of left ventricular systolic function. Because cardiac remodeling is also characterized by altered molecular expression of contractile proteins, including α- and β-MHC isoforms and ultrastructural alterations [1], it is not clear if these effects are reproduced in models of SHR with diet-induced obesity. The maximal shortening velocity, which correlates to the ATP hydrolysis rate, is significantly higher in fibers that contain a higher proportion of α-MHC isoforms [3,19]. Hearts that express predominantly α-MHC, the faster motor protein, determine higher contractile potency; up-regulation of the β-MHC isoforms, lower motor protein, configures a contributing factor to systolic and diastolic dysfunction in heart failure [20].

Clinically, essential hypertension and obesity are comorbid diseased conditions that are identified as independent risk factors for developing myocardial dysfunction and heart failure [21,22]. Because the role of diet-induced obesity is not well-characterized in essential hypertension, our primary aim was to test the hypothesis that dietary-induced obesity is associated with metabolic, endocrine, and cardiovascular disorders in SHR, which are genetically more susceptible to these disturbances when kept on a hypercaloric diet [23].

Accounting for this proposition, the present study analyzed metabolic, endocrine, and cardiovascular parameters of spontaneously hypertensive rats submitted to diet-induced obesity.

Material and Methods

Animals and experimental design
Sixty-day-old male SHR were distributed in 2 groups: a control group (C-SHR) and an obese group (OB-SHR), each consisting of 10 animals. While C-SHR received commercial Labina rat chow, OB-SHR rats were treated with 5 palatable hypercaloric diets (HD1, HD2, HD3, HD4, HD5), alternately administered [20]. Each chow type was offered for 7 days, and the experimental period was 20 weeks. The animals were individually housed under the temperature of 22°C to 24°C and humidity of 50% to 70%. A time-controlled system provided 12-hour light/dark cycles. All the animals had free access to water and chow (50 g/d).

The experimental protocol was established according to National Institutes of Health’s “Guide for the Care and Use of Laboratory Animals” published by the US National Institutes of Health (NIH Publication No. 85-23, 1996 revision) and approved by the Ethics Committee for Animal Experimentation of the Botucatu School of Medicine, UNESP, Brazil.

Diet characterization
Diets were prepared from a mixture of industrialized products and supplemented ingredients added to a previously triturated rat chow [24]. Importantly, all diets provided sufficient and similar amounts of vitamins, minerals, and essential amino acids. Detailed diets composition is described in a previous study [24]. Hypercaloric diets presented higher energetic density related to control diet (4.6 kcal/g vs 3.2 kcal/g). However, while HD2 and HD4 were richer only in lipids, HD1 and HD5 also presented important carbohydrate content, especially sucrose. Although HD3 has revealed similar energetic density (4.4 kcal/g) in relation to other hypercaloric diets, its composition was based mainly in sucrose surplus from a water solution. Hypercaloric diets were isocaloric and had ~30% more energy content than the standard diet, and they are corresponded to others interventions from studies about diet-induced obesity [25,26].

Nutritional, metabolic, and endocrine profiles of the animals
Nutritional and metabolic profile included adiposity, body weight (BW), caloric intake, feed efficiency, glycemic tolerance, and triacylglycerolemia; endocrine variables involved leptin and insulin. To analyze if dietary-induced obesity was associated with alterations in the nutritional behavior, food consumption and water intake was measured daily. Calorie intake was calculated weekly by the average weekly food consumption × dietary energetic density. Feed efficiency and the ability to transform consumed calories into body weight were determined by following the formula: mean body weight gain (g)/total calorie intake.

In relation to glycemic tolerance, after fasting for 12 to 15 hours, rats were submitted to oral glucose tolerance test...
(OGTT). Blood samples were drawn from the tail at baseline and after gavage administration of glucose (3 g/kg) [23–25]. Blood samples were collected at 0, 60, 120, and 180 minutes. Glucose levels were determined using the ACCU-CHEK GO KIT glucose analyzer (Roche Diagnostic Brazil Ltd, Brazil). Glucose tolerance was analyzed from the area under the curve of glycemic responses. After 20 weeks of experiment, after 12 to 15 hours fasting, the animals were anesthetized with sodium pentobarbital (50 mg/kg) and killed by decapitation. To biochemical and hormonal analysis, trunk blood was instantly collected in heparinized tubes; subsequently, serum was separated by centrifugation at 3000 g for 15 minutes at 4°C and then stored to −80°C to posterior analyzes. Serum contents of triacylglycerol were determined with enzymatic colorimetric kits (Kovalent Diagnosis, Rio de Janeiro/RJ, Brazil). Spectrophotometry was performed with a Micronal, model B 382 spectrophotometer. Serum insulin and leptin were measured by the ELISA method using assay kits from Linco Research Inc (St. Charles, MO, USA).

