High-fat diet induces autonomic dysfunction, cardiac remodeling and metabolic changes in ovariectomized ApoE-Ko mice

João Eduardo Izaias
Universidade São Judas Tadeu

Bruno Nascimento-Carvalho
Incor - Instituto do Coração do Hospital das Clínicas da FMUSP

Thália Leticia Brito Nascimento
Universidade São Judas Tadeu

Thayná Fabiana Ribeiro Batista
Universidade São Judas Tadeu

Bruno Durante da Silva
Incor - Instituto do Coração do Hospital das Clínicas da FMUSP

Sérgio Catanozi
Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo

Kátia De Angelis
Federal University of Sao Paulo

Kátia Bilhar Scapini
Universidade São Judas Tadeu

Maria Claudia Costa Irigoyen
Incor - Instituto do Coração do Hospital das Clínicas da FMUSP

Iris Callado Sanches (✉ prof.icsanches@gmail.com)
Universidade São Judas Tadeu

Research Article

Keywords: ApoE, Atherosclerosis, Menopause, Cardiovascular Autonomic Modulation, high-fat diet

DOI: https://doi.org/10.21203/rs.3.rs-261276/v1

License: ☝️ ☣️ This work is licensed under a Creative Commons Attribution 4.0 International License. Read Full License
Abstract

Atherosclerosis is a chronic inflammatory disease characterized by the formation of atheroma plaque in the arterial wall, process that causes long-term obstruction of the arteries. Postmenopausal women tend to have greater body adiposity, factor that corroborate to increased risk of cardiovascular events and development of atherosclerosis. Therefore, the aim of this study was to evaluate the association of experimental model of atherosclerosis with ovarian deprivation and consumption of high-fat diet in metabolic, hemodynamic, and autonomic outcomes. 21 female ApoE-Ko were divided into 3 groups (n = 7 in each): control treated with normolipidic diet (C); ovariectomized treated with normolipidic diet (Ovx); ovariectomized treated with high-fat diet (OvxHF). Hemodynamic parameters, baroreflex sensitivity and cardiovascular autonomic modulation were evaluated. Values (mean ± standard error of mean) were analyzed by One-way ANOVA, followed by Tukey's post hoc (p<0.05). The OvxHF showed increase in final body weight, adipose tissue, tachycardia at rest, in addition, there was a decrease in muscle mass, functional capacity, baroreflex sensitivity associated with less cardiac autonomic modulation. These findings provide evidence about the risk by the loss of ovarian hormones with food consumption and demonstrate the importance of adhering to prevention and treatment strategies.

Introduction

Atherosclerosis stands out as an important comorbidity and risk factor for the development of acute and chronic conditions, which impair quality of life and greater risk of cardiac events, the presence of atherosclerosis increases the risk of possible cardiovascular events by 20% in 10 years after the development of the disease. This dysfunction is a chronic inflammatory and multifactorial disease that is initiated by dysfunctions in the vascular endothelium and a possible association of impairments in the immune system. The damage of the endothelium causes deleterious effects to the blood vessel, in which there is greater pro-coagulant production that corroborates the development of atheroma plaques in the arterial wall, decreasing the vessel lumen and increasing the risk of cardiovascular events.

The Knockout model of apolipoprotein E (ApoE-KO) presents accelerated atherogenesis, so this model becomes useful to evaluate the mechanisms that involve the progression of atherosclerosis, since they occur in a similar way in an experimental model and in humans. In ApoE-Ko mice that used a Western diet, there was a marked increase in atherosclerotic lesion. Histological analyzes showed an increase in the size of the lesion and complexity, increasing the cardiovascular risk of the animals, due to the increase in plaque and atherosclerotic lesion.

Ovarian hormones are extremely important for controlling and regulating arterial pressure, acting through reflex and humoral mechanisms, and if there is a deficiency, it can lead to possible risk factors, such as arterial hypertension, dysautonomia, baroreflex dysfunction, increase in the level of lipid and triglycerides, endothelial dysfunction, as well as metabolic syndrome. All of these processes are harmful to the cardiovascular system, as well as an increase in cardiovascular incidence in postmenopausal women (FLUES et al., 2010; IRIGOYEN et al., 2005; SANCHES et al., 2018). Postmenopause is associated with
genetic and epigenetic factors may increase the risk of developing cardiovascular pathologies and associated comorbidities and corroborate the accentuated effects of atherosclerosis on hemodynamic and autonomic outcomes. In postmenopausal obese women, associated with metabolic syndrome, there is greater insulin resistance, increased leptin, and reduced adiponectin.

The combination of risk factors, such as poor eating habits and physical inactivity, may trigger changes in glycemic and lipid metabolism, increasing the chances of developing cardiovascular disease in menopausal women.

Poor eating patterns can result in cardiac remodeling, a factor that influences changes in heart size, mass and function. In experimental studies, the use of a diet rich in lipids induced changes in the myocardium in animals, causing hypertrophy, in addition to metabolic changes, such as increased cholesterol and blood lipids, as well as decreased glucose mobilization, indicating possible resistance insulin that can generate diabetes.

From the factors listed, there is a need to understand how these combined changes occur in the experimental model of atherosclerosis, as there is little evidence of the combined effects of this dysfunction associated with ovarian deprivation and a poor eating pattern. Therefore, the objective of the study is to evaluate the cardiovascular, metabolic, hemodynamic, and autonomic effects of ovarian deprivation associated with the consumption of a high-fat diet in an experimental model of atherosclerosis.

**Methods**

All procedures with the animals were in accordance with the standards described in the Guidelines for Ethical Conduct in the Care and use of Animals, the Guideline of the Committee on Care and Use of Laboratory Animal Resources of the National Research Council of the United States of America and the authors complied with the ARRIVE guidelines. This project was approved by the Research Ethics Committee for Animals (CEUA-USJT) at São Judas University 038/2019.

