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Cardioprotective action of chia (*Salvia hispanica* L.) in ovariectomized rats fed a high fat diet

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The reduction in estrogen levels is associated with the increased risk factors for cardiovascular disease development. The present study aimed to evaluate the effect of chia consumption in a standard diet (SD) or high fat diet (HFD) on ovariectomized (OVX) and non-ovariectomized (SHAM) rats, in relation to biometric measurements, oxidative stress, mineral content and ATPase enzymes in the heart. The study was conducted with 80 female Wistar rats, which received a SD or HFD for 18 weeks. During the first 7 weeks, the animals received the SD or HFD. Then, 40 rats were ovariectomized and 40 rats were SHAM operated. After recovery from surgery, the animals were allocated to 8 groups (n = 10) and they received one of the following diets for 8 weeks: SD, SD + chia, HFD and HFD + chia. In the OVX group, HFD increased weight gain, adiposity, cardiac hypertrophy, and nitric oxide (NO) and K concentration and decreased the Na^+/K^+ATPase activity. In combination with HFD, ovariectomy decreased the catalase activity, Mg, Cu and Zn concentration, total ATPase activity, and Na^+/K^+ATPase and Mg2 + ATPase activities; this group also presented higher NO, Ca, K, Fe and Mn concentration in the heart. The SHAM group fed chia presented a lower fat content in the heart. In the OVX group fed HFD, chia increased the activity of superoxide dismutase, decreased NO and maintained the content of minerals and ATPase enzymes. Thus, chia improved the biometric parameters of the heart, the antioxidant activity and maintained the content of minerals and ATPase enzymes, showing a cardioprotective action, but without reversing the deleterious effects of ovariectomy.

1. Introduction

The post-menopause period is associated with dyslipidemia, inflammation,[1] increased central fat measures[2] and reduced antioxidant defenses.[3] These are risk factors for the development of cardiovascular diseases (CVD),[1] which are the leading cause of death in the world.[4] Postmenopausal women are at higher risk of CVD than those who have not gone through menopause at the same age. These differences can be attributed to female hormones and their receptors.[5,6] Estrogen provides protection to the vascular endothelium,[7] and postmenopausal and obese women may be more susceptible to impaired endothelial function.[8] Estrogen can play an important role in inhibiting the development of cardiac hypertrophy by regulating the calcium-related pathways, nitric oxide synthase (eNOS) activity and oxidative stress.[9,10] Thus, the negative effects related to reduced estrogen level may be associated with the effects of consuming a high fat diet (HFD), such as increased oxidative stress[11] and inflammation.[12]

In this sense, a diet rich in antioxidant nutrients and bioactive compounds can protect the body from metabolic changes. Chia seed (*Salvia hispanica* L.) has high nutritional value, with high concentrations of lipids (30.17 g per 100 g), proteins (19.72 g per 100 g), total dietary fiber (37.18 g per 100 g) and minerals, in addition to bioactive compounds, which can be beneficial to human health.[13,14] Chia is a food with great potential to protect the body from CVD, since, in addition to dietary fiber, lipids and minerals, it is a rich source of proteins. *In vitro* studies have shown that digested chia seed proteins, which provide bioactive peptides during the hydrolysis process, such as albumin, globulin and glutelin, exert beneficial effects by reducing the levels of markers related to the induction of inflammation and atherosclerosis

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processes in macrophages,[15] besides producing antioxidative effects.[16]

Regarding the mineral content, we can highlight that chia seed is an excellent source of calcium (430 mg/100 g), in addition to significant concentrations of potassium, magnesium, iron and zinc.[13] The primary function of the heart is to provide a coordinated muscle contraction system to pump blood into the lungs and the body, allowing tissues to receive oxygen and nutrients. The process that unites the electrical activity and contraction of the heart muscle is regulated by many calcium-dependent systems. In addition to the rapid processes that control contraction, calcium in the heart is also involved in the activation of genes which alter the expression of proteins and in adaptive processes, such as hypertrophy.[17] Thus, since chia is a rich source of calcium, it is justified to evaluate its effect on the mineral content and activity of ATPases in the heart, through its consumption with a standard diet (SD) or HFD, in ovariectomized (OVX) rats.

The consumption of chia seed has presented a series of health benefits, including cardiovascular and liver protection.[18] The benefits of chia seed associated with CVD markers have been attributed to the high concentration of α-linolenic acid (omega 3).[19,20] The evaluation of the chia seed peptide profile in terms of its functions also demonstrated that the peptide sequences may have a beneficial biological potential due to their antioxidant and hypotensive properties.[14] Thus, chia seed is considered a potential bioactive food, as its consumption may prevent and mitigate metabolic changes, and can be used in the prevention and treatment of comorbidities associated with unbalanced diets.[21]

A previous study by our group showed that chia consumption, with the SD improved the antioxidant activity in the livers of OVX animals, while the intake of chia with the HFD in OVX rats increased the expression of superoxide dismutase (SOD) and the catalase activity.[22] However, it is necessary to clarify the effect of chia on the biometric data of animals, heart biometry, oxidative stress and mineral content of the heart in ovariectomized Wistar rats fed a HFD. Thus, the present study aimed to evaluate the effect of chia consumption with a SD or HFD on the cardiovascular health of ovariectomized Wistar rats.

2. Materials and methods

2.1. Raw materials and preparation of flours

The chia seeds (*Salvia hispanica* L.) used in this study were grown in the state of Mato Grosso (Brazil). The seeds were ground using a knife mill (Marconi Equipment, Brazil) in three replicates. Then, the chia flour obtained was packed in polyethylene aluminum bags and stored in a freezer (−18 °C ± 1 °C) until the time of the experimental diet preparation.

2.2. Animals and diets

The experimental protocol was developed by our group of researchers.[22] Eighty newly weaned, 21-day old female rats (*Rattus norvegicus*, Wistar, and *albinus* variation) were obtained from the Central Animal Facility of the Center for Biological Sciences and Health at the Federal University of Viçosa, Minas Gerais, Brazil. The animals were systematically separated into 2 groups, with 40 animals in each group, randomized by body weight, fed a SD[23] or HFD (Research Diets, New Brunswick, NJ), with modifications. They were distributed into individual stainless-steel cages in a temperature controlled environment (22 °C) with automatically controlled light and dark cycles of 12 hours. The SD contained 19% protein, 17% fat and 64% carbohydrate. The HFD contained 51% fat, 15% protein and 34% carbohydrate. The animals received deionized water and their respective experimental diets *ad libitum.* After 7 weeks of feeding, 40 female mice were ovariectomized (20 animals from each group) and 40 were sham-operated (SHAM) (20 animals from each group) and they continued to receive these diets for another 3 weeks for recovery from surgery. At 10 weeks, the OVX and SHAM animals were relocated to receive one of the following experimental diets (n = 10 per group) for 8 weeks: SD + calcium carbonate (SD + CC), SD + chia (SD + chia), HFD + calcium carbonate (HFD + CC) or HFD + chia (HFD + chia).

The diet offered to the animals satisfied 100% of calcium requirement of rodents (0.005 kg kg^{-1} diet) according to recommendations.[23] In the groups that were fed chia, the chia provided 20% of the recommended amount of calcium (0.0010 kg of calcium per kg diet) based on the composition. Chia was administered in the form of flour. The remaining recommended amount of calcium was provided by calcium carbonate (CC). The other ingredients were added in sufficient quantities to provide the recommended amounts of lipids, proteins, carbohydrates, dietary fiber and calories (Table 1).

After the experimental period, the animals were anesthetized with isoflurane (Isoforine, Cristália®, Itapira, Brazil). Blood was collected by cardiac puncture. The hearts and visceral adipose tissues were removed and weighed. The hearts were frozen in liquid nitrogen and stored at −80 °C until the time of analysis. Tibia length was measured using a 200 mm digital pachymeter (0.01 mm resolution; Model 530-312; Mitutoyo). Body weight gain and food intake were monitored weekly during the experimental period. Adiposity was calculated as a percentage, using the following formula: (visceral + gonadal + retroperitoneal + mesenteric + inguinal adipose tissues)/total body weight × 100.[24]

All experimental procedures with animals were performed in accordance with Directive 86/609/EEC of November 24, 1986, in compliance with the ethical principles for animal experimentation. The study protocol was approved by the Ethics Committee of the Federal University of Viçosa (Protocol 20/2017; date of approval: July 13th, 2017).

2.3. Biometric measurements of the heart

The hearts were removed and weighed. The fat present in the heart and the left ventricle (LV) were separated, and both were weighed separately. The cardiac hypertrophy index was obtained by calculating the proportion of heart weight (mg) to tibia length (mm). The heart volume, ventricle volume and fat volume were determined by the submersion method.[25]

Table 1 Composition of experimental diets

	Experimental diets					
	Week 1–Week 10		Week 10–Week 18			
Ingredients (g kg^{-1})	SD	HFD	SD + CC	HFD + CC	SD + chia	HFD + Chia
Albumin	179.50	179.50	179.50	179.50	133.70	133.70
Chia	0.00	0.00	0.00	0.00	232.60	232.60
Dextrinized starch	155.00	155.00	155.00	155.00	155.00	155.00
Sucrose	100.00	100.00	100.00	100.00	100.00	100.00
Lard	0.00	240.00	0.00	195.00	0.00	195.00
Soybean oil	40.00	40.00	70.20	70.20	0.00	0.00
Cellulose	50.00	50.00	86.00	86.00	0.00	0.00
Calcium-free mineral mix	35.00	35.00	35.00	35.00	35.00	35.00
Vitamin mix	10.00	10.00	10.00	10.00	10.00	10.00
L-Cystine	1.80	1.80	1.80	1.80	1.80	1.80
Choline bitartrate	2.50	2.50	2.50	2.50	2.50	2.50
Corn starch	419.95	178.45	347.50	151.00	319.40	122.90
Cholesterol	0.00	1.50	0.00	1.50	0.00	1.50
Calcium carbonate	12.50	12.50	12.50	12.50	10.00	10.00
Nutritional composition						
Total calories (kcal)	3778.00	4971.80	3760.80	4728.80	3647.40	4616.40
Caloric density (kcal g^{-1})	3.77	4.97	3.76	4.73	3.65	4.62

SD: standard diet; SD + CC: standard diet + calcium carbonate; SD + chia: standard diet + chia; HFD + CC: high fat diet + calcium carbonate; HFD + chia: high fat diet + chia.

