High-fat Diet Promotes Cardiac Remodeling in an Experimental Model of Obesity

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Abstract

Background: Although nutritional, metabolic and cardiovascular abnormalities are commonly seen in experimental studies of obesity, it is uncertain whether these effects result from the treatment or from body adiposity.

Objective: To evaluate the influence of treatment and body composition on metabolic and cardiovascular aspects in rats receiving high saturated fat diet.

Methods: Sixteen Wistar rats were used, distributed into two groups, the control (C) group, treated with isocaloric diet (2.93 kcal/g) and an obese (OB) group, treated with high-fat diet (3.64 kcal/g). The study period was 20 weeks. Analyses of nutritional behavior, body composition, glycemia, cholesterolemia, lipemia, systolic arterial pressure, echocardiography, and cardiac histology were performed.

Results: High-fat diet associates with manifestations of obesity, accompanied by changes in glycemia, cardiomyocyte hypertrophy, and myocardial interstitial fibrosis. After adjusting for adiposity, the metabolic effects were normalized, whereas differences in morphometric changes between groups were maintained.

Conclusions: It was concluded that adiposity body composition has a stronger association with metabolic disturbances in obese rodents, whereas the high-fat dietary intervention is found to be more related to cardiac morphological changes in experimental models of diet-induced obesity. (Arq Bras Cardiol. 2015; 105(5):479-486)

Keywords: Obesity; Ventricular Remodeling; Diet, High-Fat; Epidemiology, Experimental; Rats.

Introduction

Cardiac remodeling results from aggression or continuous overload conditions, and causes molecular, cellular and interstitial changes that manifest clinically as changes in the size, mass, geometry, and function of the heart. Experimental studies, including exogenous obesity models, i.e. diet-induced obesity, have reported myocardial hypertrophy and interstitial fibrosis associated with functional disorders. In the metabolic context, disturbances related to cholesterol and lipid levels, as well as to glucose metabolism have also been frequently shown as comorbidities in experimental models of high energy diet-induced obesity.

Although dietetic interventions have been frequently used in studies on obesity, the distinction between treatment responses and adiposity-related effects has been poorly investigated. High-fat and high-energy diets, per se, are associated with different tissue and systemic changes in rodents, regardless of obesity. On the other hand, numerous endocrine, paracrine and autocrine properties have been attributed to the adipose tissue, highlighting its important role on the coordination of metabolic and cardiovascular abnormalities.

This study was primarily aimed to assess the nutritional, metabolic and cardiovascular profiles of rats undergoing a high-fat, high-energy diet. The initial hypothesis was that the dietetic intervention would be associated with the manifestation of obesity, metabolic disturbances, including glycemic and dyslipidemic disorders, and evidence of cardiac remodeling in rodents. The second aim was to differentiate the effects resulting from the treatment from those related to increased adiposity. The hypothesis was that the influence of body composition on such responses surpasses the effects associated with diet.

Methods

The scientific project was evaluated and approved by the Animal Ethics Committee of the Federal University of Mato Grosso do Sul (CEUA / UFMS), and in conformity with the Brazilian College of Animal Experimentation (COBEA).
Animals and experimental protocol

Sixteen male Wistar rats, aged 30 days, were studied. Previous studies of our research group were considered for sample size determination. The animals were randomly assigned to either Control (C) group or Obese (OB) group, to receive a isocaloric diet (2.93 kcal/g) or a high-fat, high-energy diet (3.64 kcal/g) respectively, for 20 weeks. The animals were kept in individual cages at a temperature of 22 ± 2°C and humidity of 55 ± 5%, under 12 h light / 12 h dark cycles, with free access to water.

The experimental diets differed only in their lipid content, and were balanced in terms of proteins, carbohydrates, vitamins and minerals. The isocaloric diet provided 34.3% of energy from protein, 57.1% from carbohydrate, and 8.6% from fat (3.3% from unsaturated and 5.3% from saturated fat). The high-fat intervention provided 24.0% of energy from protein, 53.3% from carbohydrate, and 22.7% from fat (8.0% from unsaturated and 14.7% from saturated fat). Both diets contained soybean bran and husk, and were supplemented with vitamins and minerals.

