The hidden dangers: How obesity alters cardiac innervation and structure in Zucker rats

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Abstract. Obesity is classified as a non-communicable chronic disease, and the impetus for investigating the role of the sympathetic nervous system in obesity arises from its potential involvement in the development of cardiovascular, metabolic and renal complications commonly observed in obese individuals. Alterations in cardiac sympathetic nervous activity can cause structural and functional disruptions in the heart. For this reason, the aim of the present study was to investigate the association between heart innervation and its structure in a rat model of obesity. For this purpose, the left ventricular volume, the total volume of cardiac muscle fibers and the total volume of neurons in the stellate ganglion in obese Zucker rats were estimated using three-dimensional quantitative methods based on stereology, a more accurate and unbiased method of quantification. Cholesterol and triglyceride levels were also measured. The mean cholesterol level was 75.6 mg/dl in the control group and 145.16 mg/dl in the obese group. The mean triglyceride level was 30.36 mg/dl in the control group and 504.16 mg/dl in the obese group. The following quantitative results were obtained: A left ventricular volume of 1.25 cm^3 for the control group and 1.60 cm^3 for the obese group, a total volume of cardiac muscle fibers of 1.16 mm^3 for the control group and 2.34 mm^3 for the obese group, and a total volume of neurons in the stellate ganglion of $34.3x10^6$ μm^3 for the control group and $23.01x10^6$ μm^3 for the obese group. On the whole, the present study demonstrates that obese Zucker rats exhibit elevated cholesterol and triglyceride levels. Obesity induces cardiac structural alterations, characterized by an increase in cardiac lumen and ventricular wall thickness. In the nervous system, the total volume of neurons in the stellate ganglia decrease, suggesting a potential reduction in cardiac sympathetic activity. From a translational perspective, the present study has proven that the animal model used herein is a useful tool for investigating the interactions between the sympathetic nervous system and heart morphology in obesity. It may also prove to be useful for preventive and therapeutic measures for cardiovascular diseases in obese individuals.

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Introduction

Rodents are the most widely used animal models for the study of obesity. They play a crucial role in enhancing the understanding of the pathophysiology of obesity and in developing potential preventive measures and treatments. The Zucker fatty rat is a well-established obesity model discovered in the 1960s. This type of rat arose from a spontaneous mutation in the recessive fa/fa genotype, leading to obesity and hypertension in these animals (1,2). The novelty of the present study lies in validating the use of this animal model for evaluating the consequences of obesity on the sympathetic nervous system and its effects on heart morphology.

The rising prevalence of obesity and the concurrent increase in obesity-related illnesses have placed a significant amount of pressure on healthcare systems worldwide. While the combination of reduced amounts of exercise, increased sedentary behavior, poor diet and genetic predisposition is undoubtedly crucial in causing obesity and elevating the risk of disease, extensive research indicates that the sympathetic nervous system also plays a role in the development and progression of obesity-related diseases (3).

Obesity has multifactorial causes that facilitate the development and association of dermatological, respiratory, neurological, musculoskeletal, gastrointestinal, genitourinary, locomotor, endocrine, cardiovascular and other disorders (4-7).

When considering the effects of obesity on the cardiovascular system, it is worth highlighting hypertrophy and the loss of cardiomyocytes due to apoptosis, as well as the accumulation of intracellular lipids, which predispose to eccentric left ventricular impairment, specifically during diastole (8-10). Furthermore, alterations in the sympathetic nervous system can also lead to changes in cardiac structure, such as the hypertrophy of smooth muscle cells, contributing to an increase in heart rate, blood pressure and microvascular tone (7).

Given the high number of obese patients presenting with heart diseases, the objective of the present study was to investigate, in a classic animal model of obesity, the extrinsic sympathetic innervation by estimating the total volume of neurons in the stellate ganglion. Additionally, the present study aimed to analyze cardiac structure by examining the volume of the left ventricle and the total volume of cardiac muscle fibers.

Materials and methods

Experimental groups. A total of 12 Zucker rats, comprising 6 lean and 6 obese rats, all male and 12 weeks old were used in the present study. The present study opted to use only males to reduce variability associated with hormonal differences, which can markedly influence outcomes in studies related to cardiovascular function, sympathetic nervous system activity and metabolic processes. These rats were obtained from the Center for the Development of Experimental Models for Biology and Medicine (CEDEME) at the Federal University of São Paulo (UNIFESP), São Paulo, Brazil. To prevent and alleviate pain, discomfort and distress, the animals were euthanized following an acclimation period of 15 days. The experiment lasted for 17 days. All individuals responsible for inspecting the animals were trained veterinarians, specialized in evaluating the physiology, behavior and overall condition of the animals. All rats were housed in plastic cages (2 animals/cage) in an experimental room maintained under controlled conditions of temperature (22±2˚C) and humidity (50±10%) with 12-h light-dark cycle and *ad libitum* access to standard feed and water at the Animal Facility of the Faculty of Veterinary Medicine and Animal Science of the University of São Paulo.

