Dose-related effects of red wine and alcohol on heart rate variability

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ABSTRACT

In healthy subjects a standard drink of either red wine (RW) or ethanol (EtOH) has no effect on muscle sympathetic nerve activity (MSNA) or on heart rate (HR) whereas 2 drinks increase both. Using time and frequency domain indices of HR variability (HRV), we now tested, in 12 subjects (24-47 years, 6 men) the hypotheses that: 1) this HR increase reflects concurrent dose-related augmented sympathetic HR modulation; and 2) RW with high polyphenol content differs from EtOH in its acute HRV effects. RW, EtOH, and water were provided on 3 days, 2 weeks apart according to a randomized, single-blind design. Eight minute segments were analyzed. One alcoholic drink increased blood concentrations to 36±2mg/dL (mean±SE), and 2 drinks to 72±4 (RW) and 80 ±2mg/dL (EtOH). RW quadrupled plasma resveratrol (P<0.001). HR fell after both water drinks. Compared with respective baselines, one alcoholic drink had no effect on HR or HRV, whereas two glasses of both increased HR (RW: +5.4±1.2; EtOH: +5.7±1.2 min⁻¹; P<0.001), decreased total HRV (RMSSD) by 28-33% (P<0.05) and high frequency (HF) spectral power by 32-42% (vagal HR modulation), and increased low frequency (LF) power by 28-34% and the LF/HF ratio by 98-119% (sympathetic HR modulation) (all P≤0.01). In summary, when compared with water, one standard drink lowered time and frequency domain markers of vagal HR modulation. When compared with respective baselines, two alcoholic drinks increased HR by diminished vagal and augmented sympathetic HR modulation. Thus, alcohol exerts dose-dependent HRV responses, with RW and EtOH having similar effect.

Key Words: nervous system – sympathetic; nervous system – parasympathetic, heart rate variability, ethanol, spectral analysis
INTRODUCTION

In epidemiological studies, light to moderate alcohol intake is associated with less risk than is abstinence for coronary heart disease, ischemic stroke, and heart failure (12, 24), but the hypothesis that cardiovascular event rates are reduced by such alcohol consumption has yet to be tested prospectively in large clinical trials. The American Heart Association (AHA) Nutrition Committee does not recommend initiating alcohol consumption for cardiovascular risk reduction, but advises men who drink to limit their intake to 2 drink equivalents per day and women to 1 drink equivalent per day. Because of their similar alcohol content (15-18g), each of a 12-ounce bottle of beer (355mL), a 4-ounce glass of wine (120mL), and a 1½ ounce (44mL) shot of spirits is considered one standard “drink” (8, 18).

The potential benefits of long-term alcohol consumption have been attributed to its metabolic, anti-thrombotic, anti-coagulant, anti-oxidant or anti-inflammatory properties and, in the context of the present analysis, to its effects on hemodynamics, vascular endothelial function, and neuro-humoral regulation of the circulation. Evidence from a variety of sources has suggested that constituents of red wine, such as polyphenols, might augment these several actions. However, until we conducted a singled blinded randomized controlled trial of the hemodynamic and vascular effects of one and two standard drinks of red wine or ethanol (33), there had been no comprehensive evaluation and comparison of the acute cardiovascular and sympathoneural effects of low to moderate doses of red wine and ethanol. In those experiments one glass had no effect on heart rate (HR) or on efferent sympathetic nerve traffic directed at skeletal muscle, whereas 2 glasses increased both. Ethanol and red wine had similar effect on both responses.
The present objective was to determine, using time- and frequency-domain heart rate variability (HRV) analysis, whether the rate increase observed reflects a concordant increase in cardiac sympathetic HR modulation, a diminished vagal heart rate modulatory effect of alcohol, or both. Using water as a control for time and volume, we tested the hypotheses that: 1) the chronotropic effects of ethanol result from dose-related augmented sympathetic HR modulation; and 2) red wine with verified high polyphenol content and ethanol differ with respect to their acute effects on HRV.

METHODS

Subjects
Thirteen healthy non-smoking adult men and non-pregnant women of non-Asian ancestry were studied. Both heavy drinkers and total alcohol abstainers were excluded. Subjects with any cardiovascular or metabolic disorder were excluded, and none took any prescription or non prescription drug over the time course of the study. This protocol (33) was approved by our Institutional Research Ethics Board, and all subjects provided informed written consent.

