

Physical activity, grape juice, red wine and resveratrol effects in cardiac remodeling of *Wistar* rats submitted to a high fat diet

Efeitos da atividade física, suco de uva, vinho tinto e resveratrol na remodelação cardíaca de ratas *Wistar* submetidas a dieta hiperlipídica

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ABSTRACT

Background: The association between regular physical activity and the polyphenol-rich drinks in models with a high fat diet, has not been sufficiently elucidated. **Objective:** The objective was to study the effects of the association among regular physical activity and polyphenol-rich drinks consumption in lipid profile and cardiac remodeling of *Wistar* rats submitted to the high fat diet. **Methods:** Rats *Wistar*, 90 days, 05 groups (n=10/group): Control Group (CG); High Fat Group (HG); Resveratrol Group (RG); Grape Juice Group (JG); Red Wine Group (WG); HG, RG, JG and WG received a high fat diet (20%). The animals performed a treadmill running protocol for 60 days. After, blood was collected for biochemical evaluation and heart was removed for histological analysis. Variables were expressed as mean \pm standard deviation. ANOVA one way and Bonferroni or Tuckey's as post-test, when appropriate, $p < 0.05$. **Results:** JG show higher total cholesterol (72.0 ± 8.9 $p < 0.05$) and HDL (28.0 ± 2.0 $p < 0.05$) in relation to CG (total cholesterol = 57.8 ± 8.1 , HDL = 24.4 ± 2.9). Collagen concentration: HG (9.65%, $p < 0.05$) presented higher concentration in relation to the CG (3.68%), RG (4.73%), JG (3.57%) and WG (6.59%). **Conclusions:** It is concluded that the association between physical activity and drinks rich in polyphenols was able to protect the heart tissue from damage caused by the high fat diet.

Keywords: remodeling, resveratrol, physical activity, *Wistar* Rats.

RESUMO

Fundamento: Os efeitos da associação entre a atividade física regular e bebidas ricas em polifenóis em animais submetidos à dieta hiperlipídica ainda não foram suficientemente elucidados. **Objetivo:** O objetivo foi estudar os efeitos da associação entre atividade física regular e consumo de bebidas ricas em polifenóis no perfil lipídico e na remodelação cardíaca de ratas *Wistar* submetidas à dieta hiperlipídica. **Métodos:** Ratas *Wistar*, 90 dias, divididas em 5 grupos (n = 10 / grupo): Grupo Controle (GC); Grupo Hiperlipídico (GH); Grupo Resveratrol (GR); Grupo Suco de Uva (GS); Grupo Vinho Tinto (GV); GH, GR, GS e GV receberam uma dieta hiperlipídica (20%). Os animais realizaram um protocolo de corrida em esteira rolante por 60 dias. Em seguida, foi coletado sangue para avaliação bioquímica e o coração foi retirado para análise histológica. As variáveis foram expressas como média \pm desvio padrão. Foi utilizado ANOVA *one way* e Bonferroni ou Tuckey como pós-teste, quando apropriado, considerando-se significativos os resultados que apresentaram $p < 0,05$. **Resultados:** GS apresentou maior concentração de colesterol total ($72,0 \pm 8,9$ $p < 0,05$) e HDL ($28,0 \pm 2,0$ $p < 0,05$) em relação ao GC (colesterol total = $57,8 \pm 8,1$, HDL = $24,4 \pm 2,9$). Concentração de colágeno: GH apresentou maior concentração (9,65%, $p < 0,05$) em relação ao GC (3,68%), GR (4,73%), GS (3,57%) e GV (6,59%). **Conclusões:** Conclui-se que a associação entre atividade

física e bebidas ricas em polifenóis foi capaz de proteger o tecido cardíaco dos danos causados pela dieta hiperlipídica.

Palavras-chave: remodelação cardíaca, resveratrol, atividade física, ratos *Wistar*.

1 INTRODUCTION

Saturated fats rich diets consumption can lead to a series of adverse reactions, including hyperlipidemia, hypertension, heart failure, and coronary heart disease [1,2].

