Investigação

RED WINE, GRAPE JUICE OR RESVERATROL CONSUMPTION EFFECTS ON LIPID AND GLYCEMIC PROFILE AND HEPATIC MORPHOLOGY OF WISTAR RATS SUBMITTED TO HIGH FAT DIET AND PHYSICAL TRAINING

Nina M. A. Pimenta^{1*}, Raiza S. Ferreira-Fiochi², Letícia M. F. Cardoso², Bruna M. Ferreira¹, Isabelle W. S. M. Silva¹, Juliana A. S. Monnerat¹, Renata Beatriz da R. Ramalho¹, Cristiane C. Teixeira¹, Dr^a Vilma B. Azeredo², Dr Gilson T. Boaventura², Dr Mauricio A. Chagas⁴, Dr^a Gabrielle S. Rocha^{2,3}, Dr Sergio G. Barroso²

¹Universidade Federal Fluminense, Faculdade de Nutrição Emilia Jesus Ferreiro, Rio de Janeiro, Brazil

²Universidade Federal Fluminense, Faculdade de Nutrição Emilia Jesus Ferreiro, Departamento de Nutrição e Dietética, Rio de Janeiro, Brazil.

³Universidade Federal Fluminense, Pós-graduação em Ciências Cardiovasculares, Rio de Janeiro, Brazil. ⁴Universidade Federal Fluminense, Instituto Biomédico, Departamento de Morfologia, Rio de Janeiro, Brazil

ABSTRACT

The aim of this study was to evaluate the effects of red wine, grape juice or resveratrol consumption on lipid and glycemic profile and liver nuclear area and circularity of Wistar rats submitted to the high fat diet and physical training. Original article. Wistar rats, with 90 days, were divided into 5 groups (n=6/group): Control (GC); High Fat (HFG); Grape juice (GJG); Red Wine (WG) and Resveratrol (RG). The animals performed a running protocol. After 60 days, the animals were anesthetized, blood was collected for biochemical analyzes, sacrificed and the liver removed. Liver samples were mounted on histological slides and stained with hematoxylin and eosin. The results were expressed as mean $\pm$ standard deviation and assessed for normality with Kolgomorov-smirnov test. One way ANOVA, and Bonferroni post-test were used. It was considered significant when $p < 0.05$, using PRISMA 5.0 software. Groups consuming high fat diet had a significantly higher energetic intake compared to CG ($p = 0.0001$), however, there was no significant difference in body mass and weight gain among groups ($p = 0.95$). There was no significant difference in triglyceride and glucose levels among groups ($p = 0.66$ and $p = 0.71$, respectively). Total cholesterol and high-density lipoprotein (HDL) were higher in GJG when compared to CG ($p < 0.0001$ and $p = 0.008$, respectively). Hepatocytes nuclear areas were significantly higher in the HFT and WG ($p = 0.0029$), with no difference in nucleus circularity among

groups ($p = 0.50$). It was concluded that even in a high fat diet scenario, animals receiving resveratrol had their results matched to those receiving a balanced diet. .

Keywords: polyphenols, wine, grape juice, resveratrol, liver.

INTRODUCTION

According to the World Health Organization, in 2014, there were 1.9 billion overweight adults, with 600 million obese (WHO, 2016). It is projected that by 2025, there will be 2.3 billion overweight people and more than 700 million obese adults worldwide (ABESO, 2016). In Brazil, nutritional transition process is mainly due to current dietary pattern rich in saturated fat and sugar, with low fiber content associated to sedentary lifestyle (COUTINHO et al. 2012).

Saturated fats and obesity are associated with Non-Alcoholic Fatty Liver Disease (NAFLD) development, which is defined as a progressive inflammatory disease where lipid accumulation occurs in hepatocyte cytoplasm (FICHER et al. 2014). NAFLD may be aggravated by oxidative stress, and may progress to fibrosis, hepatic cirrhosis and late stage hepatic failure or hepatocarcinoma (FUJITA et al. 2011; FICHER et al. 2014; PAN et al. 2014).

