2021

Evening And Overnight Cardiovascular and Arterial Stiffness Responses To Simulated Binge Drinking

Grant Thivierge
Michigan Technological University, gsthivie@mtu.edu

EVENING AND OVERNIGHT CARDIOVASCULAR AND ARTERIAL STIFFNESS RESPONSES TO SIMULATED BINGE DRINKING

By

Grant S. Thivierge

A THESIS

Submitted in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

In Biological Sciences

MICHIGAN TECHNOLOGICAL UNIVERSITY

2021

This thesis has been approved in partial fulfillment of the requirements for the Degree of MASTER OF SCIENCE in Biological Sciences.

Department of Biological Sciences

Thesis Co-Advisor:	*Dr. John J. Durocher*
Thesis Co-Advisor:	*Dr. William H. Cooke*
Committee Member:	*Dr. Robert A. Larson*
Department Chair:	*Dr. Chandrashekhar P. Joshi*

Table of Contents

List of Figures

List of Tables

Acknowledgements

When I began my college career five years ago, I did not plan on completing a master's degree. Without the support of many individuals, I may not have continued to pursue this degree. I would first like to thank Dr. John Durocher. Thank you for the opportunities and guidance over the last four years. I am grateful for the opportunity to develop my skills in an independent research project as well as your help to improve my skills as a writer. It has been a long journey and it would not have been possible without your support. I would like to thank Dr. William Cooke for his support during my research. I am thankful for you being so willing to help during this past year with our ever-changing situation. I would also like to thank Dr. Robert Larson for his willingness to help guide me in the final phase of my degree. I cannot thank him enough for helping me with the short notice that was caused by these strange circumstances. I would also like to extend my thanks to Dr. Jason Carter and Ian Greenlund for allowing me to collaborate on their research projects so I could have a strong data set to write my thesis.

I would like to thank the members of the Clinical and Applied Human Physiology Laboratory. I would like to thank Sarah LewAllen, Jenna Disser, Madison Bramer, Thomas Basala, and everyone I have had the pleasure of working with. You all made my experience in research enjoyable and kept me motivated to continue. Finally, I would like to thank my family and friends for their support throughout my college career. I would like to thank Hannah Reish for her help keeping me on track in the final phase of my degree. This would not have been possible without them.

List of Abbreviations

AC	alcoholic cardiomyopathy
AF	atrial fibrillation
AIx	aortic augmentation index
AIx@75	aortic augmentation index normalized to 75 heart beats per minute
AS	arterial stiffness
BMI	body mass index
BrAC	breath alcohol concentration
cfPWV	carotid-femoral pulse wave velocity
CHD	coronary heart disease
CVD	cardiovascular disease
DAP	diastolic arterial pressure
ECG	electrocardiogram
ESS	Epworth sleepiness scale
HR	heart rate
ISI	insomnia severity index
LV	left ventricle
MAP	mean arterial pressure.
NO	nitric oxide
PP	pulse pressure
PSQI	Pittsburgh sleep quality index
PWA	pulse wave analysis
PWV	pulse wave velocity
SAP	systolic arterial pressure

Abstract

Acute alcohol consumption has been shown to increase blood pressure, while chronic heavy alcohol consumption is associated with the development of hypertension. Another factor that contributes to hypertension is arterial stiffness. While evidence suggests light to moderate alcohol consumption can decrease arterial stiffness, the impact of binge alcohol consumption on acute and overnight changes of arterial stiffness remains unknown. Twenty-seven participants (14 female; 13 male) received a binge alcohol dose or fluid control randomly one month apart. Alcohol or fluid control doses were administered in two equally divided allocates at 8 and 9 pm. A 1:3 mixture of 190 proof grain ethanol and fruit juice based on body weight and sex was administered for the alcohol dose (1g/kg men, 0.85g/kg women), while the fluid control was only fruit juice in equal volume. Arterial stiffness was recorded via applanation tonometry thirty minutes after the consumption of each dose and after waking the following morning. A tonometer was placed at the radial artery for pulse wave analysis. While gated to a three-lead electrocardiogram, a tonometer was placed at the carotid and femoral arteries for pulse wave velocity. Supine HR was higher after the 2nd dose of alcohol (Δ+4±2 bpm). The Fluid Control resulted in a lower supine HR after the 2nd dose (Δ-3±2 bpm) as well as in the morning (Δ-7±2 bpm). Our findings for carotid to femoral pulse wave velocity indicate that neither alcohol nor the fluid control significantly changed carotid to femoral pulse wave velocity. However, aortic augmentation index, when normalized to 75 beats per minute referred to as AIx75, was significantly decreased following the first alcohol dose (Δ-5±3%) as well as significantly increased in the morning (Δ+5±3%).

1 Literature Review

The purpose of the literature review is to first provide a brief synopsis of current knowledge of arterial stiffness, how it is measured, and how it can affect cardiovascular health. Second, it will summarize the adverse effects of alcohol on the cardiovascular system such as blood pressure, coronary heart disease, and stroke. Third, it will provide a summary of how alcohol can change arterial stiffness. Finally, it will include a short summary and hypothesis for this study.

1.1 Arterial Stiffness

Arterial stiffness (AS) refers to the physical properties of the arterial wall. These properties affect pressure, blood flow, and arterial diameter changes with each heartbeat; hence they have functional consequences for the artery. Elastin and collagen components have a substantial role in determining the pressure load of each heartbeat, with less contribution from arterial smooth muscle.

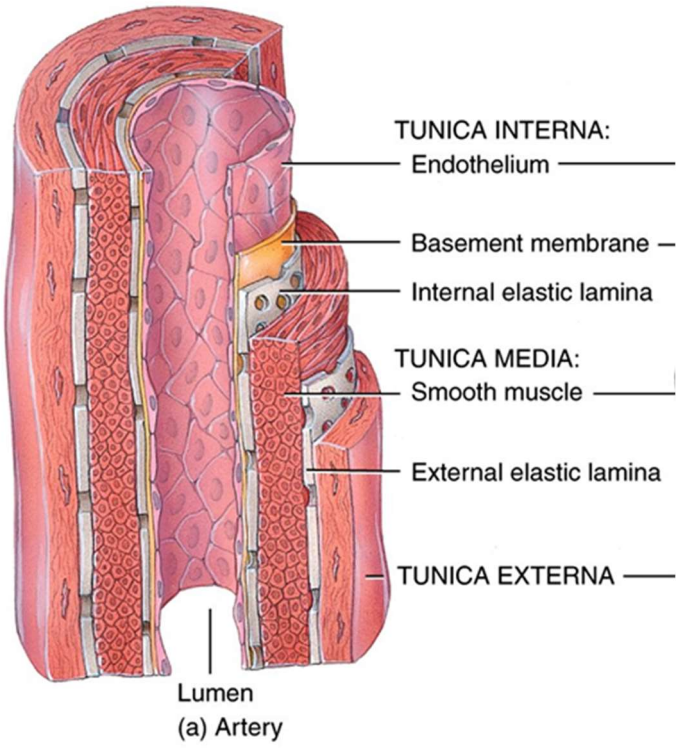

Figure 1: Arterial Structure. Image from 15th edition of Tortora and Derrickson (1).

The major factor of vascular impedance is arterial stiffness (AS). Changes in arterial pressure and blood flow are linked by vascular impedance. AS is one of the first signs of structural and functional abnormalities in the vessel wall. Pressure, flow, and vessel diameter are the primary determinants of AS and changing these variables may lead to changes in AS (2).

AIx, which calculates how much of the central pulse pressure is accounted for by the reflected pulse wave, is often used as an indirect measure of arterial stiffness. AIx is determined by arterial stiffness and the reflective properties of the arteries, which include the reflected wave's amplitude and the artery's reflectance point (3).

With age comes alterations to the cardiovascular system. Initial changes in the cardiovascular system include an increase in arterial strain and decreased distensibility due to a rise in systolic, diastolic, and mean blood pressure. These values are typically at their most extreme after the age of 50 years. These changes often lag about a decade in women compared to men. AIx, is an overall estimate of arterial stiffness that calculates how much of the central pulse pressure is accounted for by the reflected pulse wave. AIx is typically higher in women than in men and increases with age until the age of 60 years where the increase begins to level off (3).

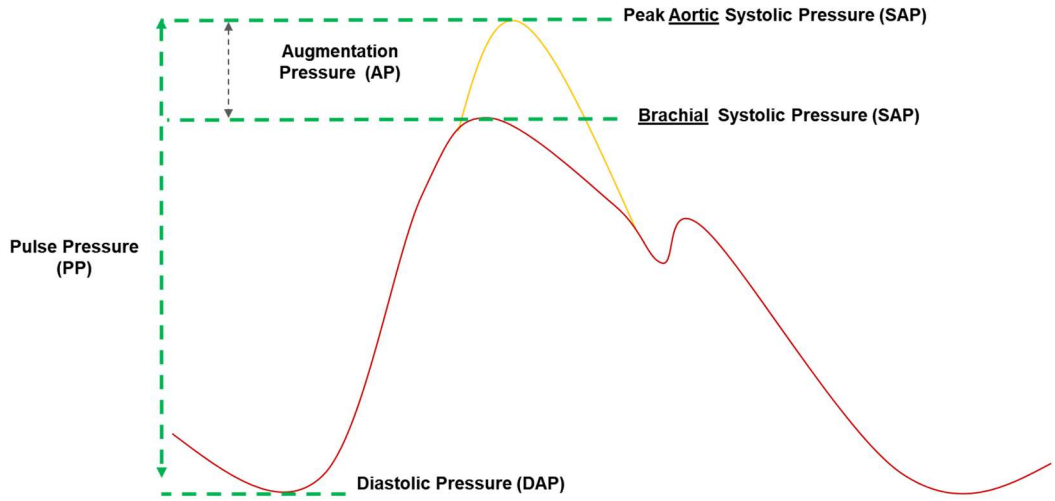

Figure 2: Aortic augmentation index (AIx). AIx is determined by arterial stiffness and wave reflection properties of the artery. It is calculated with augmentation pressure related to pulse pressure. Figure credit: Morton Harwood.

Secondary changes that begin to occur with age are aortic structural remodeling, an increase in arterial wall stiffness, and increased aortic dilatation. Aortic remodeling progresses more quickly in men than in women with a more pronounced dilatation and an increase in wall stiffness. The main manifestation of aortic remodeling is increased aortic impedance which contributes to widening pulse pressure (4).

1.1.1 Pulse Wave Analysis and Pulse Wave Velocity

The arterial pulse is an essential source of information in the clinical assessment of both cardiovascular and overall health. Only the peak and trough of the peripheral arterial pulse waveform are clinically analyzed with current standard practices of using sphygmomanometers and oscillometric devices (5). It is now possible to generate the ascending aortic pressure wave from the arterial pressure pulse noninvasively using applanation tonometry in the radial or carotid artery. Applanation tonometry pulse wave analysis, depends on radial pressure waves being found and recorded. To calibrate a tonometry device, brachial pressure is used. A computerized transfer function, or mathematical function that derives information from recorded inputs, is used to interpret an ascending aortic pressure waveform (6).

The central pulse pressure waveform can be accurately estimated, reproducibly, and noninvasively using tonometry of the radial artery. Tonometry is the "measuring of pressure or pulse", whereas applanation means "to flatten." Pulse wave analysis is performed by placing a handheld tonometer, an extremely sensitive pressure transducer, on the radial artery site and applying pressure to flatten the artery against the dense tissue underneath. The pulse waveform is transmitted from the tonometer to an analysis software which is used to derive central aortic pressure. The derivation and calculation of central pressure from a peripheral brachial blood pressure and the recording of pulse pressure with tonometry at the radial site are made possible by an algorithm (5). The most common method for measuring arterial stiffness is applanation tonometry with pulse

wave velocity, which has been well validated in large populations as a strong predictor of adverse cardiovascular outcomes (7).

Pulse wave velocity (PWV) is the gold-standard for measuring arterial stiffness. Pulse wave velocity is the speed or velocity at which a pulse from the heart travels through the circulatory system.