Nutritional and murinometric characterization

Nutritional characterization comprised evaluation of food intake (FI), energy intake (EI) and energy efficiency. Food intake was assessed daily, and EI was calculated by the formula: FI x (energy value of the diet)\(^{5,7,9,10}\). Energy provided by fatty acids was calculated by the formula: EI x (% fat provided by the diet)\(^{7}\). In order to evaluate the efficiency of conversion of dietary energy into body mass, food efficiency (FE) was calculated by weight gain (g) / total energy intake (kcal) rate\(^{7,9,10}\).

For body composition analysis, body mass was measured weekly, using a digital scale. Weight gain was calculated by subtracting the final weight from the initial weight. After euthanasia, adiposity measures were obtained considering retroperitoneal, epididymal and visceral fat masses\(^{5-9}\). Total body adiposity was calculated by the relationship between the sum of fat in each compartment (CF) and the final body mass (BM): \(\Sigma CF \times 100/(BM - \Sigma CF)\)\(^{7,10}\).

Metabolic characterization

Glucose tolerance test was performed after a 12-hour fast, and blood samples collected from caudal artery were used for glucose analysis. Intraperitoneal administration of 20% glucose (glucose monohydrate, Merck, São Paulo, Brazil) (2 g/kg) was performed, followed by measurement of glucose levels at 30, 60, 90, 120, 180 and 240 minutes\(^{7,10}\), using a glucose meter (ACCU-CHKG KIT, Roche Diagnostic Ltda, SP, Brazil).

Two days after in vivo analysis, the animals were kept under fasting for 12 hours, anesthetized intraperitoneally with sodium pentobarbital (50 mg/kg) and euthanized by decapitation. Blood samples were collected, centrifuged (3,000 rpm) and stored for further analysis. Biochemical analyses included measurements of serum glucose, triglycerides, total cholesterol, HDL, LDL, albumin and total protein, using an enzymatic method with specific kits.

Cardiovascular characterization

Cardiovascular characterization comprised determination of systolic arterial pressure, echocardiographic analysis, cardiopulmonary morphology analysis and myocardial morphometry. Systolic arterial pressure was measured by plethysmography\(^{23}\) using a sphygmomanometer (709-0610, Narco Bio-Systems ®, Austin, TX, USA) at the end of experimental period. Two days later, transthoracic echocardiography was performed to assess cardiac function and structure, according to previously described methods\(^{20-22}\).

Following the measurement of body mass, the animals were anesthetized intramuscularly with a ketamine hydrochloride (50 mg/kg) and xylazine hydrochloride (1 mg/kg) solution. For the echocardiography test, the anterior chest hair was removed and the animals were examined in the left lateral decubitus position. For determination of cardiac chambers, one-dimensional guided M-mode images were obtained with the ultrasound beam oriented by two-dimensional imaging, and the transducer in the parasternal short-axis position. Images of the left ventricle (LV) were obtained at the papillary muscle level, by positioning the M-mode cursor below the plane of the mitral valve\(^{21}\), and images of the thoracic aorta and left atrium were obtained by positioning the M-mode cursor below the plane of the aortic valve. The images were recorded with the use of a printer (UP-890, Sony Co., Japan), and the cardiac structures were measured manually using a precision pachimeter. Left ventricular end-diastolic diameter (LVDD), posterior wall diastolic thickness (PWDT) and interventricular diastolic septum thickness (IVDST) were measured at the point of maximum diameter of the ventricular cavity. Left ventricular end-systolic diameter (LVED), posterior wall systolic thickness (PWSST) and interventricular systolic septum thickness (IVSTST) were measured at the point of minimum diameter of the ventricular cavity. Left atrium (LA) was measured at its maximum diameter. Left ventricular mass (LVM) was calculated by the formula: LVM = (LVDD + PWDT + IVDST\(^{2}\)) - (LVDD\(^{2}\))\(^{2}\). The following variables were derived from the above-mentioned dimensions: relative thickness of the LV (PWDT/LVDD), LVDD/FBW, LA/FBW and LVM Index (LVM, LVM/FBW) where FBW refers to final body weight.