The animals were inspected daily, and no abnormalities or health issues were reported during the experimental period; thus, no interventions were necessary. The rats were euthanized using sodium pentobarbital (Cristália Produtos Químicos e Farmacêuticos Ltdal; 200 mg/kg, intraperitoneal injection). The animals were healthy at the time of euthanasia. Animal death was confirmed by a combination of the cessation of heartbeat and respiration, the loss of corneal and toe-pinch reflexes, paleness of the mucous membranes, or the presence of rigor mortis.

The present study was conducted following the approval of the Ethics Committee for the Use of Animals (CEUA) of the Faculty of Veterinary Medicine and Animal Science at the University of São Paulo (CEUA/FMVZ, protocol no. 7564110418).

Sample collection. All personnel involved in the experiment possessed the knowledge and skills to handle the animals in order to avoid pain and discomfort. The rats were individually weighed (g) using an electronic scale (Mettler Toledo Indústria e Comércio Ltda). Immediately after weighing, blood samples (1 ml) were collected from all the rats through tail vein puncture (11), using a venous catheter attached to a hypodermic syringe (BD Precision Glide, Becton & Dickinson Indústria Cirúrgica Ltda). These blood samples were used for the measurements of triglyceride (mg/dl) and total cholesterol (mg/dl) levels.

Blood aliquots were stored in microtubes without ethylenediaminetetraacetic acid (EDTA), individually labeled and processed at the Clinical Laboratory of the Veterinary Hospital of the University of Franca using conventional techniques (12). The samples were processed in an automated biochemical analyzer (Chemwell model-Labtest Diagnóstica) using the Liquiform Triglycerides kit and Liquiform Cholesterol kit (Labtest Diagnóstica S.A.) for triglyceride and total cholesterol measurements, respectively, both based on the enzymatic-Trinder methodology (13).

Following blood collection, the rats were euthanized using sodium pentobarbital (Cristália Produtos Químicos e Farmacêuticos Ltda; 200 mg/kg, intraperitoneal injection) (14).

Subsequently, the thoracic cavities were accessed using a conventional technique (15), to cannulate the ascending aorta for perfusion with phosphate-buffered saline (PBS; Laborclin®), followed by 4% paraformaldehyde (PFA) (Neon Reagentes Analíticos) in PBS to wash the vascular system.

Subsequently, the hearts were removed from the thoracic cavity and weighed (g) using a precision semi-analytical scale (Mettler Toledo Indústria e Comércio Ltda).

Stereological analyses

Total volume of the left ventricle. The total volumes of the left ventricles were estimated using the Cavalieri method (16-18). For this purpose, the cardiac chambers were sectioned into ~1-mm-thick fragments perpendicular to the base-apex axis and photographed (Canon® EOS 400D coupled with a digital Sigma lens 1:1,000 macro). A point system was randomly overlaid on each of the photographic images, with points counted that touched both the wall and the lumen of this cardiac compartment (Fig. 1). The total volume of the left ventricle was estimated by summing the wall volume and lumen wall; V(W) + V(L) in cm^3. The following formula was used to calculate the volume of the left ventricle:

Volume of the left ventricle: $V(LV)=[\sum p(w) \times a(pw)] + [\sum p(L) \times a(pl)] \times t$

where $\sum p$ represents the sum of points touching the left ventricular wall (w) and the lumen of left ventricle (L); a(p) represents the area associated with each point of the point grid area per point (W): 0.043 cm^2; and t represents section thickness at 20 μm.

Total volume of cardiac muscle fibers. After estimating the total volume of the left ventricle, the fragments of this cardiac compartment were sectioned into various sizes and organized in ascending order following the smooth fractionator principle (16,17) (Fig. 1). Subsequently, these fragments were fixed in a 4% formaldehyde solution (Neon Reagentes Analíticos) for 72 h at a temperature of 4˚C and then in a cryoprotective solution (30% sucrose, Neon Reagentes Analíticos) for 24 h at a temperature of 4 to 8˚C. They were embedded in Tissue Tek (Tissue-Tek O.C.T. Compound, Sakura) and frozen in liquid nitrogen at -180˚C. The blocks were cut to a thickness of 20 μm using a cryostat (CM1850; Leica Biosystems, Inc.) and

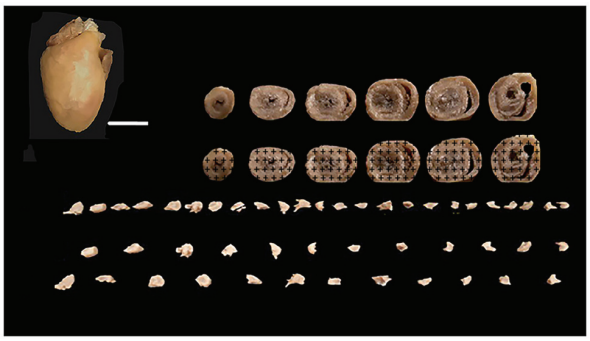

Figure 1. Heart sampling. The cardiac chambers sectioned into 1-mm-thick fragments (first row). A point system was randomly overlaid on each fragment. Points that touched both the wall and the lumen of the cardiac compartments were counted (second row). The fragments of these cardiac compartments were sectioned into various sizes and organized according to the smooth fractionator principle (third, fourth and fifth rows). Scale bar, 1 mm.

