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Effect of the intake of liquids rich in polyphenols on blood pressure and fat liver deposition in rats submitted to high-fat diet

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Abstract

Introduction: **seeking better quality of life, the number of studies on functional foods and disease prevention is growing fast. Whole red grape juice (WRGJ) and red wine (RW) stand out, which are rich in polyphenols, showing antioxidant and anti-inflammatory activity.**

Objective: **to evaluate the effect of WRGJ and RW intake and resveratrol solution (RS) on blood pressure and fat liver deposition of rats fed with high-fat diet.**

Material and methods: **during 60 days, 50 Rattus norvegicus Wistar Albino adults were divided into: control group (CG) - balanced diet, hyperlipidic group (HG), red wine group (RWG), grape juice group (JGG) and resveratrol group (RG) – hyperlipidic diet. Feed and water were offered ad libttum to all groups. WRGJ, RW and RS were offered daily to JG, WG and RG, respectively. Blood pressure was measured using tail plestimograph. The animals were anesthetized, sacrificed and the liver was removed, weighed and fat was extracted using Soxhlet extractor.**

Results: **no difference in weight gain, feed intake, liver weight and diastolic blood pressure among groups was observed. However, systolic blood pressure (mmHg) and liver fat concentration (g%) were lower (p <0.05) in JGG than in HG, WG and RG, but similar to CG.**

Conclusions: **the daily consumption of WRGJ minimizes the effects of high-fat diet on systolic blood pressure**

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EFECTO DE LA INGESTA DE LÍQUIDOS RICOS EN POLIFENOLES SOBRE LA PRESIÓN ARTERIAL Y DE LA DEPOSICIÓN DE GRASA HEPÁTICA EN RATAS SOMETIDAS A DIETA RICA EN GRASAS

Resumen

Introducción: **en busca de una mejor calidad de vida, crece el número de estudios sobre alimentos funcionales y la prevención de enfermedades. Destacan el jugo de uva roja (JU) y el vino tinto (VT), que son ricos en polifenoles, y tienen efecto antioxidante y antiinflamatorio.**

Objetivo: **evaluar el efecto de la ingesta de JU, VT y solución de resveratrol (SR) sobre la presión arterial y la deposición de grasa hepática en ratas alimentadas con una dieta rica en grasas.**

Métodos: **durante 60 días, 50 adultos de Rattus norvegicus Wistar Albino fueron divididos en grupos: control (GC) - dieta equilibrada y hiperlipídica (GH), vino tinto (GV), jugo de uva (JV) y resveratrol (GR) - dieta rica en grasas. El alimento y el agua se ofrecieron *ad libttum* a todos los grupos. JU, VT y SR se ofrecen diariamente a la GS, GV y GR, respectivamente. Se midió la presión arterial en la cola mediante manguito inflable. Los animales fueron anestesiados y sacrificados y se les extirpó el hígado, que fue pesado y del cual se extrajo la grasa con el extractor Soxhlet.**

Resultados: **no hubo diferencia en la ganancia de peso, consumo de alimento, peso del hígado y presión arterial diastólica entre los grupos. Sin embargo, la presión arterial sistólica (mmHg) y la concentración de grasa en el hígado (g%) fueron menores (p <0,05) en los GJ que en GH, GV y GR, pero similar a la GC.**

Conclusiones: **el consumo diario de JU minimiza los efectos de la dieta alta en grasas en la presión arterial sis-**

and prevents nonalcoholic fatty infiltration in the liver of animals, which was not observed in the consumption of RW or resveratrol solution.

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Key words: *Polyphenols. High-fat diet. Blood pressure. Fatty liver. Wistar rats.*

tólica y previene la infiltración grasa no alcohólica en el hígado de los animales, que no se observó en el consumo de VT o resveratrol aislado en solución.