Left ventricular systolic function was evaluated by using the following parameters:

- Percentage of mid-wall shortening: [(LVDD + ½PWDT + ½IVDST) - (LVDD + ½PWST + ½IVSTST)]/(LVDD + ½PWDT + ½IVDST);
- Percentage of endocardial shortening: [(LVDD - LVED)/LVED];
- Posterior wall shortening velocity (PWSV).

Diastolic function was evaluated by the parameters: ratio of the peak early filling velocity (E-wave) to peak atrial contraction velocity (A-wave) of transmirtal flow (E/A ratio); E-wave deceleration time (DT) and isovolumetric relaxation time (IVRT). The echocardiography was performed using a commercially available echocardiographic system with an 11.5-MHz transducer (Vivid S6, General Electric Medical Systems). All measurements were performed by the same investigator, following the American Society of Echocardiography guidelines\(^{23}\).
For evaluation of macroscopic morphology, the atrial mass, right ventricle mass (RVM), and left ventricle mass (LVM) were expressed both in absolute values and in relation to final body mass (BM) and tibia length. Morphometric analysis of myocardium, involving both myocyte cross-sectional areas (MCSAs) and interstitial collagen fraction (ICF) was performed in LV samples. Tissue fragments were fixed in 10% formalin\(^2\), embedded in paraffin, and 7-μm sections were cut and stained with hematoxylin and eosin (HE) or Picrosirius Red (PR). Sections stained with HE were used for analysis of MCSAs, encompassing 50-100 cardiomyocytes, whereas sections stained with PR were used for quantification of ICF.

### Statistical Analysis

Statistical analysis was performed using SYSTAT\(^\text{®}\), version 12.0 (Systat Software, Inc.). The Kolmogorov-Smirnov test was used to evaluate data distribution. Parametric results were analyzed using Student’s t-test and expressed as mean and standard deviation. Non-parametric findings were analyzed using the Mann-Whitney U-test, and expressed as median and interquartile range. Analysis of covariance (ANCOVA) was used to examine the effect of adiposity as an intervening variable on metabolic and cardiovascular outcomes, considering the measures related to adipose tissue as covariables. To draw statistical conclusions, the level of significance was set at 0.05.

### Results

With respect to nutritional behavior and body composition, although the C group had lower food intake and unchanged energy consumption, higher fat intake, energy efficiency, body mass, and changes in body weight and adiposity were observed in the OB group as compared with controls (Table 1).

In the context of metabolism, levels of lipid, cholesterol, protein and albumin were not significantly different between the groups. However, glucose levels were different between groups, and hyperglycemia was observed in the OB group (Table 2).

In agreement with these findings, the OB group showed altered glucose tolerance as compared with C group (Figure 1), although no changes in area under the curve were observed. In the covariance analysis, after adjusting the values for adiposity, glucose levels were found to be normal in both groups (C, 93 ± 4; OB, 94 ± 4 mg/dL, \(p > 0.05\)).

Considering the in vivo cardiovascular results, no changes in systolic arterial pressure or cardiac function were observed in response to diet. In the structural aspect, only absolute values of left atrium diameter were found to be increased in the OB group (Table 3).

Additionally, although macroscopic findings were similar between the groups, higher values of MCSAs and ICF fraction were observed in the OB group as compared with controls (Table 4).

After adjusting the morphometric values for adiposity indexes, no changes in the MCSAs (C, 95 ± 3; OB, 103 ± 3 μm\(^2\), \(p < 0.05\)) and ICF (C, 2 ± 1; OB, 5 ± 1 %, \(p < 0.05\)) values were observed.

### Discussion

In accordance with the initial hypothesis of this study, high-fat diet associates with manifestation of obesity, metabolic disturbances and cardiac remodeling in rodents. This hypothesis was largely confirmed by the results, since the occurrence of obesity was associated with hyperglycemia, altered glucose tolerance, and morphometric indicators of cardiac remodeling in rats treated with high-fat, high-energy diet. However, the second hypothesis was only partly confirmed, since although glucose changes were found to be directly related to body composition, the cardiac findings resulted from diet-related factors, independently of adiposity.