2.4. Oxidative stress

2.4.1. Homogenate preparation. To obtain a heart homogenate, 200 mg of heart sample was mixed with 1000 μL of phosphate buffer (50 mM) and 1 mM EDTA (pH 7.4). The sample was macerated and centrifuged at 12 000*g* and 4 °C for 10 min. The supernatant was removed and stored in an ultra-freezer until the time of analysis. Total protein in the heart homogenate was quantified by the Bradford method.[26]

2.4.2. Superoxide dismutase (SOD). The quantification of SOD was performed in relative units, and one unit was defined as the amount of SOD enzyme that inhibits the pyrogallol oxidation rate by 50%. The analysis was carried out with a spectrophotometer (Multiskan GO, Thermo Scientific, Ratastie, Finland) at 570 nm. The results were expressed as units of SOD activity per milligram of protein. Calculations for the absorbance value of the standard were performed, considering that it has 1 U of SOD, namely, 100% of pyrogallol oxidation.[27]

2.4.3. Malondialdehyde (MDA). The MDA formed after the addition of thiobarbituric acid was quantified based on the ability of thiobarbituric acid to react with certain compounds resulting from lipid peroxidation, such as MDA. The analysis was carried out using a spectrophotometer (Multiskan GO, Thermo Scientific, Ratastie, Finland) at 535 nm.[28,29] The concentration of MDA was calculated using the molar absorptivity coefficient $E_0 = 1.56 \times 10^5\ M^{-1}\ cm^{-1}$.[30] The results were expressed in μM of MDA per milligram of protein.

2.4.4. Catalase activity. The catalase activity is determined by its ability to cleave hydrogen peroxide (H_2O_2) in water and molecular oxygen, as described by AEBI (1984).[31] The absorbance was determined at 0, 30 and 60 seconds, at 240 nm, using a spectrophotometer (T70 + UV/VIS Spectrometer, Taylors, USA). One unit (U) of catalase is equivalent to the hydrolysis of 1 mol of H_2O_2 (ε = 39.4 L mol^{-1} cm^{-1}) per minute. The catalase activity was calculated according to the Lambert–Beer law and the results were expressed in mmol min mg^{-1} PTN.

2.4.5. Nitric oxide (NO). The heart homogenate was mixed with sulfanilamide (in 2.5% H_3PO_4) and naphthyl l ethylene diamide dihydrochloride (in 2.5% H_3PO_4). The absorbance was read using a spectrophotometer (Multiskan Go, Thermo Scientific) at 570 nm, and the results were expressed in μM.[32]

2.5. Mineral microanalysis

The mineral content in the cardiac tissue was investigated by energy-dispersive X-ray spectroscopy (EDS), using a scanning electron microscope (JEOL, JSM-6010LA) with an X-ray detector system attached to it (Silicon Drift Detector). The small fragments of the heart of each animal were dehydrated in an oven and covered with a thin film of evaporated carbon (Quorum Q150 T, East Grinstead, West Sussex, England, UK). Microanalysis EDS was performed at 350× magnification, an acceleration voltage of 20 kV and a working distance of 10 mm. The proportion of the elements calcium (Ca), sodium (Na), potassium (K), magnesium (Mg), iron (Fe), copper (Cu), zinc (Zn), and manganese (Mn) was measured by EDS, and the values were normalized and expressed as mean value and standard deviation (g 100^{-1} g).[33]

2.6. Determination of the activities of ATPase, Ca^{2+}ATPase, Na^+/K^+ATPase and Mg^{2+}ATPase

The total membrane-bound adenosine triphosphatase (ATPase) activity, and Ca^{2+}ATPase, Na^+/K^+ATPase and Mg^{2+}ATPase activities were measured in the supernatant of

100 mg of the frozen heart previously homogenized in 0.1 M Tris-HCl (pH 7.4) buffer solution and centrifuged at 3500*g* and 5 °C for 10 min. In a microcentrifuge tube, 750 μL of buffer and 250 μL of the mixture containing 0.1 M NaCl, 0.1 M KC1, 0.1 M $MgCl_2$, 0.1 M $CaCl_2$, and 0.01 M ATP solution (50 μL of each solution) were added to 50 μL of supernatant. The samples were incubated at 37 °C for 20 min, and the reaction was interrupted by adding 500 μL of icecold 10% TCA. The samples were centrifuged for 10 min at 1500*g*.[34,35] The inorganic phosphorus content in the supernatant was estimated by colorimetric determination.[36] The total ATPase activity, and Ca^{2+}ATPase, Na^+/K^+ATPase and Mg^{2+}ATPase activities were expressed as micromol of phosphorus liberated per minute per milligram of protein.

2.7. Histopathological analysis of the heart

A fragment of each left ventricle was immersed in a 4% glutaraldehyde solution for histopathological analysis. The fragments were dehydrated in a crescent ethanol series and embedded in Historesin® (Leica, Nussloch, Germany). A rotary microtome (RM 2255, Leica Biosystems, Nussloch, Germany) was used to cut the material into histological sections of 3 μm thickness, and the cut sections were mounted on glass slides. The sections were stained with Gomori's Trichrome[37] for extracellular matrix observation. We carried out a qualitative analysis of the heart microstructure.

2.8. Statistical analysis

The experiments were conducted in a completely randomized design with ten biological replicates (n = 10 animals per group). The results were subjected to analysis of variance (ANOVA) at 5% probability. To determine "*F*-value" significance, the *post hoc* Newman–Keuls test was used to compare the intra group mean values (among the groups with or without ovariectomy). *T*-tests were used to compare inter group differences (the groups fed the same diet with and without ovariectomy). Factorial ANOVA was used to delineate the effects of each variable (calcium source, different diets and ovariectomy) on biometric status, oxidative stress, mineral contents and ATPase bomb activity. Statistical analysis was performed using the GraphPad Prism software, version 6. Data with a *P*-value ≤ 0.05 were considered statistically significant.

3. Results

3.1. Biometric variables

SHAM animals fed HFD showed lower food intake than SHAM animals fed SD. In OVX animals, HFD reduced food intake when fed with chia. The total chia consumption in the OVX and SHAM groups was higher in animals that consumed the SD than in animals fed the HFD. The fatty acid content including saturated, polyunsaturated, omega 3 (n-3) and omega 6 (n-6) were determined based on the composition of the diet. We observed that saturated and n-6 fatty acid consumption was higher in the groups fed HFD, regardless of chia presence. At the same time, OVX and SHAM groups fed chia showed higher polyunsaturated and n-3 fatty acid concentration. The amount of omega 3 provided by all diets with chia was higher in relation to the groups fed diets without chia. Chia consumption provided 20% of the amount of calcium. The fiber intake was determined based on the composition of the diet. In diets containing chia seed, 100% of the recommended fiber was provided by the seed. We observe that in the SHAM group, animals fed with HFD showed lower fiber intake and calcium intake than animals fed SD. In relation to the OVX group, animals fed HFD + chia showed lower consumption of fiber and calcium.

Total energy intake did not differ among the experimental groups. HFD consumption increased weight gain and visceral adiposity in OVX animals. In SHAM animals, the type of diet consumed did not alter weight gain or visceral adiposity. Regarding surgery, OVX animals fed HFD (HFD + CC or HFD + chia) showed higher weight gain and visceral adiposity than SHAM animals fed the same diets. The heart weight, heart volume and heart weight/tibia length ratio did not differ among the experimental groups. The fat weight in the heart was not altered by the type of diet consumed in both SHAM and OVX animals, but in relation to surgery, the SHAM animals fed chia presented a lower fat weight in the heart than the OVX animals that consumed the same diet.

The volume of fat in the heart was not altered by the type of diet consumed either in SHAM animals or in OVX. In relation to surgery, the fat size in the heart of the SHAM animals fed SD + chia or HFD (HFD + CC or HFD + chia) was lower than that of the animals in the OVX group. In the SHAM group, the ventricle weight and ventricle weight/tibia length ratio were not altered by the type of diet consumed. However, the OVX animals that consumed HFD presented higher ventricle weight and ventricle weight/tibia length ratio. Regarding surgery, the OVX group that consumed chia presented lower ventricle weight and ventricle weight/tibia length ratio than the SHAM group which received the same diet. The consumption of chia or HFD did not change the ventricle volume between SHAM animals and OVX animals. In relation to surgery, the OVX animals that consumed SD + chia presented lower ventricle volume than the SHAM animals that consumed the same diet (Table 2).

In relation to factorial ANOVA, when the factor analyzed was ovariectomy (OVX and SHAM groups), we observed that there was no difference in food intake and total energy intake. The OVX group showed higher weight gain and visceral adiposity, higher weight of the heart and weight of fat and lower weight of the ventricle. When we analyzed the diet (HFD and SD), we observed that the group fed with HFD showed lower food intake and higher energy intake, higher weight gain and visceral adiposity, higher weight of the heart, weight of fat and weight of the ventricle. Finally, when we performed the analysis in relation to the calcium source (CC and chia), we observed that there was no difference among the experimental groups (Table 3).