Cardiovascular diseases (CVD) might cause morphologic changes in the heart, resulting from multiple aggressions such as injuries, pressure load, among others. These changes results culminate in a ventricular wall thickening, myocardial fibrosis and remodeling, accompanied by systolic and diastolic dysfunction, arrhythmia and adverse outcomes [3,4].

A regular physical activity has many beneficial effects, for example, it ameliorated diabetic cardiomyopathy by improving mitochondrial function and cardiac contractility, prevented hypertension and also attenuated aortic endothelial oxidative damage [5,6].

In addition to physical activity, resveratrol, a polyphenol found in grapes and red wine, has antiatherogenic and anti-CVD effects. One of the most recognized effects is its antioxidant activity, elimination of free radicals and platelet aggregation reduction, and decreasing low density lipoproteins (LDL) oxidation [7,8].

The association between regular physical activity and polyphenol-rich drinks in models with a high fat diet (HFD), has not been sufficiently elucidated in the literature. Thus, the objective of the present study was to investigate the effects of this association in lipid profile and cardiac remodeling of *Wistar* rats submitted to the high fat diet.

2 MATERIAL AND METHODS

All experimental procedures used during these experiments complied with Ethics Committee guidelines for laboratory animals use, protocol number CEUA 473/2013.

Fifty adult female Norvergicus *Wistar Albinus* rats (90 days). Throughout experimental period, animals were kept in individual polypropylene cages, in an environment with a constant temperature ($24^{\circ}\pm 2^{\circ}\text{C}$) and adequate lighting (light and dark cycle - 12 hours).

Rats were divided into 5 groups (n = 10/group): 1) Control Group (CG) receiving balanced diet, based on casein (AIN93M) (table 1); 2) High-Fat Group (HG) receiving HFD (20%), casein-based when the usual fat content is 4 - 8% (table 1).; Resveratrol Group (RG) receiving HFD (20%) and 15 mL of a resveratrol solution 4% (diluted water); 4) Grape Juice

Group (JG) receiving HFD (20%) and 15 mL of whole grape juice and 5) Red Wine Group (WG), receiving HFD (20%) and 10 mL of red wine. All animals received diet and filtered water ad libitum and followed for 60 days. In this period, animals underwent physical activity on a treadmill at a constant speed of 10m/s for 10 minutes, 5 times a week during the whole experiment.

Table 1: Composition of the control and high fat diets used in the experiment (g /100g of feed).

Ingredients	Control (%)	HFD (%)
Casein ¹	14	14
Corn starch ²	62,07	46,07
Sugar ³	10	10
Mineral Mix ¹	3,5	3,5
Vitamin Mix ¹	1	1
Soybean oil ⁴	4	-
Lard ⁵	-	20
Cellulose ⁶	5	5
B-choline ⁷	0,25	0,25
L-cystine ⁷	0,18	0,18
BHT	0,008	0,008
TOTAL	100,01	100,01

* Balanced according to the recommendations of the AIN 93 M.¹M. Cassab Comércio e Indústria Ltda, ²Maisena - Unilever Bestfoods Brasil Ltda, ³União, ⁴Liza Cargill Agricultura Ltda, ⁵Sadia – BRF Food Services ⁶Macrocel - BlanverLtda, ⁷Comércio e Indústria Farmos Ltda
Source: Prepared by the author

After 60 days, animals were anesthetized, submitted to a median laparotomy, blood was collected by cardiac puncture, centrifuged to obtain serum for the subsequent biochemical analysis, included serum concentrations of total cholesterol (TC), high density lipoproteins (HDL) and triglycerides (TG) determined on the Bioclin ® device with the following equipment specific Bioclin ® kits, Monoreagent Cholesterol - K083, HDL Direct - K071 and Monoreagent Triglycerides - K117.

Through a thoracotomy, heart was removed and were weighed on Sartorius® analytical balance and then packed in 10% formalin solution for histological analysis.