With respect to the effects of diet on nutritional behavior and adiposity, although no change in energy intake was observed on both groups, the OB group had higher fat intake and energy efficiency and, as a consequence, higher BMI, as compared to the C group. High-fat diets are classically associated with accumulation of body reserves and fat due to the high energy density of lipids\(^2\). In this context, the increase in body mass may have resulted from the development of adiposity, which corroborates with obesity, in accordance with previous findings\(^3-10\).

### Table 1 – Nutritional behavior and murinometric parameters (mean and standard deviation)

| Variables                  | Control (C)          | Obese (OB)          | \(p\) value |
|----------------------------|----------------------|---------------------|-------------|
| Food intake (g/day)        | 28.8 ± 3.0           | 22.6 ± 1.8          | 0.0002*     |
| TEI (kcal/day)             | 64.3 ± 8.6           | 82.2 ± 6.7          | 0.5864      |
| TEI · Fat (kcal)           | 708 ± 73             | 1956 ± 159          | < 0.001*    |
| Energy efficiency (kcal/g) | 0.030 ± 0.003        | 0.036 ± 0.003       | 0.0007*     |
| Final body mass (g)        | 506 ± 59             | 575 ± 60            | 0.0369*     |
| Weight change (%)          | 230 ± 35             | 269 ± 36            | 0.0415*     |
| Adiposity                  | 4.89 ± 1.26          | 8.18 ± 1.47         | 0.0003*     |

TEI: Total energy intake; TEI · Fat: Total energy intake in relation to fat consumption; *\(p < 0.05\) vs. C; Student’s t-test.
Table 2 – Biochemical profile and glucose tolerance (mean and standard deviation)

| Variables     | Group          | p-value |
|---------------|----------------|---------|
|               | Control (C)    | Obese (OB) |
| Triglyceride  | 68.8 ± 11.5    | 78.9 ± 15.5 | 0.1650 |
| Cholesterol (mg/dL) | 67.8 ± 7.5   | 72.6 ± 14.3 | 0.4138 |
| HDL (mg/dL)   | 22.5 ± 2.7     | 22.1 ± 3.0  | 0.7639 |
| LDL (mg/dL)   | 31.4 ± 4.6     | 34.8 ± 9.4  | 0.3774 |
| VLDL (mg/dL)  | 13.9 ± 2.4     | 15.8 ± 3.2  | 0.1991 |
| Protein (mg/dL) | 5.74 ± 0.32  | 5.75 ± 0.18  | 0.9700 |
| Albumin (mg/dL) | 3.23 ± 0.18   | 3.25 ± 0.07  | 0.8147 |
| Glucose (mg/dL) | 87.8 ± 6.4    | 97.0 ± 10.2 | 0.0480* |
| AUC           | 27699 ± 3109   | 29366 ± 6123 | 0.5036 |

HDL: High density lipoprotein; LDL: Low density lipoprotein; VLDL: Very low density lipoprotein; AUC: Area under the curve; AUC: Glucose tolerance *p < 0.05 vs. C; Student’s t-test.

Consequently, in the metabolic aspect, hyperglycemia and altered glucose tolerance in the OB group was mainly caused by obesity. Adipose tissue hypertrophy is associated with the production of inflammatory cytokines, which may lead to hyperglycemia, insulin resistance and, consequently, altered glucose tolerance16,17. Results of glucose measurements are in accordance with previous evidence that high-fat diet-induced obesity associates with altered glucose and insulin metabolism5,10,11. On the other hand, neither lipid levels nor cholesterol levels were affected by the treatment (Table 2). Regarding lipid concentrations, continuously increased levels of insulin may have stimulated triglyceride uptake by adipocytes, contributing to adipogenesis and inhibiting lipolysis, and hence attenuating the increase in triglyceride levels26. In addition, unchanged levels of cholesterol may be associated with quantities of cholesterol equivalent in experimental diets. Changes in cholesterol levels have been reported only by studies in which oxidized cholesterol was added to the diets27,28. These findings differ from those reported in previous approaches in the literature, and further studies aiming to better explain these results are still needed.

