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

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Spaak J, Tomlinson G, McGowan CL, Soleas GJ, Morris BL, Picton P, Notarius CF, Floras JS. Dose-related effects of red wine and alcohol on heart rate variability. Am J Physiol Heart Circ Physiol 298: H2226–H2231, 2010. First published April 23, 2010; doi:10.1152/ajpheart.00700.2009.—In healthy subjects a standard drink of either red wine (RW) or ethanol (EtOH) has no effect on muscle sympathetic nerve activity or on heart rate (HR), whereas two drinks increase both. Using time- and frequency-domain indexes of HR variability (HRV), we now tested in 12 subjects (24–47 yr, 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 wk apart according to a randomized, single-blind design. Eight-minute segments were analyzed. One alcoholic drink increased blood concentrations to 36 ± 2 mg/dl (mean ± SE), and 2 drinks to 72 ± 4 (RW) and 80 ± 2 mg/dl (EtOH). RW quadrupled plasma resveratrol (P < 0.001). HR fell after both water drinks. When 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; and EtOH, +5.7 ± 1.2 min⁻¹; P < 0.001), decreased total HRV by 28–33% (P < 0.05) and high-frequency spectral power by 32–42% (vagal HR modulation), and increased low-frequency power by 28–34% and the ratio of low frequency to high frequency by 98–119% (sympathetic HR modulation) (all, P ≤ 0.01). In summary, when compared with water, one standard drink lowered heart rate and sympathetic 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 a similar effect.

sympathetic nervous system; parasympathetic nervous system; spectral analysis

IN EPIDEMIOLOGICAL studies, light to moderate alcohol intake is associated with less risk than is abstention 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 Nutrition Committee does not recommend initiating alcohol consumption for cardiovascular risk reduction, but advises men who drink to limit their intake to two drink equivalents per day and women to one drink equivalent per day. Because of their similar alcohol content (15–18 g), each of a 12-oz bottle of beer (355 ml), a 4-oz glass of wine (120 ml), and a 1.5-oz shot of spirits (44 ml) is considered one standard drink (8, 18).

The potential benefits of long-term alcohol consumption have been attributed to its metabolic, antithrombotic, anticoagulant, antioxidant, or anti-inflammatory properties and, in the context of the present analysis, to its effects on hemodynamics, vascular endothelial function, and neurohumoral regulation of the circulation. Evidence from a variety of sources has suggested that constituents of red wine, such as polyphenols, might augment these many actions. However, until we conducted a single-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 the efferent sympathetic nerve traffic directed at the skeletal muscle, whereas two glasses increased both. Ethanol and red wine had similar effect on both responses.

The present objective was to determine, using time- and frequency-domain HR variability (HRV) analysis, whether the rate increase observed reflects a concordant increase in cardiac sympathetic HR modulation, a diminished vagal HR 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 nonsmoking adult men and nonpregnant 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 nonprescription drugs 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.

Subjects sat in a reclining chair with leg support. An antecubital vein was cannulated for blood sampling. HR was determined continuously from an electrocardiogram (ECG). Blood pressure was recorded automatically at 1 min 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 the blood was drawn, the first drink was ingested over 5 min. After ~10 min, when blood alcohol concentration (BAC) reached ~40 mg/dl, a second set of data was acquired. Once BAC had fallen to 25–30 mg/dl, the second drink was ingested. A third set of data was acquired once the BAC peaked at about 90 mg/dl. Subjects were then monitored until their BAC fell below 10 mg/dl. The water intervention protocol followed the same time course as the wine and ethanol study day protocols.

**Interventions.** The Quality Assurance Laboratory of the Liquor Control Board of Ontario (Toronto, Canada) selected a moderately priced Pinot Noir (Wolf Blass, Australia; 2001) determined to have high r-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; <1.3 g) 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 Fig. 1, in Spaak et al. (33)] were applied to calculate for each subject the concentration (BAC) reached about 90 mg/dl. The water intervention protocol followed the same time course as the wine and ethanol study day protocols.

**HR variability.** The ECG signal was sampled at 1,000 Hz and together with the breathing signal digitized and stored using LabView (National Instruments, Austin, TX) for subsequent analysis (1). After all studies were completed, the R-R 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 (HF) (predominantly vagal) HR modulation (29): the standard deviation (SD) of R-R intervals (STD); the root mean square successive difference of R-R intervals (RMSSD); and the number of successive difference of intervals that 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 (R-R tri-index), the length of R-R intervals serves as the x-axis of the plot and the number of each R-R 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 R-R interval frequency distribution diagram. The triangular interpolation of N-N interval histogram is the width of the distribution measured as a base of a triangle, approximating the N-N interval distribution. It 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 R-R interval fluctuation. Each R-R interval is plotted as a function of the previous R-R interval, and the SD of the distribution is calculated. SD-1 is a function of short-term variability (usually caused by respiratory sinus arrhythmia), whereas 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 (LF) (<0.04 Hz) spectral component] power were calculated separately for the LF (predominantly sympathetic) (0.04–0.15 Hz) and HF (0.15–0.40 Hz) spectral band, and the LF-to-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 means ± SE. A linear mixed-effects model was used to account for repeated measurement after zero, one, and two 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 three experimental sessions, but in one subject a high prevalence of premature supraventricular beats on all study days precluded power spectral density calculations. Reported are findings in six men and six women aged 35 (24–47) yr [mean (range)] whose body mass index was 23 (18.4–27.6) kg/m².

