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ACUTE EFFECTS OF ALCOHOL ON HEART RATE VARIABILITY: TIME-RELATED CHANGES AND GENDER DIFFERENCE

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ABSTRACT

Objectives: Alcohol consumption is associated with a broad array of physiologic and behavioral effects including changes in cardiac autonomic activity. In the present study, time-related acute effects of alcohol have been characterized and compared between genders. **Methods:** A total of 30 healthy subjects (15 males and 15 females) were enrolled in this study. The red wine was given to each subject at a dosage of 0.27 g of pure ethanol per kilogram of body weight. 5-min electrocardiograms (ECGs) were collected before (BR) and at 15 min (P15), 30 min (P30), 45 min (P45) and 60 min (P60) after alcohol intake. Time- and frequency-domain analysis of heart rate variability (HRV) was performed. The time-domain HRV indices include mean RR interval, pNN50, SDNN and RMSSD. The low- (LF: 0.04 to 0.15 Hz) and high-frequency (HF: 0.15 to 0.4 Hz) components along with LF/HF ratio were calculated for frequency-domain analysis of HRV. HRV was also analyzed by mathematical models, e.g. Poincaré plot, which uses a nonlinear geometric representation of change in interbeat heart rate. Poincaré plots indices, SD1, SD2, SD1/SD2 ratio and r_{RR}, were applied in this study for HRV assessment. **Results:** Alcohol intake was associated with decreased HRV in both time and frequency domains. The lowest HRV was observed 30–40 min after the intake of alcohol. The alcohol intake also caused the decrease of Poincaré plots indices (SD1 and SD1/SD2 in P30, P40 and P60, and r_{RR} in P45), accompanied with a narrower plot area. The changes of HRV indices differed by gender. The male subjects demonstrated a greater decrease of parameters measured in this study compared to the female subjects. **Conclusion:** Acute effects of alcohol ingestion resulted in reductions in HRV, indicating impaired cardiac autonomic nervous activity. Autonomic nervous activity in the females was less dampened by the alcohol compared to the males.

Keywords: Alcohol; Autonomic nerve activity; Heart rate variability; Time domain; Frequency domain; Poincaré plot.

INTRODUCTION

There are both beneficial and harmful implications regarding the role of alcohol in cardiovascular system. Although light-to-moderate drinking can protect against coronary artery disease, alcohol drinking is a major cardiovascular risk factor. It has been shown that heavy and chronic alcohol consumption can damage the cardiovascular system, resulting in maladies such as cardiomyopathy, arrhythmias, hypertension and strokes.[1] Previous studies have also suggested the effect of acute intake of alcohol on heart rate and heart rate variability (HRV). Acute consumption of moderate or high doses of alcohol resulted in significantly lower HRV than with placebo or water consumption.[2] For acute low dose alcohol consumption, the HRV did not decrease

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significantly.[3] Physiologic response to acute administration of alcohol appears to depend on the volume of co-administered fluids.[4] In the study of comparison of the HRV changes with other measures, alcohol-related changes were found either in heart rate or HRV but not in both measures.[5,6] In the study by Rossinen and colleagues,[7] neither change was significant. However, to our knowledge, the acute effect of alcohol drinking on HRV has not been extensively studied, particularly the time-related acute alcohol drinking on HRV in the healthy subjects, and also none compared the differences between genders.

HRV, the amount of fluctuation of the beat to beat differences, is known to be a reliable, noninvasive marker of autonomic nervous system activity.[8] HRV characterizes the variations in the intervals between consecutive heartbeats, that is, the variations in duration of RR intervals between consecutive QRS complexes, measured by electrocardiograph (ECG). Assessment of HRV may provide quantitative information on the modulation of cardiac vagal and sympathetic nerve input. HRV analysis is a recognized tool for the estimation of cardiac autonomic modulations.[8] Previous alcohol studies have indicated that HRV was an objective measure of an integrative and sensitive physiologic function that reflects responsivity to alcohol consumption.[9,10]

The goal of this study was to assess the time-related acute effects of alcohol on cardiac autonomic function using HRV analyses and to compare the differences between genders. Given the number of subjects and their narrow age range, the present alcohol study should be considered as a preliminary attempt for evaluation of acute effects of alcohol on HRV. Nevertheless, the findings generated in this work have provided novel information of time-related changes and gender difference in variability of heart rate response to alcohol.

MATERIALS AND METHODS

Participants

The subjects with a baseline history of cardiovascular disease were excluded from the study. The subjects were normotensive and none was taking any medication. A total of 30 healthy subjects (15 males, mean age 21.5 ± 1.7 years, and 15 females, mean age 22.5 ± 1.1 years) were included in the study. A written consent was obtained from all subjects and our local ethical committee approved the study.