It has already been demonstrated that chronic aerobic physical exercise has benefits for NAFLD treatment, decreasing hepatic lipids accumulation of (GUO et al. 2015). In addition of being an important strategy in overweight and obesity treatment and prevention, favoring body fat oxidation, leading to weight loss and lipid profile improvement (CAMBRI et al. 2006).

Due to this scenario, searching for strategies for treatment and prevention of diet rich in saturated fat associated to sedentary lifestyle harmful effects is growing. Several natural compounds present in fruits and vegetables, such as carotenoids, polyphenols and omega-3, are indicated because they contain health-promoting properties, with antioxidant and anti-inflammatory activity and insulin sensitivity, among others (PAN et al. 2014).

Among bioactive compounds present in food, resveratrol is one of the most studied, a polyphenol called trans-3,40,5-trihydroxystilbene, naturally produced by several species of plants and present, for example, in peanuts, pomegranates, soybeans and grape. In grapes, it is present in barks, so the

red wine has a higher concentration of resveratrol than white wine, which is fermented without grape bark. Several studies suggest that resveratrol has beneficial effects on health in chronic diseases prevention and treatment (CATALGOL et al. 2012).

Polyphenols present in grapes have recognized antioxidant capacity, able to modulate endogenous antioxidant system, neutralizing reactive oxygen species and inhibiting lipid peroxidation. In addition, it has also been demonstrated that these compounds present anti-inflammatory and anticancer action, with known neuroprotective activity and reduced oxidative DNA damage (PIRES et al. 2013).

The aim of the study was to evaluate the effects of red wine, grape juice or resveratrol consumption in liver nucleus area and circularity and lipid and glycemic profile of Wistar rats submitted to high fat diet and physical training.

MATERIAL AND METHODS

RESEARCH DESIGN

This study was approved by the Committee on Ethics in the Use of Animals - CEUA 473/2013. Biological assays were carried out at Laboratório de Nutrição Experimental (LABNE) of the Faculty of Nutrition of the Universidade Federal Fluminense (UFF). *Rattus norvegicus*, *albinus*, Wistar, females, 90 days, initially weighing around 230g, were used from LABNE / UFF. The animals were kept in individual polypropylene cages, in an environment with constant temperature (22°C ±2°C) and adequate lighting (light/dark; 12/12hs).

Animals were divided into 5 groups (n=6/group), with a diet based

on casein and water offered ad libitum and monitored for 60 days: Control Group (CG), receiving a balanced diet; High Fat Group (HFG), receiving high fat diet (20%); Grape Juice Group (GJG) receiving high fat diet (20%) and whole red grape juice (15mL/day); Red Wine Group (WG) receiving high fat diet (20%) and red wine (10mL/day) and Resveratrol Group (RG), receiving high fat diet (20%) and 4% resveratrol solution (15mL/day).

FORMULATION OF RATIOS

Control and high fat rations (Table 1 and 2) were based on recommendations from the American Institute of Nutrition (AIN, 1993M) for *Rattus norvegicus* and handled at LABNE/UFF. High fat diet (HFD) presented lard inclusion in its composition in substitution of soybean oil. Lard, juice and wine were purchased at local market and resveratrol was purchased from a handling pharmacy. Grape juice used was obtained from American grapes, Bordô and Jacquez, produced in Rio Grande do Sul - Brazil. Red wine was obtained from 100% Cabernet Sauvignon wine grapes, produced in São Paulo - Brazil.

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

Ingredients	Control (%)	High Fat (%)
Casein1	14	14
Start2	62,07	46,07
Sugar3	10	10
Mix of Minerals1	3,5	3,5
Mix of Vitamins1	1	1
Soybean Oil	4	-
Lard5	-	20
Cellulose6	5	5
B-coline7	0,25	0,25
L-cistine7	0,18	0,18
BHT	0,008	0,008
TOTAL (%)	100,01	100,01

*Balanced according to the recommendations of 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 Famos Ltda.

Table 2 - Macronutrient composition of the diets used in the experiment (100 g).

