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Otros

# Effect of grape juice consumption on antioxidant activity and interleukin-6 concentration in lactating rats

## *Efecto del consumo de zumo de uva en la actividad antioxidante y la concentración de interleucina-6 en ratas lactantes*

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## Abstract

**Objective:** This study evaluated the effect of grape juice consumption on the antioxidant capacity and the interleukin-6 blood level of lactating rats.

**Material and method:** Eighteen Wistar rats, lactating females adult, were divided into two groups: control group (CG) and grape juice group (GJG). The antioxidant activity was determined by the method of 2,2-diphenyl-1-picrylhydrazyl (DPPH)-kidnapping of free radicals (DPPH) and oxygen radical absorbance capacity (ORAC), and the interleukin-6 was determined by Enzyme-Linked Immunosorbent Assay (ELISA) method. The data were presented as mean and standard deviation.

**Results:** The antioxidant capacity was higher ($p < 0.05$) in the GJG (25.00 ± 3.08 µmol eq. Trolox/g) than in the CG (10.00 ± 3.11 µmol eq. Trolox/g), by the ORAC method. The interleukin-6 (pg/ml) level was lower in the grape juice group than in CG.

**Conclusion:** The consumption of grape juice during lactation improves the antioxidant capacity in lactating rats and seems capable to decrease the inflammatory activity.

**Key words:**

Lactation. Grape juice. Antioxidant activity. Interleukin-6. Wistar rats.

## Resumen

**Introducción:** este estudio evaluó el efecto del consumo de zumo de uva sobre la capacidad antioxidante y el nivel de interleucina-6 en sangre de ratas lactantes.

**Material y método:** dieciocho ratas Wistar lactantes adultas se dividieron en dos grupos: grupo control (CG) y grupo de zumo de uva (GJU). La actividad antioxidante se determinó mediante el método de secuestro de radicales libres (DPPH) y la Capacidad de Absorción Radical de Oxígeno (ORAC) por 2,2-difenil-1-picrilhidrazil (DPPH), y la interleucina-6 se determinó mediante Inmunosorbent Enzyme-linked Método de ensayo (ELISA). Los datos se presentaron como media y desviación estándar.

**Resultados:** la capacidad antioxidante fue mayor ($p < 0,05$) en el GJU (25,00 ± 3,08 µmol ecuación Trolox/g) que en el CG (10,00 ± 3,11 µmol ecuación Trolox/g), según el método ORAC. El nivel de interleucina-6 (pg/ml) fue menor en el grupo de zumo de uva que en CG.

**Conclusión:** el consumo de zumo de uva durante la lactancia mejora la capacidad antioxidante de las ratas lactantes y parece ser capaz de disminuir la actividad inflamatoria.

**Palabras clave:**

Lactancia. Zumo de uva. Actividad antioxidante. Interleucina-6. Ratas Wistar.

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## INTRODUCTION

The lactation is physiologically associated with increase in the metabolic processes, due to the high needs for energy, especially in the initial stage (first three months) when milk production is high (1). During this period, the chances to raise the production of the reactive oxygen species (ROS) and increase the interleukin-6 (IL-6) level, is high (2).

The literature shows that the increase in the metabolic processes associated with the deficiency of substances with antioxidant activity are able to increase the oxidative stress in the body (3), impairing normal physiological functions of the mother and contributing to the appearance of diseases in children (4,5).

The disease prevention is a purpose of the experts in health area. Therefore it is important to investigate the health benefits associated to the consumption of bioactive compounds. Among the food that contains these compounds, there is the whole red grape juice, which is rich in polyphenols and like a non-alcoholic beverage it can be consumed by breastfeeding women (6).

Therefore, in an attempt to maintain the balance between oxidative and antioxidative systems during lactation, is important increase the consumption of antioxidant compounds able to neutralize the reactive oxygen species produced in the metabolism of lactating women. The consumption of whole red grape juice was used in this study because it is rich in polyphenols with antioxidant properties (7), able to reduce or neutralize the adverse effects caused by free radicals and improve the antioxidant capacity of the maternal organism (8). It is important emphasize that there is few studies about the effects of the consumption of whole red grape juice on the antioxidant capacity and the IL-6 level, during lactation period. There are reports stating the benefits of regular consumption of grape juice to improve the endothelial function, in the reduction of total cholesterol, LDL cholesterol and inflammatory processes in non lactating health women (9).