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Palabras clave: *Polifenoles. Dieta rica en grasas. Presión arterial. Hígado graso. Ratas Wistar.*

List of abbreviations

WRGJ: Whole red grape juice.
RW: Red wine.
RS: Resveratrol solution.
CG: Control group.
HG: Hyperlipidic group.
WG: Red wine group.
JG: Grape juice group.
RG: Resveratrol group.
NCDs: Non-communicable chronic diseases.
NAFLD: Non-alcoholic fatty liver disease.

Introduction

Lifestyle and facilities of the modern world have influenced the eating habits of the population, encouraging the consumption of high-calorie diets with carbohydrate and / or lipid overload, known as "western" or "fast food" diets[1].

As a result of this new food trend, the consumption of diets with high fat content has increased the risk of overweight and obesity, and hence the risk of the development of non-communicable chronic diseases (NCDs) such as cardiovascular diseases and certain liver diseases[2,3,4].

Therefore, there has been an increase in the number of studies on the functional properties of foods and bioactive compounds that promote health and prevent diseases. Among these compounds, polyphenols stand out, which are found in abundance in the diet and have different physiological properties already recognized, whose relation with the prevention of diseases has been widely researched[5,6,7].

Polyphenols are known to beneficially affect a variety of inter and intracellular enzyme systems capable of modulating the immune function, inflammatory processes, vascular reactivity, antioxidant mechanisms, cell proliferation and platelet function[5,6].

In foods, various types of polyphenols can be found in vegetable sources such as fruits, vegetables, oil seeds and aromatic herbs, for example. Whole red grape juice and red wine are rich in polyphenols and studies have shown potent antioxidant and anti-inflammatory action of these foods, mainly associated with polyphenol resveratrol present in grape and its derivatives[6,7].

In addition to studies on the functional properties of foods and the action of their bioactive compounds, the pharmaceutical industry has launched in the market capsules of different polyphenols, emphasizing their antioxidant protective properties against some diseases.

The lack of adverse effects upon oral administration of certain polyphenols such as resveratrol is the first step to elucidate whether this polyphenol can be used as a phytochemical. However, further studies should be carried out with *in vitro* and *in vivo* models in order to prove the health promotion properties of polyphenols before being indicated to humans[8].

Thus, this study aims to evaluate the effect of the consumption of whole red grape juice, red wine and resveratrol solution on blood pressure and liver fat deposition of rats fed with high fat diet. The results of this study become relevant due to the possibility that the consumption of a food (grape juice) could provide protection against chronic diseases caused by the consumption of high-fat diets, showing that the consumption of foods with functional properties may be beneficial for health maintenance and protection against some diseases.

Objectives

This study aims to evaluate the effect of the consumption of whole red grape juice, red wine and resveratrol solution on blood pressure and liver fat deposition of rats fed with high fat diet.

Methods

The experiment was performed at the Laboratory of Experimental Nutrition (LABNE), *Emilia of Jesus Ferreiro* School of Nutrition, Fluminense Federal University. For the development of this work, 50 female adult *Rattus norvegicus* Wistar rats (90 days old), weighing about 200g were used. The animals were maintained in polypropylene identified individual cages for 60 days with constant temperature (22° C ± 2° C) and photoperiod of 12 hours.

The animals were divided into 5 groups (n = 10 / group): 1) Control Group (CG) - receiving control balanced diet based on casein and water; 2) Hyperlipidic

Group (HG) - receiving hyperlipidic diet based on casein and water; 3) Whole red grape juice group (JG) - receiving hyperlipidic diet based on casein, water and whole red grape juice (15 mL / day); 4) Red wine group (WG) - receiving hyperlipidic diet based on casein, water and red wine (10 mL / day), and 5) resveratrol group (RG) - receiving hyperlipidic diet based on casein, water and 15mL / day resveratrol solution.

Red wine, whole red grape juice and resveratrol solution were offered daily. Feed and filtered water were supplied weekly on free demand.