21 female mice, 6 months old, ApoE-Ko, that are animals prone to the development of atherosclerosis, were divided into 3 groups: control submitted during 9 months to normolipidic diet (C); ovariectomized submitted during 9 months to normal diet (Ovx); ovariectomized submitted during 9 months to high-fat diet (OvxHF). The animals came from the Lipid Laboratory of the Faculty of Medicine of the University of São Paulo, and were transported to the biotery of São Judas University, where they were kept in plastic boxes (5 animals per cage), in an environment with controlled temperature (22°C – 24°C) and with controlled light, in a 12-hour cycle (light / dark) and fed with water and feed “ad libitum”.

At the beginning of the protocol, the mice were anesthetized (mixture of 0.5% -2% isoflurane and 98% O₂ at a flow rate of 1.5 L / min) and were placed in the supine position, where a small incision (1 cm) was made in parallel with the body line on the skin and muscles in the lower third in the abdominal region.
The ovaries were located and ligation of the oviducts, including blood vessels, was performed. The oviducts were cut, and the ovaries removed. The musculature and skin were sutured, analgesic were administered\textsuperscript{11,15–17}.

The administration of the diets began in the 6th month of life of the mice. “AIN - 93M” diet was administered in C and Ovx groups (REEVES; NIELSEN; FAHEY, 1993), whose caloric content is 3,802.8 Kcal/kg, of which 76% were provided by carbohydrates, 14% by proteins and 10% by lipids (soy oil). For OvxHF group, “AIN-93M” adapted diet, whose caloric content is 5,362.8 Kcal/kg, of which 60% was provided by lipids (lard and soybean oil), 30% by carbohydrates and 10% proteins, as previously explained and detailed (REEVES; NIELSEN; FAHEY, 1993).

Water and feed were offered unrestricted, with feed consumption measured 3 times a week, on alternate days. At the end of the study, the average weekly consumption of each box was calculated and divided by the number of animals in the box to obtain the average individual consumption value. In addition, the caloric consumption of each animal was calculated. For normolipidic feed, 3.8 kcal/gram was calculated, for high-fat diet, 5.6 kcal/gram.

The blood glucose measurement and the oral glucose tolerance test (OGTT) were performed in the 13th month of animal life and at the end of the protocol (15th month of animal life). Blood glucose concentrations were determined on a 12-hour fast using the Roche \textsuperscript{©} Advantage\textsuperscript{®} device and its reagent strips (IRIGOYEN et al., 2005a; SANCHES et al., 2012, 2014; SOUZA et al., 2007a). Then, a glucose solution (1.4g/kg of body weight of the animal) was gavaged and blood glucose measured after 15, 30, 60, 90 and 120 minutes and, subsequently, the area under the curve was calculated\textsuperscript{11}.

The echocardiographic examination was performed at the end of the protocol (15th month) with the anesthetized animals (mixture of 0.5% -2% isoflurane and 98% O2 at a flow rate of 1.5 L / min). SEQUOIA 512 equipment (ACUSON Corporation, Mountain View, CA) was used with a 15 MHz transducer. From the visualization of the left ventricle (cross section) at the level of the papillary muscles, diastolic (DDVE) and systolic (DSVE) diameters of the left ventricle and the thickness of the interventricular septum (IVS) and the left ventricular posterior wall (PP) in systole and diastole. After the measurements were taken, the left ventricular mass was calculated, according to guidance from the American Society of Echocardiography, which estimates the left ventricle mass using the following mathematical formula: \text{LVM} = [(LVDD + SIV + PP) - (DDVE)] x1.047, where 1.047 (mg/mm\textsuperscript{3}) corresponds to myocardial density. In addition to the left ventricle mass, the left ventricular shortening force (D\% = [(DDVE-DSVE) / DDVE] x100) was calculated. The absolute values of left ventricular mass were normalized by body weight. The cardiac hypertrophy index was calculated by dividing the left ventricular mass by body weight. The images obtained through Doppler were used to calculate the parameters of the left ventricular diastolic function. Peak E wave velocities, peak A wave velocities, isovolumetric relaxation time (TRIV) and deceleration time were measured, and the E/A wave ratio was also calculated. Also using the ejection time (TE) of the left ventricular outflow tract, the circumferential shortening speed of the myocardial fiber (Vcf = [(DDVE-DSVE) / DDVE] / TE) was calculated. Although VCF is sensitive to acute changes in arterial pressure in
hemodynamic overload, at baseline conditions, in the absence of acute changes in blood pressure, the calculation of VCF provides information regarding myocardial contractility.

One day after echocardiographic evaluations, the animals were anesthetized (mixture of 0.5% - 2% isoflurane and 98% O₂ at a flow rate of 1.5 L/min) and placed in the supine position. A small incision was made in the neck, through which polyethylene catheters (cannulas; tygon P50) filled with saline were implanted. These cannulas were positioned inside the carotid artery and jugular vein to record arterial pressure (AP), heart rate (HR) and drug administration, respectively. After the correct and firm implantation of the cannulas in the carotid artery and jugular vein, these were externalized on the animal’s back in the cervical region and fixed with cotton thread on the skin. 11,16–21. Each animal was kept in a standard individual box during the systemic hemodynamic evaluations that started 24h after the cannulation.

With the animal awake, the arterial cannula was connected to an extension of 20 cm, allowing free movement of the animal through the box, during the entire period of the experiment. This extension was connected to an electromagnetic pressure transducer (Kent Instruments) which, in turn, was connected to a preamplifier (Stemtech). BP signals were recorded over a period of 30 minutes on a microcomputer equipped with a data acquisition system (WinDaq Recording and Playback Software), allowing analysis of pressure pulses, beat-to-beat, with a sampling frequency of 4KHz by channel to study the BP and HR values (DE ANGELIS et al., 2012; HEEREN et al., 2009).

