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Acute alcohol ingestion and sympathetic neural responses during orthostatic stress in humans

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ACUTE ALCOHOL INGESTION AND SYMPATHETIC NEURAL RESPONSES DURING ORTHOSTATIC STRESS IN HUMANS

By

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This thesis, **"**Acute Alcohol Ingestion and Sympathetic Neural Responses During Orthostatic Stress in Humans," is hereby approved in partial fulfillment of the requirements for the Degree of MASTER OF SCIENCE IN BIOLOGICAL SCIENCES.

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Preface

Some of the text throughout this thesis, specifically a majority of the Abstract, Methods, and Results, along with all of the figures and the table in the Results section, are used with consent from the American Physiological Society as a concerted effort with co-authors Dr. Jason Carter, Dr. John Durocher, and Robert Larson. All data collection was performed in Dr. Jason Carter's laboratory at Michigan Tech University in Houghton, Michigan. My involvement included subject recruitment, data acquisition, data analysis and statistical analysis of the study. I also had an important part in authoring the Abstract and Results sections of the article, with a lesser, but still important role in the Introduction, Methods, and Discussion sections. Please see Appendix E for documentation relating to the permission to republish this material.

Acknowledgements

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List of Abbreviations

ANS	Autonomic nervous system
BAC	Blood alcohol content
BMI	Body mass index
CO	Cardiac output
DAP	Diastolic arterial pressure
E	Epinephrine
FBF	Forearm blood flow
FVC	Forearm vascular conductance
FVR	Forearm vascular resistance
HB	Heartbeat
HR	Heart rate
LBNP	Lower body negative pressure
MAP	Mean arterial pressure
MSNA	Muscle sympathetic nerve activity
NE	Norepinephrine
NTS	Nucleus tractus solitarii
OH	Orthostatic hypotension
SAP	Systolic arterial pressure
SV	Stroke volume
TPR	Total peripheral resistance

Abstract[1]

Acute alcohol consumption has been reported to decrease mean arterial pressure (MAP) during orthostatic challenge, a response that may contribute to alcohol-mediated hypotension and eventually syncope. Muscle sympathetic nerve activity (MSNA) increases during orthostatic stress to help maintain MAP, yet the influence of alcohol on MSNA during orthostatic stress has not been determined. We hypothesized that alcohol ingestion would blunt arterial blood pressure and MSNA responses to progressive lower body negative pressure (LBNP). MAP, MSNA, and heart rate (HR) were recorded during progressive LBNP (-5, -10, -15, -20, -30, and -40 mmHg; 3 min/stage) in 30 subjects (age 24 ± 1 yrs). After an initial progressive LBNP protocol (pre-treatment), subjects were randomly assigned to consume alcohol (0.8g ethanol/kg body mass; *n*=15) or placebo (*n*=15) and then repeated the progressive LBNP protocol (post-treatment). Alcohol increased (drug × treatment, $P \leq 0.05$) resting HR (59 ± 2 to 65 ± 2 beats/min) and MSNA (13 ± 3 to 19 ± 4 bursts/min) when compared to placebo. While alcohol increased MAP (83 ± 2 to 87 ± 2 mmHg), these increases were also observed with placebo (82 ± 2 to 88 ± 1 mmHg; treatment, $P < 0.05$; drug × treatment, $P > 0.05$). During progressive LBNP, a prominent decrease in MAP was observed after alcohol (drug × time × treatment, $P < 0.05$), but not placebo. There was also a significant attenuated response in forearm vascular resistance (FVR) during progressive LBNP (drug × time × treatment, $P < 0.05$). MSNA and HR increased during all LBNP protocols, but there were no differences between treatments or groups (drugs). In summary, acute alcohol ingestion induces an attenuation in blood pressure response during an orthostatic challenge, possibly due to the effect that alcohol has on impairing peripheral blood vessel constriction.

[1] The material in this abstract was previously published in the American Journal of Physiology – Endocrinology and Metabolism.

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Chapter 1
INTRODUCTION

1.1 Homeostasis

The autonomic nervous system (ANS) is critical in preserving homeostasis throughout the body. Walter Cannon first coined this term in 1929, with "homeo" meaning like or similar and "stasis" meaning a condition. He described the human body as an open system that interacts with the environment, and hypothesized that those changes in the surrounding environment create internal disturbances of the system. "*Such disturbances are normally kept within narrow limits, because autonomic adjustments within the system are brought into action, and thereby wide oscillations are prevented and the internal conditions are held fairly constant*" (Cannon, 1929). This homeostasis is maintained by negative feedback. By comparing organisms to machines, Rosenblueth, Wiener, and Bigelow (1943), a physiologist, mathematician, and engineer, respectively, were able to recognize the importance of negative feedback in the living organism. "*The behavior of some machines and some reactions of living organisms involve a continuous feed-back from the goal that modifies and guides the behaving object* (Rosenblueth, 1943)."

When homeostasis is disrupted, the possibility of cardiovascular risks increase. Many lifestyle choices, such as alcohol consumption, contribute to this disruption of homeostasis. Alcohol has been shown to increase the incidence of both hypertension (Zilkens *et al.*, 2005; Lichtenstein *et al.*, 2006; Kloner & Rezkalla, 2007; Saremi & Arora, 2008; van de Wiel & de Lange, 2008; Klatsky, 2009; Wakabayashi, 2009) and orthostatic hypotension (Fisher, 1979; Hollister, 1992; Narkiewicz *et al.*, 2000; Medow *et al.*, 2008; Freeman *et al.*, 2011; Lanier *et al.*, 2011). While the long-term effects of moderate alcohol consumption and hypertension have been well studied and are thought to be due to sympathoexcitation (Johnson *et al.*, 1986; Grassi *et al.*, 1989; Iwase *et al.*, 1995; Randin *et al.*, 1995; Kloner & Rezkalla, 2007), the link between alcohol induced hypotension after an orthostatic stress is still unclear.

1.2 Autonomic Nervous System

Unlike the somatic nervous system, which is voluntary, the ANS operates predominately without any conscious input or voluntary control. Its role is to innervate cardiac muscle, smooth muscle, and many exocrine and endocrine glands (Cannon, 1929). It is further subdivided into two divisions, the parasympathetic and the sympathetic nervous systems, which are both constantly active to some degree. The sympathetic nervous system is perhaps best known for Cannon's infamous "fight or flight" analogy, and is responsible for mobilizing the body's systems during physical or mental stress by increasing heart rate, increasing heart contractility, and constricting blood vessels. Cannon described how an animal responds to threats and that all of the changes that occur are "*directly serviceable in making the organism more effective in the violent display of energy which fear or rage or pain may involve*" (Cannon, 1915). The sympathetic nervous system is the main division of the ANS that is responsible for vascular tone (Cannon, 1929). The parasympathetic nervous system, also known as the "rest and digest" response, is responsible for conserving energy and is usually complementary of the sympathetic nervous system (Cannon, 1929).

1.2.1 Adrenergic Receptors

Adrenergic receptors are receptors that respond to both norepinephrine and epinephrine (Furchgott, 1959). The main neurotransmitter used to transmit information to the target tissue in the sympathetic nervous system via post-ganglionic fibers is norepinephrine (NE). NE can also be released in the bloodstream along with epinephrine (E) through stimulation of the adrenal medulla. There are two classes of α receptors and three classes of β receptors (Bylund, 2007). α_1, β_1, and β_2 receptors are important to the maintenance of blood pressure. α_1 receptors are found in blood vessels and have a higher affinity for NE than E. Stimulation of these will cause vasoconstriction (Furchgott, 1959; Charkoudian & Rabbitts, 2009). β_2 receptors are also found in many blood vessels and stimulation of these adrenergic receptors will result in vasodilation (Furchgott, 1959). These receptors have a greater affinity for epinephrine. β_1 receptors, which are found on the heart, have affinity for both epinephrine and norepinephrine. If these are stimulated,

there is an increase in both heart rate and heart contractility (Furchgott, 1959). There are also α_2 and β_3 receptors. α_2 receptors are found in many membranes of adrenergic axon terminals and have the ability to inhibit NE release (Marieb & Hoehn, 2008). β_3 receptors are found in adipose tissue and stimulation will result in lipolysis (Marieb & Hoehn, 2008).

1.2.2 Muscle Sympathetic Nerve Activity

Microneurography is a technique used to record sympathetic neural traffic. It measures the post-ganglionic efferent sympathetic bursts to skeletal muscle beds, i.e. muscle sympathetic nerve activity (MSNA). Microneurography originated in Sweden in 1965-1966 by researchers Karl-Erik Hagbarth and Åke Vallbo who first developed the technique by inserting needles into their own ulnar nerves (Vallbo *et al.*, 2004). In the late 1960s and early 70s, Gunnar Wallin and Göran Sundlöf learned the microneurography system from Hagbarth and made vast contributions to the development of not only the method, but of the role of the sympathetic nervous system in health and disease (Vallbo *et al.*, 2004). This has especially played an important role in advancing our understanding of the pathophysiology of cardiovascular disease (Grassi & Esler, 1999; Charkoudian & Rabbitts, 2009). For more information about the technique itself, please refer to the muscle sympathetic nerve activity section in the methods of this thesis.

Although MSNA is a measurement of the sympathetic outflow to the vasculature of skeletal muscle, it appears to reasonably represent sympathetic activity to other vascular beds and thus be a good indicator of overall sympathetic activity . It has been demonstrated that there is a correlation between renal NE spillover and MSNA and also between cardiac NE spillover and MSNA (Wallin *et al.*, 1992; Wallin *et al.*, 1996). As outlined previously, with every sympathetic burst, NE is released by post-ganglionic sympathetic fibers and binds to vascular receptors (Morlin *et al.*, 1983). This process elicits varying levels of vasoconstriction and beat-to-beat control of arterial blood pressure. These bursts represent a collection of action potentials of several nerve fibers (Wallin, 2006) and occur in rhythm with the cardiac cycle (Sundlof, 1978). MSNA is largely modulated via the baroreflex and has a negative correlation with blood pressure,

specifically diastolic blood pressure (DAP) (Sundlof, 1978). If there is a decrease in blood pressure, there is typically an increase in MSNA which elicits vasoconstriction and a subsequent increase in blood pressure. If there is an increase in blood pressure, there will typically be a decrease in MSNA. As a result, there will be less sympathetic tone, which will decrease blood pressure (Sundlof, 1978). The relationship between MSNA and arterial blood pressure via the baroreflex is a classic example of a negative feedback system to aid in homeostasis.

1.2.3 Blood Pressure Regulation

Baroreceptors are mechanoreceptors with sensory afferent nerve endings located in blood vessels and the heart. They detect increases or decreases in blood pressure or volume by the change in stretch/deformation (Kirchheim, 1976). High-pressure arterial baroreceptors are located in the aortic arch and the carotid artery and low-pressure cardiopulmonary baroreceptors are located in the heart (Kirchheim, 1976; Mosqueda-Garcia *et al.*, 2000; Freeman, 2006). If a change in blood pressure or volume is detected, the number of signals being sent to the nucleus tractus solitarii (NTS) in the medulla via afferent pathways changes, and there is a corresponding modification in sympathetic and parasympathetic outflow (Kirchheim, 1976). As a result, there is a change in sympathetic neural activity, heart rate, heart contractility, vascular tone, and total peripheral resistance, which results in a correction of blood pressure (Charkoudian & Rabbitts, 2009).

Mean arterial pressure (MAP) is the product of cardiac output (CO) and total peripheral resistance (TPR) (Shepherd, 1987), and CO is the product of heart rate (HR) and stroke volume (SV) (Rushmer & Smith, 1959). The most immediate regulation of arterial blood pressure is through the baroreflex (Sundlof, 1978). A summary of the short-term events that take place if there is a decrease in MAP, including the ones that were already discussed, is outlined in Figure 1.2.

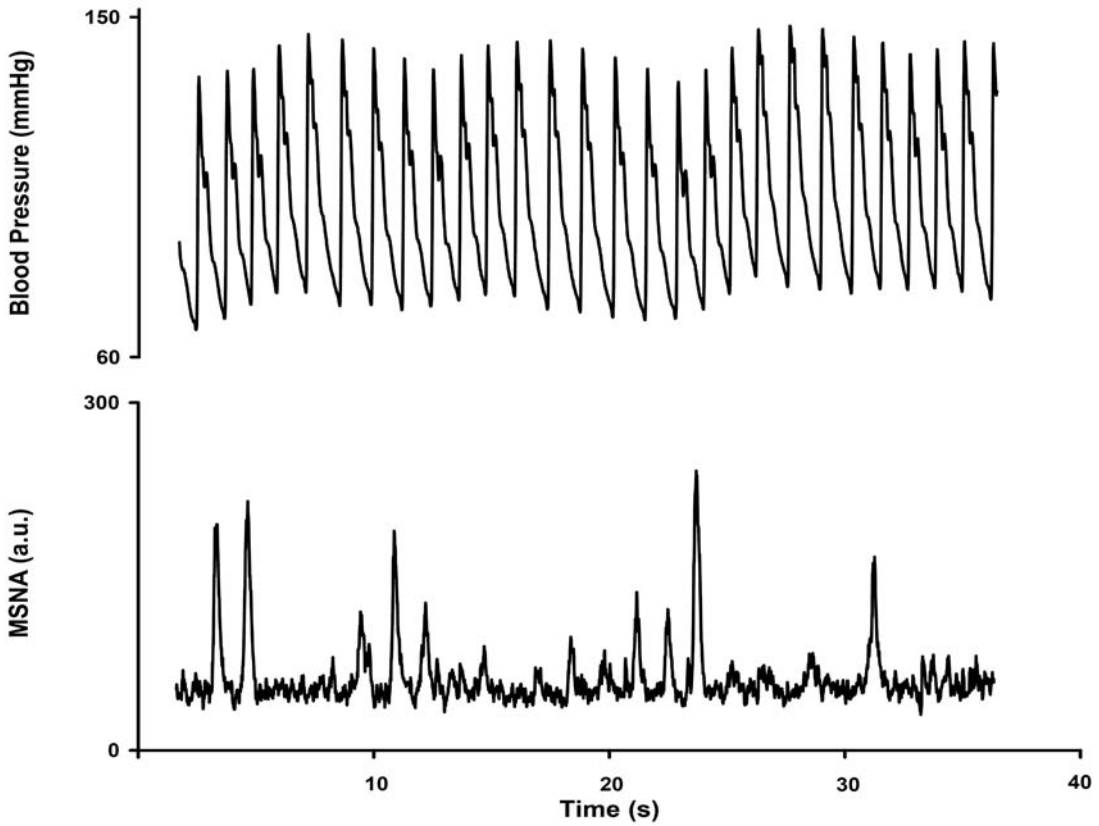

Figure 1.1 A neurogram and the corresponding blood pressure during an approximately 30 second period of baseline. Note the relationship between fluctuations in blood pressure and MSNA such that burst occur more frequently during reductions in blood pressure. MSNA, muscle sympathetic nerve activity; a.u., arbitrary units

There are several other methods the body uses to maintain a stable blood pressure, including the kidneys which participate predominantly in long-term control. The sympathetic nervous system can stimulate the kidneys to release renin, which activates the renin-angiontensin-aldosterone system (Rowell, 1993). When activated, this system can increase water and salt retention and thus, increase blood volume. Angiontensin II will also stimulate vasopressin release from the posterior pituitary gland, which can increase water and salt reabsorption. Angiontensin II and vasopressin are vasoconstrictors as well (Rowell, 1993). This is also diagrammed in Figure 1.3. Additionally, local factors can play a role in blood pressure regulation. Endothelial cells can release vasodilators

such as nitric oxide (NO) and prostaglandins, which can decrease local sympathetic tone and therefore, TPR (Wolf *et al.*, 1999).

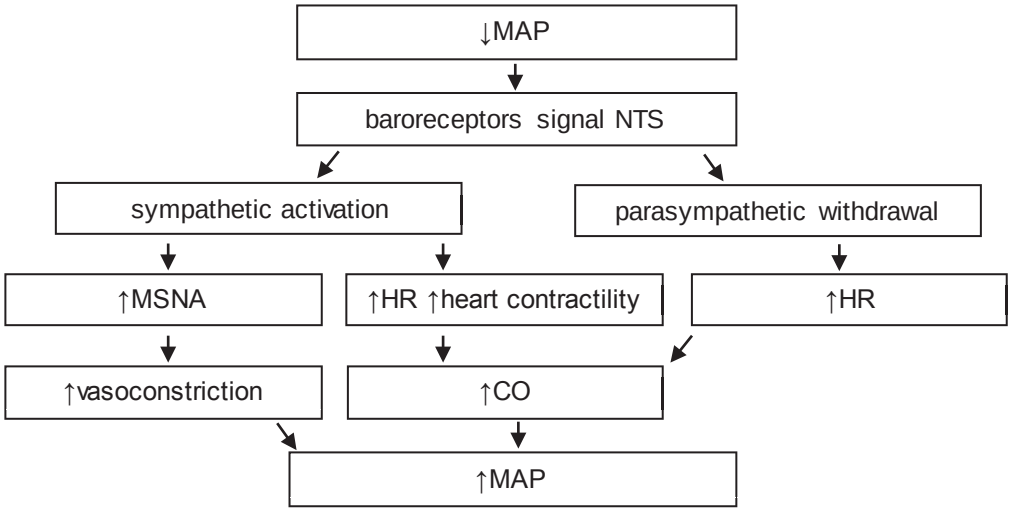

Figure 1.2 The body's short-term response to a decrease in blood pressure via the baroreflex. MAP, mean arterial pressure; NTS, nucleus tractus solitarii; MSNA, muscle sympathetic nerve activity; HR, heart rate; CO, cardiac output.

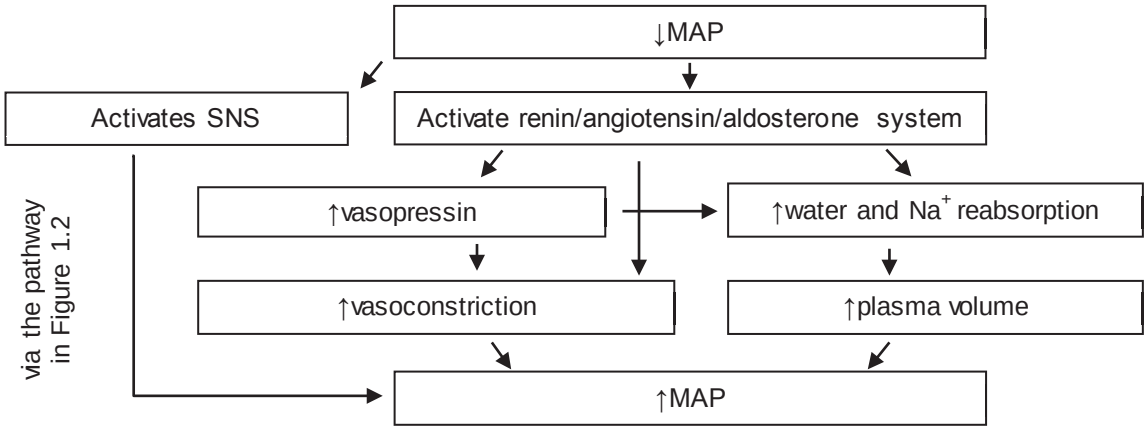

Figure 1.3 The body's long-term response to a decrease in blood pressure. MAP, mean arterial pressure; SNS, sympathetic nervous system. NA^+, sodium.

1.3 Orthostatic Hypotension

Orthostatic hypotension (OH) often results after a postural change to the upright position. One of the first physiological accounts of this came from Thomas Lewis who was a medical doctor studying British soldiers in World War I. He noticed a drop of 30 mmHg or more in blood pressure as soldiers went from supine to erect (Lewis, 1920). This idea was expanded on by Swedish physicians, Bjure and Laurell, who in the late 1920s and 30s demonstrated that patients with orthostatic hypotension had reduced stroke volume and cardiac output (Streeten, 1999).

In the past, OH was used to describe an abnormal drop in blood pressure after standing up. However, the American Autonomic Society and the American Academy of Neurology defines OH as a decrease in SAP of at least 20 mmHg or DAP of at least 10 mmHg within three minutes of standing ((NIAAA), 2012). It is more common in elderly (Vaddadi *et al.*, 2007; Freeman *et al.*, 2011), females (Ganzeboom *et al.*, 2006), astronauts that return from space (Mano & Iwase, 2003), and highly trained endurance athletes (Raven & Pawelczyk, 1993). It occurs spontaneously in 0.5% of individuals (Medow *et al.*, 2008) and is also a major risk factor for falls, which can lead to serious injuries (Naschitz & Rosner, 2007).