Diets (control and hyperlipidic) were prepared in the experiment site using ingredients described in table I, and control diet was prepared according to recommendations of the American Institute of Nutrition - 93M - focused on maintaining the nutritional needs of animals at adult age.

Red wine (Cabernet Sauvignon), whole red grape juice, resveratrol capsules (used for the preparation of the resveratrol solution) and lard were obtained from local trade. Nutrition information of the drinks offered (grape juice and red wine) was obtained from labels and is described in Frame I.

Weight and feed intake of all animals were weekly recorded using precision scales (BioPrecisa® JY 50001, precision 0.01 g). Blood pressure was weekly measured using tail plestimograph (Insight®) and expressed in mmHg. For this, animals were carefully handled so as to spontaneously come into the acrylic cylinder used for containment with internal dimensions (H x W x L) of 63 x 65 x 232 mm. Subsequently, they were kept in a heater (Insight®) at 42° C for 10 minutes. They were then placed in the apparatus for pressure measurement. Three pressure measurements were performed at each session.

At the end of the experiment, the animals were submitted to vaginal wash procedure for determining the stage of the estrus cycle and being in the "estrus"

Table I
Ingredient used for formulation of rations control and hyperlipidic offered in the experiment

Ingredients	*Ration Control g/100g ration*	*Ration Hyperlipidic g/100g ration*
*Casein **	14,0	14,0
Starch	62,1	46,07
Soy oil	4,0	-
Lard	-	20,0
Cellulose	5,0	5,0
Mix of vitamins[1]	1,0	1,0
Mix of minerals[2]	3,5	3,5
B-choline	0,25	0,25
L-cystine	0,18	0,18
Sugar	10,0	10,0
Total	100	100

Legend: *% of casein protein = 92,5% protein/100g casein; **[1]Mix of vitamins (mg/kg ration):** retinyl palmitate 2.4, cholecalciferol 0.025, bisulfite sodium benadiona 0.8, biotin 0.22, cyanocobalamin 0.01, riboflavin 6.6, thiamine hydrochloride 6.6 and tocopherol acetate 100; **[2]Mix of minerals (g/kg ration):** Copper sulfate 0.1, ammonium molybdate 0.026, sodium iodate 0.0003, potassium chromate 0.028, zinc sulfate 0.091, calcium hydrogen phosphate 0.145, ammoniated iron sulphate 2.338, magnesium sulfate 3,37, manganese sulfate 1.125, sodium chloride 4.0, calcium carbonate 9,89 and potassium diidrogenofosfato 14,75.

Frame I
Nutriotinal Information of whole red grape juice, red wine and resveratrol used in the experiment

	Whole red grape juice	*Red wine*	*Resveratrol solution*
*Carbohydrate (g / L) **	15000	2900	5200
*Protein (g / L) **	-	traces	-
*Lipid (g / L) **	-	-	-
*Ethanol (g / L) **	-	10600	-
*Resveratrol (mg / L) ***	0.381	1,323	1,53

Legend: * Source: Label each drink; ** Source: Data obtained by high performance liquid chromatography efficiency analysis (HPLC) during the experiment.

phase, animals were separated for sacrifice after fasting and a 6-h resting period.

Animals in the estrus phase were anesthetized by intraperitoneal injection of xylazine hydrochloride: ketamine (1: 1) at a dose of 0.1 mL / 200 g body weight, and then blood collection was performed.

After sacrifice, laparotomy was performed to remove the liver. The liver was weighed on a precision scale (BioPrecisa® JY 50001, precision 0.01 g) and its weight was standardized in g / 100 g body weight. It was subsequently dehydrated and macerated and the fat content was determined using Sohxlet extractor. The total fat content was expressed as g%.

The study was approved by the Ethics Committee on Animal Use, protocol number 00216/10. The experiment was conducted according to criteria established by the Brazilian College of Animal Experimentation / Brazilian Society of Sciences in Laboratory Animals.