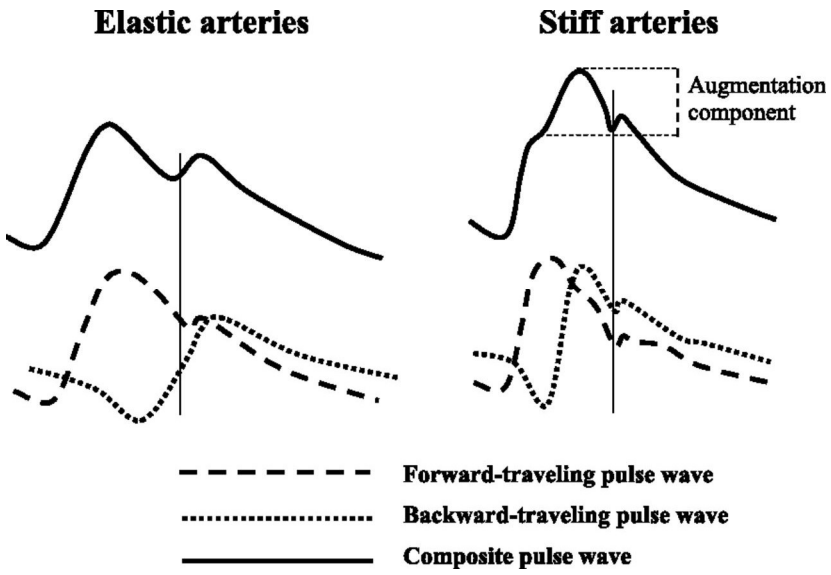

Figure 3: A comparison of elastic vs. stiff arteries. This demonstrates that pressure waves travel faster through stiffer arteries as well as stiffer arteries have higher pressure (8).

Arterial stiffness can be estimated in a non-invasive manner because increased arterial stiffness causes a higher pulse wave velocity (5). This is typically accomplished by measuring the time a pressure wave created by a heartbeat takes to reach a specific location on an artery (3). Aortic PWV is usually measured between the carotid and femoral artery. Factors that cause less distensibility, or a lack of ability to expand in response to pressure, of the vessel lead to an increased PWV (5). Atherosclerotic risk factors have been linked to vascular remodeling, which causes the aorta and other large

arteries to stiffen. The number of cardiovascular risk factors present in a patient is directly related to the increase in pulse wave velocity. In patients with various diseases that have cardiovascular implications, such as diabetes, as well as healthy elderly adults, arterial stiffness is linked to fitness, cardiovascular health, and mortality (5).

In both normotensive and hypertensive patients, an increase in PWV has been linked to the aging process, particularly after 50 years of age. An increase in AS is linked to this age-related increase in PWV. Even in healthy normotensive or hypertensive patients, this phenomenon may be responsible for an increased cardiovascular health risk (9). PWV and the aortic augmentation index (AIx) are two different measurements of arterial health that cannot be used interchangeably, despite their similarities (3).

Pulse wave velocity is often increased in patients with hypertension. How drastic of an increase in PWV depends on many parameters including baseline blood pressure, heart rate, BMI, gender, age, and smoking status. Hypertension medications may affect PWV not only by lowering blood pressure but also by improving endothelial function in the arteries. Both structural and functional changes in the artery can affect arterial stiffness (10).

PWV of the aorta is a predictor of cardiovascular events and mortality. In subjects with a higher baseline risk, arterial stiffness has a better predictive ability. As a result, PWV has the potential to be used in clinical practice as a diagnostic and preventative tool (11). Increased arterial stiffness and wave reflections are linked to impaired systolic and diastolic function in middle-aged and elderly patients. Arterial stiffness and wave

reflections may be able to provide additional information in the diagnosis and treatment of patients with heart failure (12). PWV is also associated with higher CVD mortality, CHD events, and stroke (13).

1.1.2 Arterial Stiffness and Cardiovascular Disease

The widening of pulse pressure, a decrease in arterial elasticity, and an increase in transmission pulse wave flow into the capillaries and microcirculation are all negative hemodynamic effects of stiffening the central arteries. These effects have negative consequences, which may explain why stiffness can predict cardiovascular disease risk mechanistically (2). Arterial stiffness has been linked to several cardiovascular events, including an increased risk of cardiovascular mortality, coronary heart disease, and stroke (13).

Hypertension is becoming more prevalent and common as the world's population ages. As a result, hypertension-related morbidity and mortality are on the rise. We can begin to predict cardiovascular risk and detect conditions earlier using arterial stiffness measurements (14). Arterial stiffness can be passively reduced by hypertension medication through a blood pressure dependent mechanism, where reducing blood pressure allows the arteries to reduce stiffness (15).

Arterial stiffening occurs as people age and is a result of a variety of diseases, including atherosclerosis and hypertension. Arterial stiffening is also associated with an increased risk of cardiovascular disease, such as myocardial infarction, heart failure, and stroke (16). After age 50, the link between arterial stiffness and mortality is nearly

twofold higher in women than in men (16). Reduction of arterial stiffness in these patients impacts the morbidity and mortality of older adults, as well as patients with atherosclerosis, hypertension, and disease risk. These reductions will likely improve quality of life in these populations (17). Aging causes aortic or arterial stiffening, which can be accelerated by hypertension. Reduced arterial elasticity is one of the earliest signs of negative structural and functional changes within the vessel wall (7).

Arteriosclerosis, hardening of the arterial wall, is caused by elastic lamellae within the arteries degrading over time with age and various factors. Arteriosclerosis leads to an increase in pulse pressure and systolic blood pressure. This contributes to the development of hypertension which in turn can increase arterial stiffness. Systolic hypertension is considered a consequence of the degeneration of arterial distensibility. Hypertension can hasten aortic degeneration and cause pressure wave changes like those seen with aging, but at a younger age. Increased stiffness and accelerated pulse wave velocity in the more tense aorta are responsible for this acute and reversible mechanism (6).

Pulse pressure (the difference between systolic and diastolic blood pressure), wave reflections, arterial health, and, most importantly, cardiovascular risk are all affected by increased arterial stiffness (18). This rise in pulse pressure could be linked to an increase in atherosclerosis cases. Atherosclerosis is a type of arteriosclerosis in which fatty deposits or cholesterol build up on the inner walls of the arteries. This can cause a rise in blood pressure and, as a result, a rise in arterial stiffness. Aortic pulse pressure and arterial stiffness, rather than brachial pressure, are stronger indicators of vascular

hypertrophy and the extent of carotid atherosclerosis (19). By using applanation tonometry to determine central arterial stiffness and pulse pressure we can better estimate the risk for atherosclerosis. Currently there is no known association between current alcohol intake and carotid atherosclerosis (20).

With its elasticity, the aorta limits arterial pulsatility and protects the microvasculature from potentially harmful pressure and blood flow fluctuations, also known as the Windkessel effect. This protective measure is disrupted by large artery (aortic) stiffening, which occurs with aging and various pathologic conditions, and has serious consequences for cardiovascular health (21). These consequences include hypertension, increased pressure in the capillaries and microvasculature which can lead to organ damage, and abnormal ventricular-arterial interactions that contribute to left ventricular remodeling, dysfunction, and failure. Large artery stiffness predicts cardiovascular risk independently (21). Peripheral muscular arteries are more important therapeutic targets in regressing left ventricular mass than central elastic arteries, because they are often major reflecting sites (22). Aortic pressure, rather than brachial pressure, is applied to the heart, kidneys, and major arteries supplying the brain. As a result, there is a strong case to be made that a cardiovascular event is more closely linked to central rather than brachial pressure (23).

All measures of arterial stiffness can predict increases in blood pressure in hypertensive patients. Arterial stiffness may be used as a tool to identify hypertensive patients who are at a higher risk of their hypertension worsening. Arterial tonometry is a non-invasive, low-cost test that can be done in a non-clinical setting (12, 24).

1.2 Alcohol

Alcohol is a toxin that circulates rapidly throughout the body, resulting in a variety of synchronous and synergistic effects. Alcohol consumption has been shown to lower myocardial contractility and to cause arrhythmias and dilated cardiomyopathy, which can lead to cardiovascular dysfunction and structural damage (25). Alcohol is clearly harmful to the cardiovascular system when consumed in high doses, increasing the risk of total and cardiovascular mortality, coronary and peripheral artery disease, heart failure, stroke, hypertension, dyslipidemia, and diabetes mellitus (25).

While ethanol consumption at high levels, either acutely or chronically, increases the risk of myocardial infarction and ischemic stroke, there is a consistent inverse relationship between light to moderate alcoholic beverage consumption and cardiovascular risk. The ethanol in alcoholic beverages contributes to the health benefits of moderate alcohol consumption, such as a lower risk of coronary heart disease and cardiac mortality (26, 27).

Alcohol consumption has a significant, but complex, effect on cardiovascular disease. Most major cardiovascular disease categories are negatively impacted by both irregular and chronic heavy drinking, whereas light to moderate drinking has been linked to beneficial effects on ischemic heart disease and ischemic stroke (28). The effects of alcohol on cardiovascular health vary depending on the amount and frequency of consumption. These effects are described as having a J-shaped curve, with low-to-

moderate consumption associated with lower risk than lifetime abstention, and heavy drinkers having the highest risk (29).

Light and moderate alcohol consumption may reduce the risk of death from cardiovascular disease. There is a J-shaped relationship between alcohol consumption and mortality (30). It is too early to draw any conclusions about a causal protective relationship between light to moderate alcohol consumption and mortality risk.

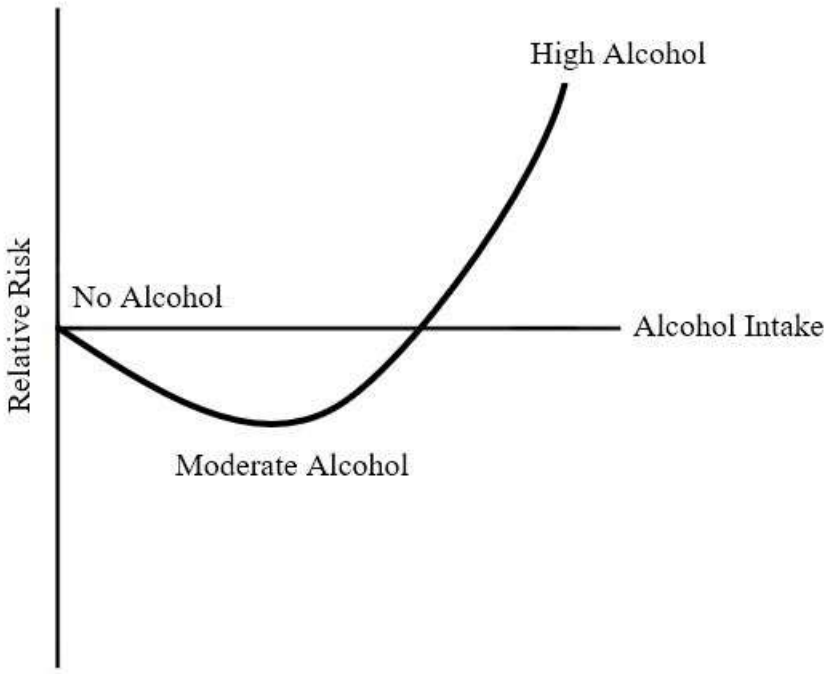

Figure 4: J-shaped curve for association of alcohol intake and risk of cardiovascular disease. Re-created based on Gardner and Mouton (31).

While moderate alcohol consumption clearly benefits the myocardium and vasculature, heavy alcohol consumption, both acute and chronic, has a negative impact

on the cardiovascular system. These negative impacts include increased blood pressure and increased sympathetic nervous system activity. Chronic heavy alcohol consumption can cause cardiac fibrosis, which exacerbates contractile dysfunction. The primary cardiac disease linked to chronic alcohol abuse is alcoholic cardiomyopathy (AC). AC is the most common cause of nonischemic dilated cardiomyopathy in the United States, and it is characterized by left-ventricular hypertrophy, chamber dilation, and a decreased ejection fraction (31). Heavy alcohol consumption, both acute and chronic, raise the risk of heart attack, stroke, and the development of arrhythmias (31). Conversely, low-to-moderate alcohol consumption is linked to a lower risk of MI, stroke, coronary artery disease, arrhythmias, and heart failure, among other cardiovascular diseases (31). Light to moderate alcohol consumption has been shown to have a clear effect on the reduction of risk factors and the prevention of cardiovascular diseases. The cardioprotective effects of moderate alcohol consumption are mediated through a variety of mechanisms (32).