When a person stands up, anywhere from 300-800 mL of blood redistributes to the lower part of the body such as the legs, buttocks, pelvis, and splanchnic region (Freeman, 2006). As a result, there is a drop in stroke volume (SV), cardiac output, and arterial blood pressure (Burke *et al.*, 1977). The baroreceptors located in the carotid arteries, aorta, and the heart quickly detect the drop in blood volume and pressure (Bradley & Davis, 2003). The heart rate increases almost immediately due to parasympathetic withdrawal and an increase in sympathetic activity (Burke *et al.*, 1977; Freeman, 2006). There is also vasoconstriction in the splanchnic, renal, and skeletal muscle regions and a subsequent increase in TPR (Burke *et al.*, 1977; Rowell, 1993; Freeman, 2006). Additionally, the contraction of the leg and abdominal muscles upon standing activates the skeletal and respiratory pumps and enhances venous return to the heart (Bradley & Davis, 2003; Medow *et al.*, 2008). An orthostatic challenge has also

been shown to increase plasma NE levels (Mosqueda-Garcia *et al.*, 2000), as well as renin, angiotensin II, aldosterone, and vasopressin (Tidgren *et al.*, 1990; Norsk, 1992), all of which help to maintain blood pressure. As a result of all the above mechanisms, arterial blood pressure is generally well maintained during orthostasis (Burke *et al.*, 1977; Sundlof & Wallin, 1978; Mano & Iwase, 2003; Freeman, 2006; Ichinose *et al.*, 2006).

OH is due to a drop in CO and/or insufficient vasoconstriction (Sharpey-Schafer, 1956; Medow *et al.*, 2008; Freeman *et al.*, 2011). It is associated with primary and secondary autonomic disorders, postural tachycardia syndrome, or reflex syncope (Medow *et al.*, 2008). OH is often exacerbated by many factors like alcohol, medications (e.g. vasodilators, diuretics, antidepressants), hypovolemia (e.g. hemorrhage, diarrhea, vomiting), eating a large meal, exercise, prolonged bed rest, time of day (i.e. morning), a warm environment, or having poor respiratory or skeletal pump functioning (Fisher, 1979; Medow *et al.*, 2008; Moya *et al.*, 2009; Freeman *et al.*, 2011). It can be symptomatic or asymptomatic (Bradley & Davis, 2003; Freeman *et al.*, 2011). It may result in presyncopal symptoms, such as dizziness, lightheadedness, blurred vision, weakness, fatigue, and nausea (Medow *et al.*, 2008) or syncope itself if there is a transient loss of consciousness (Moya *et al.*, 2009; Lanier *et al.*, 2011). If syncope results it is often neurally-mediated, with a sympathetic withdrawal and parasympathetic activation (Medow *et al.*, 2008; Moya *et al.*, 2009).

There are a few variants of OH that have recently been discussed in the literature. Initial OH is a drop in SAP of at least 40 mmHg and/or DAP of 20 mmHg within 15 seconds of standing (Wieling *et al.*, 2007; Freeman *et al.*, 2011). If often happens in young, asthenic subjects or the elderly (Moya *et al.*, 2009). In delayed OH, there is a fall in BP beyond three minutes of a postural change (Moya *et al.*, 2009). There is a decrease in venous return over time and this causes a reduced CO. It is mostly seen in older individuals (Freeman *et al.*, 2011).

1.3.1 Methods for Inducing Orthostatic Stress

Lower body negative pressure (LBNP) and head-up tilt (HUT) are two common ways to elicit an orthostatic challenge. During LBNP, a pressure gradient is created by placing the lower body into an air-tight chamber with a negative pressure compared to the surrounding air. As a result, blood is displaced from the upper body to the lower body and limbs. If done progressively in discrete stages, this action gradually unloads the baroreceptors (Freeman, 2006). The head-up tilt is similar in that the subjects lay supine and then their head is tilted, usually 60-80 degrees for up to 60 min to unload the baroreceptors (Freeman, 2006). The main difference between these two applications is that splanchnic volume increases with head up tilt whereas it decreases with LBNP (Taneja *et al.*, 2007). If done properly, these passive orthostatic challenges do not substantially activate the skeletal muscle pump (Freeman, 2006). For more information on the LBNP protocol that we used, please see the procedures section in the methods description of this thesis.

1.4 Syncope

An early French physician, Pierre Piorry described syncope in 1826 as "*the heart continues to beat, but the beats have not force enough to overcome the resistance which is given by gravity*" (Hill, 1894). Syncope is a short-lived event with rapid onset. It is due to a decrease in cerebral perfusion, which causes a brief loss of consciousness, followed by a spontaneous recovery (Mosqueda-Garcia *et al.*, 2000; Vaddadi *et al.*, 2007; Chen *et al.*, 2008; Costantino *et al.*, 2008; Moya *et al.*, 2009).

Syncope is a common problem, and has been reported to account for up to 1% of all emergency department visits and up to 3% of hospital admissions (Morichetti & Astorino, 1998; Blanc *et al.*, 2002). The Framingham Study examined over 5,000 subjects over 26 years and showed that 3% of men and 3.5% of women had at least one syncopal episode throughout that time period (Savage *et al.*, 1985), and another study showed that syncope has a lifetime incidence of 35% (Ganzeboom *et al.*, 2006). It is estimated that expenses related to syncope are approximately $2 billion a year (Sun *et al.*,

2005). Syncope is related to a higher increase in fractures, injuries, depression, and decreased quality of life (Moya *et al.*, 2009).

Syncope is not a disease itself, but often an outcome due to another underlying cause (Zaidi & Fitzpatrick, 2000). Ganzeboom et al. (2006) reported that the lifetime incidence of syncope in the general public were caused by everyday situations that affected the maintenance of orthostatic blood pressure, such as a postural changes, people taking certain medications, and people suffering from hypovolemia (Ganzeboom *et al.*, 2006). All of these situations can cause a decreased CO and/or TPR. If the systolic blood pressure drops below 50 mmHg, cerebral hypoperfusion will likely result in syncope (Sharpey-Schafer, 1956).

The most common kind of syncope that occurs due to orthostatic hypotension is vasovagal syncope, which is a type of neurally-mediated syncope (NMS). For being so common, the pathophysiology behind this type of syncope is still not fully understood (Aydin *et al.*, 2010), but it is often a result of a combination of parasympathetic activation and sympathetic withdrawal (Mosqueda-Garcia *et al.*, 2000). Figure 1.4 depicts how orthostatic stress might cause vasovagal syncope, a conceptual model commonly referred to as the ventricular theory (Mosqueda-Garcia *et al.*, 2000). It was first popularized by Sharpey-Schafer as he noticed a reflex mechanism that can overcome the baroreflex (Sharpey-Schafer, 1956). The abrupt loss of MSNA is often the last physiological incident that sets off orthostatic responses (Wallin & Sundlof, 1982; Mano & Iwase, 2003). However, this statement has recently been challenged by Cooke et al. (2009) who has shown that in some presyncopal subjects there was no withdrawal of MSNA immediately preceding syncope despite decreases in blood pressure. More research in this area is needed.

1.5 Alcohol

As mentioned previously, there are many triggers for syncope. The consumption of alcohol has been shown to attenuate blood pressure responses after a lower body negative pressure protocol (Eisenhofer *et al.*, 1984; Narkiewicz *et al.*, 2000) and this

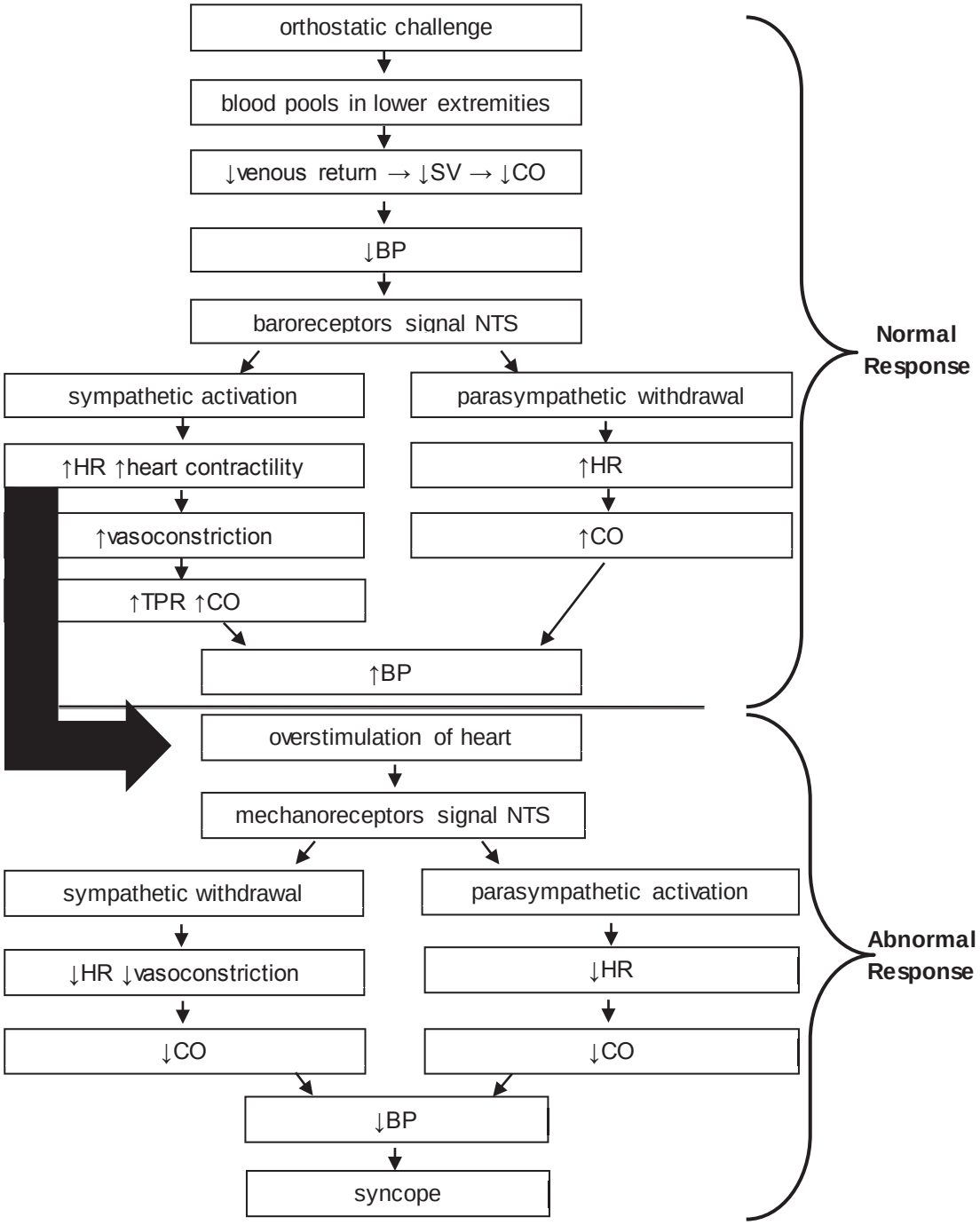

Figure 1.4 The normal response to an orthostatic challenge, such as a postural change, and the ventricular theory describing how an orthostatic challenge may contribute to syncope (abnormal response). In the abnormal response, there is a sympathetic withdrawal and a parasympathetic activation, causing a decrease in vascular tone, heart rate, and blood pressure. SV, stroke volume; CO, cardiac output; BP, blood pressure; NTS, nucleus tractus solitarii; HR, heart rate.

could theoretically result in orthostatic hypotension and eventually syncope. Ganzeboom et al. (2006) reported that almost 3% of the people who had a syncopal episode had ingested alcohol prior to losing consciousness.

Alcohol is generally regarded as the most abused drug in the United States (Wolf *et al.*, 1999) and it has been noted that its overall harm is greater than heroin, cocaine, and methamphetamine (Nutt *et al.*, 2010). It can be a huge personal, family, social, and economical burden. Each year in the United States, alcohol plays a role in over 100,000 lives lost and has an economic toll over $184.6 billion (United States. Dept. of Health and Human Services. Office of the Secretary. & National Institute on Alcohol Abuse and Alcoholism (U.S.), 2000). At any given point, approximately 20-40% of patients in urban hospitals are admitted as a result of an alcohol-related incident (United States. Dept. of Health and Human Services. Office of the Secretary. & National Institute on Alcohol Abuse and Alcoholism (U.S.), 2000). A standard drink is considered to be a 12 ounce bottle of beer, a 5 ounce glass of wine, or a 1.5 ounce shot of 80-proof spirits ((NIAAA), 2012). It is recommended that males not consume more than two drinks per day and females not consume more than one drink per day ((NIAAA), 2012). Any more than this "moderate drinking" over an extended period of time and negative side effects are most likely to occur.

Alcohol is classified as a depressant, because it decreases the excitatory actions of the neurotransmitter glutamate *and* increases the inhibitory actions of the neurotransmitter gamma-aminobutyric acid (GABA) (United States. Dept. of Health and Human Services. Office of the Secretary. & National Institute on Alcohol Abuse and Alcoholism (U.S.), 2000). Once it is consumed, roughly 20-25% of it is absorbed by the stomach, and 75-80% by the small intestine (Tateoka *et al.*, 2007). Alcohol dehydrogenase, an enzyme found in the liver, converts most of the ethanol to acetaldehyde and then aldehyde dehydrogenase converts acetaldehyde to acetate (Diamond & Messing, 1994). If the alcohol is ingested on an empty stomach it only takes one hour for 90% of it to be absorbed and for the individual to reach his or her peak alcohol level (Wolf *et al.*, 1999; Tateoka *et al.*, 2007). Alcohol readily crosses the blood-

brain barrier and intoxication can develop starting at levels 50 to 150 mg per dl (Diamond & Messing, 1994), which is roughly equivalent to readings by a portable breath test of 0.05% to 0.15%.

1.5.1 Long-Term Effects

Alcohol has been suggested to play a key role in the development of certain cardiovascular diseases. In the United States, cardiovascular diseases contributes to more deaths annually than any other groups of diseases (United States. Dept. of Health and Human Services. Office of the Secretary. & National Institute on Alcohol Abuse and Alcoholism (U.S.), 2000). The long-term effects of alcohol consumption are well documented. Moderate to heavy drinking (i.e. greater than one drink a day for women and greater than two drinks a day for men) leads to hypertension (Zilkens *et al.*, 2005; Lichtenstein *et al.*, 2006; Kloner & Rezkalla, 2007; Saremi & Arora, 2008; van de Wiel & de Lange, 2008; Klatsky, 2009; Wakabayashi, 2009), and this is likely caused, in part, by overstimulation of the sympathetic nervous system (Johnson *et al.*, 1986; Grassi *et al.*, 1989; Iwase *et al.*, 1995; Randin *et al.*, 1995; Kloner & Rezkalla, 2007). Moderate to heavy drinking can also lead to cancer, stroke, and cardiomyopathy among other health risks (Kloner & Rezkalla, 2007; Saremi & Arora, 2008; Klatsky, 2009). However, light drinking (i.e. not more than one drink a day for women and not more than 2 drinks a day for men) may lower the risk of several diseases. More specifically, cardiovascular risk associated with alcohol appears to have a J-shaped curve for mortality, coronary heart disease, blood pressure, and other cardiovascular events (Sun & Reis, 1996; Di Castelnuovo *et al.*, 2002; Kloner & Rezkalla, 2007; van de Wiel & de Lange, 2008; Klatsky, 2009). Light drinking lowers the risk for several of these cardiovascular diseases, but any more than this recommended amount and risks appear to increase. The benefits of alcohol, no matter what the type, could be due to the anticoagulant and anti-inflammatory properties they contains, as well as the ability to increase HDL levels (Sun & Reis, 1996; Di Castelnuovo *et al.*, 2002; Kloner & Rezkalla, 2007; van de Wiel & de Lange, 2008; Klatsky, 2009).

1.5.2 Short-Term Effects

Whereas the negative effects of the long-term consumption of alcohol are well documented, the short-term effects of moderate alcohol consumption remain controversial. Most results investigating the influence of short-term alcohol ingestion are documented after subjects reach mild intoxication levels. Results from these studies demonstrate that acute alcohol consumption generally increases MSNA (Zsoter & Sellers, 1977; Grassi *et al.*, 1989; Iwase *et al.*, 1995; Randin *et al.*, 1995; van de Borne *et al.*, 1997; Spaak *et al.*, 2008), along with heart rate (Giles *et al.*, 1982; Kupari, 1983; Stott *et al.*, 1987; Grassi *et al.*, 1989; Iwase *et al.*, 1995; Randin *et al.*, 1995; van de Borne *et al.*, 1997; Narkiewicz *et al.*, 2000; Yoda *et al.*, 2005; Takahashi *et al.*, 2008).

The relationship between acute alcohol consumption and blood pressure is not as clear as the MSNA studies. Many studies showed that after ingestion, resting MAP remains unchanged (Chaudhuri *et al.*, 1994; Tomaszewski *et al.*, 1995; van de Borne *et al.*, 1997; Narkiewicz *et al.*, 2000; Tateoka *et al.*, 2007; Spaak *et al.*, 2008; Takahashi *et al.*, 2008) or increases (Grassi *et al.*, 1989; Nixon *et al.*, 1989; Iwase *et al.*, 1995; Randin *et al.*, 1995)

The effect that alcohol consumption has on the vasculature is also unclear. Most studies have demonstrated that at rest, alcohol ingestion results in peripheral vasodilation (Altura *et al.*, 1979; Kupari, 1983; Johnson *et al.*, 1986; Malpas *et al.*, 1990; van de Borne *et al.*, 1997), greater increases in skin blood flow (Fewings *et al.*, 1966; Gillespie, 1967; Iwase *et al.*, 1995; Wolf *et al.*, 1999; Yoda *et al.*, 2005), and a decrease in peripheral resistance (Kupari, 1983; van de Borne *et al.*, 1997). However, other studies report no changes in flow mediated dilation after alcohol consumption (Chaudhuri *et al.*, 1994; Spaak *et al.*, 2008) and no change in forearm vascular resistance (Narkiewicz *et al.*, 2000). It may be that alcohol consumption results in *variable* effects depending on which vascular beds are being examined (Johnson *et al.*, 1986).

Several studies report an increase in blood flow to the splanchnic region after alcohol consumption (Carmichael *et al.*, 1988; Orrego *et al.*, 1988; Chaudhuri *et al.*, 1994; Israel *et al.*, 1994), as well as an augmentation to gastrointestinal blood flow and a

drop in systemic vascular resistance (Tateoka *et al.*, 2007). The vasculature of the splanchnic region has an critical role in the maintenance of blood pressure because it has the greatest volume of blood of all the regional beds (Chaudhuri *et al.*, 1994) (Taneja *et al.*, 2007).

Alcohol has also been shown to alter plasma hormone levels (Johnson *et al.*, 1986), but the results are not conclusive. Many studies have reported that acute ingestion increases plasma NE (Eisenhofer *et al.*, 1984; Ireland *et al.*, 1984; Randin *et al.*, 1995) and inhibits vasopressin release (Eisenhofer & Johnson, 1982). Alcohol may also increase plasma E (Ireland *et al.*, 1984) and has shown to have contradictory effects on plasma cortisol (Jenkins & Connolly, 1968; Linkola *et al.*, 1979; Ireland *et al.*, 1984).

1.5.3 Acute Alcohol Consumption and an Orthostatic Challenge

Previous studies have demonstrated that acute alcohol consumption leads to a decreased blood pressure response during a progressive lower body negative pressure protocol (Eisenhofer *et al.*, 1984; Narkiewicz *et al.*, 2000). Narkiewicz et al. (2000) also reported a blunted forearm vascular resistance (FVR) response in the alcohol group during progressive LBNP**.** These responses may be due to a lack of blood vessel constriction (Eisenhofer *et al.*, 1984; Narkiewicz *et al.*, 2000). However, it was not determined if the blunted vasoconstriction was due to a decreased sympathetic response during the orthostatic challenge (Narkiewicz *et al.*, 2000), or whether the sympathetic response was still present, but the dilator effect of alcohol suppresses the vasoconstriction (van de Borne *et al.*, 1997; Wolf *et al.*, 1999; Narkiewicz *et al.*, 2000).

To date, no studies have examined the influence of acute alcohol ingestion and direct neural recordings of MSNA during an orthostatic stress. It is possible that a decrease in MSNA could contribute to alcohol-mediated hypotension during an orthostatic challenge. The purpose of this study was to understand the role that MSNA may have in the regulation of the blood pressure during lower body negative pressure (LBNP) after acute alcohol consumption. **We hypothesized that alcohol ingestion would blunt arterial blood pressure and MSNA responses to progressive LBNP.**

Chapter 2
METHODS[2]

2.1 Subjects

Thirty subjects (23 males, 7 females) from the local community of Houghton, Michigan volunteered to participate in this study. There were 15 subjects in the alcohol group (12 males, 3 females: age 23 ± 1 yrs, height 180 ± 3 cm, weight 86 ± 3 kg, BMI 27 ± 1 kg/m^2) and 15 subjects in the placebo group (11 males, 4 females: age 25 ± 1 yrs, height 175 ± 2 cm, weight 76 ± 3 kg, BMI 25 ± 1 kg/m^2). Subjects were randomly assigned to either the alcohol or placebo group. All participants had no personal or family history of substance abuse or cardiovascular disease. Subjects had to be at least 21 years of age. All females were tested in the early follicular phase of their menstrual cycle, since it has been shown that MSNA can vary depending on the phase of the menstrual cycle (Carter *et al.*, 2009; Fu *et al.*, 2009). This study was approved by the Michigan Technological University Institutional Review Board (M0472) and all subjects provided written consent prior to the study.

