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Early signs of cardiovascular dysregulation in young adult binge drinkers

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Abstract

Binge drinking is widespread on American college campuses, but its effects on the cardiovascular system are poorly understood. This study sought evidence of preclinical cardiovascular changes in binge drinking young adults ($n = 24$) compared to nondrinking ($n = 24$) and social drinking ($n = 23$) peers during baseline, paced sighing (0.033Hz), and paced breathing (0.1Hz) tasks. Binge drinkers showed consistent, but often statistically nonsignificant evidence of greater sympathetic activation and reduced baroreflex sensitivity. Interestingly, the structure of group-averaged baseline heart rate spectra was considerably different between groups in the low frequency range (0.05–0.15 Hz). In particular, the binge drinking group-averaged spectra showed several spectral peaks not evident in the other groups, possibly indicating two functionally distinct subranges (0.05–0.08 and 0.08–0.15 Hz) that reflect vascular tone baroreflex activity and heart rate baroreflex activity, respectively. Vascular tone baroreflex gain and power in two peaks in the 0.05–0.08 Hz range were associated with years of drinking in the binge drinking group. Vascular dysfunction may be an early indicator of drinking-related change in the cardiovascular system.

The time course and trajectory of cardiovascular reactions to alcohol at the level of individual intoxication-withdrawal cycles, or across time as the number of intoxication cycles grows, are only partially understood. Yet, chronic heavy alcohol use is a well-established risk factor for adverse cardiovascular events in middle age, such as stroke, sudden death, myocardial infarction, and progression of atherosclerosis (Bar et al., 2006; Bau, Bau, Naujorks, & Rosito, 2005; Marques-Vidal et al., 2001; Puddey, Zilkens, Croft, & Beilin, 2001). A central question is how and when do cardiovascular reactions to acute alcohol give rise to alcohol-related system dysfunction. This is a particularly compelling question considering more than a half of college student drinkers engage in heavy drinking (i.e., binge) episodes and do so on a weekly or greater basis (Courtney & Polich, 2009; White, Kraus, & Swartzwelder, 2006). During binge episodes, blood alcohol levels exceed 0.08% and risk for adverse alcohol-related consequences is significantly elevated (Courtney & Polich, 2009; Naimi et al., 2003). This blood alcohol level is typically achieved when a

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woman consumes 4 standard drinks or a man consumes 5 standard drinks in a two-hour period (Courtney & Polich, 2009).

Multiple bodies of evidence suggest that each individual bout of intoxication precipitates multiple reversible molecular responses that impact cardiovascular functioning (Bar et al., 2006; Bau et al., 2005; Bau, Bau, Rosito, Manfroi, & Fuchs, 2007). Recently, simultaneous and noninvasive recordings of heart rate (HR), stroke volume, vascular tone, and blood pressure in young adult social drinkers revealed a complex relationship among cardiovascular processes early in the ascending limb of the blood alcohol curve that allowed the cardiovascular system to adapt to the presence of alcohol (Buckman et al., 2015). That study showed specific actions of alcohol intake on individual cardiovascular processes (e.g., increasing HR) and, more importantly, on the interrelations between processes. Evidence of reversible, interdependent responses of cardiovascular system processes to alcohol supports the adaptive capacity of the overall system that is only observable when cardiac and vascular dynamics are simultaneously examined.

A recent study of young adult binge drinkers further adds to this line of reasoning (Goslowski et al., 2013). Early clinical manifestations of cardiovascular risk were evident as lower flow-mediated and nitroglycerin-mediated dilation among young adult binge drinkers compared to same aged alcohol abstainers/very light drinkers. This suggests that even early in an individual's drinking history, repeated heavy drinking episodes may lead to endothelial dysfunction and the potential for general vasomotor dysregulation, both of which may serve as early prognostic indicators of future cardiovascular disease (Bar et al., 2006; Bau et al., 2005; Marques-Vidal et al., 2001; Puddey et al., 2001). The possibility of early stage vascular dysfunction raises the question of how repeated binge alcohol use could interfere with adaptive changes to the multifaceted and time-dependent cellular influences of alcohol on vascular endothelium (Adachi, 2016; Jochum et al., 2011), such that they are diminished or become counterproductive and serve instead to destabilize the cardiovascular system.

The present study sought to examine cardiovascular regulation in young adult (18 to 25 years of age) binge drinkers compared to that in young adult nondrinkers and social drinkers. A broad perspective on the cardiovascular system was taken by simultaneously and noninvasively measuring multiple system processes, and their interrelations, at rest and during two tasks that provoked either sympathetic (paced sighing at 0.033 Hz, or 2 sighs per minute) (Vaschillo et al., 2015) or parasympathetic (paced breathing at 0.1 Hz, or 6 breaths per minutes) activity (Lehrer et al., 2003). Individual cardiovascular functions were assessed using standard analytic approaches, including individual cardiovascular parameter averages (HR, stroke volume, vascular tone, and blood pressure) and heart rate measures (Task Force of the European Society of Cardiology and the American Society of Pacing and Electrophysiology, 1996) and the relationship of these parameters to blood pressure control via respective baroreflex branches (Vaschillo, Vaschillo, Buckman, Pandina, & Bates, 2012). These analyses were then supplemented with two exploratory strategies. The first was to plot the interrelation of parameter averages to characterize whole system functionality based on evidence that compensatory and synergistic factors are important to consider, particularly when the system is challenged (Buckman et al., 2015). The second was to measure the amplitude of power peaks in the low frequency range of the RRI power spectrum to

differentiate vascular tone and HR control systems that are active in the low frequency domain of the RRI power spectrum (Stauss, Anderson, Haynes, & Kregel, 1998). These exploratory strategies were hypothesized to increase the ability to detect subtle changes in cardiac and vascular functioning using noninvasive and real-time data collection strategies. Assessment of whether changes appeared cumulative as drinking histories lengthened was also explored. Binge drinking was broadly hypothesized to exert subtle but cumulative adverse effects on the cardiovascular system based on evidence of the effects of acute alcohol intoxication on the cardiovascular system (Buckman et al., 2015), evidence of clinical manifestations of cardiovascular risk in young binge drinkers (Adachi, 2016; Goslawski et al., 2013), and evidence of adverse effects of long-term chronic alcohol use on cardiovascular health (Puddey et al., 2001); (Bau et al., 2005); (Bar et al., 2006); (Marques-Vidal et al., 2001).