Concerning the cardiovascular aspect, the systolic pressure was not influenced by diet-induced obesity. In a recent study8, although an increase in adipose tissue has led to metabolic and hormonal changes, no changes in arterial pressure were observed at 15 and 30 week. We have previously reported that systolic pressure in rats with diet-induced obesity was affected by physical stress6 and progression of the experimental protocol, although in the end of the experiment, arterial pressure levels remained unchanged7. Therefore, the
possibility that changes in arterial pressure may have occurred in the present experimental model cannot be ruled out, and further investigations are needed to clarify this effect.

Similarly, the echocardiographic study and macroscopic analysis did not reveal significant changes in cardiac structure or function. Although different from previous studies, considering that cardiac remodeling encompasses complex processes related to cardiovascular adaptation and defense to chronic overload, the possibility that these responses were not produced during this experimental period (20 weeks) cannot be excluded. This is corroborated by a previous study, in which the pressure overload in response to diet and/or body composition...
induced cardiac injury only at 15 weeks, and cardiac remodeling was observed by 30 weeks after intervention. Also, values of microscopic morphology variables were affected by the high-fat diet in this study, suggesting evidence or cardiac remodeling.

Intriguingly, myocardial hypertrophy and interstitial fibrosis were not modulated by increased adiposity in the OB group, implicating that diet was the main determinant of cardiac remodeling. Saturated fats are the main metabolic fuel for the heart and, paradoxically, accumulation of excess lipid may stimulate mitochondrial overload and activates molecular mechanisms of cardiac remodeling. As a result, lipotoxicity and/or oxidative stress may be related to the onset of cardiac remodeling. Accumulation of products of lipid metabolism generates the production of potential intracellular substrates for non-oxidative, damaging processes, such as diacylglycerol and ceramide, which act in molecular pathways of cardiomyocyte hypertrophy, apoptosis and interstitial fibrosis. In addition, free radicals produced during lipid oxidation cause cellular damage by oxidative damage to proteins and DNA, and interact with pathways that regulate myocardial hypertrophy and interstitial remodeling. Based on the current findings, further studies need to be carried out to investigate different mechanisms associated with the development of cardiovascular disturbances using diet-induced obesity models.

Therefore, it can be concluded that increased body adiposity has a stronger association with metabolic disturbances than dietary fat overload, whereas the high-fat dietary intervention is found to be more related to cardiac changes in experimental models of exogenous obesity. Similarly, by using canonical correlation analysis, we verified that the consumption of saturated fat was the main cause of various disturbances in an experimental model of obesity induced by a high-energy, high-sucrose and high-fatty acid diet. This is the first study to differentiate the effects of obesity condition from the effects of a high-fat diet. It is clear, however, that further studies are needed in order to confirm or not the evidence here described. It is suggested that the use of different diet compositions and different fatty acids is investigated in future studies in order to clarify the effects of different components of the treatment.

Conclusion
High-fat diet was associated with obesity, hyperglycemia, and myocardial hypertrophy and interstitial fibrosis. While the metabolic disturbances were associated with obesity, cardiac remodeling were directly associated with diet.

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Author contributions
Conception and design of the research: Campos DHS, Martínez PF, Cicogna AC, Oliveira-Junior SA; Acquisition of data: Martins F, Campos DHS, Pagan LU, Okoshi K, Souza AS; Analysis and interpretation of the data: Martins F, Martínez PF, Okoshi K, Okoshi MP, Oliveira-Junior SA; Statistical analysis: Padovani CR, Oliveira-Junior SA; Obtaining financing: Oliveira-Junior SA; Writing of the manuscript: Martins F, Martínez PF, Oliveira-Junior SA; Critical revision of the manuscript for intellectual content: Martínez PF, Okoshi MP, Padovani CR, Cicogna AC.

Potential Conflict of Interest
No potential conflict of interest relevant to this article was reported.

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Study Association
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