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Resveratrol and grape juice differentially ameliorate cardiovascular autonomic modulation in L-NAME-treated rats

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ABSTRACT

Polyphenols consumption detected in red wine and grape juice may prevent or help in the treatment of hypertension. However, cardiovascular autonomic effects of polyphenols were poorly studied. Therefore, we evaluated the effects of resveratrol and grape juice treatments in hemodynamics, baroreflex sensitivity, heart rate (HR) and blood pressure (BP) variability and cardiac redox parameters. Male Wistar rats were divided in 3 groups ($n = 7$/each) and treated for 30 days: only L-NAME-treated (60 mg/kg/day by oral gavage), L-NAME + resveratrol (L-NAME + R) and L-NAME + grape juice (L-NAME + G). BP signal was directly recorded and pulse interval (PI) and systolic arterial pressure (SAP) variability were analyzed in time and frequency domains. Baroreflex sensitivity (BRS) was determined by the alpha index. Oxidized and reduced glutathione concentrations were determined in cardiac tissue. L-NAME increased BP with no differences among groups (mean BP: L-NAME = 124 ± 4, L-NAME + R = 126 ± 3 and L-NAME + G = 125 ± 4 mmHg). PI and SAP variability expressed by total variance were also similar among groups. However, normalized low frequency (LF) and high frequency (HF) components of PI variability were lower and higher, respectively, in both R and G-treated groups when compared to only L-NAME group. Interestingly, sympathetic modulation to the vessels (LF from SAP variability) and BRS were decreased and increased, respectively, only in L-NAME + R rats. Additionally, GSH/GSSG ratios were higher in L-NAME + R and L-NAME + G than in L-NAME group. Our results indicate that resveratrol and grape juice treatments can modulate autonomic function and promote cardiac redox benefits even when nitric oxide is decreased. Moreover, resveratrol influences not only cardiac but also vascular autonomic modulation.

1. Introduction

Hypertension is a clinical condition of high prevalence and difficult control. About 7.6 million deaths worldwide are attributable to elevated blood pressure (BP) levels. Among the risk factors for hypertension are age, gender, ethnicity, overweight, obesity, sedentary lifestyle and unhealthy eating habits (WHO, 2009). Furthermore, for each 20 mmHg increase in systolic BP (SBP) or 10 mmHg in diastolic BP (DBP), the risk of mortality from ischemic heart disease and stroke is doubled. On the other hand, a reduction of 5 mmHg in DBP and/or 10 mmHg in SBP may lower the risk of stroke by one-third and the risk of coronary heart disease by one-sixth (Rosendorff et al., 2007).

Moreover, two dominants vasoactive systems, endothelium and sympathetic nerves, seem to be important counterparts of blood pressure regulation in hypertension. It has been also demonstrated in rats that the inhibitory effect of L-NAME on endothelial function is entirely reversed by the treatment with NO donors (Kristek, 2000; Török and Kristek, 2002), Available data indicate that sympathetic overactivity plays a major role in the hypertension induced by chronic NO inhibition (Kunes et al., 2004; Pechánová et al., 2004a; Zicha et al., 2006). Nevertheless, we have not found in the literature any study that well covers the autonomic effects of grape juice and the association of these effects with redox parameters.

Strategies for changing dietary habits and increasing the consumption of polyphenols have been proposed to prevent or help the treatment of hypertension. In this context, the antioxidant, anti-apoptotic and anti-inflammatory properties of resveratrol, a polyphenol found in red wine and grape juice (Romero-Pérez et al., 1999), have been investigated in many studies, as it seems to prevent endothelial dysfunction.

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Indeed, a significant decrease in BP in Korean hypertensive males was found in male subjects who ingested grape juice for 8 weeks (Park et al., 2004). In addition, in nephrectomized spontaneous hypertensive rats, resveratrol consumption attenuated hypertension and prevented endothelial nitric oxide synthase uncoupling. However, when these rats were treated with resveratrol and L-NAME, the benefits were no longer observed (Bhatt et al, 2011).

In contrast, other studies have shown that red wine treatment may prevent the increase in BP, cardiovascular remodeling and the oxidative stress in L-NAME treated rats (Pechánová et al., 2004a, 2004b). Additionally, in rats with heart failure, resveratrol seems to improve survival and attenuate systolic dysfunction and BP levels (Wu et al., 2001).

