Cardiac remodeling in a rat model of diet-induced obesity

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The mechanisms by which diet-induced obesity cause remodeling and cardiac dysfunction are still unknown. Interstitial collagen and myocardial ultrastructure are important in the development of left ventricular hypertrophy, and are essential to the adaptive and maladaptive changes associated with obesity. Thus, the accumulation of collagen and ultrastructural damage may contribute to cardiac dysfunction in obesity. The purpose of the present study was to investigate cardiac function in a rat model of diet-induced obesity and to test the hypothesis that cardiac dysfunction induced by obesity is related to myocardial collagen deposition and ultrastructural damage. Thirty-day-old male Wistar rats were fed standard (control [C]) and hypercaloric diets (obese [Ob]) for 15 weeks. Cardiac function was evaluated by echocardiogram and isolated left ventricle papillary muscle. Cardiac morphology was assessed by histology and electron microscopy. Compared with C rats, Ob rats had increased body fat, systolic blood pressure and area under the curve for glucose, leptin and insulin plasma concentrations. Echocardiographic indexes indicated that Ob rats had increased left ventricular mass, increased systolic stress and depressed systolic function. Analysis of the isolated papillary muscle was consistent with higher myocardial stiffness in Ob compared with C rats. The Ob rats had an increase in myocardial collagen and marked ultrastructural changes compared with C rats. Obesity promotes pathological cardiac remodeling with systolic dysfunction and an increase in myocardial stiffness, which, in turn, is probably related to afterload elevation and cardiac fibrosis. Obesity also causes damage to myocardial ultrastructure, but its effect on myocardial function needs to be further clarified.

Key Words: Collagen; Echocardiography; Myocardial ultrastructure; Obesity; Papillary muscle; Rats

Obesity is defined as an excessive amount of body fat in relation to lean mass that compromises healthy individuals (1) and is associated with various comorbidities such as type 2 diabetes mellitus, dyslipidemias, hypertension, stroke and coronary artery disease (2,3). More importantly, obesity has long been recognized as an independent risk factor for cardiovascular disease (4). The results of various clinical studies generally demonstrate that obesity, either uncomplicated (5) or associated with comorbidities (6), presents with left ventricular (LV) diastolic dysfunction (5,6), and systolic function that is preserved (7,8), improved (9) or depressed (10).

To understand the pathophysiology of the abnormalities secondary to obesity, genetic and dietetic experimental models have been proposed

Le remodelage cardiaque dans un modèle murin d'obésité induite par le régime

Les mécanismes par lesquels l'obésité induite par le régime provoque un remodelage et une dysfonction cardiaques demeurent inconnus. Le collagène interstitiel et l'ultrastructure myocardique revêtent de l'importance dans l'apparition de l'hypertrophie ventriculaire gauche et sont essentiels aux changements adaptatifs et maladaptatifs liés à l'obésité. Ainsi, l'accumulation de collagène et d'atteinte ultrastructurale contribuent peut-être à la dysfonction cardiaque causée par l'obésité. La présente étude visait à explorer la fonction cardiaque chez un modèle murin d'obésité induite par le régime et à vérifier l'hypothèse selon laquelle la dysfonction cardiaque induite par le régime est liée à un dépôt de collagène myocardique et à une atteinte ultrastructurale. Les chercheurs ont alimenté des rats Wistar mâles de 30 jours au moyen d'un régime standard (témoins [T]) ou hypercalorique (obèses [Ob]) pendant 15 semaines. Ils ont évalué la fonction cardiaque par échocardiogramme et par le muscle papillaire isolé du ventricule gauche, et leur morphologie cardiaque au moyen d'une histologie et d'un microscope à électron. Par rapport aux rats T, les rats Ob avaient plus de masse grasse, une tension artérielle systolique plus élevée et des régions de concentrations plasmatiques de glucose, de leptine et d'insuline sous la courbe. Les indices échocardiographiques indiquaient que la masse ventriculaire gauche et le stress systolique des rats étaient accrus, tandis que leur fonction systolique était réduite. L'analyse du muscle papillaire isolé était indicatrice d'une plus grande raideur myocardique chez les rats Ob que chez les rats T. Les rats Ob présentaient une augmentation du collagène myocardique et des modifications ultrastructurelles marquées par rapport aux rats T. L'obésité favorise un remodelage cardiaque pathologique accompagné d'une dysfonction systolique et d'une augmentation de la raideur myocardique qui, à son tour, est probablement liée à une élévation de la pression diastolique et à une fibrose cardiaque. L'obésité provoque également une atteinte à l'ultrastructure myocardique, mais il faudra mieux en clarifier l'effet sur la fonction myocardique.

