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Chemical

Merlot wine and the metabolic and cardiovascular changes in LDLR - / - mice

Vinho merlot e as alterações metabólicas e cardiovasculares em camundongos LDLR -/-

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ABSTRACT

Dyslipidemia is considered a risk factor for the development of insulin resistance and cardiovascular diseases, such as left ventricular hypertrophy and remodeling of the arterial wall. Resveratrol, present in grapes, has been studied as a possible mediator of cardiovascular protection. The present study aimed to evaluate the effect of red wine from the Merlot grape (*Vitis vinifera L.*) on dyslipidemia and its involvement in male mice, knockout for the LDL receptor gene (LDLR-/-). The mice were divided into 4 experimental groups: Group S received standard rodent food; Group SV received standard feed for rodents and wine; Group HL received high fat diet; Group HLV received hyperlipidic food and wine orally for 60 days. All animals received water and food *ad libitum* during this period. After this period, they remained fasting and were then anesthetized. Blood was collected for serum analysis of triglycerides, total cholesterol, and its fractions (HDL and VLDL), C-reactive protein, glucose, and insulin to compare the data. Morphometric and histological analyzes of the left ventricle and abdominal aorta were also performed. It was found that Merlot red wine has antidyslipidemic effects on genetic dyslipidemia, associated with food, partially preventing insulin resistance, hyperinsulinemia and increased CRP; as well as cardiovascular effect, partially preventing LVH and arterial remodeling.

Keywords: Functional foods; Resveratrol; Cardiovascular diseases; Insulin; Cholesterol; Dyslipidemia

RESUMO

A dislipidemia é considerada um fator de risco para o desenvolvimento de resistência insulínica e de doenças cardiovasculares, como a hipertrofia ventricular esquerda e o remodelamento da parede arterial. O resveratrol, presente nas uvas, tem sido estudado como um possível mediador de proteção cardiovascular. O presente estudo teve como objetivo avaliar o efeito do vinho tinto da uva *Merlot* (*Vitis vinifera L*.) na dislipidemia e seus acometimentos em camundongos machos, knockout para o gene do receptor de LDL (LDLR-/-). Os camundongos foram divididos em 4 grupos experimentais: Grupo S recebeu ração padrão para roedores; Grupo SV recebeu ração padrão para roedores e vinho; Grupo HL recebeu ração hiperlipídica; Grupo HLV recebeu ração hiperlipídica e vinho, via oral, durante 60 dias. Todos os animais receberam água e ração *ad libitum* nesse período. Após este, permaneceram em jejum e, em seguida, foram anestesiados. O sangue foi coletado para análise sérica dos triglicérides, do colesterol total e suas frações (HDL e VLDL), da proteína C-reativa, glicose e insulina para comparação dos dados. Também foram realizadas análises morfométricas e histológicas do ventrículo esquerdo e da aorta abdominal. Constatou-se que o vinho tinto *Merlot* apresenta efeitos antidislipidêmicos na dislipidemia genética, associada a alimentar, prevenindo, parcialmente, a resistência insulínica, hiperinsulinemia e o aumento da PCR, assim como efeito cardiovascular, prevenindo, parcialmente, a HVE e o remodelamento arterial.

Palavras-Chave: Alimentos funcionais; Resveratrol; Doenças cardiovasculares; Insulina; Colesterol; Dislipidemia

1 INTRODUCTION

Elevated plasma levels of low-density lipoprotein cholesterol (LDLc) (hypercholesterolemia), reduced levels of high-density lipoprotein cholesterol (HDLc) and/or increased triglycerides (TG) (hypertriglyceridemia) characterize the clinical condition called dyslipidemia (Ferreira 2019). This is considered a risk factor for the development of cardiovascular diseases such as atherosclerosis (Hosvsepian et al., 2015), left ventricular hypertrophy (LVH) (Martins et al., 2020; Sarto et al., 2018; Garcia & Incerpi 2008), remodeling of arterial wall cells (Silva et al., 2015) and insulin resistance (Sarto et al., 2018).

It is known that the predominance of insulin resistance in patients with established cardiovascular disease is high (Marcadanti et al., 2013). Insulin resistance conditions have been increasingly studied, and the concern on this issue has been the subject of several studies (Oliveira et al., 2020; Valença et al., 2018; Bischoff 2017).