1.2.1 Hypertension, Coronary Heart Disease, and Stroke

In developed countries, coronary heart disease (CHD) and stroke are the leading causes of mortality, disability, and death. Most cases of CHD are caused by atherosclerosis, a vascular degeneration process triggered by oxidative stress and chronic inflammation. Smoking, arterial hypertension, hypercholesterolemia, diabetes mellitus, obesity, lack of physical activity, and genetic factors have all been linked to an increased risk of cardiovascular disease (29). Alcohol consumption of 1 to 2 drinks per day has been linked to a lower risk of CHD. Approximately 1 to 2 drinks per day may have no

effect on stroke events or may result in a slight reduction; however, higher daily alcohol levels increase the risk of all stroke events and incident stroke types (33)

1.2.2 Myocardial and Hemostatic Effects

Alcohol, which directly affects atrial properties and contributes to hypertension and obesity, is a significant risk factor for atrial fibrillation (AF). Atrial fibrillation may be the most serious side effect of excessive alcohol consumption, especially binge drinking (33). Moderate drinking over time, as well as binge drinking, increases the risk of AF and can lead to recurrent AF in those who continue to drink. Although a small amount of alcohol is thought to be cardioprotective, it has no effect on AF (33). Reduced contractility, tachycardia, and arrhythmia are all acute effects of alcohol on the myocardium. Atrial fibrillation and, in rare cases, sudden cardiac arrest and death can result from arrythmias. Cardiac output is reduced as contractility declines. Various compensatory mechanisms, such as decreased afterload, decreased peripheral vascular resistance, and lower aortic pressure, can be used to counteract this (34).

Alcohol consumption is linked to both positive and negative hemostatic and coagulation effects. Alcohol consumption lowers coagulation factors like fibrinogen, which is a cardiovascular risk marker when levels are high. Fibrinogen, in addition to being required for the formation of clots, may play a role in the development of certain cardiovascular diseases, such as vascular wall disease and atherosclerosis, due to its inflammatory properties (33). Platelet activation and aggregation may be reduced with low or moderate alcohol consumption. Significant daily alcohol consumption, on the

other hand, increases platelet aggregation and reactivity. Rebound thrombocytosis can occur because of stressful events like heavy alcohol consumption, and it can occur spontaneously after cessation of drinking. This can happen with both heavy and one-time heavy (binge) drinking. As a result, chronic alcohol consumption may increase the risk of blood loss following vascular trauma (33).

Alcoholic cardiomyopathy is a disease that affects long-term alcoholics. A dilated left ventricle (LV), reduced ventricular wall myocardial thickness, increased ventricular mass, and a reduced LV ejection fraction are all symptoms of this condition. The amount and duration of alcohol required for the development of AC are unknown. Long-term heavy alcohol consumption alters the myocardium's histological, cellular, and structural properties (33).

1.3 Alcohol consumption and Arterial Stiffness

Men should have no more than two drinks per day, while women should have no more than one drink per day, according to the American Society of Hypertension and the International Society of Hypertension (35). One cardiovascular effect of alcohol that effects arterial stiffness is a change in vessel diameter (36). Vasodilation has very little effect on central arterial stiffness however, vasodilation can change the pattern of aortic wave reflection (37). Since the chemical properties of all alcohol is the same, the type of alcohol does not appear as important as the dose (38, 39). It is believed that this vasodilation is caused by a modulation of the central vasomotor control mechanism rather than a direct effect of alcohol (ethanol) on endothelium and vascular wall. Vasodilation is

expected to result in a reduction in arterial blood pressure and an increase in heart rate (39).

A moderate drinking habit is linked to less arterial stiffness than a heavier drinking habit. The cessation of alcohol consumption has also been linked to accelerated arterial stiffness in males over time. These findings support the idea that moderate alcohol consumption is linked to lower cardiovascular risk, but they also suggest that the strength and form of this link may vary by sex (40) as discussed in section 1.3.3.

It has also been shown that those who binge drink, or even consume a moderate amount of alcohol, have a higher mean arterial stiffness than those who abstain from alcohol (41). There is a J-shaped association between alcohol consumption and carotid arterial wall thickness and carotid to femoral-PWV values as markers of vascular structure and arterial stiffness. Chronic heavy alcohol consumption may lead to increased cfPWV and arterial wall thickness (42). Binge drinking is also associated with sympathetic activation (41).

Additionally, an increase in fluid volume acts in a similar manner to high alcohol consumption (42). This change is due to an increase in blood volume. Arterial stiffness is responsible for maintaining compliance during changes in blood volume (43). Arteries act as a buffering system to prevent arterial stress or damage. When blood volume increases arteries become more compliant leading to decrease in pressure. In cases where arterial stiffness is elevated, an increase in blood volume has a compounding effect that leads to higher blood pressure (43).

1.3.1 Dose Dependent Changes

A significant reduction in arterial stiffness is associated with mild-to-moderate alcohol consumption (44). Alcohol consumption, whether in binge doses or over a lifetime, is clearly harmful to most cardiovascular health markers, causing progressive cardiovascular dysfunction and structural damage, as well as increased morbidity and mortality. High-dose alcohol consumption increases the risk of coronary and peripheral artery disease, arrhythmias, dilated cardiomyopathy, heart failure, stroke, arterial hypertension, dyslipidemia, and diabetes mellitus (25). Starting with diastolic dysfunction and progressing to systolic dysfunction and congestive heart failure, alcoholic dilated cardiomyopathy develops in a dose-dependent manner. Alcohol consumption for the benefit of cardiovascular health is not recommended in non-consumers because of the deleterious, toxic effect of high-dose alcohol in relation to cardiovascular disease. Although low-dose alcohol lowers mortality, reducing alcohol consumption has global health benefits, and the benefits and drawbacks of low-dose alcohol should be carefully considered (25).

A single alcoholic drink can increase sympathetic nerve activity, heart rate, and cardiac output, but two drinks may decrease sympathetic nerve activity, heart rate, and cardiac output (45). Hypertension, hypertrophy, heart failure, and cardiovascular death are all linked to increased heart rate and sympathetic activity. Although the chronic effects of alcohol ingestion cannot be inferred from this acute dose-response comparison, the narrow dose-response relationships shown may resemble the J-shaped relationship between alcohol consumption and cardiovascular events seen in population studies (45).

1.3.2 Sex Differences

Arterial stiffness rises with age and is linked to a higher risk of cardiovascular disease (CVD). There are sex differences in the process of aging-related arterial stiffness changes and the risk of CVD, which has been shown to increase disproportionately in postmenopausal women (16).

A consensus has not been reached on the association between alcohol and arterial stiffness between sexes. There is a potential U-shaped relationship between alcohol consumption and arterial stiffness in women. This relationship is not mediated by cardiovascular risk factors or atherosclerosis (46). A characteristic J-curve is frequently seen in men. This suggests that women may be at a higher risk than men when they consume less alcohol. Evidence for a J-shaped relationship between alcohol consumption and aortic stiffness as measured by PWV among men over 40, with the lowest PWV values for alcoholic beverage intake among moderate drinkers (47).

On the other hand, some evidence suggests that non-drinking is associated with a higher PWV in women when compared to moderate alcohol consumption (48). In comparison to moderate drinkers, non-drinkers and heavy drinkers had significantly higher PWV. This shows a significant U-shaped relationship between alcohol consumption and PWV in men, which contradicts other findings (48). This lack of a consensus shows that further analysis is required to determine if there is a sex related difference in the effect of alcohol on pulse wave velocity. Moreover, a sex-based

association between acute alcohol consumption and pulse wave velocity needs to be investigated.

1.4 Summary and Hypothesis

One major determinant of cardiovascular risk as well as health is arterial stiffness. To better understand how cardiovascular risk and health are modified we must first determine how arterial stiffness is modified. One modifier that should be investigated further is the effect of acute alcohol consumption on pulse wave velocity and arterial stiffness. Perhaps it can be verified through research that an acute or moderate amount of alcohol consumption can be beneficial to cardiovascular health. While evidence suggests light to moderate alcohol consumption can decrease arterial stiffness, the impact of binge alcohol consumption on acute changes of arterial stiffness remains unknown (45). We hypothesized that arterial stiffness would be increased after a simulated binge drinking intervention.

2 Study

2.1 Introduction

According to the American Society of Hypertension and the International Society of Hypertension men should have, on average, no more than two drinks per day, while women should have no more than one drink per day on average (35).

Light and moderate alcohol use may reduce the risk of death from cardiovascular disease. It is thought that there is a J-shaped relationship between alcohol use and death (30). Alcohol consumption of 1 to 2 drinks per day has been linked to a lower risk of CHD. 1 to 2 drinks per day may have no effect or may result in a minor reduction in stroke events; however, higher daily alcohol levels increase the risk for all stroke events and incident stroke types (33). A moderate drinking pattern is linked to less arterial stiffness than a heavier drinking pattern. The cessation of drinking has also been linked to faster vascular stiffness in men over time (40).

Acute alcohol consumption has been shown to increase blood pressure while chronic heavy alcohol consumption is associated with the development of hypertension. Another factor that contributes to hypertension is arterial stiffness. While evidence suggests light to moderate alcohol consumption can decrease arterial stiffness, the impact of binge alcohol consumption on acute and overnight changes of arterial stiffness remains unknown (46).

These findings support the idea that moderate alcohol consumption is linked to lower cardiovascular risk, but they also suggest that the strength and form of this link may differ by gender (40). We therefore tested the hypothesis that arterial stiffness would be increased after a simulated binge drinking intervention.

2.2 Methods

Initial orientation took place in the Integrative Physiology Research Laboratory. Familiarization and testing took place in the Michigan Tech Sleep Research Laboratory in the Department of Kinesiology and Integrative Physiology at Michigan Technological University. The final four subjects in this study were tested in the Montana State University Department of Health & Human Development. Prior to any testing the Institutional Review Board at each institution approved this study. All subjects were informed about the study and its risks and signed a consent form to participate in this study.

2.2.1 Subjects

Twenty-seven individuals ranging from 21-44 years of age participated in this study. Male subjects were significantly taller (176 ± 2 vs. 164 ± 2 cm) and heavier (85 ± 3 vs. 72 ± 3 kg) than female subjects ($p<0.01$ for both), but all other anthropometric and seated resting cardiovascular data were similar between males and females as presented in Table 1.

Table 1: Subject anthropometrics and seated resting cardiovascular variables.

Variable	Male n=13	Female n=14	P-Value
Age (years)	24 ± 1	28 ± 2	0.097
BMI (kg/m^2)	28 ± 1	26 ± 1	0.383
SAP (mmHg)	112 ± 2	108 ± 2	0.229
DAP (mmHg)	73 ± 2	72 ± 2	0.806
MAP (mmHg)	86 ± 2	84 ± 2	0.513
HR (bpm)	63 ± 3	69 ± 2	0.106

Values are mean ± SE. Body mass index (BMI), systolic arterial pressure (SAP), diastolic arterial pressure (DAP), mean arterial pressure (MAP), and heart rate (HR) in beats per minute (bpm).

Volunteers were recruited primarily by email and flyer. To participate in this study, individuals were non-smokers, had a BMI of less than 35 kg/m^2, not diagnosed with diabetes. Subjects were not taking any form of cardiovascular or autonomic medication and were able to refrain from use of alcohol a minimum of 24 hours prior to testing. Female subjects were not pregnant, demonstrated regular menstrual cycles, and were not on any form of hormonal contraceptive within 6 months prior to testing. Additionally, subjects did not have moderate-to-severe alcohol use disorder and were not prone to facial flushing after 1-2 drinks. Subjects could not be diagnosed with severe sleep apnea, bullous lung disease, bypassed upper airway, pneumothorax, or hypertension. All enrolled subjects met the criteria for inclusion and were in good health. Subjects read and completed a consent form and were informed they could discontinue the study at any time with no penalty.

2.2.2 Orientation

Subjects underwent a comprehensive orientation that informed the subject of the experimental design, expectations, and risks. Potential subjects reported to the Integrative Physiology Research Laboratory or Sleep Research Laboratory where researchers provided an overview of the research study. Researchers answered any questions the potential subjects had and verified subjects met the inclusion criteria.

Following study consent, subjects filled out several surveys to evaluate subjective measures of sleep and alcohol/mental health. These surveys included DSM-V for alcohol use disorder, a flushing questionnaire, the Pittsburgh Sleep Quality Index (PSQI), Epworth Sleepiness Scale (ESS), Insomnia Severity Index (ISI), depression questionnaire, state/trait anxiety inventory, and a dietary survey for planned meals with a registered dietician. Subjects used a sleep diary leading up to the intervention.

Subjects were given an accelerometer to track their sleep-wake cycle for 3-5 nights before baseline measurements at a familiarization session. Subjects were also given a ResMed nasal cannula and finger pulse oximeter device to use at home estimate sleep apnea at night, this was used once to establish if sleep apnea was present.