(11,12). Although it is clear that genetic factors contribute to the propensity of an individual to become obese (Ob), the over-consumption of a high-energy diet may promote a positive energy balance and lead to the development of overweight and obesity states (13). Administration of a high-fat diet is an experimental model that reproduces many features of human obesity (14).

Experimental studies relating cardiac function and diet-induced obesity present divergent results. Studies (15-17) in which rats were fed hypercaloric diets for eight to 14 weeks verified, by echocardiogram (15,16) and ventricular myocytes (17), that obesity did not alter cardiac function. However, Ouwens et al (18) showed higher basal contractile force and impaired recovery after an increase of workload in papillary

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muscles from rats fed a hypercaloric diet for seven weeks. Moreover, other investigators found systolic dysfunction in isolated heart and papillary muscles (19), reduction in diastolic compliance (20) and impaired mechanical function of ventricular myocytes (21) from Ob rabbits (19,20) and rats (21) fed high-fat diets for 12 weeks.

Several agents (22), such as accumulation of interstitial collagen (23) and ultrastructural damage (24), have been proposed as contributing to cardiac dysfunction in different models of heart disease. Nevertheless, the role of these factors in obesity-induced cardiac dysfunction is unknown. Carroll et al (20) suggested that collagen deposition could be related to decreased diastolic compliance in Ob rabbits. The relationship between myocardial dysfunction and ultrastructural damage in this obesity model has not been determined. 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 (24,25).

Given the scarce and divergent data in the existing literature on the subject, the purpose of the current study was to obtain further information regarding the influence of diet-induced obesity on cardiac function, and the involvement of myocardial ultrastructure and the extracellular matrix. Accordingly, the study was designed to test the hypothesis that cardiac dysfunction induced by obesity is related to myocardial collagen deposition and ultrastructural damage.

METHODS

Animal model and experimental protocol

Thirty-day-old male Wistar rats were randomly assigned to one of two groups (n=30 each): control (C) or Ob. The C group was fed a standard rat chow containing 11.2% fat, 55.5% carbohydrate and 33.3% protein. The Ob animals received a high-fat diet containing 45.2% kcal fat, 28.6% carbohydrate and 26.2% protein. Each group was fed the diet for 15 consecutive weeks. The high-fat diet was designed in the Experimental Laboratory of the Department of Clinical and Cardiology, São Paulo State University (Brazil), and contained powdered commercial Labina rat chow (Purina, Brazil), industrialized feed, protein supplement, vitamins and minerals. The high-fat diet was calorically rich (high-fat diet = 4.5 kcal/g versus standard diet = 3.3 kcal/g) due to the higher fat composition, made with saturated (17%) and unsaturated (83%) fatty acids. All rats were housed in individual cages in an environmentally controlled clean-air room at 23±3°C with a 12 h light/dark cycle and 60±5% relative humidity. Food consumption was measured daily and water intake was controlled once a week. Initial body weight and final body weight (FBW) were recorded. Weekly caloric intake was calculated as the average weekly food consumption multiplied by the caloric value of each diet. Feed efficiency (the ability to translate calories consumed into body weight) was also evaluated. The heart weight, LV weight (LVW), heart/FBW ratio, LVW/FBW ratio and papillary muscle cross-sectional area were recorded.