In this second decade of the 21st century, several strategies for the control of dyslipidemias have been described by several authors (Martins et al., 2020; Vileigas et al., 2019; Sarto et al., 2018; Silva et al., 2015; Calderon et al., 2011), a period in which Statin established itself as the most widely used pharmaceutical drug for its treatment (Veloso 2019). However, the continuous and long-term use of statins tends to cause serious side effects (Gois 2019), such as muscular, cognitive, sexual, memory, pain, or numbness problems in the extremities (Rodrigues et al., 2019).

Studies with functional foods and their medicinal properties have been growing as alternative treatments in the control of dyslipidemia (Martins et al., 2020; Vileigas et al., 2019; Sarto et al., 2018; Braga and Barleta, 2017; Silva et al., 2015; Calderon et al., 2011). The main active compounds referenced are carotenoids and polyphenols, such as isoflavones and flavonoids (Braga & Barleta, 2017).

Some researchers have demonstrated that wine is beneficial for health with protective effects on different target organs (Rosa et al., 2017; Anastácio 2012; Penna & Hecktheuer 2004). Red wine, if consumed in moderation, has the most benefits. In their manufacturing process, husks and seeds are used, increasing the concentration levels of antioxidants, among them, resveratrol, and flavonoids (Mamede & Pastore 2004). It has a beneficial action in controlling cholesterol and blood pressure, rejuvenation, preventing some types of cancer and reducing the incidence of inflammatory diseases (Bontempo 2019; Vaccari et al., 2009).

Considering the increase in the applicability of red wine as a functional food, there is a need for research that elucidates the beneficial and harmful effects of consuming this beverage. Moreover, there are only a few reports in the literature on the effects generated by red wine from the *Merlot* grape (*Vitis vinifera L.*). Thus, the present study aimed to evaluate its effect in the prevention of dyslipidemia, left ventricular hypertrophy (LVH), remodeling of the abdominal aortic wall and insulin resistance in dyslipidemic mice fed a high-fat diet.

2 MATERIAL AND METHODS

2.1 Analysis of red wine polyphenols

The wine used in the present study was the Reservado red Merlot (2019 batch) 750 ml, which was evaluated for the Total Polyphenols Index (TPI) using the absorbance method at 280nm (A280). The analysis was performed in a Multiskan Sky spectrophotometer, a 7 ml quartz cuvette was filled with wine and placed in the equipment to measure phenolics by quantifying the UV light absorption at 280nm by compounds with aromatic rings present in the sample, as previously described by Habertson and Spayd (2006). The analysis was performed at the Enolab Laboratory, located in Flores da Cunha, RS.

2.2 Animal protocol

The experiments were conducted with 40 male mice, homozygous for the LDL receptor gene (LDLR- / -), aged four months and weighing 25 ± 3 g, divided into groups of ten animals each (Table 1). The animals were kept under controlled temperature (25 ± 1 ºC) and a 12-hour light/dark cycle.

Table 1– Experimental groups and respective protocols

Group	Experimental Protocol	Denomination
1	LDLR - / - mice received standard rodent food (NuvitalTM, Nuvilab, Colombo, Brazil) for 60 days.	S
2	LDLR - / - mice received standard rodent food (NuvitalTM, Nuvilab, Colombo, Brazil) and 4.3mL/kg/day of red *Merlot* wine (*Vitis vinifera L.*) orally for 60 days.	SV
3	LDLR - / - mice received high fat food with 20% total fat, 1.25% cholesterol and 0.5% cholic acid; a total of 2.89 kcal/g (Instituto Tecnológico de Alimentos, Campinas, Brazil) for 60 days.	HL
4	LDLR - / - mice received high fat food with 20% total fat, 1.25% cholesterol and 0.5% cholic acid; a total of 2.89 kcal/g (Instituto Tecnológico de Alimentos, Campinas, Brazil) and 4.3mL/kg/day of red Merlot wine (*Vitis vinifera L.*) orally for 60 days.	HLV

Source: Authors (2022)

All animals received water and food *ad libitum*. The wine used was the Reserved red *Merlot* (2019's batch) 750mL, obtained commercially. 4.3 mL/kg/day were administered, by gavage, this volume corresponds to two glasses of wine ingested by an adult human being (Vaccari et al., 2009). The experimental procedures were conducted according to guidelines established by the National Council for the Control of Animal Experiences (CONCEA, in portuguese) and approved by the Animal Ethics Committee of the José do Rosário Vellano University (UNIFENAS, in portuguese), Alfenas-MG, Brazil (nº 35A/2015).