Protocol
Subjects attended three morning sessions, during which one of either red wine, ethanol or water was administered at random (33). They were instructed to abstain from caffeine, alcohol and flavanoid-rich drinks (such as fruit-juices) and food (fruits, dark chocolate, etc.) from the afternoon before each session onward and to eat only a light breakfast on the study morning.
Red wine, ethanol and heart rate variability

Subjects sat in a reclining chair with leg support. An antecubital vein was canulated for blood sampling. HR was determined continuously from an electrocardiogram (ECG). Blood pressure was recorded automatically at one minute intervals from the upper arm (Dinamap Pro 100, Critikon, Tampa, FL). A respiratory belt encircled the abdomen.

After 10 min of quiet rest, baseline signals were acquired during 8 min of spontaneous breathing. After blood was drawn, the first drink was ingested over 5 min. After approximately 10 min, when blood alcohol concentration (BAC) reached approximately 40mg/dL a second set of data was acquired. Once BAC had fallen to 25-30mg/dL, the second drink was ingested. A third set of data was acquired once the BAC peaked, at about 90mg/dL. Subjects were then monitored until their BAC fell below 10mg/dL. The water-intervention protocol followed the same time course as the wine and EtOH study day protocols.

Interventions

The Quality Assurance Laboratory of the Liquor Control Board of Ontario (LCBO), Toronto, Canada, selected a moderately priced Pinot Noir (Wolf Blass, Australia; 2001) determined to have high t-resveratrol (9.36 mg/L) and catechin (67.2 mg/L) concentrations and an average quercetin concentration (11.4 mg/L). Ethanol (95%), provided by the Hospital Pharmacy, was diluted with bottled Perrier to a volume and concentration equal to the wine, and flavored with a sugar-free artificial flavoring (Crystal-Light; <1.3g) for palatability. Equal volumes of Perrier were provided as water control. Concentrations of free resveratrol, catechin and quercetin in the bottle wine, in venous plasma and urine were determined by gas chromatography (32).
Blood-Alcohol Concentrations

Classical pharmacokinetics (see Figure 1, in Spaak et al (33)) were applied to calculate for each subject the volume required to achieve with one drink a BAC of 40mg/dL, e.g. for a 68 kg male, 155mL of wine with 12% alcohol content (15g of ethanol). This volume was then doubled (310mL) for the second dose. BAC was assessed using a breathalyzer (Intoxilyzer®SD-5, CMI-Inc, Owensboro, KY) (accuracy <0.5mg/dL).

Heart rate variability

The ECG signal was sampled at 1000Hz, and with the breathing signal digitized and stored using LabView (National Instruments, Austin, TX) for subsequent analysis (1). After all studies were completed, the RR-interval series, constructed from each 8 min ECG recording, were submitted to blinded HRV analysis in the time and frequency domains using Microsoft Windows®-based software (HRV Analysis 1.1; Courtesy of Biosignal Analysis and Medical Imaging Group, University of Kuopio, Finland) (25).

Time-domain analysis focused on methods appropriate to such brief recordings. These included three statistical representations of high frequency (predominantly vagal) HR modulation (29): the standard deviation of RR-intervals [STD]; the root mean square successive difference of RR-intervals [RMSSD]; and the number of successive difference of intervals which differ by more than 50 ms, expressed as a percentage of the total [pNN50]), plus geometrical methods (triangular index) and Poincaré plots. In the triangular index (RR-triindex) the length of RR-intervals serves as the x-axis of the plot and the number of each RR interval length serves as the y-axis. The length of the base of the triangle is used and approximated by the main peak of the RR interval frequency distribution diagram. The triangular interpolation of NN interval
histogram (TINN) is the width of the distribution measured as a base of a triangle, approximating the NN interval distribution. TINN relates to total spectral power in frequency domain measures, but is less sensitive to corruption by artifacts and ectopic beats (15, 35). Poincaré plots provide a nonlinear dynamic representation of RR interval fluctuation. Each RR interval is plotted as a function of the previous RR interval, and the standard deviation (SD) of the distribution is calculated. SD-1 is a function of short term variability (usually caused by respiratory sinus arrhythmia), while SD-2 quantifies longer-term variability (15, 29).

To better discriminate between sympathetic and parasympathetic contributions to HR modulation, HR spectral power in the frequency domain was quantified by fast Fourier transformation. In addition to total power, absolute and normalized (i.e. the ratio of power within a specific band to the net of total power minus power within the very low frequency [<0.04 Hz] spectral component) power were calculated separately for the low frequency (LF; predominantly sympathetic) (0.04-0.15 Hz) and high frequency (HF) (0.15-0.40Hz) spectral band, and the LF/HF ratio was derived by convention as a postulated additional representation of the strength of cardiac sympathetic HR modulation (15, 23, 35).