All experiments and procedures were performed in accordance with the *Guide for the Care and Use of Laboratory Animals*, published by the United States National Institutes of Health (26), and were approved by the Botucatu Medical School Ethics Committee (Universidade Estadual Paulista "Julio de Mesquita Filho" [UNESP], São Paulo, Brazil).

Systolic blood pressure

At the conclusion of the experiments, the systolic blood pressure (SBP) was assessed by using the noninvasive tail-cuff method (27) with a Narco BioSystems Electro-Sphygmomanometer (International Biomedical, USA). The average of two readings was recorded for each measurement.

Oral glucose tolerance test

At the end of the 15-week feeding period, an oral glucose tolerance test was performed. Blood samples were drawn from the tips of the tails of rats that fasted overnight (12 h to 15 h). Blood glucose was collected under basal conditions and after gavage administration of 3 g/kg glucose load (28). Blood samples were collected at 0 min, 60 min, 120 min,

180 min and 240 min, and analyzed using a glucometer (Accu-Chek Go Kit; Roche Diagnostics Brazil Ltda, Brazil).

Plasma analysis of hormones

At the end of treatment, animals were subjected to a 12 h to 15 h fast, anesthetized with sodium pentobarbital (50 mg/kg intraperitoneal [ip]) and euthanized by decapitation. Blood was collected in heparinized tubes, centrifuged at 3000 g for 15 min at 4°C, and then stored at -80°C. Plasma leptin and insulin concentrations were determined by ELISA (16) using specific commercial kits (Linco Research Inc, USA).

Body fat analysis

After animals had been anesthetized with sodium pentobarbital (50 mg/kg ip), decapitated and thoracotomized, the viscera were discarded, leaving only the carcass. Carcasses were dried at 100±5°C for 72 h in a ventilated Fanem dryer (Fanem, Brazil). After drying, the carcasses were wrapped in filter paper and the fat was extracted in a Soxhlet extractor (Corning Life Sciences, USA). The percentage of body fat in each carcass was calculated by the following formula:

$$((PP-PSSG)/PP) \times 100,$$

where PP is postdrying weight, PSSG is dry weight after fat extraction, and PPr is predrying weight (14).

Histology and electron microscopy

LV transverse sections of seven animals from each group were fixed in 10% buffered formalin and embedded in paraffin (29). Thick sections of 1 µm were cut from the tissue block and stained with hematoxylin and eosin, and with the collagen-specific stain picrosirius red (Sirius red F3BA in aqueous saturated picric acid). The myocyte cross-sectional area was determined for at least 100 myocytes per hematoxylin and eosin-stained slide. The myocyte cross-sectional area measurements were obtained from digitized images (40× magnification lens) collected using a video camera attached to a Leica microscope (Leica Mikroskopie & Systems GmbH, Germany) and computerized image analysis software (Image-Pro Plus 3.0, Media Cybernetics, USA). Myocyte cross-sectional area was measured using a digitizing pad, and the selected cells were cut transversely with the nucleus clearly identified in the centre of the myocyte. Interstitial collagen volume fraction (CVF) was determined for the entire picrosirius red-stained cardiac section using an automatic image analyzer (Image-Pro Plus 3.0). The components of the cardiac tissue were identified according to colour level as follows: red for collagen fibres; yellow for myocytes; and white for interstitial space. The CVF was calculated as the sum of all connective tissue areas divided by the sum of all connective tissue and myocyte areas. On average, 35 microscopic fields were analyzed using a 20× lens. Perivascular collagen was excluded from this analysis.

For ultrastructural studies, small fragments of the LV papillary muscle from three rats of each group were fixed in Karnovsky's fixative (0.12 M phosphate [pH 7.2]) for 1 h to 2 h, followed by postfixation in 1% osmium tetroxide in 0.1 M phosphate buffer for 2 h. After dehydration in a graded ethanol series, the fragments were embedded in epoxy resin. Ultra-thin sections were double-stained with uranyl acetate and lead citrate, and examined using a Philips EM 301 electron microscope (Philips, USA).