After 60 days of experiment, the rats were fasted for 8 hours and subsequently anesthetized intraperitoneally using xylazine (Bayer AG) and ketamine (Parke-Davis®), in concentrations of 6 and 40 mg/kg, respectively. Blood samples were collected by puncturing the retro-orbital venous plexus, using heparinized capillary tubes for serum analysis of triglycerides (TG), total cholesterol (CT), high density lipoprotein (HDL), C-reactive protein (CRP), glucose and insulin, which were performed at the H. Pardini laboratory (Belo Horizonte-MG, Brazil).

2.3 Serum analyzes

2.3.1 Blood serum

The serum was obtained by centrifuging the blood (1200g, 4ºC, 10 minutes). TG, CT and HDLc were measured using the colorimetric enzymatic methods, using protocols described in commercial kits (In Vitro®) by automation (Hedrick et al., 2001).

2.3.2 Serum analyzes of triglycerides

Triglycerolemia was determined by the colorimetric enzymatic method that uses four enzymes, according to Labtest's GPO-ANA triacylglycerol kit. The glycerol released by the hydrolysis of the triacylglycerol contained in the serum, catalyzed by the lipoprotein lipase, is converted by the action of glycerolkinase into glycerol-

3-phosphate, which is oxidized to dihydroxyacetone and hydrogen peroxide in the presence of glycerol phosphate oxidase. The coupling reaction between hydrogen peroxide, 4-aminoantipyrine and ESPAS is catalyzed by peroxidase, producing quinoneimine, which has a maximum absorbance of 540nm (Trinder, 1969). The Kit is composed of: 50mmol/L buffer solution, pH 6.5; Mg acetate 5mmol/L, ESPAS 1 mmol/L, 4-aminoantipyrine 0.7 mmol/L, ATP 0.3 mmol/L, glycerolkinase ≥ 800U/L, glycerolphosphate ≥2500U/L, lipoprotein lipase ≥100KU/L, peroxidase ≥ 350U/L, sodium azide 1.54mmol/L. The samples were prepared according to the manufacturer's instructions, and, after reading the absorbance at 540nm, the triglyceride concentration was calculated in mg/dL.

2.3.4 Serum analysis of total cholesterol

Cholesterolemia was determined by the enzymatic method, according to the Labtest Liquiform cholesterol kit, using an association of the oxidation reaction catalyzed by cholesterol oxidase, after hydrolysis of cholesterol esters, with an absorbance reading at 500 nm (Trinder, 1969). The dosage kit is composed of: 50 mmol/L buffer containing 0.01g/dL, pH 7.0, cholesterol esterase (> 150U/L), cholesterol oxidase (> 175U/L), peroxidase (> 1000U/L), 4-aminoantipyrine 0.5nmol/L, phenol 2.4mmol/L, standard solution 200mg/dL, and food preservatives. The samples were prepared according to the kit manufacturer's instructions, and after reading the absorbance at 500 nm, the cholesterol concentration in mg/dL was calculated.

2.3.5 Serum HDL analysis

Labtest's HDL cholesterol kit enzyme system was used for precipitation of low- and high-density lipoproteins (LDL and VLDL) and determination of HDL cholesterol in the supernatant after centrifugation (Warnick et al., 2001). The kit contains: precipitant with phosphotungstic acid 1,5nmol/L and magnesium chloride 54 mmol/L, 20mg/dL HDL standard solution and color reagent - cholesterol Liquiform Labtest. After measuring

the absorbance of the samples at 500nm, the concentration of HDL cholesterol in mg/dL was calculated.

2.3.6 C-reactive protein

CRP levels were measured by commercially available turbidimetric method. The turbidimetric method (Biotécnica Indústria e Comércio, Varginha, MG, Brazil) assesses agglutination of latex particles coated with antibody against CRP by quantifying the absorbed light (Sung et al., 2002) (detection limit> 0.4mg / dL).