After thoracotomy and abdominal incision, adipose deposits (AD) from visceral, retroperitoneal, epididymal, and carcass sites were measured [24]. The adiposity index was obtained from the sum of the weights of individual fat pads: \( \text{EAD} \times 100/\text{BW} \). Body weight (BW) was evaluated once a week.

**Cardiovascular profile**

**Blood pressure and ventricular performance**

At the conclusion of the experiments (before killing), systolic blood pressure (SBP) was assessed using a noninvasive tail-cuff method with a Narco Biosystems Eletro-Sphygmomanometer (International Biomedical, Austin, TX, USA) [27]. Each animal was individually coupled to system and the average of 2 readings was recorded for each measurement.

At follow-up (20 weeks), all animals were weighed and evaluated via a transthoracic echocardiographic examination performed with a commercially available echocardiography machine (Sonos 5500, Philips, Andover, MA, USA) equipped with a 12-MHz phased array transducer. All measurements were obtained by the same observer according to the method recommended by the American Society of Echocardiography [28].

**Morphologic studies**

The heart was removed and dissected at the time of killing. Gross morphology concerned the atria (AW), left (LW), and right ventricles (RVW) weights, as well as their respective relations with the body weight (AW/BW, LW/BW, and RVW/BW) [29]. A partial section from the left ventricle was used for histologic analysis; 7-µm sections were cut from the blocked tissue and stained with hematoxylin-eosin to determination of myocyte cross-sectional area (MA) and with the collagen-specific stain picrosirius red (Sirius red F3BA in aqueous saturated picric acid) to analyze interstitial collagen volume fraction. Detailed methods are described in a prior study [24]. Importantly, MA was determined for at least 100 myocytes per slide stained with hematoxylin-eosin [1].

For ultrastructural study, 3 animals from each group were used for analysis. Small fragments of the left ventricle papillary muscle were fixed in Karnovsky’s fixative (0.12 M phosphate, pH 7.2) for 1 to 2 hours followed by postfixation in 1% osmium tetroxide in 0.1 M phosphate buffer for 2 hours. After dehydrating in a graded ethanol series, fragments were embedded in epoxy resin. Ultrathin sections were double-stained with uranyl acetate and lead citrate and examined on an electron microscope (Phillips EM 301).

**Myosin heavy chain composition**

An additional sample (200 to 300 mg from the anterior wall of the left ventricle) was selected and frozen to myocardial MHC evaluation, realized by electrophoresis. Methods of sample preparation and electrophoresis conditions are presented with details in previous study [24].

**Statistical analyzes**

Results are expressed as descriptive measures of centralization and variability. Comparisons between groups were performed using the Student t-test or Mann-Whitney test for independent samples. Body weight evolution, in function of the experimental period, was studied by non-linear regression models. The comparison between models was performed by angular coefficient and regression constant tests [30]. The level of significance was considered to be \( P < .05 \).
Body weight profile from both groups along of experimental period is presented in Figure 1; the comparison between regression models revealed that the hypercaloric diet optimized body weight gain in relation to the control diet, from the second to the third interval to the 20th week (P<0.05, Figure 1). Regression model referent to OB-SHR group presented better prediction coefficient: 97.6% (Table 1).

Moreover, obesity was associated with enhanced calorie intake, feed efficiency, final BW, and adiposity in relation to the control group. Although insulin levels were not modified in the obese rats, OB-SHR presented higher triacylglycerol and leptin contents as well as impaired glycemic tolerance in relation to the C-SHR group (Table 2).