After recording BP, an extension of approximately 20 cm (P10) was connected to the venous cannula for injection of vasoactive drugs. With animals at rest, baroreflex sensitivity was tested by infusing increasing doses of phenylephrine (100 ng/ml, 150 ng/ml, 250 ng/ml) and sodium nitroprusside (100 ng/ml, 150 ng/ml, 250 ng/ml). The drugs were injected randomly between the animals, beginning the session with one or the other drug. Phenylephrine (Sigma Chemical Company, St. Louis, MO, USA) is potent α₁ stimulator, whose predominant action occurs in peripheral arterioles, causing vasoconstriction, was used to cause increased BP, which is followed by reflex bradycardia commanded by the preceptors. Sodium Nitroprusside (Sigma Chemical Company, St. Louis, MO, USA) is a potent vasodilator, both for arterioles and veins, whose action occurs through the activation of guanylate cyclase and increased synthesis of 3’,5’-guanosine monophosphate (cyclic GMP) in the smooth muscles of vessels and other tissues, was used to cause a fall in BP, followed by a reflex tachycardic response commanded by the pressoreceptors. The \( \alpha \) index was obtained from the division between the PI and SAP variability in the two main low frequency bands (LF) 22.

From the baseline record of the awake animals, it was possible to use the time-frequency analysis tool for pulse interval (IP) and systolic arterial pressure (SAP) variabilities. The parameters for analysis in the time domain consisted of calculating the mean values of SAP and IP, and their variability was quantified by calculating the mean of standard deviations. The variability of the pulse interval was obtained by analyzing the tachogram from the SAP record, where the frequency of beats was determined by the
interval between two systolic peaks. For this analysis, stable records of at least 5 minutes and sampling frequency of 4,000 Hz were used.\textsuperscript{23}

The analysis in the frequency domain consisted of the decomposition of the sistogram by the Fast Fourier Transform. After this mathematical remodeling, the absolute powers of the very-low frequency band of the pulse interval (VLF-IP: 0.00 - 0.4 Hz), low frequency band of the pulse interval (LF-IP: 0.4 Hz -1.50 Hz), and high frequency band of the pulse interval (HF-IP: 1.5 - 5.0Hz) were obtained.\textsuperscript{24–27} The LF component was used as an indicator of sympathetic modulation. The HF component was used as an indicator of parasympathetic modulation. The LF/HF ratio indicated the sympathetic-vagal balance.\textsuperscript{24}

At the end of the study, the following tissues were collected and weighed: Heart, left ventricle, white adipose tissue, soleus and gastrocnemius muscle. Subsequently, the % of the body weight of the tissues was calculated for comparison between the groups.

Data analysis were performed using the Graph Pad Prisma software (version 8.0). The arithmetic mean and the standard error of the mean (SEM) were calculated for all variables. The Shapiro-Wilk test was used to verify the normality of the results. After the experimental period, the values obtained were analyzed by the two-way variance test (Two-way ANOVA), followed by Turkey's post hoc. The level of significance used in all analyzes was 5%.

**Results**

Table 1 shows the animals' body composition parameters. The animals' body weight at the beginning of the protocol (1th month of diet use) was similar due to the distribution of animals performed for balance division of experimental groups. The OvxHF group presented greater weight when compared to C at the 3th month of diet use, at the 6th month of diet use, and at the end of the protocol most weight than the other groups. A reduction in the soleus muscle was observed in Ovx groups when compared to C group. There was a reduction in gastrocnemius muscle in the OvxHF group, when compared to the others. The OvxHF group showed an increase in white adipose tissue when compared to the other studied groups. The OvxHF group had lower heart weight (corrected by % of the body weight) when compared to the Ovx group. The OvxHF group had lower left ventricular weight corrected by % of body weight when compared to the other groups and finally, the relationship of body weight and heart showed that the OvxHF group presented greater weight when compared to C and Ovx.

In relation to the pattern of food consumption, the experimental group oofectomized with a hyperlipidic diet (OvxHF) showed higher consumption in relation to grams (Average daily consumption: C: 6,113 ± 0.5256; Ovx: 7,452 ± 0.5403; OvxHF: 4,010 ± 0.1548, grams, P <0.0001) (Figure 1.a) as also in relation to calories (Average calorie consumption: C: 23.23 ± 1.997; Ovx: 28.31 ± 2.053; OvxHF: 21.49 ± 0.8297, Kcal, P <0.0366) (Figure 1.b). In addition, the same experimental group had a lower running capacity at the end of the protocol compared to the control group (Final Treadmill Test: C: 711.8±47.21; Ovx: 670.2±18.23;
OvxHF: 580.0 ±41.50, **Count in seconds, P<0.0739** (Figure 1.d). There no differences in the initial treadmill test (Figure 1.c) between groups.

The OvxHF showed a higher baseline glycemia (**Blood glucose at the beginning of the protocol**: C: 127.0 ± 5.509; Ovx: 130.5 ± 10.18; OvxHF: 160.0 ± 7.726, mg/dl, P <0.0145) (Figure 2.a) and longer glucose mobilization time (**Area under the curve at the end of protocol**: C: 21880 ± 1183; Ovx: 19083 ± 1409; OvxHF: 26372 ± 521.9, **AUC**, P <0.0038) (Figure 2.d), elucidating that the association of diet and ovariectomy brought losses in the glucose mobilization of the OvxHF group. There are no differences in blood glucose and in the area under the curve at the end of the protocol (Figure 2.b and figure 2.c).

Figure 3 represents heart rate and HRV parameters in the time domain, there was an increase in heart rate (**HR**: C: 546.8 ± 25.36; Ovx: 561.4 ± 47.01; OvxHF: 663.1 ± 16.60, **bpm**, P <0.0373) in the group OvxHF when compared to C (Figure 3.a). A decrease in the standard deviation of the pulse interval between the ovariectomized groups (**SD-PI**: C: 4.553 ± 0.6668; Ovx: 2.417 ± 0.1129; OvxHF: 2.460 ± 0.4253, **ms**, P <0.0257), indicating decrease in general variability (Figure 3.b). There are no differences in the parameters of the root mean square of successive differences between normal heartbeats and variance of pulse interval (Figure 3.c and figure 3.d).

No differences were observed in heart rate variability in the frequency domain. Table 2 shows the blood pressure parameters and baroreflex sensitivity indexes, there were differences only in baroreflex sensitivity were observed through the alpha index, with a reduction in values between groups that were subjected to ovarian deprivation compared to control group.