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

Similar BACs were achieved after one and two alcoholic drinks: for wine, 35.9 ± 2 mg/dl and 71.8 ± 4 mg/dl; and for ethanol, 35.7 ± 2 mg/dl (\( P = 0.58 \)) and 80.2 ± 2 mg/dl (\( P = 0.10; \ P < 0.01 \) from baseline for both). Only wine significantly increased plasma and urine polyphenols (33), and plasma resveratrol quadrupled from 11.9 ± 2.3 to 46.7 ± 9.6 \( \mu \)g/l (\( P < 0.001 \)).

There was no significant change in blood pressure after one or two 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 ethanol caused a small increase, compared with the effects of one drink of water. The second glass of both alcoholic beverages 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 (in ms), or STD (in \( \text{min}^{-1} \)) from their respective baseline values but compared with the response to one glass of water, STD (in ms), RMSSD, and pNN50 all decreased significantly. Two glasses of either alcoholic drink caused significant suppression of all time-domain parameters except STD (in \( \text{min}^{-1} \)), RMSSD, for example, decreased by 28% after ethanol and by 33% after wine. Geometrical parameters were also suppressed by two alcoholic drinks (Table 1 and Fig. 1).

In the frequency domain, one 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 wine caused a significant suppression of HF total power (Fig. 1 and Table 1). However, two glasses of both alcohols altered HRV significantly when compared with both respective baselines and with two 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-to-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 while increasing the LF-to-HF ratio (14, 38, 39), but the acute influence of only one alcoholic drink (\( \sim 15 \) g) on HRV and its modulation by vagal and sympathetic neural influences have yet to be reported. We previously demonstrated, in this cohort of young healthy subjects, a dose-response relationship between acute increases in BAC and both HR 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

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Values are means ± SE; \( n = 12 \) subjects. HR, heart rate; RRI, R-R interval; STD, standard deviation (SD) of R-R intervals; RMSSD, root mean square of successive difference; pNN50, number of successive difference of intervals that differ by more than 50 ms, expressed as a percentage of the total; TINN, triangular interpolation of N-N interval histogram; FFT, fast Fourier transform; LF, low frequency; HF, high frequency; LF/HF, LF-to-HF ratio; nu, normalized units. \( * P < 0.05 \) compared with predrink levels. \( † P < 0.05 \), change from baseline, significantly different from the change during the water (control) intervention.
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 sinoatrial discharge, HR recordings obtained at rest before and after one and two standard drinks of red wine, ethanol, and water as a time and volume control were submitted to time- and frequency-domain analysis of HRV. Our principal findings were that alcohol diminishes time- and frequency-domain indexes of parasympathetic HR modulation in a dose-dependent manner, whereas only higher doses (2 drinks) augment frequency domain indexes of sympathetic HR modulation.

When a dose of alcohol (24 g) slightly less than that contained in two drinks was consumed daily over 1 wk, HF power was reported to increase and LF power to diminish, but HR data in that study were acquired not during its ingestion but on the subsequent morning after an overnight fast (5). Among Japanese men, a habitual moderate to high evening alcohol intake (23–45 g/day) was observed to increase both HR and the LF-to-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 yr after myocardial infarction, Janszky et al. (11) found no correlation between total alcohol consumption and either time- or frequency-domain indexes of HRV, as derived from the 24-h ambulatory ECG. Within this group, mean alcohol intake was very low (1.62 g/day). Interestingly, these authors did detect, but only in wine-drinking women, a decrease in HR and an increase in SD of normal-to-normal R-R interval (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 of 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 an analysis of the potential differences between men and women.

The observed effects of alcohol on these many representations of vagal and sympathetic HR modulation may reflect alterations in the rhythmicity of central neural outflow, a direct effect on the autonomic modulation of sinoatrial discharge or altered sinoatrial responsiveness to released neurotransmitters (23, 27). Randin et al. (30) studied nine subjects in whom ethanol (0.5 g/kg) was infused intravenously over 45 min on two separate sessions with and without 2 days of antecedent treatment with 2 mg of dexamethasone, given to inhibit an alcohol-induced stimulation of corticotrophin-releasing hormone (an effect inferred but not measured directly). Ethanol alone doubled the muscle sympathetic firing rate and increased blood pressure. After dexamethasone pretreatment, 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 an intracerebroventricular administration of an acetylcholinesterase inhibitor, suggesting that alcohol altered the central nicotinic receptor-mediated modulation of automatic 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 two 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 22%, whereas HR increased by only 11% (33), we considered the possibility that alcohol might counter the increased sympathetic HR modulation by inducing a degree of vagal restraint at the sinoatrial 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 (200 mg/dl) acutely induced 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 among 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 antioxidants (16). Flavanoids, a complex group of chemicals, are synthesized by grapes in response to adverse environmental stimuli. Some flavanoids, such as resveratrol, also exhibit anti-inflammatory (4) and central sympathoinhibitory actions. In anesthetized male rats, microinjections of resveratrol into the rostral ventrolateral medulla reduced blood pressure, HR, and renal sympathetic nerve activity. These responses were abolished by NOS inhibition (19). Flavonoids can also act as antioxidants and as phytoestrogens, stimulating endothelial NOS expression and augmenting nitric oxide bioavailability (37). In postmenopausal 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 significantly increased 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 two 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 low-
ered 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 HRV (RMSSD) in the time domain and power in the HF 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, a 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 the 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, two glasses, but not one glass, of both red wine and ethanol increased LF normalized power and the LF-to-HF ratio, observations consistent with our hypothesis that this quantity increases, in parallel, sympathetic outflow to the splanchnic node, as well as to skeletal muscle. In addition, two glasses of both alcohols decreased the total HRV and HF spectral power. Thus two 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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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

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