**Statistical Analysis**

Values are expressed as mean± standard error (SE). A linear mixed-effects model was used to account for repeated measurement after 0, 1 and 2 drinks on three different days. Comparisons were made using linear contrasts on the results of the fitted model. A linear contrast was also constructed to test for equal trends over time for the ethanol and wine days. P<0.05 was applied as the threshold for statistical significance.
RESULTS

All subjects completed all 3 experimental sessions, but in 1 subject a high prevalence of premature supra-ventricular beats on all study days precluded power spectral density calculations. Reported are findings in 6 men and 6 women, aged 35(24-47) years (mean (range)), whose body mass index was 23(18.4-27.6) kg/m².

The baseline measurements did not differ between the 3 experimental sessions.

Similar BAC were achieved after 1 and 2 alcoholic drinks: for wine 35.9±2mg/dL and 71.8±4mg/dL; for ethanol 35.7±2mg/dL (P=0.58) and 80.2±2mg/dL (P=0.10; P<0.001 from baseline for both). Only wine increased significantly plasma and urine polyphenols (33), and plasma resveratrol quadrupled from 11.9±2.3 to 46.7±9.6 μg/L (P<0.001).

There was no significant change in blood pressure after 1 or 2 drinks of water, ethanol, or wine. One and two drinks of water, in combination with the resting time, caused a small but significant reduction in HR. The first glass of wine had no effect on HR, whereas the first glass of EtOH caused a small increase, as compared to the effects of one drink of water. The second glass of both alcoholic beverage increased HR significantly (+5.4±1.2 beats/min for wine and +5.7±1.2 beats/min for ethanol, both P<0.001). However there were no differences between wine and ethanol with respect to these HR responses (P=0.49) (Table 1).

With respect to time-domain analysis, one drink of ethanol or red wine did not alter RMSSD, STD (ms), or STD (min⁻¹) from their respective baseline values but when compared to the
response to one glass of water, STD (ms), RMSSD and pNN50 all decreased significantly. Two glasses of either alcoholic drink caused significant suppression of all time domain parameters except STD (min⁻¹). RMSSD, for example, decreased by 28% after ethanol and by 33% after wine. Geometrical parameters also were suppressed by two alcoholic drinks (Table 1, Figure 1).

In the frequency domain, 1 alcoholic drink did not alter any spectral power parameters from their respective baseline. When compared with the response to one glass of water, the glass of red caused significant suppression of HF total power (Fig. 1; Table 1). However, 2 glasses of both alcohols altered HRV significantly when compared both to respective baselines, and to 2 glasses of water: HF spectral power decreased by between 32 and 42%, and LF spectral power increased by between 28 and 34%, and the LF/HF ratio increased by between 98 and 119% (all P ≤ 0.01; see Table 1).

DISCUSSION

It is known that acute alcohol intoxication with 0.7-1.0 g/kg reduces RMSSD and HF spectral power, whilst increasing the LF/HF ratio (14, 38, 39), but the acute influence of only 1 alcoholic drink (approximately 15 g) on HRV and its modulation by vagal and sympathetic neural influences have yet to be reported. We demonstrated previously, in this cohort of young healthy subjects, a dose-response relationship between acute increases in blood alcohol concentration and both heart rate and muscle sympathetic nerve activity (MSNA) (33). The present analysis was conducted to determine whether the acute chronotropic response to low- to moderate alcohol intake in these individuals resulted from a concordant increase in cardiac sympathetic modulation of HR, a reduction in vagal HR modulation, or a combination of these two neural
actions. Because such methods permit, in healthy subjects, general inferences concerning parasympathetic and sympathetic modulation of sino-atrial discharge, heart rate recordings obtained at rest before, and after 1 and 2 standard drinks of red wine, ethanol, and water as a time and volume control were submitted to time- and frequency domain analysis of heart rate variability (HRV). Our principal findings were that alcohol diminishes time and frequency domain indices of parasympathetic HR modulation in a dose-dependent manner, whereas only higher doses (2 drinks) augment frequency domain indices of sympathetic HR modulation.