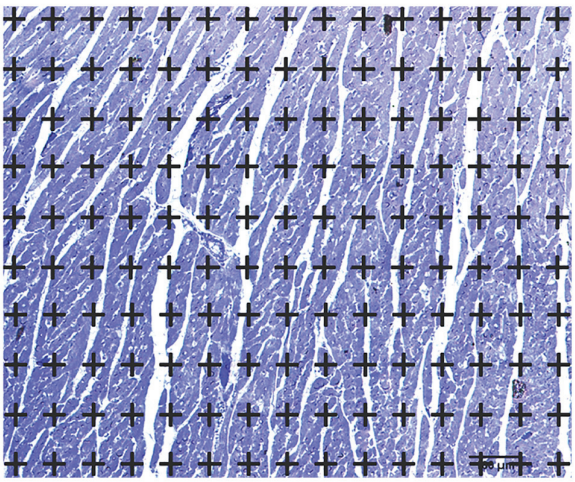

Figure 2. Photomicrograph of cardiac heart fiber. The image shows an example of the frame used to counting the points hitting the heart muscular fibers. Toluidine blue staining was used. Scale bar, 100 μm.

the slides were stained with toluidine blue (MilliporeSigma), at room temperature for 2 min, for observation under a light microscope (Nikon Eclipse 80i; Nikon Corporation) at the Advanced Imaging Diagnostics Center of the School of Veterinary Medicine and Animal Science at the University of São Paulo (CADI-FMVZ USP). The sections were sampled following the SURS pattern and over each histological section, a point-counting system was overlaid (Fig. 2). The points touching the muscle fibers (Vv FM) were counted. The ratio between the number of points hitting the muscle fiber (FM) and the entire myocardium (M) was estimated as follows:

$$Vv\ (FM)=\Sigma p\ (FM)/\Sigma p\ (M)$$

To estimate the total volume of muscle fibers (VFM) in mm^3, the following formulas were applied:

$$V(FM)=Vv\ (FM)\ xV(W)$$

where V(FM) represents the total volume of muscle fibers (in mm^3), Vv (FM) represents the volume fraction of muscle fibers and V(W) represents the total volume of the left ventricle wall estimated previously.

Total volume of neurons in the stellate ganglia. To generate uniform and random vertical sections, the right and left stellate ganglia (Fig. 3) were rotated along their major axis and subsequently processed for cryostat sections (CM1850; Leica Biosystems, Inc.). The sections were sampled following the SURS pattern and a point system was overlaid on each histological section.

To estimate the total volume of neurons in the stellate ganglia, the Vv (neuron) was multiplied by the total volume of the stellate ganglia (Vge), previously calculated using the Cavalieri's Principle. For the calculation of Vge, a random point system was overlaid on the histological sections of the stellate ganglia:

$$V(ge)=\Sigma p\ [a(p)\ x\ t\ x\ k]$$

where Σp represents the summation of points that touched the stellate ganglia; a(p) represents the area associated with each point of the point grid=330 μm^2; t represents the thickness of the section=15 μm; and k represents the distance=5 sections.

For the estimation of the total volume of neurons in the stellate ganglia, the following formula was applied:

$$V(NGe)=Vv\ (neuron)\ xVge.$$

Vv was estimated as follows:

$$Vv\ (Neuron)=\Sigma p\ (N)/\Sigma p\ (G)$$

where Σp (N) represents the summation of points that intersected the neurons and Σp (G) represents the summation of points that intersected the stellate ganglia.

Statistical analysis. The frequency distribution of the estimated parameters was conducted using the Shapiro-Wilk test. Non-parametric data were compared using the Mann-Whitney U test and parametric data were compared using the unpaired Student's t-test. Correlation analyses were performed using Spearman's correlation analysis. These analyses were performed using the statistical software GraphPad Prism 8.4.3 (Dotmatics). The results are expressed as the group median followed by the coefficient of variation in parentheses. A P-value <0.05 was considered to indicate a statistically significant difference.

Results

Body weight. The body weight of the rats was 279.58 g (0.01) and 452.12 g (0.09) for the Zucker lean and fat rats, respectively. When comparing the body weights of the Zucker lean and Zucker fat rats, the difference between groups was significant (P<0.05; Fig. 4A).