Echocardiographic study

Echocardiography was performed on 16 C rats and 13 Ob rats at the end of the experimental period to evaluate LV morphology and function using a commercially available Sonos 2000 echocardiograph (Hewlett-Packard Medical Systems, USA) equipped with a 7.5 MHz phased array transducer. Imaging was performed at a 60° sector angle and 3 cm imaging depth (30). Rats were anesthetized by ip injection with a mixture of ketamine (50 mg/kg) and xylazine (1 mg/kg). Two-dimensionally targeted M-mode echocardiograms were obtained from short-axis views of the left ventricle at or just below the tip of the mitral valve leaflets, and were recorded on a black-and-white thermal printer (Sony UP-890 MD, Somatechnology, USA) at a sweep speed

of 100 mm/s. All LV traces were manually measured with a caliper by the same observer according to the leading-edge method of the American Society of Echocardiography (31). Measurements were recorded as the mean of at least five consecutive cardiac cycles. LV end-diastolic dimension (LVDD), posterior wall thickness in diastole (PWTd) and anterior wall thickness in diastole (AWTd) were measured at the maximum diastolic dimension. LV end-systolic dimension (LVSD), posterior wall thickness in systole (PWTs) and anterior wall thickness in systole (AWTs) were measured at the maximum anterior motion of the posterior wall. Left atrial dimension, aortic dimension, early peak transmitral flow velocity to late peak transmitral flow velocity ratio, early peak transmitral flow velocity to late peak transmitral flow velocity ratio, and heart rate (HR) were also measured. Relative wall thickness (RWT) was determined by PWTd/LVDD. LV mass (LVM) was calculated using the following formula (32):

$$([LVDD + AWTd + PWTd]^3 - LVDD^3) \times 1.04,$$

where the value 1.04 indicates the specific density of the myocardium. Indexes of LV systolic function were assessed by calculating endocardial fractional shortening (FS):

$$FS\% = ([LVDD - LVSD]/LVDD \times 100);$$

$$\text{midwall FS\%} = ([LVDD + \frac{1}{2}PWTd + \frac{1}{2}AWTd] - [LVSD + PWTs + \frac{1}{2}AWTs]) / [LVDD + \frac{1}{2}PWTd + AWTd]; \text{ and}$$

$$LV \text{ wall systolic stress} = SBP \times (LVSD/2)/PWTs.$$

Isolated muscle preparation

Myocardial performance was evaluated by studying isolated papillary muscle from the left ventricle as previously described in detail (33). Briefly, at the time of study, rats were anesthetized with sodium pentobarbital (50 mg/kg ip) and euthanized by decapitation. The hearts were quickly removed and placed in oxygenated Krebs-Henseleit solution at 28°C. LV papillary muscles from C (n=21) and Ob rats (n=22) were dissected, mounted between two spring clips, placed vertically in a chamber containing Krebs-Henseleit solution at 28°C and gassed with 95% O₂ and 5% CO₂. The composition of the Krebs-Henseleit solution was as follows: 118.5 mM NaCl; 4.69 mM KCl; 1.25 mM CaCl₂; 1.16 mM MgSO₄; 1.18 mM KH₂PO₄; 5.50 mM glucose; and 24.88 mM NaCO₃. The lower spring clip was attached to a 120T-20B force transducer (Kyowa Electronic Instruments Co, Japan) by a thin steel wire (1/15,000 inch), which passed through a mercury seal at the bottom of the chamber. The upper spring clip was connected by a thin steel wire to a rigid lever arm, above which, a micrometer stop was mounted for adjusting the muscle length. The muscle preparation was placed between two parallel platinum electrodes and stimulated at a frequency of 0.2 Hz, using square-wave pulses of 5 ms duration. Voltage was set to a value 10% greater than the minimum required to produce a maximal mechanical response.