Method

Participants

Seventy-one college student volunteers (51% women) were enrolled in the study (21.9 (*SD*: 2.15) years old, range 18–25) based on having one of three specific drinking histories. First, binge drinkers (BD) were included and defined as individuals who drank at least 4 (women) or 5 (men) drinks within ~ 2 hours (thus achieving a blood alcohol concentration of ~ 0.08%) at least 5 times per month ($n = 24$). Second, social drinkers (SD) were included and defined as individuals who regularly drank (at least 2 times per month) but drank no more than 2 (women) or 3 (men) drinks per occasion ($n = 23$). Third, nondrinkers (ND) were included and defined as individuals who consumed no alcohol in the past 3 months and had drank no more than 2 drinks per occasion, with less than 4 occasions per year ($n = 24$). Drinking group definitions were adapted from the National Institute on Alcoholism and Alcohol Abuse (NIAAA) definitions (<https://www.niaaa.nih.gov/alcohol-health/overview-alcohol-consumption/moderate-binge-drinking>). Specifically, the BD group definition matched the quantities defined by NIAAA and included frequency of use to align with SAMHSA's definition of heavy drinking. The SD designation blends NIAAA's definition of "moderate drinking" and "low-risk drinking" to best differentiate this drinking pattern from binge/heavy drinking. The ND group definition allowed for negligible alcohol exposure, which often occurs during adolescence/early adulthood, but both quantity and frequency limits ensured that all participants were very low risk for the subsequent development of alcohol use disorders.

Exclusion criteria were current use of medication affecting the cardiovascular system, a history of cardiac abnormality, high blood pressure ($> 140/90$ mmHg), medical condition that can directly or indirectly influences vascular tone or cardiovascular functioning, or a body mass index indicative of obesity. Individuals with past year treatment for any psychiatric disorder were also excluded, as well as individuals who reported nicotine product use in the prior 3 months, regular (monthly) use of psychoactive substances (other than alcohol), or who had a lifetime diagnosis of a substance use disorder. For women, pregnancy and lactation were additional exclusion criteria.

Participants were recruited through university and community bulletin boards, electronic postings, and flyers. The racial composition was: White (60.5%), Asian (22.5%), Black or African American (11.3%), and mixed or other (5.7%); 16.9% self-reported being Hispanic or Latino. Study participants, males and females, were grouped based on drinking profiles. Groups were matched on age, sex, and ethnicity/race.

This experiment was approved by the Rutgers University Institutional Review Board for the Protection of Human Subjects Involved in Research. Participants were compensated \$25 for their time.

Procedure

An initial telephone screening interview was conducted to assess eligibility for study inclusion. Upon arrival at the laboratory, all eligible participants provided written informed consent. Each participant completed one laboratory session that lasted approximately 2 hours and started between 10 a.m. and 2 p.m. to minimize biological circadian variations. After completion of a series of questionnaires (demographic, family health history, alcohol and other drug use and the Substance Use Risk Profile scale (Woicik, Stewart, Pihl, & Conrod, 2009)), participants were seated in a comfortable chair located 2.5 m in front of a large TV screen in a sound attenuated, dimly lit room. ECG, beat-to-beat blood pressure, and thoracic respiration were continuously collected while participants completed five 5-minute tasks (baseline task, 3 paced sighing tasks at 0.02, 0.033, and 0.066 Hz, and a paced breathing task at 0.1 Hz). The primary aims of the larger study were to examine the vascular tone control system in its working frequency range of 0.02–0.08 Hz (Stauss et al., 1998) and characterize the effects of drinking on this system. This paper focuses on the latter aim through comparison of baseline (at rest), 0.033 Hz sighing (sympathetic challenge), and 0.1 Hz breathing (parasympathetic challenge). The 0.033 Hz frequency was selected as it was midrange in the testing frequency and we have previously published on this stimulation frequency rate (Vaschillo, Lehrer, Rishe, & Konstantinov, 2002; Vaschillo, Zingerman, Konstantinov, & Menitsky, 1983). Study design and sample sizes were determined to allow medium effect size group differences in standard cardiovascular indices (Task Force of the European Society of Cardiology and the American Society of Pacing and Electrophysiology, 1996).

Self-report questionnaires

Participants provided self-report information during the initial telephone screening interview that was reviewed upon arrival at the laboratory. Biological sex and date of birth from this screening was used in the present study. Participants then completed several surveys, including a 15-item alcohol use questionnaire. Age of first use was used in the present study.

Physiological tasks

Participants were first presented with a low-demand cognitive task (Jennings, Kamarck, Stewart, Eddy, & Johnson, 1992) that provides a more standardized assessment than an uncontrolled resting state. A rectangle presented in the center of the TV screen changed color every 10 sec for 5-min, and participants were asked to silently count the number of rectangles that were blue. This task served as the baseline (BL) task and was completed first.

Three paced sighing tasks (PS) were then completed in counterbalanced order. In these tasks, participants voluntarily sighed when the TV screen turned red; between sighs, participants breathed normally. Participants were instructed to inhale fully and quickly by mouth, but not too deeply, and exhale in a relaxed way. Before recording, participants underwent a short training to become familiarized with the sighing pattern. The red screen presentation was programmed using E-Prime software (Psychology Software Tools, Inc., Pittsburgh, PA) with an accuracy of ± 1 ms. Three pacing frequencies were used in the overall study design: 0.02 Hz (one sigh per 50 s), 0.033 Hz (one sigh per 30 s), and 0.066 Hz (one sigh per 15 s). For this study, paced sighing at 0.033 Hz was used as a sympathetic challenge (i.e., to elicit a robust response from the sympathetic nervous system)(Vaschillo et al., 2015).

A single paced breathing task (PB) involved breathing at a rate of six breathing cycles per minute (0.1 Hz) by following a visual pacer (Easy Air, Biofeedback Foundation of Europe, Montreal, Canada) presented on the TV screen. This pace is much slower than normal breathing (12–20 breathes per minute). Before recording, participants underwent a short training to ensure that they breathed slowly, but not too deeply, to avoid possible hyperventilation. This task is well-established to elicit a vagal response and synchronize neurocardiac signaling with respiratory sinus arrhythmia (Lehrer, Vaschillo, & Vaschillo, 2000). It was used as a parasympathetic challenge.