Experimental Protocols

All subjects were taken to a quiet, dimly lit, 22°C to 24°C room. All participants were instructed to refrain from alcohol- and caffeine-containing beverages and strenuous exercise for 24 h prior to the study. Subjects attended two morning sessions scheduled at least one week apart, during which one of either red wine or water was administered at random.

All the tests were carried out while the subject rested in the 135° sitting position on a comfortable chair. For each subject, ECG signals were recorded for 5 min as baseline records (BRs) following a resting period of at least 15 min. After completion of BRs, subjects were asked to imbibe red wine with a calculated volume of alcohol (e.g. 0.27 g of ethyl alcohol per kilograms body weight). Subjects were instructed to drink at an even pace in 3 min. ECG signals were recorded for four 5-min durations at 15 min (P15), 30 min (P30), 45 min (P45) and 60 min (P60) after alcohol drinking. The design of experimental protocols was shown in Fig. 1. The water intervention protocol followed the same time course as the wine study day protocols.

Data Acquisition System

The ECG signals were collected by a multi-channel physiological signal recording system (MP150, BioPac, Goleta, California, USA). The three electrodes for the lead II ECG signal collection were placed on the right wrist, the left wrist and the right leg of the subjects, respectively. The ECG signals were amplified, sent to analog-digital hardware (MP150, Biopac Systems, Goleta, California), and recorded at 1k Hz (Acqknowledge, Biopac Systems, Goleta, California). The bandwidth of the ECG amplifiers was 0.13 to 100 Hz with 50-Hz notch. Beat-to-beat cardiac interval values were automatically measured for each sinus beat and

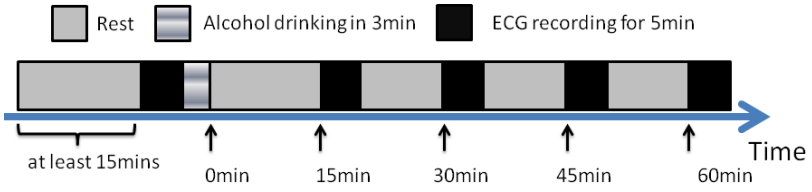

Fig. 1 Diagram of experimental protocols.

exported for further analysis using the MATLAB software (MathWorks Inc., MA, USA). All ECG data used for subsequent analysis in this study were free of any form of morphologically abnormal beat.

HRV Analysis

Time-domain analysis. The time-domain analysis focuses on the heart rhythm and its variations. The most frequently used measurements included the mean RR interval, the standard deviation of all RR intervals (SDNN), the root mean square of successive differences (RMSSD) and the number of successive difference of intervals that differ by more than 50 ms, expressed as a percentage of the total (pNN50). Changes in time-domain measures give an indication of the magnitude of the change in autonomic tone.

Frequency-domain analysis. Frequency-domain analysis, i.e. power spectral analysis, provides data on how power (variance) distributes as a function of frequency.[8] Frequency-domain analysis is recommended for short-term (2–5 min) recordings of RR intervals under physiologically stable conditions.[8] In this study, frequency-domain HRV indices are determined by calculating the power spectral density of the RR time series using a nonparametric fast Fourier transform (FFT) algorithm. The HRV power spectrum is typically parsed into four frequency ranges as follows: (i) ultra low frequency, ULF (<0.003 Hz), (ii) very low frequency (0.003 to 0.04 Hz), VLF, (iii) low frequency (0.04 to 0.15 Hz), LF and (iv) high frequency (0.15 to 0.4 Hz) or HF. LF and HF power components are taken into account in this study. Spectral analysis methods are more sensitive for extracting information regarding sympathetic and parasympathetic tone.

Poincaré plot. The Poincaré plot is based on the notion of different temporal effects of changes in the parasympathetic and sympathetic modulation of the heart rate on the subsequent RR intervals. Poincaré plot as a nonlinear method is a scatter outline of the current RR interval plotted against the preceding RR interval. The plot delivers not only an outline but also a detail of beat-to-beat behavior of cardio-physiology. The computerized analysis entails fitting an ellipse to the plot, with its center coinciding with the center point of the markings (see Fig. 2). The dispersion of the points perpendicular to the line-of-identity measures the width of the Poincaré cloud and reflects the level of short-term HRV. This measure is equivalent to the standard deviation of the successive differences of the RR intervals, i.e. standard deviation of successive differences (SDSD).[11,12] The dispersion of points along the line-of-identity measures