Left ventricular (LV) histological analyzes were performed according to Vicente *et al.*, (2016) [9]. Soon after the removal, LV was fixed in buffered formalin (formalin Millonig) for 24 hours and were processed with a technique for inclusion in paraffin. After inclusion, the blocks of paraffin containing the pieces of LV were cut in the microtome Laica® RM 2125RT in sections of 5µm and mounted on standard slides for optical microscopy.

Slides containing sections of the LV tissue were stained with hematoxylin and eosin to detect the nuclei and cytoplasm of the myocytes and Picro-sirius red for fibrillar staining of the collagen in the extracellular matrix.

For analysis of the volumetric density (Vv), the images were captured by a 14-inch LG television set, where by counting points overlapping an M42 test system, counting of the area occupied by collagen (Vv (i%)) was performed.

For the measurement of left ventricular thickness the images were captured by the BELView program in the Belphotonics microscope in a 0.5x magnification. After that, analysis of the LV wall thickness was performed using the ImageJ® program. All images were scanned in .tiff format.

2.1 STATISTICAL ANALYSIS

Variables were expressed as mean ± standard deviation. Normality evaluation was assessed using the Shapiro-Wilk test. For comparison among groups, one-way analysis of variance (ANOVA) test was used with Bonferroni or Tuckey`s as post-test, when appropriate. A $p < 0.05$ was considered significant. GraphPad software (version 5.00 for Windows XP, GraphPad Software) was used to perform statistical analysis.

2.2 RESULTS

Table 2 displays results of animals metabolic characteristics. It's possible to observe higher TC and HDL in JG in relation to the CG. TG and weight didn't differ among groups. The groups that received a HFD had a higher energy consumption than the CG.

Table 2: Animals Metabolic Characteristics

	CG	HG	RG	JG	WG
Weight	289.5±15.0	286.3±4.9	289.2±14.1	284.2±13.9	286.3±14.8
Calories intake (kcal/mouse/d)	54.7±2.2	68.0±6.0	72.0±6.9	65.2±5.2	69.9±7.1
Total Cholesterol	57.8 ± 8.1	55.42 ± 7.9	54.1 ± 7.6	72.0 ± 8.9*	55.4 ± 7.9
HDL	24.4 ± 2.9	23.1 ± 3.6	23.5 ± 2.8	28.0 ± 2.0*	25.1 ± 5.0
Triglycerides	57.7 ± 21.5	47.0 ± 13.6	54.2 ± 15.4	55.2 ± 19.9	51.2 ± 16.3

Groups (n=10): Control (CG); Hightfat (HG); Resveratrol (RG); Grape juice (JG) and Red wine (WG). Variables were expressed as mean ± standard deviation. A $p < 0.05$ was considered significant (*) when compared to CG. Anova *one way* and Bonferroni as post-test for Total Cholesterol, HDL e Triglycerides and Anova *one way* and Tukeys as post-test for Weight and Calories intake.

Source: Prepared by the author

Figure 1a shows LV thickness and heart weight analysis and figure 1b shows collagen deposition in cardiac tissue results. Regarding heart weight analyzes, RG heart mass was lower

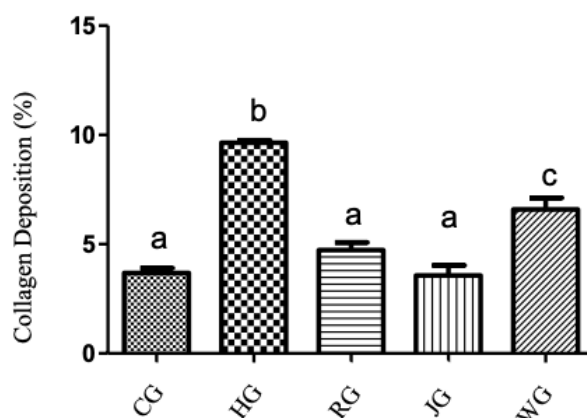
in relation to the CG. LV thickness analysis did not present significant differences. However, it's possible to observe a higher concentration of collagen in HG when compared to others groups. RG and JG presented a percentage of collagen similar to CG.