The muscles were kept contracting isotonically with light loads for 60 min, and then loaded to contract isometrically and stretched to the maximum of their length-tension curves. After a 5 min period during which preparations performed isotonic contractions, muscles were again placed under isometric conditions, and the peak of the length-tension curve ($L_{\max}$) was carefully determined. A 15 min period of stable isometric contraction was imposed before the experimental period and one isometric contraction was then recorded.

The following parameters were measured from the isometric contraction: peak developed tension (DT [g/mm²]); resting tension (RT [g/mm²]); maximum rate of tension development (+dT/dt [g/mm²/s]); and maximum rate of tension decline (-dT/dt [g/mm²/s]). The myocardial stiffness was determined by the ratio between the muscle length variation and RT. RT was analyzed in muscle length corresponding to 90%, 92%, 94%, 96%, 98% and 100% of the $L_{\max}$. RT-length curves were plotted using potential regression analysis:

$$\log(RT) = -51.1118 + 25.5425 \log(L_{\max}) \text{ for C group};$$

$$\log(RT) = -58.1992 + 29.1455 \log(L_{\max}) \text{ for Ob group}.$$

TABLE 1

General and nutritional characteristics of the rats

Parameters	Group	
	Control (n=28)	Obese (n=25)
Initial body weight, g	105±10	111±12
Systolic blood pressure, mmHg	108±9	121±15*
Food consumption, g/day	21.9±1.5	17.0±1.8*
Water intake, mL/day	40.3±3.9	42.4±4.6
Caloric intake, kcal/day	72.4±4.9	85.6±9.6*
Feed efficiency, g/kcal	0.044±0.003	0.050±0.005*

Data presented as mean ± SD. *P<0.05 versus controls. Student's *t* test for independent samples

The parameters used to characterize papillary muscle were length (mm), weight (mg) and cross-sectional area (mm²). After the end of each experiment, the muscle length at $L_{\max}$ was measured with a catheterometer (Gaertner Scientific Corporation, USA), and the muscle between the two clips was blotted dry and weighed. The cross-sectional area was calculated from the muscle weight and length by assuming uniformity and a specific gravity of 1.0. All force data were normalized for the muscle cross-sectional area.

Statistical analysis

General, nutritional, hormonal and morphological characteristics, and cardiac function evaluation were expressed as mean ± SD and the comparison between groups was analyzed using Student's *t* test. The glucose profile of the groups was compared by ANOVA for repeated measures. When significant differences were found (P<0.05), the post hoc Bonferroni multiple comparisons test was performed. Myocardial stiffness curves were compared by the angular coefficient test and linear regression constant. The level of significance considered was 5%.

RESULTS

General characteristics of the rats

Table 1 and Figure 1A show the influence of obesity on the general characteristics of the animals. Although the Ob rats ingested less food than the C rats, the caloric intake, feed efficiency and FBW were greater in the Ob than the C rats. Furthermore, the percentage of carcass body fat was significantly increased (Figure 1B; 112.5±33.5%) in the Ob rats in relation to the C rats. The comorbidities associated with obesity – glucose intolerance (Figure 1C) and insulin resistance (Figure 1D) – were also observed in the study. The final SBP (Table 1), plasma leptin and insulin concentrations were higher in the Ob than in the C rats (Figure 1D).

Cardiac function

Table 2 and Figure 2 summarize cardiac function data obtained from the echocardiographic study.

The echocardiographic evaluation showed that Ob rats had increased LVM, LVSD and PWTd compared with C rats (Table 2 and Figure 2A). No significant differences existed in HR, early peak transmitral flow velocity to late peak transmitral flow velocity ratio, left atrial dimension, aortic dimension or relative wall thickness between the groups (Table 2). LVDD was increased in Ob rats in relation to C rats, but this difference was not statistically significant (P=0.056) (Figure 2A). The indexes of LV systolic function, observed both within the heart (P=0.07) and at the midwall FS (P<0.05), demonstrate depressed systolic function compared with C rats (Figure 2B). LV wall systolic stress was elevated in the Ob rats compared with the C rats (Figure 2C).