Therefore, the aim of the present study was to evaluate the effect of the consumption of whole red grape juice, during exclusive breastfeeding period on the antioxidant capacity and interleukin-6 level, in lactating rats.

## METHODS AND MATERIALS

### ANIMALS

Ninety days old lactating female Norvegicus Wistar albino rats were obtained from the Experimental Nutrition Laboratory of the Department of Nutrition and Dietetics of the Federal Fluminense University, RJ, Brazil. Eighteen rats (260 g) were housed in individual standard cages of polypropylene with environment with constant temperature (24 °C ± 2 °C) and adequate lighting cycle (light-dark of 12/12 hours) during two weeks (period of exclusive breastfeeding), with free access to food and water.

The litter was reduced to six (three female an three male) to allow adequate and homogeneous access of all of them to breast milk. The animals were divided into two groups (n = 9 / group): a) control group (CG) received commercial feed Nuvilab CR-1; and b) grape juice group (GJG) received commercial feed Nuvilab CR-1 and whole red grape juice (15 mL), daily. The whole red grape juice was obtained in the local market and the type of grape used in juice production was the *American* type.

The animals were weighed on an electronic scale once a week. By weighing the animals, it was possible to assess the initial and final body weight of each animal. With this data, it was obtained the average variation of weight (VW) of each group studied (in grams).

The amount of feed offered and waste were weighed to obtain food consumption (FC), and the ingestion of water and juice were analyzed to obtain the consumption and waste, daily.

After six hours (6) of fasting, all animals (rats and offspring) were anesthetized and sacrificed and blood samples were collected in heparinized bottle and gently homogenized to prevent coagulation. It was centrifuged at 3000 rpm for 20 minutes to obtain the plasma and aliquots were separated and frozen at -70 °C for further analyses.

This study was submitted to the Ethics Committee responsible for research in laboratory animals of the Federal Fluminense University and has been approved, protocol number 404/2014. All animals were handled in accordance with the ethical principles adopted by the Brazilian Society of Science in Laboratory Animals (SBCAL) and all efforts were made to minimize animal suffering.

### BIOCHEMICAL ANALYSES IN GRAPE JUICE

#### Total polyphenols

Total polyphenols of the whole red grape juice were determined by the Folin-Ciocalteu method (10): 2 mL of each sample were diluted in acetone solution at 70%. This solution was filtered and diluted with 10mL of distilled water. After that, 0.5 mL of this solution was added 2.5 mL of Folin-Ciocalteu solution (10%). The mixture was incubated for 2 min at room temperature, and 2 mL of sodium carbonate (75 g,$L^{-1}$) was added. The mixture was incubated for 15 min at 50 °C and finally cooled in a water-ice bath. The absorbance readings was immediately measured at spectrophotometer (Biospectro SP 220®) with specific absorbance at 760 nm. Total phenolic content was expressed in milligram of gallic acid equivalent (GAE) per liter (GAE mg/L). All samples were analysed in triplicate.

#### Trans-resveratrol

The determination of trans-resveratrol concentration in the samples was by high-performance liquid chromatography (HPLC) according Malovaná y cols. (2001) (11). After extraction, the samples were analyzed in triplicate, and injected into the HPLC system with eluting gradient. It was used the integrated high performance liquid chromatograph LC-20AT (Shimadzu), C18 column Shimpack VP-ODS (Shimadzu®) of 5µ, C18 pre-column GVP-Shimpack 5µ

ODS (4.6 x 10 mm) of the same manufacturer for separation and array diode detector SPD-M20A for identification and quantification of substances. The loop used was 20 µL. Spectrophotometric detection was performed in the UV region and quantification was performed at 306 nm at 25 minutes elution time. The concentration of trans-resveratrol was expressed in mg/L (milligrams per liter). All samples were analysed in triplicate.

### Antioxidant activity

#### *2,2-diphenyl-1-picrylhydrazyl (DPPH) Method*

The antioxidant capacity was determined by the modified 2,2-Diphenyl-1-picrylhidrazyl (DPPH) radical method (12) which is based on the quantification of free radical-scavenging activity. Stock solution of DPPH (100 µL; 0,1 µM) was diluted with ethanol (900 µL) and absorbance read at 517 nm. 100 µL of the sample plasm were added to 900 µL of the DPPH solution for 30 min in the dark, and the decrease of the absorbance was measured at 517 nm in spectrophotometer (Bioespectro® SP 220). All samples were analysed in triplicate. The results are expressed as a percentage of free radical scavenging (% FRS) using the following equation:

$$\%FRS = 100 - \frac{(Ab_{sample} - Ab_{blank}) \times 100}{Ab_{blank}}$$

*Legend: percentage of free radical scavenging (% FRS); absorbance of sample ($Ab_{sample}$); absorbance of blank ($Ab_{blank}$).*