Physiological assessment

A PowerLab Acquisition System (ADInstruments, Colorado Springs, CO) and Finometer MIDI (Finapres, Amsterdam) were used to collect electrocardiogram (ECG), beat-to-beat blood pressure, and thoracic respiration. The sampling rate for all data collection was 2000 Hz. A standard lead II was used for ECG measurement. A cuff-sensor for continual beat-to-beat blood pressure measurement was attached to the second phalange of the right middle finger. Respiratory data were collected from a belt with a transducer that was set around the upper part of the chest, just below the underarms. Respiration was calibrated before the start of physiological records using a standard 800 ml bag into which participants were asked to breathe in and out five times. Physiological data were collected during all tasks.

Analysis of physiological data

Recorded physiological data were exported to WinCPRS software (Absolute Aliens Oy, Turku, Finland) to measure R wave to R wave intervals of ECG (RRI), systolic and diastolic arterial blood pressure, stroke volume, and pulse transit time. Blood pressures were measured as the peak and valley, respectively, of each finger pulse recorded by the Finometer MIDI. The ModelFlow methodology for cardiac output measurement (Bogert & van Lieshout, 2005) was used to measure beat-to-beat stroke volume from the same finger pulse. Pulse transit time for each heart beat was measured as the time interval between the R-spike of the ECG and the peak of the corresponding finger pulse recorded by the Finometer MIDI and was used as a proxy measure of vascular tone. Higher pulse transit time corresponds to lower vascular tone (Naka, Tweddel, Doshi, Goodfellow, & Henderson, 2006; Naschitz et al., 2004). Beat-to-beat data were transformed into equidistant waveform through cubic interpolation and 4 Hz resampling.

Average functioning of individual cardiovascular parameters was measured as mean RRI, stroke volume, pulse transit time, and SAP for each task. Variability in cardiovascular parameters included heart rate variability (HRV), which was measured in the time domain as the root of the mean squared differences of successive intervals (RMSSD) and in frequency domain as total power of the RRI spectrum in frequency range of (Total HRV, 0.001–0.05 Hz) and spectral power in the high (HF HRV, 0.15–0.5 Hz) and low (LF HRV, 0.05–0.15 Hz) frequency ranges of the RRI spectrum. Average baseline RRI spectra were then created by group (ND, SD, BD). Power peaks in the low frequency range of averaged spectra were identified and the amplitude of these peaks were calculated for each participant.

HR, stroke volume, and vascular tone baroreflex gains were calculated to separately evaluate the activity of each baroreflex branch respectively. Cross-spectral analysis of simultaneously recorded beat-to-beat RRI, stroke volume, pulse transit time, and systolic arterial pressure (SAP) was used to calculate transfer functions with SAP as the input, and RRI, stroke volume, or pulse transit time as the outputs (SAP-RRI, SAP-SV, and SAP-PTT transfer functions, respectively). HR, stroke volume, and vascular tone baroreflex gains were estimated as the means of the corresponding transfer function in the low frequency range (0.05–0.15 Hz), where coherence between SAP and RRI, stroke volume, or pulse transit time was > 0.5 (Vaschillo et al., 2012). Thus baroreflex gain was estimated as the magnitude of change in RRI (ms), stroke volume (ml), and pulse transit time (ms) in response to one unit of change in SAP (mmHg).

Statistical analysis

Data from two participants were excluded from the study after physiological recording due to excessive coughing and a high frequency of extra systoles. Initial analyses included a 3-group (BD, SD, and ND) repeated measures analysis of variance (SAS 9.3, SAS Institute, Cary, NC). In these analyses, the ND and SD groups did not differ in any mean or variability measure from any physiological domain. Subsequent repeated measures analyses focused on differences between the SD and BD groups to specifically gauge the differential impact on the cardiovascular system of moderate, low risk drinking compared to heavy episodic binge drinking. Repeated measures analysis of variance was used to calculate group differences in mean HR, stroke volume, SAP, and pulse transit time; multiple measures of HRV; and gain in the HR, stroke volume, and vascular tone baroreflex branches. A Bonferroni correction for multiple testing was used. Based on performing 10 repeated measures ANOVAs, alpha was set at $p < .005$.

Specifically within the BD group, linear regression was used to assess the relationship of years of alcohol use to vascular tone baroreflex gain and to power in LF spectral peaks in baseline RRI spectra. Years of alcohol use was estimated by subtracting current age from the age of first drinking episode. Cardiovascular parameters were log transformed prior to repeated measures analysis of variance and linear regression analysis. Normalized baseline RRI spectra were used in the latter analyses. Regression analysis also was used to describe the pattern of relationships between cardiovascular parameters. The coefficient of determination (R^2) were computed between average HR, stroke volume, pulse transit time, and SAP for each task in each group (ND, SD, BD) and significant R^2 were plotted.

Results

Average cardiovascular parameter functioning

There were no significant main effects of drinking group or group by task interaction effects on mean RRI, stroke volume, pulse transit time, or SAP. There was, however, a significant main effect of task (BL, PS, PB) on mean RRI, $F(2,90) = 8.78$, $p < 0.0006$, and mean SAP, $F(2,90) = 10.14$, $p < 0.0003$. Posthoc means comparisons of RRI showed significant differences between baseline and paced sighing, $F(1,45) = 24.40$, $p < 0.0001$ (Figure 1A). Posthoc means comparisons of SAP showed significant differences between baseline and paced sighing, $F(1,45) = 29.52$, $p < 0.0001$ and between paced sighing and paced breathing, $F(1,45) = 10.68$, $p < .002$ (Figure 1D). Neither mean stroke volume (Figure 1B) nor mean pulse transit time (Figure 1C) showed task differences that were significant using the Bonferroni-corrected significance levels.

The relationships between average HR, stroke volume, pulse transit time, and SAP were then compared within each group. Figure 2 shows significant R^2 values between parameters. Two overarching patterns were observed. First, cardiac and vascular indices were more likely to be significantly related in the BD group compared to the ND and SD group. This is notable for the HR-vascular tone R^2 , the stroke volume-vascular tone R^2 , and vascular tone-SAP R^2 group values. Second, this pattern was observed at baseline and in response to both the sympathetic (paced sighing) and parasympathetic (paced breathing) challenge.