2.6.7 Glucose

Blood samples were placed in Vacutainer® tubes and centrifuged at 3,300 rpm (1,950 G) for 10 minutes. The plasma obtained was kept refrigerated (4 ° C) until it was used to measure the plasma glucose concentration by the enzymatic glucose oxidase method, with a commercial colorimetric kit (Doles®). For each test performed, 1 ml of the enzyme reagent was used in a test tube for 0.01 ml of the analyzed serum sample, in duplicate, which after homogenization were placed in a water bath at 37 º C for 10 minutes. After this time, the absorbance was read on a spectrophotometer at 505 nm. The glucose value, in mg/dL, was calculated in relation to the standard glucose solution provided by the kit (100 mg/dL).

2.3.8 Insulin

To determine the serum insulin concentration, the commercial kit “Rat/Mouse Insulin ELISA kit” (Merck Millipore, USA) was used. This kit is sensitive for insulin determination, using the “sandwich ELISA” method (Enzyme-Linked Immunoabsorbent Assay). Briefly, in this technique, the antibody (anti-insulin) was immobilized on the microplate. The sample (10 µL) containing the insulin was added, reacting with the immobilized anti-insulin antibody. After washing the wells, a second anti-insulin antibody linked to a peroxidase was added, allowing the reaction with the antibody/

insulin complex present in the microplate. After the second free antibody was washed away, the 3,3 ‘, 5,5’-tetramethylbenzidine (TMB) substrate was added, and the colored reaction product was measured in the microplate reader at 450 and 590nm.

2.3.9 Evaluation of the homeostasis model (HOMA-IR)

The Homa index (HOMA-IR) was calculated using the formula: {Homa-ir = [rapid insulinemia (mU/L) x rapid glycemia (mmol/L)]/22.5} to determine insulin resistance.

2.4 Morphometric and histological analyzes

After a thoracotomy, the heart was removed, dissected and the left ventricle was weighed (mg). The LVH index was calculated by the proportion of left ventricular weight (mg) and body weight (g). The left ventricle was fixed for 24 hours in 10% formalin. Then, it was included in paraffin for histological sections of four micrometers thick, according to Junqueira et al. (1979). The histological sections were stained with picrosirius red (PR) and with hematoxylin/eosin (HE) for quantitative analysis of collagen and for morphometric analysis of cardiomyocytes, respectively. The sections stained with PR were analyzed with polarized light. Each micrograph was analyzed using the LGMC-IMAGE version 1.0 software, through which fractional collagen percentages for the areas marked in red were obtained (Shirani et al., 2000). From the HE-stained histological sections, four photomicrographs (400x) were obtained from the same pre-fixed point of the cross sections of the ventricle of each mouse, using the digital camera coupled to the Leica IM50 program (version 1.20). The diameters of 8 to 12 cardiomyocytes from each histological section were measured, totaling 12 sections in each animal (Armstrong et al., 1998).

The abdominal aorta was fixed together with the ventricles, both included in paraffin, histological sections of the aorta were stained with PR. The measurement of the luminal, medium and intimate areas was performed using the LGMC-IMAGE version 1.0 software. The average area is that between the external and internal elastic lamina. The intima area is the one between the inner face of the inner elastic lamina and the endothelial surface of the lumen.

Histological sections of the aorta artery stained with PR were also used for quantitative analysis of collagen. Four photomicrographs were obtained from the same pre-fixed point of the cross sections of the aortas of each animal using the digital camera coupled to the Leica IM50 program (version 1.20). The sections stained with PR were analyzed with polarized light. Each photomicrograph was analyzed using the LGMC-IMAGE version 1.0 software, through which fractional collagen percentages were acquired (Armstrong et al., 1998), referring to the areas marked in red by the PR. The percentages of collagen were quantified in the middle and intima areas of the artery wall. In the quantification of the total area, the adventitial area is included.

All histological analyzes were performed by a single examiner, using the double-blind method.

2.5 Statistical analysis

The data were expressed as mean ± standard error of the mean (SEM). The analysis of variance (ANOVA) was followed by the Tukey test to compare the means between the different groups. The differences were considered significant at $p < 0.05$. All statistical treatments were performed using the Graphpad Instat TM statistical software, version 3.05 for Windows (San Diego, CA, USA).