These benefits have been explained by the positive effect of resveratrol and other polyphenols on endothelial nitric oxide synthase expression (Chu et al., 2011; Petrovski et al., 2011). However, other mechanisms such as redox balance and autonomic modulation and barorreflex sensitivity have been poorly explored.

The autonomic functional indexes such as heart rate variability, systolic arterial pressure variability and baroreflex sensitivity (BRS) have been regarded as important markers of mortality and organ damage (La Rovere et al., 1998; Miao and Su, 2002). Indeed, the interaction between antioxidant enzymes, inflammatory conditions redox parameters and autonomic nervous system has been shown in recent studies (Zhang et al., 2010; Lee et al., 2011). Nevertheless, we have not yet found any study that well covers the autonomic effects of grape juice and the association of these effects with redox parameters.

Therefore, the aim of this study was to evaluate the effects of resveratrol and grape juice treatments on oxidized and reduced glutathione in cardiac tissue, BRS and cardiovascular autonomic modulation and whether NO blockade interfere in these effects. The demonstration of an improvement in these parameters would offer a new tool for early prevention of cardiovascular disease.

2. Methods

2.1. Animals

Experiments were performed on male Wistar rats (251 ± 10 g), with 12 weeks of age, from the Animal Shelter at Federal University of Pelotas, Rio Grande do Sul, Brazil, receiving standard laboratory chow and water ad libitum. The animals were housed in cages with 3 or 4 animals each in a temperature-controlled room (22 °C) with a 12-h dark–light cycle. All surgical procedures and protocols used were in accordance with the Guidelines for Ethical Care of Experimental Animals approved by the International Animal Care and Use Committee.

The rats were randomly assigned into 1 of 3 groups according to the following treatments regimen ($n = 7$ each): control group (only L-NAME), resveratrol (L-NAME + R) and grape juice treated (L-NAME + G), all accompanied for 30 days. Concentrations of resveratrol were calculated in order to obtain the same proportion found in grape juice (Wood CellarHouse®), and were established after analyzing the juice composition. In this analysis, we determined the amount of 0.062 mg of resveratrol per 100 ml of juice. The choice of the volume to be administered to the animals was determined based on the beneficial effects found in the daily intake of 500 ml of grape juice/day for a healthy individual. For the resveratrol and grape juice treatments, 2 ml of the solutions were daily administered by gavage. L-NAME was diluted in ~1 mL of water and also given by gavage (60 mg/kg/day).

2.2. Cardiovascular assessments

After the protocol period, 2 catheters filled with 0.06 mL saline were implanted into the femoral artery and vein (PE-10) in anesthetized animals (Ketamine 80 mg/kg + Xylazine 12 mg/kg) for direct measurements of BP.

Rats were studied 1 day after catheter placement; they were conscious and allowed to move freely during the experiments. An arterial cannula was connected to a strain-gauge transducer (P23Db, Gould-Statham, Oxnard, CA), and AP signals were recorded over a 30-minute period by a microcomputer equipped with an analog-to-digital converter board (Windaq, 2 kHz sampling frequency; Dataq Instruments, Inc., Akron,OH). The recorded data were analyzed on a beat-to-beat basis to quantify changes in mean AP and heart rate (HR) (Farah et al., 1999; Harthmann et al., 2007; Souza et al., 2007).

2.3. Heart rate and blood pressure variabilities

Time-domain analysis consisted of calculating the variance from pulse interval (PI) and systolic arterial blood pressure (SAP) respective time series. For frequency domain analysis, the whole 20-min time series of PI and SAP were cubic-spline-interpolated (250 Hz) and -decimated to be equally spaced in time. Following linear trend removal, power spectral density was obtained by the autoregressive method over 16,384 points with a Hanning window (512) and 50% overlapping. Spectral power for very low- (VLF 0–0.20 Hz), low- (LF 0.20–0.75 Hz), and high- (HF 0.75–3.0 Hz) frequency bands was calculated by means of power spectrum density integration within each frequency bandwidth, using a customized routine (MATLAB 6.0, Mathworks) (Soares et al., 2004).