The results were presented using descriptive statistics such as mean and standard deviation. One-way ANOVA was used to compare means among groups and Tukey test was used as post test. A 5% significance level was considered. To support the proposed analyses, Kurtosis and Skewness tests were used to verify the normality of data. Significance level of 5% was adopted using the GraphPad InStat software (version 3.10, 2009) to perform the analyses.

Results

At the end of the experiment, it was observed that all groups were homogeneous with respect to initial and final body weight. No difference in weight gain among groups was observed throughout the study; however, a trend of lower feed intake was observed in groups receiving high-fat diet compared to the control group, according to data presented in table II.

No difference in liver weight of animals was observed (Table II). However, there was lower ($p < 0.05$) fat deposition in the liver of animals receiving grape juice in relation to HG, WG and RG, but similar to the control group (Fig. 1).

Throughout the experiment, the blood pressure of animals was measured and it was found that diastolic blood pressure did not differ among groups, although the group receiving grape juice presented the lowest numerical value for this variable. However, animals from JG had lower systolic blood pressure level ($p < 0.05$) compared to HG, WG and RG groups, but similar to the CG (Fig. 2).

Discussion

Grape and its derivatives have several polyphenols able to promote health benefits due to their functional properties, and resveratrol is a compound highlighted in many studies[10,11]. Grapes are not the only source of these polyphenols, but also grape juice, wine and currently isolated compound capsules[11]. The resveratrol concentration in whole red grape juice (0.381mg / L) and red wine (1.323mg / L) used in this study are in agreement with literature that reports resveratrol concentration in grape juice produced in Brazil of 0.19-0.90mg / L[10] and in red wine of 0.82-5.75mg / L[12].

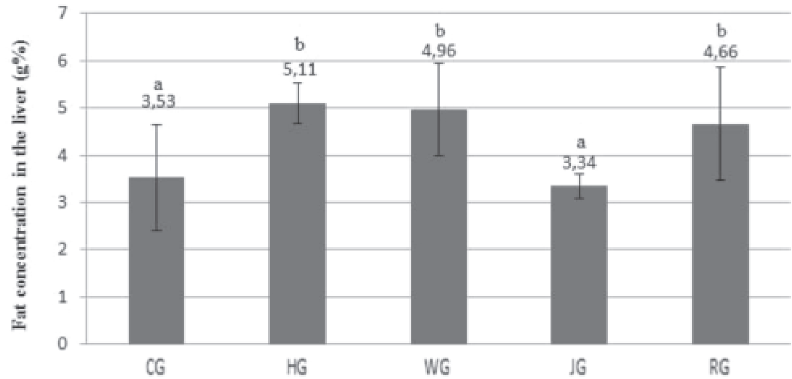
Although diets were offered *ad libitum*, a trend of lower feed intake of groups receiving high-fat diet throughout the experiment was observed. The same was observed in the study by Moura et al[13], who found that lower feed intake of group receiving diet rich in saturated fat, compared to the group consuming balanced diet, which corroborated our results. The trend of lower food consumption by animals receiving high-fat diet combined with grape juice and red wine consumption can be explained by the fact that these foods are extra source of energy for animals, increasing their satiety.

Table II

Means and standard deviation of initial and final body weight, body weight variation, feed intake and liver weight of the different study groups