3 RESULTS

In the analysis of the TPI of the wine used in the experiment, an average index of 39.23 nm was observed. LDLR - / - mice that were fed with a high-fat diet (HL group) developed severe mixed dyslipidemia followed by a reduction in serum HDLc levels. In the lipidemic profile, it was observed that *Merlot* wine partially prevents the increase in triglycerides, total cholesterol, and the decrease in HDLc in the HLV Group compared to the HL and S groups (Table 2).

The hyperlipidic diet in LDLR - / - mice (HL group), in addition to inducing severe mixed dyslipidemia, generated insulin resistance with hyperinsulinemia and increased serum CRP levels (Table 2) when compared with Groups S and SV. However, *Merlot* wine partially prevented insulin resistance and increased serum levels of CRP without altering the serum glucose level in mice in the HLV group when compared with the HL and S groups (Table 2).

Table 2 – Serum profile of triglycerides (TG), total cholesterol (TC), HDL cholesterol (HDLc), C-reactive protein (CRP), glucose, insulin and HOMA Index (HOMA-IR)

Groups	**S**	**SV**	**HL**	**HLV**
N	7	9	6	8
TG (mg/dL)	165±10^{c}	146±12^{c}	297±24^{a}	206±9^{b}
TC (mg/dL)	263±8^{c}	163±15^{c}	870±15^{a}	403±21^{b}
HDLc (mg/dL)	68±3^{a}	59±3^{a}	20±1^{c}	39±1^{b}
CRP (mg/dL)	6±0,9^{c}	5,9±0,6^{c}	14±1^{a}	9,7±1^{b}
Glucose (mMol/L)	5,6±0,2^{a}	5,8±0,3^{a}	6,0±0,3^{a}	5,9±0,5^{a}
Insulin (mU/L)	2,7±0,2^{c}	2,5±0,2^{c}	6,1±0,8^{a}	4,5±0,4^{b}
Homair	0,7±0,07^{c}	0,6±0,03^{c}	1,6±0,03^{a}	1,2±0,1^{b}

Values were expressed as mean ± SEM. Different letters indicate significant differences between groups ($p < 0.05$ - Tukey's test). S - standard food; SV - standard food and wine; HL - hyperlipidic diet; HLV - hyperlipidic food and wine; mg/dL - milligrams per deciliter; mMol/L - millimol per liter; mU/L - milliunits per liter.
Source: Authors (2022)

The hyperlipidic diet, associated with genetic dyslipidemia, also triggered LVH in mice in the LH Group, determined by the increase in the proportion of left ventricular weight (mg) by mouse weight (g), characterized by an increase in the diameter of cardiomyocytes and the percentage of interstitial collagen in the ventricular myocardium. The ingestion of *Merlot* wine had a beneficial effect, partially preventing the establishment and evolution of LVH in the left ventricular morpho-histological parameters in the HLV group when compared with the HL group (Table 3, Figure 1).