In the cardiovascular context, dietary obesity increased indexes of the left ventricle’s weight (LVW), interstitial collagen fraction (ICF; P<0.01), and cardiomyocyte cross-sectional area (MA; P<0.05; Table 3). In relation to *in vivo* variables, OB-SHR presented higher left ventricular diastolic thickness (LVDT) in relation to C-SHR group. Although obesity did not

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<th>Table 1. Body weight (BW) non-linear regression models in function to experimental period.</th>
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<td><strong>Groups</strong></td>
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<td>C-SHR</td>
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<td>OB-SHR</td>
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<td>* P&lt;0.001 versus different weeks (W).</td>
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<th>Table 2. Nutritional, metabolic and endocrine evaluation.</th>
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<td><strong>Variables</strong></td>
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<tr>
<td>Body weight (g)</td>
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<tr>
<td>Adiposity (%)</td>
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<tr>
<td>Calorie Intake (Kcal/day)</td>
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<td>Feed Efficiency (g/Kcal)</td>
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<td>Glycemia (AUC)</td>
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<td>Triacylglycerol (mg/dL)</td>
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<td>Leptin (ng/dL)</td>
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<td>Insulin (ng/dL)</td>
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<td>Values expressed as mean ± standard error; AUC – area under curve of response to oral glucose tolerance test; * p&lt;0.05, ** p&lt;0.01 vs. C-SHR; t-Student test. Insulin values expressed in median ± interval between 25th and 75th percentiles; Mann-Whitney test.</td>
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<th>Table 3. Post-mortem cardiac morphological evaluation.</th>
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<td><strong>Variables</strong></td>
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<td>AW (g)</td>
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<td>AW/BW (mg/g)</td>
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<td>RVW (g)</td>
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<td>LVW (g)</td>
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<tr>
<td>LVW/BW (mg/g)</td>
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<td>ICF (%)</td>
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<td>MA (µm²)</td>
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<td>Values expressed as mean ± standard error. AW, RVW, and LVW – atria, right, and left ventricles weights (g), respectively; AW/BW, RVW/BW and LV/BW – ratios between AW, RVW, and LVW and body weight, respectively; ICF – interstitial collagen fraction; MA – myocyte cross-sectional area; * p&lt;0.05, ** p&lt;0.01 vs. C-SHR; t-Student test. AW, AW/BW, RVW, RVW/BW, and LVW values expressed in median ± interval between 25th and 75th percentiles; Mann-Whitney test.</td>
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<th>Table 4. Blood pressure and echocardiography study.</th>
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<td><strong>Variables</strong></td>
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<td>Heart rate (beats/min)</td>
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<tr>
<td>SBP (mmHg)</td>
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<td>LVEDd (mm)</td>
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<tr>
<td>LVESd (mm)</td>
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<td>LVDT (mm)</td>
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<tr>
<td>LVDT/LVEDd</td>
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<td>LA/AO</td>
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<td>EFS (%)</td>
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<tr>
<td>EF</td>
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<tr>
<td>PWSV (mm/s)</td>
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<tr>
<td>E-wave (cm/s)</td>
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<tr>
<td>A-wave (cm/s)</td>
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<td>E/A</td>
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<tr>
<td>EDT (ms)</td>
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<td>IVRT (ms)</td>
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<td>Values expressed as mean ± standard error; SBP – systolic blood pressure; LVEDd – left ventricular end-diastolic diameter; LVESd – left ventricular end-systolic diameter; LVDT – diastolic thickness of the left ventricle; LVDT/LVEDd – ratio between LVDT and LVEDd; LA/AO – ratio between LA and diameter of aortic artery; EFS – endocardium fraction shortening; EF – ejection fraction; PWSV – posterior wall shortening velocity; E/A – ratio between the E and A-waves evaluated in transmural flow; EDT – E-wave deceleration time; IVRT – isovolumetric relaxation time; * p&lt;0.05, ** p&lt;0.01 versus C; t-Student test. LVEDd and IVRT values expressed in median ± interval between 25th and 75th percentiles; Mann-Whitney test.</td>
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**RESULTS**

Body weight profile from both groups along of experimental period is presented in Figure 1; the comparison between regression models revealed that the hypercaloric diet optimized body weight gain in relation to the control diet, from the second to the third interval to the 20th week (P<0.05, Figure 1). Regression model referent to OB-SHR group presented better prediction coefficient: 97.6% (Table 1). Moreover, obesity was associated with enhanced calorie intake, feed efficiency, final BW, and adiposity in relation to the control group. Although insulin levels were not modified in the obese rats, OB-SHR presented higher triacylglycerol and leptin contents as well as impaired glycemic tolerance in relation to the C-SHR group (Table 2).
change systolic blood pressure (SBP) or systolic function, it reduced the values of A-waves evaluated in transmitral flow and the isovolumetric relaxation time of the left ventricle, while it increased the ratio between E and A-waves (P<0.05; Table 4).