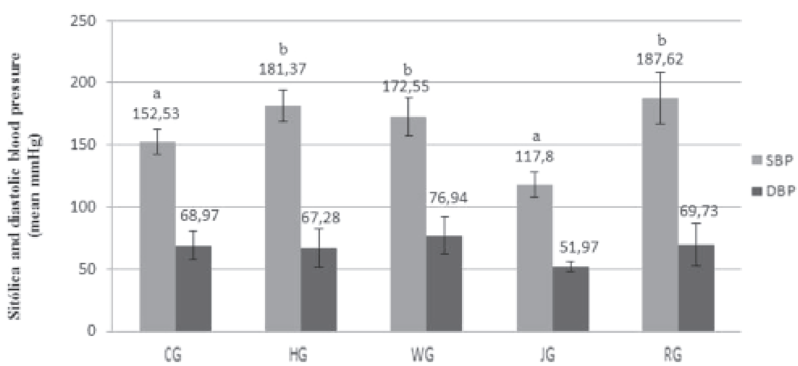
	Initial body weight (g)	*Final body weight (g)*	*Body weight variation (g)*	*Feed intake (g/100gBW)*	*Liver weight (g/100gBW)*
CG	228,57±31,05	306,51±23,83	77,93±47,75	6,01±1,34	2,91±0,19
HG	211,63±37,07	298,00±55,25	86,37±51,79	5,15±1,16	2,77±0,16
WG	220,24±34,21	300,26±46,97	80,02±61,28	4,67±1,61	2,79±0,24
JG	219,68±32,59	296,47±39,28	76,79±54,15	4,84±1,29	2,59±0,26
RG	242,80±27,85	284,60±22,26	41,80±13,92	5,95±0,38	2,55±0,11
*ρ valor**	0,3942	0,8864	0,5947	0,0674	0,019

Legend: Different letters represent significant differences between the groups ($p<0.05$); Body weight (BW); Control Group (CG) - the balanced feed; Hyperlipidic group (**HG**) - fat diet; Group whole red grape juice (**JG**) - fat diet + whole red grape juice, red wine Group (**WG**) - fat diet + red wine, resveratrol group (**RG**) - fat diet + resvertatrol solution.
Statistical analysis: one-way ANOVA and Tukey for mean comparison between groups.



Legend: Different letters represent significant differences between the groups (p<0.05); Control group (**CG**) - the balanced feed; Hyperlipidic group (**HG**) - fat diet; Group whole red grape juice (**JG**) - fat diet + whole red grape juice, red wine Group (**WG**) - fat diet + red wine, resveratol group (**RG**) - fat diet + resveratrol solution.
Statistical analysis: one-way ANOVA and Tukey for mean comparison between groups.

Fig. 1.— Mean concentration of fat in the liver of different groups (g%).



Legend: Different letters represent significant differences between the systolic blood pressure of the groups (p<0.05); Systolic blood pressure (**SBP**); Diastolic blood pressure (**DBP**); Control Group (**CG**) - the balanced feed; Hyperlipidic group (**HG**) - fat diet; Group whole red grape juice (**JG**) - fat diet + whole red grape juice, red wine Group (**WG**) - fat diet + red wine, resveratol group (**RG**) - fat diet + resveratrol solution.
Statistical analysis: one-way ANOVA and Tukey for mean comparison between groups.

Fig. 2.— Means of systolic and diastolic pressure (mmHg) animals throughout the study.

Studies have shown that the consumption of high-fat diet is correlated with increase in the obesity prevalence in Brazil and worldwide. Increased body weight due to the use of this type of diet predisposes individuals to the development of NCDs[14,15]. However, obesity development was not observed this study, since groups receiving high-fat diet showed no difference in weight gain compared to the group receiving balanced diet.

Studies with rats comparing the intake of high-protein and high-fat diets (66% lard) with balanced diet observed that the weight of the liver of animals fed with high-fat diet was not different from that of control group[15,16,17], which corroborates the findings of this study, since no difference in animals' liver weight was observed, regardless of type of diet consumed. However, literature shows that high consumption of saturated fatty acids is related to inflammatory processes and lipotoxicity of some organs, among them liver, resulting in non-alcoholic fatty liver disease (NAFLD)[13,16,17,18,19].