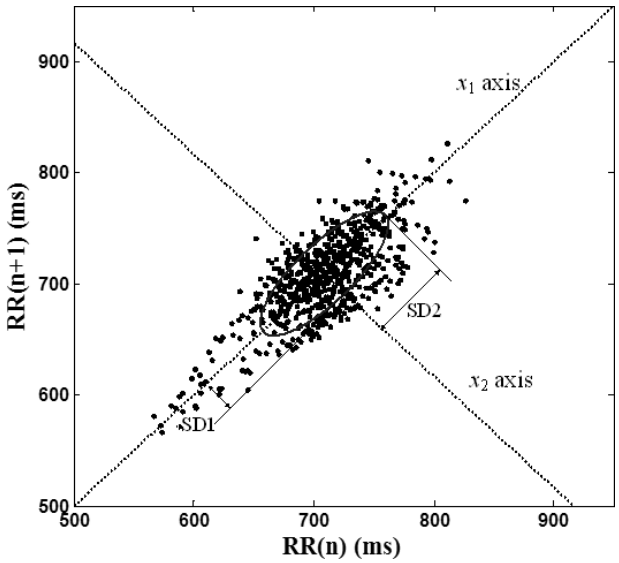

Fig. 2 A typical Poincaré plot. An ellipse is fitted to the data points and the Poincaré plot indices are calculated by estimating the short diameter (SD1), the long diameter (SD2) and the ratio of the short and long diameters (SD1/SD2 ratio) of the fitted ellipse. SD1 is the standard deviation of the distances of points from x_1 axis, SD2 is the standard deviation of the distances of points from x_2 axis.

the length of the cloud and is thought to indicate the level of long-term HRV which reflects the standard deviation of the RR interval, i.e. SDNN.[11] The width (SD1) and the length (SD2) of the Poincaré plot are related to the standard HRV measures in the following manner:

$$\mathrm{SD1}^2 = \frac{1}{2}\mathrm{SDSD}^2, \tag{1}$$

$$\mathrm{SD2}^2 = 2\mathrm{SDNN}^2 - \frac{1}{2}\mathrm{SDSD}^2. \tag{2}$$

SD1 and SD2 determine the width and length of the fitted ellipse to characterize the shape of the plot mathematically (see Fig. 2). Many researchers use SD1, SD2 and SD1/SD2 ratio to measure the heart activity.[13–16]

In our study, the correlation coefficient r_{RR} of the Poincaré plot is applied to characterize its shape, providing insight into autonomic disorders. For the Poincaré plot, the correlation coefficient can be expressed in terms of the autocovariance function:

$$r_{\mathrm{RR}} = \frac{\phi_{\mathrm{RR}}(1)}{\phi_{\mathrm{RR}}(0)} = \frac{\mathrm{SD2}^2 + \mathrm{SD1}^2}{\mathrm{SD2}^2 - \mathrm{SD1}^2}. \tag{3}$$

Poincaré plots analysis may provide additional prognostic information and insight into autonomic alterations.[17] The patterns of Poincaré plots can reflect a normal or reduced cardiac autonomic modulation. Generally, the narrower plot area indicates the increased sympathetic activity accompanied with decreasing RR interval.

Statistical Analysis

All data were presented as mean ± standard deviation (SD). The significance of difference between pre alcohol intake and post alcohol intake within groups was compared using one-way ANOVA with repeated measures. The significance of differences among groups was compared using one-way ANOVA with post hoc multiple comparison procedures. The statistical analyses were run in MATLAB software (MathWorks Inc., MA, USA). A $p < 0.05$ was considered statistically significant.

RESULTS

The present study investigated the effects of the low-dose alcohol consumption on HRV by comparing the parameters in pre and post intake of red wine. Equal volumes of water were provided as control. The BR did not differ between the alcohol and the water experiments.

Figure 3 shows RR interval series extracted from ECG signal for one subject in BR, P15, P30, P45 and P60 in alcohol experiment. Larger RR intervals with greater fluctuations were observed in BR. In fact, RR interval series graphs were also examined in other subjects and similar patterns of fluctuations were observed. These RR interval time series gave the qualitative information of variability of heart beats.

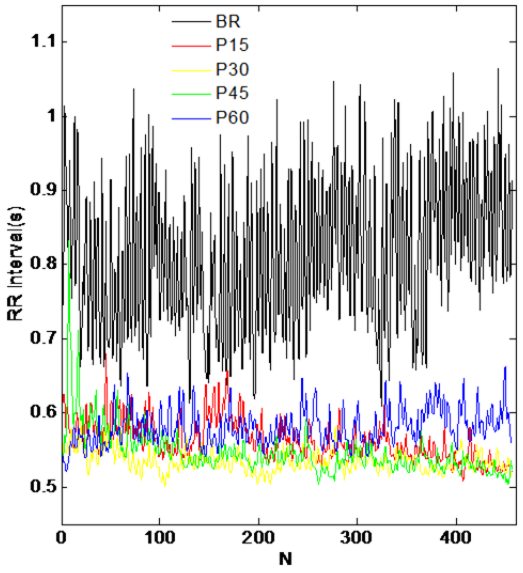

Fig. 3 RR interval series from one participant for five sessions (e.g. BR, P15, P30, P45 and P60). x-axis is the RR interval series and all signals are based on 450 sample RR intervals. y-axis is the values of RR interval. The signal recorded before alcohol drinking (BR) presents larger intervals and fluctuations than other sessions (P15, P30, P45 and P60).