2.2 Procedures

Subjects arrived at the laboratory at 7:30 AM after undergoing an overnight fast and from abstaining from alcohol for a minimum of 72 hours and from caffeine and exercise for at least 12 hours. Upon arrival, subjects were given two granola bars (Nature Valley, General Mills Sales) to eat and water if needed in an attempt to control for the amount of food in the gastrointestinal tract during testing. Subjects filled out the subject information sheet and consent form and took a preliminary breath test. Their height and weight were then recorded. Following a five minute resting period, three blood pressures

[2] The material in this chapter was previously published in the American Journal of Physiology – Endocrinology and Metabolism.

Carter JR, Stream SF, Durocher JJ & Larson RA. (2011). Influence of acute alcohol ingestion on sympathetic neural responses to orthostatic stress in humans. *American journal of physiology Endocrinology and metabolism* **300,** E771-778.

were taken with an automated sphygmomanometer in the seated position with each measurement separated by a minute. After these measurements, the participants were asked to use the restroom to void their urine and to change into shorts. Subjects were then instructed to lay supine in the LBNP chamber with their lower torso inside the chamber. All participants wore a neoprene skirt that created a vacuum right below the subjects' iliac crest, which would allow venous blood to be shifted from the upper torso to the lower extremities (Mano & Iwase, 2003; Freeman, 2006). This simulates an orthostatic stress. Subjects were then prepped to record heart rate, blood pressure, blood flow, and MSNA.

Subjects underwent five minutes of resting baseline data, followed by a progressive LNBP protocol that consisted of three minutes each at -5, -10, -15, -20, -30, and -40 mmHg (pre-treatment). Following this treatment, subjects remained supine and consumed either the alcohol or placebo through a straw, depending on what group they were randomly assigned to. Participants had 15 minutes to drink at which time their heads were supported at an incline to make it easier to ingest the liquid. Following this 15 minutes, there was a 30 minute waiting period to allow for blood alcohol levels to reach their peak. After this 45 minute period in which no measurements were recorded, subjects repeated the 5 minute baseline and the progressive LBNP protocol (post-treatment). Table 2.1 outlines the experimental timeline. Subjects were carefully monitored for presyncopal symptoms and continuously asked for feedback. If any signs did occur such as a drop in systolic blood pressure less than 80 mmHg, profuse sweating, light headedness, or nausea, the experiment was stopped immediately.

The participants were blinded to as which treatment they would be receiving. The alcohol group consumed 2.5 mL/kg body mass of 40% vodka (0.8 g ethanol/kg body mass) diluted in a 1:4 mixture of Crystal Light (Kraft Foods Global, Inc.) and the placebo group ingested 12.5 mL/kg body mass of Crystal Light. The amount of liquid was the same whether the subject drank the alcohol or placebo. Crystal light was chosen to mask

Table 2.1
Timeline of the experimental protocol.

Baseline	Progressive LBNP	Treatment (Alcohol or Placebo)	Baseline	Progressive LNBP
5 min	18 min	15 min ingestion 30 min rest	5 min	18 min

the taste of the alcohol and at the same time be low in sugar. The rims of all the glasses were also wiped with vodka in another attempt to disguise the drink. The amount of alcohol used in this experiment would be roughly equivalent to four or five drinks for the average participant, considering that 1.5 ounces of vodka is equivalent to one standard drink (Lichtenstein *et al.*, 2006; (NIAAA), 2012). This was consistent with what other studies had used.

2.3 Measurements

2.3.1 Heart Rate

Heart rate was measured using a three-lead electrocardiogram. Two electrodes were placed on the shoulders and one was placed on the lower left rib cage. Baseline heart rates were successfully recorded on all 30 subjects for both pre- and post-treatment. However, one placebo subject reached presyncope during the post-treatment LBNP protocol, making our heart rate measurements for the entire study (baseline plus LBNP), n=15 for alcohol and n=14 for placebo.

2.3.2 Blood Pressure

After an initial five minute resting period when subjects first arrived to the laboratory, three blood pressures each separated by a minute, were recorded in the seated position using an automated sphygmomanometer (Omron HEM-907XL; Omron Health Care). The automated sphygmomanometer was also used to record three supine blood pressures (each separated by a minute) immediately before the pre-treatment and post-

treatment protocols. The average of these three blood pressures was used to determine baseline values. Beat-to-beat blood pressure was also recorded throughout the entire experiment using the Finometer (Finapres Medical Systems, Amsterdam, The Netherlands). The Finometer is a noninvasive blood pressure acquisition. It was used to precisely record the relative changes in the blood pressure and the readings from the automated sphygmomanometer were used for absolute baseline measurements. Blood pressures were recorded as systolic, diastolic, and mean arterial pressures. Mean arterial pressures were calculated as DAP plus one-third of the pulse pressure (SAP minus DAP). All blood pressures were taken on the right limbs of the subjects. Baseline measurements were obtained on all 30 subjects for both pre-and post-treatment. For the entire study (baseline plus LBNP), n= 15 for alcohol and n=14 for placebo (due to the presyncopal participant).

2.3.3 Muscle Sympathetic Nerve Activity

Microneurography is a technique that allows direct measurement of autonomic function, more specifically, the sympathetic nerve activity to the vasculature of muscle beds (Freeman, 2006). Microneurography was performed on the right leg of each subject. Multifiber recordings of MSNA were obtained by inserting a sterilized tungsten microelectrode into the superficial peroneal nerve located in the popliteal region behind the knee. A reference electrode was placed roughly two centimeters from this recording electrode. The nerve signal was amplified (80,000 gain), band-pass filtered (700-2,000 Hz), and integrated at a time constant of 0.1 seconds to obtain a mean voltage display of nerve activity. This signal has a latency period of approximately 1.3 seconds after an R-wave occurs. MSNA recordings were considered satisfactory when spontaneous pulse synchronous bursts did not change during auditory stimulation or stroking of the skin and increased during end-expiratory apnea.

MSNA can be quantified by: 1) burst frequency, often recorded as bursts per minute 2) burst per 100HB, since MSNA is linked to the cardiac cycle, and 3) total MSNA, which is a combination of burst frequency and the area under each burst. Microneurography is fast acting and measurements can be taken instantaneously. It is

safe, can be recorded for an extended period of time, burst pattern and activity is similar among different nerve sites, and it is highly reproducible from day to day (Freeman, 2006). 18 individuals (9 placebo and 9 alcohol) had successful MSNA recordings that were obtained throughout the entire experiment (baseline plus LBNP). 25 subjects (13 alcohol and 12 placebo) had successful MSNA recordings for the baselines of both pre-treatment and post-treatment.

2.3.4 Alcohol Content

The subject's alcohol level was measured using a portable breath analyzer (Alco-Sensor III, Intoximeters) borrowed from Michigan Tech Public Safety. The portable breath analyzer uses the breath alcohol content to estimate the blood alcohol content. The laboratory was given demonstrations and adequate instruction on how to properly use the portable breath analyzer. Upon arrival to the laboratory, the subjects were given a breath test to ensure that they did not have any alcohol already in their system. The subjects were also given a breath test immediately before starting the post-treatment and also at the end of the post-treatment protocol. Blood alcohol readings were obtained for all 30 participants. Following completion of the study, alcohol subjects were given snacks and water and were continuously monitored. When their blood alcohol content measured less than 0.075% they were allowed to exit the laboratory. All participants had to have their transportation arranged ahead of time and the subjects in the alcohol group were required to sign a voluntary waiver in which they agreed to abstain from operating a motor vehicle for 24 hours upon completion of the study.

2.3.5 Blood Flows

Forearm blood flow (FBF) was measured using venous occlusion plethysmography (EC6; D.E. Hokanson, Bellevue, WA) during the baseline and LBNP stages of both protocols. Mercury-in-silastic strain gauges were placed around the maximal circumference of the subject's left forearm. Cuffs were placed on the left wrist and also on the upper left arm of the subject. The wrist cuff was inflated to 220 mmHg to arrest circulation to the hand. The upper arm cuff was continuously inflated (8 seconds) to 60 mmHg and deflated (7 seconds) for a 15 second cycle. When inflated, the upper

cuff occluded venous blood flow but still allowed arterial blood flow. FBF was measured in milliliters per 100 milliliters per minute and used to calculate vascular resistance and vascular conductance. Forearm vascular resistance (FVR) was calculated as MAP divided by FBF, whereas forearm vascular conductance (FVC) was the reciprocal of this. Baseline measurements were obtained on 15 alcohol subjects and 14 placebo subjects. Blood flows were successfully measured on all 15 of the alcohol subjects for the duration of the experiment, and due to the presyncopal subject, measurements were obtained on 13 placebo participants.

2.4 Data Analysis

Data were imported and analyzed in the WinCPRS software program (Absolute Aliens, Turku, Finland). R-waves were identified from the electrocardiogram and marked in the time series. MSNA bursts were automatically detected on the basis of amplitude using a signal-to-noise ratio of 3:1 within a 0.5 second search window centered on a 1.3 second expected burst peak latency from the previous R-wave. Potential bursts were displayed and edited by one trained investigator that was blinded to the intervention. The average burst area occurring during baseline was normalized to a mean value of 100. MSNA was expressed as bursts per minute, bursts per 100 heart beats, and total MSNA (i.e. the sum of normalized burst areas per minute).

2.5 Statistical Analysis

All collected data were analyzed using commercial software (SPSS 15.0, SPSS, Chicago, IL). Subject characteristics for alcohol and placebo groups were compared using independent t-tests. Repeated measures ANOVA with time (progressive LBNP stages) and treatment (pre- vs. post-treatment) as the within factor variables and drug (alcohol vs. placebo) as the between-factor variable to analyze values at rest (drug × treatment interaction) and throughout the LBNP protocol (drug × time × treatment interaction). When significant drug × time × treatment interactions were observed, each group was analyzed separately as time × treatment, and a priori post hoc analyses of treatment were performed when a time × treatment interaction was significant. One-tailed

analyses were performed on blood pressure, MSNA, and blood flow responses based on our directional hypotheses, which were guided by prior literature. Means were considered significantly different when $P \leq 0.05$. Results are expressed as means ± SE.

Chapter 3
RESULTS[3]

3.1 Baseline Responses

Pre- and post-treatment baseline values for alcohol and placebo are shown in Table 3.1. SAP, DAP, and MAP all increased significantly from pre-treatment to post-treatment in both alcohol and placebo (treatment, $P < 0.05$; drug × treatment, $P > 0.05$). HR, MSNA bursts/min, and BAC were elevated in alcohol post-treatment (treatment, $P < 0.05$), but not placebo. HR, MSNA bursts/min, and BAC were also significantly different in the alcohol group than compared with the placebo group (drug × treatment, $P \leq 0.05$). Blood flows remained the same in both treatments and there was no difference between alcohol and placebo.

[3] The material in this chapter was previously published in the American Journal of Physiology – Endocrinology and Metabolism.

Carter JR, Stream SF, Durocher JJ & Larson RA. (2011). Influence of acute alcohol ingestion on sympathetic neural responses to orthostatic stress in humans. *American journal of physiology Endocrinology and metabolism* **300,** E771-778.

Table 3.1
Pre- and post-treatment baseline values for alcohol and placebo groups.

	Alcohol		Placebo	
Variable	Pre-treatment	Post-treatment	Pre-treatment	Post-treatment
SAP, mmHg	120 ± 3	124 ± 4*	117 ± 2	123 ± 2*
DAP, mmHg	64 ± 2	69 ± 2*	64 ± 2	71 ± 2*
MAP, mmHg	83 ± 2	87 ± 2*	82 ± 2	88 ± 1*
HR, beats/min	59 ± 2	65 ± 2*†	59 ± 3	58 ± 3
MSNA, burst/min	13 ± 3	19 ± 4*†	15 ± 2	15 ± 2
MSNA, bursts/100 HB	23 ± 5	30 ± 5	25 ± 3	26 ± 3
Total MSNA (a.u.)	6330 ± 1312	8535 ± 1479	6630 ± 750	6958 ± 806
FBF, unit	2.1 ± 0.2	2.1 ± 0.2	2.1 ± 0.3	2.0 ± 0.2
FVR, mmHg/unit	45 ± 4	47 ± 3	46 ± 5	49 ± 4
FVC*100, unit/mmHg	2.5 ± 0.2	2.4 ± 0.2	2.5 ± 0.3	2.3 ± 0.2
BAC, %	0.00 ± 0.00	0.08 ± 0.01*†	0.00 ± 0.00	0.00 ± 0.00

Values are means ± SE (n = 15 for alcohol and n = 15 for placebo unless otherwise noted). SAP, systolic arterial pressure; DAP, diastolic arterial pressure; MAP, mean arterial pressure; HR, heart rate; MSNA muscle sympathetic nerve activity (n = 13 for alcohol and n = 12 for placebo); HB, heart beats; a.u., arbitrary units; FBF, forearm blood flow; unit = ml/100ml/min; FVR, forearm vascular resistance; FVC forearm vascular conductance (n = 15 for alcohol and n = 14 for placebo, all forearm variables); BAC, blood alcohol content. *$P < 0.05$ pre- vs. post-treatment baseline data in respective groups. †$P \leq 0.05$ for alcohol vs. placebo groups. (Carter *et al.*, 2011).

3.2 Progressive LBNP Responses

3.2.1 Hemodynamic Responses

Figure 3.1 and 3.2 demonstrate the responses showed by SAP, DAP, MAP (Figure 3.1) and HR (Figure 3.2) during progressive LBNP. Arterial pressures were significantly attenuated after alcohol but not placebo (drug × time × treatment, $P < 0.05$). HR responses increased during progressive LBNP, but there was no difference between pre- and post-treatment.

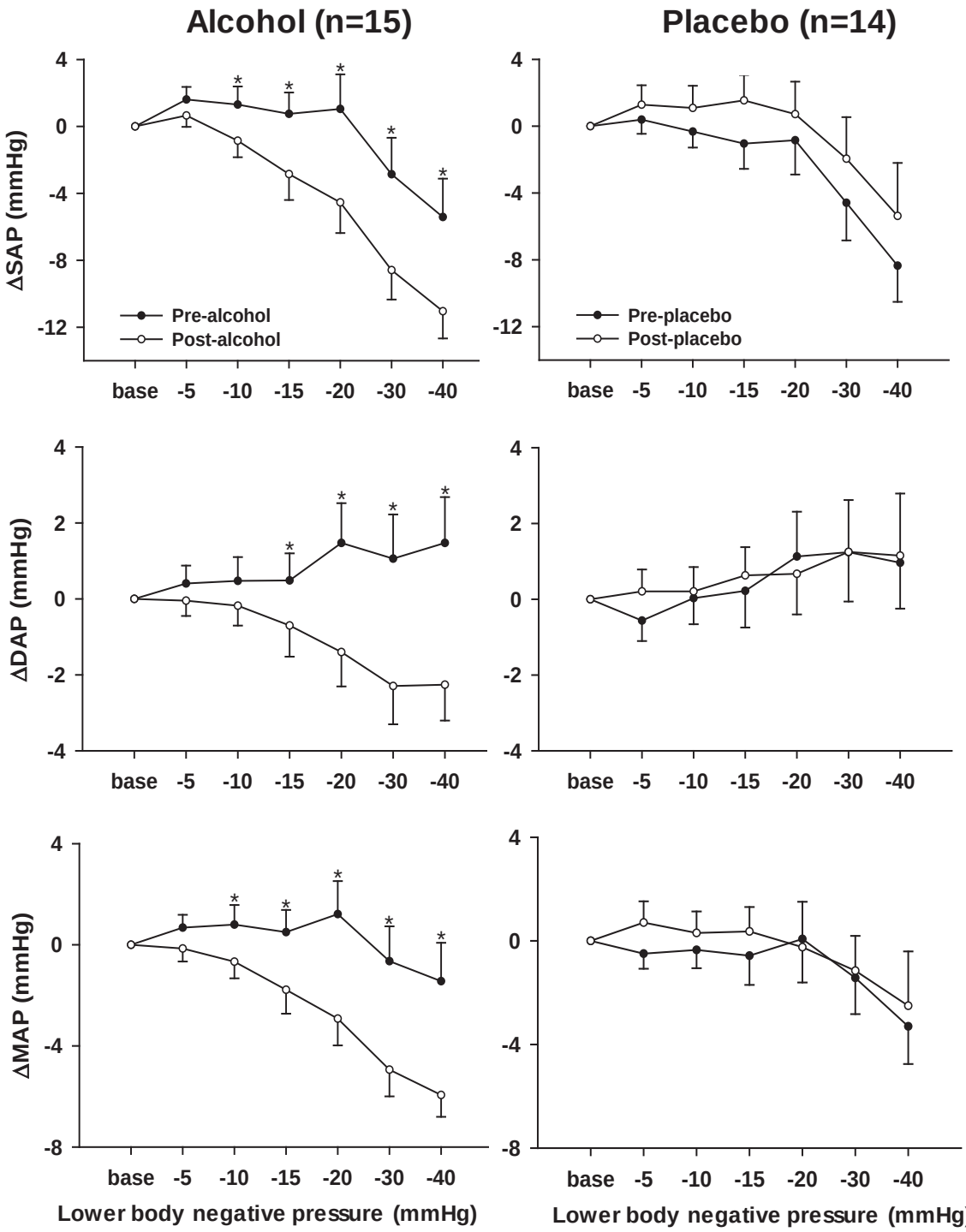

Figure 3.1 Changes (Δ) in SAP, DAP, and MAP during progressive LBNP. *n*=15 for alcohol and *n*=14 for placebo. Alcohol blunted arterial blood pressure responses. *P < 0.05 vs. corresponding post-alcohol value. SAP, systolic arterial pressure; DAP, diastolic arterial pressure; MAP, mean arterial pressure. (Carter *et al.*, 2011).

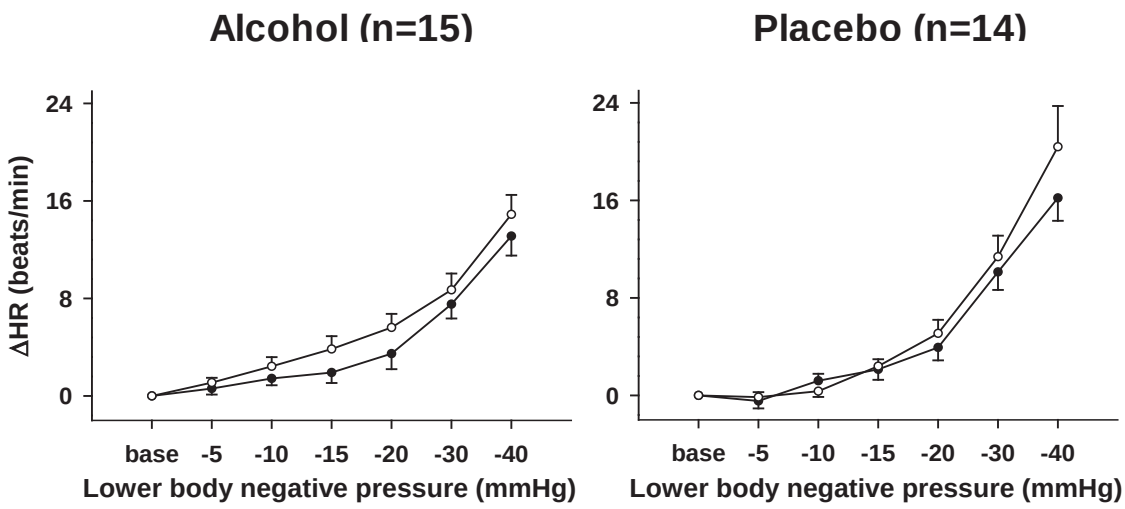

Figure 3.2 Changes (Δ) in HR during progressive LBNP. *n*=15 for alcohol and *n*=14 for placebo. Progressive LBNP elicited similar increases in HR during both treatments (pre- vs. post-treatment) and groups (alcohol vs. placebo). Drug × time × treatment interactions were $P > 0.05$ for HR, whereas the time effect was $P < 0.001$ for each treatment in both groups. HR, heart rate. (Carter *et al.*, 2011).

3.2.2 Sympathetic Responses

Figure 3.3 portrays similar findings to the HR response. MSNA responses all increased during progressive LBNP for both alcohol and placebo, but there was no difference between pre- and post-treatments.