Electrophoresis separation between α- and β-MHC isoforms is presented in Figure 2A and 2B; OB-SHR showed higher relative content of β-MHC (C-SHR: 41.2±3.0 vs OB-SHR: 46.3%±3.2%, P=0.002; Figure 2A) and β/α-MHC ratio (C-SHR: 0.71±0.09 vs OB-SHR: 0.87±0.12, P=0.003; Figure 2B) than the C-SHR group. Ultrastructural analysis showed that C-SHR presented normal cardiomyocyte morphology, including sarcolemma, myofibrils, mitochondria, central nucleus, nucleolus, and sarcoplasmic reticulum (Figure 3A,B). OB-SHR exhibited focal alterations in the cardiomyocytes: Z-line disorganization, swelling, severe degradation and polymorphisms of mitochondria with disorganized or absence of cristae, and lipid droplets. Moreover, many cells showed areas with loss of myofilaments and mitochondria (Figure 3C–E) as well as T-tubules dilation (Figure 3F).

**DISCUSSION**

Hypertension and obesity are comorbid diseases that have been identified as independent risk factors for development of myocardial dysfunction and heart failure [21,22,31]. Importantly, these risk factors are increasing in prevalence at an alarming rate and, in general, blood pressure is strongly correlated with body mass index [22,31,32]. The major findings from the present study supported the issue that diet-induced obesity accentuated cardiac remodeling from hypertension, causing diastolic dysfunction of restrictive filling pattern in young SHR rats with age of 7–8 months.

In the present research, obesity was induced from hypocaloric intervention, obtained by adding a mixture of industrialized products to a standard diet, resulting in enhanced content of fatty acids, mainly unsaturated fats, and cholesterol combined to sucrose overload. The dietaries composition were based in models of obesity cafeteria diet-induced, such as are highly energetic, tasty and contain different shapes and are therefore much closer to the food consumed in general by humans [33].

The OB-SHR group was constituted by obese animals, as shown by superior values of BW and adiposity in relation to their control counterparts. However, although body weight is associated with adiposity, it can underestimate differences in fat content. The OB-SHR group showed increased BW and total adiposity by 14.0% and 56.3%, respectively. Moreover,
increased adiposity was derived from higher calorie intake and feed efficiency of OB-SHR in relation to C-SHR group. The nutritional results confirm biometric findings of other studies, which showed that high-fat and hypercaloric interventions promoted obesity in SHR [17,18].

In association with nutritional and biometric characterization, OB-SHR exhibited higher levels of triacylglycerol and leptin compared to C-SHR. Previous studies showed that increased adiposity is directly associated with leptinemia [34,35]. Taking into account that leptin promotes lipolysis, reducing the uptake of TG in adipocytes [35], it is probable that hyperleptinemia increased serum TG levels in OB-SHR as compared to the C-SHR group. Importantly, hypertriacylglycerolemia also could be associated with insulin resistance [35]; insulin stimulates the uptake of TG in adipocytes, contributing to adipogenesis, and inhibits lipolysis, therefore preventing the increase of serum triacylglycerols [35]. In this study, it is probable that OB-SHR presented insulin resistance, since obesity was associated with reduced glycemic tolerance although insulin levels were not modified. This insulin profile could be derived from fasting periods, since rats were maintained in fasting for periods of 12–15 hours.

Clinically, elevated anthropometric indexes have been associated with hypertriacylglycerolemia, glycemic disorders as well as arterial hypertension in obese patients [22,37].