Variability in cardiovascular functioning using HRV indices and group-averaged spectra

Similar to the mean cardiovascular parameters, measures of HRV showed no significant group or group by task interaction effects, but significant task effects were seen in total HRV, $F(2,90) = 189$, $p < 0.0001$ (Figure 3A), LF HRV, $F(2,90) = 239$, $p < 0.0001$ (Figure 3B), and RMSSD, $F(2,90) = 45.32$, $p < 0.0001$ (Figure 3D). Task differences in HF HRV did not reach Bonferroni-adjusted significance levels (Figure 3C). Posthoc comparisons of total HRV showed significant differences between baseline and paced breathing, $F(1,45) = 217$, $p < 0.0001$ and paced sighing and paced breathing, $F(1,45) = 319$, $p < 0.0001$. Posthoc comparisons of LF HRV showed significant differences between baseline and paced sighing, $F(1,45) = 9.10$, $p < 0.0042$, baseline and paced breathing, $F(1,45) = 284$, $p < 0.0001$, and paced sighing and paced breathing, $F(1,45) = 400$, $p < 0.0001$. Posthoc comparisons of RMSSD showed significant differences between baseline and paced breathing, $F(1,45) = 42.14$, $p < 0.0001$, and baseline and paced sighing, $F(1,45) = 85.43$, $p < 0.0001$ (Figure 3D).

Baseline RRI spectra then were averaged within the ND, SD, and BD groups (Figure 4). Qualitative comparison of these group-averaged baseline RRI frequency spectra revealed extensive group differences in the spectral structure in the low frequency range (0.05–0.15 Hz). Specifically, three distinct power peaks appeared to differentiate the groups: at ~ 0.066 Hz, ~ 0.08 Hz, and ~ 0.1 Hz. These three spectral peaks were more prominent in the binge drinking group (Figure 4C) compared to social drinking (Figure 4B) and nondrinking (Figure 4A) groups. Neither the very low frequency range (0.001–0.05 Hz) nor the high frequency (0.15–0.35 Hz) range showed substantial differences between the groups.

Association of power peaks in the RRI spectra to duration of drinking in binge drinkers

Comparison of Figure 4C to Figures 4A and 4B also suggests greater variance in the BD group compared to the ND and SD groups. To investigate the possibility that this heterogeneity in part was due to differences in drinking history, the power of LF peaks was regressed on the duration of alcohol use in the BD group (Figure 5). Duration of alcohol use was calculated as the difference between age at the experimental session and self-reported age of first use [Men: age at experimental session ($M: 21.5$, $SD: 1.6$), age of first drink ($M: 16.9$, $SD: 2.3$), years of drinking ($M: 4.6$, $SD: 2.3$, range: 1–9). Women: age ($M: 19.7$, $SD: 1.6$), age of first drink ($M: 16.3$, $SD: 1.2$), years of drinking ($M: 3.6$, $SD: 1.8$, range: 2–9)]. A significant negative association between years of drinking and peaks in baseline RRI spectra was observed in the BD group. Specifically, the 0.066 Hz power peaks, $F(1,22) = 14.7$, $p < 0.0001$; $R^2 = 0.40$ (Figure 5A), and the 0.08 Hz power peaks, $F(1,22) = 8.36$, $p < 0.006$; $R^2 = 0.17$ (Figure 5B), were significantly negatively associated with duration of drinking; the 0.1 Hz power peak (Figure 5C) was not. Similar analyses in the SD group revealed no significant relationships.

To further illustrate this relationship, we graphed individual normalized baseline RRI spectra from representative participants with different drinking history lengths (Figure 6). The left panels show the amplitude of the spectral peak at 0.066 Hz in male binge drinking participants who were estimated to have been drinking for 1 year (top), 3 years (middle) and 9 years (bottom). The right panels show the amplitude of the spectral peak at 0.066 Hz in female binge drinking participants who were estimated to have been drinking for 2 years (top), 3 years (middle) and 9 years (bottom). Note that 2 years was the shortest duration of use reported for any female in the BD group.

Baroreflex functioning across drinking groups

Group differences in baroreflex gain did not achieve significance using Bonferroni-adjusted significance levels, nor did any interaction terms. There were, however significant task effect for all three measures of baroreflex gain. The significant HR baroreflex gain task effect, $F(2,90) = 50.42$, $p < 0.0001$, was related to gain being higher during paced breathing versus baseline, $F(1,45) = 45.27$, $p < 0.0001$, and paced sighing, $F(1,45) = 92.91$, $p < 0.0001$ (Figure 7A). The significant stroke volume baroreflex gain task effect, $F(2,90) = 8.57$, $p < 0.001$, was related to gain being higher during paced breathing compared to baseline, $F(1,45) = 9.59$, $p < 0.003$, and paced sighing, $F(1,45) = 19.73$, $p < 0.0001$ (Figure 7B). The significant vascular tone baroreflex gain task effect, $F(2,90) = 6.22$, $p < 0.004$, was related to gain being higher during paced breathing, $F(1,45) = 8.64$, $p < 0.005$, compared to baseline (Figure 7C).

Results of exploratory regression analysis (Figure 8) revealed a significant negative association between the number of years of drinking in the BD group and vascular tone baroreflex gain in paced sighing task, $F(1,22) = 7.6$, $p < 0.014$; $R^2 = 0.24$, and paced breathing task, $F(1,22) = 6.15$, $p < 0.021$; $R^2 = 0.22$; there was no association in baseline.

Discussion

This study used a holistic perspective of the cardiovascular system in ostensibly healthy young adults who either did not drink, engaged in social drinking (but not binge drinking), or routinely engaged in binge drinking, to characterize subtle cardiovascular changes that may occur early in an individual's drinking history. Cardiovascular dysfunction was captured with: (a) average functionality at rest and in response to acute sympathetic (paced sighing at 0.033 Hz) and parasympathetic (paced breathing at 0.1 Hz) challenges; (b) variability in HR, which is a widely-used measure of adaptive cardiac responsivity; and, (c) sensitivity of the baroreflex, which reflects system functionality across cardiac and vascular systems and brain - body interconnection (Bates & Buckman, 2013). Cardiovascular dysfunction was also explored by developing new measures that were hypothesized to enhance detection of subtle preclinical changes. These measures included (a) the interrelation of distinct cardiac and vascular functional domains (i.e., HR, stroke volume, vascular tone, and blood pressure), and (b) the amplitude of the power peak at 3 discrete frequencies within the low frequency range of the RRI spectrum. This combination of standard and exploratory measures of cardiovascular capacity suggests that binge drinking may instigate cardiovascular dysfunction, particularly as it relates to vasomotor dynamics, but that standard indices of cardiovascular function may not be sufficiently sensitive to detect subtle preclinical changes.