The results of time-domain analysis of HRV were summarized in Table 1. Water drink did not alter any time-domain parameters from their respective baseline. Compared to the BR, subjects had lower values of RR interval after alcohol drinking ($p < 0.05$ in P30, and $p < 0.01$ in P45 and P60). This was accompanied with lower ($p < 0.05$) values of pNN50 and RMSSD. SDNN was lower in post alcohol intake, but no statistically significant differences were found. Compared with response to intake of water, alcohol caused significant suppression of pNN50 and RMSSD in P30, P45 and P60.

The results of frequency-domain analysis of HRV were summarized in Table 2. For water experiment, drink of water caused significant increase of LF in P45. For alcohol experiment, significantly lower HF and LF power values were observed in P30 (all $p < 0.01$) and P45 ($p < 0.01$ for LF and $p < 0.05$ for HF). No significant differences were observed for LF/HF in pre and post alcohol drinking. Compared with response to intake of water, alcohol caused significant suppression of HF and LF power values in P30, P45 and P60.

The present quantitative Poincaré plots were used to analyze instantaneous beat-to-beat variance of the RR intervals (SD1), the long-term continuous variance of all RR intervals (SD2), and their ratios (SD1/SD2). For the alcohol experiment as presented in Table 3, SD1 significantly decreased in P30, P45 and P60 (all $p < 0.05$), accompanied with significant decreases of SD1/SD2 ratio in P30, P45 and P60 (all $p < 0.01$). r_{RR} decreased following alcohol intake, but only found smaller in P45 with significant difference ($p < 0.05$). Compared with response to intake of water, alcohol caused significant suppression of all Poincaré plots parameters although in different sessions (e.g. SD1 in P15, P30, P45, P60, SD2 in P45, SD1/SD2 in P30, P45, and P60 and r_{RR} in P30 and P45).

Figure 4 shows the Poincaré plot measured before and after alcohol intake for one representative subject. The difference in variability in pre and post alcohol intake was apparent. Visually, the Poincaré plot area was larger in BR [see Fig. 4(A)] compared to other plots [see Figs. 4(B)–4(E)].

Few studies compared the differences between genders before and after alcohol intake. Male and female demonstrated distinct patterns of change in standard HRV indices in response to the alcohol consumption, as shown in Fig. 5. Negative changes (from BR) in time-domain parameters were observed in RR interval, pNN50, RMSSD and SDNN. There were no gender differences for the changes in RR interval, RMSSD and SDNN in response to the alcohol consumption. However, the male subjects showed greater decrease

Table 1. The Time-Domain Parameters of HRV Before and After Alcohol Drinking.

	RR Interval (ms)		pNN50 (%)		RMSSD (ms)		SDNN (ms)	
	Water	**Alcohol**	**Water**	**Alcohol**	**Water**	**Alcohol**	**Water**	**Alcohol**
BR	801.1 ± 95.7	828.6 ± 78.2	26.2 ± 13.0	27.4 ± 21.9	46.9 ± 13.4	51.5 ± 30.5	63.5 ± 18.0	60.4 ± 19.5
P15	836.0 ± 56.3	771.8 ± 132.4	27.6 ± 9.1	15.9 ± 11.6	49.5 ± 19.3	37.4 ± 19.1	64.1 ± 17.5	52.8 ± 21.5
P30	838.6 ± 62.9	738.6 ± 117.4[a]	32.8 ± 11.2	11.9 ± 12.2[a,c]	51.2 ± 17.5	29.0 ± 15.6[a,c]	62.3 ± 16.3	47.2 ± 17.9
P45	846.7 ± 70.5	732.3 ± 96.2[b]	29.4 ± 8.9	11.2 ± 9.2[a,c]	53.3 ± 19.2	29.8 ± 13.0[a,c]	65.6 ± 19.3	49.1 ± 16.1
P60	850.0 ± 59.8	752.3 ± 78.3[b]	31.5 ± 10.6	10.6 ± 11.9[a,c]	53.4 ± 20.1	31.4 ± 10.3[a,c]	60.9 ± 14.5	48.7 ± 13.3

[a]$p < 0.05$ vs. BR.
[b]$p < 0.01$ vs. BR.
[c]$p < 0.05$ vs. water (control) intervention.