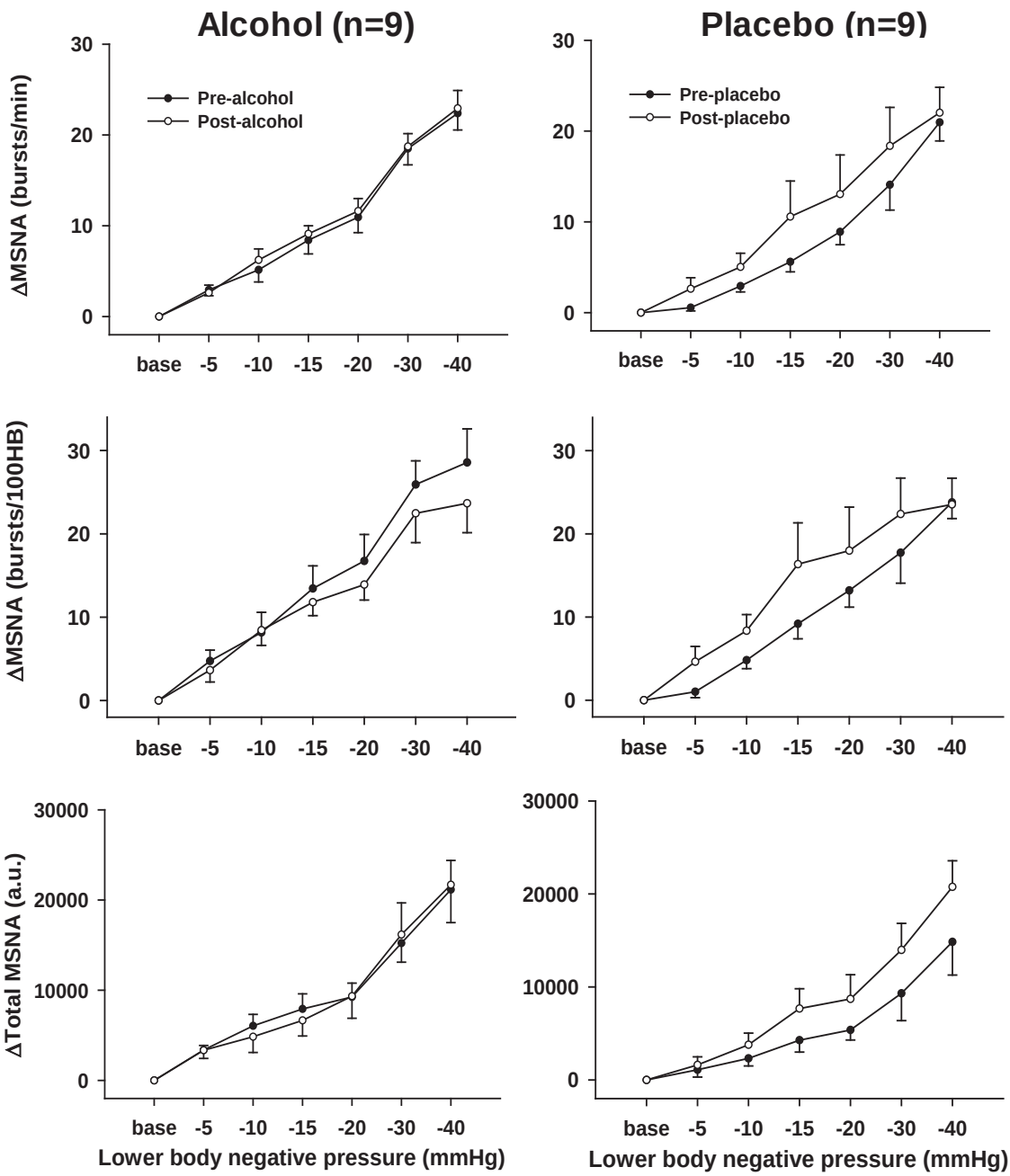

Figure 3.3 Changes (Δ) in MSNA qualified as bursts/min, bursts/100 HB, and total MSNA during progressive LBNP. Progressive LBNP elicited similar increases in MSNA during both treatments (pre- vs. post-treatment) and groups (alcohol vs. placebo). Drug × time × interactions were $P > 0.05$ for all MSNA variables, whereas the time effect was $P < 0.001$ for each treatment in both groups. MSNA, muscle sympathetic nerve activity; HB, heartbeat; a.u., arbitrary units. (Carter *et al.*, 2011).

3.2.3 Vasculature Responses

Lastly, Figure 3.4 demonstrates the responses showed by FBF, FVR, and FVC to progressive LBNP. Increases in FVR were significantly blunted (drug × time × treatment, $P < 0.05$) during progressive LBNP following the consumption of alcohol, whereas FVR responses were not altered by placebo. Changes in FBF and FVC were not different between pre- and post-treatment in the alcohol or placebo groups during progressive LBNP.

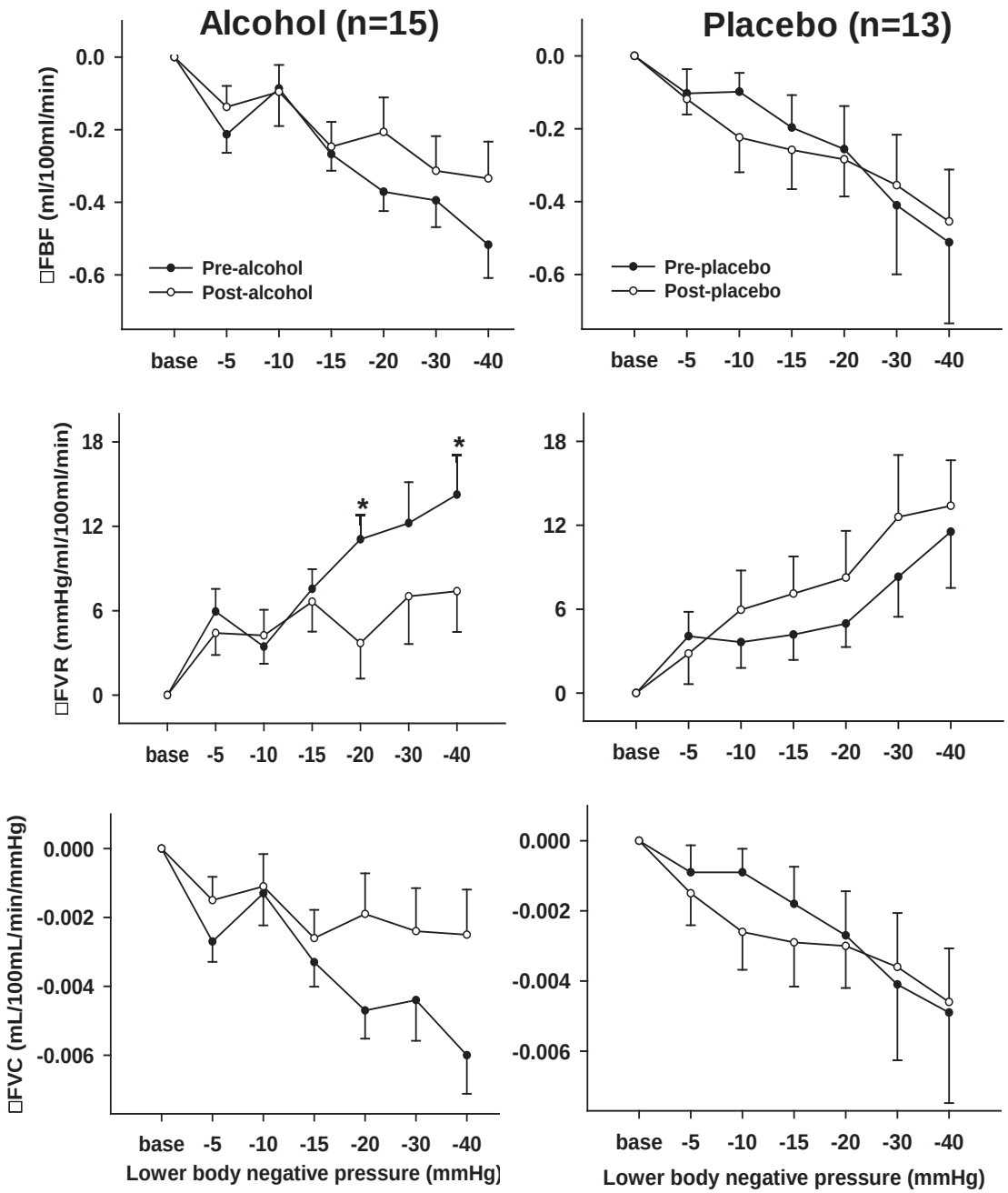

Figure 3.4 Changes (Δ) FBF, forearm vascular resistance (FVR), and forearm vascular conductance (FVC) during progressive LBNP. Increases in FVR were significantly blunted by alcohol (group × time × treatment, $P < 0.05$). Drug × time × treatment interactions were $P > 0.05$ for both FBF and FVC, whereas the time effect was $P < 0.01$ for each treatment in both groups. *$P < 0.05$ vs. corresponding post-alcohol value. FBF, forearm blood flow; FVR, forearm vascular resistance; FVC, forearm vascular conductance. (Carter *et al.*, 2011).

Chapter 4
DISCUSSION

We investigated sympathetic neural and cardiovascular responses to lower body negative pressure both before and after ingestion of alcohol or placebo. There are three primary findings. First, alcohol consumption increased resting HR and MSNA burst frequency. Second, alcohol blunted the arterial blood pressure responses during progressive LBNP, yet there was no difference in MSNA responses pre-and post-treatment. Third, alcohol compromises increases in FVR during progressive LBNP, but has no effect on FVC.

Previous studies have shown that acute alcohol consumption raises resting HR (Zsoter & Sellers, 1977; Giles *et al.*, 1982; Kupari, 1983; Stott *et al.*, 1987; Grassi *et al.*, 1989; Iwase *et al.*, 1995; Randin *et al.*, 1995; van de Borne *et al.*, 1997; Narkiewicz *et al.*, 2000; Yoda *et al.*, 2005; Takahashi *et al.*, 2008), resting MAP (Grassi *et al.*, 1989; Iwase *et al.*, 1995; Randin *et al.*, 1995), and MSNA (Grassi *et al.*, 1989; Iwase *et al.*, 1995; Randin *et al.*, 1995; van de Borne *et al.*, 1997; Spaak *et al.*, 2008). This study also demonstrated increases in resting HR and MSNA bursts/min after alcohol, but not placebo consumption. In contrast, both placebo and alcohol demonstrated increases in resting blood pressure after drink ingestion.

Stott et al. (1987) also reported increases in arterial blood pressure in both placebo and alcohol groups after acute ingestion. It would be easy to assume that the increase in arterial pressure is due to an increase in plasma volume, which would increase CO and thus, MAP. This may or may not be the case in both the present study and others (Stott *et al.*, 1987). Jordan (2000) demonstrated that if a 175 pound (79 kg) person consumed 500 mL of water, the total plasma volume would only change by one percent. Almost all of the water would be dispersed into the intra- and extracellular space and would only increase plasma volume by a total of 35 ml (Jordan, 2002). However, our subjects, on average, consumed 1,000 mL of water. Most studies that look at water drinking and blood pressure responses examined a maximum of 500 mL consumption (Jordan *et al.*, 2000; Endo *et al.*, 2002; Jordan, 2002; Claydon *et al.*, 2006; Callegaro *et al.*, 2007).

Jordan et al. (2000) found that in patients with severe autonomic failure, drinking 480 mL of tap water vs. 240 mL caused a much greater increase in SAP. The effects of water consumption may be dose dependent, and the effect of more than 500 mL of water ingestion should be examined further.

Arterial blood pressure reactivity to acute water ingestion appears to be quite variable. Jordan et al. (2000) found that 480 mL of tap water caused a significant increase in SAP in patients with severe autonomic failure or in the older control group, but not the younger control group. HR did not increase in the younger control group either. Callegaro et al. (2007) displayed that in normotensive subjects who ingested 500 mL of water, SAP, DAP, and MSNA all increased significantly compared with baseline measurements. Claydon et el. (2006) showed that in patients who consumed 500 mL of water, MAP significantly increased, but HR remained the same. Endo et al. (2002) found that after 500 mL of water ingestion significantly increased MAP and HR, and significantly decreased MSNA. It is evident that the research is inconclusive. However, collectively it appears as if drinking a large amount of water in a short amount of time can elicit acute increases in arterial blood pressure. However, how this influences HR or MSNA remains debatable. Many of these studies suggest that the increase in blood pressure may be due to enhanced sympathetic activation (Jordan *et al.*, 2000; Jordan, 2002; Claydon *et al.*, 2006; Callegaro *et al.*, 2007), and this may be stimulated by the osmolality of the liquid being ingested (Brown *et al.*, 2005; Claydon *et al.*, 2006; May & Jordan, 2011).

Another potential explanation for the increases in arterial blood pressure in both the alcohol and placebo groups could be distension in the lower torso. Many of our subjects stated that they wish they could have voided their urine before the post-treatment protocol began; however this was not an option with the microneurography technique. Hvarness et al. (1999) demonstrated that a “full bladder” and the urge to urinate increased mean blood pressure in 12 healthy females. Fagius et al. (1989) not only demonstrated an increase of blood pressure with bladder distension, but also an increase in MSNA as well. Since alcohol inhibits vasopressin release (Eisenhofer & Johnson, 1982), it might be

reasonable to assume that the alcohol group would have a greater gastric distention due to a greater urinary volume. However, Jones (1990) observed that the greatest urinary output after alcohol consumption occurs one to two hours after ingestion, which would have occurred following the completion of the experiment. Although we did not measure gastric or bladder distension in this study, it is reasonable to assume similar distention in both groups (i.e., alcohol vs. placebo) because the total amount of liquid consumption was similar. We believe the physiological data and comparisons are valid given this placebo-based time control approach.

The increase in resting HR in the alcohol group appears to be due to increase in sympathetic activity and does not seem to be caused by withdrawal of the parasympathetic nervous system. Specifically, Eisenhofer et al. (1985) treated a group of subjects with atropine (a parasympathetic blockade) after ingestion of ethanol or placebo. At rest, HR was increased in the alcohol group when compared with placebo. After atropine, both alcohol and placebo elicited similar increases in HR and there was no difference between the two groups. Since the ethanol group did not demonstrate a greater increase in HR after atropine infusion, the increase in HR after ethanol ingestion is unlikely to be vagally mediated (Eisenhofer *et al.*, 1985).

Alcohol appears to elicit a paradoxical vasoconstriction and vasodilation. Specifically, acute alcohol consumption increases sympathetic activity to both the heart and blood vessels, but this sympathoexcitation appears to be opposed by peripheral vasodilation (van de Borne *et al.*, 1997; Wolf *et al.*, 1999). Our subjects demonstrated no change in resting FVR, despite the increase in MSNA. It may be that alcohol consumption results in variable effects depending on where the vascular beds are located throughout the body (Johnson *et al.*, 1986). However, others report that alcohol elicits peripheral vasodilation at rest (Altura *et al.*, 1979; Kupari, 1983; Johnson *et al.*, 1986; Malpas *et al.*, 1990; van de Borne *et al.*, 1997). Although MSNA is increasing, it is possible that the vasoconstriction is being attenuated by the direct vasodilator effect of alcohol on the peripheral blood vessels, and that is why there is no change in resting FVR.

How exactly alcohol causes peripheral vasodilation remains uncertain, but there are many studies that have looked at alcohol's effect on the peripheral vasculature. It has been reported that alcohol might depress α-adrenoceptor-mediated vasoconstriction by reducing the sensitivity of the α-adrenoceptors (Takeda & Momose, 1983; Eisenhofer *et al.*, 1984). Norepinephrine is an α-agonist, and is important for increasing vasoconstriction in peripheral blood vessels. Eisenhofer et al. (1984) studied subjects after they were given NE infusions alone or NE plus ethanol. The group who received NE plus ethanol had an increase in DAP, but this response was blunted compared to the group who had only received NE infusions. The ethanol plus NE group demonstrated higher levels of plasma NE. This indicates that ethanol increases plasma NE, perhaps through sympathetic activation. More importantly, this study displays that although blood pressure increases, its response is blunted and this may be due to the direct actions of alcohol on the periphery. Different subjects in this same study were also given methoxamine (which is an α_1 agonist) or methoxamine plus ethanol. DAP and SAP increases were significantly attenuated in the methoxamine plus ethanol group. Since methoxamine is an α_1 agonist, these results reveal that alcohol has an inhibitory effect on α-adrenoceptor mediated vasoconstriction (Eisenhofer *et al.*, 1984). Similarly, Takeda et al. (1983) looked at the effects of ethanol in the guinea-pig. They noticed that contractile responses to stimulation of adrenergic nerves were reduced as well as the contractile responses to NE infusion (Takeda & Momose, 1983).

Other studies also support the idea of peripheral vasodilation due to alcohol's effect on the blood vessels. Gillespie et al. (1967) studied six patients who had at least one sympathectomized upper limb. After oral ingestion of whiskey, these subjects had a robust increase in skin blood flow. This demonstrates that the vasodilator properties associated with alcohol consumption are not produced by central inhibition (Gillespie, 1967). Altura et al. (1979) looked at the vasculature effects of ethanol in young male rats. Ethanol inhibited the vasoconstrictor effects of NE, angiotensin, and vasopressin; ethanol also had a direct effect on the smooth muscle itself. They concluded that alcohol caused peripheral vasodilation by direct relaxation of blood vessels, and blunted the

vasoconstrictor responses of vasoactive substances (Altura *et al.*, 1979). Finally, Randin et al. (1995) provided subjects alcohol plus phentolamine, which is an α-adrenergic blockade. After this infusion, blood pressure and calf vascular resistance actually decreased and HR still increased. This finding reinforces the significance of sympathetic activation in response to alcohol ingestion (Randin *et al.*, 1995), as well as the role of α-adrenoceptors in blood pressure regulation. In conclusion, the effects of alcohol on the vasculature seem to depend upon both sympathetic activation to the heart and blood vessels, and peripheral vasodilation (van de Borne *et al.*, 1997; Wolf *et al.*, 1999). The peripheral vasodilation that occurs appears to be due to an impairment of the sensitivity of α-adrenoceptors. Nevertheless, at rest alcohol still exerts increases HR and MSNA.

The second main finding is that alcohol ingestion blunts arterial blood pressure responses to progressive lower body negative pressure, despite an increase in HR and MSNA. This is consistent with what other studies have reported (Eisenhofer *et al.*, 1984; Narkiewicz *et al.*, 2000). Eisenhofer et al. (1984) demonstrated a greater drop in SAP during four stages of LBNP after ethanol ingestion, despite greater increases in plasma NE. HR increased in both the alcohol and placebo group throughout LBNP, and there was no difference between groups. Due to the increase in NE and the drop in blood pressure, the authors concluded that ethanol exhibits inhibitory effects on vasoconstriction responses, specifically reduced responsiveness to α-adrenoceptor agonists (Eisenhofer *et al.*, 1984).

Narkiewicz et al. (2000) reported similar results after alcohol ingestion. At rest, HR increased compared to placebo, but there was no change in arterial blood pressure or FVR. However, they only used 400 mL of liquid compared to the roughly 1,000 that our study had used, which might explain the differences in resting arterial blood pressure findings between studies. During progressive LBNP, Narkiewicz et al. (2000) reported that MAP significantly decreased throughout the four stages in the alcohol group. FVR responses were also significantly blunted when compared to the placebo and this is similar to what we had demonstrated. Similar to Eisenhofer et al. (1984), HR increased throughout the LBNP protocol, but there was no different between alcohol or placebo.

Narkiewicz et al. (2000) concluded that this alcohol-induced hypotension was due to an impairment of vasoconstriction. However, they did not measure MSNA or plasma catecholamines, thus they could not conclude with certainty that the vasoconstriction was attenuated due to decreased sympathetic activity (Narkiewicz *et al.*, 2000).

Our study was the first to examine MSNA responses during graded LBNP after acute ethanol ingestion. MSNA typically increases during progressive LBNP through a baroreflex response (Sundlof & Wallin, 1978), and peripheral vasoconstriction often depends on the degree of MSNA activation (Fu *et al.*, 2002; Mano & Iwase, 2003; Wallin, 2006). TPR must increase in order to maintain a stable blood pressure. Our study confirms that reductions of blood pressure during LBNP are more dramatic after alcohol, but these responses do not appear to be related to any attenuation in MSNA or HR; we demonstrate similar increases of MSNA and HR during LBNP before and after alcohol consumption. However, the greater decreases in blood pressure after alcohol were associated with greater reductions in FVR. As mentioned previously, alcohol seems to elicit sympathetic activation that is directly opposed by the dilator effects of alcohol. The similar increases in MSNA and HR both pre- and post-treatment in both alcohol and placebo groups demonstrate that the sympathetic reactivity to LBNP after alcohol is intact. This is also supported by the enhanced increase in NE in the alcohol group during LBNP in the study done by Eisenhofer et al. (1984). However, the blunted FVR response that we and Narkiewicz et al. (2000) report exists despite preserved increases in MSNA, a finding we attribute to the impairment on α-adrenoceptors discussed previously (Takeda & Momose, 1983; Eisenhofer *et al.*, 1984). We suggest that a decrease in α-adrenergic receptor sensitivity attenuates vasoconstrictor effect of increased sympathetic activity (Wallin, 2006), and that this resulted in the more dramatic reductions in arterial blood pressure during LBNP after ethanol ingestion.