Figure 4 shows the sympathetic vascular modulation, have an increase in variance (**VAR-SAP**: C: 15.91 ± 2,605; Ovx: 28.03 ± 3,281; OvxHF: 29.15 ± 4,381, **mmHg²**, P <0.0350) and standard deviation of systolic blood pressure (**SD-SAP**: C 15: 3,898 ± 0.3786; Ovx15: 5,270 ± 0.3161; OvxHF: 5,295 ± 0.3653, **mmHg**, P <0.0350) of the OvxHF group compared to the C group. No differences were observed in low-frequency of systolic arterial pressure.

Tables 3 shows echocardiography data. There was a decrease in A’ wave in OvxHF group in relation to C group, which represents the peak speed of atrial contraction. And there is also the difference in the AET parameter, which represents the left ventricular ejection time, and the OvxHF group had a longer time to perform blood ejection when compared to the other groups, indicating impaired function. In the other parameters analyzed, there were no differences between groups.

Table 4 shows the morphometric parameters that were quantified during the echocardiography analysis. The only difference observed was the higher LV mass value (mg) in which the Ovx group had a greater left ventricular mass when compared to the C group.

**Discussion**
The consumption of a diet rich in lipids, in addition to potentiating the deleterious effects, reduces the functional capacity, causes hemodynamic and cardiac function impairments, in addition to impairing the sympathetic vascular modulation.

Regarding the effect of ovarian hormone deprivation in APOE-Ko model, Ovx group showed reduction in soleus muscle, decreased baroreflex control, and lower cardiac autonomic control. When ovarian deprivation is combined with a high-fat diet (vs. Control), an increase in body weight is observed associated with an increase in adiposity, reduced functional capacity, impaired glucose mobilization capacity, increased heart rate, decreased baroreflex control associated with reduced cardiac and vascular autonomic control, and decreased left ventricular ejection time (indicator of functional impairment of the heart).

It has already been shown that the association of ovarian deprivation with the consumption of a high-fat diet promotes negative changes in body composition, with an increase in body weight associated with greater adiposity. However, this study is the first to present these adaptations in the APOE-KO model.

Ovarian deprivation alone (group Ovx) did not induce changes in body composition, this can be explained by the fact that ApoE-Ko mice are deficient in apolipoprotein E. The ApoE has a present fraction in the HDL structure, which has the function to remove excess cholesterol from tissues. This does not occur due to their absence and possibly low levels of HDL, resulting in increasing local tissue inflammation and reducing adiponectin, which have catabolic function in the endocrine system, which in fact shows why there was no difference in body weight.

It was shown that groups with ovarian deprivation have lower weight of soleus muscle corrected by body weight. These findings corroborate the ovarian deprivation induces reduction of bone mass, causing damage and can promote diseases such as osteoporosis and sarcopenia.

The high-fat fed mice showed lower consumption in relation to grams (vs. C and Ovx) and lower caloric consumption when compared to the Ovx group. The changes relating to consumption occurred because the diet rich in lipids promotes greater satiety to the animal by higher energy density, as well as for the changes in leptin concentrations, this acting as signaling in adipose tissue and the central nerve system, reducing animal food intake.

Regarding the maximum treadmill run test performed at the 07th month of diet administration use, was noticed that the groups showed no difference and in the maximum treadmill run test performed at the end of the protocol, the OvxHF group showed lower performance when compared to group C. In fact, greater body weight associated with the accumulation of white adipose tissue impairs physical performance, in addition to this lower performance being related to reduced mobility and development of motor disabilities.

In relation to basal glucose at 07th month of diet administration use is evident that the OvxHF group showed higher blood glucose compared to the other groups, it is given by the fact that the increase in
adipose tissue causes increase the demand for insulin\textsuperscript{31,32}. This ends up becoming more and more resistant, and therefore, there is an increase in glycemia as evidenced, however, there are no differences at the end of the protocol, this can be explained by the genetic alteration of the animals, in which it makes them develop morbidities associated with weight gain and diseases resulting from obesity. Finally, another difference that occurs is the area under the curve at the end of the protocol which shows that the OvxHF group had more time for mobilization of glucose compared to Ovx. Saturated fats consumed by OvxHF group plays a role in inhibition of plasma cleaning of LDL and allows greater entry of cholesterol in these particles, culminating in excessive intake\textsuperscript{31–33}.

Regarding the weight of the heart, left ventricle, it can be seen that the Ovx high-fat-fed mice, had less weight in these parameters. These can certainly be linked to possible cardiac hypertrophy, greater accumulation of fatty acids in cardiomyocytes, resulting in greater interference of lipid oxidation \textsuperscript{34}.

Related to heart rate, there is an increase in the OvxHF group when compared to C. The increase in weight associate to increased adipose tissue contributed to the increase in heart rate, as well how the decrease in general variability. This increase in HR is related to the presence of cardiac hypertrophy\textsuperscript{34–36}. The Body mass gain in OvxHF group requires an increase in cardiovascular needs, caused by the accumulation of adipose tissue, result in increase in cardiac output, which may lead to dilation and left ventricular hypertrophy\textsuperscript{36}.

This neural control is closely linked to HR and baroreflex activity from afferent communication, through a complex interaction of stimulus and inhibition, the sympathetic and parasympathetic pathways are stimulated and modify HR\textsuperscript{37}. The increase in HR is a consequence of the greater action of the sympathetic pathway and the lower parasympathetic activity, that is, vagal inhibition, induced by the use of a diet rich in lipids.

Just as there is the standard deviation of systolic blood pressure and variability of systolic blood pressure that correspond to sympathetic arterial modulation, there was a statistical difference between groups C vs. OvxHF, in which ovarian deprivation associated with the consumption of a diet rich in lipids showed an increase in vascular sympathetic modulation. This fact can influence the increase in blood pressure values as well as brings\textsuperscript{38} who used SHR OVX rats, and demonstrated that the association of hypertension and ovarian deprivation increases blood pressure in ovarietomized rats and there is a marked increase in vascular sympathetic modulation and cardiac sympathetic-vagal balance. Therefore, although there was no pressure difference, due to the study model proposed in this study, we have a difference in vascular variability indicating cardiovascular damage.