Histology and electron microscopy

Although Ob rats had a larger heart weight (Figure 3A) and LV weight (Table 2) than the C rats, the heart/FBW (Figure 3B), LV/FBW (C=1.91±0.17 mg/g, Ob=1.84±0.16 mg/g; P>0.05) and myocyte cross-sectional area (Figure 3C) were similar in both groups. Interstitial CVF (Figure 3D) was higher in Ob rats than in C rats.

Figure 1 **A** Final body weight (control n=28, obese n=25); **B** Carcass body fat (control n=28, obese n=25); **C** Blood glucose levels following an oral glucose load in control (white triangles; n=8) and obese animals (black diamonds; n=8); **D** Plasma insulin and leptin (n=13). Data presented as mean $\pm$ SD. * $P < 0.05$ versus control. Student's t test for independent samples; repeated measures two-way ANOVA and Bonferroni's posthoc analysis

TABLE 2
Echocardiography data

Parameters	Group	
	Control (n=16)	Obese (n=13)
HR, beats/min	302 $\pm$ 41	293 $\pm$ 28
LVDD, mm	8.18 $\pm$ 0.53	8.52 $\pm$ 0.35
LVSD, mm	3.54 $\pm$ 0.44	3.90 $\pm$ 0.42*
PWTd, mm	1.42 $\pm$ 0.06	1.50 $\pm$ 0.05*
AO, mm	3.88 $\pm$ 0.23	3.86 $\pm$ 0.33
LA, mm	5.56 $\pm$ 0.60	5.52 $\pm$ 0.39
LV mass, g	0.84 $\pm$ 0.10	0.96 $\pm$ 0.07*
Mitral E/A	1.41 $\pm$ 0.24	1.49 $\pm$ 0.36
RWT	0.17 $\pm$ 0.01	0.18 $\pm$ 0.01

Data presented as mean $\pm$ SD; * $P < 0.05$ versus control. Student's t test for independent samples. AO Aortic dimension; HR Heart rate; LA Left atrium; LV Left ventricle; LVDD Left ventricular end-diastolic dimension; LVSD Left ventricular end-systolic dimension; Mitral E/A Early peak transmitral flow velocity to late peak transmitral flow velocity ratio; PWTd Posterior wall thickness in diastole; RWT Relative wall thickness

The ultrastructural study of the LV myocardium of the C rats revealed normal morphological aspects that included fibres containing sarcoplasm filled with myofibrils, well-defined sarcomeres, mitochondria with lamellar cristae, plasma membranes with straight undulating aspects and nuclei with loose chromatin (Figure 4). Electron microscopy revealed marked ultrastructural abnormalities in cardiomyocytes from Ob rats, changes in mitochondria, absence and/or disorganization of myofilaments, dilated sarcoplasmic reticulum vesicles and the presence of large amounts of lipid droplets between the myofibrils (Figure 4).

Myocardial function

Figures 4A to 4E show the myocardial performance evaluated by studying isolated papillary muscle. The mechanical function of isolated papillary muscle under basal conditions was similar in all parameters

Figure 2 **A** Representative echocardiographic M-mode images obtained in control (n=16) and obese (n=13) rats, respectively. In these images, the left ventricular end-diastolic and end-systolic dimension, and posterior wall thickness in diastole are observed. **B** Echocardiographic indexes of systolic function. **C** Left ventricular (LV) wall systolic stress. Data presented as mean $\pm$ SD. * $P < 0.05$ versus control. Student's t test for independent samples. FS Fractional shortening