2.2.3 Familiarization & Intervention

If the at-home tests showed a consistent sleep time of more than 6 hours and showed no sign of sleep apnea, subjects were scheduled for a familiarization night and two formal testing sessions. For familiarization subjects arrived at the sleep lab at 4 pm.

Familiarization did not include any alcohol or fluid control treatment. It was designed to familiarize the subjects with the PSG process, overnight beat-to-beat blood pressure recordings from the Finometer, and arterial stiffness measurements.

On the days the of interventions, subjects reported to the laboratory for a standardized breakfast at 7:30 AM. After breakfast, they were given a box lunch to eat at noon. Subjects reported to the Sleep Research Laboratory 4 or 4:30PM. Urine samples were obtained to determine specific gravity as a marker of hydration status, and breathalyzer was taken to confirm absence of recent alcohol. Female subjects also took a pregnancy test to confirm that they were not pregnant as per the inclusion criteria.

At approximately 4:10 PM, subjects had surface electrodes placed on the shoulders and the lower rib cage to record electrical activity of the heart (electrocardiogram). They were also instrumented with the finger plethysmography which combined with the electrocardiogram were used to continuously monitor beat-to-beat blood pressure. Applanation tonometry was utilized to estimate central arterial blood pressure and arterial stiffness.

Applanation tonometry recording occurred with subjects lying down in a relaxed supine position with the palm of their right hand supinated using a SphygmoCor CPVH system. Measurements were collected for PWA, AIx, and AIx@75 by placing the SphygmoCor tonometry probe directly on the radial artery and flattening the artery against the dense underlying connective tissue and bone. Recordings were taken for at least 10 full cardiac cycles to ensure an accurate waveform was recorded. Acceptable

recordings required an operator index of 75 or higher on the SphygmoCor software. Duplicate recordings were taken and averaged for analysis.

When testing resumed at Montana State University, applanation tonometry measures were taken using the SphygmoCor XCEL system. This system uses a standard brachial blood pressure cuff to estimate the central aortic pressure waveform. PWA measures use a standard brachial cuff to measure blood pressure and capture a brachial waveform. This waveform is then used to estimate the aortic waveform as well as AIx. PWV is measured using a carotid tonometer, like the standard SphygmoCor system, simultaneously with a leg pressure cuff to capture pressure waveforms at the carotid and femoral sites. The distance between pulse sites is measured and used to calculate the velocity of the pressure wave, and the suprasternal notch is used to estimate the location of the aorta.

Carotid femoral pulse wave velocity (cfPWV), an estimate of central arterial stiffness, were recorded using the femoral artery and carotid artery sites. These sites were palpated and marked with tape or a marker dot, respectively. The straight-line distance of each of these sites relative to the suprasternal notch of the sternum was measured and recorded to the closest millimeter. The three electrodes used for the electrocardiogram were re-used for recording R waves. These recorded R-waves were gated to the pulses recorded at the carotid and femoral sites to calculate the time it takes a pulse wave to arrive at each specified site. PWV was determined simply by the time it takes a pulse to reach each of the sites. Data were deemed acceptable if the recordings between the carotid and femoral sites were within 1 m/s of each other and the standard deviation of

each recording was less than 10%. Following applanation tonometry, a blood draw was performed by a registered nurse. Blood draws were performed after the baseline measures, after the 2nd dose of alcohol, and in the morning. The blood was stored in a freezer until it was sent to Quest diagnostics for analysis.

Dinner was provided at approximately 5:00 PM. At 7:00 PM subjects completed a brief series of questionnaires and psychomotor performance tests as reported in Greenlund et al (49). At 7:30 PM, quiet resting baseline measurement with the ECG and finger plethysmography were performed.

At 8:00 PM, subjects began the beverage consumption protocol. Oral alcohol (190-proof ethanol) was given in two divided doses of 1.0 g/kg of body weight for men, administered at 1-hour intervals (8 and 9 PM). Women received a reduced dose (.85 g/kg) to account for the reduced capacity of women to metabolize alcohol compared to men, and so that women would achieve a similar breath alcohol concentration to the BrAC in men. The alcohol beverage was diluted in a 1:3 mixture with cranberry juice or orange juice, depending on the subject's preference. The fluid control session consisted of the cranberry or orange juice plus 1% alcohol added as a taste mask, in the same volume. All beverages were sprayed with an alcohol mist that has a strong alcoholic scent to ensure consistency between the placebo and alcohol dose. Beverages were administered double blind and served in opaque lidded cups. At both times, the drinks were divided into thirds, and subjects had 5 min to consume each drink. The breathalyzer was administered in 15 min intervals from 8 to 11 PM. Arterial stiffness and cfPWV measurements were repeated in 1-hour intervals at 8:45 and 9:45 PM. At 9:15 PM, subjects were

instrumented with PSG. Subjects repeated the breath alcohol content (BrAC) measures, affective questionnaires, and psychomotor performance tests at 10:00 PM.

At 10:45 PM, subjects were instrumented with end-tidal and transcutaneous CO2 monitors via nasal cannula and digital skin sensor, respectively. Subjects rested quietly in bed, with lights-out at 11:00 PM. From 11:00 PM to 7:00 AM, subjects were continuously recorded via PSG, finger plethysmography, end-tidal/transcutaneous CO2, and electrocardiogram.

Subjects were woken up at 7:00 AM, and blood alcohol content was assessed via breathalyzer and venous blood sample, specific gravity via urine sample, and vascular stiffness via applanation tonometry. PSG de-instrumentation followed the morning urine sample. The arterial stiffness measures began at approximately 7:15 AM. At 7:30 AM, affective questionnaires and psychomotor performance tests were assessed, after which subjects participated in an autonomic function test. Additional information on polysomnography and autonomic function testing can be found in the protocols by Greenlund et al. (50)

2.2.4 Statistical Analysis

Statistical data analyses were completed using SPSS. Repeated measures analysis of variance (ANOVA) were conducted to assess changes in all cardiovascular variables at four-time intervals: pre-supper baseline, post drink 1, post drink 2, and morning. These variables include SAP, DAP, HR, MAP, PP, AIx, AIx@75, and cfPWV. We first checked that each of these variables had a normal distribution. Mauchly's Test of

Sphericity was performed for each variable to determine whether sphericity could be assumed. When sphericity had been violated, the Greenhouse-Geisser method was used to determine the adjusted p-value. Additionally, post-hoc paired t-tests were performed for variables that had a significant change based on their ANOVA testing. Data were considered significantly different when $p<0.05$.

2.3 Results

Supine systolic arterial pressure as well as diastolic arterial pressure did not significantly change within any subject effect (Table 2).

Table 2: Average supine arterial pressure (SAP), diastolic arterial pressure (DAP), and mean arterial pressure (MAP). No significant changes were seen at any time interval.

Variable	Condition	Pre-Supper	Post Dose 1	Post Dose 2	Morning
SAP (mmHg)	BA	111±2	112±2	110±2	112±2
	FC	112±2	112±2	113±2	112±2
DAP (mmHg)	BA	62±2	62±2	60±1	64±1
	FC	62±2	63±2	64±2	63±2
MAP (mmHg)	BA	78±2	79±2	77±2	80±2
	FC	79±1	79±1	77±3	77±3

Supine HR (bpm) was significantly changed within each condition. Supine HR was significantly decreased after the second fluid control dose ($P<0.01$), as well as the morning after the fluid control ($P<0.01$) when compared to the pre-supper baseline.

Supine HR was increased significantly at the third time period or after the 2nd dose of alcohol (P<0.01) but was not significantly changed in the morning.

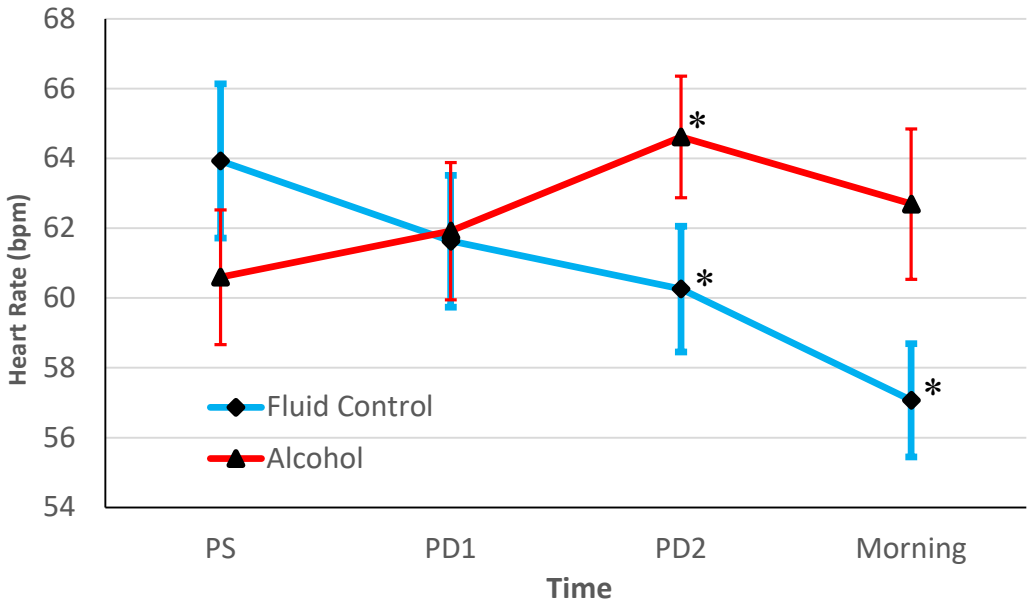

Figure 5: Supine heart rate (HR) responses. *P<0.05 vs. the pre-supper baseline for that condition.

Aortic augmentation index normalized to 75 beats per minute showed a significant time effect. AIx@75 was significantly decreased following the first alcohol dose (PD1) (P=0.015) and significantly increased in the morning after the alcohol intervention (P<0.01) as shown in Figure 3. There was no change in AIx@75 from baseline values with the fluid control.

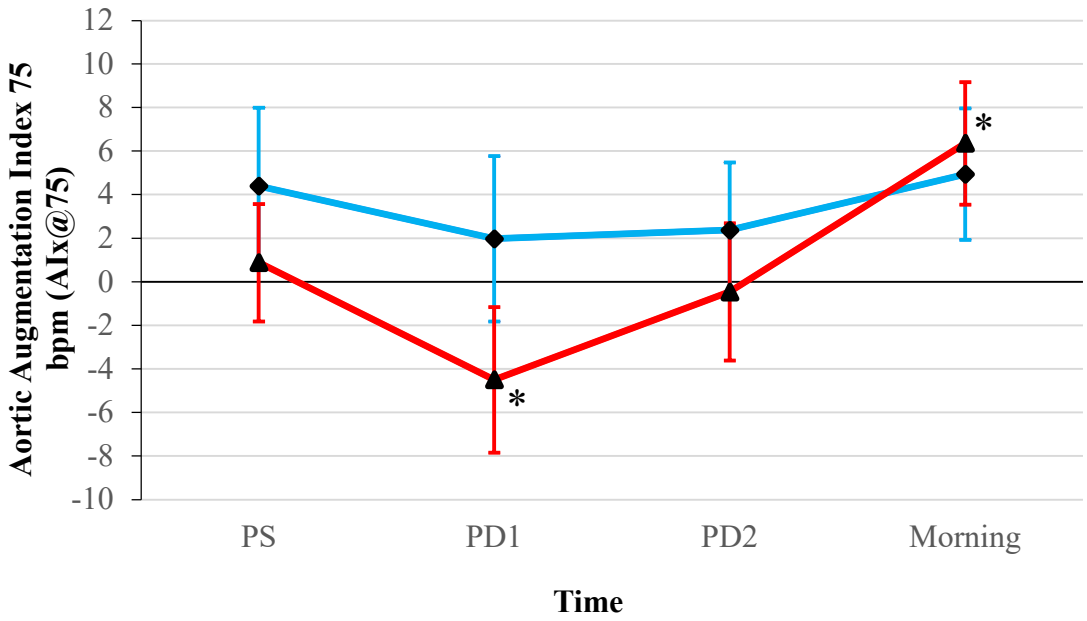

Figure 6: Aortic Augmentation Index (AIx@75) responses. *P<0.05 vs. the pre-supper baseline for that condition.

There was no significant change in cfPWV as shown below in Figure 7 (time x condition P=0.054).