When a dose of alcohol (24 g) slightly less than that contained in 2 drinks was consumed daily over 1 week, HF power was reported to increase and LF power to diminish, but HR data in that study was acquired not during its ingestion, but on the subsequent morning, after an overnight fast (5). Amongst Japanese men, habitual moderate to high evening alcohol intake (23-45 g/d) was observed to increase both HR and the LF/HF ratio during sleep, but not after awakening the following morning (28). In this particular study, potential causes of higher nocturnal cardiac sympathetic tone, such as alcohol-induced obstructive sleep apnea (2), or a full bladder were not controlled for.

In a cohort of 102 women studied 1 year after myocardial infarction, Janszky et.al. found no correlation between total alcohol consumption and either time- or frequency domain indices of HRV, as derived from the 24 hour ambulatory electrocardiogram. Within this group, mean alcohol intake was very low (1.62 g/d). Interestingly, these authors did detect, but only in wine-drinking women, a decrease in HR and an increase in SDNN, total power, VLF and LF, but no significant effect on HF power. There were no such associations between either ethanol or beer
consumption and these particular representations HRV. These investigators did not study men or healthy controls but their findings did suggest potential differences between wine and other alcoholic beverages with respect to HR modulation (11). In our material, the small sample size precluded analysis of potential differences between men and women.

The observed effects of alcohol on these several representations of vagal and sympathetic heart rate modulation may reflect alterations in the rhythmicity of central neural outflow, a direct effect on autonomic modulation of sino-atrial discharge, or altered sino-atrial responsiveness to released neurotransmitters (23, 27). Randin et al. studied 9 subjects in whom ethanol, 0.5g/kg, was infused intravenously over 45min on two separate sessions, with and without 2 days of antecedent treatment with 2mg of dexamethasone, given to inhibit alcohol-induced stimulation of corticotrophin releasing hormone (CRH) (an effect inferred but not measured directly) (30). Ethanol alone doubled muscle sympathetic firing rate and increased blood pressure. After dexamethasone pre-treatment, ethanol lowered mean arterial pressure and induced calf vasodilation, but had no effect on MSNA (30).

In a rat model of hemorrhagic shock, alcohol intoxication disturbed neurohumoral cardiovascular regulation, but this could be reversed by intracerebroventricular administration of an acetylcholinesterase inhibitor, suggesting that alcohol altered central nicotinic receptor mediated modulation of automomic outflow (20). The present findings are therefore consistent with centrally mediated sympathoexcitatory and vagolytic effects of alcohol. Also, any reductions in preload and arterial baroreceptor unloading induced by 2 drinks of alcohol would exert,
reflexively, concordant effects on efferent parasympathetic and cardiac sympathetic nerve discharge (6).

Because red wine and ethanol increased MSNA by approximately 22%, whereas HR increased by only 11% (33), we considered the possibility that alcohol might counter increased sympathetic HR modulation by inducing a degree of vagal restraint at the sino-atrial node. Both red wine and ethanol have been demonstrated to increase acutely, plasma nitric oxide concentrations in healthy human subjects (21), and in experimental preparations neuronal nitric oxide synthase (NOS) gene transfer into the right atrium or efferent vagus has been shown to augment cardiac parasympathetic function (10, 22). There is also evidence from studies in healthy volunteers that nitric oxide can potentiate cardiac vagal drive in sympathomimetic settings (3), a finding that might have been relevant to the effects of the second dose of alcoholic beverages in the present context. Indeed, in states of simultaneously high sympathetic and parasympathetic activity some vagal withdrawal is required before the adrenergic activation can be appreciated by an increase in HR (34). Despite this body of literature, suggesting the potential for augmented vagal tone, in the present series a single dose of alcohol was sufficient to attenuate vagal HR modulation, presumably via a central or reflex efferent action. Of note, in healthy humans, an intravenous infusion of ethanol in a forearm artery to locally high levels (200mg/dL) induced acutely vasoconstriction, yet at the same time augmented endothelium-dependent and -independent vasodilation but not by increasing nitric oxide bioavailability (36).