Using the standard tools of cardiovascular assessment described above, a consistent nonsignificant trend of reduced cardiovascular efficiency and/or sensitivity was noted. These trends, although not statistically significant, nonetheless may be noteworthy because the participants were all 18–25 years old with no known or observable health problems and the binge drinking group reported drinking for only 1–9 years. Thus, even if they engaged in frequent bingeing episodes, these young adults were still relatively early in their lifetime drinking trajectory and any evidence of cardiovascular differences between social drinking and binge drinking young adults suggests that high-quantity drinking episodes may instigate specific physiological responses. We therefore developed two new data approaches to enhance detection of change in the biological mechanisms that drive cardiovascular dynamics and integration.

Mean cardiovascular functioning and pattern of interrelation

Binge and social drinkers demonstrated similar mean HR (i.e., inverse of RRI), stroke volume, vascular tone (i.e., inverse of pulse transit time), and blood pressure at baseline and in response to the sympathetic paced sighing and parasympathetic paced breathing challenges (Figure 1). Average cardiovascular functioning is thought to reflect the metabolic state of the body and thus averages may not be sufficiently sensitive to detect the level of preclinical change that this study sought to observe. These types of changes may become more evident when there are overt signs of metabolic issues, such as obesity, which were absent in the present sample. Alternatively, changes in average cardiovascular functioning may be diffused across multiple parameters and only evident when the relationship between parameters is examined (Buckman et al., 2015).

To assess this latter possibility, the pattern of significant associations between HR, stroke volume, vascular tone, and SAP was compared across groups. This analytic approach allowed visualization of the interdependence of multiple cardiovascular parameters and suggested that repeated binge drinking episodes may provoke persistent coupling between cardiovascular parameters. Figure 2 shows that the binge drinking group had the greatest number of significant relationships between pairs of mean parameters compared to either of the other groups. There were modest differences in the social drinking group compared to the nondrinking group. In addition, in the BD group, the strength of relationships, particularly between vascular tone and blood pressure, vascular tone and stroke volume, and stroke volume and blood pressure were greater in the paced sighing (sympathetic challenge) task and somewhat lower in the paced breathing (parasympathetic challenge) task. The baroreflex system typically links cardiovascular parameters through its regulation of system variability, not parameter averages. We speculate that baroreflex regulation of parameter averages could lead to greater parameter coupling, as seen in this sample. This would suggest a role for the baroreflex in the metabolic control of cardiovascular functions, which may serve as an early indicator of whole system disruption from repeated high-quantity drinking episodes.

Cardiovascular variability and structure of group-averaged RRI spectra

Variability in biosignals is thought to reflect regulatory processes in system functioning that dynamically detect the response of the system to changes in inner state and external environment. Statistical analysis revealed no group differences in any of the measures of HRV assessed (Figure 3); however, there was a consistent, nonsignificant trend (often the realm of 0.5 log units of difference) towards reduced variability in the binge drinker group compared to the social drinker group in all measures. In addition to this trend, there were extensive group differences in the structure of the low frequency range of baseline RRI spectra (Figure 4). Variability in the low frequency range (0.05–0.15 Hz) is not well understood, but speculated to reflect elements of sympathetic nervous system activity, baroreflex functioning, and vascular dynamics (Berntson et al., 1997; Task Force of the European Society of Cardiology and the American Society of Pacing and Electrophysiology, 1996; Trubachev, Gorbunov, Trubacheva, Nemtseva, & Ogorodnikova, 2013). In addition to HR control system activity in this range, there is evidence that vascular tone control systems, which have a working frequency range of ~ 0.02–0.08 Hz (Stauss et al., 1998), are active in this range. We thus further compared the baseline RRI spectra in each group to examine the possibility of differentiating control mechanisms that underlie HRV in the low frequency range. Specifically, we focused on power peaks in the low frequency range that were evident when group-averaged spectra were generated. Three robust peaks at 0.066, 0.081, and 0.1 Hz were observed in the BD group-averaged baseline RRI spectrum, but absent in the nondrinkers group and subtle/inconsistent in the social drinkers group (Figure 4). In addition, there was greater within-group variability in spectral peaks in the binge drinker group than in the other groups.

We and others have previously described a power peak at 0.1 Hz in the RRI spectrum that reflects the resonance frequency of the HR baroreflex branch (Vaschillo et al., 1983); (Saul et al., 1991); (van de Vooren et al., 2007); (Vaschillo et al., 2002). In fact, this power peak,

when measured during a 0.1 Hz paced breathing task as was used in this study, is a proxy estimate (correlation coefficient > 0.7) of HR baroreflex gain, when blood pressure measurement is unavailable (Davies, Colhoun, Coats, Piepoli, & Francis, 2002). Moreover, this peak robustly responds to many forms of rhythmical stimulation (emotional, physical, or other) of the cardiovascular system (Vaschillo et al., 2008), even in cases where overall power in the LF range of the RRI spectrum does not significantly change. This illustrates the value of measuring spectral peaks in the RRI spectrum and supports the use of specific peak amplitudes to measure specific mechanisms of variability (Stauss et al., 1998). This differs from the more standard use of overall power in the LF range, which is likely a composite index that reflects multiple mechanisms of variability.

We hypothesize that the 0.066 Hz and 0.081 Hz peaks are related to mechanisms of vascular tone regulation. Thus, HRV in the low frequency range may be comprised of two functionally distinct subranges: a 0.05–0.08 Hz range that is related to vascular tone baroreflex activity and the 0.08–0.15 Hz range that is related to HR baroreflex activity (Figure 4). Further studies are needed to more clearly differentiate mechanisms of HR and vascular tone control underlying HRV because this observation may contribute to the long-standing debate in the field of psychophysiology about the functional significance of low frequency heart rate oscillations. (Task Force of the European Society of Cardiology and the American Society of Pacing and Electrophysiology, 1996).