In respect of cardiac parasympathetic modulation, the OVX groups showed a statistical difference in SD-PI, which is a value for heart rate variability and presents the general activity of the ANS, this lower value when compared to C, showing less general variability\textsuperscript{39}. The depression of the estrogen levels of the ovarietomized groups may be responsible for the reduction of HRV and decrease of the alpha index, reducing the control of the baroreflex, considering that inside the mechanisms, the estrogen acts in the
regulation of blood pressure and also in the body temperature, in addition to acting on the organism as a whole, from sexual maturation function, to physical development, in which it plays an important role as well as its cardio-protective function\textsuperscript{37,40}.

In this scenario, autonomic changes seem to be the first changes observed, suggesting that changes in the autonomic nervous system precede metabolic and hemodynamic disorders, and the increase in vascular sympathetic modulation is a possible indicator of pressure change in the medium term. \textsuperscript{41}.

The data related to echocardiography, we have the decrease in the parameters of A' and increase in the AET, which elucidate that the OvxHF group takes longer to perform the LV ejection, these parameters are related to the increase in HR of the OvxHF group, indicating a possible cardiac hypertrophy from the calculation of the ratio of final body weight/LV corrected by body weight, therefore, indicating impairment of its function in the left ventricle. There are studies that indicate that obesity causes changes in cardiac function and remodeling. As a result, there are dysfunctions that were presented in the OSD 15 group, which are confirmed in the framework of obese individuals, corroborating the increased risk of heart failure \textsuperscript{36}.

The combined effects of ovarian deprivation and high fat diet resulted in weight gain and adiposity. In fact, ovarian deprivation associated with weight gain is already expected to induce impairments in glycemic metabolism associated with an imbalance in baroreflex control, impairments that overload the sympathetic nervous system\textsuperscript{42}. Atherosclerosis alone induces increased inflammation associated with vascular damage\textsuperscript{43}. The adaptations that must have been enhanced by the increase in adiposity. Exacerbated inflammation causes damage to the inflammatory cholinergic reflex\textsuperscript{44}. Adaptations that potentiate the imbalance in the autonomic nervous system. It is established in the literature that an imbalance in autonomic control can promote cardiac overload\textsuperscript{45}. The increase in weight also tends to promote an increase in overload\textsuperscript{35}. When there is a chronic imbalance in some control mechanism, there is also a tendency to damage the structure, which would justify a possible cardiac hypertrophy of the left ventricle and a reduction in the ejection fraction and a decrease in functional capacity.

**Conclusion**

There is a previous condition of atherosclerosis, with reduced lean mass, less autonomic cardiac control and baroreflex sensitivity. The findings of this study indicate that ovarian deprivation associate to high fat diet use causes potential risks in metabolism, autonomic and hemodynamic to potentiating the deleterious effects, reduces the functional capacity, causes hemodynamic and cardiac function impairments, in addition to impairing the sympathetic vascular modulation. Thus, we highlight the importance of adopting a healthy lifestyle to minimize cardiometabolic risk factors in this population.

**References**
1. Da Silva Dias, D. et al. Impact of combined exercise training on the development of cardiometabolic and neuroimmune complications induced by fructose consumption in hypertensive rats. *PLoS One* **15**, 1–17 (2020).

2. Maria Cristina Batista dos Santos, José Alexander de Mesquita Vieira, Bruno N. César & Maria Rita Carvalho Garbi Novaes. Hábitos e perfil socioeconômico do paciente aterosclerótico no Brasil. *Rev. Bras. Ciências da Saúde* **22**, 247–256 (2011).

3. Xavier H. T., I. et al. V Diretriz Brasileira De Dislipidemias E Prevenção. *Arq. Bras. Cardiol.* **101**, (2013).

4. Vieira Da Motta, N. A., Fumian, M. M., Pereira De Castro, J. & Ferreira De Brito, F. C. Inflamação e Aterosclerose: Novos Biomarcadores e Perspectivas Terapêuticas Inflammation and Atherosclerosis: New Biomarkers and Outlooks for Treatment. *Rev. Bras. Cardiol.* **26**, 390–399 (2013).

5. Lo Sasso, G. et al. The Apoe −/− mouse model: a suitable model to study cardiovascular and respiratory diseases in the context of cigarette smoke exposure and harm reduction. *J Transl Med* **14**, 146 (2016).

6. Meir, K. S. & Leitersdorf, E. Atherosclerosis in the apolipoprotein E-deficient mouse: A decade of progress. *Arterioscler. Thromb. Vasc. Biol.* **24**, 1006–1014 (2004).

7. Flues, K. et al. Exercise training associated with estrogen therapy induced cardiovascular benefits after ovarian hormones deprivation. *65*, 267–271 (2010).

8. Sanches, I. C. et al. Combined aerobic and resistance exercise training attenuates cardiac dysfunctions in a model of diabetes and menopause. *PLoS One* **13**, 1–15 (2018).

9. Irigoyen, M.-C. C. et al. Exercise Training Improves Baroreflex Sensitivity Associated With Oxidative Stress Reduction in Ovariectomized Rats. *Hypertension* **46**, 998–1003 (2005).

10. Lobo, R. A. Metabolic syndrome after menopause and the role of hormones. *Maturitas* **60**, 10–18 (2008).

11. Sanches, I. C. et al. Cardiometabolic benefits of exercise training in an experimental model of metabolic syndrome and menopause. *Menopause* **19**, 562–568 (2012).

12. Rodrigues, P. & Chagas, E. A relação entre composição corporal e perfil bioquímico em mulheres sedentárias pós-menopausa. *Rev. Bras. Prescrição e Fisiol. do Exerc.* **9**, 64–71 (2015).

13. Martins, F. et al. Dieta hiperlipídica promove remodelação cardíaca em modelo experimental de obesidade. *Arq. Bras. Cardiol.* **105**, 479–486 (2015).