Table 2 Biometric data of the experimental animals

	Non-ovariectomized (SHAM)				Ovariectomized (OVX)			
Groups	SD + CC	SD + Chia	HFD + CC	HFD + Chia	SD + CC	SD + Chia	HFD + CC	HFD + Chia
Total food intake (g)	740.31 ± 61.15^{a}	789.77 ± 87.58^{a}	624.49 ± 78.22^{b}	633.48 ± 66.31^{b}	730.04 ± 70.88^{a}	743.45 ± 94.30^{a}	669.43 ± 116.07^{ab}	619.80 ± 37.18^{b}
Total chia intake (g)	—	183.70 ± 8.89^{+}	—	147.34 ± 7.98	—	172.93 ± 9.73^{+}	—	144.16 ± 8.65
Total energy intake (kcal)	2783.55 ± 229.93^{a}	2882.66 ± 319.68^{a}	2953.83 ± 370.00^{a}	2926.70 ± 306.34^{a}	2744.94 ± 266.50^{a}	2713.61 ± 344.19^{a}	3018.33 ± 304.10^{a}	2863.40 ± 71.78^{a}
Total fiber intake (g)	63.67 ± 5.26^{a}	67.92 ± 7.53^{a}	53.71 ± 6.73^{b}	54.48 ± 5.70^{b}	62.78 ± 6.10^{a}	63.94 ± 8.11^{a}	57.57 ± 9.98^{ab}	53.30 ± 3.20^{b}
Total calcium intake (g)	3.38 ± 0.28^{a}	3.66 ± 0.27^{a}	2.89 ± 0.36^{b}	2.90 ± 0.30^{b}	3.34 ± 0.32^{a}	3.36 ± 0.43^{a}	3.09 ± 0.54^{ab}	2.83 ± 0.17^{b}
Total fatty acid intake (g)								
Saturated	8.24 ± 0.81^{b}	5.27 ± 0.72^{c}	61.70 ± 6.44^{a}	59.23 ± 3.58^{a}	8.12 ± 0.79^{b}	4.95 ± 0.67^{c}	66.15 ± 7.83^{a}	61.14 ± 6.39^{a}
Polyunsaturated	33.81 ± 3.28^{c}	68.30 ± 7.39^{a}	41.22 ± 8.88^{b}	67.31 ± 4.03^{a}	33.77 ± 3.20^{c}	61.93 ± 6.86^{a}	47.78 ± 5.98^{b}	61.80 ± 7.20^{a}
n-3	2.36 ± 0.24^{b}	37.42 ± 3.02^{a}	3.58 ± 0.87^{b}	38.63 ± 1.24^{a}	2.33 ± 0.86^{b}	35.22 ± 3.76^{a}	3.97 ± 0.96^{b}	37.02 ± 2.04^{a}
n-6	25.81 ± 2.31^{b}	10.45 ± 1.21^{c}	36.76 ± 2.76^{a}	41.40 ± 3.56^{a}	24.89 ± 3.45^{b}	9.78 ± 1.26^{c}	38.03 ± 1.45^{a}	38.74 ± 3.75^{a}
Weight gain (g)	176.70 ± 19.92^{a}	178.45 ± 23.49^{a}	202.60 ± 23.79^{a}	206.4 ± 26.94^{a}	191.37 ± 15.08^{b}	197.78 ± 14.78^{b}	261.98 ± 23.61^{a*}	263.60 ± 22.10^{a*}
Visceral adiposity (g)	1.13 ± 0.32^{a}	1.20 ± 0.25^{a}	1.25 ± 0.44^{a}	1.33 ± 0.39^{a}	1.02 ± 0.28^{b}	1.27 ± 0.29^{b}	1.82 ± 0.33^{a*}	1.74 ± 0.29^{a*}
Heart weight (g)	1.78 ± 0.13^{a}	1.71 ± 0.07^{a}	2.12 ± 0.26^{a}	2.09 ± 0.31^{a}	1.74 ± 0.16^{a}	2.09 ± 0.39^{a}	2.31 ± 0.41^{a}	2.44 ± 0.31^{a}
Heart volume (mL)	0.87 ± 0.11^{a}	0.90 ± 0.08^{a}	0.96 ± 0.18^{a}	1.01 ± 0.12^{a}	0.80 ± 0.04^{a}	0.90 ± 0.09^{a}	0.91 ± 0.04^{a}	0.91 ± 0.12^{a}
Heart weight (mg)/tibia length (mm)	45.53 ± 3.22^{a}	42.96 ± 1.49^{a}	51.91 ± 5.64^{a}	53.16 ± 9.43^{a}	43.89 ± 5.23^{a}	52.14 ± 10.99^{a}	58.37 ± 10.26^{a}	60.26 ± 7.08^{a}
Fat weight (g)	0.42 ± 0.12^{a}	0.24 ± 0.09^{a}	0.32 ± 0.04^{a}	0.33 ± 0.04^{a}	0.46 ± 0.18^{a}	0.50 ± 0.19^{a*}	0.72 ± 0.29^{a}	0.73 ± 0.08^{a*}
Fat volume (mL)	0.37 ± 0.11^{a}	0.23 ± 0.09^{a}	0.28 ± 0.05^{a}	0.30 ± 0.04^{a}	0.57 ± 0.19^{a}	0.54 ± 0.17^{a*}	0.80 ± 0.16^{a*}	0.69 ± 0.05^{a*}
Ventricle weight (g)	0.65 ± 0.10^{a}	0.64 ± 0.05^{a*}	0.73 ± 0.10^{a}	0.76 ± 0.05^{a*}	0.56 ± 0.05^{b}	0.55 ± 0.03^{b}	0.67 ± 0.05^{a}	0.65 ± 0.05^{a}
Ventricle volume (mL)	0.60 ± 0.05^{a}	0.59 ± 0.04^{a*}	0.66 ± 0.10^{a}	0.69 ± 0.06^{a}	0.51 ± 0.05^{ab}	0.49 ± 0.04^{b}	0.62 ± 0.07^{a}	0.61 ± 0.05^{ab}
Ventricle weight (mg)/tibia length (mm)	17.58 ± 2.63^{a}	16.16 ± 1.29^{a*}	18.05 ± 2.37^{a}	19.42 ± 2.03^{a*}	14.09 ± 1.60^{b}	13.56 ± 0.89^{b}	16.97 ± 1.29^{a}	16.17 ± 1.28^{a}

SD + CC: standard diet + calcium carbonate; SD + chia: standard diet + chia; HFD + CC: high fat diet + calcium carbonate; HFD + chia: high fat diet + chia; n-3: omega 3; n-6: omega 6.* Significant mean inter group differences according to the *t*-test ($p < 0.05$). Different small letters (a and b) indicate significant mean differences among the groups according to the Newman–Keuls test at 5% threshold of probability. + Significant mean intra group differences according to the *t*-test ($p < 0.05$). Data are expressed as mean ± standard deviation.

Table 3 Effects of different factors (calcium source, different diets and ovariectomy) on biometric variables in Wistar rats (n = 10 per group) for 126 days

		Variables						
Factor	Levels	Total food intake (g)	Total energy intake (kcal)	Weight gain (g)	Visceral adiposity (g)	Heart weight (g)	Fat weight (g)	Ventricle weight (g)
Ovariectomy	OVX	690.67 ± 95.49	2835.0 ± 288.8	228.74 ± 38.77*	1.46 ± 0.43*	2.14 ± 0.40*	0.62 ± 0.18*	0.60 ± 0.07
	SHAM	697.01 ± 100.17	2886.6 ± 303.2	191.03 ± 24.94	1.22 ± 0.34	1.92 ± 0.26	0.33 ± 0.09	0.70 ± 0.08*
Diet	HFD	636.79 ± 78.79	2940.5 ± 285.3*	233.63 ± 36.70*	1.53 ± 0.42*	2.23 ± 0.32*	0.52 ± 0.23*	0.70 ± 0.07*
	SD	750.89 ± 79.31*	2781.1 ± 286.8	186.13 ± 18.65	1.15 ± 0.28	1.82 ± 0.25	0.42 ± 0.16	0.60 ± 0.07
Source of calcium	CC	691.06 ± 93.35	2875.1 ± 301.8	208.19 ± 37.59	1.30 ± 0.45	1.98 ± 0.34	0.48 ± 0.20	0.66 ± 0.08
	CHIA	696.62 ± 102.19	2846.5 ± 291.9	211.58 ± 37.95	1.38 ± 0.36	2.08 ± 0.37	0.47 ± 0.21	0.65 ± 0.09

OVX: ovariectomized group; SHAM: not ovariectomized group; HFD: high fat diet; SD: standard diet; CC: calcium carbonate; Kcal: kilocalories. * Significant mean differences according to the ANOVA factorial test ($p < 0.05$). Data are expressed as mean ± standard deviation.

3.2. Oxidative stress

In the SHAM group, the activity of catalase, an enzyme whose function is the dismutation of H_2O_2 in oxygen and water, acting in the cellular defense against oxidative damage by H_2O_2,[38] was not altered by the consumption of either HFD (HFD + CC × SD + CC) or chia (SD + CC × SD + chia and HFD + CC × HFD + chia). In the OVX group, the diet did not alter the catalase activity. Regarding surgery, the catalase activity was higher in OVX animals that consumed SD + chia and lower in animals that consumed HFD + CC compared to SHAM animals that consumed the same diets.

SOD is the first line of defense among antioxidant enzymes against oxygen free radicals, catalyzing the dismutation of the superoxide anion in oxygen (O_2) and hydrogen peroxide (H_2O_2), which is less reactive, thus preventing the generation of highly reactive OH radicals.[39] The SOD activity, both for OVX and SHAM animals, did not differ between the animals that consumed SD and HFD (SD + CC × HFD + CC) and between the animals that consumed SD + CC and SD + chia (SD + CC × SD + chia). Among the OVX animals that consumed HFD (HFD + CC × HFD + chia), those that consumed chia showed higher levels of SOD than those that did not consume chia. However, this difference was not observed in SHAM animals. Regarding surgery, OVX animals that consumed SD + chia showed reduced SOD activity, compared to SHAM animals fed the same diet.