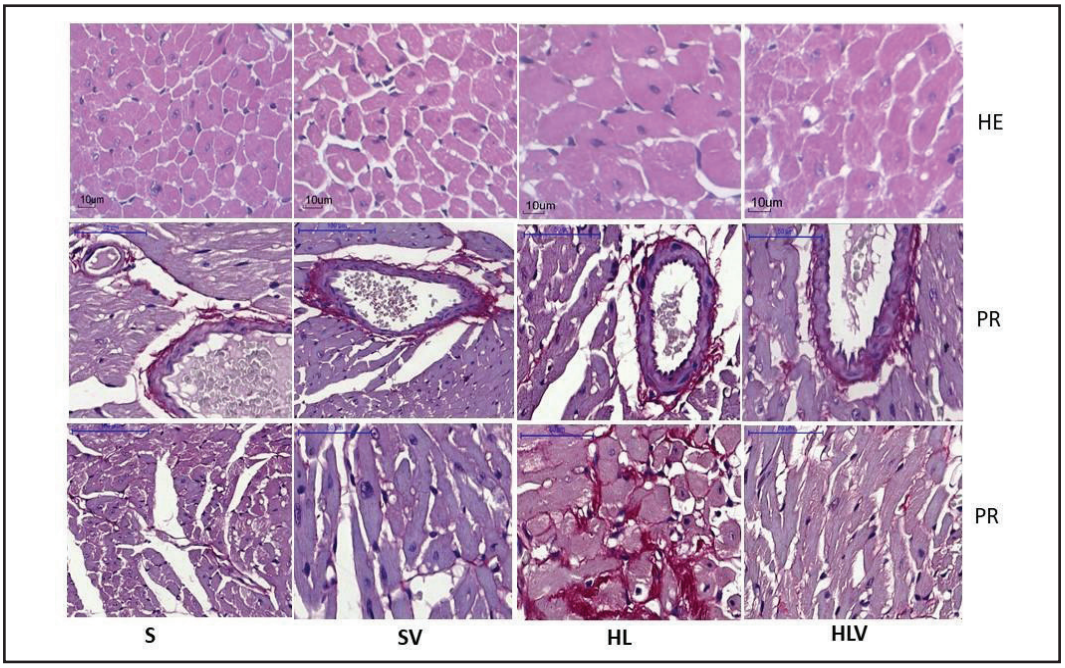
Table 3 – Morphological histological parameters of the left ventricle and abdominal aorta

	Groups	S	SV	HL	HLV
	N	**7**	**9**	**6**	**8**
Left ventricle	Diameter of cardiomyocytes (um)	18±1,3^{c}	18±1,8^{c}	24±2,2^{a}	21±1,1^{b}
	Ratio of LV weight (mg)/ mouse weight (g)	3,4±0,08^{b}	3,3±0,07^{b}	4,9±0,09^{a}	4,0±0,06^{a,b}
	% of interstitial collagen in the ventricular myocardium	3,3±0,3^{c}	3,2±0,4^{c}	8,0±1,0^{a}	5,6±1,0^{b}
Abdominal aorta	**Medium area μm²**	72840±1831^{c}	73422±1024^{c}	90564±1618^{a}	81433±1799^{b}
	Intimal area μm²	6323±630^{c}	6429±798^{c}	11839±1022^{a}	9024±832^{b}
	% of collagen in the aortic wall	12,7±0,9^{c}	11,9±1,0^{c}	20,9±1,2^{a}	15±1,8^{b}

Values were expressed as mean ± SEM. Different letters indicate significant differences between groups (p <0.05 - Tukey's test). S - standard food; SV - standard food and wine; HL - hyperlipidic diet; HLV - hyperlipidic food and wine; VE - Left ventricle; one - micrometer; μm2 - square micrometers, mg - milligram; g - gram.

Source: Authors (2022)