Given the fact that in freely moving animals the proportional contribution of the very low frequency component may increase in 20-min-long recordings, data were also submitted to another processing routine, where the 20-min recordings of the decimated PI signal (2048 points) were segmented in 2.5-min periods, and only steady segments were processed as above described.

Spectral power within the low frequency component and high frequency component bands in each animal along the 20-min recordings were averaged across the 2.5-min segments and used for analysis (Soares et al., 2004). The baroreflex sensitivity was evaluated by alpha index. The alpha index analysis evaluates short-term changes in the systolic blood pressure and in the PI interval. The coherence between the PI and the SAP signal variability was assessed by means of a cross-spectral analysis. The alpha index in the LF band was calculated only when the magnitude of the squared coherence between the PI and SAP signals exceeded 0.5 (range, 0–1). After coherence calculation, the alpha index was obtained from the square root of the ratio between PI and SAP variability in the two major LF bands (Pagani et al., 1988).

2.4. Determination of oxidized and reduced glutathione concentration

To determine oxidized and reduced glutathione concentrations, cardiac tissue was deproteinized with 2 mol/L perchloric acid, centrifuged for 10 min at 1000 g, and the supernatant was neutralized with2 mol/L potassium hydroxide. The medium reaction contained 100 mmol/L phosphate buffer (pH 7.2), 2 mmol/L nicotinamide dinucleotidephosphate acid, 0.2 U/mL glutathione reductase, and 70 μmol/L5,5′ dithiobis (2-nitrobenzoic acid). For the determination of reduced glutathione,supernatant was neutralized with 2 mol/l potassium hydroxide in order to react with 70 μmol/l 5,5′ dithiobis (2-nitro benzoicacid), and the absorbance values were measured at 420 nm (Akerboom and Sies, 1981).

2.4.1. Statistical analysis

Data are reported as means ± SEM, and ANOVA (one-way) was used to compare groups, followed by the Student–Newman–Keuls test. Pearson correlation was used to study the association between variables.

Table 1
Hemodynamics variables in L-NAME, L-NAME + R and L-NAME + G groups.

Measurement/Group	L-NAME	L-NAME + R	L-NAME + G
Systolic BP (mmHg)	174 ± 2	176 ± 3	173 ± 3
Diastolic BP (mmHg)	138 ± 3	141 ± 2	140 ± 4
Mean BP (mmHg)	124 ± 4	126 ± 3	125 ± 4
Heart rate (bpm)	380 ± 6	377 ± 9	382 ± 8

Results are shown in mean ± SEM. BP = blood pressure. R = resveratrol, G = grape juice.

3. Results

3.1. Hemodynamic evaluations

The direct BP evaluation performed at the end of the protocol was not significantly different among groups, as well as the HR (Table 1). These results indicate that hemodynamic changes induced by L-NAME treatment were not modified by resveratrol or grape juice treatments.

3.2. PI and SAP variabilities in time and frequency domains

The results of PI and SAP variabilities are presented in Fig. 1. The PIV and SAPV in time domain expressed by variance were similar among groups. Analyzed in the frequency domain, HR showed that absolute LF and absolute HF power components of PIV were similar among groups. However, the normalized LF component of PIV was lower in L-NAME + R and L-NAME + G when compared to the L-NAME group. In addition, the normalized LF component of PIV was similar between LNAME + R and LNAME + G groups. The HF component of PIV was increased in L-NAME + R and L-NAME + G in comparison with L-NAME. However, no significant difference was observed between normalized HF component of L-NAME + R and L-NAME + G rats. Consequently, sympathovagal balance was significantly decreased in L-NAME + R and L-NAME + G groups when compared to the L-NAME group. The LF component of SAPV was lower in L-NAME + R than L-NAME group while L-NAME-G rats were not different from L-NAME animals. Similarly, BRS (alpha index) was improved only in L-NAME + R rats and no differences were observed between L-NAME + G and L-NAME rats.