Figure 1: Left ventricle thickness and heart weight and collagen deposition

(A)

	CG	HG	RG	JG	WG
Heart weight (corrected) (g)	25.6 ± 0.9	22.7±2.7	21.7±2.0*	23.7±2.0	22.2±4.1
Left ventricle thickness (mm)	3.3±0.3	3.6±0.2	3.5±0.3	3.0±0.3	3.2±0.2

(B)



Groups (n=10): Control (CG); Hightfat (HG); Resveratrol (RG); Grape juice (JG) and Red wine (WG). Variables were expressed as mean ± standard deviation, Anova *one way* and Tukey's as post-test. A $p < 0.05$ was considered significant. (A) Left ventricle thickness (mm) and heart weight. Animals heart mass were corrected according to the length of the tibia. (*) when compared to CG, $p < 0.05$. (B) Collagen deposition (%). Different letters for different results, $p < 0.05$.

Source: Prepared by the author

3 DISCUSSION

Physical activity and drinks rich in polyphenols association may have contributed to weight control even in the face of greater energy consumption (table 2). As observed in the study by Shen *et al.*, (2015) [10], animals consuming HFD and supplemented with polyphenols showed an improvement in body composition, with increase in lean mass and weight reduction, in addition to other biochemical parameters.

Concerning biochemical parameters, GS showed higher TC and HDL concentrations when compared to control group. Yu *et al.*, (2017) observed a TC increase in groups receiving higher concentrations of grape marc in their diets, however, also observed a slight increase in HDL in these groups, although without statistical significance [11].

It was expected a TC decreased levels and increased HDL concentration after drinks rich in polyphenols consumption as reported by several studies in the literature [12,13], however, the present study experimental results do not support this, requiring further research on these parameters.

The LV analysis pointed to a remodeling process, related to morphological changes in the heart, resulting in myocyte hypertrophy, interstitial fibrosis, collagen degeneration and changes in its mass due to hypertrophy [4].

In the present study, animals heart mass were corrected according to the length of the tibia, with a lower mass of GR compared to the CG, with no difference among other groups. LV thickness analysis did not show any difference between the groups in relation to control. These results corroborate the study by Chen *et al.*, (2019), suggesting aerobic exercise ability to reverse cardiac remodeling, with no changes in LV weight and thickness [14].

However, after tissue collagen concentration evaluation, it is observed the GH group has a higher concentration than the other groups. This fact may be associated with a HFD without polyphenols drinks rich consumption.

In the study by He *et al.*, (2020) it was observed the hyperlipidic diet can lead to a remodeling situation with collagen deposition between cells, a contributing factor to cardiac dysfunction [15].

Terra *et al.*, (2013) also observed a picture of remodeling in animals consuming a HFD, however, in their study, such animals were submitted to physical activity with no polyphenol supplementation. The group consuming a HFD had a remodeling picture not observed in the group with a balanced diet. Such findings lead us to believe physical activity alone has not been able to protect tissue from the damage of a HFD [16].

Therefore, it can be suggested in the present study, physical activity with polyphenols rich drinks contributed to the fact during the studied period there was no ventricular remodeling. GR and GS behavior were similar to CG group, with no statistical difference. The GV group had higher collagen concentrations GC group, however, these concentrations are still lower than the GH group.

Finally, it was observed during the 60 days protocol, the HFD began to cause damage cardiac tissue with collagen fibers increase in GH group, characterizing the onset of fibrosis in this tissue, even with physical activity. However, an exacerbation of this fibrosis was not observed in the groups consuming polyphenols rich drinks different from what was observed in the literature [14-16].

It is concluded, although lipid profile results have shown to be divergent from other findings in the literature, it is observed physical activity and drinks rich in polyphenols consumption together were able to protect cardiac tissue from damage caused by the HFD. Thus, the importance of further studies assessing the benefits of such an association is highlighted.

4 CONCLUSIONS

The association between physical activity and drinks rich in polyphenols had a protective effect against cardiac remodeling, reducing the concentration of collagen in the left ventricle. This association didn't show significant effects in the lipid profile.

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