Figure 3) Comparison of control versus obese rats. **A** Heart weight. **B** Heart/final body weight ratio. **C** Myocyte cross-sectional area (40× magnification lens); representative hematoxylin and eosin-stained left ventricular cross-sections from control and obese rats are shown. **D** Interstitial collagen volume fraction of myocardium (20× magnification lens) from control and obese rats; representative picrosirius red-stained left ventricular section from control and obese rats are shown. Arrows represent the interstitial collagen volume fraction of control and obese rats. Data presented as mean ± SD. * $P < 0.05$ versus control group. Student's *t* test for independent samples

Figure 4) Left ventricular papillary muscle ultrastructural study. Electron micrographs of the myocardial cells of control and obese rats: myofibrils (M), mitochondria (m), plasma membrane (arrow), disorganization of myofibrils (dm), lipid droplets (L), endothelial cell (E) and nucleus with nucleolus (N). Magnifications: ×7750 (top control), ×13,250 (bottom control), ×13,650 (top obese), ×13,250 (bottom obese). **A** Resting tension behaviour as a function of muscle length variation. **B** to **E** Baseline data from isolated papillary muscle of control and obese rats. **B** Peak developed tension (DT) normalized per cross-sectional area; **C** Resting tension (RT) normalized per cross-sectional area. **D** Maximum rate of tension development normalized per cross-sectional area (+dT/dt); **E** Maximum rate of tension decline normalized per cross-sectional area (-dT/dt). Data presented as mean ± SD. * $P < 0.05$ versus control group. Student's *t* test for independent samples. L_{max} Peak of the length-tension curve

(peak developed tension, RT, maximum rate of tension development and maximum rate of tension decline) between groups (Figures 4B to 4E). However, the myocardial stiffness was higher in Ob rats than in C rats (Figure 4A). There were no significant differences between C and Ob animals in the cross-sectional area of LV papillary muscle ($C=0.88\pm0.20$, $Ob=0.96\pm0.19$; $P>0.05$).

DISCUSSION

The hypercaloric diet used in the present study was of sufficient intensity and duration to promote obesity in rats. The Ob rats had an increase in body weight and body fat of 26% (Figure 1A) and 112.5% (Figure 1B), respectively, in relation to the C rats. Moreover, we also observed comorbidities associated with obesity, such as impaired glucose tolerance (Figure 1C), hyperinsulinemia, hyperleptinemia (Figure 1D) and elevated blood pressure (Table 1). These results are in agreement with several studies (14,15,21,34) that induced obesity in rats through a hypercaloric diet.

The analysis of cardiac morphology indicated that obesity induced increases in LVM (Table 2), but did not promote changes in LVW/FBW ratio or left myocyte dimensions (Figure 3C). Although Carroll and Tyagi (35) verified that diet-induced obesity caused ventricular hypertrophy in rabbits, Carroll et al (15) did not show cardiomyocyte hypertrophy in rats.

In the present investigation, obesity led to an increase in interstitial collagen deposition in the left ventricle (Figure 3D). There is little information about the cardiac collagen fraction in experimental obesity studies (15,36). While Carroll et al (15) did not find a histological increase of cardiac collagen in a rat model of diet-induced obesity, it was verified in another study (35) in rabbits that a high-fat diet for a 12-week period causes fibrosis in coronary vessels, as well as accumulation of collagen in the cardiac interstitium. The mechanisms underlying the accumulation of collagen in Ob animals remain unknown; Brands et al (36) suggested that the higher collagen concentration is linked to abnormalities in insulin metabolism. Insulin growth factor induces transforming growth factor beta-1, which directly stimulates collagen expression (37). However, given that obesity also has been associated with elevation of cytokines, endothelin and renin-angiotensin-aldosterone, it is probable that some of these factors are involved in the development of cardiac fibrosis (38,39).