	Control Diet (100 g)	High Fat Diet (100 g)
Energy (kcal)	331,85	428,14
Protein (g)	11,96	11,94
Carbohydrate (g)	61,59	48,74
Lipids (g)	4,18	20,59

PHYSICAL TRAINING

During the 60 days, all the animals enrolled a treadmill running protocol with 10 meters per minute speed of 10min, 5 days/week (BORBOREMA, 2013).

BLOOD AND ORGAN COLLECTION

After 60 days of experimentation, animals were fasted for 6 hours and anesthetized with intraperitoneal injection of Thiopentax® (thiopental sodium, 0.1 ml/100 g body weight). Animals were submitted to the median laparotomy procedure, blood was collected for biochemical analysis and sacrificed by exsanguination. Each animal liver was removed. Lipidogram and glycemia analysis were determined in the Bioclin® apparatus of LABNE/UFF.

MORPHOLOGICAL ANALYSIS

This procedure was carried out in the Laboratório de Biomorfologia Celular e Extracelular of the Department of Morphology of the UFF Biomedical Institute.

Liver samples were fixed in buffered formalin solution (Millonig formalin) for 24 hours. Materials were submitted to dehydration, diaphanization and inclusion in histological paraffin procedures. After inclusion, paraffin blocks containing liver fragments were cut into the Laica® RM 2125RT microtome in 5µm sections and mounted on standard slides for optical microscopy. The sections were dewaxed and hydrated and sent to staining with hematoxylin and eosin for core staining.

Image Analysis

Slides were captured and digitized in .tiff format by the CellSens Olympus® program with Olympus BX-51 microscope, 40x objective. Subsequently, they were analyzed using ImageJ® software, which provided nuclear area (µm²) and core circularity quantitative information. Circularity was calculated using nucleus area and perimeter using the formula: $4\pi(\text{area}/\text{perimeter}^2)$.

STATISTICAL ANALYSIS

The results were expressed through descriptive statistics as mean and standard deviation. Data were evaluated for their normality with the Kolgomorov-smirnov test. For comparison we used one way ANOVA, and Bonferroni post-test, and $p < 0.05$ was considered significant. PRISMA 5.0 software was used.

RESULTS

Body weight and energy intake

Figure 1 shows animals body weight after the 60 days of experiment. There were no significant differences when compared to CG ($p=0.95$).

Figure 1: Weight of animals (g) after 60 days of experiment. Groups (n=6): Control (CG); High fat (HFG); Grape juice (GJG); Red Wine (WG) and Resveratrol (RG). Results presented as mean ± standard deviation.

Table 3 shows of the animals weight gain after 60 days of experimentation. There was no significant difference when compared to CG ($p=0.47$).

Weight Gain (g)	CG	HFG	GJG	WG	RG	p value
Mean $\pm$ Standard Deviation	55.58 $\pm$ 6.08	56.00 $\pm$ 9.50	48.25 $\pm$ 8.92	53.75 $\pm$ 18.34	61.42 $\pm$ 13.66	0.47

Table 3: Weight gain of animals (g) after 60 days of experiment. Groups (n=6): Control (CG); High fat (HFG); Grape juice (GJG); Red Wine (WG) and Resveratrol (RG). Results presented as mean $\pm$ standard deviation.

Figure 2 shows energy intake of the groups. The animals receiving a hyper fat diet (HFG, GJG, WG and RG) had higher energy consumption when compared to CG, who received a balanced diet ($p = 0.0001$).

Figure 2: Energy intake in kilocalories per day (kcal/day) of the groups (n=6): Control (CG); High fat (HFG); Grape juice (GJG); Red Wine (WG) and Resveratrol (RG). Results presented as mean $\pm$ standard deviation. Considered significant when $p<0.05$ (*).

Biochemical analyzes

Figure 3 (A, B, C, D) shows animals biochemical analyzes. It was observed that GJG had higher serum levels of total cholesterol and HDL-c when compared to CG ($p<0.0001$ and $p=0.008$, respectively). Regarding triglyceride levels and serum glucose, no significant differences were observed among groups when compared to control ($p=0.66$ and $p=0.71$, respectively).