Biochemical analysis. The cholesterol level for the Zucker lean rats was 75.6 mg/dl (0.06) and 145.16 mg/dl (0.01) for the Zucker fat rats. The triglyceride level for the Zucker lean rats was 30.36 mg/dl (0.41) and 504.16 mg/dl (0.42) for the fat rats. For both cholesterol

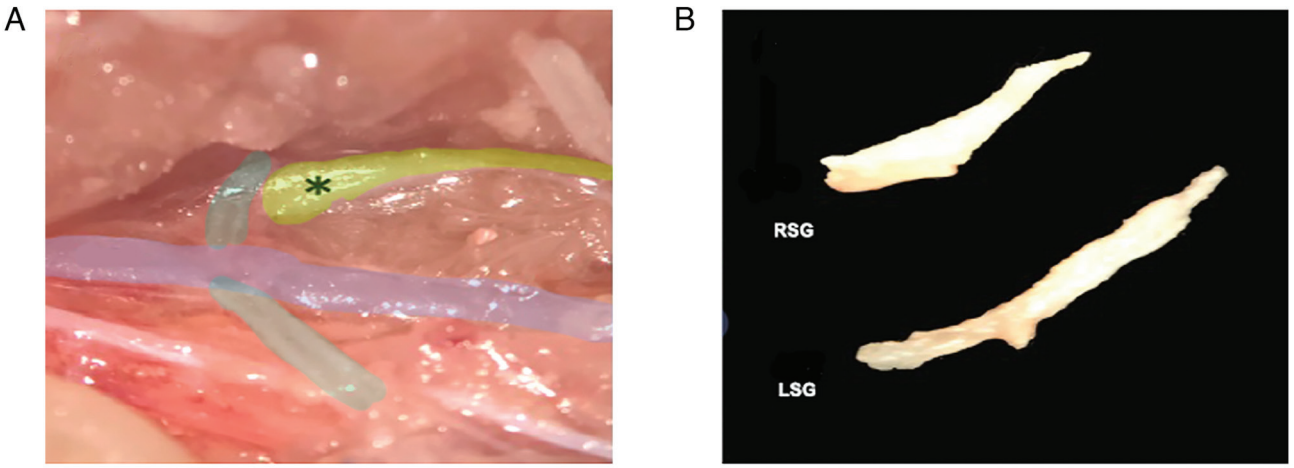

Figure 3. Stellate ganglia in Zucker rats. (A) The stellate ganglia *in situ* is indicated by the '*' symbol, the common carotid artery is indicated in blue and the subclavian artery is indicated in green. (B) Right stellate ganglia and left stellate ganglia are shown. RSG, right stellate ganglia; LSG, left stellate ganglia.

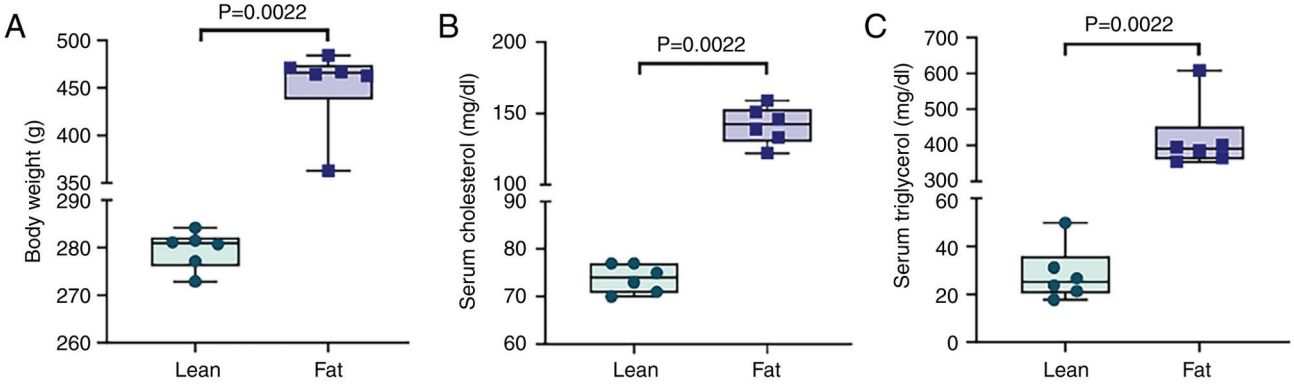

Figure 4. Graphs of (A) body weight, (B) serum cholesterol levels and (C) serum triglyceride levels in Zucker lean and Zucker fat rats.

and triglyceride measurements, the differences between groups were statistically significant (P<0.05; Fig. 4B and C).

Stereological analysis

Total volume of ventricular lumen. The ventricular lumen volume was 0.36 cm^3 (0.32) and 0.75 cm^3 (0.18) for the Zucker lean and fat rats, respectively. The total volume of ventricular lumen differed significantly when comparing the groups (P<0.05; Figs. 5A, 6A and B).