The levels of malondialdehyde, used as a biomarker of lipid peroxidation since malondialdehyde is a secondary product of lipid peroxidation,[40] did not differ among the groups, either in relation to diet or surgery.

NO is essential for endothelial functioning, which is related to vasodilation. Therefore, it is considered a cardioprotective molecule, despite being a free radical. However, under conditions of oxidative stress, high levels of superoxide can react with NO to form peroxynitrite, a reactive nitrogen species (RNS) that causes changes that can lead to endothelial dysfunction.[41] Among SHAM animals, the NO levels were lower in animals that consumed HFD than those that consumed SD. In OVX animals, the consumption of HFD increased the levels of NO, but when this diet was associated with the consumption of chia, the amount of NO decreased and was comparable to that observed in animals fed SD. Regarding surgery, the OVX group that consumed SD + CC presented a lower amount of NO, compared to the SHAM group fed the same diet. However, when chia was fed with SD, the NO levels were comparable between the SHAM and OVX groups. The OVX animals fed HFD + CC showed higher NO concentration, but when chia was fed with HFD, the amount of NO was comparable between the SHAM and OVX groups (Fig. 1).

In the factorial ANOVA, when the factor analyzed was ovariectomy (OVX and SHAM groups), we observed that the OVX group showed reduced SOD activity. When we analyzed the diet (HFD and SD), we observed that the group fed with a HFD showed higher catalase activity and SOD activity and higher levels of malondialdehyde. Finally, when we performed the analysis in relation to the calcium source (CC and chia), we observed that the group fed with chia showed higher SOD activity (Table 4).

3.3. Mineral microanalysis

Calcium, sodium and potassium are essential electrolytes for suitable excitability of the cardiac muscle fiber membrane and contractile performance of the heart. The analysis of the concentration of minerals in the heart showed that among OVX animals the type of diet did not alter the calcium concentration. In SHAM animals, chia consumption did not alter the calcium concentration in the heart, but those who were fed HFD + CC presented lower calcium concentration than the SD + CC group. The HFD + chia group was able to maintain the calcium concentration in relation to animals fed SD. Regarding surgery, SHAM animals fed SD + chia or HFD + CC presented lower concentration of calcium in the heart, than OVX animals that received the same diet. In both OVX and SHAM animals, the type of diet did not change the sodium concentration in the heart. Regarding surgery, OVX animals fed HFD + chia presented lower sodium concentration in the heart. OVX animals fed HFD + CC showed higher concentration of potassium in the heart than those fed the other diets. In SHAM animals, the consumption of HFD (HFD + CC or HFD + chia) decreased potassium concentration when com-

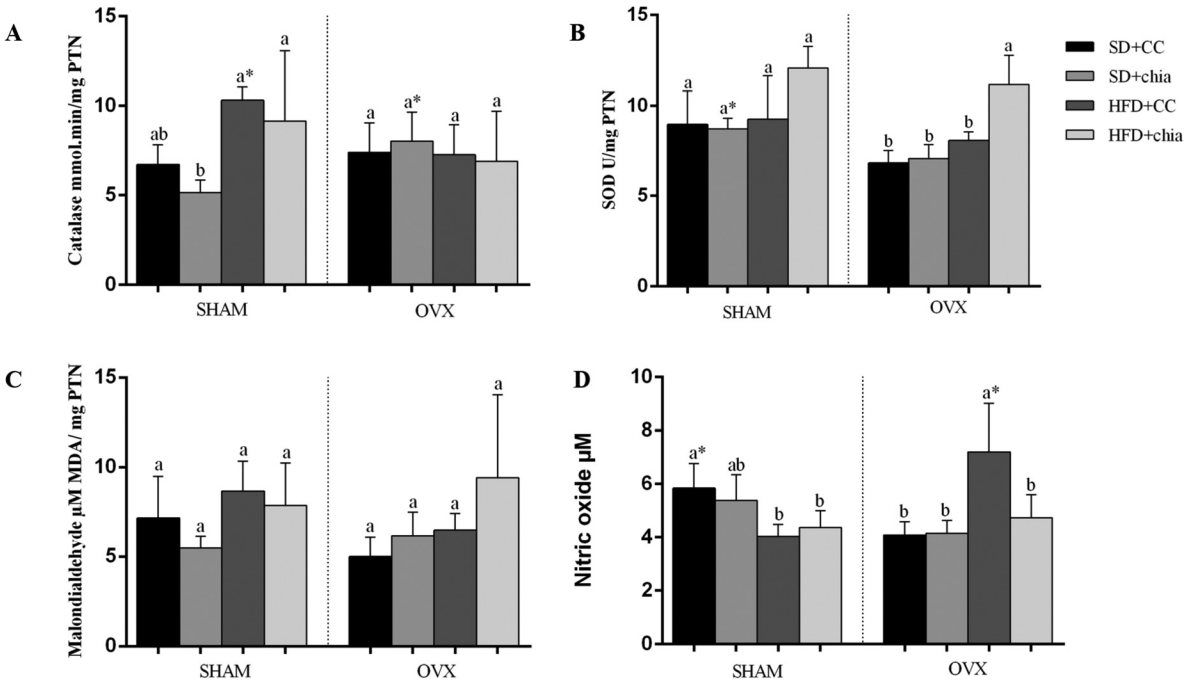

Fig. 1 Oxidative stress analysis. SD + CC: standard diet + calcium carbonate; SD + chia: standard diet + chia; HFD + CC: high fat diet + calcium carbonate; HFD + chia: high fat diet + chia; PTN: protein; SHAM: non-ovariectomized group and OVX: ovariectomized group; SOD: superoxide dismutase. Graphs show (A) catalase, (B) superoxide dismutase, (C) malondialdehyde and (D) nitric oxide. * Significant mean inter group differences according to the *t*-test ($p < 0.05$). Different small letters (a and b) indicate significant mean differences among the groups according to the Newman–Keuls test at 5% threshold of probability. Data are expressed as mean ± standard deviation.

Table 4 Effects of different factors (calcium source, different diets and ovariectomy) on oxidative stress in Wistar rats (n = 10 per group) for 126 days

		Variables			
Factor	Levels	Catalase (mmol min mg^{-1} PTN)	SOD (U mg^{-1} PTN)	Malondialdehyde (µM mg^{-1} PTN)	Nitric oxide (µM)
Ovariectomy	OVX	7.40 ± 1.68	8.28 ± 1.93	6.75 ± 2.62	5.04 ± 1.55
	SHAM	7.83 ± 2.82	9.74 ± 1.91*	7.28 ± 1.99	4.90 ± 0.97
Diet	HFD	8.41 ± 2.65*	10.12 ± 2.04*	8.09 ± 2.46*	5.08 ± 1.53
	SD	6.82 ± 1.59	7.89 ± 1.30	5.95 ± 1.58	4.86 ± 0.98
Source of calcium	CC	7.92 ± 1.80	8.27 ± 1.57	6.82 ± 1.94	5.29 ± 1.59
	CHIA	7.31 ± 2.72	9.74 ± 2.20*	7.22 ± 2.67	4.65 ± 0.77

OVX: ovariectomized group; SHAM: not ovariectomized group; HFD: high fat diet; SD: standard diet; CC: calcium carbonate; Ca: calcium; PTN: protein; U: units. * Significant mean differences according to the ANOVA factorial test ($p < 0.05$).

pared to SD consumption (SD + CC or SD + chia). Regarding surgery, the SHAM animals that consumed HFD (HFD + CC or HFD + chia) presented lower concentration of potassium in the heart than the OVX animals that consumed the same diet. Magnesium is a mineral that participates in cardiac electrophysiology, and it significantly affects cardiac ion channels.[42] Neither SHAM nor OVX animals present a change in magnesium concentration in the heart. Regarding surgery, the OVX groups that did not consume chia (SD + CC or HFD + CC) presented lower concentration of magnesium in the heart than SHAM animals fed the same diets.

Iron, copper, zinc and manganese contribute as cofactors for enzymes that act in the cardiac oxidative defense. The iron concentration in the heart was not altered by the consumption of chia or HFD in both SHAM and OVX groups. Regarding surgery, OVX animals that consumed HFD + CC showed higher concentration of iron in the heart than SHAM animals that consumed the same diet. The concentration of copper in the heart of OVX animals was not altered by the diet consumed. In SHAM animals that consumed HFD (HFD + CC or HFD + chia), the concentration of copper was higher. The consumption of chia did not change the concentration of the mineral. Regarding surgery, the OVX animals fed HFD (HFD + CC or HFD + chia) presented lower copper concentration in the heart.