#### *Oxygen Radical Absorbance Capacity (ORAC) method*

The ORAC assay was performed according to Ou et al., 2001 (13), adapted for the analysis in plasma, for this were prepared: phosphate-saline buffer solution (PBS, pH 7.4), fluorescein solution, and Trolox standard solution 2, 2'-azobis (2-amidinopropane) dihydrochloride (AAPH). The Trolox standard was prepared at eight concentrations (3.25; 6.25; 12.5; 18.75; 25.00; 31.25; 37.5; 50,00 µg/mL). For blank and control PBS aliquots were used. The Trolox standard and samples were added to the plate, in differents concentrations and in duplicate. Immediately after, 120 µL of the fluorescein solution were added and then added 60 µL of AAPH solution to all wells except control. For the calculation, the Trolox standard equation was obtained. The ORAC method determines the scavenging capacity of an antioxidant against the peroxyl radical introduction of 2,2'-azobis (2-amidinopropane) dihydrochloride (AAPH) at 37 °C. It was determined using fluorescein as a fluorescent molecule in a microplate fluorometer (Fluostar Optima - BMG Labtech). The reading had the duration of 1 hour and 40 minutes. The results are expressed in micromol of Trolox equivalent/g.

The analysis of cholesterol, HDL (high-density lipoprotein) cholesterol, triglycerides, calcium, magnesium, phosphorus, protein and albumin were made by colorimetric method in a BioClin® BS-120 Chemistry Analizer automated machine. BioClin® commercial kits were used as well as specific wavelengths for each biochemical indicator.

The concentration of interleukin-6 was determined by Enzyme-linked Immunosorbent Assay (ELISA). It was used kit of the brand DRG. The reading was performed at 450 nm in a microplate reader, brand Thermoplate Reader, and the result was expressed in pg/mL.

## STATISTICAL ANALYSIS

Data were presented using descriptive statistics such as mean and standard deviation. Analysis of mean comparison within the group (before and after) were made using the hypothesis paired student *t* test and the analysis between groups it was used the unpaired student *t* test. A significance level of 5% was accepted. The GraphPadinStat software (version 3.10, 2009) was used for analysis.

## RESULTS

The initial, final and body weight variation data is shown in table I. It can be observed that the lactating rats groups maintained similar body weight and weight gain, during the study. However, the offspring of the grape juice group showed a tendency to higher weight gain during the exclusive breastfeeding period (Table I).

Table II presents the biochemical data of the lactating rats. There was no difference in the glucose, cholesterol, HDL cholesterol, triglycerides, calcium, phosphorus, magnesium, protein, and albumin plasma level, between groups.

The total polyphenols contents of the whole red grape juice used in the study was 1090.1 mg EAG L-1 and 0.381 mg/L of trans-resveratrol.

**Table I.** Initial, final and weight gain of animals during the experiment

| Variables | Control group | Grape juice group | p-value |
|---|---|---|---|
| *Lactating rats* | | | |
| Initial weight (g) | 268,13 ± 26,00 | 276,14 ± 35,21 | 0,2229 |
| Final weight (g) | 283,88 ± 22,51 | 280,43 ± 36,13 | 0,1207 |
| Weight gain (g) | 15,75 ± 19,08 | 4,28 ± 20,47 | 0,2810 |
| *Offsprings (g) / 6* | | | |
| Initial weight (g) | 8,08 ± 0,90 | 6,61 ± 0,77 | 0,0593 |
| Final weight (g) | 33,26 ± 4,55 | 35,06 ± 6,01 | 0,0560 |
| Weight gain (g) | 26,17 ± 4,79 | 28,44 ± 5,70 | 0,0540 |