The 0.066 and 0.081 Hz power peak amplitudes were associated with the number of years of drinking in the binge drinking group; the ~ 0.1 Hz peak amplitude, however, was not (Figure 5). This association is notable because the participants in this study were young and overtly healthy, and had relatively short drinking histories (1–9 years). Future studies should examine these relationships with individuals with longer drinking histories as well.

Baroreflex functioning

The sensitivity in three branches of the baroreflex was calculated using well-established measures (Vaschillo et al., 2012). Only nonsignificant group differences were noted, but a significant negative association was observed between vascular tone baroreflex gain during the challenge tasks and years of drinking in the binge drinker group (Figure 8). (Goslowski et al., 2013) reported complex vascular endothelial impairments in binge drinkers compared to nonbinge drinkers. We speculate that the decreasing amplitude of the 0.066 Hz peak as years of drinking increased may reflect progressive destruction of endothelial control mechanisms.

At rest, endothelial functioning is likely the primary mechanism of vascular tone control. Under conditions of increased loading due to greater circulation, the vascular tone baroreflex may become activated as endothelial mechanisms become impaired (Nobrega et al., 2014). (Buckman et al., 2015) recently found that vascular tone baroreflex gain was elevated during acute alcohol intoxication compared to a predrinking baseline, further suggesting that vascular tone baroreflex sensitivity is elevated to support alcohol-induced increases in circulation. It may be that heavy drinking episodes disrupt both the endothelial and vascular tone baroreflex control mechanisms. This is plausible as disruption of vessel endothelium can lead directly to lower sensitivity of the baroreceptors that sit within the vessel wall.

Conclusion

Binge drinking on college campuses is widespread and has been linked to risk of physical injury, sexual assault perpetration and victimization, vandalism, and death (Adan, Benaiges, & Forero, 2016; Bouchery, Harwood, Sacks, Simon, & Brewer, 2011; Broughton & Molasso, 2006; Mouilso, Fischer, & Calhoun, 2012; Mundt, Zakletskaia, & Fleming, 2009). This study further identified several potential signs of early cardiovascular change in a sample of young binge drinkers. Specifically, the newly developed measures in this study identified measurable, preclinical, alcohol-induced cardiovascular changes within the first 9 years of binge drinking. This proposition has compelling public health implications, but should be considered in light of study limitations. The sample was relatively small and powered to detect medium effect size differences. The number of years of binge drinking was not directly measured. Years of drinking were estimated as the difference between current age and age at the time of first use (not including sips of alcohol). There were no significant differences between nondrinkers and social drinkers; thus, initial analyses focused on social drinkers versus binge drinkers to delineate the impact of heavy drinking episodes from more moderate alcohol use events on the cardiovascular system. With longer drinking profiles, however, it is unclear whether differences between nondrinkers, social drinkers, and binge drinkers would remain the same. Future studies should be powered to allow for detection of subtle group differences as may occur in a young, ostensibly healthy sample. Future study also should aim to include greater detail on drinking history, specifically related to the frequency of bingeing and the severity of these binge episodes (i.e., number of drinks per occasion). These studies would further benefit from including either a broader range of drinking years or a prospective study design to better capture the timeline of cardiovascular disruption caused by binge drinking episodes. Nonetheless, these findings warrant further examination in independent samples and across different conditions that are known to impact cardiovascular function. The ability to efficiently detect subtle preclinical changes in the cardiovascular system and develop new strategies for understanding the mechanisms underlying HR and vascular tone control systems can contribute importantly to improving health.

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References

- Adachi H. Impact of heavy alcohol consumption on impaired endothelial function. *Journal of Atherosclerosis and Thrombosis*. 2016; 23(9):1028–1029. <https://doi.org/10.5551/jat.ED041>. [PubMed: 27052785]
- Adan, A., Benaiges, I., Forero, DA. Heavy episodic drinking or binge drinking: A booming consumption pattern. In: Preddy, V., editor. *Neuropathology of Drug Addictions and Substance Misuse*. Vol. 1. London: Academic Press; 2016. p. 389-397.
- Bar KJ, Boettger MK, Boettger S, Groteluschen M, Neubauer R, Jochum T, Voss A. Reduced baroreflex sensitivity in acute alcohol withdrawal syndrome and in abstained alcoholics. *Drug and Alcohol Dependence*. 2006; 85(1):66–74. <https://doi.org/10.1016/j.drugalcdep.2006.03.014>. [PubMed: 16650658]