Among the OVX animals, no difference was observed in zinc concentration for the different diets. Among the SHAM

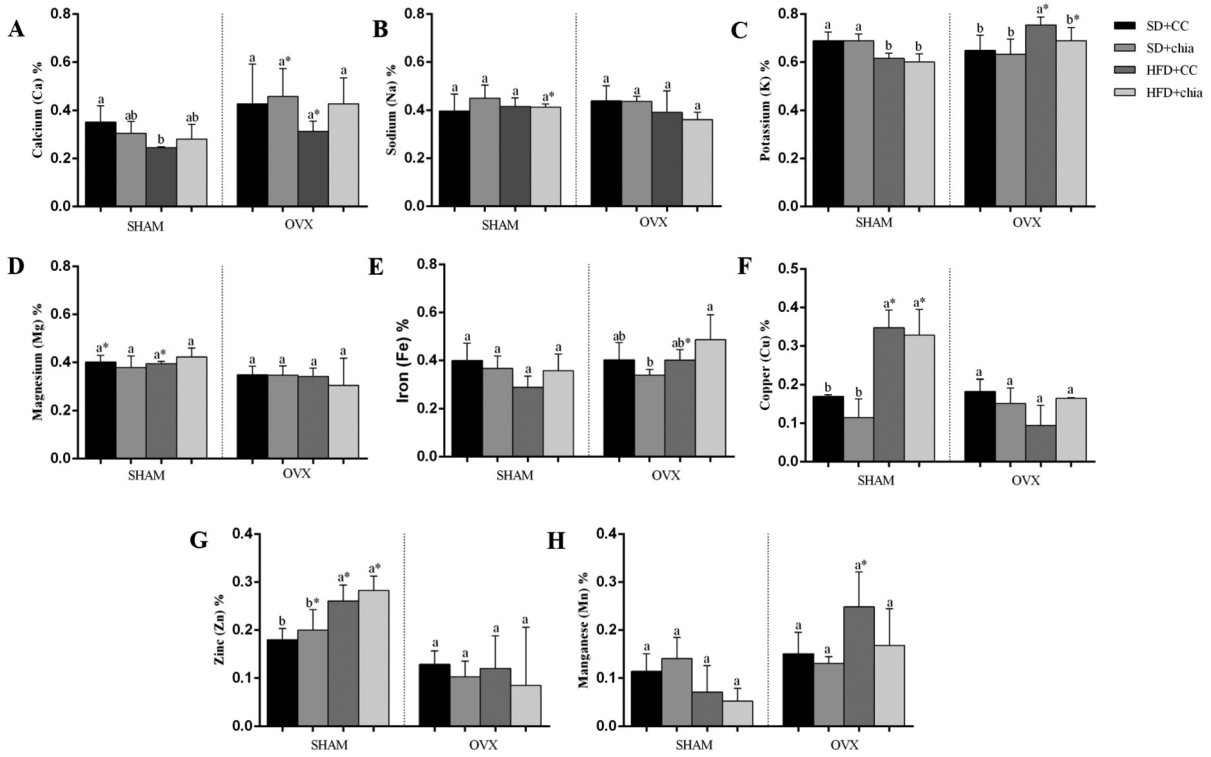

Fig. 2 Mineral proportion in the heart of Wistar rats fed chia and HFD. SD + CC: standard diet + calcium carbonate; SD + chia: standard diet + chia; HFD + CC: high fat diet + calcium carbonate; HFD + chia: high fat diet + chia; SHAM: non-ovariectomized group and OVX: ovariectomized group. Graphs show (A) calcium content, (B) sodium content, (C) potassium content, (D) magnesium content, (E) iron content, (F) copper content, (G) zinc content and (H) manganese content. * Significant mean inter group differences according to the *t*-test ($p < 0.05$). Different small letters (a and b) indicate significant mean differences among the groups according to the Newman–Keuls test at 5% threshold of probability. Data are expressed as mean ± standard deviation.

animals, those that consumed HFD (HFD + CC or HFD + chia) presented higher concentration of zinc in the heart than the animals that received SD. Regarding surgery, the OVX animals which consumed SD + chia or HFD (HFD + CC or HFD + chia) showed lower zinc concentrations than the SHAM animals fed the same diets. The manganese concentration was not altered by the type of diet consumed in both SHAM and OVX animals. Regarding surgery, OVX animals fed a HFD + CC diet showed higher concentration of manganese in the heart (Fig. 2)

In relation to factorial ANOVA, when the factor analyzed was ovariectomy (OVX and SHAM groups), we observed that the OVX group showed higher concentration of calcium, iron and manganese, and lower concentration of magnesium, copper and zinc. When we analyzed the diet (HFD and SD), we observed that the animals fed with HFD showed higher concentration of copper and zinc, and lower concentration of calcium and sodium. Finally, when we performed the analysis in relation to the calcium source (CC and chia), we observed that there was no difference between the groups (Table 5).

3.4. Determination of the activities of total ATPase, Ca^{2+}ATPase, Na^{+}/K^{+}ATPase and Mg^{2+}ATPase

ATPases are transport enzymes present in all cell membranes, and they contribute to osmotic balance by regulating ion concentrations within cells, so as to maintain cellular homeostasis.[43] Among OVX animals, the diet did not alter the total ATPase activity. However, in the SHAM group, the total ATPase activity was higher in animals that consumed HFD + CC. Nevertheless, when this diet was combined with chia, the activity was reduced. Regarding surgery, OVX animals fed HFD without chia presented lower total ATPase activity than SHAM animals fed the same diet.

Ca^{2+}ATPase activity was not altered either by the type of diet consumed or by ovariectomy. The consumption of HFD + CC by OVX animals decreased Na^{+}/K^{+}ATPase activity compared to the SD consumption. In SHAM animals, Na^{+}/K^{+}ATPase activity was lower in the hearts of rats fed SD + chia than in rats fed SD without chia. Regarding surgery, OVX animals fed HFD + CC presented lower Na^{+}/K^{+}ATPase activity. When the animals were fed SD + chia, they presented higher Na^{+}/K^{+}ATPase activity than the SHAM animals fed the same diet.

Mg^{2+}ATPase activity was not altered by the consumption of chia or HFD in the OVX group or in the SHAM group (SD + CC × HFD + CC, SD + CC × SD + chia, and HFD + CC × HFD + chia). However, regarding surgery, OVX animals fed HFD (HFD + CC or HFD + chia) showed lower activity of Mg^{2+}ATPase than the SHAM group fed the same diets. OVX animals fed SD + chia presented higher Mg^{2+}ATPase activity (Fig. 3).

Table 5 Effects of different factors (calcium source, different diets and ovariectomy) on minerals in Wistar rats (n = 10 per group) for 126 days

		Variables							
Factor	Levels	Calcium (g per 100 g)	Sodium (g per 100 g)	Potassium (g per 100 g)	Magnesium (g per 100 g)	Iron (g per 100 g)	Copper (g/100 g)	Zinc (g/100 g)	Manganese (g/100 g)
Ovariectomy	OVX	0.40 ± 0.10*	0.40 ± 0.06	0.67 ± 0.06	0.33 ± 0.05	0.40 ± 0.07*	0.14 ± 0.04	0.10 ± 0.04	0.17 ± 0.06*
	SHAM	0.29 ± 0.05	0.41 ± 0.04	0.64 ± 0.05	0.39 ± 0.03*	0.35 ± 0.06	0.23 ± 0.10*	0.22 ± 0.04*	0.09 ± 0.05
Diet	HFD	0.31 ± 0.08	0.39 ± 0.05	0.65 ± 0.07	0.36 ± 0.06	0.38 ± 0.08	0.23 ± 0.11*	0.18 ± 0.09*	0.13 ± 0.09
	SD	0.38 ± 0.10*	0.42 ± 0.05*	0.66 ± 0.05	0.37 ± 0.04	0.37 ± 0.05	0.15 ± 0.03	0.15 ± 0.04	0.13 ± 0.03
Source of calcium	CC	0.33 ± 0.09	0.40 ± 0.06	0.67 ± 0.06	0.37 ± 0.03	0.36 ± 0.06	0.19 ± 0.10	0.17 ± 0.06	0.14 ± 0.07
	CHIA	0.36 ± 0.10	0.41 ± 0.04	0.65 ± 0.05	0.36 ± 0.07	0.38 ± 0.07	0.18 ± 0.09	0.16 ± 0.08	0.12 ± 0.05

OVX: ovariectomized group; SHAM: not ovariectomized group; HFD: high fat diet; SD: standard diet; CC: calcium carbonate. * Significant mean differences according to the ANOVA factorial test ($p < 0.05$). Data are expressed as mean ± standard deviation.

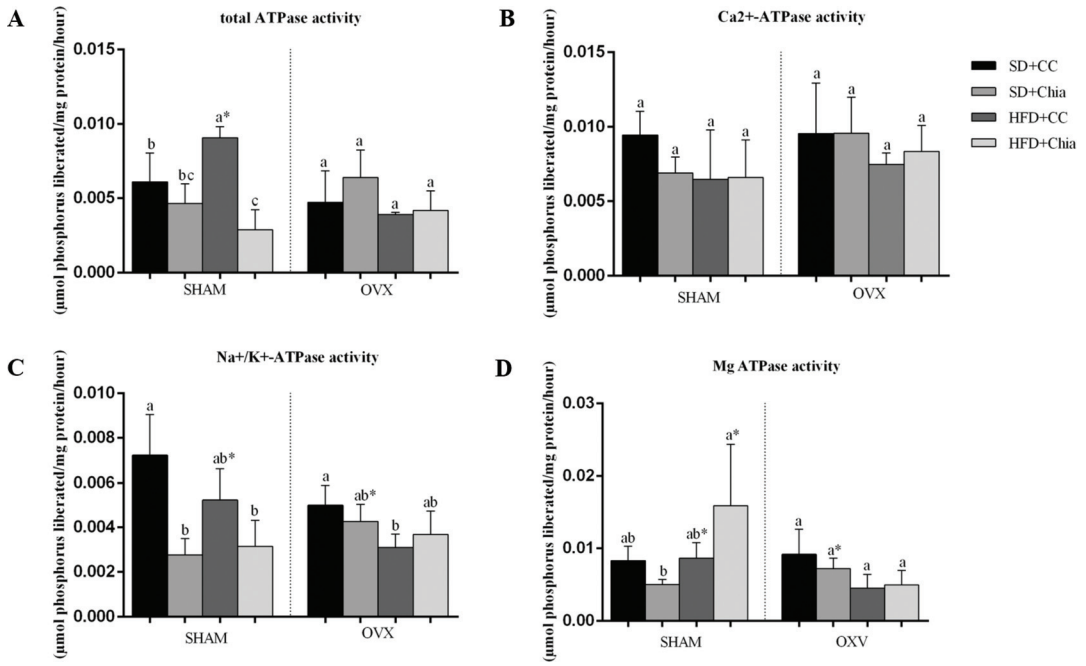

Fig. 3 The activities of total ATPase, Ca^{2+} ATPase, Na^{+}/K^{+} ATPase and Mg^{2+} ATPase in the heart of control and experimental animals. SD + CC: standard diet + calcium carbonate; SD + chia: standard diet + chia; HFD + CC: high fat diet + calcium carbonate; HFD + chia: high fat diet + chia; SHAM: non-ovariectomized group and OVX: ovariectomized group. The graphs show (A) total ATPase, (B) Ca^{2+} ATPase, (C) Na + /K^{+}ATPase and (D) Mg^{2+} ATPase. * Significant mean inter group differences according to the t-test ($p < 0.05$). Different small letters (a and b) indicate significant mean differences among the groups according to the Newman–Keuls test at 5% threshold of probability. Data are expressed as mean ± standard deviation.