Although its specific composition will vary significantly between different grape varieties, different areas, and from year to year, red wine contains about 2 g/L of polyphenolic compounds,
primarily flavanoids and stilbenes (16). The latter are potent anti-oxidants (16). Flavanoids, a complex group of chemicals, are synthesized by grapes in response to adverse environmental stimuli. Some flavanoids, such as resveratrol, exhibit also anti-inflammatory (4) and central sympatho-inhibitory actions. In anesthetized male rats, microinjections of resveratrol into the rostral ventrolateral medulla reduced blood pressure, heart rate, and renal sympathetic nerve activity. These responses were abolished by NOS inhibition (19). Flavonoids also can act as anti-oxidants and as phyto-estrogens, stimulating endothelial NOS expression and augmenting nitric oxide bio-availability (37). In post-menopausal women, estrogen replacement therapy augments total HRV and reduces HRV indexes of sympathetic activation, likely via this mechanism (31). Red wine polyphenols with estrogen-like effects might have similar cardiac autonomic actions when consumed chronically.

Plasma resveratrol concentrations increased significantly after both the first and the second dose of red wine (33). Similar effects of red wine and ethanol on sympathetic contributions to HRV might have been expected from our previous demonstration of virtually identical increases in muscle sympathetic nerve firing rate after 2 drinks of both beverages (33), but one might have anticipated, from this prior literature, a differential effect of red wine and ethanol on vagal contributions to HRV. Moreover, red wine increased plasma dopamine concentrations 28-fold (P<0.001) and plasma ACTH by 82% (P<0.006); whereas ethanol had no effect on dopamine and lowered ACTH by 30% (33). Despite this verified rapid absorption of red wine polyphenols and these higher plasma dopamine and ACTH concentrations, in the present analysis ethanol and red wine were found to have essentially similar overall effects on HRV. Two glasses of both red wine and ethanol diminished total HR variability (RMSSD) in the time domain, and power in the
high frequency spectral band, but there was no significant difference between the effects of these
two beverages with respect to either index of tonic vagal HR modulation.

The present study evaluated the acute, rather than the chronic effects of red wine and ethanol on
HRV. In humans, chronic ingestion may be required before any autonomic actions of red wine
polyphenols become evident. It is possible that the sample size of 12 subjects might have
obscured true differences between the actions of red wine and ethanol. With the present paired
comparison study design such differences would be small. Also differences in HRV might have
emerged if higher doses of flavanoids were administered. However the intent of the present
protocol was to replicate the effects of light to moderate wine consumption.

In summary, 2 glasses, but not 1 glass, of both red wine and ethanol increased LF normalized
power and the LF/HF ratio, observations consistent with our hypothesis that this quantity
increases, in parallel, sympathetic outflow to the sino-atrial node, as well as to skeletal muscle.
In addition, 2 glasses of both alcohols decreased total HRV and HF spectral power. Thus 2
glasses of either wine or ethanol increase HR, suppress HRV, cause sympathetic activation and
inhibit tonic vagal HR modulation. Although each in the present context represents an acute
response to alcohol consumption, in longitudinal studies such autonomic alterations have been
associated with increased cardiovascular risk and mortality (7, 9, 13, 17, 26). Longer term
randomized interventional studies are required to determine whether the present observations
provide fundamental mechanistic insight into the relationship between chronic alcohol
consumption and cardiovascular event rates. Importantly, most of these potentially adverse
effects on HRV may be avoided if intake is limited to one glass.
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FIGURE LEGENDS

**Figure 1:**

One glass (Post-1) of either wine (▲) or ethanol alone (■) did not alter any HRV indices as compared to water (○). Two drinks (Post-2) of either wine or ethanol caused significant increases in the sympathetic index (LF normalized power; 0.04-0.15 Hz), and significant decreases in the parasympathetic index (HF normalized power; 0.15-0.40 Hz), increased the ratio (LF/HF), and reduced the root mean square successive difference (RMSSD; a time-domain measure). All values shown are mean±SE; **P<0.01; *P<0.05 as compared to pre-drink levels. † indicate a change from baseline significant (p<0.05) different from the change during the water (control) intervention.
### Table 1. Heart rate variability results.

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<th>Water</th>
<th>Wine</th>
<th>Ethanol</th>
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<td></td>
<td>Predrink mean ±SE</td>
<td>Postdrink1 mean ±SE</td>
<td>Postdrink2 mean ±SE</td>
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<tr>
<td></td>
<td>Predrink mean ±SE</td>
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<td>HR (min⁻¹)</td>
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<td>RRI (s)</td>
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<td>STD (ms)</td>
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<td>pNN50 (%)</td>
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<td>SD2 (ms)</td>
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<td>FFT Normalized power spectra</td>
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Values are mean±SE, n=12. * p<0.05 as compared to predrink levels. † indicate a change from baseline significant different (p<0.05) from the change during the water (control) intervention.
Spaak et al. Figure 1