Figure 3: Animals Biochemical analyzes, groups (n=6): Control (CG); High fat (HFG); Grape juice (GJG); Red Wine (WG) and Resveratrol (RG). Results presented as mean $\pm$ standard

deviation. Considered significant when $p<0.05$ (*). Figure 3A: Total Cholesterol (mg/dL), 3B: HDL-Cholesterol (mg/dL), 3C: Triglycerides (mg/dL), 3D: Glucose (mg/dL).

Morphological Analysis

Figure 4 shows animals hepatocytes nuclear area. HFG and WG presented a significantly higher nuclear area when compared to CG ($p = 0.0029$).

Figure 4: Hepatocytes nuclear area, in μm^2 , after 60 days of treatment, groups (n=6): Control (CG); High fat (HFG); Grape juice (GJG); Red Wine (WG); and Resveratrol (RG). Results are presented as mean $\pm$ standard deviation. Considered significant when $p<0.05$ (*).

Figure 5 shows liver nuclear circularity. There was no significant difference among groups when compared to CG ($p=0.50$).

Figure 5: Degree liver nucleus circularity, after 60 days of treatment, the groups (n=6): Control (CG); High fat (HFG); Grape juice (GJG); Red Wine (WG) and Resveratrol (RG). Results are presented as mean $\pm$ standard deviation.

Figure 6: Liver rats photomicrograph, captured with a 40x objective. A: Control Group; B: High Fat Group; C: Grape Juice Group; D: Red Wine Group; E: Resveratrol Group. The images were stained with hematoxylin and eosin. Scale Bar = 50 μ m.

DISCUSSION

According to data presented, it was observed that a higher energetic intake in groups receiving HFD than animals that receiving a balanced diet, although body mass of the animals at the end of the experiment did not present significant difference among groups.

In literature it is already well known that intake of a diet rich in saturated fats can result in an increased body mass, inducing insulin resistance, increased free fatty acid concentrations and hepatic alterations (CARMIEL-HAGGAI et al. 2005; MOURA et al. 2012; PINTO JUNIOR and SERAPHIM, 2012). However, studies showing body mass gain were performed using diets with higher

lipid percentages or with a longer time of intervention, using 32% of lipids (MOURA et al. 2012) or with 14 weeks intervention (PINTO JUNIOR and SERAPHIM, 2012).

Da Rocha et al. (2016) observed physical exercise contribution on body mass maintenance, even in the face of HFD ingestion. It corroborates results found in the present study, since all animals begun the study with similar body mass and performed a treadmill running protocol, and even groups receiving HFD had body mass similar to CG.

HFD can cause changes in the lipid profile, causing an increase in total and LDL cholesterol and a reduction in HDL-cholesterol (MOURA et al. 2012; BUETTNER et al. 2006). This was not observed in the present study, however, GJG presented an increase in serum concentrations of total cholesterol and HDL-cholesterol.

Mann et al. (2014) have shown that a moderate-intensity aerobic exercise improvement lipid profile, increasing HDL-cholesterol and decreasing LDL-cholesterol and triglycerides. In addition, grape products have already demonstrated lipid profile modulation, increasing serum HDL-cholesterol levels (EVANS et al. 2014). Therefore, it is suggested that, based in this study results, physical exercise associated to polyphenols present in grape juice, was able to increase the HDL-cholesterol concentration in GJG. Probably, the higher total cholesterol levels in GJG are due to HDL increased fraction.

There were no significant difference among groups regarding plasma concentrations of triglycerides and glucose ($p=0.66$ and $p=0.71$, respectively). Even in the presence of a HFD, animals had no change in these parameters, suggesting ability of this treatment (exercise and polyphenol rich drinks) in 60 days in possible damages prevention caused by HFD (MANN et al. 2014; ANDRADE et al. 2014; CHANG et al. 2016).

In addition to biochemical changes, HFD may lead to hepatic

alterations, such as steatosis, inflammation and fibrosis, and may contribute to the NAFLD progression (CARMIEL-HAGGAI et al. 2005).