In relation to factorial ANOVA, when the factor analyzed was ovariectomy (OVX and SHAM groups), we observed that there was no difference between the experimental groups. When we analyzed the diet (HFD and SD), we observed that the group fed HFD showed lower Ca^{2+}ATPase and Na^{+}/K^{+}ATPase activities. Finally, when we performed the analysis in relation to the calcium source (CC and chia), we observed that the group fed chia presented lower total ATPase activity and Na^{+}/K^{+}ATPase activity (Table 6).

3.5. Histopathological analysis of the heart

The SHAM group fed SD + CC presented a highly organized myocardium structure, well vascularized by thin capillary blood vessels and large vessels surrounded by a thin and well delimited collagen matrix. The epicardium region presented a thin layer of collagen matrix, as expected. This same phenotype was observed in the animals of the SHAM SD + chia, OVX SD + CC and OVX SD + chia groups, with little or no variation. In some animals, thicker bundles of extracellular matrix were observed among the cardiomyocytes, however they were isolated cases that were present in all four groups.

In the groups fed HFD with calcium carbonate (SHAM and OVX) the regions of large vessels showed a thicker collagen layer upon beam irradiation between the cardiomyocytes, entering the regions of the myocardium. This characteristic was more frequent, with thickening of the fibers and greater cellularity in the matrix. The groups treated with chia combined with HFD (SHAM and OVX) presented well-defined

Table 6 Effects of different factors (calcium source, different diets and ovariectomy) on ATPase bombs activity in Wistar rats (n = 10 per group) for 126 days

Factor	Levels	VariablesTotal ATPase activity (μM P mg^{-1} PTN)	CaATPase activity (μM P mg^{-1} PTN)	NaKATPase activity (μM P mg^{-1} PTN)	MgATPase activity (μM P mg^{-1} PTN)
Ovariectomy	OVX	0.0048 ± 0.0016	0.1397 ± 0.0022	0.0040 ± 0.0009	0.0040 ± 0.0009
	SHAM	0.0056 ± 0.0025	0.1176 ± 0.0024	0.0045 ± 0.0021	0.0045 ± 0.0021
Diet	HFD	0.0050 ± 0.0025	0.1155 ± 0.0021	0.0037 ± 0.0012	0.0084 ± 0.0062
	SD	0.0054 ± 0.0017	0.1418 ± 0.0023*	0.0048 ± 0.0018*	0.0074 ± 0.0025
Source of calcium	CC	0.0059 ± 0.0023*	0.1317 ± 0.0026	0.0051 ± 0.0018*	0.0051 ± 0.0018
	CHIA	0.0045 ± 0.0017	0.1256 ± 0.0021	0.0034 ± 0.0009	0.0034 ± 0.0009

OVX: ovariectomized group; SHAM: not ovariectomized group; HFD: high fat diet; SD: standard diet; CC: calcium carbonate; Ca: calcium; P: phosphorus; PTN: protein.* Significant mean differences according to the ANOVA factorial test ($p < 0.05$). Data are expressed as mean ± standard deviation.

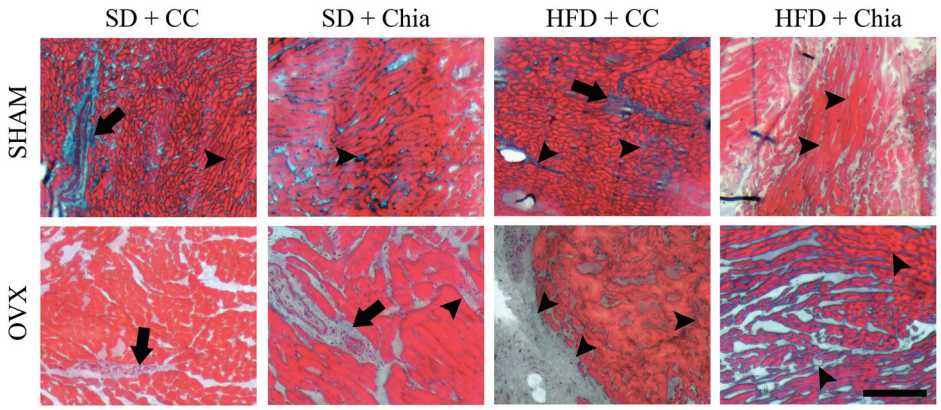

Fig. 4 Representative Gomori's Trichrome stained photomicrographs of the left ventricle of the rats, ovarectomized and the respective SHAM control groups treated with standard diet, high fat diet, calcium carbonate and chia. SD + CC: standard diet + calcium carbonate; SD + chia: standard diet + chia; HFD + CC: high fat diet + calcium carbonate; HFD + chia: high fat diet + chia; SHAM: non-ovariectomized group and OVX: ovariectomized group. Thick arrows indicate the perivascular collagen matrix and the arrowheads indicate the collagen bundles in the cardiac muscle. Reference bar = 200 μm.

matrix regions around the larger caliber vessels, with no beam irradiation between the cardiomyocytes. Little or no thickening of the matrix surrounding the cardiomyocytes was observed (Fig. 4).

4. Discussion

Post-menopause is a period associated with hormonal changes, with consequences on the antioxidant and cardiovascular system. Chia is a good source of polyunsaturated fatty acids (mainly n-3 fatty acids), bioactive compounds, and minerals, whose effect on the heart of adult ovariectomized Wistar rats is under investigation in relation to biometrics, oxidative stress and mineral content. We used an animal model of ovariectomized rats to mimic cardiovascular changes, body composition and oxidative processes caused by hormonal loss in the post-menopausal period, associated with the consumption of a HFD. Our results showed that the combination of ovariectomy with HFD consumption affected biometric parameters, oxidative stress, mineral content and ATPase pump activity, while chia consumption had positive effects on these factors.

It is important to highlight that the diet offered to the animals satisfied 100% of calcium requirement of rodents. However, in the groups that were fed chia, the chia provided 20% of the recommended amount of calcium. The animals received similar amounts of energy even with the intake of different amounts of diet. The higher satiety and higher energy density may justify the lower food intake observed in animals fed HFD (HFD + CC and HFD + chia) in the SHAM group and with HFD + chia in the OVX group, as previously noted.[22] Despite the lower food intake, the OVX groups fed HFD (HFD + CC and HFD + chia) presented greater weight gain and greater visceral adiposity in relation to animals fed SD and SHAM animals. It is suggested that ovariectomy makes rats more likely to accumulate fat due to the lack of hormonal protection. Therefore, HFD can aggravate the effect of ovariectomy, with increased body mass and fat accumulation,[44,45] which corroborates our results for biometric parameters of the heart. The total chia consumption in the OVX and SHAM groups was higher in animals that consumed SD diet than in

animals fed HFD. It is important to highlight that chia is a good source of omega 3.[13] Toko *et al.* (2020) showed that oral administration of omega 3 prevented the development of heart failure by suppressing inflammation. Inflammation is a key factor in the development of hypertrophy and cardiac dysfunction, and the imbalance of metabolites derived from omega 6 and omega 3 causes amplification and continuation of inflammation in the long term, leading to tissue damage. Thus, accelerating the resolution of inflammation contributes to maintaining organ homeostasis.[46]

Lepczyński *et al.* (2021) evaluated the effects of feeding HFD with different fatty acid compositions and all HFD, regardless of the composition of fatty acid, showed a comparable pattern of changes in cardiac proteins, but mice fed with HFD rich in saturated fatty acids manifested more severe cardiac hypertrophy and fibrosis lesions. The animals that consumed the highest amount of omega 3 fatty acid presented less pronounced changes.[47] Creus *et al.* (2016) demonstrated the reversal of dyslipidemia when chia seeds were administered in the diet of rats fed a diet rich in sucrose, which led to a reduction in the storage of lipids in the heart, reaching values similar to those observed in the heart of rats fed with a standard diet. These results were related to the content of omega 3 in the chia seeds.[48]

The OVX and SHAM groups fed chia showed higher polyunsaturated and n-3 fatty acid concentration. The study by Pandurangan *et al.* (2020) showed that chia seed extract controlled the accumulation of lipids in adipocytes, suppressed lipogenesis and hypertrophy of adipocytes, favoring the suppression of the formation of pro-inflammatory cytokines in adipose tissue. The authors attributed these anti-obesity and immunoregulatory effects to the availability of a 3 : 1 ratio of omega 3 and omega 6 fatty acids.[49]

HFD consumption in the OVX group increased the ventricle weight and the ventricle weight/tibia length ratio, which corroborates our results of histopathological analysis that showed a thicker collagen layer upon irradiation between the cardiomyocytes, indicating ventricular hypertrophy. Cardiac hypertrophy in previous studies was associated with both obesity[50] and estrogen deficiency.[51] Regarding obesity, hypertrophy may be caused by factors that increase cardiac load by cardiac compression.[52,53] Besides, inflammatory conditions and fibrosis can affect cardiac remodeling.[54] In ovariectomized rats, estrogen has been shown to decrease hypertrophy induced by pressure overload by an estrogen-dependent mechanism, which increases calcineurin degradation[55] and decreases the left ventricular mass and ventricular weight/body weight ratio.[56]