Table 2. The Frequency-Domain Parameters of HRV Before and After Alcohol Drinking.

	LF (ms^2)		HF (ms^2)		LF/HF	
	Water	**Alcohol**	**Water**	**Alcohol**	**Water**	**Alcohol**
BR	601.0 ± 96.5	589.2 ± 57.6	398.3 ± 102.3	469.3 ± 80.7	1.51 ± 0.08	1.29 ± 0.12
P15	695.2 ± 102.6	552.5 ± 108.4	456.3 ± 96.3	434.0 ± 102.8	1.52 ± 0.13	1.28 ± 0.13
P30	685.2 ± 96.5	499.8 ± 92.4[b,c]	516.6 ± 71.6	382.9 ± 78.3[b,c]	1.38 ± 0.14	1.30 ± 0.11
P45	729.1 ± 112.3[a]	507.5 ± 85.5[b,c]	556.9 ± 89.3	396.1 ± 73.5[a,c]	1.36 ± 0.09[a]	1.34 ± 0.09
P60	688.2 ± 103.5	525.9 ± 73.6[c]	527.1 ± 56.2	406.5 ± 65.2[c]	1.31 ± 0.12	1.33 ± 0.06

[a]$p < 0.05$ vs. BR.
[b]$p < 0.01$ vs. BR.
[c]$p < 0.05$ vs. water (control) intervention.

Table 3. The Poincaré Plot Parameters of HRV Before and After Alcohol Drinking.

	SD1(ms)		SD2 (ms)		SD1/SD2		r_{RR}	
	Water	**Alcohol**	**Water**	**Alcohol**	**Water**	**Alcohol**	**Water**	**Alcohol**
BR	38.1 ± 9.2	36.4 ± 21.6	72.9 ± 25.6	76.8 ± 19.6	0.52 ± 0.10	0.45 ± 0.13	1.69 ± 0.58	1.72 ± 0.88
P15	43.2 ± 10.6	25.6 ± 13.8[c]	79.8 ± 16.3	65.7 ± 25.9	0.54 ± 0.16	0.36 ± 0.10	1.71 ± 0.51	1.41 ± 0.30
P30	43.6 ± 12.2	21.6 ± 11.4[a,c]	82.1 ± 10.9	61.9 ± 20.0	0.53 ± 0.06	0.32 ± 0.09[b,c]	1.73 ± 0.56	1.32 ± 0.19[c]
P45	39.8 ± 9.5	22.0 ± 9.7[a,c]	77.3 ± 15.3	63.5 ± 15.0[c]	0.49 ± 0.12	0.31 ± 0.07[b,c]	1.67 ± 0.11	1.23 ± 0.12[a,c]
P60	35.4 ± 7.2	20.5 ± 8.1[a,c]	73.6 ± 14.2	62.7 ± 22.9	0.47 ± 0.16	0.34 ± 0.08[b,c]	1.69 ± 0.55	1.29 ± 0.22

[a]$p < 0.05$ vs. BR.
[b]$p < 0.01$ vs. BR.
[c]$p < 0.05$ vs. water (control) intervention.

(all $p < 0.05$) in pNN50 in the four recordings, i.e. P15, P30, P45 and P60.

For the changes in frequency-domain parameters, the male subjects showed greater decreases in LF for P15, P30 and P45 ($p < 0.05$) compared to the female subjects, however no gender differences were noted in changes of HF. For the changes in LF/HF, the gender differences were statistically significant (all $p < 0.05$), and the female subjects showed positive changes following alcohol ingestion whereas the male subjects show negative or little (<1%) changes.

For the changes of SD1 and SD2 in Poincaré plot, acute alcohol intake caused a greater decrease in the males compared to the females (SD1 in P15, SD2 in P15, P45 and P60, all $p < 0.05$) or no significant differences between genders.

DISCUSSION

Time- and Frequency-Domain Analysis

Some previous studies have evaluated HRV response to acute alcohol consumption in healthy nonalcoholic volunteers. Changes in HRV induced by low-dose alcohol consumption were uncertain. In Gonzalez Gonzalez and colleagues' study,[18] acute ingestion of a low dose of alcohol (0.3 g/kg) resulted in appreciable changes in the magnitude of the short-term RR fluctuations in power