14. Getz, G. S. & Reardon, C. A. ApoE knockout and knockin mice: the history of their contribution to the understanding of atherogenesis. *J. Lipid Res.* **57**, 758–766 (2016).

15. Latour, M. G., Shinoda, M., Lavoie, J. & Martin, G. Metabolic effects of physical training in ovariectomized and hyperestrogenic rats. *J. Appl. Physiol.* **7**, 235–241 (2001).

16. Sanches, I. C., Conti, F. F., Sartori, M., Irigoyen, M. C. & De Angelis, K. Standardization of resistance exercise training: Effects in diabetic ovariectomized rats. *Int. J. Sports Med.* **35**, 323–329 (2014).

17. Irigoyen, M.-C. et al. Exercise Training Improves Baroreflex Sensitivity Associated With Oxidative Stress Reduction in Ovariectomized Rats From the Hypertension Unit (*Hypertension* **46**, 998–1003).
18. Sanches, I. C., Sartori, M., Jorge, L., Irigoyen, M. C. & De Angelis, K. *Tonic and reflex cardiovascular autonomic control in trained-female rats*. Brazilian Journal of Medical and Biological Research vol. 42 www.bjournal.com.br (2009).

19. De Angelis, K. *et al.* Exercise training changes autonomic cardiovascular balance in mice. 2174–2178 (2012) doi:10.1152/japplphysiol.00870.2003.

20. Heeren, M. *et al.* Exercise improves cardiovascular control in a model of dislipidemia and menopause. *Maturitas* 62, 200–4 (2009).

21. Souza, S. B. C. *et al.* Role of exercise training in cardiovascular autonomic dysfunction and mortality in diabetic ovariectomized rats. *Hypertension* 50, 786–791 (2007).

22. Da Palma, R. K. *et al.* Resistance or aerobic training decreases blood pressure and improves cardiovascular autonomic control and oxidative stress in hypertensive menopausal rats. *J. Appl. Physiol.* 121, 1032–1038 (2016).

23. De Angelis, K. *et al.* Exercise training changes autonomic cardiovascular balance in mice. *J Appl Physiol* 96, 2174–2178 (2004).

24. Thireau, J., Zhang, B. L., Poisson, D. & Babuty, D. Heart rate variability in mice: A theoretical and practical guide. *Exp. Physiol.* 93, 83–94 (2007).

25. Gehrmann, J. *et al.* Phenotypic screening for heart rate variability in the mouse. *Am. J. Physiol. - Hear. Circ. Physiol.* 279, 733–740 (2000).

26. Pelat, M. *et al.* Rosuvastatin decreases caveolin-1 and improves nitric oxide-dependent heart rate and blood pressure variability in apolipoprotein E/- mice in vivo. *Circulation* 107, 2480–2486 (2003).

27. Sheng Zhong Duan, Christie, M., Milstone, D. S. & Mortensen, R. M. Go but not Gi2 or Gi3 is required for muscarinic regulation of heart rate and heart rate variability in mice. *Biochem Biophys Res Commun* 357, 139–143 (2007).

28. Canella, D. S. *et al.* Consumption of vegetables and their relation with ultra-processed foods in Brazil. *Rev. Saude Publica* 52, 1–11 (2018).

29. Eguchi, R. *et al.* Efeitos do exercício crônico sobre a concentração circulante da leptina e grelina em ratos com obesidade induzida por dieta. *Rev. Bras. Med. do Esporte* 14, 182–187 (2008).

30. Murphy, R. A. *et al.* Weight change, body composition, and risk of mobility disability and mortality in older adults: A population-based cohort study. *J. Am. Geriatr. Soc.* 62, 1476–1483 (2014).

31. De Francischi, R. P. P. *et al.* Obesity: Updated information about its etiology, morbidity and treatment. *Rev. Nutr.* 13, 17–28 (2000).

32. Ramos, H. & Méndez, J. Diabetes Mellitus Experimental. *Cienc. Vet.* 348–371 (1994).

33. Santos, C. R. B., Portella, E. S., Avila, S. S. & Soares, E. D. A. Fatores dietéticos na prevenção e tratamento de comorbidades associadas à síndrome metabólica. *Rev. Nutr.* 19, 389–401 (2006).

34. Lacerda, D. dos S., Bock, P. M. & Funchal, C. Consumo exacerbado de lipídeos provoca dano celular em algumas doenças metabólicas e cardiovasculares. *Nutrire* 40, 200–213 (2015).
35. Filho, F. S. F. R. et al. Obesidade, Hipertensão Arterial e Suas Influências Sobre a Massa e Função do Ventriculo Esquerdo. Arq Bras Endocrinol Metab 44, 64–71 (2000).

36. Valezi, A. C. & Machado, V. H. S. Emagrecimento e desempenho cardíaco. ABCD. Arq. Bras. Cir. Dig. (São Paulo) 24, 131–135 (2011).

37. Vanderlei, L. C. M., Pastre, C. M., Hoshi, R. A., Carvalho, T. D. de & Godoy, M. F. de. Noções básicas de variabilidade da frequência cardíaca e sua aplicabilidade clínica. Rev. Bras. Cir. Cardiovasc. 24, 205–217 (2009).

38. da Palma, R. K. et al. Resistance or aerobic training decreases blood pressure and improves cardiovascular autonomic control and oxidative stress in hypertensive menopausal rats. J. Appl. Physiol. 121, 1032–1038 (2016).

39. Taralov, Z. Z., Terziyski, K. V. & Kostianev, S. S. Heart Rate Variability as a Method for Assessment of the Autonomic Nervous System and the Adaptations to Different Physiological and Pathological Conditions. Folia Med. (Plovdiv). 57, 173–180 (2015).

40. Charkoudian, N., Hart, E. C. J., Barnes, J. N. & Joyner, M. J. Autonomic control of body temperature and blood pressure: influences of female sex hormones. Clin. Auton. Res. 27, 149–155 (2017).