The ultrastructural study of the LV papillary muscle showed that the myocardium from Ob rats presented marked ultrastructural abnormalities in cardiomyocytes (Figure 4), such as absence and/or disorganization of myofilaments, dilated sarcoplasmic reticulum vesicles, and the presence of large amounts of lipid droplets between the myofibrils and changes in mitochondria. The mechanism by which an excess in lipids may cause cell death in myocardium is not fully known. It has been proposed (40) that overaccumulation of the lipid enlarges the intracellular pool of fatty acyl-coenzyme A beyond the oxidative requirements of the cell, thereby providing substrate for potentially destructive nonoxidative pathways, such as *de novo* ceramide formation (41) and lipid peroxidation (42,43). Ouwens et al (18) found mitochondrial degeneration, including matrix dilution, cristolysis and mitochondria-associated lamellar bodies in cardiac sections of Ob rats; however, they did not relate these morphological changes to cardiac function.

The purpose of the present investigation was to study the changes in LV and myocardial performance using the combination of a noninvasive method, an echocardiogram and isolated papillary muscle preparation. The isolated preparation measured the capacity of the cardiac muscle to develop force, and shorten independent changes in loads, HR, chamber and wall geometry that could modify mechanical performance of the heart *in vivo* (25). Obesity did not promote myocardial systolic dysfunction in isolated papillary muscle, although it presented an increase of myocardial collagen and ultrastructural damage. Although the disarrangements in

the myocyte can hamper coordinated transmission of muscular contraction and reduce myocardial performance (24), the focal myocardial damage observed in Ob rats was not sufficient to promote alterations in myocardial contractile or relaxation function analyzed by papillary muscle in the present study.

The echocardiographic study showed that obesity did not change the diastolic function (Table 2), but induced LV systolic dysfunction observed by reduction of midwall FS (Figure 2B) and an increase of end-systolic diameter (Figure 2A). The damage to LV ejection capacity can be due to myocardial contractility depression and/or changes in the cardiac load conditions (30). The echocardiogram does not permit the exact identification of the cause of systolic dysfunction. The additional experiment with papillary muscle may allow better understanding of the LV changes observed in the present study. Given that obesity in this experiment did not alter the systolic function in papillary muscle, the alterations in ventricular systolic function observed *in vivo* may be due to changes in afterload. Afterload is a mechanical parameter directly influenced by ventricular pressure and diameter, and inversely related to wall thickness. Thus, it is possible that LV mechanical overload pressure, due to an increase of wall systolic stress, could explain the systolic dysfunction observed in Ob rats.

One important finding of the present study was the discrepancy between diastolic function evaluated by echocardiogram and isolated muscle preparation. While echocardiography did not show diastolic dysfunction, the papillary muscle revealed an increase in myocardial stiffness (Figure 4A). The reduction of diastolic compliance is more subtle in LV function than that of myocardial preparation, and often does not become apparent until hearts are challenged metabolically or with increased work loads. Passive myocardial stiffness is determined by structural properties of the cardiac tissue itself. The accumulation of interstitial cardiac collagen observed in the present study could have been a factor that contributed to the stiffer papillary myocardium in Ob rats. Because the collagen analysis was realized in the LV wall, we cannot assert that there was a collagen increase in the papillary muscle. It has been suggested that myocardial fibrosis may restrict myofibrillar motion and, thereby, impair diastolic function (44). Specifically, a two- or threefold increase in CVF adversely affects diastolic stiffness, promoting diastolic dysfunction, whereas a fourfold increase in CVF or more is associated with diastolic stiffness and systolic dysfunction (23). The ultrastructural feature in cardiomyocytes of Ob rats may contribute to the reduction of diastolic compliance, although further study is warranted.

SUMMARY

The present investigation showed that obesity promotes pathological cardiac remodeling with LV systolic dysfunction and increases myocardial stiffness, probably related to overload pressure and myocardial fibrosis, respectively. Obesity also caused damage to the myocardial ultrastructure, but its effect on myocardial function needs to be further clarified.

Limitations

The procedure used to measure body fat may have underestimated the total body fat in the rats of the present study. Other measures, such as dual-energy x-ray absorptiometry or fat pad weights, could determine the actual level of obesity.

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