The SHAM animals fed chia presented lower weight and volume of fat in the heart than the OVX animals that consumed the same diet, which indicates that chia in the presence of estrogen in the SHAM group may reduce the fat content in the heart. This fact revealed a cardioprotective action, but the deleterious effects of ovariectomy could not be reversed. The nutritional composition of chia, with a large amount of α-linolenic acid (omega 3), has been related to its benefits associated with CVD markers,[19–21] since the consumption of α-linolenic acid can be beneficial and is associated with a moderately lower risk of CVD.[21,57] Peptides are also related to the cardioprotective benefits of chia, due to their antioxidant activity and the reduction of inflammatory and atherosclerotic markers.[15,16]

Oxidative stress, an unbalanced condition between the production and decomposition of reactive oxygen species (ROS),[58] is directly related to CVD.[59] Chia consumption is associated with higher antioxidant activity of the catalase enzyme and increased SOD expression in the liver of Wistar OVX rats.[22] In our study, ovariectomy showed lower catalase activity when associated with HFD compared to the SHAM group fed the same diet. However, when chia was added to this diet, the catalase activity was comparable to that of the SHAM group. When ovariectomy was associated with HFD + chia, increased SOD concentration was observed, which suggests a compensatory mechanism for the increased levels of ROS observed in the OVX group fed HFD. In addition, OVX animals fed HFD + CC showed higher NO production, which may increase the production of RNS.[11,60] However, when chia with HFD was fed to the OVX group, the NO concentration returned to levels comparable to those in the control group.

It is known that estrogen positively regulates the eNOS activity that may be involved in cardiovascular protection by estrogen observed in women.[61–64] HFD and ovariectomy can lead to increased generation of ROS and reduced antioxidant capacity. Besides, when these conditions were associated with chia consumption, a protective mechanism that increased SOD and reduced NO was observed, which indicates potential of chia to inhibit and/or reduce the damage caused by the action of ROS. The antioxidant capacity of chia is determined by its nutritional composition in relation to the profile of peptides,[16] phenolic acids and lipophilic compounds, such as carotenoids, tocopherols, phospholipids and alpha linolenic acid, and the interactions and synergistic activity between these components.[21]

We evaluated the relationship between minerals and antioxidant enzymes. Redox metabolism presents minerals acting as cofactors for antioxidant enzymes, whose activity requires the availability and mobilization of these elements.[65] Imbalance in mineral levels can alter the expression and synthesis of the antioxidant enzymes that maintain redox balance and prevent oxidative stress.[66] The variation in the content of the minerals copper, zinc and manganese, cofactors of the SOD enzyme, and iron, cofactor of the catalase enzyme, was not necessarily accompanied by the corresponding variations in these enzymes in the heart. However, in the OVX group fed SD + chia, reduced Zn concentration was accompanied by a reduced amount of SOD. Thus, enzyme reduction is justified by the reduction of this mineral, which would be mobilized for the antioxidant system. In the OVX groups, the diet did not change the calcium concentration in the heart. But in SHAM animals, HFD decreased the calcium concentration in the heart, and when the animals were fed chia, the calcium concentrations were comparable to those in animals fed CC. These results corroborate the

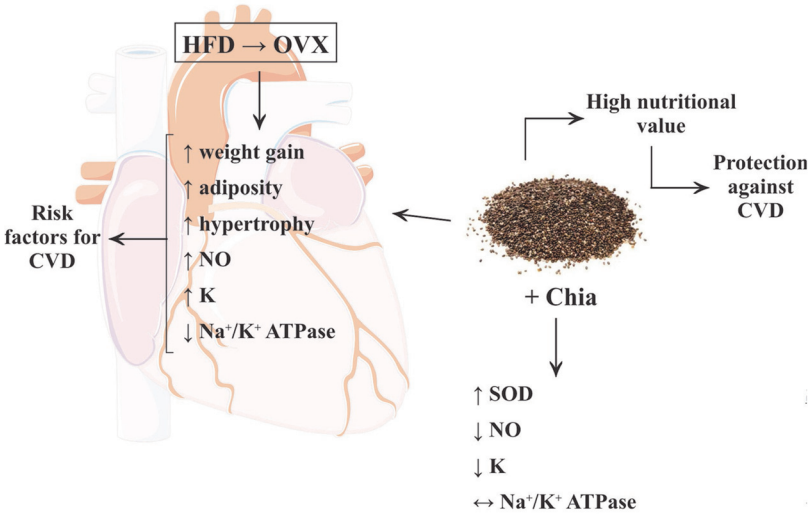

Fig. 5 Effect of addition of chia to a high fat diet on the heart of ovariectomized Wistar rats. HFD: high fat diet; OVX: ovariectomy; CVD: cardiovascular disease; NO: nitric oxide; K: potassium; ↑: increased; ↓: decreased; ↔: maintained.

findings of another study of ours[67] which has reported that HFD decreases calcium retention.

Our results demonstrated that the OVX group fed HFD presented reduced total ATPase activity, Na^+/K^+ATPase and Mg^{2+}ATPase compared to SHAM animals fed the same diet, which suggests that HFD is an aggravating factor for the effects of ovariectomy, and that the consumption of chia did not reverse this effect. Hyperlipidemia and oxidative stress are associated with the disturbance of membrane fluidity and stability, which affects the activity of membrane-associated enzymes.[43,68,69] Na^+/K^+ATPase is responsible for the electrochemical gradient of sodium and potassium ions, which maintain a low intracellular concentration of Na and high intracellular level of K. This enzyme helps in maintaining the potential of the cell membrane and regulates vascular tone. Changes in its activity can worsen the cardiovascular outcome.[43,70,71] Magnesium contributes to cardiac electrophysiology, and both extracellular and intracellular magnesium affect cardiac ion channels and, consequently, the duration of the action potential, cellular excitability and contractility.[42] Ca^{2+}ATPase is the main active calcium transport protein in the plasma membrane, and it maintains the normal levels of intracellular calcium and removes calcium from the cell, thus reducing free intracellular calcium. Changes in calcium homeostasis have been linked to heart failure.[72–74] In muscle contraction, Ca^{2+} is released from the sarcoplasmic reticulum and Ca^{2+}ATPase pumps back the released Ca^{2+} to cause relaxation.[75] Although the SHAM group fed HFD had lower calcium concentration in the heart, our study observed no changes in the Ca^{2+}ATPase activity, which indicates that calcium homeostasis was not altered; thus, the contraction function was not affected by the treatments, in relation to the mineral content.

In our study, the consumption of HFD by OVX animals resulted in deleterious effects to the heart in relation to ventricular hypertrophy, increased animal weight gain and visceral adiposity, increased oxidative stress and reduced activity of ATPase enzymes, which can impair the cardiovascular outcome. Chia consumption presented a cardioprotective effect in relation to the fat content in the heart of SHAM animals fed with a SD and HFD, and potential antioxidant capacity to reduce the damage caused by oxidative stress due to a HFD diet in OVX animals (Fig. 4). The analysis of minerals demonstrated that reduced Zn concentration was accompanied by reduced amount of SOD in the OVX group fed SD + chia. The variation in the other minerals analyzed was not necessarily accompanied by variations in antioxidant enzymes or ATPases (Fig. 5).

5. Conclusion

The HFD consumption exacerbated the deleterious effects of ovariectomy on the heart. The consumption of chia, a source of polyunsaturated fatty acids, with HFD by OVX rats improved the antioxidant activity, which indicates its potential to inhibit and/or reduce the damage caused by the action of ROS by increasing SOD and reducing NO. Chia reduced the heart fat content when consumed by SHAM animals. The contents of minerals and ATPase enzymes in the heart were maintained in OVX animals that consumed chia, which demonstrates its cardioprotective activity. However, the deleterious effects of ovariectomy were not reversed.

Compliance with ethical standards

All procedures in this study involving animals were performed in accordance with the ethical standards of the Federal University of Viçosa and the UK. Animals (Scientific Procedures) Act, 1986.

Conflicts of interest

The authors declare that they have no conflicts of interest.