41. Bernardes, N. et al. Barorex Impairment Precedes Cardiometabolic Dysfunction in an Experimental Model of Metabolic Syndrome: Role of Inflammation and Oxidative Stress. Sci. Rep. 8, 1–10 (2018).

42. Peppa, M., Koliaki, C. & Dimitriadis, G. Body Composition as an Important Determinant of Metabolic Syndrome in Postmenopausal Women. Endocrinol. Metab. Syndr. 02, 1–4 (2012).

43. Ulleryd, M. A. et al. The association between autonomic dysfunction, inflammation and atherosclerosis in men under investigation for carotid plaques. PLoS One 12, 1–12 (2017).

44. Pavlov, V. A., Wang, H., Czura, C. J., Friedman, S. G. & Tracey, K. J. The Cholinergic Anti-inflammatory Pathway: A Missing Link in Neuroimmunomodulation. Mol. Med. 9, 125–134 (2003).

45. Bernardes, N. et al. Excessive consumption of fructose causes cardiometabolic dysfunctions through oxidative stress and inflammation. Can. J. Physiol. Pharmacol. 95, 1078–1090 (2017).

Tables

| Table 1. Weight parameters |
|---------------------------|


| Parameters (grams)                                      | C                  | Ovx                | OvxHF              | p      |
|--------------------------------------------------------|--------------------|--------------------|--------------------|--------|
| Initial body weight                                    | 21.92±0.446        | 21.48±0.0624       | 21.5±0.460         | 0.7821 |
| Body weight at 3 months of diet use                    | 23.93±0.614        | 25.69±0.855        | 27.31±0.717        | 0.0100 |
| Body weight at 6 months of diet use                    | 25.55±0.631        | 26.54±0.589        | 32.08±0.945        | <0.0001|
| Body weight at the end of protocol (9th month)         | 27.24±0.863        | 26.07±0.821        | 36.86±1.560        | <0.0001|
| Soleus (% of body weight)                              | 0.37±0.001         | 0.0073±0.0000209   | 0.0251±0.00004903  | 0.0002 |
| Gastrocnemius (% of body weight)                       | 0.52±0.000431      | 0.51±0.0002882     | 0.32±0.0003301     | 0.0023 |
| White adipose tissue (% of body weight)                | 2.44±0.005597      | 2.65±0.00592       | 7.61±0.01603       | 0.0062 |
| Heart weight correct for % body weight                 | 0.61±0.0002858     | 0.79±0.001431      | 0.49±0.0004696     | 0.0305 |
| Left ventricle correct for % body weight               | 0.52±0.0006676     | 0.42±0.0007066     | 0.19±0.0002195     | 0.0008 |
| Body weight and heart ratio                             | 4587±336.1         | 3739±683.3         | 7709±641           | 0.0002 |

C = sedentary control group treated with a 15-month normolipid diet; Ovx = Sedentary ovariectomized group treated with a 15-month normolipid diet; OvxHF = Sedentary ovariectomized group treated with a 15-month high lipid diet.; Different letter indicates statistically different groups (Two-way ANOVA + Tukey test, p<0.05). a p<0.05 vs. C; b p<0.05 vs. OvxHF. Data are reported as mean ± SEM. N=07 Animal/group.

**Table 2. Hemodynamic evaluations, autonomics and barorereflex sensitivity**
| Parameters                                | C         | Ovx      | OvxHF    | P        |
|------------------------------------------|-----------|----------|----------|----------|
| Systolic arterial pressure (mmhg)        | 138.8±3.341 | 150.7±4.532 | 148.3±4.174 | 0.1433  |
| Diastolic arterial pressure (mmhg)       | 94.89±2.840 | 106.4±4.882 | 105.4±5.145 | 0.1795  |
| Mean arterial pressure (mmhg)            | 117±2.923  | 128.3±4.413 | 126.6±4.454 | 0.1531  |
| Low-frequency of pulse interval (ms)     | 2.609±0.742 | 0.5467±0.149 | 0.4433±0.228 | 0.0914  |
| High-frequency of pulse interval (ms)    | 4.003±0.863 | 1.61±0.423  | 2.633±0.916  | 0.2249  |
| Power total                              | 12.68±3.625 | 3.32±1.095  | 3.603±1.350  | 0.1398  |
| Lf/hf                                    | 0.348±0.078 | 0.45±0.080  | 0.365±0.062  | 0.6508  |
| Bradycardic response (bpm/mmhg)         | 0.7586±0.202 | 1.268±0.420 | 1.415±0.267 | 0.2040  |
| Tachycardic response (bpm/mmhg)         | 0.9343±0.264 | 0.975±0.534 | 1.357±0.323 | 0.6286  |
| Alpha index                              | 1.542±0.227 | 0.76±0.222a | 0.654±0.053a | 0.0082  |

C = sedentary control group treated with a 15-month normolipid diet; Ovx = Sedentary ovariectomized group treated with a 15-month normolipid diet; OvxHF = Sedentary ovariectomized group treated with a 15-month high lipid diet.; Different letter indicates statistically different groups (two-way ANOVA + Tukey test, p<0.05). a p<0.05 vs. C. Data are reported as mean ± SEM. N=07 Animal/group.