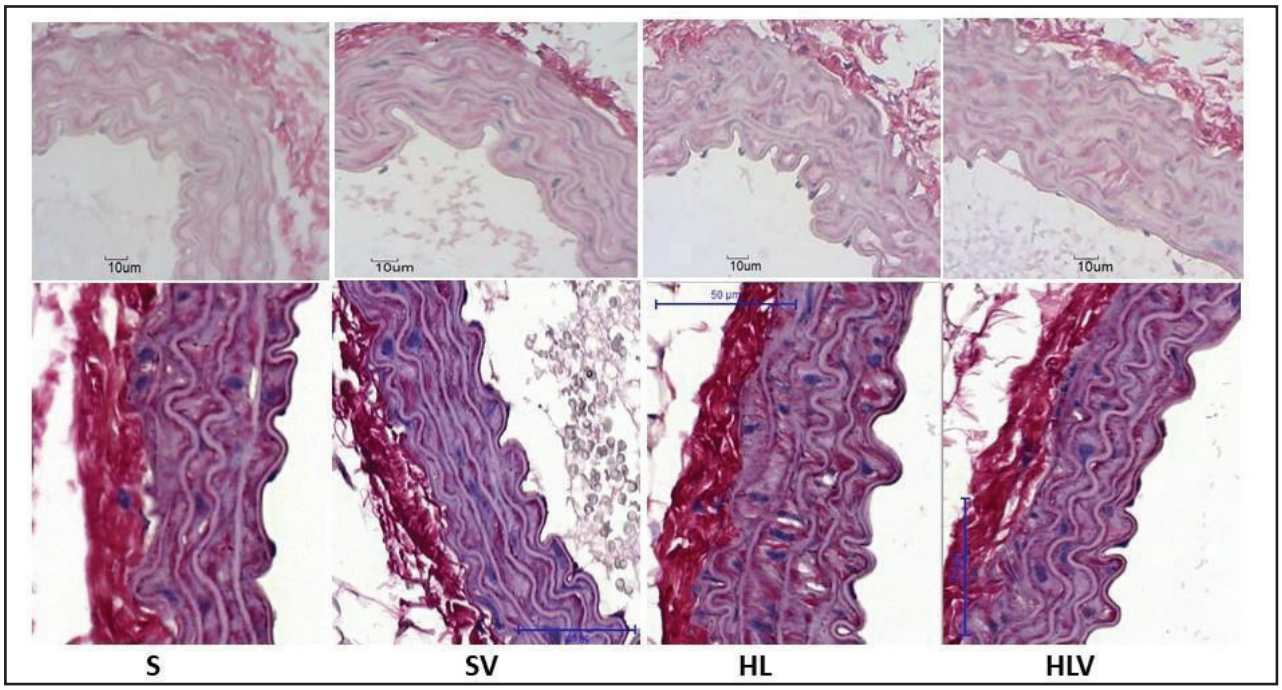
Figure 1 – Photomicrographs of histological slides from the left ventricular myocardium of LDLR - / - mice stained with hematoxylin and eosin (HE), showing the diameter of the cardiomyocytes (upper panel) and picrosirius red (PR), showing the perivascular collagen deposit in red (middle panel) and interstitial (bottom panel)



Source: Elaborated by the author (2022)

In the morphometric and histological analysis of the abdominal aorta of mice in Group HL, an arterial remodeling was observed, characterized by an increase in the intima and middle areas with a greater collagen deposit in the arterial wall when compared with Group S (Table 2, Figure 2). Arterial remodeling in the aortic wall of S and SV mice did not differ. *Merlot* wine partially prevented arterial remodeling, preventing the increase in the intima and middle areas and the deposit of collagen in the arterial wall when compared to the HL Group mice (Table 3, Figure 2). It was also observed that the mice in Group SV showed a better organization of elastic fibers than the other groups studied (Figure 2).

Figure 2 – Photomicrographs of histological slides from the abdominal aorta wall of LDLR - / - mice stained with red picrosirius red, showing the organization of collagen fibers and collagen deposit in red



Source: Elaborated by the author (2022)

Our results showed that all animals experienced weight gain (dados não mostrados). Besides, we noted that fluid and solid food intake remained within the expected standard patterns.

4 DISCUSSION

Grapes are considered to be one of the greatest sources of phenolic compounds when compared to other fruits and vegetables (Freire et al., 2020). The TPI, observed in the red wine of the *Merlot* grape (*Vitis vinifera L.*) in our study, presented an average index of 39.23 nm, which corresponds to the average found in the wines evaluated in the study by Fogaça et al. (2012). The TPI of wine depends a lot on the grape variety, its ripeness, geographic origin, soil type and sun exposure, in addition to the technology applied in production such as storage (Capella 2017; Fogaça et al., 2012; Oliveira 2016).

In the present study, it was observed that LDLR - / - mice fed a high-fat diet had severe mixed dyslipidemia with decreased HDLc and increased serum levels of CRP, followed by insulin resistance, which is characterized by increased serum levels of insulin and HOMA-IR in the HL group in relation to the other studied groups. In addition, mice in this group showed cardiovascular structural changes such as LVH and remodeling in the abdominal aorta wall. In LDLR - / - mice fed with high-fat food associated with the ingestion of *Merlot* wine, group HLV, it partially prevented dyslipidemia, decreased HDLc and insulin resistance, increased serum CRP levels and cardiovascular changes.

Studies have shown that high-fat foods in LDLR - / - mice results in the development of severe dyslipidemia with decreased HDLc followed by LVH (Martins et al., 2020; Sarto et al., 2018; Garcia et al., 2008) and atherosclerosis (Silva et al., 2015), due to the inflammatory process caused by severe dyslipidemia, associated with decreased nitric oxide bioavailability and serum HDLc levels with decreased antioxidant and anti-inflammatory functions (Garcia et al., 2008). In addition, LDLR - / - mice fed with high-fat food (HL group) in the present study developed insulin resistance with hyperinsulinemia and a remodeling of the abdominal aorta wall.