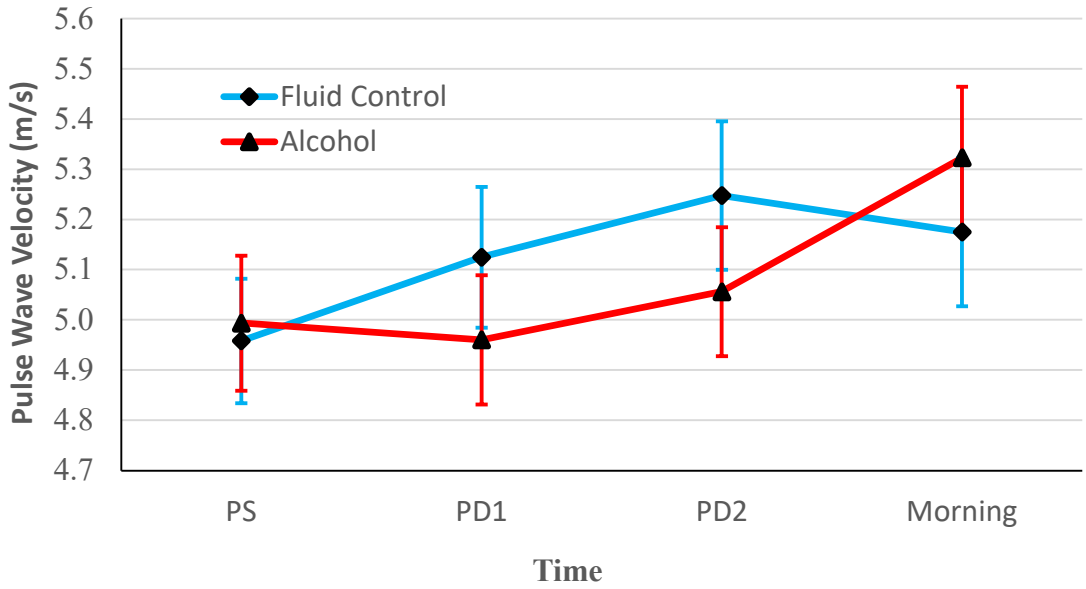

Figure 7: Carotid femoral pulse wave velocity (cfPWV) responses.

2.4 Discussion

To our knowledge, this study is the first to examine the acute and overnight cardiovascular implications of binge alcohol consumption. Blood pressure remained unchanged, but supine heart rate was significantly decreased after administration of the 2nd fluid control dose as well as in the morning after the fluid control. Supine HR was increased significantly after the 2nd dose of alcohol. Aortic augmentation index normalized to 75 beats per minute was significantly decreased following the first dose of alcohol and significantly increased in the morning after the alcohol intervention. No significant change was detected for cfPWV.

Previous studies suggest that binge alcohol consumption is associated with transient increases in blood pressure by as much as 7 mmHg (33). This change in blood pressure is seen in people who show chronic binge drinking habits. Previous research suggests that in healthy adults with low to moderate alcohol consumption habits, blood pressure changes may not occur with acute alcohol consumption (33, 51). This is supported by our study because blood pressure values did not significantly change after the simulated binge drinking session in our young healthy adult subjects.

The observed decrease in supine heart rate during the fluid control intervention aligns with previous research that after increased fluid consumption heart rate often decreases (31, 33) due to blood volume increasing. When blood volume is increased through excess fluid intake the heart compensates by reducing heart rate to maintain a relatively constant cardiac output. After the 2nd dose of alcohol, heart rate was increased.

This supports previous research that alcohol consumption can increase heart rate. When alcohol is consumed it can cause a negative inotropic effect on the heart leading to a decrease in cardiac output (33). This can be compensated for by increasing heart rate.

Aortic augmentation index normalized to 75 beats per was significantly decreased following the first alcohol dose and significantly increased in the morning after the alcohol intervention. When comparing AIx@75 between alcohol abstainers and binge drinkers a significant decrease has been observed in binge drinkers (41). Chronic drinkers on the other hand show a significant increase in AIx@75 compared to both those who occasionally binge drink and those who abstain from alcohol (33, 41). This pattern is consistent with the J-shaped association previously established between alcohol and cardiovascular health.

No significant change in cfPWV was detected, however there may be a notable effect with a larger sample size. Chronic heavy alcohol consumption may lead to increased cfPWV and arterial wall thickness (42). Additionally, those who abstain from alcohol have shown lower carotid to femoral pulse wave velocity compared to those who binge drink or are chronic drinkers (41). While our findings are not conclusive on this matter our data suggests that binge drinking may have a similar effect to chronic drinking when it comes to pulse wave velocity in the short term. This further indicates that there may be more negative cardiovascular implications of binge drinking than originally thought and more research is required.

One potential mechanism that may explain how alcohol is effecting arterial stiffness is the mechanism by which nitric oxide (NO) effects arteries (52). NO is produced by an

enzyme nitric oxide synthase. NO can cause vasodilatation, increased blood flow, lowered blood pressure, and aids in the prevention of atherosclerosis and other vascular issues (52). When alcohol is ingested in small quantities, alcohol increases the production of nitric oxide synthase leading to higher amounts of free NO in the blood reducing blood pressure and causing vasodilation. At higher doses, alcohol has the opposite effect and reduce the level of NO or inhibit NO receptors. This leads to higher pressure and vasodilation does not occur (52). This may be the mechanism responsible for the changing AIx values occurring in our study. After the first dose of alcohol, AIx is lowered while higher doses of alcohol show AIx returning to near its baseline levels after the second dose of alcohol.

3 Summary, Limitations, and Future Directions

3.1 Summary

Our results suggest that binge alcohol consumption attenuated AIx@75 after the first dose in the evening, increased supine HR after the second dose, and increased AIx@75 in the morning. Additionally, carotid to femoral pulse wave velocity did not show significant changes with the current sample size. Our preliminary findings align with evidence on chronic drinkers and suggest that acute arterial stiffness and cardiovascular responses to alcohol are dose dependent and suggest that binge drinking 2 to 2.5 drinks and having a BrAC of around 0.045 can have short term beneficial effects on AIx75.

3.2 Limitations

While this research provides novel insights and considerations for binge alcohol consumption and cardiovascular health, there are some limitations associated with this study. First, a sample size of less than 30 subjects may limit the current results. Additionally, the absence of a 24-48 hour follow up may be a limitation. Having data on the longitudinal effects of a binge drinking session may bring further insight to the interaction between alcohol and the cardiovascular system. Due to the increase observed in AIx@75 the morning after a binge drinking session it may be beneficial to have a repeat measure of all hemodynamic variables (AIx@75, BP, HR, etc.) 24-48 hours after the morning recordings. Lastly, this study's cohort was comprised of mostly college-aged healthy adults. This limits the scope of our study in determining if binge alcohol

consumption would have the same effect in older individuals who are more prone to arterial stiffness due to more advanced age.

3.3 Future Directions

There are a variety of directions that this research can expand upon in the future. A larger sample size would be beneficial in determining if there is a significant change to cfPWV as related to binge alcohol consumption. A larger sample size may also help to determine if there are any possible sex differences. Additionally, it may be beneficial to examine if the type of alcohol used may influence cardiovascular health. While previous research shows there is not a difference between types of alcoholic beverages, these studies were conducted longitudinally and not in short binge drinking sessions (26, 27, 38). Lastly, it may be beneficial to test hemodynamics as well as arterial stiffness measures again after the 24- and 48-hour period post binge drinking. While this study shows an immediate effect as well as some morning aftereffects, it is unclear how long these effects remain.

4 References

1. **Tortora GJ, and Derrickson BH**. *Principles of Anatomy and Physiology 15th Edition*. Wiley, 2016.
2. **Townsend RR, Wilkinson IB, Schiffrin EL, Avolio AP, Chirinos JA, Cockcroft JR, Heffernan KS, Lakatta EG, McEniery CM, Mitchell GF, Najjar SS, Nichols WW, Urbina EM, Weber T, and American Heart Association Council on H**. Recommendations for Improving and Standardizing Vascular Research on Arterial Stiffness: A Scientific Statement From the American Heart Association. *Hypertension* 66: 698-722, 2015.
3. **Janner J, Godtfredsen N, Ladelund S, Vestbo J, and Prescott E**. Aortic augmentation index: reference values in a large unselected population by means of the SphygmoCor device. *American Journal of Hypertension* 23: 180-185, 2010.
4. **AlGhatrif M, and Lakatta EG**. The conundrum of arterial stiffness, elevated blood pressure, and aging. *Curr Hypertens Rep* 17: 12, 2015.
5. **Nelson MR, Stepanek J, Cevette M, Covalciuc M, Hurst RT, and Tajik AJ**. Noninvasive measurement of central vascular pressures with arterial tonometry: clinical revival of the pulse pressure waveform? *Mayo Clin Proc* 85: 460-472, 2010.
6. **O'Rourke MF, Pauca A, and Jiang XJ**. Pulse Wave Analysis. *British Journal of Clinical Pharmacology* 51: 507-522, 2001.
7. **Cavalcante JL, Lima JA, Redheuil A, and Al-Mallah MH**. Aortic stiffness: current understanding and future directions. *J Am Coll Cardiol* 57: 1511-1522, 2011.
8. **Karpetas A, Sarafidis PA, Georgianos PI, Protogerou A, Vakianis P, Koutroumpas G, Raptis V, Stamatiadis DN, Syrganis C, Liakopoulos V, Efstratiadis G, and Lasaridis AN**. Ambulatory recording of wave reflections and arterial stiffness during intra- and interdialytic periods in patients treated with dialysis. *Clin J Am Soc Nephrol* 10: 630-638, 2015.
9. **Diaz A, Tringler M, Wray S, Ramirez AJ, and Cabrera Fischer EI**. The effects of age on pulse wave velocity in untreated hypertension. *J Clin Hypertens (Greenwich)* 20: 258-265, 2018.
10. **Lovic D, Kallistratos MS, Poulimenos L, Skyrlas A, Stojanov V, Jakovljevic B, Zdravkovic M, Koracevic G, Chamodraka E, Kouremenos N, Pittaras A, Kokkinos P, Tsinivizov P, and Manolis A**. Pulse Wave Velocity: Retrospective Analysis in a Balkan Normotensive and Hypertensive Population: A Study of 9923 Patients. *Angiology* 69: 59-64, 2018.
11. **Vlachopoulos C, Aznaouridis K, and Stefanadis C**. Prediction of cardiovascular events and all-cause mortality with arterial stiffness: a systematic review and meta-analysis. *J Am Coll Cardiol* 55: 1318-1327, 2010.
12. **Weber T, O'Rourke MF, Ammer M, Kvas E, Punzengruber C, and Eber B**. Arterial stiffness and arterial wave reflections are associated with systolic and diastolic function in patients with normal ejection fraction. *Am J Hypertens* 21: 1194-1202, 2008.
13. **Sutton-Tyrrell K, Najjar SS, Boudreau RM, Venkitachalam L, Kupelian V, Simonsick EM, Havlik R, Lakatta EG, Spurgeon H, Kritchevsky S, Pahor M, Bauer D, Newman A, and Health ABCS**. Elevated aortic pulse wave velocity, a marker of