Table 3. Flow Doppler echocardiography
| Parameters                  | C                    | Ovx                  | OvxHF                | P       |
|-----------------------------|----------------------|----------------------|----------------------|---------|
| A' (velocity mm/s)          | 28.98±2.664          | 26.38±4.202          | 19.16±1.875          | 0.0329  |
| E' (velocity mm/s)          | 23.41±2.483          | 19.81±2.162          | 18.67±1.859          | 0.2761  |
| Ivct (time ms)              | 25.84±3.718          | 21.72±4.354          | 24.27±3.501          | 0.7871  |
| Ivrt (time ms)              | 17.88±1.172          | 15.09±1.103          | 16.88±1.542          | 0.4431  |
| A (velocity mm/s)           | 657.2±79.23          | 510.3±82.6           | 593.8±69.83          | 0.4877  |
| Mv decel (time ms)          | 16.23±1.725          | 12.21±1.633          | 18.15±2.304          | 0.1992  |
| E (velocity mm/s)           | 855.9±45.2           | 832.4±116.6          | 745.4±52.15          | 0.4006  |
| Aet (time ms)               | 47.91±1.158          | 47.42±1.302          | 55.03±1.669          | 0.0018  |
| S' (velocity mm/s)          | 19.45±0.9734         | 19.01±1.387          | 18.81±1.326          | 0.9242  |
| Wave ratio a'/e'            | 1.338±0.1869         | 1.422±0.2665         | 1.074±0.1204         | 0.3708  |
| Wave ratio e'/a'            | 0.874±0.136          | 0.8977±0.2734        | 1.024±0.1079         | 0.743   |
| Wave ratio e/a              | 1.517±0.2696         | 1.772±0.3507         | 1.418±0.2426         | 0.7148  |
| Wave ratio e/e'             | 38.32±4.432          | 42.07±4.15           | 42.73±5.033          | 0.7721  |
| Body weight and heart ratio | 4587±336.1           | 3739±683.3           | 7709±641            | 0.0002  |

C = sedentary control group treated with a 15-month normolipid diet; Ovx = Sedentary ovariectomized group treated with a 15-month normolipid diet; OvxHF = Sedentary ovariectomized group treated with a 15-month high lipid diet.; Different letter indicates statistically different groups (Two-way ANOVA + Tukey test, p<0.05). a p<0.05 vs. C; b p<0.05 vs. Ovx. Data are reported as mean ± SEM. N=07 animal/group.

**Table 4. Morphometric Parameters**
| Parameters          | C     | Ovx   | OvxHF  | P      |
|---------------------|-------|-------|--------|--------|
| Ivs;d (mm)          | 0.534±0.03064 | 0.5402±0.0397 | 0.5799±0.03289 | 0.5599 |
| Ivs;s (mm)          | 0.888±0.02562 | 0.9044±0.04258 | 0.9959±0.04121 | 0.0859 |
| Diameter;d (mm)     | 4.081±0.2202 | 4.73±0.0934 | 4.497±0.1867 | 0.1077 |
| Diameter;s (mm)     | 3.042±0.2175 | 3.676±0.09041 | 3.344±0.1812 | 0.1336 |
| Lvpw;d (mm)         | 0.6603±0.04195 | 0.7582±0.06187 | 0.7159±0.03175 | 0.3219 |
| Lvpw;s (mm)         | 0.82±0.03765 | 0.9564±0.06091 | 0.9386±0.04989 | 0.1132 |
| Lvef (%)            | 51.21±2.69 | 44.77±2.151 | 50.64±2.477 | 0.2646 |
| Lvsf (%)            | 25.93±1.568 | 22.28±1.24 | 25.83±1.536 | 0.2839 |
| LV mass (mg)        | 83.85±7.935 | 120.9±13.11 a | 109.5±8.216 | 0.0324 |
| LV mass cor (mg)    | 75.98±9.472 | 104.1±4.743 | 94.32±9.758 | 0.1455 |
| LV vol;s(ul)        | 38.64±6.734 | 57.45±3.319 | 40.29±2.026 | 0.0615 |
| Ao root (mm)        | 1.914±0.04682 | 1.956±0.05913 | 2.101±0.05931 | 0.0506 |
| La (mm)             | 3.091±0.1011 | 3.222±0.2183 | 3.097±0.1311 | 0.8045 |
| Ao/la               | 0.6234±0.02254 | 0.6204±0.05255 | 0.6871±0.0326 | 0.2778 |

C = sedentary control group treated with a 15-month normolipid diet; Ovx = Sedentary ovariectomized group treated with a 15-month normolipid diet; OvxHF = Sedentary ovariectomized group treated with a 15-month high lipid diet.; Different letter indicates statistically different groups (Two-way ANOVA + Tukey test, p<0.05). a p<0.05 vs. C. Data are reported as mean ± SEM. N=07 Animal/group.

### Figures

**A** Average daily consumption

**B** Average caloric consumption

**C** Initial treadmill test

**D** Final treadmill test

**Figure 1**
Average daily consumption (1.a); Average calorie consumption (1.b); Initial treadmill test (1.c); Final Treadmill Test (1.d) of the sedentary control group treated with a 15-month normolipid diet (C), sedentary ovariectomized group treated with a 15-month normolipid diet (Ovx), and sedentary ovariectomized group treated with a 15-month high lipid diet (OvxHF). a \( p \leq 0.05 \) vs. C; b \( p \leq 0.05 \) vs. Ovx.

Figure 2

Blood glucose at the beginning of the protocol (2.a); Blood glucose at the end of the protocol (2.b); Area under the curve at the beginning of the protocol (2.c); Area under the curve at the end of the protocol (2.d) of the sedentary control group treated with a 15-month normolipid diet (C), sedentary ovariectomized group treated with a 15-month normolipid diet (Ovx), and sedentary ovariectomized group treated with a 15-month high lipid diet (OvxHF). a \( p \leq 0.05 \) vs. C; b \( p \leq 0.05 \) vs. Ovx.
Figure 3

Heart rate (3.a) Standard deviation of the pulse interval (3.b); Square root of the mean of the difference in the consecutive heartbeat interval (3.c); Variance of the pulse interval (3.d) of the sedentary control group treated with a 15-month normolipid diet (C), sedentary ovariectomized group treated with a 15-month normolipid diet (Ovx), and sedentary ovariectomized group treated with a 15-month high lipid diet (OvxHF). a \( p \leq 0.05 \) vs. C;

Figure 4
Standard deviation of systolic arterial pressure (4.a) Variance of the systolic arterial pressure (4.b) Low-frequency of the systolic arterial pressure (4.c) of the sedentary control group treated with a 15-month normolipid diet (C), sedentary ovariectomized group treated with a 15-month normolipid diet (Ovx), and sedentary ovariectomized group treated with a 15-month high lipid diet (OvxHF). a p≤ 0,05 vs. C;