3.3. Cardiac redox parameters

GSSG values were lower in L-NAME + R (0.0023 ± 0.0008 mmol/gtec) and L-NAME + G (0.0026 ± 0.0008 mmol/gtec) than in L-NAME (0.0052 ± 0.0005 mmol/gtec) group. However, GSH values were similar among the groups L-NAME + G (0.10 ± 0.01 mmol/gtec), L-NAME + R (0.095 ± 0.011 mmol/gtec) and L-NAME (0.08 ± 0.01 mmol/gtec). Consequently, GSH/GSSG ratios were higher in L-NAME + R, (41 ± 5) and LNAME + G (39 ± 4) than in L-NAME group (18 ± 3).

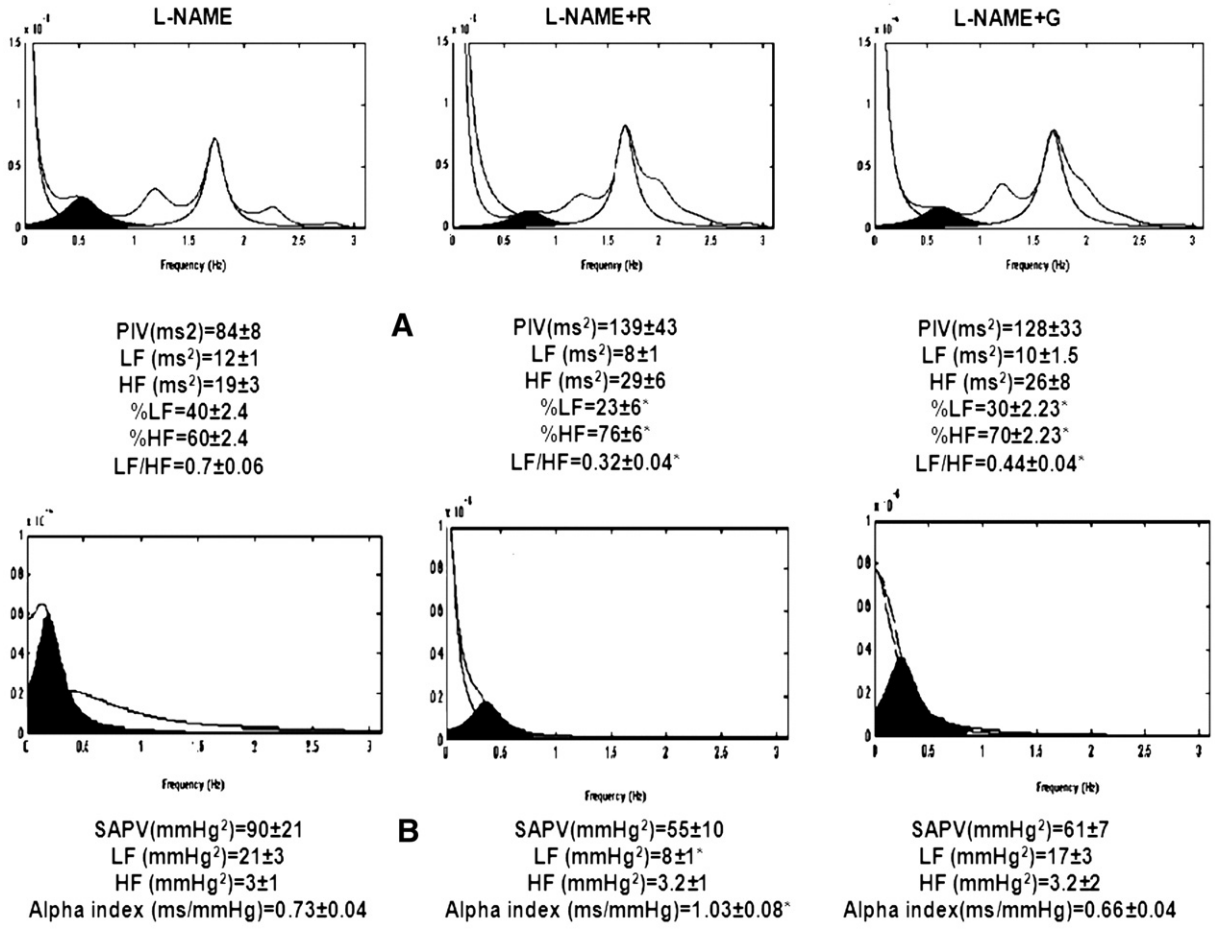

Fig. 1. (A) Pulse interval variability (PIV) and systolic arterial pressure variability (SAPV). There was no differences between PIV and SAPV in time domain and absolute values in frequency domain among groups. (B)Normalized index of PIV in frequency domain and baroreflex index (alpha index) were altered after treatment. Results in mean ± SEM, $^{*}p < 0.05$ vs. L-NAME.