Regarding structural cardiac evaluation, dietary-induced obesity promoted cardiomyocyte hypertrophy, interstitial remodeling, ultrastructural alterations, and synthesis β-MHC isoforms. Several bioactive molecules secreted by the adipose tissue, such as rennin-angiotensin-aldosterone system, endothelin, catecholamine, and inflammatory cytokines [34,35], could be responsible for increased myocyte size, myosin changes, and interstitial remodeling in obese animals. Furthermore, lipotoxicity [38] and oxidative stress [39] are not disregarded in these structural alterations. Excessive accumulation of lipids within non-adipose tissue increases the intracellular pool of long-chain fatty acyl-CoA, thereby providing fatty acid substrates for non-oxidative processes, including triacylglycerol, diacylglycerol, and ceramide synthesis, which can cause cardiomyocyte hypertrophy, apoptotic cell death, and interstitial fibrosis [38,39]. On the other hand, oxidative stress constituted a causative mechanism of endothelial dysfunction and cardiac remodeling in rabbits kept on cholesterol atherogenic diet [40]. Since our dietary model present sucrose supplements, it is not possible discard the sugar as cause of cardiac remodeling; previous studies documented accentuated myocardial hypertrophy, and synthesis β-MHC isoforms in conditions of pressure overload and sugar support [41,42]. Therefore, an important limitation of the current study is the multifactorial universe of causes which could be associated with cardiac remodeling in this experimental model of obesity.

It is noteworthy that ultrastructural abnormalities constituted an interesting finding. These disturbances were indicated by cardiomyocyte and organelle degradation, including Z-line disorganization, swelling, severe degradation and polymorphisms of mitochondria with disorganized or absence of cristae, and lipid droplets. Disorganization and lack of myofibrils, myofilaments, and Z discs, and disconnection among myocytes can hamper the coordinated transmission of muscular contraction and reduce myocardial performance [43,44]. Among the ultrastructural alterations, T-tubular dilatation constitutes an instigating result. Some authors documented that cardiomyocytes present increased T-tubular surface to maintain a normal surface-to-volume relationship and mechanical performance during remodeling [45,46]. One benefit of increasing myocyte surface is to enhance ionic diffusion capabilities, mainly Ca\(^{2+}\) handling, activating contractility of reminiscent sarcomeres [46]. The mechanisms underlying the expression and maintenance of the T-tubules are not clear [47]. The ability of the tubular system to maintain its remarkable degree of structure despite the forces exerted during the normal contractile cycle depends on the integrity of focal adhesion molecules, membrane, and basal lamina-associated proteins [47]. Eventual injuries of these structures could affect T-tubular morphology with alteration in its form. Further investigations about morphology and composition of T-tubules may clarify the causes of this dilatation.

Associated to ultrastructural disorders, up-regulation of β-MHC isoforms configured other important indicative of cardiac remodeling. In general, this change in MHC isoforms is associated with reduced contractile shortening velocity and consequent energetic economy to myocardial performance in pathological conditions [1,3,19]. It was demonstrated that β-MHC isoforms promote higher duration of displacement and force transients than α-MHC isoforms [3,19]. In current study, although in vivo contractile shortening velocity was not affected by modified MHC composition, these changes coupled with the increased collagen deposition, cardiac hypertrophy and myocardial ultrastructural damage configure potential mechanisms of diastolic dysfunction of restrictive filling pattern [6,8,20], confirmed from reduced A-wave, and IVRT accompanied by increase of E/A-waves ratio in OB-SHR group. These parameters are influenced by disturbances in relaxation and compliance phases [20]. On other hand, the lower heart rate could optimize the mitral blood inflow during the passive relaxation, reducing the values of A-wave, and, therefore, enhancing the E/A ratio [20]. Decreased heart rate is an unexpected finding, since dietary obesity induces sympathetic nervous system hyperactivity in the SHR [17], causing tachycardia. Other studies will can to elucidate the causal mechanisms related to these results.

**Conclusions**

In conclusion, conjugated results sustain the initial hypothesis in this study; diet-induced obesity promoted additional cardiac remodeling in essential hypertension, indicated by cardiomyocyte hypertrophy, interstitial fibrosis, synthesis of β-MHC isoforms and ultrastructural alterations, with occurrence of diastolic dysfunction of restrictive filling pattern in young SHR. The possible mechanisms responsible for cardiac alterations are indefinite and further studies are necessary to elucidate them. These results suggest that nutritional and behavioral conducts focused in feeding and lifestyle could be beneficial in treatment of the obesity, hypertension and cardiovascular diseases in humans.

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