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References

1 O. Taleb-belkadi, H. Chaib, L. Zemour, A. Fatah, B. Chafi and K. Mekki, *Gynecol. Endocrinol.*, 2016, **32**, 982–985.
2 A. Ambikairajah, E. Walsh, H. Tabatabaei-Jafari, *et al.*, *Am. J. Obstet. Gynecol.*, 2019, **221**, 393–409.
3 R. López-Grueso, J. Gambini, K. M. Abdelaziz, *et al.*, *Antioxid. Redox Signaling*, 2014, **20**, 236–246.
4 G. A. Roth, C. Johnson, A. Abajobir, F. Abd-Allah, S. F. Abera, G. Abyu, *et al.*, *J. Am. Coll. Cardiol.*, 2017, **70**, 1–25.
5 C. L. Blenck, P. A. Harvey, J. F. Reckelhoff and L. A. Leinwand, *Circ. Res.*, 2016, **118**, 1294–1312.
6 V. Regitz-Zagrosek and G. Kararigas, *Physiol. Rev.*, 2017, **97**, 1–37.
7 Y. B. Somani, J. A. Pawelczyk, M. J. De Souza, *et al.*, *Am. J. Physiol.: Heart Circ. Physiol.*, 2019, **317**, H395–H404.
8 T. M. Suboc, K. Dharmashankar, J. Wang, R. Ying, A. Couillard, M. J. Tanner and M. E. Widlansky, *Physiol. Rep.*, 2013, **1**, 1–10.
9 E. Murphy, *Circ. Res.*, 2012, **109**, 687–696.
10 J. Wu, F. Dai, C. Li, *et al.*, *J. Cardiovasc. Transl. Res.*, 2019, **13**(1), 73–84.
11 L. L. Ventura, N. C. Fortes, H. C. Santiago, M. V. Caliari, M. A. Gomes and D. R. Oliveira, *PeerJ*, 2017, **5**, e2967.
12 B. P. Da Silva, R. C. L. Toledo, M. Grancieri, M. E. C. Moreira, N. R. Medina, R. R. Silva, N. M. B. Costa and H. S. D. Martino, *Food Res. Int.*, 2019, **116**, 592–599.
13 B. P. Da Silva, P. C. Anunciação, J. C. S. Matyelka, *et al.*, *Food Chem.*, 2017, **221**, 1709–1716.
14 M. Grancieri, H. S. D. Martino and E. G. Mejia, *Compr. Rev. Food Sci. Food Saf.*, 2019a, **18**, 480–499.
15 M. Grancieri, H. S. D. Martino and E. G. Mejia, *Food Chem.*, 2019b, **289**, 204–214.
16 M. Grancieri, H. S. D. Martino and E. G. Mejia, *Mol. Nutr. Food Res.*, 2019c, **63**, 1–15.
17 A. T. Derek, in *Calcium Signaling*, ed. S. Islam, Spinger, Switzerland, 2nd edn, 2020, pp. 395–435.
18 D. Melo, T. B. Machado and M. B. P. P. Oliveira, *Food Funct.*, 2019, **10**, 3068–3089.
19 L. T. Toscano, M.Sc dissertation, Universidade Federal da Paraíba, 2014.
20 M. A. Fortino, *et al.*, *Prostaglandins, Leukotrienes Essent. Fatty Acids*, 2017, **116**, 19–26.
21 B. N. Enes, L. P. D. Moreira, B. P. Da Silva, *et al.*, *J. Food Sci.*, 2020, **85**, 226–239.
22 B. P. Da Silva, R. C. L. Toledo, M. D. V. Mishima, *et al.*, *Food Funct.*, 2019b, **10**, 4036–4045.
23 P. Reeves, F. Nielsen and G. Fahey, *J. Nutr.*, 1993, 1939–1951.
24 S. S. Pereira, L. G. Teixeira, E. C. Aguilar, R. O. Matoso, F. L. P. Soares, A. V. M. Ferreira and J. I. Alvarez-Leite, *Anim. Sci. J.*, 2012, **83**, 549–555.
25 W. Scherle, *Mikroskopie*, 1970, **26**, 57–60.
26 M. M. Bradford, *Anal. Biochem.*, 1976, **72**, 248–254.
27 S. Marklund, *Handbook of Methods for Oxygen Radical Research*, 1985, pp. 243–247.
28 H. I. Kohn and M. Liversedge, *J. Pharmacol. Exp. Ther.*, 1944, **82**, 292–300.
29 L. A. Pyles, E. J. Stejskal and S. Einzig, *Proc. Soc. Exp. Biol. Med.*, 1993, **202**, 407–419.
30 J. A. Buege and S. D. Aust, *Methods Enzymol.*, 1978, **52**, 302–310.
31 H. Aebi, *Methods Enzymol.*, 1984, **105**, 121–126.
32 L. C. Green, D. A. Wagner, J. Glogowski, *et al.*, *Anal. Biochem.*, 1982, **126**, 131–138.
33 L. C. M. Ladeira, E. C. Dos Santos, G. E. Valente, J. Da Silva, T. A. Santos and I. R. S. C. Maldonado, *Biol. Trace Elem. Res.*, 2019, **196**, 168–172.
34 K. S. Al-Numair, C. Veeramani, M. A. Alsaif, *et al.*, *Pharm. Biol.*, 2015, **53**, 1372–1378.
35 M. N. Sertorio, A. C. F. Souza, D. S. S. Bastos, F. C. Santos, L. O. G. Ervilha, K. M. Fernandes, L. L. De Oliveira and M. Machado-Neves, *Environ. Sci. Pollut. Res.*, 2019, **26**, 12459–12469.
36 C. H. Fiske and Y. Subbarow, *J. Biol. Chem.*, 1925, **66**, 375–400.
37 E. Da Silva, *et al.*, *Pathol., Res. Pract.*, 2016, **212**, 325–334.
38 C. Glorieux and P. B. Calderon, *Biol. Chem.*, 2017, **398**, 1095–1108.
39 G. Bresciani, I. B. M. Da Cruz and J. González-Gallego, *Adv. Clin. Chem.*, 2015, **68**, 87–130.
40 A. Ayala, M. F. Muñoz and S. Argüelles, *Oxid. Med. Cell. Longevity*, 2014, **2014**, 360438.
41 B. Halliwell and J. M. C. Gutteridge, *Free radicals in biology and medicine*, Oxford University Press, Oxford, 2015.
42 J. Li, M. K. Hovey, C. A. Andrews, A. Quddus, *et al.*, *J. Women's Health*, 2020, **29**, 7–12.
43 L. Sousa, M. T. C. Pessoa, T. G. F. Costa, *et al.*, *Ann. Hematol.*, 2018, **97**, 377–385.
44 J. Hong, R. E. Stubbins, R. R. Smith, A. E. Harvey and N. P. Núñez, *Nutr. J.*, 2009, **8**, 1–5.

45 A. Ludgero-Correia, M. B. Aguila, C. A. Mandarim-de-Lacerda, *et al.*, *Nutrition*, 2012, **28**, 316–323.
46 H. Toko, H. Morita, M. Katakura, *et al.*, *Sci. Rep.*, 2020, **10**, 1–11.
47 A. Lepczyński, M. Ożgo, K. Michałek, *et al.*, *Nutrients*, 2021, **13**, 1–19.
48 A. Creus, M. Ferreira, M. Oliva, *et al.*, *J. Clin. Med.*, 2016, **5**, 18.
49 S. B. Pandurangan, S. A. Al-Maiman, L. N. Al-Harbi, *et al.*, *Foods*, 2020, **9**(3), 368.
50 T. G. Alexescu, A. Cozma, A. Sitar-Tăut, *et al.*, *Rom. J. Intern. Med.*, 2016, **54**, 161–172.
51 P. Rouet-Benzineb, R. Merval and E. Polidano, *Physiol. Rep.*, 2018, **6**, e13912.
52 M. A. Alpert, *Am. J. Med. Sci.*, 2018, **6**, 225–236.
53 B. C. Bernardo, K. L. Weeks, L. Pretorius and J. R. Mcmullen, *Pharmacol. Ther.*, 2010, **128**, 191–227.
54 I. Manabe, *Circ. J.*, 2011, **75**, 2739–2748.
55 C. Donaldson, S. Eder, C. Baker, M. J. Aronovitz, A. D. Weiss, M. Hall-Porter, *et al.*, *Circ. Res.*, 2009, **104**, 265–275.
56 M. Van Eickels, C. Grohe, J. P. Cleutjens, B. J. Janssen, H. J. Wellens and P. A. Doevendans, *Circulation*, 2001, **104**, 1419–1423.
57 A. Pan, M. Chen, R. Chowdhury, *et al.*, *Clin. Nutr.*, 2012, **96**, 1262–1273.
58 A. Agita and M. Thaha, *Acta Med. Indones.*, 2017, **49**, 158–165.
59 B. Skibska and A. Goraca, *Oxid. Med. Cell. Longevity*, 2015, **2015**, 313021.
60 S. Novella, A. P. Dantas, G. Segarra, P. Medina and C. Hermenegildo, *Front. Physiol.*, 2012, **3**, 165.
61 S. Nuedling, S. Kahlert, K. Loebbert, P. A. Doevendans, R. Meyer, H. Vetter and C. Grohe, *Cardiovasc. Res.*, 1999, **43**, 666–674.
62 J. Sun, E. Picht, K. S. Ginsburg, D. M. Bers, C. Steenbergen and E. Murphy, *Circ. Res.*, 2006, **98**, 403–411.
63 J. Lin, C. Steenbergen, E. Murphy and J. Sun, *Circulation*, 2009, **120**, 245–254.
64 S. Chakrabarti, O. Lekontseva, A. Peters and S. T. Davidge, *Cardiovasc. Res.*, 2010, **85**, 796–805.
65 K. O. Soetan, C. O. Olaiya and O. E. Oyewole, *Afr. J. Food Sci.*, 2010, **4**, 200–222.
66 A. C. F. Souza, S. C. Marchesi, G. D. A. Lima, *et al.*, *Biol. Trace Elem. Res.*, 2018, **183**, 305–313.
67 M. D. V. Mishima, B. P. Da Silva, R. C. L. Toledo, *et al.*, *J. Am. Coll. Nutr.*, 2020, **30**, 1–11.
68 P. V. A. Babu, K. E. Sabitha, *et al.*, *Pharmacol. Res.*, 2006, **162**, 157–164.
69 K. Srikanthan, J. Shapiro and K. Sodhi, *Molecules*, 2016, **21**, 1172.
70 J. Palacios, E. T. Marusic, N. C. Lopez, M. Gonzalez and L. Michea, *Am. J. Physiol.: Heart Circ. Physiol.*, 2004, **286**, 1793–1800.
71 R. F. Ribeiro Junior, J. Fiorim, *et al.*, *Vasc. Pharmacol.*, 2017, **99**, 23–33.
72 K. F. Frank, B. Bolck, K. Brixius, *et al.*, *Basic Res. Cardiol.*, 2002, **97**, 72–178.
73 B. Pieske, L. S. Maier, V. Piacentino, *et al.*, *Circulation*, 2002, **106**, 447–453.
74 K. Toischer, S. E. Lehnart, G. Tenderich, *et al.*, *Basic Res. Cardiol.*, 2010, **105**, 279–287.
75 C. Toyoshima, *Biochim. Biophys. Acta*, 2009, **1793**, 941–946.