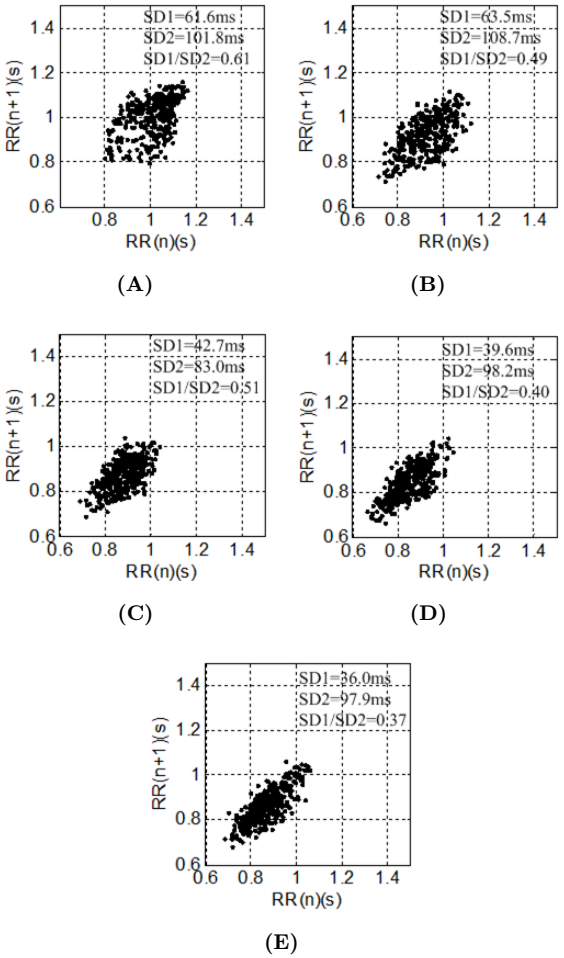

Fig. 4 Representative Poincaré plot RRn vs. RRn+1 demonstrating cardiovascular variations in BR **(A)**, P15 **(B)**, P30 **(C)**, P45 **(D)** and P60 **(E)**.

spectral of the HRV signal. However, Murata and colleagues[6] failed to show a statistically significant change in the HRV power spectrum indices after consumption of 0.54 to 0.66 g/kg of ethanol. Study from Spaak and colleagues investigated the effects of the low-dose alcohol consumption on HRV.[3] Their studies demonstrated that 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. Yet, acute consumption of moderate to heavy doses of alcohol resulted in a significant change in HRV.[2,3] Generally, the principal findings of HRV changes associated with acute alcohol consumption were that only higher doses augment frequency-domain indices of sympathetic HR modulation. However, the time-related effects of acute alcohol intake were neglected in these studies. In the present study, we monitored the effect of acute alcohol intake on the HRV via recording ECG signal of four time-periods for the duration of 1 h.