Dyslipidemia is a determinant of oxidative stress (Sarto et al., 2018) and, consequently, there is a decrease in HDLc levels (Dornas et al., 2009) due to increased hepatic removal of oxidized HDLc. Thus, oxidative stress generated by dyslipidemia in

mice of the HL group may have oxidized insulin receptors, generating insulin resistance, determined by the increase in HOMA-IR. Dyslipidemia and hyperinsulinemia are essential factors for the development of LVH (Garcia & Incerpi, 2008). Hypercholesterolemia can act in the myocardial K_{ATP} channels, changing its function and expression, presenting itself as a trigger for cardiac hypertrophy (Genda et al., 2002). Hyperinsulinemia can cause myocardial protein biosynthesis and, directly or indirectly, cardiac hypertrophy (Samuelsson et al., 2006). The decrease in serum HDLc levels associated with severe mixed dyslipidemia and the inflammatory process, characterized in the present study by the increase in CRP, are the common denominators of arterial remodeling in atherogenesis. Changes in the lipid profile of mice in the HL group associated with insulin resistance and the inflammatory process support LVH and arterial remodeling to accommodate the atheroma plaque in the present study.

Mice that received high-fat food associated with red wine from the *Merlot* grape (*Vitis vinifera L.*), Group HLV, when compared to Group HL, showed an increase in HDLc levels and a decrease in TG and insulin, predictive factors for the metabolic syndrome (Jama 2001; Alberti & Zimmet, 1998). Therefore, the decrease in HDLc and triglycerides, associated with the fall in insulin levels contributed to the prevention of insulin resistance and cardiovascular diseases in mice of the HLV Group. A reduction in CRP, a biomarker of inflammation commonly found at high levels in metabolic syndrome, was also observed (Ridker 2000), contributing to the reduction of the risk of cardiovascular diseases such as LVH and remodeling of the abdominal aorta wall.

The prevention of LVH and remodeling of the abdominal aorta wall in mice of the HLV Group can be attributed to the polyphenols present in the red wine of the *Merlot* grape (*Vitis vinifera L.*), among them the flavonoid resveratrol. This flavonoid is a mediator of cardiovascular protection independent of alcohol (Pinto 2019), acting as an inhibitor of lipoprotein oxidation, anti-inflammatory, platelet aggregation, synthesis of pro-atherogenic ecosystems, cell proliferation, also causing vasorelaxation and suppression of pro-coagulant tissue factor induction (Turner et al., 1999). Thus, the

antioxidant activity of resveratrol contributed to the reduction of oxidative stress markers in the HLV Group. The lower occurrence of oxidative stress prevented HDLc oxidation and its hepatic removal, with a consequent increase in HDLc levels, which has antioxidant and anti-inflammatory functions (Holvoet, 2008; Christison et al., 1996).

Procházková et al. (2011) demonstrated that flavonoids have antioxidant activity by eliminating free radicals, using the metal chelating property, suppressing the enzymes involved in the synthesis of free radicals and stimulating antioxidant enzymes, mechanisms that may have prevented the reduction of nitric oxide bioavailability and, consequently, prevented the left ventricular and aorta remodeling in the mice of the HLV Group in this study.

The antioxidant and anti-inflammatory effect of resveratrol (Matos et al., 2012), associated with the prevention of decreased HDLc levels was also decisive in preventing insulin resistance in mice of the HLV Group. The red wine of the *Merlot* grape (*Vitis vinifera L.*) showed an anti-lipid-lowering effect, avoiding an increase in TG, TC, HOMA-IR and a decrease in HDLc, partially preventing insulin resistance, oxidative stress, and increased CRP, and consequently, LVH and arterial remodeling.

In conclusion, red Merlot wine can be used as an alternative in the prevention of dyslipidemia, insulin resistance and cardiovascular changes within the experimental protocol and the animal model of this study. We suggest further studies with red Merlot wine in other models of dyslipidemia and in other species, as well as the association with other anti-lipidic products

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