Total volume of ventricular wall. The ventricular wall volume was 0.67 cm^3 (0.3) and 0.88 cm^3 (0.17) or the Zucker lean and Zucker fat rats, respectively. When comparing the groups, the total volume of the ventricular wall did not differ significantly between the groups (P>0.05; Figs. 5B, 6A and B).

Total volume of left ventricle. The left ventricle volume was 1.24 cm^3 (0.27) and 1.60 cm^3 (0.12) for the Zucker lean and Zucker fat rats, respectively. When comparing the groups, the total volume of left ventricle differed significantly (P<0.05; Fig. 5C).

Total volume of cardiac muscular fiber. The cardiac muscular fiber was 1.16 mm^3 (0.09) for the Zucker lean rats and 2.34 mm^3 (0.20) for the Zucker fat rats. The total volume of cardiac muscular fiber differed significantly between the groups (P<0.05; Figs. 5D and 7).

Total volume of neurons in stellate ganglia. The volume of neurons in stellate ganglia was $34.29x10^6$ μm^3 (0.20) and $23.01x10^6$ μm^3 (0.16). The total volume of neurons in the stellate ganglia differed significantly between the Zucker lean and Zucker fat rats (P<0.05; Fig. 5E).

Correlation analysis. A correlation analysis was performed between the following parameters: body weight, left ventricle volume, and neuron volume in the stellate ganglia (Fig. 7).

Discussion

Obesity frequently leads to hyperlipidemia, and both are significant factors for cardiovascular diseases. These conditions affect the structure and function of the heart, as well as its autonomic innervation. The implications of these alterations are extensive, encompassing a range of conditions from ventricular hypertrophy to autonomic dysfunctions, which can predispose individuals to arrhythmias (19-21).

In the present study, a positive correlation was observed between cholesterol and triglycerides levels, and heart structure. The left ventricle and volume wall increased by ~30% in Zucker fat rats, and the ventricular lumen volume almost doubled compared with the lean rats. In their study, Aung *et al* (22), using magnetic resonance analysis, revealed a connection