**Table II.** Biochemical data of the lactating rats at the end of the study

| Variables | RV | GC | GSU | p-value |
|---|---|---|---|---|
| Blood glucose (mg/dL) | 64,00-108,00*** | 87,25 ± 7,10 | 87,57 ± 10,39 | 0,1716 |
| Cholesterol (md/dL) | 37,00-85,00** | 70,66 ± 0,11 | 68,39 ± 0,06 | 0,1376 |
| HDL (mg/dL) | 11,40 ± 37,00** | 33,46 ± 0,38 | 35,40 ± 0,39 | 0,4971 |
| Triglycerides (mg/dL) | 20,00-114,00* | 49,89 ± 0,74 | 51,61 ± 0,76 | 0,4760 |
| Calcium (mg/dL) | 9,50-11,50* | 11,27 ± 0,03 | 11,53 ± 0,08 | 0,4116 |
| Phosphorus (mg/dL) | - | 6,53 ± 0,08 | 6,66 ± 0,80 | 0,4501 |
| Magnesium (mg/dL) | - | 2,34 ± 0,05 | 2,42 ± 0,05 | 0,4903 |
| Protein (g/dL) | 4,70-8,20* | 4,90 ± 0,30 | 5,19 ± 0,19 | 0,1119 |
| Albumin (g/dL) | 2,70-5,10* | 2,99 ± 0,05 | 3,00 ± 0,11 | 0,0741 |

*Non-paired student´s t test. CG: control group; GJG: grape juice group; RV: reference value.*

Regarding the antioxidant activity in the plasm of lactating rats, it was observed by DPPH method, that the grape juice group (18.612 ± 3.106 µg/mL) presented similar antioxidant activity in relation to control group (CG) (17.191 ± 1.337 µg/mL) (Fig. 1). However, the antioxidant capacity by ORAC method in plasm, showed that the group of rats which received the grape juice presented higher antioxidant activity ($p < 0.05$) (25.00 ± 3.08 µmoL eq. Trolox/g) than the control group (10.00 ± 3.11 µmoL eq. Trolox/g). Even though, with respect to offspring, it was observed a tendency ($p = 0.06$) to higher antioxidant capacity in the offspring of grape juice group (14.00 ± 3.65 µmoL eq. Trolox/g) compared to control group (10.00 ± 2.23 µmoL eq. Trolox/g) (Fig. 2).

The IL-6 level was numerically lower in grape juice group (39.63 ± 2.53) compared to the control group (46.47 ± 14.38), but without statistical significance (p-value > 0.05).

## DISCUSSION

The literature show that phenolic compounds which are present in foods such as grapes and their derivatives, act as important natural antioxidant agents. The increase in total antioxidant capacity in plasm after consumption of food rich in phenolics compounds indicates an *in vivo* increase of the antioxidant defense status (14), it can be a favorable response to neutralize the production of free radicals in the body.

Some studies have shown that grape is a source of antioxidant bioactive compounds (15), however, its consumption is little studied when it comes to the form of its derivative (red juice), mainly in special biological moments such as pregnancy and lactation. In this research, we analyzed the antioxidant capacity in the plasma of lactating rats and their offspring, and the concentration of IL-6 in rats after ingestion of red grape juice. Similar studies have not been found in literature related to the design of this research, which made it difficult to discuss the results, so studies with other types of food or different experimental designs were used.

Weight gain (g), water consumption (mL/100 gPc/day) and feed intake (g/100 gPc/day) were suitable for the biological moment studied and they had similar results among the groups; which can be explained by the fact that animals are able to regulate their ingestion from the amount of energy consumed (16). However,

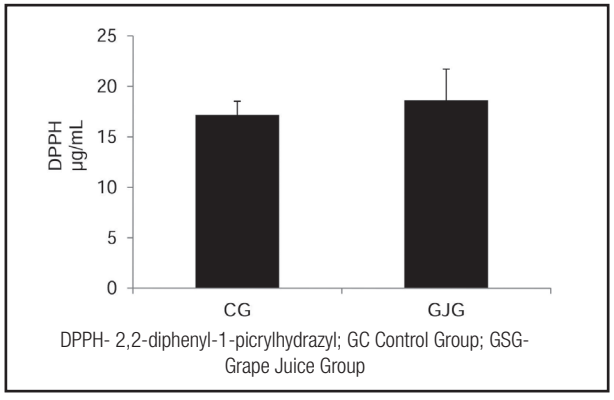


**Figure 1.**
Antioxidant activity by the DPPH method between groups.

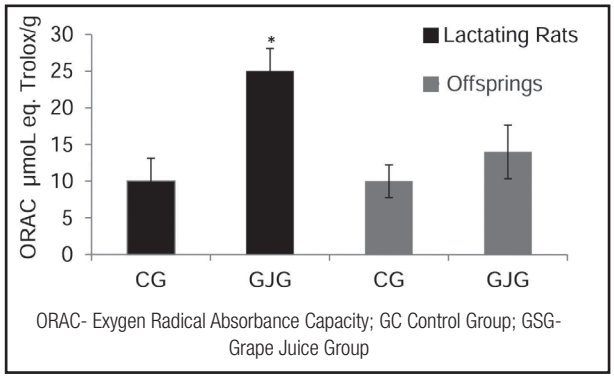


**Figure 2.**
Antioxidant capacity by ORAC method between groups of lactating rats and their offsprings.

the offspring weight of the GJG showed a tendency to be greater than the CG. Maybe the grape juice consumption by mothers can have indirect effects on weight gain of their offsprings, during exclusive breastfeeding.