- Bates, ME., Buckman, JF. Integrating body and brain systems in addiction neuroscience. In: Miller, P., editor. *Biological Research on Addiction: Comprehensive Addictive Behaviors and Disorders*. Vol. 2. New York: Elsevier; 2013. p. 187-198.
- Bau PF, Bau CH, Naujorks AA, Rosito GA. Early and late effects of alcohol ingestion on blood pressure and endothelial function. *Alcohol*. 2005; 37(1):53–58. <https://doi.org/10.1016/j.alcohol.2005.10.034>. [PubMed: 16472719]
- Bau PF, Bau CH, Rosito GA, Manfroi WC, Fuchs FD. Alcohol consumption, cardiovascular health, and endothelial function markers. *Alcohol*. 2007; 41(7):479–488. <https://doi.org/10.1016/j.alcohol.2007.08.004>. [PubMed: 17980786]
- Berntson GG, Bigger JT Jr, Eckberg DL, Grossman P, Kaufmann PG, Malik M, van der Molen MW. Heart rate variability: origins, methods, and interpretive caveats. *Psychophysiology*. 1997; 34(6): 623–648. [PubMed: 9401419]
- Bogert LWJ, van Lieshout JJ. Non-invasive pulsatile arterial pressure and stroke volume changes from the human finger. *Experimental Physiology*. 2005; 90(4):437–446. <https://doi.org/10.1113/expphysiol.2005.030262>. [PubMed: 15802289]
- Bouchery EE, Harwood HJ, Sacks JJ, Simon CJ, Brewer RD. Economic costs of excessive alcohol consumption in the U.S., 2006. *American Journal of Prevention Medicine*. 2011; 41(5):516–524. <https://doi.org/10.1016/j.amepre.2011.06.045>.
- Broughton EA, Molasso WR. College Drinking: Content Analysis of 30 Years of Research. *Journal of College Student Development*. 2006; 47(6):609–627. <https://doi.org/10.1353/csd.2006.0064>.
- Buckman JF, Eddie D, Vaschillo EG, Vaschillo B, Garcia A, Bates ME. Immediate and complex cardiovascular adaptation to an acute alcohol dose. *Alcoholism: Clinical and Experimental Research*. 2015; 39(12):2334–2344. <https://doi.org/10.1111/acer.12912>.
- Courtney KE, Polich J. Binge drinking in young adults: Data, definitions, and determinants. *Psychological Bulletin*. 2009; 135(1):142–156. <https://doi.org/10.1037/a0014414>. [PubMed: 19210057]
- Davies LC, Colhoun H, Coats AJ, Piepoli M, Francis DP. A noninvasive measure of baroreflex sensitivity without blood pressure measurement. *American Heart Journal*. 2002; 143(3):441–447. <http://dx.doi.org/10.1067/mhj.2002.121263>. [PubMed: 11868049]
- Goslawski M, Piano MR, Bian JT, Church EC, Szczurek M, Phillips SA. Binge drinking impairs vascular function in young adults. *Journal of the American College of Cardiology*. 2013; 62(3): 201–207. <https://doi.org/10.1016/j.jacc.2013.03.049>. [PubMed: 23623907]
- Jennings JR, Kamarck T, Stewart C, Eddy M, Johnson P. Alternate cardiovascular baseline assessment techniques: vanilla or resting baseline. *Psychophysiology*. 1992; 29:742–750. <http://dx.doi.org/10.1111/j.1469-8986.1992.tb02052.x>. [PubMed: 1461961]
- Jochum T, Weissenfels M, Seeck A, Schulz S, Boettger MK, Voss A, Bar KJ. Endothelial dysfunction during acute alcohol withdrawal syndrome. *Drug and Alcohol Dependence*. 2011; 119(1–2):113–122. <https://doi.org/10.1016/j.drugalcdep.2011.06.002>. [PubMed: 21719213]
- Lehrer PM, Vaschillo E, Vaschillo B. Resonant frequency biofeedback training to increase cardiac variability: rationale and manual for training. *Applied Psychophysiology and Biofeedback*. 2000; 25:177–191. [PubMed: 10999236]
- Lehrer PM, Vaschillo E, Vaschillo B, Lu SE, Eckberg DL, Edelberg R, Hamer RM. Heart rate variability biofeedback increases baroreflex gain and peak expiratory flow. *Psychosomatic Medicine*. 2003; 65(5):796–805. [PubMed: 14508023]
- Marques-Vidal P, Arveiler D, Evans A, Amouyel P, Ferrieres J, Ducimetiere P. Different alcohol drinking and blood pressure relationships in France and Northern Ireland: The PRIME Study. *Hypertension*. 2001; 38(6):1361–1366. <http://dx.doi.org/10.1161/hy1101.095328>. [PubMed: 11751718]
- Mouilso ER, Fischer S, Calhoun KS. A prospective study of sexual assault and alcohol use among first-year college women. *Violence and Victims*. 2012; 27(1):78–94. <https://doi.org/10.1891/0886-6708.27.1.78>. [PubMed: 22455186]
- Mundt, MP, Zakletskaia, LI., Fleming, MF. Extreme College Drinking and Alcohol-Related Injury Risk. *Alcoholism, Clinical and Experimental Research*. 2009. <https://doi.org/10.1111/j.1530-0277.2009.00981.x>

- Naimi TS, Brewer RD, Mokdad A, Denny C, Serdula MK, Marks JS. Binge drinking among US adults. *JAMA*. 2003; 289(1):70–75. <http://dx.doi.org/10.1001/jama.289.1.70>. [PubMed: 12503979]
- Naka KK, Tweddel AC, Doshi SN, Goodfellow J, Henderson AH. Flow-mediated changes in pulse wave velocity: a new clinical measure of endothelial function. *European Heart Journal*. 2006; 27(3):302–309. <http://doi.org/10.1093/eurheartj/ehi619>. [PubMed: 16267075]
- Naschitz JE, Bezobchuk S, Mussafia-Priselac R, Sundick S, Dreyfuss D, Khorshidi I, Gaitini L. Pulse transit time by R-wave-gated infrared photoplethysmography: review of the literature and personal experience. *Journal of Clinical Monitoring and Computing*. 2004; 18(5–6):333–342. <http://dx.doi.org/10.1007/s10877-005-4300-z>. [PubMed: 15957624]
- Nobrega AC, O’Leary D, Silva BM, Marongiu E, Piepoli MF, Crisafulli A. Neural regulation of cardiovascular response to exercise: role of central command and peripheral afferents. *BioMed Research International*. 2014; 2014:478965. <https://doi.org/10.1155/2014/478965>. [PubMed: 24818143]
- Puddey IB, Zilkens RR, Croft KD, Beilin LJ. Alcohol and endothelial function: a brief review. *Clinical and Experimental Pharmacology and Physiology*. 2001; 28(12):1020–1024. <http://dx.doi.org/10.1046/j.1440-1681.2001.03572.x>. [PubMed: 11903307]
- Saul JP, Berger RD, Albrecht P, Stein SP, Chen MH, Cohen RJ. Transfer function analysis of the circulation: unique insights into cardiovascular regulation. *The American Journal of Physiology*. 1991; 261(4 Pt 2):H1231–1245. [PubMed: 1928405]
- Stauss HM, Anderson EA, Haynes WG, Kregel KC. Frequency response characteristics of sympathetically mediated vasomotor waves in humans. *The American Journal of Physiology*. 1998; 274(4 Pt 2):H1277–1283. [PubMed: 9575932]
- Task Force of the European Society of Cardiology and the American Society of Pacing and Electrophysiology. Heart rate variability: Standards of measurement, physiological interpretation, and clinical use. *Circulation*. 1996; 93:1043–1065. [PubMed: 8598068]
- Trubachev VV, Gorbunov AV, Trubacheva VS, Nemtseva MS, Ogorodnikova MS. Plasticity of heart rate modulation of young adults during paced breathing with different frequencies. *Russ Fiziol Zh Im I M Sechenova*. 2013; 99(12):1450–1459. [PubMed: 25464775]
- van de Vooren H, Gademan MG, Swenne CA, TenVoorde BJ, Schalij MJ, Van der Wall EE. Baroreflex sensitivity, blood pressure buffering, and resonance: what are the links? Computer simulation of healthy subjects and heart failure patients. *Journal of Applied Physiology*. 2007; 102(4):1348–1356. <https://doi.org/10.1152/japplphysiol.00158.2006>. [PubMed: 17185500]
- Vaschillo E, Lehrer P, Rishe N, Konstantinov M. Heart rate variability biofeedback as a method for assessing baroreflex function: a preliminary study of resonance in the cardiovascular system. *Applied Psychophysiology and Biofeedback*. 2002; 27(1):1–27. [PubMed: 12001882]
- Vaschillo EG, Bates ME, Vaschillo B, Lehrer P, Udo T, Mun EY, Ray S. Heart rate variability response to alcohol, placebo, and emotional picture cue challenges: Effects of 0.1-Hz stimulation. *Psychophysiology*. 2008; 45:847–858. [PubMed: 18513359]
- Vaschillo EG, Vaschillo B, Buckman JF, Nguyen-Louie T, Heiss S, Pandina RJ, Bates ME. The effects of sighing on the cardiovascular system. *Biological Psychology*. 2015; 106:86–95. <https://doi.org/10.1016/j.biopsycho.2015.02.007>. [PubMed: 25720947]
- Vaschillo EG, Vaschillo B, Buckman JF, Pandina RJ, Bates ME. Measurement of vascular tone and stroke volume baroreflex gain. *Psychophysiology*. 2012; 49(2):193–197. <https://doi.org/10.1111/j.1469-8986.2011.01305.x>. [PubMed: 22092290]
- Vaschillo EG, Zingerman AM, Konstantinov MA, Menitsky DN. Research of the resonance characteristics for cardiovascular system. *Human Physiology*. 1983; 9:257–265.
- White AM, Kraus CL, Swartzwelder H. Many college freshmen drink at levels far beyond the binge threshold. *Alcoholism: Clinical and Experimental Research*. 2006; 30(6):1006–1010. <https://doi.org/10.1111/j.1530-0277.2006.00122.x>.
- Woicik PA, Stewart SH, Pihl RO, Conrod PJ. The Substance Use Risk Profile Scale: A scale measuring traits linked to reinforcement-specific substance use profiles. *Addictive Behaviors*. 2009; 34(12):1042–1055. <https://doi.org/10.1016/j.addbeh.2009.07.001>. [PubMed: 19683400]