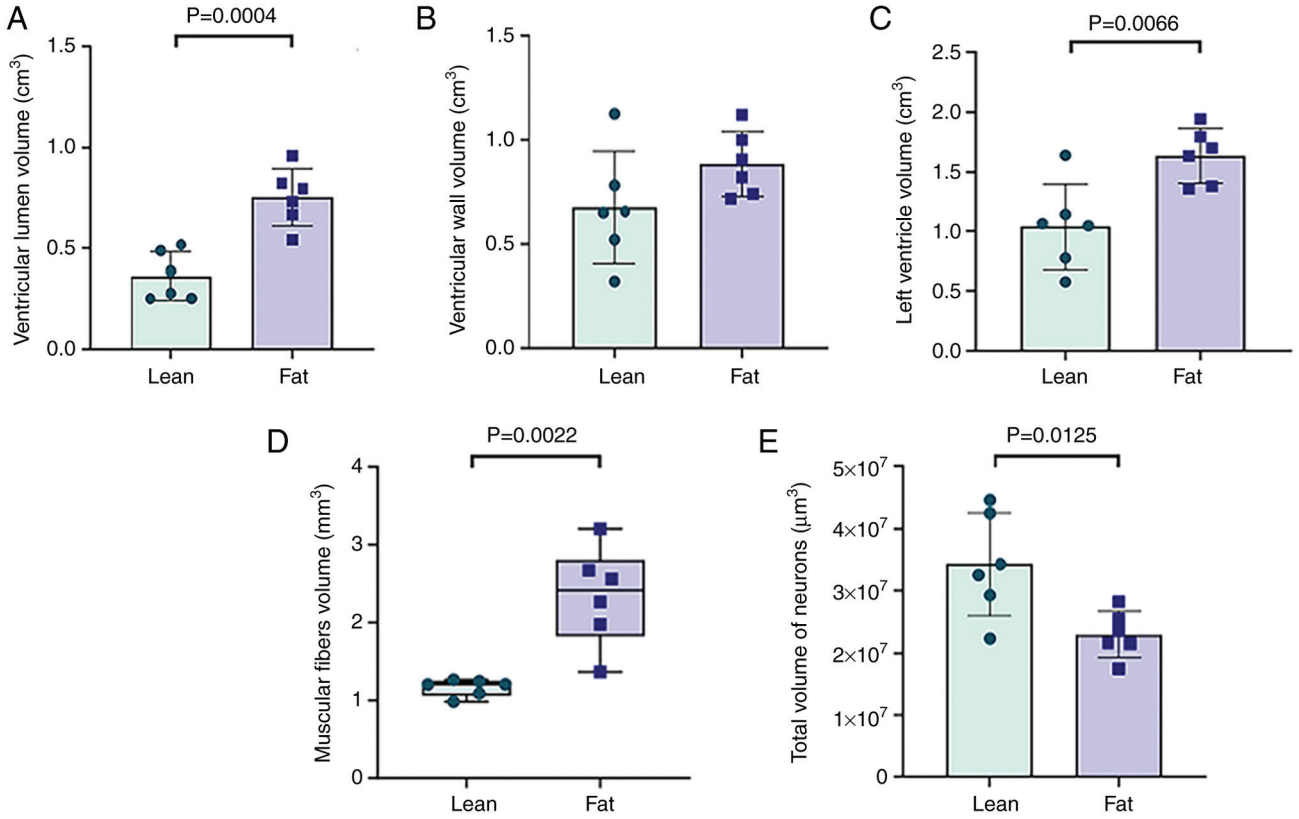

Figure 5. Graphs of (A) ventricular lumen volume, (B) ventricular wall volume, (C) left ventricular volume, (D) muscular fibers volume and (E) total volume of neurons in Zucker lean and Zucker fat rats.

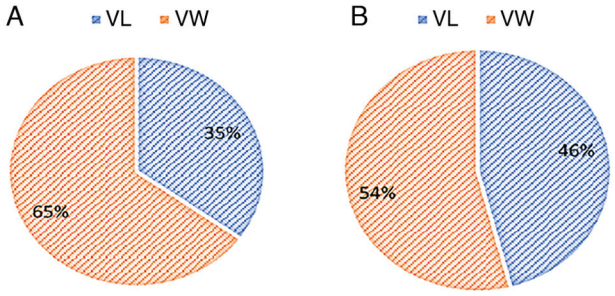

Figure 6. Perceptual value of the ventricular wall and ventricular lumen in (A) Zucker lean rats and (B) Zucker fat rats. VL, ventricular lumen; VW, ventricular wall.

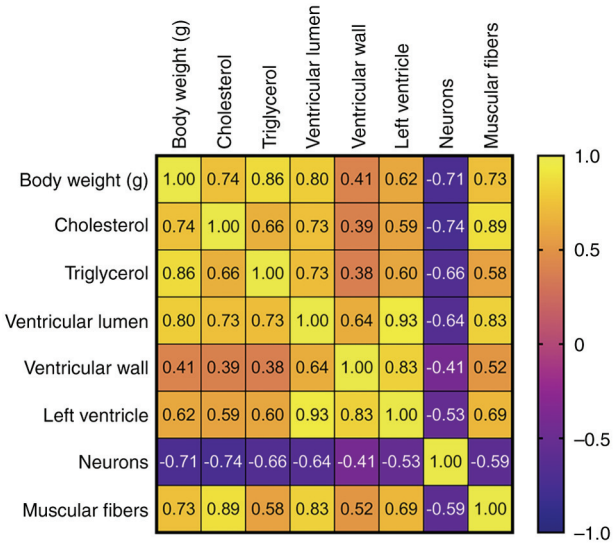

Figure 7. Spearman's correlation analysis between the following parameters: Body weight, left ventricle volume and neurons volume in the stellate ganglia.

between low-density lipoprotein (LDL) cholesterol and triglyceride levels, and unfavorable alterations in cardiac structure and function, particularly in relation to left ventricular mass. These results suggest that LDL cholesterol and triglycerides play a causative role in shaping cardiac morphology, beyond their well-established role in atherosclerosis.

Aurigemma *et al* (23), stated that obesity is one of the primary factors involved in the development of cardiovascular diseases, primarily affecting cardiac structure and function, given that the high metabolic demands of lean and visceral adipose body mass predispose to increased cardiac output and workload.

In the present study, a 2-fold increase in the total volume of cardiac muscle fibers was observed in the obese animals. When considering these parameters, it is evident that there is a structural alteration of the left ventricle, as evidenced by the enlargement of the ventricular lumen and the increase in the total volume of cardiac fibers in the ventricular wall. This structural reorganization of the left ventricle in obese animals is consistent with the findings of echocardiographic research that indicates eccentric ventricular hypertrophy in obese patients (24). The increase in preload raises diastolic stress on the ventricular wall, resulting in an increase in the radius of

the left ventricular cavity and, consequently, an increase in the thickness of the left ventricle (Law of Laplace) (25). Body mass and left ventricular mass are significantly elevated in obese individuals. The left ventricular hypertrophy was accompanied by a significant increase in cardiomyocyte lipid droplets and total myocyte volume. It has also been shown that obesity leads to noteworthy structural changes in cardiomyocytes and capillaries, but no structural modifications have been observed in myocardial innervation (6).