3.4. Correlation analysis

Correlation was carried out by associating autonomic and cardiac redox parameters (Fig. 2). A positive correlation was found between GSH/GSSG ratio and PIV ($r = 0.88$ $p = 0.0001$). Additionally, a positive correlation was found between GSH/GSSG and alpha index (0.73 $p = 0.004$). The sympathovagal balance and absolute LF component of SAPV showed a negative correlation with GSH/GSSG ($r = 0.7$ $p = 0.004$ and $r = 0.63$ $p = 0.01$ respectively).

4. Discussion

The present study have shown that L-NAME rats undergoing resveratrol and grape juice treatments did not present any significant attenuation of hypertension compared to those which had not undergone this treatment. However, resveratrol and grape juice treatments promoted autonomic and oxidized and reduced glutathione in cardiac tissue improvements. These results may be explained in the light of other findings in the literature showing the positive effects of resveratrol and other polyphenols (Petrovski et al., 2011) on endothelial nitric oxide synthase. In the present study, the benefits of the treatments might not be associated with these positive effects, since all animals received L-NAME, a known blocker of the isoforms of nitric oxide synthases. Moreover, resveratrol may act on endothelial cells through different mechanisms, for instance, the activation of Sirt 1 (silent mating type information regulation 2 homolog) and KLF2, (Krüppel-like factor 2), the phosphorylation mediated by AMPK (adenosine monophosphate (AMP)-activated protein Kinase) and ERK1/2 (extracellular – signal-regulated kinase) and by enhancing antioxidant enzymes expression and lowering reactive oxygen species (Schmitt et al., 2010). In this context, and due to the inhibition of NO, we believe that these other forms of resveratrol action may partially account for our results in both treated groups, LNAME + R and LNAME + G.

As far as we know, this is the first study showing beneficial effects of resveratrol and grape juice in autonomic modulation. Our results clearly demonstrate a decrease in sympathovagal balance in both treated groups, and an additional decrease in SBP sympathetic modulation and an increase in the alpha index only in resveratrol group. This finding is relevant because several other studies have reported that increased sympathetic modulation may lower BRS, and decrease vagal modulation to the heart.

These effects could be associated not only to increases in both mortality and morbidity, but also to the end organ damage (Miao and Su, 2002; Mostarda et al., 2009; Mostarda et al., 2011). Therefore, strategies which improve BRS, and as such, attenuate autonomic disturbance, can contribute to greater longevity and less target-organ damage in hypertension.

The mechanisms by which resveratrol and grape juice would promote these changes seem to be different, since sympathetic modulation of SAP and BRS were improved only in L-NAME + R group. Therefore we can hypothesize that while resveratrol acts in both cardiac and vascular autonomic modulation, grape juice acts primarily in cardiac modulation. Moreover, in our protocol no significant difference between GSH/GSSG ratio was observed in neither of the treated groups. This result indicates that other changes in the