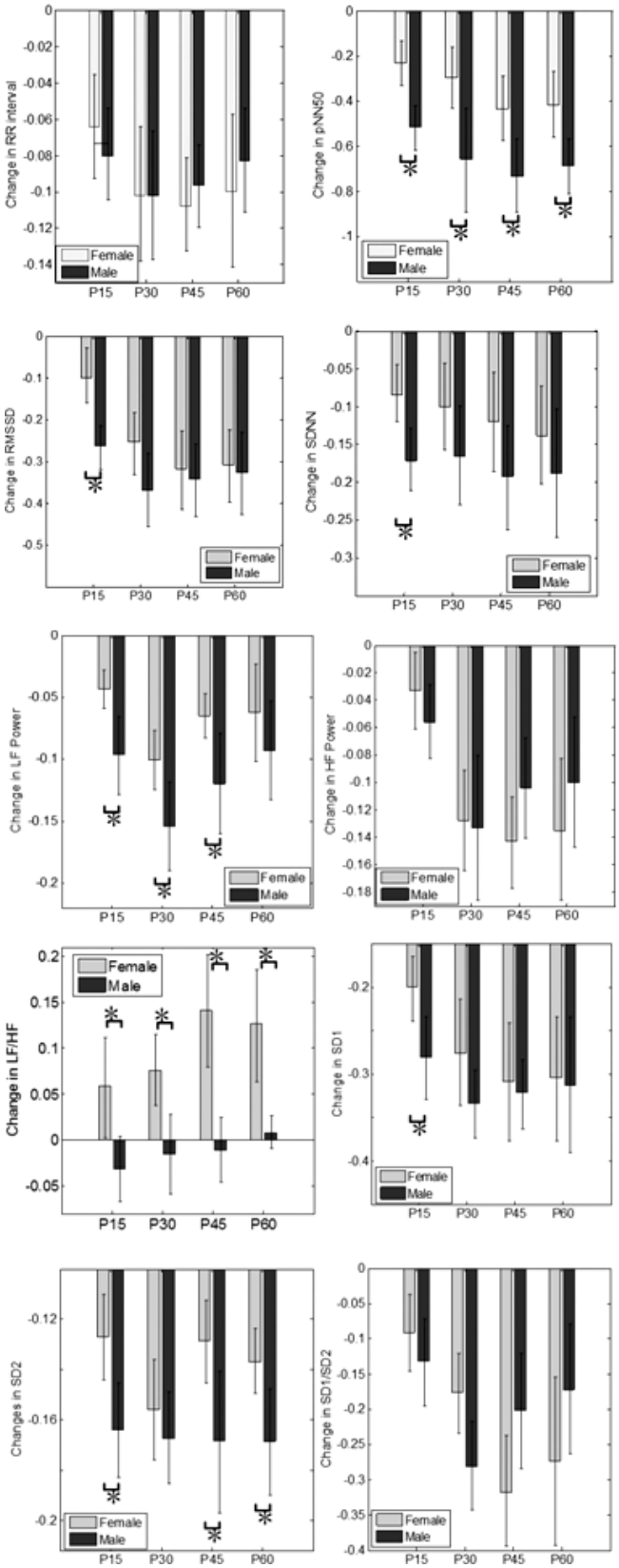

Fig. 5 Mean changes from BR in RR interval, pNN50, RMSSD, SDNN, LF, HF, LF/HF, SD1, SD2 and SD1/SD2. Significant differences between genders were marked with *.

In this work, the series of RR interval in five sessions were presented (see Fig. 3) for one representative subject. Both fluctuations and values in the interval between heart beats were obviously larger before wine drinking compared to the sessions of post wine drinking. Smaller RR intervals with lower fluctuation after wine intake indicated the higher sympathetic activity and reduced HRV.[8] Significantly decreased pNN50 and RMSSD indicate the suppressive parasympathetic activity since both pNN50 and RMSSD are the markers of parasympathetic nerve activity. SDNN, the marker of both sympathetic and parasympathetic nerve activities, have no significant differences following alcohol intake. In addition, the statistic differences of all the time-domain parameters were observed 30 min after alcohol intake. The present findings demonstrated that acute effects of low dose alcohol intake suppressed the parasympathetic nerves and stimulated the sympathetic nerves, and this influence was strongest 30–45 min after alcohol intake. Also, the reduced HRV, assessed by the time-domain measures in our experiment, indicated impaired autonomic nervous activity to the heart due to alcohol intake.

Spectral analysis of HRV is a useful, noninvasive technique to study the short-term (2–5 min) autonomic modulation of HR under physiologically stable conditions.[8,19] For the frequency-domain parameters of HRV, the power value of the HF content is considered as a pure measure of parasympathetic activity, while the power value of the LF content is reflective of both sympathetic modulation and parasympathetic tone.[8,20] The suppressive effects of alcohol on LF and HF observed in this study suggested that alcohol may suppress the parasympathetic nerve activity of both the LF and HF components. Also, the shortening of RR interval after alcohol intake, as seen in Fig. 3, would account for a reduced beat-to-beat variability in the LF band. Moreover, our results demonstrated that even a low dose drink could induce the suppression of the autonomic nerve activity, and the obvious variation could be detected in 30 min after alcohol intake and diminished in about 60 min.

LF/HF ratio was considered as an index of sympathetic activity as well as the balance between the sympathetic and parasympathetic nerves.[20–22] Although no significant difference was observed, this value did increase following alcohol intake. This did not necessarily mean that sympathetic nerve activity is enhanced, but it did point out the possibility that the sympathetic nerves do achieve dominance over the parasympathetic nerves following alcohol consumption. The decrease in all frequency-domain measures without the change in the LF/HF ratio suggested entirely impaired autonomic nervous activity to the heart, and may reflect diminished afferent autonomic activity as well.[23]

Quantitative Poincaré Plot

Poincaré plot is a simple but powerful graphical tool to describe the dynamics of a system. In this study, Poincaré plot parameters were investigated to reflect autonomic function changes associated with alcohol drinking. Because vagal effects on the sinus node are known to develop faster than sympathetically mediated effects, the instantaneous beat-to-beat variability of the RR interval time series (SD1) is mediated by vagal efferent activity. Moreover, a linear progressive reduction was observed in SD1 during incremental doses of atropine.[14] Thus, SD1 could be considered as an indicator of parasympathetic activity. While for SD2, previous studies suggested that SD2 is influenced by both parasympathetic and sympathetic tone.[14,24] SD1/SD2 ratio, which increased during the exercise after a complete parasympathetic blockade, could be used as an indicator of sympathetic activity.