It is well described in the literature, in the face of trauma, a compensatory liver hyperplasia related to increase on cell multiplication and division (PETROIANU et al. 2004), which may lead to an increase in the nuclear area, suggesting more tissue work and greater liver metabolism demand (GAYOTTO and ALVES, 2001). In this study, it was observed increased nuclear area on HFG and WG compared to the CG.

The increase in the nuclear area in the group receiving the HFD demonstrates it's capacity to cause nuclear tissue damage similar to those found in other study demonstrating mitochondrial alterations with increased size and density (SILVA and ESCANHOELA, 2009).

In WG, increase in nucleus size may be a result of alcohol effect associated with the HFD. Alcohol causes oxidative stress, leading to increased concentrations of reactive oxygen species and, possibly, decreasing resveratrol and other polyphenols present in the beverage antioxidant action (SID et al. 2013). In addition, hepatic enzyme alanine aminotransferase and gamma glutamyl transferase serum levels have been shown to increase in moderately alcohol-consuming subjects compared with abstainers, indicating hepatic injury (ALATALO et al. 2008). Concomitant administration of HFD and alcohol results in increased oxidative stress with increased liver damage, as reported by Alatalo et al. (2008).

Therefore, grape juice and the resveratrol solution seem to have shown protection against possible hepatic nuclear damage caused by high fat diet, since GJG and RG did not present significant difference when compared to the CG. Alturfan et al. (2012) observed resveratrol capacity to revert oxidative stress

and oxidative damage to DNA, reducing liver enzymes (indicators of hepatic impairment), with an increase in antioxidant enzymes concentration and lipid peroxidation markers and collagen content reduction.

In spite of differences in nuclear area, no significant difference were observed among groups regarding nucleus circularity ($p=0.50$). This finding corroborates those published by Malta et al. (2010) who observed, in rats treated with dipyrone, hepatocytes nuclei with increased size, but without form alteration.

Nevertheless, visually, it was possible to observe a higher cytoplasm vacuolization HFG and GJG, indicated in figure 6, being less present in CG, WG and RG. Vacuoles formation may be related to hepatocytes lipids microvesicular deposition (SILVA and ESCANHOELA, 2009).

Pan et al. (2014) demonstrated resveratrol, in models of NAFLD, obesity or hepatic steatosis, effects on suppressing lipids, cholesterol and triglycerides accumulation, inhibiting inflammatory cells infiltration, reducing lipogenesis and oxidative stress, acting on antioxidant enzymes regulation. Bujanda et al. (2008) also observed reduction in the degree of hepatic steatosis associated with a decrease in the production of tumor necrosis factor alpha in rats treated with resveratrol.

Ahn et al. (2008) investigated resveratrol protective effect on hepatic metabolism in NAFLD models and observed effects on genes related to lipid metabolism modulation, increasing sirtuin 1 enzyme expression, an important cellular metabolism modulator, with a protective effect on HFD induced damage.

In addition to polyphenols in wine beneficial effects, moderate alcohol consumption leads to increased adiponectin plasma levels, stimulating fatty acid catabolism and inhibiting gluconeogenesis, promoting hepatic lipid content reduction (SIERKSMA et al. 2004; MAIA-FERNANDES et al. 2008). These findings suggest adiponectin possible contribution to lower vacuolization observed in WG, acting as a compensatory mechanism against oxidative damage caused by moderate alcohol consumption.

It is possible to conclude better results of grape juice on biochemical analysis and better resveratrol solution regarding hepatic morphology, and even in a HFD scenario, animals receiving resveratrol had their results matched to those receiving a balanced diet.

The importance of this study to scientific community was to demonstrate detrimental effects to liver tissue of moderate red wine consumption even though it presents cardiovascular health benefits (WOLLIN and JONES, 2001). Therefore, this study does not encourage alcohol consumption to non-alcoholics.

In clinical practice, in natura food consumption its simplest form, such as grape or whole grape juice, should be encouraged to achieve bioactive compounds present in food beneficial effects. Although the isolated compound as a supplement also has health benefits, food consumption also ensures an increase in fruit intake, as suggested by Dietary Guidelines for the Brazilian Population (BRASIL, 2014).

Further research is needed with dietary sources of resveratrol, rather than just the isolated compound.

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