arterial stiffness, predicts cardiovascular events in well-functioning older adults. *Circulation* 111: 3384-3390, 2005.
14. **Dumor K, Shoemaker-Moyle M, Nistala R, and Whaley-Connell A**. Arterial Stiffness in Hypertension: an Update. *Curr Hypertens Rep* 20: 72, 2018.
15. **Zanoli L, Lentini P, Briet M, Castellino P, House AA, London GM, Malatino L, McCullough PA, Mikhailidis DP, and Boutouyrie P**. Arterial Stiffness in the Heart Disease of CKD. *J Am Soc Nephrol* 30: 918-928, 2019.
16. **DuPont JJ, Kenney RM, Patel AR, and Jaffe IZ**. Sex differences in mechanisms of arterial stiffness. *Br J Pharmacol* 176: 4208-4225, 2019.
17. **Zieman SJ, Melenovsky V, and Kass DA**. Mechanisms, pathophysiology, and therapy of arterial stiffness. *Arterioscler Thromb Vasc Biol* 25: 932-943, 2005.
18. **Safar ME**. Arterial stiffness as a risk factor for clinical hypertension. *Nat Rev Cardiol* 15: 97-105, 2018.
19. **Roman MJ, Devereux RB, Kizer JR, Lee ET, Galloway JM, Ali T, Umans JG, and Howard BV**. Central pressure more strongly relates to vascular disease and outcome than does brachial pressure: the Strong Heart Study. *Hypertension* 50: 197-203, 2007.
20. **Demirovic J, Nabulsi A, Folsom A, Carpenter M, Szklo M, Sorlie P, and Barnes R**. Alcohol Consumption and Ultrasonographically Assessed Carotid Artery Wall Thickness and Distensibility. *Circulation* 88: 2787-2793, 1993.
21. **Chirinos JA, Segers P, Hughes T, and Townsend R**. Large-Artery Stiffness in Health and Disease: JACC State-of-the-Art Review. *J Am Coll Cardiol* 74: 1237-1263, 2019.
22. **Hashimoto J, Westerhof B, Westerhof N, Imai Y, and O'Rourke MF**. Different role of wave reflection magnitude and timing on left ventricular mass reduction during antihypertensive treatment. *Journal of Hypertension* 26: 1017-1024, 2008.
23. **McEniery CM, Cockcroft JR, Roman MJ, Franklin SS, and Wilkinson IB**. Central blood pressure: current evidence and clinical importance. *Eur Heart J* 35: 1719-1725, 2014.
24. **Coutinho T, Bailey KR, Turner ST, and Kullo IJ**. Arterial stiffness is associated with increase in blood pressure over time in treated hypertensives. *J Am Soc Hypertens* 8: 414-421, 2014.
25. **Fernandez-Sola J**. Cardiovascular risks and benefits of moderate and heavy alcohol consumption. *Nat Rev Cardiol* 12: 576-587, 2015.
26. **Krenz M, and Korthuis RJ**. Moderate ethanol ingestion and cardiovascular protection: from epidemiologic associations to cellular mechanisms. *J Mol Cell Cardiol* 52: 93-104, 2012.
27. **Ronksley PE, Brien SE, Turner BJ, Mukamal KJ, and Ghali WA**. Association of alcohol consumption with selected cardiovascular disease outcomes: a systematic review and meta-analysis. *BMJ* 342: d671, 2011.
28. **Rehm J, and Roerecke M**. Cardiovascular effects of alcohol consumption. *Trends Cardiovasc Med* 27: 534-538, 2017.
29. **Minzer S, Losno RA, and Casas R**. The Effect of Alcohol on Cardiovascular Risk Factors: Is There New Information? *Nutrients* 12: 2020.

30. **Braillon A**. Alcohol: Cardiovascular Disease and Cancer. *J Am Coll Cardiol* 71: 582-583, 2018.
31. **Gardner JD, and Mouton AJ**. Alcohol effects on cardiac function. *Compr Physiol* 5: 791-802, 2015.
32. **Castaldo L, Narvaez A, Izzo L, Graziani G, Gaspari A, Minno GD, and Ritieni A**. Red Wine Consumption and Cardiovascular Health. *Molecules* 24: 2019.
33. **Piano MR**. Alcohols Effects on the cardiovascular system. *Alcohol Research: Current Reviews* 38: 23, 2017.
34. **Voskoboinik A, Prabhu S, Ling LH, Kalman JM, and Kistler PM**. Alcohol and Atrial Fibrillation: A Sobering Review. *J Am Coll Cardiol* 68: 2567-2576, 2016.
35. **Day E, and Rudd JHF**. Alcohol use disorders and the heart. *Addiction* 114: 1670-1678, 2019.
36. **Dengerink H, Mead J, and Bertilson H**. Individual differences in response to alcohol. Vasoconstriction and vasodilation. *Journal of Studies on Alcohol* 39: 12-18.
37. **Nichols WW**. Clinical measurement of arterial stiffness obtained from noninvasive pressure waveforms. *Am J Hypertens* 18: 3S-10S, 2005.
38. **Arranz S, Chiva-Blanch G, Valderas-Martinez P, Medina-Remon A, Lamuela-Raventos RM, and Estruch R**. Wine, beer, alcohol and polyphenols on cardiovascular disease and cancer. *Nutrients* 4: 759-781, 2012.
39. **Krnic M, Modun D, Budimir D, Gunjaca G, Jajic I, Vukovic J, Salamunic I, Sutlovic D, Kozina B, and Boban M**. Comparison of acute effects of red wine, beer and vodka against hyperoxia-induced oxidative stress and increase in arterial stiffness in healthy humans. *Atherosclerosis* 218: 530-535, 2011.
40. **O'Neill D, Britton A, Brunner EJ, and Bell S**. Twenty-Five-Year Alcohol Consumption Trajectories and Their Association With Arterial Aging: A Prospective Cohort Study. *J Am Heart Assoc* 6: 2017.
41. **Hwang CL, Piano MR, Thur LA, Peters TA, da Silva ALG, and Phillips SA**. The effects of repeated binge drinking on arterial stiffness and urinary norepinephrine levels in young adults. *J Hypertens* 38: 111-117, 2020.
42. **Gonzalez-Sanchez J, Garcia-Ortiz L, Rodriguez-Sanchez E, Maderuelo-Fernandez JA, Tamayo-Morales O, Lugones-Sanchez C, Recio-Rodriguez JI, Gomez-Marcos MA, and Investigators EVA**. The Relationship Between Alcohol Consumption With Vascular Structure and Arterial Stiffness in the Spanish Population: EVA Study. *Alcohol Clin Exp Res* 44: 1816-1824, 2020.
43. **Avolio A**. Arterial Stiffness. *Pulse (Basel)* 1: 14-28, 2013.
44. **Nishiwaki M, Yamaguchi T, Nishida R, and Matsumoto N**. Dose of Alcohol From Beer Required for Acute Reduction in Arterial Stiffness. *Front Physiol* 11: 1033, 2020.
45. **Spaak J, Merlocco AC, Soleas GJ, Tomlinson G, Morris BL, Picton P, Notarius CF, Chan CT, and Floras JS**. Dose-related effects of red wine and alcohol on hemodynamics, sympathetic nerve activity, and arterial diameter. *Am J Physiol Heart Circ Physiol* 294: H605-612, 2008.
46. **Mattace-Raso F, Van Der Cammen T, Van Der Elzen A, Schalekamp M, Asmar R, Reneman R, Hoeks A, Hofman A, and Witteman J**. Moderate Alcohol

Consumption Is Associated With Reduced Arterial Stiffness in Older Adults: The Rotterdam Study. *The Journals of Gerontology Series A: Biological Sciences and Medical Sciences* 60: 1479-1483, 2005.
47. **Sierksma A, Muller M, Van Der Schouw Y, Grobbee D, Hendricks H, and Bots M**. Alcohol consumption and arterial stiffness in men. *Journal of Hypertension* 22: 357-362, 2004.
48. **Sasaki S, Yoshioka E, Saijo Y, Kita T, Okada E, Tamakoshi A, and Kishi R**. Relation between alcohol consumption and arterial stiffness: A cross-sectional study of middle-aged Japanese women and men. *Alcohol* 47: 643-649, 2013.
49. **Greenlund IM, Cunningham HA, Tikkanen AL, Bigalke JA, Smoot CA, Durocher JJ, and Carter JR**. Morning Sympathetic Activity after Evening Binge Alcohol Consumption. *American Journal of Physiology* 320: 2020.
50. **Greenlund IM, Bigalke JA, Tikkanen AL, Durocher JJ, Smoot CA, and Carter JR**. Evening Binge Alcohol Disrupts Cardiovagal Tone and Baroreflex Function During Polysomnographic Sleep. *Sleep* 2021.
51. **Carter JR, Stream SF, Durocher JJ, and Larson RA**. Influence of acute alcohol ingestion on sympathetic neural responses to orthostatic stress in humans. *Am J Physiol Endocrinol Metab* 300: E771-778, 2011.
52. **Toda N, and Ayajiki K**. Vascular actions of nitric oxide as affected by exposure to alcohol. *Alcohol Alcohol* 45: 347-355, 2010.

A Appendix A: Raw Data

A.1 Demographic Information

Subject Number	Sex	Height	Weight	BMI	Age
1	Male	182.9	89.5	26.8	21
2	Male	160	88.6	34.6	26
3	Male	172.7	84.7	28.4	22
4	Female	167.64	66.67	23.7	22
5	Female	178	80.9	25.5	22
6	Female	154.94	75.29	31.4	24
7	Male	175.26	73.94	24.1	22
8	Male	180.34	73.03	22.5	34
9	Female	158.75	59.87	23.8	22
10	Male	180	71	21.9	23
11	Male	186.69	98.88	28.4	22
12	Male	181.61	110.7	33.6	21
13	Male	172.7	74.55	25.0	22
14	Female	157.48	55.79	22.5	22
15	Female	177.8	82.73	26.2	21
16	Male	185.42	91.36	26.6	22
17	Male	175.26	93.6	30.5	26
18	Female	165.1	87.73	32.2	37
19	Female	152.4	45.91	19.8	21
20	Female	165.1	64.09	23.5	38

21	Female	165.1	84.09	30.8	21
22	Female	163.2	79.09	29.7	29
23	Female	165.1	75	27.5	28
24	Female	165	72	26.4	44
25	Male	171	83.5	28.6	23
26	Male	159	74	29.3	22
27	Female	164	73	27.1	36

A.2 Pre-Supper Brachial Hemodynamics; Fluid Control

Subject Number	SAP (mmHg)	DAP (mmHg)	Supine HR (bpm)	MAP (mmHg)	Aortic PP (mmHg)
1	107	48	50	67.7	37.5
2	111	65	63	80.3	26.5
3	115	67	80	83.0	27.5
4	97	54	45	68.3	26.5
5	110	67	54	81.3	28.0
6	106	77	85	86.7	22.0
7	125	62	62	83.0	38.0
8	104	51	45	68.7	34.5
9	116	70	67	85.3	32.0
10	111	55	64	73.7	35.0
11	124	57	62	79.3	43.0
12	121	66	63	84.3	35.5
13	118	64	73	82.0	33.0
14	109	56	61	73.7	39.5
15	114	60	43	78	31.0
16	109	53	45	71.7	36.5
17	112	52	58	72	35.5
18	98	59	57	72	29.0
19	112	62	72	78.7	34.0
20	131	84	67	99.7	38.5

21	108	57	75	74.0	29.5
22	99	69	73	79.0	22.5
23	114	70	72	84.7	25.5
24	113	73	72	86.3	29.5
25	117	64	79	81.7	34.5
26	99	62	73	74.3	26.5
27	121	71	66	87.7	37.5

A.3 Post Dose One Brachial Hemodynamics; Fluid Control

Subject Number	SAP (mmHg)	DAP (mmHg)	Supine HR (bpm)	MAP (mmHg)	Aortic PP (mmHg)
1	110.0	42.0	52.0	64.7	.
2	109.0	62.0	52.0	77.7	29.0
3	111.0	62.0	63.0	78.3	.
4	104.0	65.0	57.0	78.0	26.0
5	100.0	61.0	55.0	74.0	.
6	109.0	81.0	83.0	90.3	20.5
7	142.0	73.0	73.0	96.0	39.0
8	105.0	55.0	46.0	71.7	29.0
9	111.0	66.0	54.0	81.0	35.0
10	106.0	58.0	51.0	74.0	29.0
11	133.0	61.0	64.0	85.0	47.5
12	117.0	65.0	63.0	82.3	32.0
13	114.0	62.0	64.0	79.3	31.0
14	112.0	60.0	57.0	77.3	38.0
15	108.0	53.0	47.0	71.3	32.5
16	110.0	54.0	48.0	72.7	37.5
17	115.0	65.0	57.0	81.7	28.0
18	93.0	56.0	63.0	68.3	26.5
19	108.0	59.0	64.0	75.3	33.0
20	127.0	79.0	55.0	95.0	41.5
21	105.0	61.0	73.0	75.7	25.5

22	101.0	69.0	66.0	79.7	24.0
23	113.0	66.0	72.0	81.7	27.5
24	116.0	77.0	75.0	90.0	25.5
25	109.0	61.0	76.0	77.0	35.5
26	102.0	63.0	72.0	76.0	26.0
27	121.0	71.0	62.0	87.7	42.5