While blunted α-adrenergic receptor sensitivity remains a viable explanation for our findings, another interpretation could be that alcohol alters sympathetic baroreflex function. MSNA is mediated by the arterial baroreflex. Since there was a greater drop in blood pressure during LBNP after alcohol consumption, it is reasonable to speculate that

there should have been an augmentation in MSNA during graded LBNP (i.e., classic negative feedback control). Others suggest an impairment of the baroreflex function after alcohol (Zsoter & Sellers, 1977; Abdel-Rahman *et al.*, 1987; Narkiewicz *et al.*, 2000). A decrease in baroreflex sensitivity could have contributed to the lack of MSNA adjustment in our subjects, despite the larger drop in blood pressure. Theoretically, this could lead to attenuated vasoconstriction during progressive LBNP, eventually leading to a greater decrease in blood pressure. However, with this being said, we found alcohol to cause an increase in resting MSNA burst frequency between pre- and post-treatment and this is consistent with the literature. Therefore, we do not believe alcohol would cause an augmentation in sympathetic outflow at rest and attenuation in sympathetic outflow during progressive LBNP. No matter the mechanism, it is evident acute alcohol ingestion can lead to hypotension upon an orthostatic challenge, which might precede syncope.

The last important finding in our study was that alcohol blunts FVR responses to LBNP after ethanol ingestion. This is similar to what Narkiewicz et al. (2000) demonstrated. However, Narkiewicz et al. (2000) did not report FVC. We did examine FVC, but found no changes at rest or during progressive LBNP between alcohol and placebo. Thus, caution should be taken when interpreting the blunted FVR results reported in the present study and others (Narkiewicz *et al.*, 2000). Changes in conductance appear to be a better indicator of regional vascular responses in maintaining blood pressure than changes in resistance (O'Leary, 1991). More specifically, when CO is not in a steady state (i.e. progressive LBNP), changes in resistance may not be as reliable as changes in conductance (O'Leary, 1991).

4.1 Summary

In summary, acute alcohol ingestion increased resting HR and MSNA burst frequency. During progressive LBNP, alcohol elicits more of a dramatic decrease in arterial blood pressure, despite similar increases in MSNA both pre- and post-alcohol. This can be interpreted two ways: 1) Alcohol reduces the sensitivity of α-adrenoceptors, causing a peripheral vasodilation despite the increase in MSNA. During progressive LBNP, when TPR must increase to maintain a stable blood pressure, the vasodilator

effects of alcohol attenuates the sympathetic-mediated vasoconstriction, which eventually results in a significant drop in arterial blood pressure. 2) Since arterial blood pressure and MSNA are inversely related, the more dramatic drop in arterial pressure in the alcohol group during LBNP should have been accompanied by an increase in MSNA. Thus, it remains plausible that alcohol decreases baroreflex sensitivity. Finally, although FVR responses were blunted during LBNP after alcohol ingestion, these responses should be interpreted with caution because FVC is more of a reliable measurement when CO is not in a steady state.

4.2 Clinical Relevance

This study is important in advancing our mechanistic understanding of how acute alcohol ingestion can lead to orthostatic hypotension, and eventually syncope. After consuming even one or two drinks, one should be cautious when moving from a supine or seated position to standing and postural changes should be done in a slow, careful manner. This is particularly significant if one has a history of neurally-mediated syncope, or is currently taking any medications that may cause low blood pressure, or is suffering from hypovolemia. Alcohol ingestion can potentially exacerbate any pre-existing conditions that may lead to OH.

4.3 Limitations and Future Work

Limitations of this study include the fact that our subjects had to consume a very large amount of liquid in a short amount of time. Many of them complained about having the urge to urinate before the post-treatment protocol even started. If the subjects did undergo gastric/bladder distension, we did not have the equipment available to monitor. However, the experimental approach (i.e., double-blinded, placebo-based) still allows for appropriate comparisons to test the stated hypotheses. Another limitation is that we did not measure any hormone levels throughout the experimental protocol. Measurements of NE and E would have given us valuable insight in to understanding what might have been happening at the peripheral level. Also, we did not attempt to elicit presyncope for the safety of our subjects. Therefore, we don't know what would have happened to

MSNA responses had the LBNP protocol been extended, but we can assume that responses would have followed the trend that we had observed.

Future investigations of acute alcohol ingestion and orthostatic stress might include ethanol infusions instead of oral ingestion. This would take out the possibility of gastric/bladder distension and also be more time efficient. However, this eliminates the role of gastric absorption, which may be important. Additionally, future work might also examine the responses of alcohol or placebo in combination with infusion of an α-agonist prior to progressive LBNP. If alcohol does decrease α-adrenoceptor sensitivity, then looking at the responses of α-agonists versus α-agonists plus ethanol would provide a better understanding of what occurs at the periphery. Lastly, having our subjects fill out a questionnaire about their alcohol consumption, similar to the one used by the National Institute on Alcohol Abuse and Alcoholism (Alcoholism, 2003), would provide a quantification of the drinking patterns of our subjects. We could then use their alcohol habits to see if there is a correlation with how they respond to orthostatic stress after acute alcohol consumption.

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Appendix A-1: Raw data for subject characteristics

Placebo

Subject	Sex	Age (years)	Height (cm)	Weight (kg)	BMI (kg/m^2)
1	F	21	177.5	48.2	15.3
2	M	23	169.5	71.0	24.7
3	M	23	176.0	77.2	24.9
4	M	33	183.0	94.1	28.1
5	M	23	183.0	79.3	23.7
6	F	28	162.0	61.8	23.5
7	M	30	169.5	74.0	25.8
8	M	26	187.0	95.4	27.3
9	M	28	172.0	78.0	26.4
10	M	23	180.5	73.0	22.4
11	F	21	165.0	62.8	23.1
12	M	21	175.0	80.5	26.3
13	F	24	178.0	78.7	24.8
14	M	26	170.0	71.5	24.7
15	M	23	183.0	94.0	28.1

Alcohol

Subject	Sex	Age (years)	Height (cm)	Weight (kg)	BMI (kg/m^2)
16	F	21	164.5	75.7	28.0
17	M	24	182.0	83.5	25.2
18	F	22	166.0	78.0	28.3
19	M	22	172.0	73.6	24.9
20	M	21	196.0	105.6	27.5
21	F	30	165.5	64.3	23.5
22	M	22	186.0	78.3	22.6
23	M	23	182.0	77.6	23.4
24	M	24	182.5	92.0	27.6
25	M	22	179.0	91.0	28.4
26	M	34	170.0	75.5	26.1
27	M	21	186.0	107.5	31.1
28	M	22	186.0	107.3	31.0
29	M	21	186.0	97.0	28.0
30	M	21	191.0	81.5	22.3

Appendix A-2: Raw data for resting seated blood pressures taken with an automated sphygmomanometer upon subject arrival.

Placebo

Subject	SAP (mmHg)	DAP (mmHg)	MAP (mmHg)
1	112.5	81.3	91.7
2	128.0	65.7	86.5
3	121.0	73.5	89.3
4	132.8	79.5	97.3
5	119.7	71.7	87.7
6	119.8	75.0	89.9
7	128.5	79.5	95.8
8	125.7	67.0	86.6
9	134.3	71.0	92.1
10	141.0	82.8	102.2
11	111.0	71.5	84.7
12	112.0	77.3	88.9
13	94.3	65.0	74.8
14	106.3	56.0	72.8
15	135.0	64.5	88.0

Alcohol

Subject	SAP (mmHg)	DAP (mmHg)	MAP (mmHg)
16	101.0	70.3	80.5
17	139.8	85.0	103.3
18	109.3	66.5	80.8
19	138.7	83.3	101.8
20	131.7	80.3	97.4
21	97.5	65.3	76.0
22	119.3	65.0	83.1
23	113.5	55.3	74.7
24	115.5	68.8	84.4
25	149.8	96.5	114.3
26	132.3	81.0	98.1
27	137.8	85.3	102.8
28	124.5	81.3	95.7
29	126.8	66.5	86.6
30	118.0	68.0	84.7

Appendix A-3: Raw data for baseline blood pressures taken with an automated sphygmomanometer in the supine position

Placebo

	Pre-treatment			Post-treatment		
Subject	**SAP (mmHg)**	**DAP (mmHg)**	**MAP (mmHg)**	**SAP (mmHg)**	**DAP (mmHg)**	**MAP (mmHg)**
1	107.7	71.0	83.2	111.0	73.3	85.9
2	116.7	49.3	71.8	122.0	56.0	78.0
3	115.7	64.7	81.7	114.3	66.0	82.1
4	114.7	60.3	78.4	114.0	73.0	86.7
5	106.7	57.0	73.6	120.8	70.0	86.9
6	111.7	66.3	81.4	127.0	76.7	93.5
7	122.3	84.3	97.0	126.7	86.7	100.0
8	128.7	63.7	85.4	134.0	66.0	88.7
9	122.7	63.7	83.4	127.3	68.0	87.8
10	126.7	68.3	87.8	133.0	70.7	91.5
11	117.0	76.7	90.1	111.3	75.0	87.1
12	115.0	63.7	80.8	121.3	75.0	90.4
13	104.7	59.7	74.7	125.7	81.7	96.4
14	119.3	56.7	77.6	122.0	66.7	85.1
15	117.3	55.3	76.0	128.0	63.7	85.1

Alcohol

	Pre-treatment			Post-treatment		
Subject	**SAP (mmHg)**	**DAP (mmHg)**	**MAP (mmHg)**	**SAP (mmHg)**	**DAP (mmHg)**	**MAP (mmHg)**
16	105.3	68.3	80.6	101.7	69.0	79.9
17	125.0	64.7	84.8	139.0	64.3	89.2
18	107.0	63.7	78.1	104.3	63.0	76.8
19	118.3	60.7	79.9	138.3	80.3	99.6
20	129.7	65.0	86.6	123.3	78.3	93.3
21	92.3	64.3	73.6	93.3	68.3	76.6
22	128.3	58.7	81.9	125.3	61.3	82.6
23	113.7	54.7	74.4	117.7	61.0	79.9
24	117.3	62.0	80.4	124.3	72.3	89.6
25	135.0	78.7	97.5	146.7	77.7	100.7
26	114.7	66.3	82.4	117.0	68.0	84.3
27	135.0	68.3	90.5	130.0	79.3	96.2
28	122.0	61.0	81.3	139.7	65.7	90.4
29	138.7	71.0	93.6	144.3	58.7	87.2
30	112.0	57.0	75.3	121.3	67.0	85.1

Appendix A-4: Raw data for blood alcohol content. Measured in %, which is equal to g/100 mL.

Placebo

Subject	Pre-treatment	Post-treatment
1	0.000	0.000
2	0.000	0.000
3	0.000	0.000
4	0.000	0.000
5	0.000	0.000
6	0.000	0.000
7	0.000	0.000
8	0.000	0.000
9	0.000	0.000
10	0.000	0.000
11	0.000	0.000
12	0.000	0.000
13	0.000	0.000
14	0.000	0.000
15	0.000	0.000

Alcohol

Subject	Pre-treatment	Post-treatment
16	0.000	0.122
17	0.000	0.086
18	0.000	0.111
19	0.000	0.100
20	0.000	0.078
21	0.000	0.078
22	0.000	0.078
23	0.000	0.076
24	0.000	0.062
25	0.000	0.088
26	0.000	0.077
27	0.000	0.061
28	0.000	0.036
29	0.000	0.076
30	0.000	0.119

Appendix A-5: Raw data for SAP (mmHg) during LBNP

Placebo

Pre-treatment

Subject	Base	-5 mmHg	-10 mmHg	-15 mmHg	-20 mmHg	-30 mmHg	-40 mmHg
1	107.7	104.7	104.6	102.3	100.3	94.7	91.9
2	116.7	118.1	119.0	119.8	114.3	104.6	95.2
3	115.7	112.7	111.4	111.3	111.6	106.8	99.3
4	114.7	113.5	114.0	113.7	111.9	110.1	104.0
5	107.6	111.8	113.0	110.5	108.1	105.4	103.2
6	111.7	112.4	110.7	108.0	107.6	105.7	102.7
7	122.3	122.2	121.3	120.0	121.4	118.7	112.0
8	128.7	130.7	125.7	126.5	123.5	114.7	111.1
9	122.7	123.3	120.0	123.5	121.4	121.5	118.1
10	126.7	129.9	125.6	122.0	119.4	117.8	111.8
11	117.0	118.8	119.9	119.6	117.7	109.2	106.4
12	115.0	115.1	115.6	114.8	114.3	110.2	108.3
13	104.7	111.5	112.9	119.9	128.9	126.5	119.6
14	122.0	116.4	118.1	118.7	119.1	115.9	117.4
15	117.3	116.3	116.5	108.4	116.8	112.4	111.1

Post-treatment

Subject	Base	-5 mmHg	-10 mmHg	-15 mmHg	-20 mmHg	-30 mmHg	-40 mmHg
1	111.0	109.5	108.1	106.3	103.3	98.8	95.6
2	122.0	122.0	120.6	117.6	111.9	.	.
3	114.3	113.9	116.9	116.3	115.0	114.5	109.6
4	114.0	115.8	115.6	114.3	114.3	110.4	107.3
5	120.8	119.7	118.8	121.3	120.6	109.4	102.9
6	127.0	124.8	123.7	121.5	116.4	112.8	109.4
7	126.7	126.9	126.2	123.6	125.0	119.8	109.0
8	134.0	139.8	143.1	137.4	131.9	128.2	120.4
9	127.3	129.2	128.4	131.2	129.7	128.6	123.6
10	133.0	133.4	134.1	132.3	132.9	128.4	126.6
11	111.3	117.4	118.0	119.8	122.3	127.9	133.5
12	121.3	115.2	117.7	117.4	115.5	113.7	109.9
13	125.7	131.3	124.6	136.7	142.1	138.8	134.4
14	122.0	132.3	133.6	134.2	131.2	131.0	128.9
15	128.0	125.2	122.9	125.7	126.3	126.8	130.1

Alcohol

Pre-treatment

Subject	Base	-5 mmHg	-10 mmHg	-15 mmHg	-20 mmHg	-30 mmHg	-40 mmHg
16	105.3	101.4	109.4	106.7	103.1	99.5	100.1
17	125.0	129.0	120.3	127.1	129.3	129.7	127.2
18	107.0	112.4	113.5	114.4	111.8	110.2	107.3
19	118.3	121.7	124.8	127.2	133.8	134.5	134.1
20	129.7	131.6	128.0	123.0	114.1	111.5	105.9
21	92.3	95.9	99.3	99.7	101.2	96.3	90.5
22	128.3	133.4	131.2	130.4	134.1	126.8	121.9
23	113.7	111.9	111.2	108.5	108.9	101.5	98.2
24	117.3	115.3	115.6	116.9	115.4	110.2	111.7
25	135.0	139.5	135.3	137.3	143.8	138.2	132.9
26	114.7	115.5	118.9	115.2	123.3	115.2	110.6
27	135.0	135.3	139.2	134.2	130.5	126.0	128.9
28	122.0	121.5	116.9	116.2	118.8	116.8	112.2
29	138.7	138.5	135.6	133.5	130.8	129.3	124.5
30	112.0	115.6	114.7	115.3	111.1	105.7	107.1

Post-treatment

Subject	Base	-5 mmHg	-10 mmHg	-15 mmHg	-20 mmHg	-30 mmHg	-40 mmHg
16	101.7	100.5	100.8	94.5	91.6	91.8	91.9
17	139.0	137.6	137.8	138.4	136.2	133.3	133.4
18	104.3	106.6	104.2	102.1	100.4	95.8	95.5
19	138.3	139.3	133.5	140.4	139.9	138.7	133.6
20	123.3	122.9	117.1	111.5	107.6	106.5	101.7
21	93.3	99.4	99.2	99.4	99.5	94.2	89.7
22	125.3	131.1	126.6	119.3	118.4	116.6	112.3
23	117.7	118.0	114.0	105.4	106.0	99.8	107.1
24	124.3	124.0	123.1	122.2	122.5	123.0	116.8
25	146.7	148.8	148.3	149.8	145.4	140.4	140.2
26	117.0	116.7	115.5	112.8	111.0	106.8	99.8
27	130.0	128.9	125.7	124.9	123.9	120.6	119.0
28	139.7	135.7	134.0	129.3	122.2	116.9	113.8
29	144.3	145.0	145.9	147.3	148.4	133.5	132.0
30	121.3	121.5	127.7	126.2	125.1	119.5	113.7

Appendix A-6: Raw data for DAP (mmHg) during LBNP

Placebo

Pre-treatment

Subject	Base	-5 mmHg	-10 mmHg	-15 mmHg	-20 mmHg	-30 mmHg	-40 mmHg
1	71.0	69.5	71.1	70.9	70.5	70.2	70.7
2	49.2	50.1	49.5	50.1	48.0	45.7	42.4
3	64.7	62.1	62.6	63.6	63.9	62.3	56.5
4	60.3	59.4	60.9	61.4	62.9	63.4	62.2
5	57.5	61.1	63.0	62.0	62.7	64.3	64.5
6	66.3	64.7	65.8	66.9	67.5	66.7	65.9
7	84.3	82.7	82.3	82.8	84.6	86.6	84.7
8	63.7	64.6	66.3	64.9	63.6	64.3	65.8
9	63.7	63.2	61.7	63.8	63.6	65.7	65.6
10	68.3	68.1	67.7	66.5	66.9	65.6	65.0
11	76.7	75.8	75.8	75.5	74.3	73.2	76.1
12	63.7	63.5	63.6	62.9	63.3	62.4	61.4
13	59.7	62.7	64.0	69.9	74.2	74.8	72.7
14	56.7	52.6	52.7	51.6	52.8	53.7	55.4
15	55.3	54.0	54.8	52.3	56.9	56.1	56.3

Post-treatment

Subject	Base	-5 mmHg	-10 mmHg	-15 mmHg	-20 mmHg	-30 mmHg	-40 mmHg
1	73.3	71.4	70.7	70.0	69.8	70.3	70.6
2	56.0	55.5	54.9	53.6	51.2	.	.
3	66.0	66.0	67.1	67.8	66.4	66.8	64.3
4	73.0	74.2	74.0	72.7	73.6	72.5	72.6
5	70.0	68.2	68.8	70.2	68.9	66.1	66.6
6	76.7	75.9	75.2	74.4	73.3	73.3	72.7
7	86.7	86.7	86.6	86.3	86.9	86.1	82.9
8	66.0	69.4	71.9	70.3	69.2	70.1	67.1
9	68.0	68.2	67.5	70.0	69.6	71.2	70.7
10	70.7	71.5	71.8	72.0	73.1	72.0	74.0
11	75.0	77.3	77.4	78.9	81.8	87.7	91.4
12	75.0	71.5	72.3	71.5	68.1	69.2	68.0
13	81.7	84.3	82.8	87.6	90.5	91.6	91.7
14	66.7	69.7	68.6	66.8	67.2	67.7	68.4
15	63.7	61.1	60.7	62.8	63.5	65.4	67.6

Alcohol

Pre-treatment

Subject	Base	-5 mmHg	-10 mmHg	-15 mmHg	-20 mmHg	-30 mmHg	-40 mmHg
16	68.3	65.2	69.3	69.1	68.6	67.5	70.6
17	64.7	66.2	64.2	66.5	67.0	69.5	70.0
18	63.7	65.8	66.4	67.5	67.2	68.5	68.1
19	60.7	62.1	65.6	66.2	70.2	71.6	73.3
20	65.0	65.9	64.5	61.7	58.8	58.1	57.0
21	64.3	66.8	68.2	69.0	70.2	70.0	67.7
22	58.7	59.1	58.2	57.8	59.4	58.0	58.5
23	54.7	52.4	51.7	50.5	50.6	49.4	51.0
24	62.0	59.7	60.2	61.1	60.8	61.3	64.1
25	78.7	82.0	77.7	78.8	82.2	79.2	77.6
26	66.3	67.0	68.5	66.7	72.1	68.9	69.2
27	68.3	69.2	71.0	69.5	68.6	68.7	70.0
28	61.0	60.8	58.1	59.6	63.3	63.0	61.4
29	71.0	70.7	69.6	69.2	68.4	67.7	67.8
30	57.0	57.6	58.3	58.5	59.1	58.9	60.2