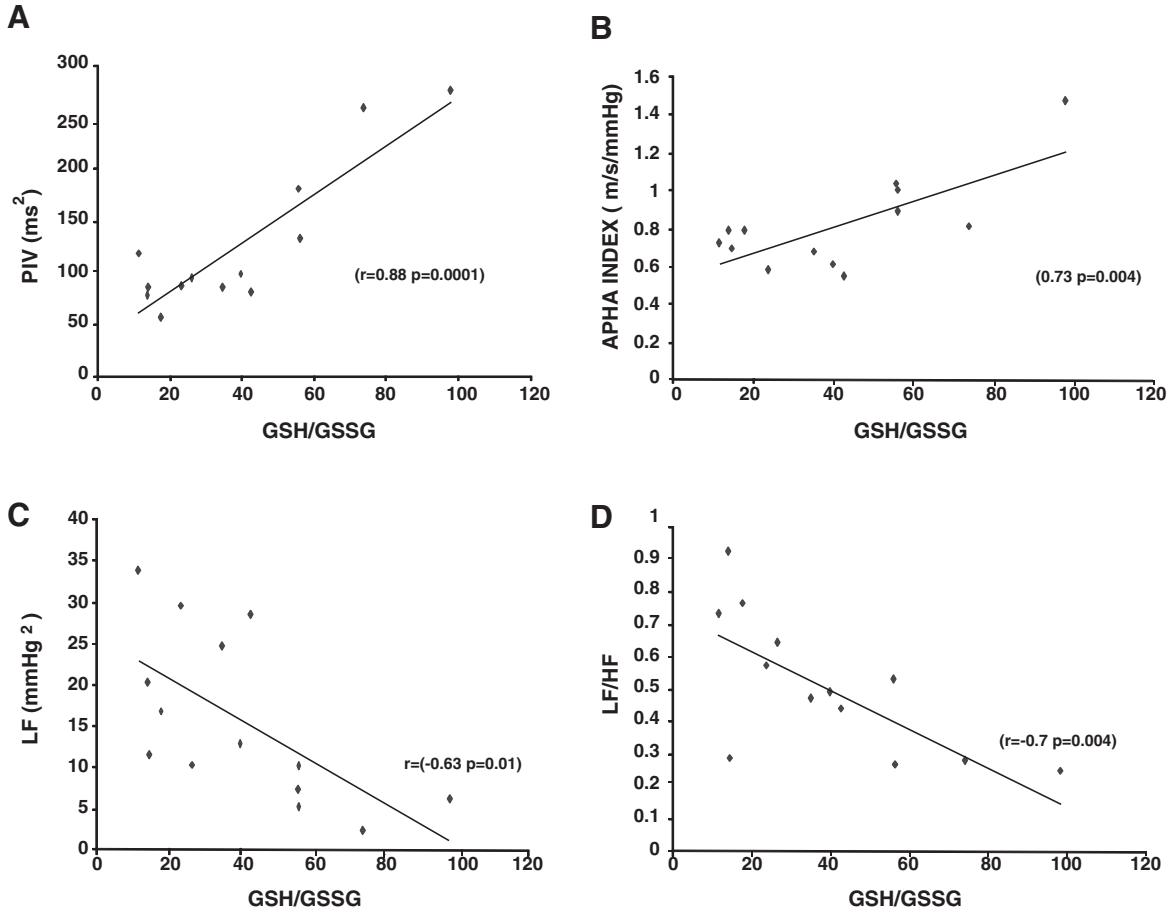

Fig. 2. The graphs are showing the association between GSH/GSSG ratio and autonomic indexes: A) PIV (pulse interval variability), B) alpha index, C) LF SAPV (low frequency component of systolic arterial pressure variability), and D) LF/HF (sympathovagal balance).

antioxidant enzymes such as glutathione peroxidase, superoxide dismutase and thioredoxin may be related to these autonomic improvements. Another possibility is the fact that resveratrol may attenuate autonomic modulation by reducing both the expression and activity of NADPH oxidase (Spanier et al., 2009).

Indeed, it has been demonstrated that NADPH activation is associated with increased sympathetic nervous system modulation in physiopathological conditions such as in central inflammation (Zhang et al., 2010). Similarly, other researchers have suggested that xanthine oxidase inhibition promotes an increase in the left ventricular function, a decrease in the catecholamine levels, an attenuation of arrhythmias and sympathetic hiperinnervation in post infarcted hearts (Lee et al., 2011). The positive correlation found between PIV with GSH/GSSG and baroreflex index with GSH/GSSG reinforces this hypothesis. In fact, the negative correlation between the sympathovagal balance and sympathetic modulation with GSH/GSSG suggests an interaction between autonomic modulation and cardiac redox parameters.

In conclusion, our results indicate that resveratrol and grape juice treatment can promote cardiovascular and redox benefits even when the nitric oxide is decreased by the inhibition of nitric oxide synthase. The additional improvement observed in sympathetic modulation to the vessels (LF component of SAPV) in the rats treated only with resveratrol may be attributed to the improvement in BRS, which was only observed in this group.

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