A.4 Post Dose Two Brachial Hemodynamics; Fluid Control

Subject Number	SAP (mmHg)	DAP (mmHg)	Supine HR (bpm)	MAP (mmHg)	Aortic PP (mmHg)
1	111.0	43.0	50.0	65.7	41.0
2	106.0	60.0	56.0	75.3	27.0
3	117.0	67.0	62.0	83.7	28.5
4	106.0	63.0	55.0	77.3	27.5
5	115.0	73.0	53.0	87.0	27.0
6	109.0	79.0	80.0	89.0	22.5
7	126.0	67.0	67.0	86.7	36.0
8	106.0	59.0	46.0	74.7	28.5
9	115.0	74.0	65.0	87.7	29.5
10	112.0	58.0	53.0	76.0	33.0
11	132.0	63.0	64.0	86.0	43.5
12	129.0	70.0	62.0	89.7	33.0
13	107.0	59.0	63.0	75.0	29.5
14	107.0	50.0	58.0	69.0	40.0
15	102.0	53.0	45.0	69.3	31.0
16	109.0	51.0	45.0	70.3	41.0
17	116.0	60.0	56.0	78.7	31.0
18	102.0	64.0	59.0	76.7	28.5
19	111.0	59.0	59.0	76.3	34.0
20	134.0	85.0	53.0	101.3	44.0
21	96.0	58.0	76.0	70.7	21.0

22	103.0	70.0	70.0	81.0	25.5
23	112.0	70.0	59.0	84.0	28.5
24	110.0	73.0	69.0	85.3	27.5
25	112.0	64.0	79.0	80.0	32.5
26	106.0	66.0	63.0	79.3	29.5
27	132.0	74.0	60.0	93.3	40.5

A.5 Morning Brachial Hemodynamics; Fluid Control

Subject Number	SAP (mmHg)	DAP (mmHg)	Supine HR (bpm)	MAP (mmHg)	Aortic PP (mmHg)
1	109.0	46.0	47.0	67.0	36.5
2	106.0	70.0	60.0	82.0	22.0
3	111.0	71.0	55.0	84.3	25.0
4	104.0	51.0	48.0	68.7	36.0
5	109.0	68.0	63.0	81.7	27.0
6	101.0	69.0	65.0	79.7	27.5
7	120.0	58.0	53.0	78.7	38.5
8	108.0	55.0	48.0	72.7	32.0
9	102.0	60.0	49.0	74.0	30.0
10	115.0	57.0	47.0	76.3	41.0
11	130.0	62.0	60.0	84.7	42.0
12	126.0	70.0	58.0	88.7	33.5
13	112.0	70.0	59.0	84.0	30.0
14	102.0	53.0	58.0	69.3	36.0
15	115.0	60.0	46.0	78.3	33.5
16	98.0	46.0	41.0	63.3	34.0
17	118.0	63.0	52.0	81.3	37.5
18	104.0	65.0	61.0	78.0	30.0
19	116.0	64.0	53.0	81.3	38.5
20	125.0	75.0	55.0	91.7	44.5
21	106.0	62.0	79.0	76.7	25.5

22	108.0	73.0	63.0	84.7	26.5
23	110.0	66.0	61.0	80.7	27.0
24	111.0	74.0	67.0	86.3	27.5
25	115.0	66.0	65.0	82.3	36.5
26	105.0	68.0	59.0	80.3	30.0
27	125.0	72.0	69.0	89.7	43.0

A.6 Pulse Wave Analysis (AIx); Fluid Control

Subject Number	Pre-Supper AIx	Post Dose 1 AIx	Post Dose 2 AIx	Morning AIx
1	8	.	1	-18
2	2	5.5	4.5	4
3	-2	.	-0.5	8
4	7	14.5	10.5	11.5
5	4.5	.	6.5	9
6	28	28	27.5	34
7	-7	-5	1.5	6.5
8	6	-13	4	2
9	21.5	24	22.5	19
10	-8	-21	-18	10
11	4	14.5	8.5	-1
12	-5	-15.5	-14	-7
13	1	-1	-7	17.5
14	24.5	22	17.5	22.5
15	-13.5	-9	-1	5
16	12.5	-5.5	19.5	9.5
17	-6.5	-9.5	-4	16
18	20.5	19	19	22.5
19	17	14.5	11	24
20	29	33	33	34.5
21	-17	-9	-26	0.5

22	24.5	26.5	25.5	25
23	-2	2	20.5	6.5
24	30	17.5	20.5	25
25	27.5	18	17.5	28.5
26	18	19.5	19.5	29.5
27	36.5	26	31.5	38

A.7 Pulse Wave Analysis (AIx@75); Fluid Control

Subject Number	Pre-Supper AIx@75	Post Dose 1 AIx@75	Post Dose 2 AIx@75	Morning AIx@75
1	-4.5	.	-11	-33.5
2	-5	-4.5	-3.5	-3.5
3	0	.	-8	-1
4	-8	2	-1	-3
5	-6	.	-3	-1.5
6	28	34.5	29	28.5
7	-11	-6	-3.5	-3.5
8	-8	-27	-9	-12.5
9	21.5	15.5	18.5	10
10	-14.5	-32	-28	-3.5
11	-2.5	10.5	4	-7.5
12	-11.5	-21.5	-21.5	-15.5
13	-0.5	-8.5	-13.5	8
14	17.5	13	10	12
15	-28	-22	-15.5	-10.5
16	-2.5	-21	6	-7
17	-15.5	-19.5	-12.5	3
18	10	13	11	14
19	9	8.5	3	12.5
20	23	24	23.5	24
21	-17.5	-9.5	-24.5	1

22	21	21.5	22.5	18.5
23	-5	4.5	15	-0.5
24	29.5	17	18.5	22
25	29	18	18.5	24.5
26	14.5	17	14.5	23.5
27	32	20	24.5	35

A.8 Pulse Wave Velocity; Fluid Control

Subject Number	Pre-Supper cfPWV(m/s)	Post Dose 1 cfPWV(m/s)	Post Dose 2 cfPWV(m/s)	Morning cfPWV(m/s)
1	4.45	3.9	4.6	4.45
2	4.8	5.5	5.55	5.1
3	4.7	4.75	5.35	4.6
4	4.55	4.15	4.6	4.3
5	5.6	4.65	5.55	5.3
6	5.2	6.65	5.8	5.9
7	5.55	5.45	6.1	5.65
8	4	5.05	4.8	4.45
9	4.5	5.1	5.35	4.75
10	4.4	4.8	4.8	4.95
11	4.4	5.15	5.1	6.15
12	5.45	5	5	5.05
13	4.75	5.6	5.75	5.75
14	4.5	4.45	4.65	4.6
15	4.4	4.1	4.75	4.8
16	4.6	4.75	4.35	3.85
17	5.85	.	.	.
18	5.9	.	.	.
19	5.15	4.8	4.7	5.45
20	6.4	5.75	7.7	7.1
21	4.8	5.3	4.55	5.5

22	5.95	6.25	5.6	6.05
23	5.75	5.9	5.65	5.4
24	5.7	5.9	5.55	5.45
25	.	.	.	.
26	4.6	4.55	4.5	4.4
27	4.8	5.5	5.6	5.2

A.9 Pre-Supper Brachial Hemodynamics; Alcohol Intervention

Subject Number	SAP (mmHg)	DAP (mmHg)	Supine HR (bpm)	MAP (mmHg)	Aortic PP (mmHg)
1	107	41	52	63	40.5
2	99	57	63	71	28.0
3	117	62	72	80.33	34.0
4	99	54	47	69	31.5
5	96	65	58	75.33	21.0
6	115	84	97	94.33	.
7	122	65	60	84	34.5
8	109	60	48	76.33	31.5
9	97	54	49	68.33	32.5
10	116	61	54	79.33	31.5
11	135	62	66	86.33	50.5
12	112	57	58	75.33	35.0
13	112	59	68	76.67	33.5
14	106	53	65	70.67	32.5
15	114	62	47	79.33	33.5
16	107	53	43	71.00	36.0
17	117	60	64	79	38.5
18	105	60	60	75.00	35.0
19	94	57	67	69.33	21.0
20	121	77	55	91.67	36.5

21	99	55	70	69.67	25.5
22	98	67	58	77.33	24.5
23	111	68	74	82.33	24.0
24	116	78	76	90.66	28.5
25	131	74	78	93	37.5
26	105	69	61	81	25.0
27	124	71	60	88.66	39.5

A.10 Post Dose One Brachial Hemodynamics; Alcohol Intervention

Subject Number	SAP (mmHg)	DAP (mmHg)	Supine HR (bpm)	MAP (mmHg)	Aortic PP (mmHg)
1	108	39	50	62	41.0
2	110	54	63	72.67	34.0
3	105	58	59	73.67	27.5
4	102	57	50	72	28.0
5	98	62	60	74	22.5
6	116	81	78	92.67	.
7	133	74	63	93.67	33.5
8	105	55	51	71.67	31.5
9	122	66	61	84.67	42.0
10	123	64	50	83.67	33.0
11	132	66	68	88.00	37.5
12	128	68	62	88	36.5
13	117	62	80	80.33	33.0
14	112	55	63	74	36.0
15	103	49	46	67.00	34.0
16	108	52	49	70.66	39.5
17	117	58	60	77.67	34.5
18	102	57	70	72	32.5
19	96	54	63	68.00	24.0
20	120	74	52	89.33	40.5
21	99	59	79	72.33	22.5

22	.	.	.	.	.
23	109	63	66	78.33	26.5
24	122	80	71	94	28.5
25	117	70	80	85.66	33.0
26	106	67	71	80	30.0
27	121	65	61	83.66	44.5

A.11 Post Dose two Brachial Hemodynamics; Alcohol Intervention

Subject Number	SAP (mmHg)	DAP (mmHg)	Supine HR (bpm)	MAP (mmHg)	Aortic PP (mmHg)
1	101	40	54	60.33	36.5
2	109	57	67	74.33	31.0
3	104	57	65	72.67	29.5
4	102	56	56	71.33	27.0
5	97	60	59	72.33	23.0
6	118	85	77	96	22.0
7	131	72	65	91.67	36.0
8	110	60	53	76.67	31.0
9	114	60	60	78	37.0
10	118	60	55	79.33	32.5
11	136	69	68	91.33	42.5
12	107	57	74	73.67	31.5
13	111	57	83	75.00	30.0
14	112	54	70	73.33	40.0
15	101	49	52	66.33	33.5
16	106	49	50	68.00	35.0
17	110	52	70	71.33	33.5
18	102	61	73	74.67	27.5
19	104	61	62	75.33	26.5
20	114	71	55	85.33	32.0

21	106	60	78	75.33	26.0
22	95	58	67	70.33	.
23	111	66	66	81.00	27.5
24	115	74	78	87.66	29.5
25	114	64	74	80.6	34.5
26	111	69	61	83	28.0
27	120	63	65	82	42.5

A.12 Morning Brachial Hemodynamics; Alcohol Intervention

Subject Number	SAP (mmHg)	DAP (mmHg)	Supine HR (bpm)	MAP (mmHg)	Aortic PP (mmHg)
1	107	47	46	67.0	37.0
2	114	66	70	82.0	29.0
3	111	71	55	84.3	25.0
4	99	51	53	67.0	32.5
5	98	67	64	77.3	23.0
6	123	87	90	99.0	.
7	133	70	78	91.0	45.5
8	110	63	49	78.7	35.0
9	105	60	50	75.0	34.0
10	117	58	48	77.7	34.0
11	123	65	59	84.3	38.0
12	128	70	82	89.3	36.5
13	109	67	73	81.0	28.5
14	113	54	59	73.7	40.0
15	107	57	51	73.7	33.5
16	100	48	50	65.3	36.0
17	107	58	67	74.3	35.0
18	114	70	69	84.7	37.5
19	105	63	58	77.0	27.5
20	121	76	52	91.0	42.0
21	106	62	75	76.7	26.0

22	102	70	69	80.7	.
23	114	64	74	80.7	28.5
24	114	71	77	85.3	30.5
25	114	67	77	82.6	35.0
26	102	59	63	73.3	29.0
27	123	70	62	87.6	39.0

A.13 Pulse Wave Analysis (AIx); Alcohol Intervention

Subject Number	Pre-Supper AIx	Post Dose 1 AIx	Post Dose 2 AIx	Morning AIx
1	-12.5	-11.5	-7.5	-15.5
2	14.5	-11	-2	6
3	-5.5	-0.5	7.5	8
4	17	-1.5	-0.5	14.5
5	11	-14	-1.5	15.5
6	.	.	17	.
7	7	2	5.5	18.5
8	3	-8	-12	18
9	19	22.5	17.5	21.5
10	-15	-16.5	-19	-7.5
11	17	-8	7.5	7.5
12	7.5	-10	1	10.5
13	4	-9.5	-11.5	11.5
14	5	0.5	21	16
15	9.5	-5	6	12.5
16	9	17.5	-8.5	8.5
17	14	-9.5	-1.5	20.5
18	23	17	15	32
19	-2	-7.5	5.5	15.5
20	28	32	21.5	39
21	-18	-22	-19.5	-5.5