Post-treatment

Subject	Base	-5 mmHg	-10 mmHg	-15 mmHg	-20 mmHg	-30 mmHg	-40 mmHg
16	69.0	69.5	70.6	68.5	67.7	69.0	71.3
17	64.3	63.0	62.4	62.3	62.4	62.4	64.0
18	63.0	64.2	64.3	63.0	63.0	62.6	62.8
19	80.3	81.0	80.7	83.5	81.9	81.2	78.5
20	78.3	77.4	75.6	73.7	73.5	72.5	72.1
21	68.3	70.9	71.2	71.7	71.9	70.2	69.4
22	61.3	63.1	61.8	59.4	57.2	57.5	57.5
23	61.0	59.6	58.0	53.9	53.2	50.5	55.7
24	72.3	70.0	70.6	70.8	71.1	72.7	72.7
25	77.7	79.5	80.2	81.7	79.9	77.7	78.4
26	68.0	68.3	67.7	67.5	66.4	65.0	63.6
27	79.3	79.2	78.5	78.7	78.2	78.2	76.5
28	65.7	62.8	62.9	61.1	57.8	56.0	54.9
29	58.7	58.2	57.6	58.2	59.3	54.8	53.8
30	67.0	66.8	69.4	69.7	69.7	69.5	69.1

Appendix A-7: Raw data for MAP (mmHg) during LBNP

Placebo

Pre-treatment

Subject	Base	-5 mmHg	-10 mmHg	-15 mmHg	-20 mmHg	-30 mmHg	-40 mmHg
1	83.2	80.8	81.7	79.8	78.3	75.2	74.2
2	71.8	73.0	72.5	72.5	69.5	64.4	59.8
3	81.7	78.3	78.5	78.8	79.0	76.6	70.2
4	78.4	77.6	79.1	79.1	79.6	79.3	76.2
5	74.3	78.5	80.4	78.6	78.1	78.5	77.6
6	81.4	79.7	81.3	82.6	83.7	81.5	79.1
7	97.0	96.1	94.9	94.6	96.2	96.6	92.7
8	85.4	86.3	85.7	84.5	83.2	79.9	79.1
9	83.4	82.8	80.3	82.2	81.5	83.0	81.5
10	87.8	88.1	87.0	84.9	84.2	82.4	80.1
11	90.1	90.1	90.6	90.1	88.6	83.4	84.3
12	80.8	81.0	80.7	79.7	79.4	77.6	76.6
13	74.7	77.5	78.2	85.8	91.5	90.8	85.5
14	77.6	74.0	74.0	72.7	73.7	73.3	74.5
15	76.0	74.1	74.5	70.4	75.8	73.7	74.0

Post-treatment

Subject	Base	-5 mmHg	-10 mmHg	-15 mmHg	-20 mmHg	-30 mmHg	-40 mmHg
1	85.9	83.4	82.3	80.6	78.8	76.4	75.1
2	78.0	77.7	76.9	74.6	71.1	.	.
3	82.1	81.9	83.4	83.9	82.6	82.3	79.3
4	86.7	87.6	87.0	85.2	85.5	83.0	81.7
5	86.9	85.7	86.5	87.3	85.4	79.9	78.5
6	93.5	92.9	92.0	91.0	89.0	87.7	84.5
7	100.0	100.2	99.8	98.6	99.2	96.1	88.9
8	88.7	92.9	95.2	91.3	88.0	86.9	84.0
9	87.8	88.5	87.3	90.0	88.7	89.4	87.5
10	91.5	91.9	91.8	91.2	92.0	89.7	91.0
11	87.1	90.8	90.9	92.0	94.6	99.8	103.7
12	90.4	86.7	87.4	86.7	82.7	83.5	81.0
13	96.4	102.0	97.3	104.1	108.3	106.9	105.2
14	85.1	90.8	89.9	87.7	86.0	85.2	85.1
15	85.1	81.8	80.7	82.7	83.0	84.3	86.6

Alcohol

Pre-treatment

Subject	Base	-5 mmHg	-10 mmHg	-15 mmHg	-20 mmHg	-30 mmHg	-40 mmHg
16	80.6	77.7	83.3	82.0	80.5	78.3	81.1
17	84.8	86.2	83.0	86.2	85.8	86.8	86.7
18	78.1	80.9	81.8	83.1	82.1	81.8	81.0
19	79.9	82.2	86.3	87.2	92.0	92.7	93.7
20	86.6	87.6	85.7	82.2	77.7	75.9	73.1
21	73.6	77.1	78.9	79.4	80.4	77.3	72.4
22	81.9	82.8	81.6	81.5	83.3	80.0	78.3
23	74.4	72.7	72.7	71.1	71.8	68.8	68.2
24	80.4	77.8	78.3	79.3	78.4	76.7	78.8
25	97.5	100.9	96.6	97.3	101.9	98.0	95.1
26	82.4	83.1	84.9	82.6	88.7	83.7	81.7
27	90.5	91.1	93.2	90.7	89.0	87.7	88.7
28	81.3	81.3	77.7	78.4	81.8	80.8	78.1
29	93.6	93.4	91.8	90.7	89.4	88.0	86.2
30	75.3	76.3	77.1	76.7	76.3	74.7	76.2

Post-treatment

Subject	Base	-5 mmHg	-10 mmHg	-15 mmHg	-20 mmHg	-30 mmHg	-40 mmHg
16	79.9	80.0	80.7	77.4	75.8	76.4	77.7
17	89.2	87.7	87.8	87.9	86.2	85.8	85.9
18	76.8	76.5	76.4	73.9	73.4	71.4	71.5
19	99.6	99.4	96.7	100.5	99.4	98.8	96.0
20	93.3	92.6	89.3	86.9	85.7	84.7	83.8
21	76.6	79.7	80.0	79.7	79.5	75.6	72.6
22	82.6	87.0	84.4	80.7	78.5	78.1	76.9
23	79.9	78.7	76.2	71.1	70.4	66.1	72.4
24	89.6	86.9	87.5	85.9	86.4	86.9	83.4
25	100.7	102.3	102.3	103.7	101.0	97.8	96.8
26	84.3	84.3	83.1	82.2	80.5	77.5	73.6
27	96.2	95.6	94.2	93.7	93.1	92.7	91.1
28	90.4	87.0	86.8	84.2	80.2	77.8	76.5
29	87.2	86.5	86.8	88.0	89.2	82.0	80.5
30	85.1	85.0	89.2	88.9	88.3	85.7	83.6

Appendix A-8: Raw data for HR (bpm) during LBNP

Placebo

Pre-treatment

Subject	Base	-5 mmHg	-10 mmHg	-15 mmHg	-20 mmHg	-30 mmHg	-40 mmHg
1	60.4	63.8	65.7	67.5	70.3	80.3	88.0
2	64.8	64.1	62.1	62.6	65.1	71.1	80.9
3	65.2	61.3	63.7	63.6	65.3	75.2	77.3
4	53.9	54.6	56.2	59.8	63.5	64.6	69.2
5	50.0	50.4	51.5	54.2	58.3	61.2	66.0
6	45.2	44.9	46.6	46.5	47.0	47.3	50.4
7	63.3	61.9	64.3	66.0	67.8	72.2	79.5
8	52.8	54.4	54.2	55.1	54.8	68.9	73.7
9	50.1	50.2	49.6	47.6	49.8	52.4	54.6
10	78.3	73.5	82.4	78.4	81.4	82.9	90.6
11	71.1	68.6	69.1	69.8	69.7	81.6	92.3
12	66.1	66.1	66.4	66.7	68.7	76.2	84.5
13	40.2	42.3	43.0	47.2	49.3	59.8	68.9
14	57.9	58.0	60.2	62.4	63.7	67.0	73.3
15	64.6	62.7	63.1	64.2	64.6	71.3	77.6

Post-treatment

Subject	Base	-5 mmHg	-10 mmHg	-15 mmHg	-20 mmHg	-30 mmHg	-40 mmHg
1	58.5	59.2	60.7	62.8	69.1	79.6	87.8
2	62.9	64.4	62.8	66.4	73.6	.	.
3	61.0	60.0	62.1	62.1	63.3	67.3	72.2
4	54.0	55.5	55.9	56.0	58.3	61.2	65.7
5	47.7	44.9	46.3	51.7	54.6	62.2	68.2
6	42.4	43.4	42.5	43.2	46.0	48.0	54.3
7	58.6	59.6	60.4	62.7	63.6	69.5	80.7
8	55.2	54.4	57.9	62.0	66.6	79.2	103.1
9	52.3	50.3	49.7	52.0	53.3	56.7	61.6
10	76.2	77.3	74.4	79.4	80.6	85.1	94.7
11	73.4	73.7	74.9	78.1	84.9	93.0	118.0
12	67.8	69.7	69.4	68.8	66.1	73.0	77.0
13	43.3	42.8	42.8	44.7	45.5	54.7	64.2
14	58.0	58.4	58.4	59.2	66.7	72.6	77.2
15	65.9	63.2	63.8	65.2	67.0	71.5	75.2

Alcohol

Pre-treatment

Subject	Base	-5 mmHg	-10 mmHg	-15 mmHg	-20 mmHg	-30 mmHg	-40 mmHg
16	78.8	76.0	82.2	78.5	78.1	84.3	94.8
17	51.4	52.8	56.4	55.2	56.7	62.9	64.8
18	63.4	61.6	61.3	61.9	64.7	72.5	86.5
19	56.2	57.5	57.7	59.8	60.8	63.4	68.5
20	60.0	59.2	59.5	58.2	58.4	62.4	66.1
21	65.0	65.8	65.6	65.5	66.6	74.1	83.5
22	55.2	55.5	56.7	56.2	58.0	61.8	66.8
23	56.0	57.0	57.1	57.2	55.6	61.9	72.1
24	55.6	54.6	55.1	54.1	54.4	61.9	66.3
25	59.9	63.7	59.4	63.0	67.2	65.9	67.6
26	58.8	60.0	60.1	60.2	61.4	64.3	69.3
27	62.1	61.3	63.0	64.7	69.2	74.4	78.6
28	48.9	52.1	53.6	60.4	67.0	69.0	73.9
29	66.4	69.9	71.1	70.6	70.1	68.1	70.1
30	54.4	54.3	54.8	55.4	56.0	58.2	60.0

Post-treatment

Subject	Base	-5 mmHg	-10 mmHg	-15 mmHg	-20 mmHg	-30 mmHg	-40 mmHg
16	78.6	81.6	83.9	83.4	87.5	95.1	101.5
17	59.2	61.2	57.9	57.6	62.2	61.9	66.9
18	66.0	65.7	65.7	64.0	70.2	71.2	83.0
19	68.2	70.2	76.0	80.0	84.8	87.9	97.5
20	66.1	66.5	67.0	72.4	72.2	76.7	83.9
21	63.8	64.6	63.2	64.5	65.9	71.6	77.7
22	59.1	58.3	59.1	59.3	59.6	63.0	70.0
23	59.5	62.7	61.6	61.0	63.9	67.5	73.7
24	55.8	55.3	57.3	64.6	60.2	65.9	75.3
25	66.3	69.4	73.1	73.1	73.9	72.4	75.7
26	60.8	61.4	61.4	63.1	64.2	68.5	72.8
27	65.9	68.6	71.1	73.6	76.3	79.2	81.6
28	65.5	66.7	70.7	72.5	75.1	78.5	81.9
29	73.4	71.8	73.8	74.9	74.4	74.6	77.8
30	61.7	62.4	64.6	63.7	63.8	66.4	74.1

Appendix A-9: Raw data for MSNA burst frequency (bursts/min) during LBNP

Placebo

Pre-treatment

Subject	Base	-5 mmHg	-10 mmHg	-15 mmHg	-20 mmHg	-30 mmHg	-40 mmHg
1	13.0	12.7	18.7	22.7	25.3	29.3	36.0
2	24.2	21.3	26.7	27.0	29.3	31.0	35.3
3	.	.	.	.	.	.	.
4	12.6	12.3	15.3	18.3	26.0	28.7	32.7
5	.	.	.	.	.	.	.
6	15.4	16.7	17.0	21.3	19.7	21.7	31.3
7	11.8	12.3	16.0	18.0	20.0	13.7	27.3
8	.	.	.	.	.	.	.
9	18.2	19.3	19.7	22.3	21.3	24.0	30.0
10	7.2	.	.	.	.	.	.
11	15.0	17.0	17.3	18.7	21.7	39.7	45.7
12	17.0	.	.	.	.	.	.
13	9.0	9.0	14.0	18.7	21.3	35.0	37.7
14	6.4	8.3	10.3	13.0	20.7	24.3	29.3
15	25.2	24.0	24.7	24.0	30.7	37.0	45.3

Post-treatment

Subject	Base	-5 mmHg	-10 mmHg	-15 mmHg	-20 mmHg	-30 mmHg	-40 mmHg
1	15.8	15.7	17.3	21.3	24.7	36.3	44.7
2	23.6	26.7	31.7	29.0	31.3	.	.
3	.	.	.	.	.	.	.
4	9.4	11.0	14.0	9.7	6.7	16.0	26.3
5	.	.	.	.	.	.	.
6	16.6	19.0	19.0	24.7	25.7	30.3	38.0
7	18.0	16.0	20.3	22.7	23.7	26.0	24.3
8	.	.	.	.	.	.	.
9	13.4	16.3	16.7	21.3	22.7	28.7	32.0
10	11.6	2.0	4.3	6.0	8.7	7.7	9.0
11	9.8	21.0	26.3	50.0	52.3	58.3	46.3
12	23.0	.	.	.	.	.	.
13	9.0	12.7	12.7	16.7	19.0	21.7	30.3
14	9.2	11.0	14.7	24.1	32.7	35.0	35.8
15	20.8	23.0	26.3	26.7	32.0	35.0	42.5

Alcohol

Pre-treatment

Subject	Base	-5 mmHg	-10 mmHg	-15 mmHg	-20 mmHg	-30 mmHg	-40 mmHg
16	5.6	5.3	5.7	10.0	12.7	12.0	18.7
17	7.0	11.0	17.3	19.7	24.0	31.0	40.7
18	13.4	15.3	18.0	18.0	19.3	28.3	28.3
19	14.2	18.3	26.3	27.0	28.3	31.7	33.3
20	6.2	10.0	11.7	10.3	18.7	22.7	27.7
21	.	.	.	.	.	.	.
22	21.4	21.7	22.3	23.3	23.7	34.0	38.7
23	.	.	.	.	.	.	.
24	5.4	.	.	.	.	.	.
25	15.6	.	.	.	.	.	.
26	37.4	37.7	39.0	46.0	48.7	51.0	61.3
27	16.8	21.0	17.3	26.3	29.0	43.3	38.0
28	7.4	.	.	.	.	.	.
29	12.4	14.3	17.3	18.7	18.7	30.0	35.7
30	2.4	8.3	8.3	17.7	19.3	25.7	29.0

Post-treatment

Subject	Base	-5 mmHg	-10 mmHg	-15 mmHg	-20 mmHg	-30 mmHg	-40 mmHg
16	11.0	.	.	.	.	.	.
17	20.8	22.7	27.0	29.3	32.7	43.7	41.7
18	9.0	13.0	17.0	21.0	21.0	27.3	34.0
19	45.2	46.3	55.0	55.7	61.7	66.3	69.7
20	13.6	20.0	25.0	25.0	29.3	37.3	35.5
21	.	.	.	.	.	.	.
22	17.8	22.0	17.7	22.0	20.3	23.7	26.3
23	.	.	.	.	.	.	.
24	19.4	.	.	.	.	.	.
25	34.4	.	.	.	.	.	.
26	32.8	30.3	41.7	39.7	43.0	44.3	59.0
27	12.4	16.0	17.0	24.7	26.3	35.3	40.7
28	10.2	.	.	.	.	.	.
29	13.8	17.3	17.7	22.3	23.3	34.0	38.3
30	2.6	4.0	6.0	10.3	15.0	24.7	29.3

Appendix A-10: Raw data for MSNA burst incidence (bursts/100 HB) during LBNP

Placebo

Pre-treatment

Subject	Base	-5 mmHg	-10 mmHg	-15 mmHg	-20 mmHg	-30 mmHg	-40 mmHg
1	21.7	20.0	28.6	33.7	36.2	36.5	40.9
2	37.6	33.3	43.0	43.3	45.4	43.7	43.8
3	.	.	.	.	.	.	.
4	23.5	22.7	27.4	30.9	41.1	44.3	47.3
5	.	.	.	.	.	.	.
6	34.4	37.3	36.7	46.0	41.8	46.1	62.3
7	18.8	20.0	25.1	27.6	29.7	19.1	34.5
8	.	.	.	.	.	.	.
9	36.7	38.9	40.1	47.2	43.2	46.2	55.2
10	9.4	.	.	.	.	.	.
11	21.2	25.0	25.2	27.2	31.3	48.8	49.6
12	25.9	.	.				
13	22.6	21.8	33.1	40.9	44.8	59.3	54.9
14	11.1	14.5	17.2	21.0	32.6	36.5	40.0
15	39.3	38.3	39.2	37.5	47.4	52.1	58.6

Post-treatment

Subject	Base	-5 mmHg	-10 mmHg	-15 mmHg	-20 mmHg	-30 mmHg	-40 mmHg
1	27.2	26.7	28.7	34.2	35.9	45.8	51.0
2	37.7	41.7	50.5	43.9	42.7	.	.
3	.	.	.	.	.	.	.
4	17.4	19.9	25.1	17.4	11.4	26.2	40.1
5	.	.	.	.	.	.	.
6	39.3	43.8	44.9	56.9	55.8	63.2	69.9
7	30.9	27.0	33.9	36.4	37.2	37.5	30.3
8	.	.	.	.	.	.	.
9	25.9	32.9	33.8	41.0	42.8	50.9	52.2
10	15.4	2.6	5.9	7.6	10.9	9.1	9.6
11	13.4	28.6	35.3	64.1	61.8	63.2	39.4
12	34.0	.	.	.	.	.	.
13	21.5	30.6	30.2	38.5	42.9	40.1	47.6
14	16.0	18.9	25.1	40.9	49.2	48.4	47.4
15	31.6	36.5	41.4	41.0	48.0	49.3	57.1

Alcohol

Pre-treatment

Subject	Base	-5 mmHg	-10 mmHg	-15 mmHg	-20 mmHg	-30 mmHg	-40 mmHg
16	7.1	7.1	6.9	12.8	16.3	14.2	19.7
17	13.7	20.9	31.0	35.8	42.4	49.5	62.9
18	21.3	24.9	29.5	29.2	30.1	39.2	32.9
19	25.4	32.0	45.9	45.3	46.7	50.3	48.8
20	10.5	16.9	19.7	17.9	32.2	36.6	41.9
21	.	.	.	.	.	.	.
22	38.9	39.2	39.4	41.7	41.0	55.4	58.0
23	.	.	.	.	.	.	.
24	9.8	.	.	.	.	.	.
25	26.2	.	.	.	.	.	.
26	64.0	62.8	65.0	76.2	79.3	79.7	88.5
27	27.4	34.4	27.8	40.9	42.2	58.6	48.7
28	15.3	.	.	.	.	.	.
29	18.8	20.6	24.4	26.5	26.7	44.1	51.2
30	4.4	15.4	15.2	31.9	34.5	44.3	48.6

Post-treatment

Subject	Base	-5 mmHg	-10 mmHg	-15 mmHg	-20 mmHg	-30 mmHg	-40 mmHg
16	14.3	.	.	.	.	.	.
17	35.1	37.2	46.8	51.2	52.7	70.8	62.5
18	13.7	19.8	25.9	32.8	30.0	38.5	41.1
19	66.5	66.2	72.7	69.9	73.1	75.7	71.6
20	20.7	30.2	37.5	34.7	40.9	49.1	41.7
21	.	.	.	.	.	.	.
22	30.2	37.9	29.9	37.1	34.1	37.8	37.8
23	.	.	.	.	.	.	.
24	34.9	.	.	.	.	.	.
25	52.0	.	.	.	.	.	.
26	54.1	49.7	67.9	63.0	67.2	64.9	81.2
27	19.0	23.5	24.3	33.8	34.8	44.9	50.2
28	15.6	.	.	.	.	.	.
29	18.9	24.3	24.0	29.9	31.4	45.7	49.6
30	4.2	6.4	9.3	16.2	23.4	37.2	39.8

Appendix A-11: Raw data for total MSNA (arbitrary units) during LBNP

Placebo

Pre-treatment

Subject	Base	-5 mmHg	-10 mmHg	-15 mmHg	-20 mmHg	-30 mmHg	-40 mmHg
1	4609	5354	7779	8912	8274	12598	15826
2	10573	9006	11640	11261	12168	15278	17325
3	.	.	.	.	.	.	.
4	5687	6042	6502	8353	13203	16495	21440
5	.	.	.	.	.	.	.
6	5487	5959	7103	8878	7615	8695	13855
7	6360	6977	9385	10928	11074	4578	10736
8	.	.	.	.	.	.	.
9	8273	13476	12975	17148	16477	18335	22880
10	4152	.	.	.	.	.	.
11	6846	11308	12606	14710	15229	36415	47145
12	7563	.	.				
13	4886	4851	7266	12641	13441	17510	25745
14	3213	3842	5401	6291	9144	10436	13153
15	11909	9297	9129	7930	11168	16020	20148