In the present study, SD1 decreased after alcohol drinking, indicating the withdrawal of parasympathetic activity. Moreover, the significant decreases of SD1 initially occurred in P30, demonstrating that it took about 30 min for the alcohol to induce the withdrawal of parasympathetic activity. SD2 decreased after alcohol drinking but there were no significant differences, confirming that this parameter was influenced by both parasympathetic and sympathetic modulations and thus SD2 are not specific indices. After alcohol drinking, SD1/SD2 ratio decreased significantly instead of increasing as sympathetic activation did. This decrease was due to the important reduction of SD1 compared to SD2, which highlights the parasympathetic withdrawal that occurred after alcohol drinking. There is no evidence that justifies a claim for a better performance of Poincaré plot parameters over temporal and frequency indices. However, the Poincaré plot parameters are interchangeable HRV measures, especially in assessing parasympathetic tone to the sinus node.[14,15]

Moreover, an association between sympathetic activity and the shape of the Poincaré plot has been established, where the narrower the pattern observed, the larger the sympathetic activity.[17,25] The visual pattern found in the present study agrees with that association (see Fig. 4). Before alcohol drinking, when the parasympathetic activity was predominant as the subjects were in the 135° sitting position, the Poincaré plot

is more scattered with larger SD1 and SD2. After alcohol drinking, the Poincaré scatter gram became narrower with smaller SD1 and SD2 as the parasympathetic activity decreased and sympathetic activity increased.

r_{RR} provides insight into autonomic disorders. Otzenberger *et al.*[26] use r_{RR} to evaluate the dynamic beat-to-beat interval behavior and the sympathovagal balance. In this study, r_{RR} are significantly smaller in P45 compared to BR ($p < 0.05$), reflecting the augmentation of sympathetic activity during this period.

Gender Differences in Acute Alcohol Intake

Some researchers investigated the heart rate and their variability in men or in women following chronic drinking.[10,27] Only a few studies included women in the acute alcohol drinking experiments. Sufke *et al.*[28] evaluated HRV in patients with pure ethanol intoxication (6 females and 8 males). Spaak's group[3] investigated the dose-related effects of red wine and alcohol on HRV (6 females and 6 males). Vaschillo *et al.*[29] explored the alcohol-related physiological response to emotional contexts (16 females and 20 males). However, we have not found any previous studies compared the differences of HRV changes associated with acute alcohol consumption between genders. In the present study, measured as a change from the BR, the male subjects showed greater decreases in pNN50 (all $p < 0.05$, Fig. 5) than the female subjects in response to alcohol intake, suggesting that the suppression of parasympathetic activity was greater in males. Although the male subjects did show greater decreases for RMSSD and SDNN in post alcohol drinking, significant gender difference was only found in P15 ($p < 0.05$). In Vaschillo *et al.*'s study,[29] reduced SDNN and pNN50 by alcohol were reported in men and women subjects. However, the authors did not report the results by gender. Decreased LF in frequency-domain parameter was greater in males than in females demonstrating that autonomic nervous activity in the females was less dampened by the alcohol compared to the males. Taking together, we interpret all these findings as showing that the male subjects are more sensitive to the effects of acute alcohol intake.

Interestingly, for the changes of LF/HF ratios, positive changes were observed in the females, yet negative or less than 1% (in P60) changes in the males. Study by Evans *et al.*[30] suggested a predominance of sympathetic vascular regulation in men compared with a dominant parasympathetic influence on heart rate regulation in women. Therefore, the present findings demonstrated that the intervention of alcohol attenuated the dominant of parasympathetic activity in females and also tended to attenuate the dominant of sympathetic activity in males.

Greater decreases of SD1 and SD2 in males were observed. We believe that Poincaré plots in the males have characteristic of narrower plot area compared to the females due to the greater suppression of autonomic nervous activity although not all Poincaré plots for each subjects were showed here to give a visible comparison. Although male and female subjects were unequal when it comes to the effects of alcohol, low dose acute red wine drinking decreased HRV parameters in both male and female.

Limitations

There are some limitations to consider. First, blood alcohol concentration (BAC) was not reported in this study. This means that we have no information about possible association between the changes of HRV and BAC. Therefore, BAC levels are suggested in the future work to provide additional information to understand the findings. Another limitation of the present study is that the amount of alcohol given to each of the subjects was not changed. Further studies are necessary to improve understanding of the dose-related effects of alcohol on cardiovascular response, in which any such findings may be useful in linking HRV change in legal situations (e.g. drink driving levels of alcohol intake). Third, the present study does not include information regarding influences of acute alcohol consumption on VLF and ULF HRV power spectrum, which remains an area open for future investigation. Also, replication research with larger samples is needed to confirm the present findings.

CONCLUSION

In summary, the findings in this study indicate the physiological response to time-related acute alcohol consumption by nonalcoholic individuals and demonstrate that HRV is a sensitive indicator of autonomic regulation. Acute effects of alcohol ingestion result in reduction of HRV, suggesting impaired cardiac autonomic nervous activity. Autonomic nervous activity in the females was less dampened by the alcohol compared to the males.

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COMPETING INTERESTS

The authors declare that they have no competing interests.

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