22	27	.	.	.
23	-5	-11.5	-11	0
24	18	29	25.5	27.5
25	20	14.5	18.5	25.5
26	17.5	16	15	16.5
27	31	35	33	29.5

A.14 Pulse Wave Analysis (AIx@75); Alcohol Intervention

Subject Number	Pre-Supper AIx@75	Post Dose 1 AIx@75	Post Dose 2 AIx@75	Morning AIx@75
1	-24	-24	-18.5	-30.5
2	6	-15	-3.5	2
3	-9	-8	3	-1
4	4	-14	-10.5	2
5	1.5	-22	-7.5	7
6	.	.	20.5	.
7	-2.5	-3.5	1	14
8	-10.5	-18.5	-23.5	3
9	7	15	16.5	9.5
10	-24	-27	-25.5	-18.5
11	12	-10.5	6.5	0.5
12	0.5	-12	-6	12
13	-0.5	-9.5	-7	5
14	-1	-5	18	5
15	-4.5	-18	-6	1.5
16	-7	4.5	-20	-5
17	6.5	-16.5	-5.5	15
18	16	13.5	14	26
19	-9	-13.5	-1	4.5
20	18	21	13.5	28
21	-21.5	-20.5	-20	-7.5

22	20	.	.	.
23	-10	-13.5	-15	-3
24	16.5	27	28.5	28
25	21	16.5	18	25.5
26	12.5	12.5	10.5	12
27	24	28.5	28.5	24

A.15 Pulse Wave Velocity; Alcohol Intervention

Subject Number	Pre-Supper cfPWV(m/s)	Post Dose 1 cfPWV(m/s)	Post Dose 2 cfPWV(m/s)	Morning cfPWV(m/s)
1	4.25	4	4.2	4.65
2	5.2	4.95	6.1	4.9
3	5	4.65	4.45	4.6
4	4.4	4.2	4.3	4.85
5	5.15	5.1	4.4	5.15
6	.	5.3	5.35	5.8
7	5.45	5.65	5.5	6.7
8	4.6	4.55	5.4	4.85
9	3.7	4.3	3.95	4.45
10	5.8	4.7	5.25	5.7
11	4.3	6.15	5.25	5.8
12	5.7	5.55	5.45	5.85
13	4.7	4.75	5.8	5.1
14	4.65	4.2	4.6	5.8
15	4.65	4.3	4.7	5.05
16	4.35	4.2	4.65	4.05
17	5.55	.	.	.
18	5.85	5.7	6.35	5.7
19	4.95	4.75	4.9	6.4
20	5.1	5.4	5.25	6.8
21	4.55	5.2	4.85	5.1

22	5.8	5.5	4.9	5.25
23	6.55	5.95	5.15	5.3
24	5.5	5.7	6.1	5.3
25	.	.	.	.
26	4.45	4.45	4.65	4.65
27	5.2	5.15	5.2	5.75

A.16 Blood Alcohol Content

Subject Number	Post Drink 1 BAC	Post Drink 2 BAC	Morning BAC
1	0.057	0.101	0
2	0.05	0.113	0
3	0.052	0.092	0
4	0.053	0.099	0
5	0.057	0.11	0
6	0.064	0.123	0
7	0.05	0.11	0
8	0.044	0.099	0
9	0.041	0.085	0
10	0.034	0.082	0
11	0.016	0.056	0
12	0.056	0.097	0.009
13	0.032	0.075	0
14	0.043	0.078	0
15	0.039	0.082	0
16	0.035	0.083	0
17	0.041	0.131	0.019
18	0.053	0.094	0
19	0.036	0.095	0
20	0.045	0.069	0
21	0.067	0.134	0

22	0.045	0.086	0
23	0.031	0.076	0
24	0.021	0.066	0
25	0.046	0.102	0
26	0.046	0.095	0
27	0.050	0.093	0

B Appendix B: Summary Statistics

B.1 Supine Systolic Arterial Pressure

Mauchly's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Greenhouse-Geisser
Cond	1.000	0.000	0		1.000
Time	0.761	5.922	5	0.314	0.849
Cond * Time	0.889	2.557	5	0.768	0.937

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Time	Sphericity Assumed	13.897	3	4.632	0.178	0.911
	Greenhouse-Geisser	13.897	2.540	5.455	0.178	0.884
	Huynh-Feldt	13.897	3.000	4.632	0.178	0.911
	Lower-bound	13.897	1.000	13.897	0.178	0.677
Cond * Time	Sphericity Assumed	61.520	3	20.507	0.872	0.460
	Greenhouse-Geisser	61.520	2.810	21.891	0.872	0.454
	Huynh-Feldt	61.520	3.000	20.507	0.872	0.460
	Lower-bound	61.520	1.000	61.520	0.872	0.360
Cond * Time * Sex	Sphericity Assumed	65.420	3	21.807	0.928	0.432
	Greenhouse-Geisser	65.420	2.810	23.279	0.928	0.428
	Huynh-Feldt	65.420	3.000	21.807	0.928	0.432

Lower-bound	65.420	1.000	65.420	0.928	0.346

B.2 Supine Diastolic Arterial Pressure

Mauchly's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Greenhouse-Geisser
Cond	1.000	0.000	0		1.000
Time	0.540	12.761	5	0.026	0.698
Cond * Time	0.875	2.758	5	0.738	0.919

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Time	Sphericity Assumed	47.182	3	15.727	0.695	0.558
	Greenhouse-Geisser	47.182	2.094	22.527	0.695	0.511
	Huynh-Feldt	47.182	2.425	19.458	0.695	0.530
	Lower-bound	47.182	1.000	47.182	0.695	0.413
Cond * Time	Sphericity Assumed	86.641	3	28.880	2.746	0.050
	Greenhouse-Geisser	86.641	2.758	31.415	2.746	0.055
	Huynh-Feldt	86.641	3.000	28.880	2.746	0.050
	Lower-bound	86.641	1.000	86.641	2.746	0.112
Cond * Time * Sex	Sphericity Assumed	17.724	3	5.908	0.562	0.642
	Greenhouse-Geisser	17.724	2.758	6.427	0.562	0.628

Huynh-Feldt	17.724	3.000	5.908	0.562	0.642
Lower-bound	17.724	1.000	17.724	0.562	0.462

Paired Samples Test

95% Confidence Interval

	Mean	Std. Deviation	Std. Error Mean	Lower	Upper	t	df	Sig. (2-tailed)
PS-PF1	-0.4444	5.39468	1.03821	-2.5785	1.68962	-0.428	26	0.672
PS-PF2	-1.3703	4.49913	0.86586	-3.1501	0.40943	-1.583	26	0.126
PS-M	-0.7037	5.25368	1.01107	-2.781	1.37458	-0.696	26	0.493
PS-PD1	0.16667	5.72308	1.16822	-2.2499	2.58331	0.143	23	0.888
PS-PD2	1.76000	5.43354	1.08671	-0.4828	4.00285	1.620	24	0.118
PS - M	-1.4800	6.14492	1.22898	-4.0165	1.05650	-1.204	24	0.240

B.3 Supine Heart Rate

Mauchly's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Greenhouse-Geisser
Cond	1.000	0.000	0		1.000
Time	0.537	13.488	5	0.019	0.703
Cond * Time	0.636	9.827	5	0.081	0.794

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Time	Sphericity Assumed	165.886	3	55.295	1.904	0.137
	Greenhouse-Geisser	165.886	2.109	78.657	1.904	0.158
	Huynh-Feldt	165.886	2.428	68.321	1.904	0.150
	Lower-bound	165.886	1.000	165.886	1.904	0.181
Cond * Time	Sphericity Assumed	526.666	3	175.555	10.101	0.000
	Greenhouse-Geisser	526.666	2.382	221.063	10.101	0.000
	Huynh-Feldt	526.666	2.792	188.646	10.101	0.000
	Lower-bound	526.666	1.000	526.666	10.101	0.004

Cond * Time * Sex	Sphericity Assumed	71.606	3	23.869	1.373	0.258
	Greenhouse-Geisser	71.606	2.382	30.056	1.373	0.262
	Huynh-Feldt	71.606	2.792	25.648	1.373	0.260
	Lower-bound	71.606	1.000	71.606	1.373	0.253

Paired Samples Test

	95% Confidence Interval							
	Mean	Std. Deviation	Std. Error Mean	Lower	Upper	t	df	Sig. (2-tailed)
PS-PF1	2.29630	7.17804	1.38141	-0.5432	5.13583	1.662	26	0.108
PS-PF2	3.66667	6.51035	1.25292	1.09126	6.24207	2.927	26	0.007
PS-M	6.85185	8.87392	1.70779	3.34145	10.3622	4.012	26	0.000
PS-PD1	-1.3200	6.26312	1.25262	-3.9052	1.26529	-1.054	24	0.302
PS-PD2	-4.1153	6.16653	1.20936	-6.6061	-1.6246	-3.403	25	0.002
PS - M	-2.1923	8.41437	1.65019	-5.5909	1.20633	-1.329	25	0.196

B.4 Aortic Augmentation Index @ 75 BPM

Mauchly's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Greenhouse-Geisser
Cond	1.000	0.000	0		1.000
Time	0.467	14.247	5	0.014	0.653
Cond * Time	0.666	7.622	5	0.179	0.836

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Time	Sphericity Assumed	1666.699	3	555.566	16.293	0.000
	Greenhouse-Geisser	1666.699	1.960	850.143	16.293	0.000
	Huynh-Feldt	1666.699	2.280	730.995	16.293	0.000
	Lower-bound	1666.699	1.000	1666.699	16.293	0.001
Cond * Time	Sphericity Assumed	152.335	3	50.778	0.974	0.411
	Greenhouse-Geisser	152.335	2.508	60.732	0.974	0.400
	Huynh-Feldt	152.335	3.000	50.778	0.974	0.411
	Lower-bound	152.335	1.000	152.335	0.974	0.335

Cond * Time * Sex	Sphericity Assumed	82.153	3	27.384	0.525	0.666
	Greenhouse-Geisser	82.153	2.508	32.753	0.525	0.635
	Huynh-Feldt	82.153	3.000	27.384	0.525	0.666
	Lower-bound	82.153	1.000	82.153	0.525	0.477

Paired Samples Test

	95% Confidence Interval							
	Mean	Std. Deviation	Std. Error Mean	Lower	Upper	t	df	Sig. (2-tailed)
PS-PF1	2.41667	9.48760	1.93665	-1.5895	6.42293	1.248	23	0.225
PS-PF2	1.14815	8.23082	1.58402	-2.1078	4.40415	0.725	26	0.475
PS-M	-1.4259	9.88657	1.90267	-5.3369	2.48507	-0.749	26	0.460
PS-PD1	5.38000	10.27769	2.05554	1.1375	9.62242	2.617	24	0.015
PS-PD2	1.34000	8.82317	1.76463	-2.3020	4.98203	0.759	24	0.455
PS - M	-5.4800	6.83051	1.36610	-8.2994	-2.6605	-4.011	24	0.001

B.5 Carotid to Femoral Pulse Wave Velocity

Mauchly's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Greenhouse-Geisser
Cond	1.000	0.000	0		1.000
Time	0.800	4.394	5	0.495	0.867
Cond * Time	0.794	4.539	5	0.475	0.859

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Time	Sphericity Assumed	2.088	3	0.696	3.121	0.032
	Greenhouse-Geisser	2.088	2.600	0.803	3.121	0.040
	Huynh-Feldt	2.088	3.000	0.696	3.121	0.032
	Lower-bound	2.088	1.000	2.088	3.121	0.092
Cond * Time	Sphericity Assumed	0.949	3	0.316	2.681	0.054
	Greenhouse-Geisser	0.949	2.576	0.368	2.681	0.064
	Huynh-Feldt	0.949	3.000	0.316	2.681	0.054

	Lower-bound	0.949	1.000	0.949	2.681	0.116
Cond * Time * Sex	Sphericity Assumed	0.432	3	0.144	1.220	0.310
	Greenhouse-Geisser	0.432	2.576	0.168	1.220	0.309
	Huynh-Feldt	0.432	3.000	0.144	1.220	0.310
	Lower-bound	0.432	1.000	0.432	1.220	0.282