Post-treatment

Subject	Base	-5 mmHg	-10 mmHg	-15 mmHg	-20 mmHg	-30 mmHg	-40 mmHg
1	6621	7145	8314	10411	12512	19687	25596
2	11589	15417	19663	19400	22309	.	.
3	.	.	.	.	.	.	.
4	5181	7001	9932	9790	4993	11408	23153
5	.	.	.	.	.	.	.
6	8238	11740	12786	17832	19654	27377	37019
7	9481	8183	10674	12310	11241	11186	11993
8	.	.	.	.	.	.	.
9	4964	5613	6807	8947	11968	17447	22266
10	6889	600	1307	1683	3361	2740	3384
11	4292	11439	16912	24271	27004	32647	32984
12	11292	.	.	.	.	.	.
13	2501	4842	5780	8303	10097	12129	22366
14	5185	6379	9652	21558	24413	29710	34710
15	7264	5967	6941	9440	10283	17971	30495

Alcohol

Pre-treatment

Subject	Base	-5 mmHg	-10 mmHg	-15 mmHg	-20 mmHg	-30 mmHg	-40 mmHg
16	3079	3477	3307	6815	7658	6359	11534
17	3634	9159	12808	16770	19524	25519	33452
18	6606	7456	8613	8625	7231	8367	10866
19	5669	9174	14275	16480	16307	20127	25614
20	3189	6280	7395	5623	11579	14100	18213
21	.	.	.	.	.	.	.
22	11814	15188	24073	17029	19966	32140	40229
23	.	.	.	.	.	.	.
24	2688	.	.	.	.	.	.
25	8201	.	.	.	.	.	.
26	17536	22691	26836	33397	30548	37507	54065
27	7135	10364	10013	14418	16176	23810	21987
28	4553	.	.	.	.	.	.
29	3823	5261	5863	7267	8488	15707	20878
30	1114	5253	5130	12235	13918	20250	25578

Post-treatment

Subject	Base	-5 mmHg	-10 mmHg	-15 mmHg	-20 mmHg	-30 mmHg	-40 mmHg
16	12009	.	.	.	.	.	.
17	6695	12347	16770	18687	20967	29334	31806
18	4495	8252	10724	13996	16537	23867	31742
19	19648	25210	31862	32259	36694	44524	54592
20	7761	13831	18189	16468	19905	29585	30339
21	.	.	.	.	.	.	.
22	10165	16305	8892	10348	8281	11423	18275
23	.	.	.	.	.	.	.
24	6911	.	.	.	.	.	.
25	17147	.	.	.	.	.	.
26	9560	8749	9930	9693	10328	10384	13294
27	6517	9223	11539	17726	25467	37435	49485
28	5948	.	.	.	.	.	.
29	5881	6548	4605	7412	9074	14939	19209
30	1696	2055	3471	5706	9201	16484	18937

Appendix A-12: Raw data for FBF (ml/100ml/min) during LBNP

Placebo

Pre-treatment

Subject	Base	-5 mmHg	-10 mmHg	-15 mmHg	-20 mmHg	-30 mmHg	-40 mmHg
1	1.6	1.5	1.5	1.5	1.4	1.4	1.6
2	2.0	2.0	1.9	1.7	1.6	1.6	1.5
3	3.0	2.8	2.7	3.0	2.8	2.6	2.6
4	1.2	1.0	1.1	1.1	1.1	1.0	1.0
5	1.7	1.8	1.7	1.5	1.6	1.4	1.1
6	1.8	1.5	1.7	1.7	1.8	1.7	1.7
7	1.7	1.5	1.9	1.7	1.7	1.6	1.6
8	1.8	1.7	1.8	2.0	1.8	2.0	1.9
9	2.1	2.0	1.9	1.6	1.5	1.3	1.1
10	5.2	5.0	5.1	4.1	3.7	2.8	2.3
11	1.0	0.8	0.8	0.8	0.8	0.8	0.7
12	1.7	1.4	1.3	1.4	1.5	1.4	1.1
13	.	.	.	.	.	.	.
14	2.3	2.0	1.9	1.8	1.6	1.3	1.4
15	2.0	2.6	2.4	2.2	2.3	2.5	2.4

Post-treatment

Subject	Base	-5 mmHg	-10 mmHg	-15 mmHg	-20 mmHg	-30 mmHg	-40 mmHg
1	1.5	1.5	1.6	1.5	1.6	1.3	1.3
2	2.5	2.6	2.6	2.5	2.7	.	.
3	3.2	2.6	3.1	2.8	3.0	2.9	2.7
4	1.1	1.1	1.1	1.1	1.1	1.1	1.1
5	2.0	1.4	1.3	1.5	1.1	1.2	1.2
6	1.6	1.5	1.6	1.4	1.4	1.1	1.0
7	1.7	2.3	1.8	1.7	1.8	2.0	1.5
8	1.7	1.5	1.6	1.6	1.5	1.3	1.1
9	1.3	1.6	1.6	1.5	1.5	1.3	1.2
10	3.7	3.4	2.6	2.4	2.6	2.2	1.8
11	1.6	1.5	1.4	1.3	1.2	1.2	1.7
12	1.5	1.3	1.0	1.0	1.0	0.8	0.9
13	.	.	.	.	.	.	.
14	1.8	1.6	1.5	1.3	1.3	1.3	1.2
15	2.8	2.6	2.5	3.1	2.8	3.2	3.1

Alcohol

Pre-treatment

Subject	Base	-5 mmHg	-10 mmHg	-15 mmHg	-20 mmHg	-30 mmHg	-40 mmHg
16	1.8	1.6	1.8	1.6	1.3	1.2	1.2
17	1.6	1.3	1.5	1.4	1.4	1.2	1.3
18	1.4	1.2	1.2	1.1	1.0	0.9	0.9
19	3.0	2.6	2.3	2.5	2.3	2.3	2.5
20	1.7	1.5	1.6	1.4	1.4	1.5	1.3
21	1.1	0.9	1.0	1.0	1.0	0.9	0.9
22	1.3	1.5	1.2	1.1	1.0	0.9	1.0
23	2.8	2.3	2.9	2.8	2.1	3.0	2.2
24	2.0	1.6	1.7	1.5	1.4	1.5	1.2
25	2.2	1.6	2.1	2.1	1.9	1.8	2.2
26	1.3	1.2	1.5	1.3	1.3	1.5	1.3
27	3.2	3.0	3.2	2.7	2.8	2.6	2.6
28	1.8	1.7	1.6	1.6	1.4	1.4	1.2
29	4.2	4.1	4.7	3.8	4.0	3.6	3.0
30	2.3	2.2	1.9	1.6	1.7	1.4	1.1

Post-treatment

Subject	Base	-5 mmHg	-10 mmHg	-15 mmHg	-20 mmHg	-30 mmHg	-40 mmHg
16	1.6	1.5	1.4	1.3	1.2	1.9	1.4
17	1.6	1.6	1.6	1.6	2.3	1.2	1.5
18	1.6	1.4	1.4	1.2	1.3	1.2	1.1
19	2.5	2.4	2.2	2.1	2.2	2.0	2.3
20	1.6	1.5	1.3	1.1	1.2	1.1	1.0
21	1.3	1.2	1.1	1.0	1.1	0.9	1.0
22	1.3	1.2	1.2	1.2	1.4	1.2	1.1
23	2.2	2.4	2.1	2.1	2.0	2.0	2.2
24	1.7	1.3	1.4	1.3	1.4	1.1	1.3
25	2.6	2.7	3.5	2.9	2.8	2.6	2.5
26	2.3	1.7	1.5	1.5	1.3	1.2	1.5
27	2.2	1.8	2.0	2.1	2.0	2.3	2.0
28	1.3	1.3	1.3	1.3	1.3	1.5	1.3
29	4.2	4.2	4.0	3.8	3.5	3.7	4.2
30	3.0	2.8	3.4	2.7	2.7	2.3	1.5

Appendix A-13: Raw data for FVR (mmHg/ml/100ml/min) during LBNP

Placebo

Pre-treatment

Subject	Base	-5 mmHg	-10 mmHg	-15 mmHg	-20 mmHg	-30 mmHg	-40 mmHg
1	52.8	53.8	54.9	53.2	54.6	53.7	45.5
2	35.1	37.2	37.4	42.9	44.0	39.2	40.0
3	27.4	27.9	28.6	26.3	28.1	29.1	26.5
4	64.3	79.0	73.5	69.7	74.8	82.6	78.2
5	42.6	44.3	48.6	51.9	50.4	54.7	70.5
6	44.6	51.8	46.5	49.4	45.8	48.0	47.3
7	56.9	62.5	50.9	57.2	56.1	60.7	59.1
8	47.0	50.3	48.7	42.2	46.7	40.9	42.6
9	40.0	41.6	42.6	52.4	54.3	65.4	74.1
10	17.0	17.7	17.1	20.6	22.7	29.3	34.7
11	93.5	109.0	110.5	110.8	107.6	107.6	119.0
12	48.7	56.3	62.3	56.2	52.5	55.8	73.0
13	.	.	.	.	.	.	.
14	34.3	36.4	38.1	39.3	44.8	57.3	55.0
15	37.1	28.7	31.2	31.4	32.5	29.5	30.6

Post-treatment

Subject	Base	-5 mmHg	-10 mmHg	-15 mmHg	-20 mmHg	-30 mmHg	-40 mmHg
1	55.8	55.1	53.0	55.6	50.8	57.8	58.1
2	30.9	29.5	29.4	29.7	26.7	.	.
3	25.7	30.9	27.3	30.4	27.4	27.9	29.5
4	75.7	79.3	79.0	76.9	79.9	77.0	77.0
5	42.9	60.3	64.4	59.1	75.1	68.2	66.6
6	59.7	62.1	58.6	63.3	62.8	82.7	81.7
7	57.5	43.7	56.4	56.9	54.8	48.4	60.6
8	52.8	60.2	60.9	57.6	60.3	65.5	74.7
9	65.2	56.1	54.1	58.8	59.3	66.7	73.3
10	25.0	27.4	35.1	37.4	35.5	41.4	49.6
11	53.2	58.7	63.2	71.5	77.7	83.5	62.5
12	60.5	69.3	86.7	83.2	81.7	108.0	95.0
13	.	.	.	.	.	.	.
14	48.3	55.9	59.7	67.9	65.3	63.7	70.2
15	30.6	31.0	32.1	27.0	30.0	26.0	28.3

Alcohol

Pre-treatment

Subject	Base	-5 mmHg	-10 mmHg	-15 mmHg	-20 mmHg	-30 mmHg	-40 mmHg
16	45.8	49.6	46.4	52.9	62.0	63.0	69.1
17	52.1	63.9	55.3	62.5	61.0	74.3	66.6
18	57.6	66.3	70.6	76.6	83.6	93.2	86.8
19	27.0	31.0	37.0	34.3	39.8	41.0	37.0
20	51.5	59.6	54.1	57.2	55.6	51.2	58.5
21	68.1	81.3	76.8	81.1	79.0	84.2	78.6
22	62.9	56.3	66.4	74.3	83.7	87.6	79.1
23	26.6	32.3	24.8	25.2	34.8	23.2	30.9
24	41.2	47.7	46.7	51.9	55.6	49.8	66.3
25	44.0	64.2	46.3	46.3	54.7	55.1	43.1
26	61.2	67.9	57.9	64.4	66.6	57.3	64.9
27	28.5	30.8	29.5	33.4	32.4	33.2	34.5
28	45.5	47.6	47.7	49.7	57.7	57.8	66.0
29	22.3	22.5	19.5	24.1	22.3	24.4	29.1
30	32.9	35.1	39.8	46.6	44.7	55.1	70.7

Post-treatment

Subject	Base	-5 mmHg	-10 mmHg	-15 mmHg	-20 mmHg	-30 mmHg	-40 mmHg
16	50.2	55.0	56.5	60.1	61.3	40.3	54.2
17	54.4	53.6	56.2	56.0	37.9	73.9	55.5
18	48.4	56.0	53.7	63.3	58.0	60.5	67.3
19	39.9	41.5	43.3	47.5	44.8	48.9	41.2
20	58.5	60.9	67.5	78.3	69.2	75.4	86.2
21	60.9	68.2	74.4	77.7	73.7	81.5	75.9
22	62.2	71.5	70.6	67.1	57.3	62.9	72.1
23	36.7	32.4	35.8	34.2	35.6	32.6	33.6
24	53.7	69.4	63.6	64.5	59.7	76.0	63.7
25	39.2	37.7	29.5	35.3	35.6	38.1	38.0
26	37.2	50.4	54.9	54.9	60.8	65.5	50.1
27	43.0	53.8	47.5	44.6	45.4	40.1	45.4
28	69.6	67.3	65.5	62.7	60.9	52.3	57.0
29	20.6	20.4	21.5	23.4	25.3	22.0	19.2
30	28.3	30.8	25.9	32.6	32.9	37.9	54.0

Appendix A-14: Raw data for FVC (ml/100ml/min/mmHg)*100 during LBNP

Placebo

Pre-treatment

Subject	Base	-5 mmHg	-10 mmHg	-15 mmHg	-20 mmHg	-30 mmHg	-40 mmHg
1	1.9	1.9	1.8	1.9	1.8	1.9	2.2
2	2.9	2.7	2.7	2.3	2.3	2.6	2.5
3	3.7	3.6	3.5	3.8	3.6	3.4	3.8
4	1.6	1.3	1.4	1.4	1.3	1.2	1.3
5	2.3	2.3	2.1	1.9	2.0	1.8	1.4
6	2.2	1.9	2.1	2.0	2.2	2.1	2.1
7	1.8	1.6	2.0	1.7	1.8	1.6	1.7
8	2.1	2.0	2.1	2.4	2.1	2.4	2.3
9	2.5	2.4	2.3	1.9	1.8	1.5	1.3
10	5.9	5.6	5.9	4.9	4.4	3.4	2.9
11	1.1	0.9	0.9	0.9	0.9	0.9	0.8
12	2.1	1.8	1.6	1.8	1.9	1.8	1.4
13	.	.	.	.	.	.	.
14	2.9	2.8	2.6	2.5	2.2	1.7	1.8
15	2.7	3.5	3.2	3.2	3.1	3.4	3.3

Post-treatment

Subject	Base	-5 mmHg	-10 mmHg	-15 mmHg	-20 mmHg	-30 mmHg	-40 mmHg
1	1.8	1.8	1.9	1.8	2.0	1.7	1.7
2	3.2	3.4	3.4	3.4	3.7	.	.
3	3.9	3.2	3.7	3.3	3.7	3.6	3.4
4	1.3	1.3	1.3	1.3	1.3	1.3	1.3
5	2.3	1.7	1.6	1.7	1.3	1.5	1.5
6	1.7	1.6	1.7	1.6	1.6	1.2	1.2
7	1.7	2.3	1.8	1.8	1.8	2.1	1.6
8	1.9	1.7	1.6	1.7	1.7	1.5	1.3
9	1.5	1.8	1.8	1.7	1.7	1.5	1.4
10	4.0	3.7	2.9	2.7	2.8	2.4	2.0
11	1.9	1.7	1.6	1.4	1.3	1.2	1.6
12	1.7	1.4	1.2	1.2	1.2	0.9	1.1
13	.	.	.	.	.	.	.
14	2.1	1.8	1.7	1.5	1.5	1.6	1.4
15	3.3	3.2	3.1	3.7	3.3	3.9	3.5

Alcohol

Pre-treatment

Subject	Base	-5 mmHg	-10 mmHg	-15 mmHg	-20 mmHg	-30 mmHg	-40 mmHg
16	2.2	2.0	2.2	1.9	1.6	1.6	1.4
17	1.9	1.6	1.8	1.6	1.6	1.3	1.5
18	1.7	1.5	1.4	1.3	1.2	1.1	1.2
19	3.7	3.2	2.7	2.9	2.5	2.4	2.7
20	1.9	1.7	1.8	1.7	1.8	2.0	1.7
21	1.5	1.2	1.3	1.2	1.3	1.2	1.3
22	1.6	1.8	1.5	1.3	1.2	1.1	1.3
23	3.8	3.1	4.0	4.0	2.9	4.3	3.2
24	2.4	2.1	2.1	1.9	1.8	2.0	1.5
25	2.3	1.6	2.2	2.2	1.8	1.8	2.3
26	1.6	1.5	1.7	1.6	1.5	1.7	1.5
27	3.5	3.3	3.4	3.0	3.1	3.0	2.9
28	2.2	2.1	2.1	2.0	1.7	1.7	1.5
29	4.5	4.4	5.1	4.2	4.5	4.1	3.4
30	3.0	2.8	2.5	2.1	2.2	1.8	1.4

Post-treatment

Subject	Base	-5 mmHg	-10 mmHg	-15 mmHg	-20 mmHg	-30 mmHg	-40 mmHg
16	2.0	1.8	1.8	1.7	1.6	2.5	1.8
17	1.8	1.9	1.8	1.8	2.6	1.4	1.8
18	2.1	1.8	1.9	1.6	1.7	1.7	1.5
19	2.5	2.4	2.3	2.1	2.2	2.0	2.4
20	1.7	1.6	1.5	1.3	1.4	1.3	1.2
21	1.6	1.5	1.3	1.3	1.4	1.2	1.3
22	1.6	1.4	1.4	1.5	1.7	1.6	1.4
23	2.7	3.1	2.8	2.9	2.8	3.1	3.0
24	1.9	1.4	1.6	1.5	1.7	1.3	1.6
25	2.6	2.6	3.4	2.8	2.8	2.6	2.6
26	2.7	2.0	1.8	1.8	1.6	1.5	2.0
27	2.3	1.9	2.1	2.2	2.2	2.5	2.2
28	1.4	1.5	1.5	1.6	1.6	1.9	1.8
29	4.8	4.9	4.7	4.3	4.0	4.5	5.2
30	3.5	3.2	3.9	3.1	3.0	2.6	1.9

Appendix B-1: Mean values plus/minus SE for pre-treatment

Variable	Group	N	Baseline	-5 mmHg	-10 mmHg	-15 mmHg
SAP	Placebo	14	117 ± 2	117 ± 2	116 ± 2	116 ± 2
mmHg	Alcohol	15	120 ± 3	121 ± 3	121 ± 3	120 ± 3
DAP	Placebo	14	65 ± 2	65 ± 2	65 ± 2	65 ± 2
mmHg	Alcohol	15	64 ± 2	65 ± 2	65 ± 2	65 ± 2
MAP	Placebo	14	82 ± 2	82 ± 2	82 ± 2	82 ± 2
mmHg	Alcohol	15	82 ± 2	83 ± 2	84 ± 2	83 ± 2
HR	Placebo	14	59 ± 3	58 ± 2	60 ± 3	61 ± 3
bpm	Alcohol	15	60 ± 2	60 ± 2	61 ± 2	61 ± 2
MSNA	Placebo	9	14 ± 2	15 ± 2	17 ± 1	20 ± 1
bursts/min	Alcohol	9	15 ± 3	18 ± 3	20 ± 3	23 ± 3
MSNA	Placebo	9	26 ± 3	27 ± 3	30 ± 3	35 ± 3
bursts/100 HB	Alcohol	9	25 ± 6	30 ± 5	33 ± 5	39 ± 6
Total MSNA	Placebo	9	6363 ± 842	7456 ± 1075	8683 ± 877	10643 ± 1182
a.u.	Alcohol	9	6724 ± 1692	10092 ± 1881	12778 ± 2603	14649 ± 2747
FBF	Placebo	13	2.1 ± 0.3	2.0 ± 0.3	2.0 ± 0.3	1.9 ± 0.2
unit	Alcohol	15	2.1 ± 0.2	1.9 ± 0.2	2.0 ± 0.2	1.8 ± 0.2
FVR	Placebo	13	47 ± 5	51 ± 7	50 ± 7	51 ± 6
mmHg/unit	Alcohol	15	45 ± 4	50 ± 4	48 ± 4	52 ± 5
FVC*100	Placebo	13	2.5 ± 0.3	2.4 ± 0.3	2.4 ± 0.4	2.3 ± 0.3
unit/mmHg	Alcohol	15	2.5 ± 0.2	2.3 ± 0.2	2.5 ± 0.2	2.2 ± 0.2

unit = ml/100ml/min

Variable	Group	N	-20 mmHg	-30 mmHg	-40 mmHg
SAP	Placebo	14	116 ± 2	112 ± 2	108 ± 2
mmHg	Alcohol	15	121 ± 3	117 ± 3	114 ± 4
DAP	Placebo	14	66 ± 2	66 ± 2	66 ± 2
mmHg	Alcohol	15	66 ± 2	65 ± 2	66 ± 2
MAP	Placebo	14	82 ± 2	81 ± 2	79 ± 2
mmHg	Alcohol	15	84 ± 2	82 ± 2	81 ± 2
HR	Placebo	14	62 ± 3	69 ± 3	75 ±3
Bpm	Alcohol	15	63 ± 2	67 ± 2	73 ± 2
MSNA	Placebo	9	23 ± 1	28 ± 3	35 ± 2
bursts/min	Alcohol	9	26 ± 3	33 ± 3	37 ± 3
MSNA	Placebo	9	39 ± 2	43 ± 4	49 ± 3
bursts/100 HB	Alcohol	9	42 ± 5	51 ± 4	54 ± 5
Total MSNA	Placebo	9	11736 ± 1029	15676 ± 2001	21214 ± 3642
a.u.	Alcohol	9	15971 ± 2349	21947 ± 3012	27876 ± 4324
FBF	Placebo	13	1.8 ± 0.2	1.7 ± 0.2	1.6 ± 0.2
unit	Alcohol	15	1.7 ± 0.2	1.7 ± 0.2	1.6 ± 0.2
FVR	Placebo	13	52 ± 6	55 ± 6	58 ± 7
mmHg/unit	Alcohol	15	56 ± 5	57 ± 5	59 ± 5
FVC*100	Placebo	13	2.3 ± 0.3	2.1 ± 0.2	2.0 ± 0.2
unit/mmHg	Alcohol	15	2.1 ± 0.2	2.1 ± 0.3	1.9 ± 0.2