Thus, although animals did not present obesity, high-fat diet caused accumulation of hepatic fat compatible with the diagnosis of NAFLD, except in the group that consumed grape juice. Although the dietary intake of groups receiving high-fat diet with grape juice and red wine was similar, the liver fat accumulation in animals fed with red wine was higher. There is controversy about the effect of red wine on the liver fat deposition. A study has shown that red wine polyphenols are able to minimize oxidative stress and hepatic steatosis in rats fed with high-fat diet when consumed daily and moderately[20]. However, according to our results, the consumption of wine seems to have adversely influenced the benefits of the drink polyphenols, which can be explained by the fact that alcohol can cause an increase in the amount of free radicals, early elimination of polyphenols in the blood and accelerated elimination of these bioactive compounds[21,22], which was not observed for groups receiving grape juice, which appears to be related to the inhibition of the synthesis and deposition of liver fat in rats fed with high-fat diet that according to some researchers, is probably due to its content of polyphenols[16,21,22] and not necessarily to the resveratrol content alone. In addition, the absence of alcohol and probably the better interaction and effect of different bioactive compounds present in grape juice should be stressed.

Similar to red wine, resveratrol solution was not capable of minimizing fat liver deposition in the study animals. Chachay et al[23] investigated eight-week administration of resveratrol in humans with NAFLD and found that the compound alone did not significantly improve any characteristic of the disease when compared to placebo, but led to increased concentration of liver enzymes, increased oxidative stress in the liver of this group. This is probably because foods have other important constituents that make the action of polyphenols, particularly resveratrol, more effective than when the compound is consumed alone. The consumption of polyphenols through capsules should be done with caution because it can generate overdose and, in the case of resveratrol, there are still questions about its effective and safe doses and what are the effects of its consumption on the long term[24].

Fat accumulation in the liver predisposes individuals to other risk factors for NCDs such as hypertension[14]. Rats fed with high-fat diet after 12 weeks showed a significant increase in systolic blood pressure compared to those fed with low-fat diet[19]. These findings corroborate the results observed in this study, where animals with higher fat liver deposition also had higher systolic blood pressure, although no difference in the diastolic blood pressure among groups was observed.

According to results, in addition to the benefits found in the consumption of whole red grape juice with respect to hepatic steatosis, the drink also seems to show a protective effect on systolic blood pressure.

It has been reported that purple and green grape juice compounds are cardioprotective[21,22,25,26,27], mainly because polyphenols present in the beverage present antioxidant and ant-inflammatory capacity. Moreover, polyphenols found in whole red grape juice prevent the increase in low-density lipoprotein concentrations and prevent oxidation, minimizing the formation of atheromatous plaques on the arterial walls and preventing platelet aggregation and oxidative damage[28,29]. Another study has shown that the greatest benefit of grape juice is its ability to act on the endothelium by increasing the nitric-oxide synthesis and preserve endothelium-dependent vasodilation due to its antioxidant role[25].

Regarding resveratrol, some findings have shown that the compound appears to prevent endothelial dysfunction by increasing nitric-oxide synthesis expression in the endothelium, in addition to acting in the redox process[28,29]. However, according to Leifert and Abeywardena[30], the ingestion of high doses of resveratrol generates compound peaks 1.5 hours after consumption, but urinary excretion is rapid, leading to a reduction of 77% polyphenols up to 4 hours. These findings may explain the fact that the consumption of resveratrol solution has not promoted protection on blood pressure values in this study. This is because the solution provided higher compound concentration compared to red wine and grape juice. However, one should take into account the possibility of beneficial interactions between nutrients and bioactive compounds in the diet, leading to promotion of protection against diseases, which does not seems to occur when using the bioactive compound in the form of capsule. Often, the action of a substance can be enhanced by its association with other compounds present in the food matrix and when consumed alone may not have the expected effect.

Conclusions

The results of this study suggest that the consumption of whole red grape juice seems to be able to minimize the effects of high-fat diet, reducing the risk of developing hypertension and nonalcoholic fatty liver disease, while the consumption of red wine and resveratrol alone did not show any benefit in preventing these diseases.

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