Herein, although there was a negative correlation between the volume of neurons in the stellate ganglion and the parameters related to cardiac morphology, cholesterol, triglycerides and body weight, it is known that excess weight is associated with the dysfunction of the sympathetic nervous system. The results of the present study indicated a 32% reduction in the total volume of neurons in the stellate ganglion in obese rats, suggesting a potential decrease in cardiac sympathetic activity since the cervicothoracic ganglion is the primary component of extrinsic cardiac sympathetic innervation. Such a reduction was also reported by Bray (26), who used the acronym 'MONA LISA (most obesities known are low in sympathetic activity)' to describe the hypothesis that the majority of known cases of obesity are associated with a low sympathetic activity. According to the study by Davy and Orr (27), a low sympathetic nervous system activity has been suggested as a risk factor for weight gain and the development of obesity. Landsberg (28), however, reported the activation of the sympathetic nervous system and its association with hypertension and obesity.

Davy and Orr (27) and Landsberg (28) described that a low activity of the sympathetic nervous system functions as a risk factor for weight gain, obesity and hypertension. Conversely, the activation of this system is a characteristic feature of metabolic and cardiovascular diseases. Various medications may focus on addressing the metabolic disruptions associated with obesity that result in dysfunction of both cardiac and non-cardiac organs. These include targeting the excessive reliance on circulating free fatty acids as an energy source, using drugs, such as partial inhibitors of fatty acid oxidation, inhibitors of carnitine palmitoyltransferase 1, and antioxidants specifically aimed at the mitochondria (29). Promising treatment agents, such as semaglutide, a glucagon-like peptide-1 receptor agonist, have been shown to result in significantly greater reductions in symptoms related to heart failure, physical limitations and weight loss compared to a placebo over a period of 1 year (30,31). The present study strongly supports the integration of comprehensive cardiovascular assessments and sympathetic nervous system monitoring into the standard clinical management of obese patients. This approach highlights a multifaceted strategy that integrates lifestyle modifications, pharmacological interventions, neuromodulation techniques, and surgical options. These strategies aim to address both the structural changes in the heart, such as increased ventricular wall thickness and the alterations in sympathetic nervous system activity that contribute to heightened cardiovascular risk.

In conclusion, the quantitative assessment of cardiac structure and extrinsic sympathetic innervation in obese Zucker rats is of utmost importance for a better understanding of cardiovascular alterations that occur in obesity. The results obtained in the present study suggest that obese Zucker rats exhibit structural changes in the heart that may affect cardiac function, such as an increase in ventricular wall thickness. Additionally, sympathetic innervation is also affected, which may lead to a higher susceptibility to obesity. Therefore, therapeutic interventions aimed at reducing obesity and controlling the sympathetic nervous system can be considered as preventive and therapeutic measures to prevent or treat cardiovascular diseases in obese individuals. The present study provides results which may enhance the understanding the cardiovascular and autonomic nervous system alterations associated with obesity, with findings that are broadly relevant to human health. However, further research in human subjects is warranted in order to fully validate the translational potential of these findings.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

DWE Designed the study, performed the experiments and analyzed the data. FGGD, JCL, SPG, REGR and BCS analyzed the data and wrote the manuscript. THDCS designed the study, analyzed the data and wrote the manuscript. All authors have read and approved the final manuscript. THDCS and SPG confirm the authenticity of all raw data.