unit = ml/100ml/min

Appendix B-2: Mean values plus/minus SE for post-treatment

Variable	Group	N	Baseline	-5 mmHg	-10 mmHg	-15 mmHg
SAP	Placebo	14	123 ± 2	124 ± 2	124 ± 2	124 ± 3
mmHg	Alcohol	15	124 ± 4	125 ± 4	124 ± 4	122 ± 5
DAP	Placebo	14	72 ± 2	73 ± 2	73 ± 2	73 ± 2
mmHg	Alcohol	15	69 ± 2	69 ± 2	69 ± 2	68 ± 2
MAP	Placebo	14	89 ± 1	90 ± 2	89 ± 2	90 ± 2
mmHg	Alcohol	15	87 ± 2	87 ± 2	87 ± 2	86 ± 2
HR	Placebo	14	58 ± 3	58 ± 3	59 ± 3	61 ± 3
Bpm	Alcohol	15	65 ± 2	66 ± 2	67 ± 2	69 ± 2
MSNA	Placebo	9	14 ± 2	16 ± 1	19 ± 2	24 ± 4
bursts/min	Alcohol	9	19 ± 4	21 ± 4	25 ± 5	28 ± 4
MSNA	Placebo	9	25 ± 3	29 ± 3	33 ± 2	41 ± 4
bursts/100 HB	Alcohol	9	29 ± 7	33 ± 6	38 ± 7	41 ± 6
Total MSNA	Placebo	9	5970 ± 714	7590 ± 821	9755 ± 1153	13651 ± 2002
a.u.	Alcohol	9	8046 ± 1680	11391 ± 2218	12887 ± 2867	14699 ± 2675
FBF	Placebo	13	2.0 ± 0.2	1.8 ± 0.2	1.7 ± 0.2	1.7 ± 0.2
unit	Alcohol	15	2.1 ± 0.2	1.9 ± 0.2	2.0 ± 0.2	1.8 ± 0.2
FVR	Placebo	13	50 ± 4	53 ± 4	56 ± 5	57 ± 5
mmHg/unit	Alcohol	15	47 ± 4	51 ± 4	51 ± 4	54 ± 4
FVC*100	Placebo	13	2.2 ± 2.5	2.1 ± 0.2	2.0 ± 0.2	2.0 ± 0.2
unit/mmHg	Alcohol	15	2.4 ± 0.2	2.2 ± 0.3	2.3 ± 0.3	2.1 ± 0.2

unit = ml/100ml/min

Variable	Group	N	-20 mmHg	-30 mmHg	-40 mmHg
SAP	Placebo	14	123 ± 3	121 ± 3	117 ± 4
mmHg	Alcohol	15	120 ± 5	116 ± 4	113 ± 4
DAP	Placebo	14	73 ± 2	74 ± 2	74 ± 3
mmHg	Alcohol	15	68 ± 2	67 ± 2	67 ± 2
MAP	Placebo	14	89 ± 2	88 ± 2	87 ± 2
mmHg	Alcohol	15	85 ± 2	83 ± 2	82 ± 2
HR	Placebo	14	63 ± 3	70 ± 3	79 ± 5
Bpm	Alcohol	15	70 ± 2	73 ± 2	80 ± 2
MSNA	Placebo	9	27 ± 4	32 ± 4	36 ± 3
bursts/min	Alcohol	9	30 ± 5	37 ± 4	42 ± 5
MSNA	Placebo	9	43 ± 5	47 ± 4	48 ± 4
bursts/100 HB	Alcohol	9	43 ± 6	52 ± 5	53 ± 5
MSNA	Placebo	9	14685 ± 2442	19951 ± 2720	26731 ± 2617
total area	Alcohol	9	17384 ± 3177	24219 ± 3985	29742 ± 4781
FBF	Placebo	13	1.7 ± 0.2	1.6 ± 0.2	1.5 ± 0.2
unit	Alcohol	15	1.9 ± 0.2	1.7 ± 0.2	1.7 ± 0.2
FVR	Placebo	13	59 ± 5	63 ± 6	64 ± 5
mmHg/unit	Alcohol	15	51 ± 4	54 ± 5	54 ± 5
FVC*100	Placebo	13	1.9 ± 0.2	1.9 ± 0.3	1.8 ± 0.2
unit/mmHg	Alcohol	15	2.2 ± 0.2	2.1 ± 0.2	2.1 ± 0.3

unit = ml/100ml/min

Appendix C-1: Repeated Measures ANOVA for baseline measurements

Baseline SAP

Mauchley's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Huynh-Feldt
treatment	1.000	0.000	0	.	1.000

Test of Within-Subjects Effects

Source	Type III Sum of Squares	df	Mean Square	F	Sig. (1-tailed)
treatment*drug	3.902	1.000	3.902	0.119	0.37

Baseline DAP

Mauchley's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Huynh-Feldt
treatment	1.000	0.000	0	.	1.000

Test of Within-Subjects Effects

Source	Type III Sum of Squares	df	Mean Square	F	Sig. (1-tailed)
treatment*drug	22.355	1.000	22.355	0.842	0.18

Baseline MAP

Mauchley's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Huynh-Feldt
treatment	1.000	0.000	0	.	1.000

Test of Within-Subjects Effects

Source	Type III Sum of Squares	df	Mean Square	F	Sig. (1-tailed)
treatment*drug	15.073	1.000	15.073	0.753	0.20

Baseline HR

Mauchley's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Huynh-Feldt
treatment	1.000	0.000	0	.	1.000

Test of Within-Subjects Effects

Source	Type III Sum of Squares	df	Mean Square	F	Sig. (2-tailed)
treatment*drug	63.370	1.000	63.670	9.812	0.004

Baseline MSNA (bursts/min)

Mauchley's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Huynh-Feldt
treatment	1.000	0.000	0	.	1.000

Test of Within-Subjects Effects

Source	Type III Sum of Squares	df	Mean Square	F	Sig. (1-tailed)
treatment*drug	96.148	1.000	96.148	2.781	0.05

Baseline MSNA (bursts/100HB)

Mauchley's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Huynh-Feldt
treatment	1.000	0.000	0	.	1.000

Test of Within-Subjects Effects

Source	Type III Sum of Squares	df	Mean Square	F	Sig. (1-tailed)
treatment*drug	141.750	1.000	141.750	1.724	0.10

Baseline Total MSNA

Mauchley's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Huynh-Feldt
treatment	1.000	0.000	0	.	1.000

Test of Within-Subjects Effects

Source	Type III Sum of Squares	df	Mean Square	F	Sig. (1-tailed)
treatment*drug	1.789E+07	1.000	1.789E+07	1.728	0.10

Baseline FBF

Mauchley's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Huynh-Feldt
treatment	1.000	0.000	0	.	1.000

Test of Within-Subjects Effects

Source	Type III Sum of Squares	df	Mean Square	F	Sig. (1-tailed)
treatment*drug	0.023	1.000	0.023	0.163	0.35

Baseline FVR

Mauchley's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Huynh-Feldt
treatment	1.000	0.000	0	.	1.000

Test of Within-Subjects Effects

Source	Type III Sum of Squares	df	Mean Square	F	Sig. (1-tailed)
treatment*drug	17.859	1.000	17.859	0.199	0.33

Baseline FVC

Mauchley's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Huynh-Feldt
treatment	1.000	0.000	0	.	1.000

Test of Within-Subjects Effects

Source	Type III Sum of Squares	df	Mean Square	F	Sig. (1-tailed)
treatment*drug	3.473E-06	1.000	3.473E-06	0.144	0.35

Appendix C-2: Repeated Measures ANOVA for blood pressures

ΔSAP

Mauchley's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Huynh-Feldt
Treatment	1.000	0.000	0	.	1.000
Time	0.001	167.270	20	0.000	0.288
Treatment * Time	0.062	69.047	20	0.000	0.536

Test of Within-Subjects Effects

Source	Type III Sum of Squares	df	Mean Square	F	Sig. (1-tailed)
Treatment	2071.313	1.000	2071.313	13.407	0.001
Time	3385.479	1.730	1956.979	23.772	0.000
Treatment * Time	54.903	3.216	17.072	0.903	0.225
Treatment * Time * Drug	243.467	3.216	75.704	4.006	0.005

ΔDAP

Mauchley's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Huynh-Feldt
Treatment	1.000	0.000	0	.	1.000
Time	0.001	162.545	20	0.000	0.328
Treatment * Time	0.012	110.417	20	0.000	0.367

Test of Within-Subjects Effects

Source	Type III Sum of Squares	df	Mean Square	F	Sig. (1-tailed)
Treatment	2673.830	1.000	2673.830	19.781	0.000
Time	10.327	1.968	5.246	0.274	0.379
Treatment * Time	62.708	2.203	28.470	3.264	0.021
Treatment * Time * Drug	45.451	2.203	20.635	2.366	0.049

ΔMAP

Mauchley's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Huynh-Feldt
Treatment	1.000	0.000	0	.	1.000
Time	0.002	156.346	20	0.000	0.335
Treatment * Time	0.023	93.828	20	0.000	0.413

Test of Within-Subjects Effects

Source	Type III Sum of Squares	df	Mean Square	F	Sig. (1-tailed)
Treatment	2297.701	1.000	2297.701	27.084	0.000
Time	598.921	2.008	298.341	10.546	0.000
Treatment * Time	88.504	2.478	35.713	2.916	0.025
Treatment * Time * Drug	69.373	2.478	27.993	2.285	0.049

Appendix C-3: Repeated Measures ANOVA for heart rate

ΔHR

Mauchley's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Huynh-Feldt
Treatment	1.000	0.000	0	.	1.000
Time	0.000	205.027	20	0.000	0.274
Treatment * Time	0.011	111.207	20	0.000	0.345

Test of Within-Subjects Effects

Source	Type III Sum of Squares	df	Mean Square	F	Sig. (2-tailed)
Treatment	1229.545	1.000	1230	14.825	0.002
Time	12205.339	1.645	7420	119.257	0.000
Treatment * Time	96.019	2.069	46.417	2.305	0.108
Treatment * Time * Drug	44.995	2.069	21.751	1.080	0.348

Appendix C-4: Repeated Measures ANOVA for MSNA

ΔMSNA bursts/min

Mauchley's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Huynh-Feldt
Treatment	1.000	0.000	0	.	1.000
Time	0.002	84.597	20	0.000	0.379
Treatment * Time	0.003	80.634	20	0.000	0.531

Test of Within-Subjects Effects

Source	Type III Sum of Squares	df	Mean Square	F	Sig. (1-tailed)
Treatment	698.501	1	698.501	2.311	0.074
Time	14049.910	2	6178.816	117.767	0.000
Treatment * Time	56.723	3.186	17.806	0.693	0.285
Treatment * Time * Drug	41.724	3.186	13.097	0.510	0.344

ΔMSNA bursts/100 HB

Mauchley's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Huynh-Feldt
Treatment	1.000	0.000	0	.	1.000
Time	0.011	61.973	20	0.000	0.542
Treatment * Time	0.029	48.942	20	0.000	0.671

Test of Within-Subjects Effects

Source	Type III Sum of Squares	df	Mean Square	F	Sig. (1-tailed)
Treatment	385.914	1.000	385.914	0.910	0.177
Time	18620.876	3.251	5727.526	74.564	0.000
Treatment * Time	155.472	4.028	38.600	0.974	0.215
Treatment * Time * Drug	131.529	4.028	32.656	0.824	0.258

ΔTotal MSNA

Mauchley's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Huynh-Feldt
Treatment	1.000	0.000	0	.	1.000
Time	0.000	106.951	20	0.000	0.325
Treatment * Time	0.001	100.994	20	0.000	0.304

Test of Within-Subjects Effects

Source	Type III Sum of Squares	df	Mean Square	F	Sig. (1-tailed)
Treatment	1.992E+08	1.000	1.992E+08	0.634	0.219
Time	1.003E+10	1.952	5.138E+09	95.252	0.000
Treatment * Time	9.348E+07	1.821	5.133E+07	0.771	0.231
Treatment * Time * Drug	5.401E+07	1.821	2.965E+07	0.445	0.314

Appendix C-5: Repeated Measures ANOVA for forearm blood flows

ΔFBF

Mauchley's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Huynh-Feldt
Treatment	1.000	0.000	0	.	1.000
Time	0.004	133.278	20	0.000	0.316
Treatment * Time	0.102	54.266	20	0.000	0.668

Test of Within-Subjects Effects

Source	Type III Sum of Squares	df	Mean Square	F	Sig. (1-tailed)
Treatment	0.668	1.000	0.668	0.911	0.175
Time	7.989	1.898	4.209	15.850	0.000
Treatment * Time	0.333	4.009	0.083	1.170	0.164
Treatment * Time * Drug	0.086	4.009	0.022	0.303	0.438

ΔFVR

Mauchley's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Huynh-Feldt
Treatment	1.000	0.000	0	.	1.000
Time	0.028	85.145	20	0.000	0.507
Treatment * Time	0.058	67.851	20	0.000	0.611

Test of Within-Subjects Effects

Source	Type III Sum of Squares	df	Mean Square	F	Sig. (1-tailed)
Treatment	580.244	1.000	580.244	0.862	0.181
Time	5043.282	3.045	1656.502	19.884	0.000
Treatment * Time	194.336	3.664	53.044	0.908	0.228
Treatment * Time * Drug	441.426	3.664	120.487	2.062	0.049

ΔFVC

Mauchley's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Huynh-Feldt
Treatment	1.000	0.000	0	.	1.000
Time	0.006	123.620	20	0.000	0.335
Treatment * Time	0.124	49.595	20	0.000	0.730

Test of Within-Subjects Effects

Source	Type III Sum of Squares	df	Mean Square	F	Sig. (1-tailed)
Treatment	0.000	1.000	0.000	2.676	0.057
Time	0.001	2.012	0.000	10.874	0.000
Treatment * Time	7.456E-05	4.380	1.702E-05	1.921	0.053
Treatment * Time * Drug	2.468E-05	4.380	5.63E-06	0.636	0.326

Appendix D-1: Post-hoc paired t-tests for baseline measurements

SAP (Alcohol)

Pairing	Mean	95% Confidence Interval		t	df	Sig. (1-tailed)
		Lower	Upper			
Pre-baseline vs Post-baseline	4.797	-9.443	-0.150	2.210	14	0.022

SAP (Placebo)

Pairing	Mean	95% Confidence Interval		t	df	Sig. (1-tailed)
		Lower	Upper			
Pre-baseline vs Post-baseline	5.860	-9.693	-2.027	3.279	14	0.003

DAP (Alcohol)

Pairing	Mean	95% Confidence Interval		t	df	Sig. (1-tailed)
		Lower	Upper			
Pre-baseline vs Post-baseline	4.653	-8.851	-0.456	2.378	14	0.016

DAP (Placebo)

Pairing	Mean	95% Confidence Interval		t	df	Sig. (1-tailed)
		Lower	Upper			
Pre-baseline vs Post-baseline	7.160	-10.569	-3.751	4.505	14	0.000

MAP (Alcohol)

Pairing	Mean	95% Confidence Interval		t	df	Sig. (1-tailed)
		Lower	Upper			
Pre-baseline vs Post-baseline	4.700	-8.055	-1.345	3.005	14	0.005

MAP (Placebo)

Pairing	Mean	95% Confidence Interval		t	df	Sig. (1-tailed)
		Lower	Upper			
Pre-baseline vs Post-baseline	6.773	-10.069	-3.477	4.408	14	0.001

HR (Alcohol)

Pairing	Mean	95% Confidence Interval		t	df	Sig. (2-tailed)
		Lower	Upper			
Pre-baseline vs Post-baseline	5.187	-7.799	-2.574	4.259	14	0.002

MSNAbursts/min (Alcohol)

Pairing	Mean	95% Confidence Interval		t	df	Sig. (1-tailed)
		Lower	Upper			
Pre-baseline vs Post-baseline	-5.985	-12.516	0.547	-1.996	12	0.035

Appendix D-2: Post-hoc Paired T-tests

ΔMAP (Alcohol)

Pairing	Mean	95 % Confidence Interval		t	df	Sig. (1-tailed)
		Lower	Upper			
Pre -5 mmHg vs Post -5 mmHg	0.827	-0.288	1.942	1.590	14	0.067
Pre -10 mmHg vs Post -10 mmHg	1.467	-0.320	3.253	1.761	14	0.050
Pre -15 mmHg vs Post -15 mmHg	2.280	0.458	4.102	2.683	14	0.009
Pre -20 mmHg vs Post -20 mmHg	4.133	1.336	6.930	3.170	14	0.004
Pre -30 mmHg vs Post -30 mmHg	4.293	1.528	7.058	3.330	14	0.003
Pre -40 mmHg vs Post -40 mmHg	4.500	1.594	7.406	3.321	14	0.003

ΔSAP (Alcohol)

Pairing	Mean	95% Confidence Interval		t	df	Sig. (1-tailed)
		Lower	Upper			
Pre -5 mmHg vs Post -5 mmHg	0.960	-0.451	2.371	1.489	14	0.084
Pre -10 mmHg vs Post -10 mmHg	2.160	-0.432	4.752	1.787	14	0.048
Pre -15 mmHg vs Post -15 mmHg	3.600	1.019	6.180	2.992	14	0.005
Pre -20 mmHg vs Post -20 mmHg	5.587	1.353	9.820	2.831	14	0.070
Pre -30 mmHg vs Post -30 mmHg	5.727	1.850	9.603	3.168	14	0.004
Pre -40 mmHg vs Post -40 mmHg	5.633	1.793	9.474	3.146	14	0.004

ΔDAP(Alcohol)

Pairing	Mean	95% Confidence Interval		t	df	Sig. (1-tailed)
		Lower	Upper			
Pre -5 mmHg vs Post -5 mmHg	0.453	-0.440	1.346	1.089	14	0.148
Pre -10 mmHg vs Post -10 mmHg	0.653	-0.464	1.771	1.254	14	0.116
Pre -15 mmHg vs Post -15 mmHg	1.187	0.035	2.338	2.211	14	0.022
Pre -20 mmHg vs Post -20 mmHg	2.873	0.844	4.903	3.036	14	0.005
Pre -30 mmHg vs Post -30 mmHg	3.353	1.110	5.592	3.212	14	0.003
Pre -40 mmHg vs Post -40 mmHg	3.730	1.239	6.228	3.210	14	0.003

ΔFVR(Alcohol)

Pairing	Mean	95% Confidence Interval		t	df	Sig. (1-tailed)
		Lower	Upper			
Pre -5 mmHg vs Post -5 mmHg	1.529	-3.679	6.737	0.630	14	0.270
Pre -10 mmHg vs Post -10 mmHg	-0.797	-5.421	3.827	-0.370	14	0.359
Pre -15 mmHg vs Post -15 mmHg	0.906	-3.331	5.144	0.459	14	0.327
Pre -20 mmHg vs Post -20 mmHg	7.381	0.595	14.167	2.333	14	0.018
Pre -30 mmHg vs Post -30 mmHg	5.204	-4.477	14.885	1.153	14	0.134
Pre -40 mmHg vs Post -40 mmHg	6.866	-0.083	13.814	2.119	14	0.026

Appendix E: Copyright clearance

Figure 4.1 A screenshot of the American Physiological Society's copyright permission page. This can be found on their web page at http://www.theaps.org/mm/Publications/Copyright-and-Permissions

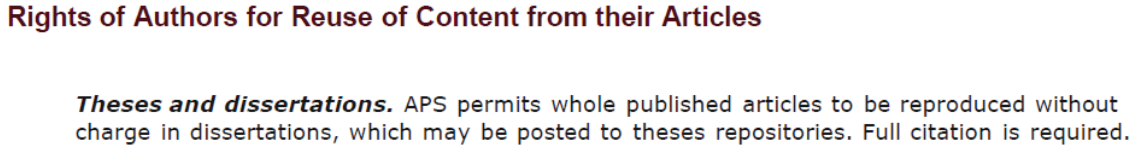

Figure 4.2 A screenshot of the American Physiological Society's copyright permission page stating the rights of authors to reuse published articles in theses. This can be found on their web page at http://www.theaps.org/mm/Publications/Copyright-and-Permissions