Figure 1.

Average ($\pm$ standard error) cardiovascular parameters at rest and in response to challenge in the binge drinking and social drinking groups. (A) R-R interval from the ECG, (B) stroke volume, (C) pulse transit time, and (D) systolic arterial pressure were averaged within group during a low-demand baseline task (BL), a paced sighing sympathetic challenge (PS), and a paced breathing parasympathetic challenge (PB). ^b significant effect of task.

Figure 2.

Interrelation of Average Heart Rate (HR), Stroke Volume (SV), Vascular Tone (VT), and Systolic Arterial Pressure (SAP) in the nondrinking, social drinking, and binge drinking groups. Solid lines denote significant positive relationships; dashed lines denote significant negative relationships. R^2 values are presented on each line to demonstrate the strength of the relationship between cardiac and vascular parameters. The binge drinking group showed considerable differences from both the nondrinking and social drinking groups, which were only negligibly different from one another.

Figure 3.

Average (+/- standard error) heart rate variability (HRV) indices at rest and in response to challenge in the binge drinking and social drinking groups. (A) Total HRV, (B) high frequency (HF) HRV, (C) low frequency (LF) HRV, and (D) the root mean square of the standard deviation (RMSSD) showed task differences when compared across a low-demand baseline task (BL), a paced sighing sympathetic challenge (PS), and a paced breathing parasympathetic challenge (PB). The overall pattern suggested a tendency for reduced vagus activity in the BD group compared to the SD group, although the interactions were not statistically significant. ^b significant effect of task.

Figure 4.

Group-averaged baseline RRI spectra. The average spectra of the (A) nondrinking group, (B) social drinking, and (C) binge drinking groups are presented with the mean shown in red and the standard error shown as dashed lines around the mean. In the low frequency range (green + gray shaded region) of the RRI spectra, peaks at 0.066 Hz, 0.081, and ~ 0.1 Hz were noted in the binge drinking (C) group. These peaks are thought to reflect activity of vascular tone branch of the baroreflex (0.066 and 0.081 Hz; green shaded region) and the HR branch of the baroreflex (~ 0.1 Hz; gray shaded region).

Figure 5.

The association of baseline RRI spectra peak powers at (A) 0.066, (B) 0.081, and (C) ~0.1 (0.098) Hz with self-reported years of alcohol use in binge drinking young adults. The number of years of drinking was measured as the difference between age at the laboratory session and the self-reported age of first use. Significant associations were noted at 0.066 Hz and 0.081 Hz. * $p < .05$.

Figure 6.

Normalized baseline RRI spectra of six binge drinking young adults that varied in the years of alcohol use. Male binge drinking young adults who reported drinking for 1 year, 3 years, and 9 years are shown on the left. Female binge drinking young adults who reported drinking for 2 years (no 1 year duration reported), 3 years, and 9 years are shown on the right. The power at the 0.066 Hz peak appeared to be lowest in the two individuals who self-reported 9 years of drinking.

Figure 7. Average ($\pm$ standard error) baroreflex gain in the heart rate (HR, top), stroke volume (SV, middle), and vascular tone (VT, bottom) branches in binge and social drinking groups. BL - Baseline task. PS - Paced Sighing at 0.033 Hz task. PB - Paced Breathing at 0.1 Hz task. ^b significant effect of task.

Figure 8.

Association of vascular tone baroreflex gain in binge drinking young adults with self-reported years of alcohol use. Gain in the vascular tone branch of the baroreflex during (A) baseline, (B) paced sighing, and (C) paced breathing tasks was regressed on the number of years of drinking as measured as the difference from age at the laboratory session and the self-reported age of first use. Significant associations were noted in response to paced sighing and paced breathing, but not at baseline. * $p < .05$.