Ethics approval and consent to participate

The present study was conducted following the approval of the Ethics Committee for the Use of Animals (CEUA) of the Faculty of Veterinary Medicine and Animal Science at the University of São Paulo, São Paulo, Brazil (CEUA/FMVZ, protocol no. 7564110418). The present study complied with the ARRIVE and AVMA euthanasia guidelines 2020.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Bray GA: The Zucker-fatty rat: A review. Fed Proc 36: 148-153, 1977.
2. Martins T, Castro-Ribeiro C, Lemos S and Ferreira T: Murine models of obesity. Obesities 2: 127-147, 2022.
3. Lambert GW, Schlaich MP, Eikelis N and Lambert EA: Sympathetic activity in obesity: A brief review of methods and supportive data. Ann N Y Acad Sci 1454: 56-67, 2019.
4. Pinheiro ARDO, Freitas SFTD and Corso ACT: An epidemiological approach to obesity. Rev Nutr Campinas 17: 523-533, 2004.
5. Corrêa L, Souza VB and Rahim ST: The relationship between obesity and depression in adults: A review of Brazilian literature in the last 10 years. Universidade do Sul de Santa Catarina, Tubarão, SC, 2018 (In Brazil).
6. Gruber C, Kohlstedt K, Loot AE, Fleming I, Kummer W and Mühlfeld C: Stereological characterization of left ventricular cardiomyocytes, capillaries, and innervation in the nondiabetic, obese mouse. Cardiovasc Pathol 21: 346-354, 2012.
7. O'Brien PD, Hinder LM, Callaghan BC and Feldman EL: Neurological consequences of obesity. Lancet Neurol 16: 465-477, 2017.
8. Peterson LR, Waggoner AD, Schechtman KB, Meyer T, Gropler RJ, Barzilai B and Dávila-Román VG: Alterations in left ventricular structure and function in young healthy obese women: Assessment by echocardiography and tissue Doppler imaging. J Am Coll Cardiol 43: 1399-1404, 2004.
9. Schipke J, Banmann E, Nikam S, Voswinckel R, Kohlstedt K, Loot AE, Fleming I and Mühlfeld C: The number of cardiac myocytes in the hypertrophic and hypotrophic left ventricle of the obese and calorie-restricted mouse heart. J Anat 225: 539-547, 2014.
10. Trivedi PS and Barouch LA: Cardiomyocyte apoptosis in animal models of obesity. Curr Hypertens Rep 10: 454-460, 2008.
11. Lee G and Goosens KA: Sampling blood from the lateral tail vein of the rat. J Vis Exp: e52766, 2015.
12. Thrall MA, Weiser G, Allison RG and Campbell TW (eds): Veterinary hematology and clinical biochemistry. 2nd edition. Wiley-Blackwell, 2012.
13. Matos SL, de Paula H, Pedrosa ML, dos Santos C, de Oliveira EL, Chianca DA Jr and Silva ME: Dietary models for inducing hypercholesterolemia in rats. Braz Arch Biol Technol 48: 203-209, 2005.
14. Flecknell PA, Richardson CA and Popovic A: Laboratory animals. In: Tranquilli WJ, Thurmon JC and Grimm KA (eds). Lumb & Jones´ Veterinary Anesthesia and Analgesia. 4th edition. Iowa: Blackwell Publishing, pp765-784, 2007.
15. Fossum TW: Small animal surgery. 4th edition. Elsevier, Rio de Janeiro, pp1640, 2014.
16. Gundersen HJ, Jensen EB, Kiêu K and Nielsen J: The efficiency of systematic sampling in stereology-reconsidered. J Microsc 193: 199-211, 1999.
17. Gundersen HJG: The smooth fractionator. J Microsc 207: 191-210, 2002.
18. Mandarim-de-Lacerda CA: Stereological tools in biomedical research. An Acad Bras Cienc 75: 469-486, 2003.
19. Powell-Wiley TM, Poirier P, Burke LE, Després JP, Gordon-Larsen P, Lavie CJ, Lear SA, Ndumele CE, Neeland IJ, Sanders P, *et al*: Obesity and cardiovascular disease: A scientific statement from the American heart association. Circulation 143: e984-e1010, 2021.
20. Limpitikul WB and Das S: Obesity-related atrial fibrillation: Cardiac manifestation of a systemic disease. J Cardiovasc Dev Dis 10: 323, 2023.
21. Vekic J, Stefanovic A and Zeljkovic A: Obesity and dyslipidemia: A review of current evidence. Curr Obes Rep 12: 207-222, 2023.
22. Aung N, Sanghvi MM, Piechnik SK, Neubauer S, Munroe PB and Petersen SE: The effect of blood lipids on the left ventricle: A mendelian randomization study. J Am Coll Cardiol 76: 2477-2488, 2020.
23. Aurigemma GP, de Simone G and Fitzgibbons TP: Cardiac remodeling in obesity. Circ Cardiovasc Imaging 6: 142-152, 2013.
24. Rocha IE, Victor EG, Braga MC, Barbosa e Silva O and Becker Mde M: Echocardiography evaluation for asymptomatic patients with severe obesity. Arq Bras Cardiol 88: 52-58, 2007.
25. Ferreira Filho PRP: Hypertrophy patterns and left ventricular geometry by transthoracic echocardiography. Braz J Echocardiogr Cardiovasc Imaging 25: 103-115, 2012 (In Brazil).
26. Bray GA: Obesity, a disorder of nutrient partitioning: The MONA LISA hypothesis. J Nutr 121: 1146-1162, 1991.
27. Davy KP and Orr JS: Sympathetic nervous system behavior in human obesity. Neurosci Biobehav Rev 33: 116-124, 2009.
28. Landsberg L: Diet, obesity and hypertension: An hypothesis involving insulin, the sympathetic nervous system, and adaptive thermogenesis. Q J Med 61: 1081-1090, 1986.
29. Noordali H, Loudon BL, Frenneaux MP and Madhani M: Cardiac metabolism-a promising therapeutic target for heart failure. Pharmacol Ther 182: 95-114, 2018.
30. Kosiborod MN, Petrie MC, Borlaug BA, Butler J, Davies MJ, Hovingh GK, Kitzman DW, Møller DV, Treppendahl MB, Verma S, *et al*: Semaglutide in patients with obesity-related heart failure and type 2 diabetes. N Engl J Med 390: 1394-1407, 2024.
31. Chao AM, Tronieri JS, Amaro A and Wadden TA: Semaglutide for the treatment of obesity. Trends Cardiovasc Med 33: 159-166, 2023.