The consumption of food rich in antioxidant bioactive compounds has been studied, and different methods have been applied to determine the antioxidant capacity of these types of food. And for antioxidant activity in plasm after its ingestion there are few studies. After extensive reading on the subject, the DPPH and ORAC methods were chosen to evaluate the antioxidant activity in plasma of lactating rats and their offspring after a cronic ingestion of whole red grape juice.

Regarding the content of total polyphenols, the whole red grape juice used in the present study presented 1090.1 mg EAG $L^{-1}$ of total polyphenols, this value is in accordance with the study conducted by Malacrida & Motta (2005) (17), that observed values between 600 and 2410 mg in EAG $L^{-1}$. The *trans*-resveratrol content founded in grape juice, in the present study, was 0.381 mg/L and it is in accordance with the study conducted by Sautter et al., 2005 (18), that showed that the *trans*-resveratrol level in grape juice, produced in Brazil, can vary from 0.19 to 0.90 mg/L.

These results shows that the whole red grape juice used in the experiment presents concentration of total polyphenols and *trans*-resveratrol consistent with results obtained from other studies conducted in Brazil and in others countries, which demonstrates the potential antioxidant of this fruit and their byproducts.

In the present study when analyzing the antioxidant capacity of plasma of lactating rats, by DPPH method, no difference was observed among groups. However, by using ORAC method, the antioxidant capacity of rats that received grape juice daily, was significantly higher. In accordance with Albert-Fridanza et al. (2002) (19) it can be explained by the fact that this method is more accurate and sensitive to the analysis in plasm. Which shows that a proper choice of method has fundamental importance and may be related to the matrix of the sample to be analyzed.

The potential for antioxidant protection of the whole red grape juice was identified after its cronic ingestion, in the present study; and this protection may be related to its composition, rich in bioactive compounds like phenolic compounds (resveratrol, for example) (18).

A study conducted by Di Renzo et al. (2007) (20), using the ORAC method to evaluate the antioxidant capacity in plasm of adults subjects, after consumption of red wine, vegetables and organic fruit observed an antioxidant activity in plasm (2.75 µmoL eq. Trolox/g) lower than that found in the present study. While Rodrigues et al. (2013) (21), using the method of reactive substances to the thiobarbituric acid (TBA), using adults rats, divided into 3 groups (control, organic grape juice and conventional grape juice) noted that although the organic juice has higher polyphenol content than the conventional grape juice, both were able to reduce the oxidative damage induced by (pentylenetetrazole) PTZ in plasm of the Wistar rats.

As discussed in literature, the grapes and their byproducts have antioxidant activity and also anti-inflammatory activity (18). Thus, the results of this study may suggest possible protective anti-inflammatory action due to the polyphenols content present in grape juice, as its consumption shows a trend of lower production of IL-6. So, although there is no statistical difference in concentration of IL-6 among groups studied, it may be suggested that if the whole red grape juice had been consumed for a longer period, probably there would be a statistical significance.

Other studies, although in different biological moment, also showed that grape juice consumption is able to reduce the concentration of IL-6 (22), reduce cardiovascular diseases and platelet aggregation due to reduction of oxidative stress. It is suggested that the decrease in chances of developing chronic non-communicable diseases in humans can occur through daily consumption from 5 to 7.5 mL/kg of whole red grape juice, during one week (23).

The biochemical analysis showed that all the biochemical indicators studied were within the range of normality, according to the ©Canadian Council on Animal Care (1993) (24), and studies by Melo et al., 2012 (25); Lima et al., 2014 (26), for both groups.

The result of the study of the antioxidant capacity in plasma from lactating rats, indicates that grape juice is an important source of poliphenols in diet and, it can help preventing a variety of chronic diseases due to its capacity to reduce oxidative stress and maybe reduce the IL-6 production.

With the information provided, it is observed the importance of adequate nutritional counseling during the lactation period in order to reduce the risks caused by the action of free radicals (27).

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